Green biorefinery – the production, isolation and polishing of amino acids from grass silage juice at pilot and laboratory scale

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Nur Geduld! Mit der Zeit wird aus Gras Milch
(unbekannter Verfasser)

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Abstract

As humankind is confronted with numerous problems, such as climate change and the depletion of fossil fuels, alternative resources and sources of supply have to be found. Not only for the supply of energy, but also for the supply of basic compounds; which are called platform chemicals and widely needed by the chemical industry for the production of commodities used on a daily basis. In this context, the application of comprehensive biorefinery concepts would support not only the usage of biomass for energy purposes, but also for the generation of biobased compounds. First and foremost, Austrian research on this issue was focussed on the extraction of valuable compounds from grass and grass silage and therefore on the so-called “Green biorefinery”. In the last few decades, different aspects regarding the usage of grass and grass silage were investigated in Austria. Based on these research activities, the first Austrian Green biorefinery demonstration plant was established in Utzenaich (Upper Austria) in 2008. The overall target of the demonstration plant on a pilot-scale was to test whether the generation of lactic acid and amino acids from the raw material of grass silage is possible on a pre-industrial level.

The plant, which was used for the generation of the valuable compounds, consisted of a cascade of different downstream technologies. Next to membrane technologies, an ion exchanger device constisting of five columns, was used. This process represented the core process for the production of amino acids from grass silage juice. The presented work focusses on investigating the applicability and feasibility of this ion exchange process equipped with a strong cation exchanger resin for the extraction of amino acids from pre-treated silage juice. For instance, assumed process operation methods were implemented and gradually improved. Furthermore, the behaviour of starting materials obtained from different pre-treament steps of the Green biorefinery demonstration plant and two strong cation exchange resins were investigated in the ion exchange process. Next to this process parameter, other parameters such as varying flow rates at the loading step of the ion exchanger process were tested. The process parameters were varied and observed, as the yields of amino acids per batch run should be improved to provide an efficient production process and well defined amino acid products.

Basically, it was possible to produce three different, purified amino acid solutions by applying the presented ion exchange process:

- Product 1A: solutions of amino acids enriched with acidic amino acids
- Product 1B: solutions of amino acids enriched with neutral amino acids
- Product 2: solutions of amino acids enriched with neutral alkaline amino acids (contained also ammonia)

Independent of the starting material and the ion exchange resin used, yields of 6.33 to 8.30 kg of amino acids per batch run were obtained. In general, higher yields were obtained when using the resin Dowex Monosphere C-350 and applying starting material, which had previously been ultrafiltered and nanofiltered. On average, it was feasible to transfer 77 % of the amino acids introduced to product fractions. The remaining amino acids (33 %) were, in turn, found in fractions where their extraction would be more complex. For instance, some amino acids were eluted from columns with a very low yield or found within the pre- or post-product fraction, which was much more diluted than the product fractions. The ratio between inorganic cations and amino acids in the ion exchange feed mainly influenced the yield per batch run. The reason of this effect relied to the current process operation method of the ion exchanger, as the breakthrough of inorganic cations from column 2 to column 3 indicated the point to stop the loading. Therefore, higher amino acid concentrations in the feed led to higher amino acid yields as the volume used for loading depended on the amount of inorganic cations in the feed. Furthermore, it was noted that variations of other process

parameters had only a low impact. For instance, only small differences were observed by comparing the resins' performances regarding the yields of amino acids and the separation behaviour. By applying the resin with a smaller mean particle size, no considerable improvements regarding the separations between inorganic cations and amino acids and the individual amino acid groups themselves were observed.

In addition to experiments at the Green biorefinery demonstration plant, experiments were also performed in order to polish the amino acid products obtained at laboratory scale. For instance, methods for decolourisation of the amino acid product solutions were tested, where the best results were obtained with activated carbon. Furthermore, possible routes for further fractionation of the existing amino acid product solutions were tested and combined. Among others, it was possible to produce a powder enriched with aspartic acid and a powder consisting mainly of branched amino acids (leucine, isoleucine and valin) by the means of a rotary evaporator and crystallisation.

Zusammenfassung

Nicht nur für die Versorgung mit Energie, sondern auch für die Versorgung mit Grundstoffen, den sogenannten Plattformchemikalien, die beispielsweise in der chemischen Industrie Anwendung finden, müssen angesichts des drohenden Klimawandels und dem zu Neige gehen von fossilen Resourcen, Alternativen gefunden werden. Eine Möglichkeit, um Biomasse neben der Nutzung zur Energiegewinnung auch einer stofflichen Verwertung zuzuführen, besteht in der Anwendung von umfassenden Bioraffineriekonzepten. Österreichische Forschungsarbeiten in diesem Gebiet spezialisierten sich vor allem auf die Gewinnung von Wertstoffen aus Gras und Grassilage und damit auf das Konzept der sogenannten “Grünen Bioraffinerie”. Basierend auf umfassenden Forschungstätigkeiten in diesem Bereich, wurde im Jahr 2008 die erste österreichische “Grüne Bioraffinerie” in Form einer Demonstrationsanlage am Standort Utzenaich in Oberösterreich eröffnet. Ziel dieser Anlage war es die Gewinnung von Milchsäure und Aminosäuren aus Grassilagesaft im vor-industriellen Maßstab zu untersuchen.

Die Anlage, die zur Gewinnung der Wertstoffe vorgesehen war, bestand aus einer Kaskade von verschiedenen Downstream-Technologien. Neben Membrantechnologien kam auch ein Ionentauscher bestehend aus fünf Säulen, der den Kernprozess für die Gewinnung von gereinigten Aminosäurelösungen darstellte, zum Einsatz. Die vorliegende Arbeit beschäftigte sich nun intensiv mit der Anwendbarkeit des Ionentauschprozesses, welcher mit einem starken Kationenaustauscherharz betrieben wurde, zur Gewinnung von Aminosäuren aus vorbehandeltem Grassilagesaft. Beispielsweise wurden im Vorfeld generierte Prozessbetriebsweisen umgesetzt und sukzessive verbessert, aber auch das Verhalten von Startmaterialien aus unterschiedlichen Vorbehandlungsstufen wurde am Ionentauscher getestet. Weiters wurde auch die Anwendung von unterschiedlichen, starken Kationenaustauscherharze untersucht. Daneben wurden weitere Prozessparameter, wie zum Beispiel die Flussraten im Beladungsschritt des Ionentauschprozesses, variiert. Das Ziel hinter all diesen Prozessvariationen bestand darin, die Ausbeute pro Batch-Versuch zu optimieren, um so effizient wie möglich produzieren zu können und möglichst definierte Aminosäurenprodukte zu erzeugen.

Grundsätzlich konnten unter Verwendung des vorliegenden Ionentauschprozesses drei unterschiedliche, gereinigte Aminosäurelösungen hergestellt werden:

- Produkt 1A: Aminosäurelösung angereichert mit sauren Aminosäuren
- Produkt 1B: Aminosäurelösung angereichert mit neutralen Aminosäuren
- Produkt 2: Aminosäurelösung angereichert mit neutralen und basischen Aminosäuren (enthält daneben auch Ammoniak)

Die Aminosäurenausbeuten pro Batch-Versuch lagen, abhängig vom eingesetzten Ausgangsmaterial für den Ionentauscherprozess sowie vom verwendeten Harz, im Bereich von 6,33 bis 8,30 kg. Höhere Ausbeuten wurden dabei unter Verwendung des Harzes Dowex Monosphere C-350 und mit Silagesaft, der vor der Verwendung am Ionenaustauscher sowohl ultra- als auch nanofiltriert wurde, erzielt. Durchschnittlich war es möglich, dass 77 %, der im Prozess eingesetzten Aminosäuren, in Produktfraktionen überführt wurden. Der restliche Teil der eingesetzten Aminosäuren (33 %) wurde hingegen in Fraktionen überführt, die nur mit erheblichen Aufwand gewonnen werden können, da entweder die Ausbeute von den eluierten Säulen zu gering war (Säule 1 und Säule 2) oder die Fraktionen zu verdünnt waren (Vor-Produkt- und Nach-Produktphase). Als Haupteinflussfaktor auf die Aminosäuren-Ausbeute pro Batchversuch wurde das Verhältnis zwischen anorganischen Kationen und Aminosäuren im Ionentauscher-Ausgangsmaterial identifiziert. Da bei der vorliegenden Prozessführungsmethode der Durchbruch der anorganischen Kationen von Säule 2 auf Säule 3 den jeweiligen Endpunkt der Beladung

darstellte, führten vergleichsweise höhere Aminosäurekonzentrationen im Ausgangsmaterial auch zu den höheren Ausbeuten, da sich das aufgetragene Volumen immer durch die Konzentration an anorganischen Kationen ergab. Weiters wurde auch festgestellt, dass der Einfluss der übrigen untersuchten Prozessparameter nur von geringer Bedeutung war. So zeigten Vergleiche der Ergebnisse der Harze nur geringfügige Unterschiede in der Ausbeute und auch eine Verbesserung der Trennung, einerseits zwischen anorganischen Kationen und Aminosäuren, um einen besser definierten Beladungsstopp zu erzielen, und anderseits zwischen den einzelnen Aminosäuregruppen, konnte durch Einsatz des Harzes mit der geringeren Partikelgröße nicht umgesetzt werden.

Neben den Versuchen an der Demonstrationsanlage umfasste die vorliegende Arbeit auch Experimente im Labormaßstab, die eine weitere Verbesserung der Produkte, welche an der *GBR Oberösterreich* produziert wurden, zum Ziel hatten. Beispielsweise wurden Möglichkeiten zur Entfärbung der Produktlösungen untersucht, wofür sich schlussendlich Aktivkohle als Ziel führend herausstellte. In weiterer Folge wurden auch mögliche Routen zur weiteren Fraktionierung der Aminosäurelösungen untersucht. Mit Hilfe eines Rotationseindampfers sowie Kristallisationen konnte zum Beispiel ein Pulver reich an Asparaginsäure sowie ein Pulver, welches zu einem großen Teil aus den verzweigtkettigen Aminosäuren (BCAAs) Leucin, Isoleucin und Valin besteht, hergestellt werden.

Table of contents

Abbreviations

AA	amino acid
AAs	amino acids
Ala	alanine
AM	arithmetic mean
Amm	ammonia
Arg	arginine
Asn	asparagine
Asp	aspartic acid
BCAAs	branched chain amino acids
CP	checkpoint
CPs	checkpoints
CS	chemical score
Cys	cysteine
DC	displacement chromatography
DS	dry substance
eq	mol equivalents
GABA	γ-amino butyric acid
GBR	Green biorefinery
Glu	glutamic acid
Gly	glycine
His	histidine
HPLC	High performance liquid chromatography
IE	ion exchanger
Ile	isoleucine
LA	lactic acid
L-DOPA	L-3,4-dihydroxyphenylalanine
Leu	leucine
Lys	lysine
Met	methionine
n.a.	not analysed
NF	nanofiltration unit
NFs	nanofiltration units
NF2	2nd nanofiltration unit
n.s.	not specified

Phe	phenylalanine
pI	isoelectrical point
Pro	proline
RO	reversed osmosis
Ser	serine
SD	standard deviation
Thr	threonine
TLC	thin layer chromatography
Trp	tryptophan
Tyr	tyrosine
UF	ultrafiltration unit
Val	valine
w/o	without

1 Introduction

"Challenges require new ideas and strategies" – In this day and age, humankind is confronted with numerous, mostly self-made problems, such as climate change and its unpredictable effects, environmental pollution or the shortage of fossil resources. These problems have to be solved immediately in order to provide future generations with a life worth living. The capability to make at least a small contribution towards a new, sustainable future is seen in the extensive use of biomass, applying so called biorefinery concepts.

1.1 Green biorefinery

1.1.1 Fundamentals of biorefineries

Several definitions exist in literature that try to characterise the term "biorefinery". For instance, in the "Energy, Environmental, and Economics (E^3) Handbook" of the United States Department of Energy from 1997, the following explanation for biorefineries is quoted:

> *A biorefinery is an overall concept of a processing plant where biomass feedstocks are converted and extracted into a spectrum of valuable products, based on the petrochemical refinery (U. S. Department of Energy 1997)*

Another definition was produced by the *IEA Bioenergy Task 42* "Biorefineries" about 10 years later:

> *Biorefining is the sustainable processing of biomass into a spectrum of marketable Biobased Products and Bioenergy*
> *Biobased Products: chemicals & materials, but also human food & animal feed*
> *Bioenergy: fuels, power and/or heat (IEA Bioenergy)*

Generally, it can be said that the main goal of all definitions for biorefineries found in literature is summarised very well by the following, more precise explanation according to Cherubini:

> *The biorefinery concept embraces a wide range of technologies able to separate biomass resources (wood, grasses, corn…) into their building block (carbohydrates, proteins, triglycerides…) which can be converted to value added products, biofuels and chemicals. A biorefinery is a facility (or a network of facilities) that integrates biomass conversion processes and equipment to produce transportation biofuels, power, and chemicals from biomass (Cherubini 2010).*

Analogously to petroleum refineries, the target of biorefineries should be the production of multiple products, such as fuels, energy, materials and chemicals, but using CO_2-neutral, renewable, biological feedstock instead of fossil resources. Therefore, the aim of biorefinery concepts is also to upgrade biological raw materials by converting them into different products (Cherubini 2010, Lindorfer 2009, Kamm, Gruber et al. 2006). An overview of the basic principles of a biorefinery that was designed by Kamm and Kamm (Kamm, Kamm 2004) is shown consequently (Figure 1-1).

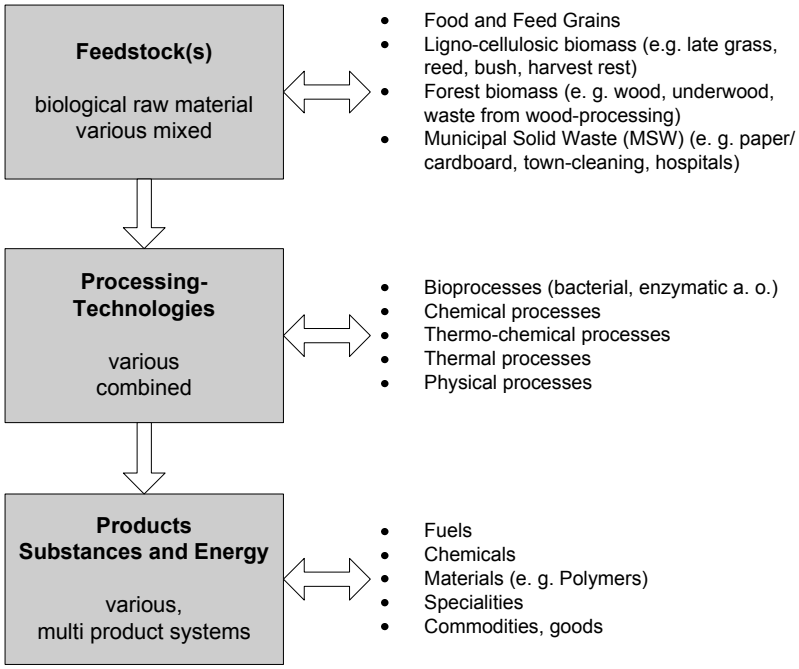


Figure 1-1: Basic principles of a biorefinery according to Kamm and Kamm (Kamm, Kamm 2004)

Next to the term “biorefinery”, the term “biomass” should also be specified. According to the Energy, Environmental, and Economics (E^3) Handbook” of the United States Department of Energy from 1997, biomass is defined as follows:

> *Biomass is defined in the Energy Security Act (P.L. 96-294) as any organic matter that is available on a renewable basis, including agricultural crops and agricultural wastes and residues, wood and wood wastes and residues, animal wastes, municipal wastes, and aquatic plants (U. S. Department of Energy 1997)*

Generally, despite its numerous structures and components biomass itself can be classified on the basis of four different basic structures (Kamm, Gruber et al. 2006):

- Carbohydrates (sugar, starch, cellulose and hemicelluloses)
- Lignin (polyphenols)
- Triglycerides and lipids (vegetable oils and animal fat)
- Proteins

Furthermore, biomass, which is used in biorefineries, is first and foremost accumulated in the following sectors (Cherubini 2010):

- Agriculture (dedicated crop and residues)
- Forestry
- Industry (process residues and leftovers) and households (municipal solid waste and wastewaters)
- Aquaculture

In fact, the term “biorefinery” was only established in the early 1990s, as in this decade the utilisation of renewable resources had begun to boom, although the fractionation of biomass to obtain valuable ingredients had been done before. For instance, in as early as 1773 a

French chemist extracted proteins from lucerne. More comprehensive concepts used to obtain compounds from biomass were then realised in the 20th century. In the 1970s, for example, technologies were developed for extracting protein-xanthophyll-fractions from green plants. Furthermore, concepts for the usage of fast-growing and lignin-containing grasses were generated in China and in the United States of America at the beginning of the 1980s. At the same time, concepts, which combine the production of silage, crude protein and biogas from green fodder and residues, were also emerging (Kamm, Gruber et al. 2006, Kamm 2008).

Nowadays, different biorefinery concepts at different stages of development are known of. Based on the numerous applied raw materials, technologies and products, there are many different possibilities for classifying them. For instance, according to the IEA Bioenergy Task 42 Biorefinery energy-driven and product-driven biorefineries can be distinguished (IEA Bioenergy):

- *Energy-driven biorefineries*

The main goal of energy-driven biorefineries is the production of one or more secondary energy carriers – fuels, power and/or heat – from biomass. Process residues are upgraded and valorised to Biobased Products to maximise the economic profitability of the full biomass-to-products chain.

- *Product-driven biorefineries*

The main goal of product-driven biorefineries is the production of one or more Biobased Products – chemicals, materials, food and/or feed – from biomass. Process residues are used for the production of Bioenergy for internal/external use to maximise the economic profitability of the full biomass-to-products chain.

Incidentally, it has to be noted that renewable resources for the generation of biobased substances are limited to biomass, in particular to plant biomass. Various renewable resources (e.g. wind, sun, biomass, etc.), in turn, can be used for producing energy (Kamm, Kamm 2004).

Furthermore, biorefinery concepts are classified by the character of the feedstock used. This form of classification is widespread in research and development. According to Kamm et al. (Kamm, Gruber et al. 2006) four different biorefinery systems are known:

- Lignocellulosic feedstock biorefinery

In this biorefinery concept “nature-dry” raw material, such as cereals (straw, chaff), ligno-cellulosic biomass (e.g. reed, reed grass), biomass from forests and paper- and cellulosic municipal solid waste, is used. Interesting products, among numerous other products, of this biorefinery concept could be hydroxyfurfural and furfural. The latter is a starting material for the production of Nylon 6,6 and Nylon 6.

- Whole crop biorefinery

In this biorefinery concept whole plants of cereals (e.g. rye, wheat, maize) are processed. The products produced depend on the respective starting material and on the form of processing. For instance, possible final products, among others, could be ethanol, bio-plastic, organic acids, dextrins etc.

- Two-platform biorefinery concept

This biorefinery concept combines a “sugar-platform” and a “syngas platform”. Within the sugar platform, sugar or starch is biochemically converted into fuels, chemicals or substances. The syngas platform, in turn, comprises of the thermochemical use of lignocellulosic raw materials.

- Green biorefinery concept

In this biorefinery concept “nature-wet” biomasses such as green grass, alfalfa, clover or immature cereals are used. This concept is discussed in detail in the following chapter.

Concluding this chapter, it must be mentioned that biorefinery concepts and the generation of different products from one biological raw material is currently an important and active research issue. Therefore, discovering new raw materials for biorefineries or the development of new concepts can occur currently.

1.1.2 Fundamentals on Green biorefineries

As the work presented was carried out at a “Green biorefinery demonstration plant”, the Green biorefinery (GBR) concept will be discussed in more detail.

1.1.2.1 Definition and raw materials of the Green biorefinery

According to Kamm and Kamm (Kamm, Kamm 2004), in 1997 the term “Green biorefinery” was explained in the following way:

> *Green biorefineries represent complex (to fully integrated) systems of sustainable, environment- and resource-friendly technologies for the comprehensive (holistic) utilisation and the exploitation of biological raw materials in the form of green and residue biomass from a targeted sustainable regional land utilisation.*

That means that in GBRs different biobased products should be produced from regionally harvested, green biomass by applying different, environmentally friendly technologies. In other words, next to organic feedstock, the method of growing, harvesting and processing the resource should also be important factors to consider. In 2004, Kromus et al. (Kromus, Wachter et al. 2004) published a more specific definition of Green biorefinieries:

> *A Green Biorefinery is a concept to utilize green (grassland) biomass as raw material for the production of biobased products like proteins, lactic acids, fibres and energy (via biogas).*

Next to the holistic definitions of the term GBR, it is also possible to define the words included in the term separately: For instance, to “*refine*” originally comes from the French word “raffiner”, which means “to improve something or to purify”. “*Green*” in the context of plants means high concentrations of chlorophyll, nutrients and water and “*bio*” is the Greek word for life (Kamm, Kamm 1999).

Considering the raw materials for GBRs, according to literature different kinds of “green biomass” can be used as raw material for them. First and foremost, the large group of green plant materials (green grass from meadow, willow, extensive willow management) are potential raw materials, but wild fruit and crops, alfalfa and clover as well as immature cereals and plant shoots could also be used. Other possible raw materials can be green harvesting residues from agriculturally cultivated crops, such as sugar beet leaves, hemp scrapes and leaves, residues from flax processing or residues from fresh vegetable production. Furthermore, some waste biomass, such as residues from the plant production or food industry, also has the potential to be used (Kamm, Gruber et al. 2006). Indeed, for large scale GBRs the raw material grass from permanent pasture has enormous potential. Today

the main purpose of grassland cultivation is still the production of fodder for animals, but due to a significant reconstruction of the dairy farming and the meat production sector, grass biomass is becoming more and more a surplus raw material in many European countries (Mandl 2010, Kamm, Gruber et al. 2006). Based on this fact, the GBR concept is also seen as a new opportunity for farmers or at least a way to generate additional income. Furthermore, some other positive side effects, such as the conservation and the improvement of cultivated landscape or the generation of new jobs in rural areas, are mentioned in literature (Kromus, Wachter et al. 2004).

As green biomass contains a high amount of water, the first fractionation step within a GBR mostly comprises of the mechanical separation (press) of the fibre-rich press cake and the nutrient-rich press juice. The press cake mainly consists of cellulose and starch. Next to this bulk materials dyes, pigments and other organic substances can also be ingredients of the fibre-rich fraction. The press juice can contain proteins, free amino acids (AAs), sugars, organic acids, minerals, dyes, enzymes and other organic substances, whereas the effective ingredients and concentrations of them are dependent on the raw material (Kamm, Kamm 1999).

1.1.2.2 International developments and investigations of Green biorefineries

As aforementioned, the extraction of proteins and dyes from green leaves has been a well-known method for centuries and was conducted as early as the 18th century. In the 20th century, research was done into the application of leaf protein concentrates for human nutrition. In particularly, in times of crisis or war, where maintaining the nutrition of people was difficult, research into the extraction of plant proteins, which could be a valuable protein source, was intensified. Indeed, at this time plants for the production of leaf proteins on a large-scale were not successful due to technical problems. In the 1960s the extraction of plant proteins was rediscovered and the development of production plants began again. Finally, the first industrial process for leaf protein extraction, the so-called Rothamsted process, was launched. In the 1970s, this process was further enhanced into the Proxan and Alfaproxan procedure, which are used for the generation of protein-xanthophyll concentrates. The industrial usage of the dyes chlorophyll and carotene, in turn, was implemented even earlier. Already in 1930 the first commercial production of chlorophyll and carotene from alfalfa was established in the United States of America. Chlorophyll was applied in different products, such as toothpastes, shampoos, chewing gums, candies and pharmaceuticals (Kromus, Narodoslawsky et al. 2002, Kamm, Gruber et al. 2006).

However, the purity of the protein concentrates produced from green biomass was too low to meet the quality standards for human nutrition and therefore this application was not possible. Nevertheless, since the late 1980s the French company France Lucerne has run large plants for the production of proteins from plants for feeding animals. At the same time, many plants used for drying fodder to produce fodder pellets were equipped with a press, as this pre-treatment step reduced the energy needed for drying. The Swede R. Carlsson was the first to discover the potential of the so produced press juice. He noticed that this juice from grass biomass, which is rich in proteins, could be an ideal raw material for fermentation and first introduced the term “Green biorefinery” in this context (Kromus, Narodoslawsky et al. 2002).

Based on this green or brown juice produced by pressing green biomass prior to drying, Pauli Kiel and co-workers started to design and to implement the first GBR concepts. First, a fermentation method using lactic acid bacteria to conserve the press juice was established (Andersen, Kiel 2000). Afterwards, research into using this juice for the fermentative production of L-lysine was performed and implemented on a demonstration-scale in Denmark (Thomsen, Bech et al. 2004, Mandl 2010).

In the middle of the 1990s, German researchers also started to work on this issue. For instance, since 1996 research into GBRs and the usage of renewable raw material for the production of chemicals has taken place at the University of Potsdam and at the Institute for agricultural Engineering in Bornim supervised by Birgit and Michael Kamm (Kromus, Narodoslawsky et al. 2002). All over Germany, some green biorefinery pilot plants with different concepts and products have already been established (Mandl 2010). A further demonstration plant with a target producing proteins, fermentation media, animal feed, and biogas from green plants is already planned or implemented (no data available) in Havelland near Berlin (Kamm, Hille et al. 2010). In the last few years, German researchers have also focussed on the usage of press juice or press cake for fermentative purposes, to produce, for instance, aminium lactate and L-lysine-l-lactate (Leiß, Venus et al. 2010) or L-lysine and ethanol (Sieker, Neuner et al. 2010).

Furthermore, in Switzerland strategies for exploiting grass have been implemented in the last decade. The concept comprises of the production of biogas, grass fibres and a feed component from grass. Nowadays, it is particularly successful in the usage of fibres and the production of insulation material for buildings. A pilot plant for the production of these fibres is running in Obre (Switzerland). Another country, where GBR pilot plants have already been established is the Netherlands. In 2004, a pilot plant for the production of proteins out of fresh grass was implemented, but a further up-scaling failed (Mandl 2010). Within the project “Grassa!”, the Dutch work on GBRs was resumed in 2008 (Projekt Grassa!).

Another country with on-going research into GBR concepts is Ireland. For instance, GBR studies into scenarios for the “Green Island” have been published (O'Keeffe, Schulte et al. 2011). Moreover, results of work that have already been put into practise were published: biorefining steps for ryegrass and silage at a pilot scale to extrude fibre cake for the production of nanofibrillated cellulose (Sharma, Carmichael et al. 2012).

1.1.2.3 Austrian developments and investigations into Green biorefineries

Not only researchers in other European countries, but also Austrian researchers were involved in the early stages of development and investigations regarding GBRs. Already, by 1990, Steinmüller and co-workers had performed experiments with grass to obtain ethanol and to explore the grass fibres for paper production. Furthermore, first experiments, where grass silage was pressed, were conducted and a theoretical study on the processing of the obtained lactic acid was developed in the following years. In the late 1990s, an international symposium on “Green Biorefinery” was held twice in Styria. At these conferences the prospective potential of the GBR concept in Austria was also highlighted and in the following years the research activities on this issue were intensified in Austria as well (Kromus, Narodoslawsky et al. 2002).

In 2003, an overview of the *Austrian GBR concept* was published. In contrast to other concepts, the Austrian concept favoured working with fermented grass (grass silage) as raw material instead of fresh grass. Silage was preferred, as the grass undergoes a conservation process and is therefore available the whole year. Within the concept, lactic acid and AAs have been seen as the key products (Kromus, Wachter et al. 2004).

In connection with this Austrian GBR concept, different projects and therein studies and experiments were conducted in between 2000 to 2008. For instance, Danner and co-workers from the IFA-Tulln published results on the extraction and purification of lactic acid from silages in 2000 (Danner, Madzingaidzo et al. 2000). Furthermore, within the frame of the Austrian project series “Fabrik der Zukunft” a lot of investigations were conducted. For instance, among others the following investigations were performed:

- Press experiments with grass silage were performed
- The ingredients of the press juice were analysed
- Possible applications of the grass fibres (e. g. insulation material, degradable pots for breeding plants etc.) were investigated and studied
- Different downstream processes were tested and applied to separate the valuable compounds from the silage juice

Detailed results of these complex research activities are summarised in the respective project reports, which are published under www.nachhaltigwirtschaften.at. Within the frame of these research activities, the research group of Senad Novalin also published different results in relation to downstream processes conducted with grass silage juice. For instance, the application and performance of pressure driven membrane processes, such as ultra- and nanofiltration (Koschuh, Thang et al. 2005) or electrodialysis (Thang, Koschuh et al. 2005) for extracting valuable compounds from grass silage juice have been investigated.

1.1.3 Green biorefinery demonstration plant in Upper Austria

Based on the results of the intensive research into GBRs in Austria, a project consortium under the lead of DI Dr. H. Steinmüller implemented the building and the operation of a "Green biorefinery demonstration plant" in Upper Austria. Next to an existing biogas plant, the "Green biorefinery Upper Austria" was established in Utzenaich (district Ried/Innkreis) in autumn 2008. The overall target of the demonstration plant on a pilot-scale was to test, if the generation of LA and AAs from the raw material of grass silage is also possible on an up-scaled level. Furthermore, the process should be optimised and results for further, possible scaling-ups should be generated.

As already suggested in the Austrian green biorefinery concept in 2004 (Kromus, Wachter et al. 2004), silage from permanent pastures and clover-enriched grass were used as raw material at the *GBR Upper Austria*. Ensiling is a fermentative conservation process for vegetable biomass under anaerobic conditions. During the process microorganisms, in particular lactic acid bacteria, degrade sugar molecules from the plants' cell walls and build lactic acid. The formation of lactic acid reduces the pH value of the grass and therefore other metabolic activities are stopped and the grass can be stored. As grass is a biological resource, different factors affect the ensiling process and therefore the quality of the silage produced. For instance, the time of harvesting affects the chemical structure of the plants. The so called formation of panicles of grasses is seen as the ideal point for harvesting, as older plants contain more cellulose leading to silage with lower quality. The weather has also to be considered, as sunny periods prior to the harvest are important for obtaining a higher sugar content within the plants. Moreover, too wet conditions also can easily lead to bad-quality fermentation processes and therefore to bad-quality silage. Next to the point of harvesting, it is important to cut the grass at an adequate height above the ground to avoid the input of soil and dirt. Soil and dirt within grass support the growth of clostridia, which lead, in turn, to the build up of unwanted butyric acid within the silage. Furthermore, air has to be removed carefully from the grass while filling the silo, otherwise acetic acid bacteria are built leading to an aerobic digestion and therefore to low quality-silage as well. For supporting the ensiling process, so called ensiling starters are often used. For instance, lactic acid bacteria are sprayed on the grass during the wrapping of the silo bales (Flatnitzer 2009, Buchgrabner 1998, Wilhelm, Wurm 1999) However, ideally the ensiling process lasts about 4 to 6 weeks and depending on the climate conditions about 3 to 5 harvests per year are possible.
During the fermentation process the products, which are extracted in the subsequent downstream process, are built. AAs are built through the degradation of proteins by proteinases, which are set free from the plants themselves after cutting the grass (Thimann 1980). As aforementioned, the second product, LA, is formed through lactic acid bacteria through the degradation of sugars. Normally, LA is produced by fermentation or through

chemical synthesis. Traditionally, LA is used in food preservation and processing (e. g. as acidulant, flavouring, pH buffering agent or inhibitor of bacterial spoilage) or in cosmetics. Next to these traditional applications, LA is increasingly used for the production of plastics (PLA) and for the production of ethyl lactate that is a nontoxic and degradable "green solvent". Therefore, the demand for LA, in particular from renewable resources, has grown more and more in the last few decades (Datta, Henry 2006, Lindorfer 2009).

1.1.3.1 Process description of the *GBR Upper Austria* – Basic process

Similar to all theoretical concepts suggested for the use of green, wet biomass, the first step (primary raffination) at the *GBR Upper Austria* comprised of a mechanical fractionation by applying a screw press. The grass silage was separated into a press cake and a press juice. Subsequently, the cellulosic press cake was directly used as substrate within the nearby biogas plant to produce biogas and therefore energy. The silage juice, in turn, was processed in the sophisticated downstream process of the *GBR Upper Austria* to separate LA and AAs.

First and foremost, at the *GBR Upper Austria* technologies where no or only low amounts of chemicals are needed were tried to be selected. Based on results from preliminary projects, a complex process was then designed for the generation of LA and AAs from grass silage juice. This process, which is subsequently called "basic process", was partly based on a patent (Gruene-bioraffinerie.at GmbH 2008) of the company Gruene-bioraffinerie.at GmbH and the process thereof was run on a licence from this company. Within the process, the silage juice was first purified by an ultrafiltration (UF) step. In this filtration step, larger particles were separated and the juice was cleared. Afterwards, the amount of inorganic cations in the cleared juice was reduced by the application of a softening step equipped with a strong cation exchanger resin. The pre-treated silage juice was then applied to the so called "hybrid process", which was the core process of the proposed down-streaming. The hybrid process, which was a cyclic process and not a linear process, consisted of a double-stage nanofiltration (NF) device used for the separation of LA and AAs, a tank for concentrating the solution, two electrodialysis lines and a reversed-osmosis (RO) device. First, the ultrafiltered and softened silage juice was nanofiltered, where large amounts of the LA were transferred into the NF Permeates at both NF-stages and the bulk of AAs remained in the NF retentate. The NF Permeates, enriched with LA, were then treated by the first electrodialysis process, where the amount of inorganic ions, in particularly potassium, was reduced a second time. After this treatment, the NF Permeates were collected in a tank, the so-called steady-state-vessel. Within this vessel, the solution with LA was concentrated by reducing the amount of water through the application of the RO device and at the same time LA was extracted from the solution through the second electrodialysis step. The final LA product was therefore found in the so-called concentrate of the second electrodialysis unit. The water extracted by the RO device, in turn, was used again for dilution purposes at the NF step. Therefore, any water used for the process was recycled. The NF Retentate enriched with AAs was further treated by an ion exchange (IE) process, where purified AA solutions were generated. In order to summarise the process description an overview of the principle streams of the basis process are shown in Figure 1-2. In addition, in Figure 1-3 shows a detailed flow diagram of the hybrid process.

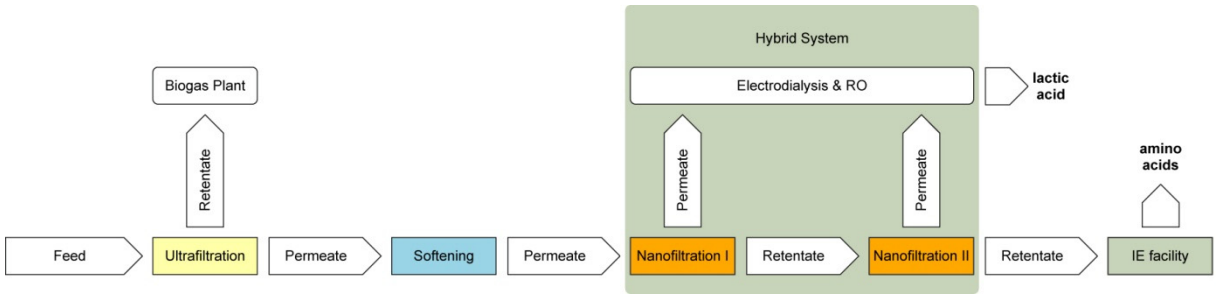
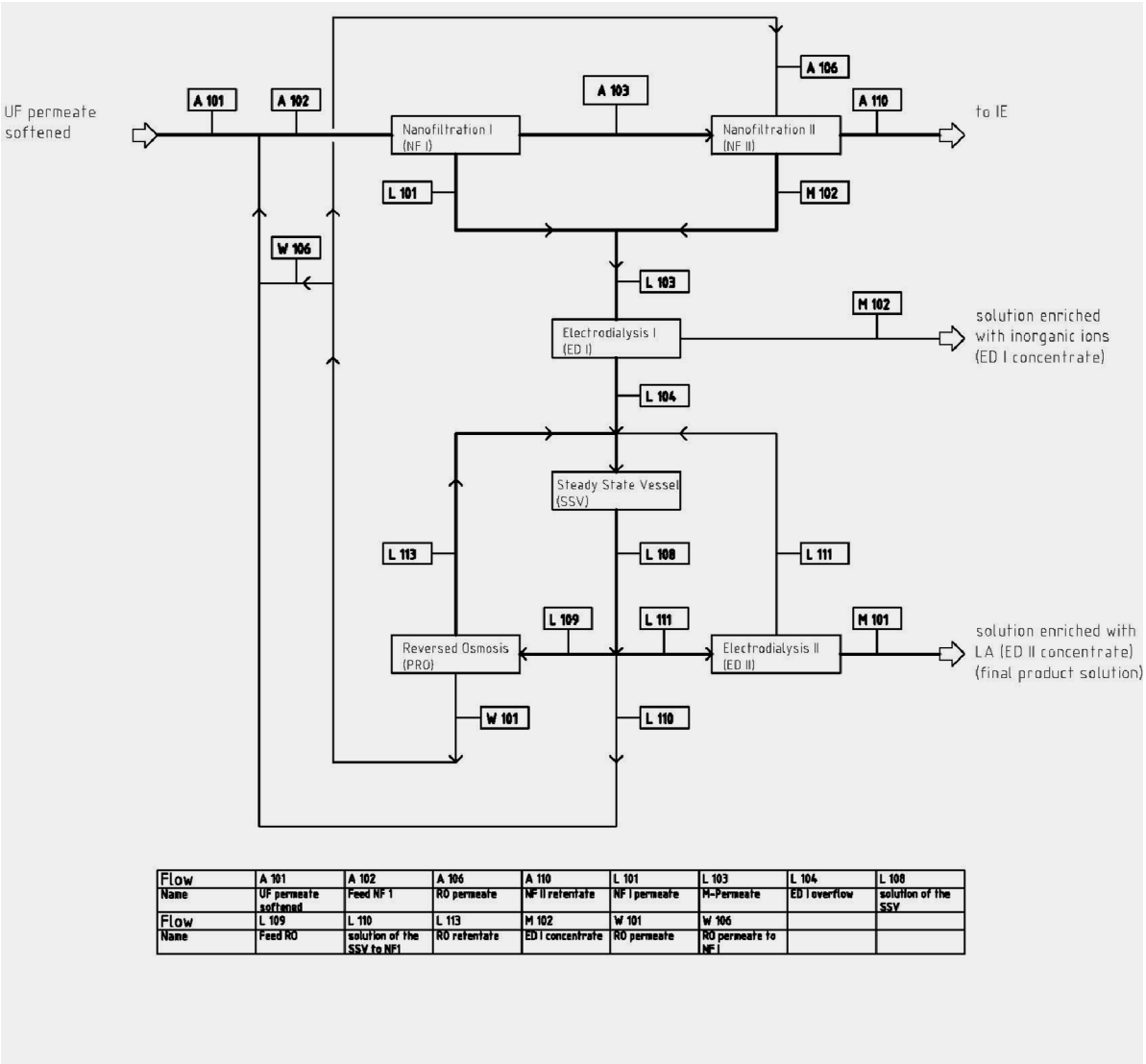


Figure 1-2: Overview of the "basic process" applied at the *GBR Upper Austria* based on the licence of the Gruene-bioraffinerie.at GmbH (Gruene-bioraffinerie.at GmbH 2008)



Flow	A 101	A 102	A 106	A 110	L 101	L 103	L 104	L 108
Name	UF permeate softened	Feed NF 1	RO permeate	NF II retentate	NF I permeate	M-Permeate	ED I overflow	solution of the SSV
Flow	L 109	L 110	L 113	M 102	W 101	W 106		
Name	Feed RO	solution of the SSV to NF1	RO retentate	ED I concentrate	RO permeate	RO permeate to NF I		

Figure 1-3: Flow diagram of the hybrid process based on the licence of the Gruene-bioraffinerie.at GmbH (Gruene-bioraffinerie.at GmbH 2008); figure made by Ecker (Ecker 2012)

Generally, it was found that by applying the basic process, it was feasible to generate liquors enriched with LA and AA as product solutions. Indeed, previous expectations of the process and of the quality of the generated products were not completely fulfilled. Firstly, the throughput per hour at the UF step was lower than expected, as it was only possible to filter 100 l silage juice per hour in the long run instead of the expected 200 l per hour. In order to solve this problem, different additional pre-treatment steps (e. g. microfiltration, centrifugation, sedimentations, etc.) were tested, but no improvements were observed. Secondly, the separation of AAs and LA in the NF device was less exact than expected. This means that some amounts of AAs were transferred to the NF Permeates, on the one hand, and some amounts of LA remained in the NF Retentate, on the other hand. Consequently, the AAs in the NF Permeates were lost for the AA generation at the IE device, but also polluted the LA enriched solution. The LA in the NF Retentate, in turn, did not pollute the final AA product, as a further separation between organic acids and AAs occurred at IE device, but was lost for the final LA product. Thirdly, by applying the nanofiltered LA solution to the electrodialysis, which was used for the final product concentration, a very poor LA extraction performance was observed. That means that this process was quite ineffective. Furthermore, the separation between LA and other organic acids (e. g. acetic acid and butyric acid) resulting from the raw grass silage was not realised. Therefore, these organic acids were also found in the final LA product. However, detailed descriptions of the basic process and the results that were obtained were discussed by Ecker (Ecker 2012) and Ecker et al. (Ecker, Schaffenberger et al. 2012).

1.1.3.2 Process description of the *GBR Upper Austria* – Innovative process

As aforementioned, by applying the basic process at the *GBR Upper Austria* some previously assumed expectations were not fulfilled. In order to improve the LA and AA production, a new process constellation, which was called “innovative process”, was therefore suggested and consequently studied.

Within the innovative process the mechanical fractionation at the screw press, the filtration at the UF and the softening were performed similarly to the basis process. Thus, instead of the hybrid process another process constellation was implemented: Firstly, the ultrafiltered and softened grass silage juice was directly treated at the IE device to separate AAs and organic acids earlier and more effectively. The AAs remained on the IE resin and the organic acids were transferred to IE effluent. The IE effluent, which was rich in LA, other organic acids and sugars, was then further processed by a NF device to separate the sugars and the organic acids. A liquor enriched with sugars were found in the NF Retentate and the organic acids were transferred to the NF Permeate. Optionally, it was possible to further concentrate the LA/organic acid solution by applying a RO device. In order to summarise the process description an overview of the principle streams of the innovative process is shown in Figure 1-4.

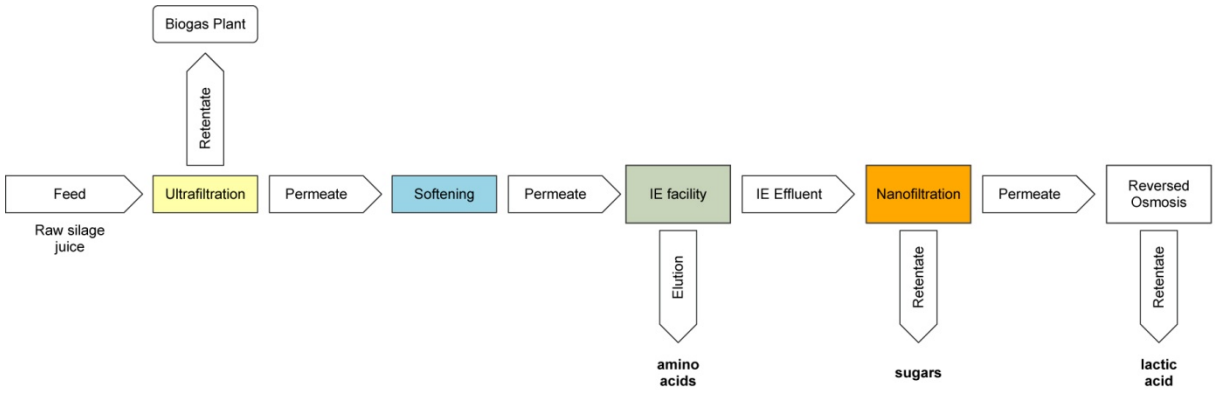
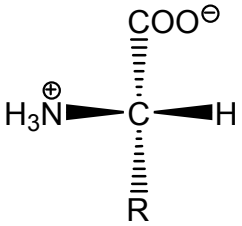


Figure 1-4: Overview of the “innovative process” applied at the *GBR Upper Austria*

Generally, it can be summarised that better results were obtained by applying the innovative process, as different advantages compared to the basic process were identified: Firstly, by applying the innovative process it was possible to generate a third product, a solution enriched with sugars, from the raw material grass silage. The process costs incurred can therefore be shared by three possible products. Secondly, the process constellation used in the innovative process was simplified, which would lead to reduced investment costs and reduced permanent operation costs. Furthermore, the separation between LA and AAs was improved in the innovative process. Therefore, the quality of the LA product was improved, as only traces of AAs were found in this product. Simultaneously, it was possible to increase the overall yield of LA, as the IE effluent was further processed and they were not discarded as in the basic process. A comprehensive description of the innovative process and its results as well as a comparison of the basic and innovative process, were performed by Ecker (Ecker 2012).

1.2 Amino acids

Organic compounds which contain a carboxyl and a minimum of one amino group are called amino acids (AAs). Depending on the position of these functional groups α-, β- or γ-AAs are distinguished. In nature, α-AAs with one amino group and one carboxyl group on adjacent carbon-atoms (Formula 1-1) are the most common and most important species. For instance, the primary structure of proteins consists of α-AAs. Therefore, AAs are also called the building blocks of life. Currently, about 20 AAs, called proteinogenic AAs which build the primary structure of proteins, are known. As the proteinogenic AAs are essential for life, as well as being important compounds in various branches of industry further explanations are focussed on them in this work. However, next to the proteinogenic AAs various non-proteinogenic AAs, from natural origins, are known as well (Jakubke 1982, Drauz, Grayson et al. 2005). All proteinogenic AAs except glycine are chiral, whereas only L-enantiomers are found in proteins. D-AAs are more unusual in nature and have only been found in cell walls of some bacteria and in some plant cells (Drauz, Grayson et al. 2005).

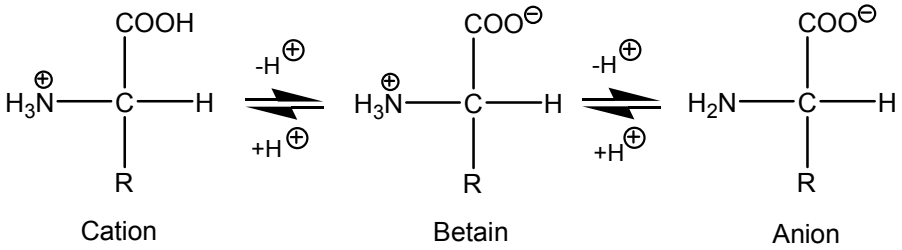


Formula 1-1: General structure of α-AAs

1.2.1 Properties of amino acids

α-AAs build white, crystalline, non-volatile solids, which decompose at between 250-300 °C. Based on their zwitterionic structure α-AAs, which are also called betaines or inner salts, are non-volatile and stable at higher temperatures. Furthermore, the good solubility of almost all of them in water and their insolubility in less polar solvents, such as alcohols or ethers, are based on their betaine structure. The dissolving of α-AAs in water is energetically preferred, as in water the zwitterionic form is built and causes an energy gain of 44.8-51.5 kJ/mol. Additionally, the structure of the side chain influences the solubility of the AAs (Jakubke 1982, Hoppe, Martens 1984).

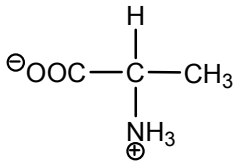
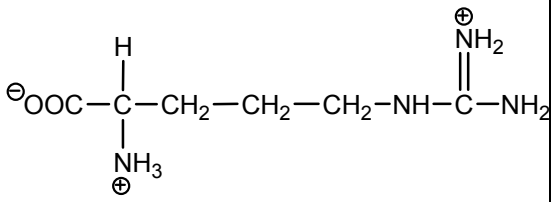
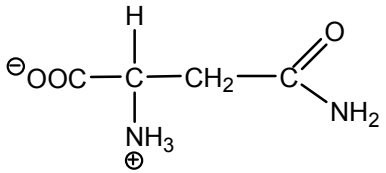
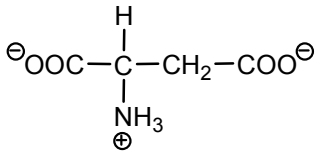
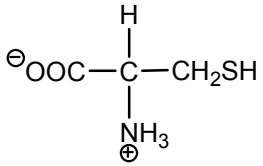
Due to their dipolar structure, AAs show a special behaviour in the presence of acids and bases. Depending on the pH, AAs can react either as proton acceptor (base) or as proton donor (acid). Therefore, in an acidic environment (pH<4) AAs exist mainly as cations and in an alkaline environment (pH>9) AAs exist mainly as anions as demonstrated in Formula 1-2 (Jakubke 1982, Drauz, Grayson et al. 2005, Hoppe, Martens 1984).

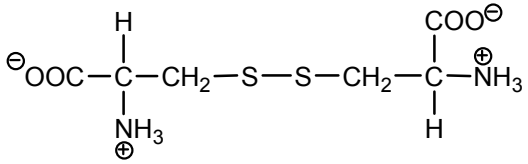
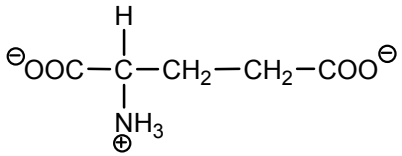
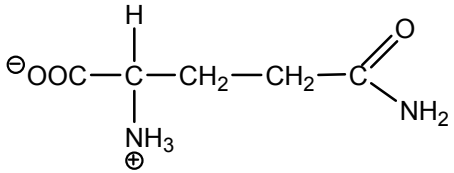
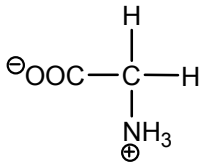
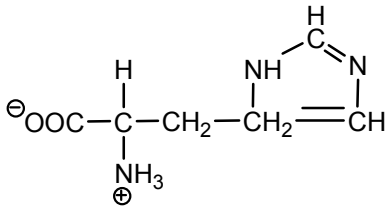


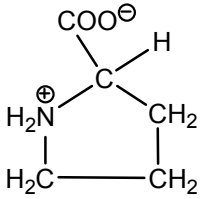
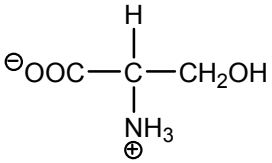
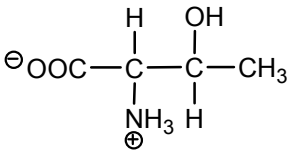
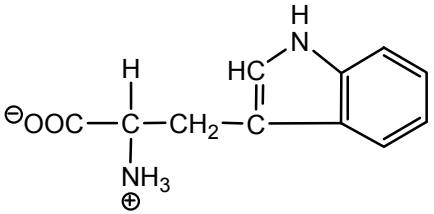
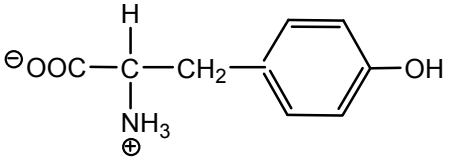
Formula 1-2: Acid/alkaline behaviour of AAs in aqueous solutions

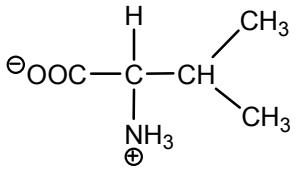
At least two pKa values can be determined for each AA. Additionally, for AAs with a charged side chain a third pKa value exists. Based on the pKa values the isoelectric points (pI) are calculated. At the pI the carboxyl group is deprotonated and the amino group is protonated. Therefore, on the surface the AAs are uncharged at this pH and exist as betaines (Jakubke 1982). In Table 1-1, the pKa values and further data of all proteinogenic AAs are summarised.

Table 1-1: Overview of data and properties of proteinogenic AAs (Jakubke 1982, Drauz, Grayson et al. 2005, Hoppe, Martens 1984, Kyte, Doolittle 1982, Voet, Voet et al. 2002)

Amino acid Abbreviation	Chemical structure	Molar Mass [g/mol]	pK_1 α-COOH	pK_2 α-NH_3^+	pK_3 side chain	pI	Solubility in H_2O [g/l]		De-composition temperature [°C]	Hydro-phobicity of R [-]
							25 °C	100 °C		
L-Alanine Ala		89.09	2.34	9.69		6.01	166.5	373	314	1.8
L-Arginine Arg		174.20	2.01	9.04	12.48 (Guanidino-)	10.8	148.7		244	-4.5
L-Asparagine Asn		132.13	2.02	8.80		5.41	298	551	236	-3.5
L-Aspartic acid Asp		133.10	2.10	9.82	3.86 (β-COOH)	2.98	5.0	69	270	-3.5
L-Cysteine Cys		121.16	1.71	10.78	8.27 (Sulfhydryl-)	5.02	160		240	2.5

Amino acid Abbreviation	Chemical structure	Molar Mass [g/mol]	pK_1 α-COOH	pK_2 α-NH_3^+	pK_3 side chain	pI	Solubility in H_2O [g/l]		De-composition temperature [°C]	Hydro-phobicity of R [-]
							25 °C	100 °C		
L-Cystine		240.30	1.04 2.05	8.00 10.25		5.02	0.11	1.1	260	2.5
L-Glutamic acid Glu		147.13	2.10	9.47	4.07 (γ-COOH)	3.08	8.43	140	224-225	-3.5
L-Glutamine Gln		146.15	2.17	9.13		5.65	36		185-186	-3.5
Glycine Gly		75.07	2.35	9.78		6.06	249.9	671.7	262	-0.4
L-Histidine His		155.16	1.77	9.18	6.10 (Imidazol-)	7.64	38.2		287	-3.2

Amino acid Abbreviation	Chemical structure	Molar Mass [g/mol]	pK_1 α-COOH	pK_2 α-NH_3^+	pK_3 side chain	pI	Solubility in H_2O [g/l]		De-composition temperature [°C]	Hydro-phobicity of R [-]
							25 °C	100 °C		
L-Proline Pro		115.13	2.00	10.64		6.30	1550	239	220-222	-1.6
L-Serine Ser		105.09	2.21	9.15		5.68	360		228	-0.8
L-Threonine Thr		119.12	2.71	9.62		6.16	90.3		253	-0.7
L-Tryptophan Trp		204.23	2.38	9.39		5.88	11.4	49.9	281	-0.9
L-Tyrosine Tyr		181.19	2.20	9.11	10.07 (Phenol-)	5.63	0.45	5.6	297-298	-1.3

Amino acid Abbreviation	Chemical structure	Molar Mass [g/mol]	pK_1 α-COOH	pK_2 α-NH_3^+	pK_3 side chain	pI	Solubility in H_2O [g/l]		De-composition temperature [°C]	Hydro-phobicity of R [-]
							25 °C	100 °C		
L-Valine Val		117.15	2.32	9.62		5.96	88.5	188	315	4.2

1.2.2 Classification of amino acids

Proteinogenic AAs can be classified in different ways. Most classifications of AAs are based on varying properties of the AA side chains. Subsequently, different classifications and the corresponding AAs are presented (Jakubke 1982, Voet, Voet et al. 2002).

1.2.2.1 Side-chain structure

Based on the chemical structures of the AA side chains, different groups of AAs can be classified as shown in Table 1-2.

Table 1-2: Classification of AAs based on the chemical structure of the side chains

Aliphatic AAs	Aromatic AAs	Heterocyclic AAs	AAs with Hydroxy-group	AAs with second Amino-group	Sulfur-containing AAs	Secondary Amino-group
Alanine Glycine Isoleucine Leucine Valine Proline Methionine	Phenylalanine Tryptophan Tyrosine	Histidine	Serine Threonine Tyrosine	Asparagine Glutamine	Cysteine Cystine Methionine	Proline Hydroxyproline

Furthermore, a group of AAs with similarities in the side chain structure are known as branched-chain amino acids (BCAAs). These are the essential AAs leucine, isoleucine and valine.

1.2.2.2 Polarity

As shown in Table 1-3, AAs can also be categorised based on the polarity of their side chains.

Table 1-3: Classification of AAs based on the side chains' polarity

Non-polar side chains	Polar, uncharged side chains	Polar, charged side chains
Glycine Alanine Valine (hydrophob) Leucine (hydrophob) Isoleucine (hydrophob) Methionine (hydrophob) Proline (hydrophob) Phenylalanine (hydrophob) Tryptophan	Serine Threonine Asparagine Glutamine Cysteine Tyrosine	Lysine Arginine Histidine Aspartic acid Glutamic acid

1.2.2.3 Position of the isoelectric point (pI)

The side chains of AAs contain different functional groups, which result in variable pKa values and pIs. Based on the position of the pI three different groups of AAs (acidic, alkaline, neutral AAs) can be distinguished. The corresponding AAs are summarised in Table 1-4.

Table 1-4: Classification of AAs based on the position of the pI

Acidic AAs	Neutral AAs	Alkaline AAs
Aspartic acid (Aspartate)	Glycine	Lysine
Glutamic acid (Glutamate)	Alanine	Histidine
	Valine	Arginine
	Leucine	
	Isoleucine	
	Methionine	
	Proline	
	Phenylalanine	
	Tryptophan	
	Serine	
	Threonine	
	Asparagine	
	Glutamine	
	Tyrosine	
	Cysteine	
	Cystine	

1.2.3 Classification of amino acids according to nutritional aspects

The functions of AAs in human and animal metabolism are manifold. In most cases AAs are brought to the organisms as proteins. During digestion the proteins are split by enzymes and free AAs are released. Among other things AAs in the organisms are used to build proteins (structural proteins, enzymes etc.) or oligo- or polypeptides (hormones). Some AAs or their metabolites are directly used as hormones. For instance, serotonin a metabolite of tryptophan is an important neurotransmitter (Drauz, Grayson et al. 2005).

1.2.3.1 Essential and non-essential amino acids

In contrast to plants, humans, animals and some bacteria are not able to synthesise all AAs, which are necessary for the metabolism. These AAs have to be brought to the organism via nutrition and are called essential AAs (Jakubke 1982, Drauz, Grayson et al. 2005). As summarised in Table 1-5, different AAs are essential for different organisms. Furthermore, the recommended daily intake of AAs for adults according to FAO/WHO/UNU is given in Table 1-6.

Table 1-5: Essential (+) and semi-essential (±) AAs (Drauz, Grayson et al. 2005)

	Baby	Adult	Rat	Chicken	Cat	Salmon
L-Arginine	±	±	±	+	+	+
L-Cysteine	+ (?)					
Glycine				+		
L-Histidine	+	±	+	+	±	+
L-Isoleucine	+	+	+	+	+	+
L-Leucine	+	+	+	+	+	+
L-Methionine	+	+	+	+	+	+
L-Phenylalanine	+	+	+	+	+	+
L-Threonine	+	+	+	+	+	+
L-Tryptophan	+	+	+	+	+	+
L-Tyrosine	+(?)					
L-Valine	+	+	+	+	+	+

Table 1-6: Recommended daily intake of essential AAs for adults (WHO, FAO et al. 2007)

Amino acid	2007 FAO/WHO/UNU		1985 FAO/WHO/UNU	
	mg/kg per day	mg/g protein	mg/kg per day	mg/g protein
Histidine	10	15	8-12	15
Isoleucine	20	30	10	15
Leucine	39	59	14	21
Lysine	30	45	12	18
Methionine + Cysteine	15	22	13	20
Methionine	10	16	-	-
Cysteine	4	6	-	-
Phenylalanine + Tyrosine	25	38	14	21
Threonine	15	23	7	11
Tryptophan	4	6	3.5	5
Valine	26	39	10	15

1.2.3.2 Evaluation of proteins and of the amino acid pattern

Considering nutritional aspects, the quality of a protein depends on its AA pattern. Generally, the significance of animal protein for the human organism is higher than the significance of plant proteins, because animal protein has a higher rate of essential AAs on average. Furthermore, the pattern of these essential AAs in animal protein is more adequate for human nutrition than the AA pattern of plant proteins. The limiting AA in plant protein is often lysine. For instance, the amount of lysine in wheat, rye, barley, oat and maize is low. Methionine is the limiting AA in meat, milk, soy beans and other beans (Drauz, Grayson et al. 2005).

An important criterion to evaluate the AA pattern of proteins is the biological value of proteins. This value can be determined experimentally with different methods, whereas in all cases it is examined how much protein in the body is displaced by protein supplied by nutrition. Additionally, the calculation (according to Formula 1-3) of the so called chemical score (CS) is another way to estimate the biological value of the protein's AA pattern. For determining the CS the AA profile of the respective protein is referred to the AA profile of a reference protein (egg or FAO/WHO provisional scoring pattern). The composition of the reference protein from the FAO/WHO is shown in Table 1-7. By using the CS to evaluate a protein, it has to be considered that this method only provides an estimation for the biological value, as the quality of the protein intake is not included. However, it can be used to identify the limiting AA (Drauz, Grayson et al. 2005).

$$Chemical\ Score = \frac{Rate\,of\,AA\,test\,protein}{Rate\,of\ AA\ reference\ protein}\,x\,100$$

Formula 1-3: Calculation of the chemical score (CS) for proteins

Table 1-7: Composition of the reference protein according to FAO/WHO for calculating the chemical score for proteins (WHO, FAO et al. 1973)

Amino acid	g/100 g protein
L-Isoleucine	4.0
L-Leucine	7.0
L-Lysine	5.5
L-Methionine + L-Cystine*	3.5
L-Phenylalanine + L-Tyrosine**	6.0
L-Threonine	4.0
L-Tryptophan	1.0
L-Valine	5.0
Sum	36

*Cystine can partly cover the total demand on S-containing AAs
**Tyrosine can partly cover the total demand on aromatic AAs

There are recommendations on the ideal AA pattern for optimal nutrition in animal breeding as well. As various AAs are essential for different animals, the protein requirements for each species differ. In animal nutrition the ideal AA pattern refers always to lysine, whereas the amount of lysine is set to 100 per cent. Table 1-8 shows typical areas for the relative amounts of essential AAs found in literature for breeding pigs and laying hens.

Table 1-8: Recommended AA pattern for pigs and laying hens (Boisen, Hvelplund et al. 2000, Bregendahl 2008)

Essential AA	Breeding pigs [%]	Laying hens [%]
L-Lysine	100	100
L-Methionine + L-Cystine*	50-63	81-94
L-Threonine	60-75	66-80
L-Tryptophan	15-19	19-23
L-Isoleucine	50-61	79-94
L-Leucine	72-110	n. s.
L-Valine	64-70	86-101
L-Histidine	- / 26-36	n. s.
L-Phenylalanine und L-Tyrosine	88-122	n. s.
L-Arginine	- / 31-42	- / 86-101

*Cystine can partly cover the total demand on S-containing AAs

1.2.3.3 AA profile of some food

The AA profiles of some protein sources are summarised in Table 1-9 for comparing the present patterns with the AAs in the silage juice or in the AA products produced at the *GBR Upper Austria*.

Table 1-9: AA profile of some protein and AA sources (Souci, Fachmann et al. 2008)

Compound	soy flour [mg/100g]	wheat [mg/100g]	dried egg [mg/100g]	whey powder [mg/g]
Protein sum (N x 6,25)	40.8 g/100 g	11.4 g/100 g	46 g/100 g	11.8 g/100 g
L-Alanine	1680	510	3210	610
L-Arginine	3140	620	3130	390
L-Aspartic acid	4480	690	5290	1490
L-Cystine	590	290	1080	170
L-Glutamic acid	7830	4080	6390	2400
L-Glycine	1680	720	1890	290
L-Histidine	950	280	1190	290
L-Isoleucine	1900	540	3220	850
L-Leucine	2880	920	4500	1400
L-Lysine	2560	380	3250	1150
L-Methionine	580	220	1640	230
L-Phenylalanine	1860	640	2790	490
L-Proline	2340	1560	2090	430
L-Serine	2080	710	4080	640
L-Threonine	1610	430	2630	1020
L-Tryptophan	480	150	810	250
L-Tyrosine	1450	410	2080	470
L-Valine	1970	620	3900	910

1.2.4 Production of amino acids

Next to the essential functions of AAs for the organism, about 100 years ago it was discovered that AAs, in particular glutamic acid or monosodium glutamate respectively, can be used as flavour enhancing compounds for food. Based on this discovery, processes and methods for the production of AAs have been developed. In particular, Japanese researchers and companies have been leading the development of new production methods for AAs in the last 60 years. Nowadays, AAs are extremely important products in various branches of industry especially in the food and flavour industry. Furthermore, AAs are applied for medical purposes (infusions or dietary food), in the cosmetic sector, for animal feed additives or as precursors in the chemical industry (Hoppe, Martens 1984, Crueger, Crueger 1989, Eggeling, Pfefferle et al. 2001). In Table 1-10 data of the market value in 2004 broken down by field of use are presented, whereas the feed sector is the most important one. Additionally, an overview of typical applications for AAs and their estimated market volume is given in Table 1-11.

Table 1-10: Market value by field of use, 2004 (Drauz, Grayson et al. 2005)

Use	Value [%]
Animal nutrition	56
Human nutrition	32
Speciality*	12

*Pharmaceuticals, cosmetics, agrochemicals and industrial uses

Table 1-11: Overview of typical applications and production amounts of AAs (Drauz, Grayson et al. 2005, Crueger, Crueger 1989)

Amino acid	Application	Quantity [t/a]
L-Alanine	Flavour enhancing compound	1500
D,L-Alanine	Flavour enhancing compound (drinks)	n/a
L-Arginine	Infusion solutions, therapeutic agents, cosmetics	3000
L-Asparagine	Therapeutic agent	200
L-Aspartic acid	Therapeutic agent, flavour enhancing compound, Aspartam®	15000
L-Cysteine	Bread production, antioxidant, therapeutic agent	7000
L-Glutamic acid	Flavour enhancing compound	1 690 000
L-Glutamine	Therapeutic agent	2000
Glycine	Sweetener	16 000
L-Histidine	Therapeutic agent	300
L-Hydroxyproline	Precursor for synthesis	100
L-Isoleucine	Infusion solution	500
L-Leucine	Infusion solution	800
L-Lysine	Feed additive, infusion solution	850 000
D,L-Methionine	Feed additive	600 000
L-Methionine	Therapeutic agent	400
L-Phenylalanine	Infusion solution, therapeutic agent, Aspartame®	14 000
L-Proline	Infusion solution	800
L-Serine	Cosmetics	300
L-Threonine	Feed additive	85 000
L-Tryptophan	Infusion solution	2000
L-Tyrosine	Infusion solution, starting material for L-DOPA	150
L-Valine	Infusion solution	1100
Others	-	14000
Total		**3 300 000**

Considering the market volumes, glutamic acid is the most important AA followed by lysine and methionine, which are important for feeding. These three AAs are produced on a large scale, therefore the prices per kg are rather low as shown in Figure 1-5. In contrary, AAs with lower production capacities per year, such as isoleucine or alanine reach higher prices.

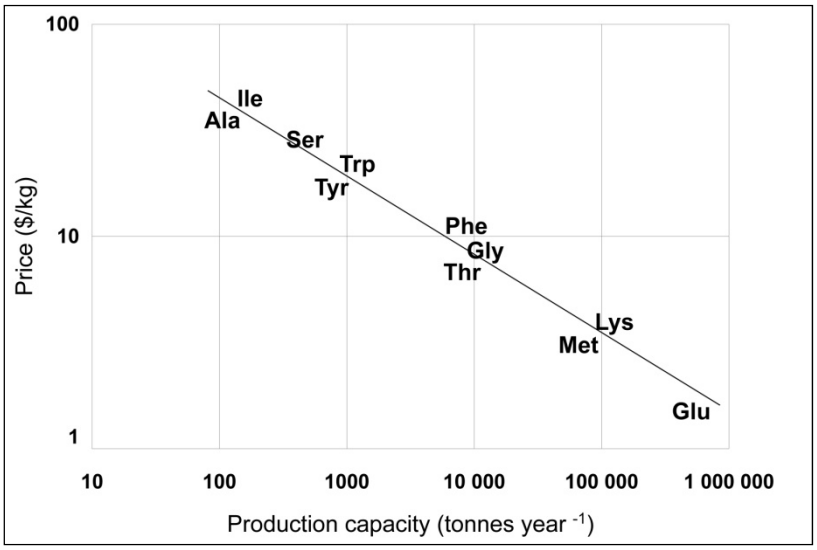


Figure 1-5: Estimated prices of AAs ($/kg) in 2001 (Eggeling, Pfefferle et al. 2001)

1.2.5 Overview of the production methods of amino acids

Different methods for the production of AAs are known. Depending on the specific demand for each AA and on specific production costs, different production methods are currently applied on an industrial scale. In principal, the production methods can be divided into three different sections (Hoppe, Martens 1984, Crueger, Crueger 1989, Eggeling, Pfefferle et al. 2001):

1. Isolation from protein hydrolyzates
2. Chemical synthesis
3. Microbiological methods consisting of:
    - 3.1 Direct fermentation
    - 3.2 Conversion of precursors by bacteria
    - 3.3 Enzymatic synthesis

In the following section, these production methods will be discussed in detail.

1.2.5.1 Isolation from protein hydrolyzate

In 1908, the flavour-enhancer L-glutamic acid was discovered by a Japanese researcher. Only one year later the Japanese company Ajinomoto started to isolate this AA from vegetable proteins such as soy or wheat protein and the industrial production of monosodium glutamate has began. The isolation of AAs from protein hydrolyzate was the first production method which was applied on a larger scale. For the following 50 years, this method was the most important production method for monosodium glutamate (Hoppe, Martens 1984, Crueger, Crueger 1989, Eggeling, Pfefferle et al. 2001).

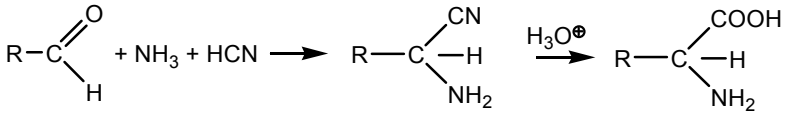
For extracting the AAs, the proteins (e.g. keratin, collagen or vegetable proteins) are treated with acid. Furthermore, hydrolysing methods with bases or enzymes are known, but only hydrolyses with acids are commercially relevant. By applying the acid the peptide bond within the proteins is destroyed and AAs are set free. Subsequently, the hydrolyzate is neutralised followed by different fractionation steps, such as evaporation, filtration or chromatographic methods. Generally, the fractionation of AAs from protein hydrolyzates is based on different properties of the AAs, such as differences in solubility or different side-chain charges (Drauz, Grayson et al. 2005, Hoppe, Martens 1984).

1.2.5.2 Chemical synthesis

Chemical synthesis is another strategy for producing AAs. There are a manifold number of methods available for synthesising AAs, where petrochemical substances are used as starting material. By using petrochemical educts, the quantitative restrictions, which occur by using natural raw materials, are reduced and larger amounts of AAs can be produced more quickly. Nevertheless, the chemical synthesis of AAs has a disadvantage, because the reactions always result in racemates. As L-AAs are particularly in demand, expensive separation and racemisation processes have to be applied after the synthesis to remove and convert the D-AA. For this reason, only some syntheses of AAs are economically feasible on an industrial scale. However, in the case of synthesising glycine or methionine, the formation of racemates is insignificant, as glycine is achiral and both enantiomers of methionine show the same biological value (Hoppe, Martens 1984).

Established syntheses on an industrial scale are the reactions of Strecker and Bucherer or the combined Strecker/Bucherer synthesis, respectively. In the Strecker synthesis aldehydes are educts. Hydrogen cyan and ammonia are added to the aldehydes to build α-

aminonitriles. Finally, the hydrolysis of the α-aminonitriles results in AAs. In explanation, Formula 1-4 shows the schema of the Strecker synthesis. Generally, the synthesis method of Bucherer can be seen as an extension of the Strecker synthesis by the addition of carbon dioxide (Hoppe, Martens 1984).



Formula 1-4: Strecker synthesis - general schema (Hoppe, Martens 1984)

The most important production process of AAs on an industrial scale using chemical synthesis is the production of L- and D-methionine. The economical feasibility of this process, which is based on the reaction of Bucherer, was realisable through the inexpensive production of its starting material acrolein from propene. Within the synthesis process of methionine, first methyl mercaptan is added to acrolein and 3-methylmercapto-propionaldehyd is formed. In further steps hydrogen cyan, ammonia and carbon dioxide are used to finally produce a racemic mixture of methionine. The chemical yields of this process are beyond 90 % (Hoppe, Martens 1984, Knapp 2004). Although, this production process of L- and D-methionine is well established, it is not without controversy. Firstly, the use of hydrogen cyan certainly contains some risks for humans and environment at the production site (Kumar, Gomes 2005). Secondly, methionine is an essential AA, which is very important in animal feeding. Due to the petrochemical raw materials, methionine, which is produced by synthesis at the moment, cannot be used for biological farming. Therefore, research is done on alternative production routes (e.g. fermentation) for methionine (Hartwich 2008).

1.2.5.3 Microbiological production methods

The production of AAs by the application of microbiological routes can be distinguished into three different methods (Hoppe, Martens 1984, Crueger, Crueger 1989):

1. Direct fermentation using bacteria
2. Precursor conversion using bacteria
3. Enzymatic synthesis

Direct fermentation

Generally, processes, where chemical products are produced through metabolic activities of microorganisms, are called fermentation. Microorganisms are wide spread and can easily adapt to different environmental conditions. In particular, at very adequate conditions, they are able to produce various organic substances very quickly. In the last 50 years, different fermentation techniques have been developed and applied to produce nutrition, feeding, pharmaceuticals and chemicals directly by overproduction of bacteria. During the production process through microorganisms very complex metabolic processes occur within the cells. Additionally, microorganisms are reproducing themselves by cell splitting within the fermentation, which automatically result in a higher amount of “production units”. Through the high adaptability of microorganisms, it is possible to produce different products by using the same microorganisms and fermentative devices. Generally, fermentation processes are conducted at mild conditions at normal pressure and temperatures between 30 to 40 °C. Therefore, high pressure installations are not necessary and result in lower investment costs (Hoppe, Martens 1984, Crueger, Crueger 1989, Drauz, Grayson et al. 2005).

For the production of AAs different carbon sources are applied: e.g. glucose, fructose, molasses or starch hydrolyzate. Fermentation processes of AAs are run in batch fermenters. Yet continuous fermentation processes are not established on an industrial scale. The fermentation process, which is done in fermenters with volumes up to 500 m^3, lasts 3 to 4 days. Before starting the production process, the inocula preparation at labaratory scale has to be done. The inocula quality influences productivity, therefore it has to be tested regularly. In the next step the inoculum is propagated in a seed train. This procedure is performed in shaking flasks or in bioreactors depending on the scale of the production bioreactor to produce a sufficient amount of inoculums for the production process. The production process itself and its optimisation depends on many different parameters, such as pH, aeration, feed rate of carbon source or process temperature. Due to high sugar degradation rates and high respiratory activities, high quantities of oxygen have to be provided to the fermentation system. Hence, the optimal aeration rate depends on the strain. The pH-value is often stabilised by adding gaseous ammonia to the fermentation broth (Crueger, Crueger 1989, Hermann 2003). Figure 1-6 gives an overview on steps within a fermentation process.

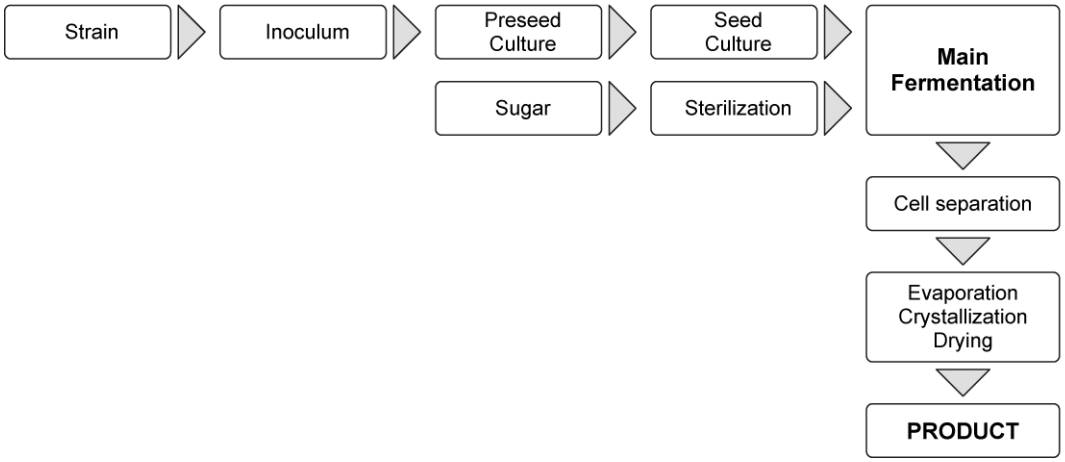


Figure 1-6: Overall flow diagram of fermentation and downstream processing (Leuchtenberger 1996)

Generally, by using a fermentation process only L-AAs are produced, which presents a decisive advantage compared to the AA production by chemical synthesis. No further separation of enantiomers has to be done afterwards. Currently, fermentation is the most economic production method for bulk AAs, such as L-glutamate, L-lysine hydrochloride or L-threonine (Drauz, Grayson et al. 2005, Hoppe, Martens 1984).

Production strains

In 1957, Dr. S. Udaka and Dr. S. Kinoshita at Kyowa Hakko Kogyo discovered a specific soil bacterium grown on mineral salts, which excreted L-glutamate. Afterwards, a number of bacteria with similar properties were isolated as well, which are summarised by the species name *Corynebacterium glutamicum* (Eggeling, Pfefferle et al. 2001), e. g. *Brevibacterium flavum, Brevibacterium lactofermentum* or *Microbacterium ammoniaphilum*. These bacteria are all gram-positive, non-spore-forming, nonmotile and require biotin for growth, therefore they were summarised as *Corynebacterium*. Besides this *Corynebacterium glutamicum*, only some other strains are able to excrete sufficient amounts of the desired AAs directly. That means, only some so called wild type strains are used for the production of AAs (Hoppe, Martens 1984, Crueger, Crueger 1989). These wild type strains have large enzyme capacities and are able to overproduce AAs.

The following AAs are produced by wild type strains (Hoppe, Martens 1984):

- L-glutamic acid
- D,L-alanine
- L-valine

As in the most wild type strains the overproduction of AAs is regulated and hindered, different strategies to force other production strains to overproduce and to excrete AAs have been developed and applied (Crueger, Crueger 1989):

1. Use of mutants
2. Recombination
3. rDNA-technology

By combining these methods, higher yields of AAs (or depending on the strains other products) can be generated. One method to force the overproduction of desired products is the usage of mutants. For the production of AAs auxotrophic mutants or regulatory mutants are applied. Auxotrophic mutants are not able to build an essential compound, mostly the final product, required for its growth, as they are missing an enzyme. Therefore, an intermediate compound, e. g. the desired AA, is accumulated. Regulatory mutants, on the other hand, produce a surplus of the final product, as they are established with a non-feedback regulated key enzyme. By the recombination of strains, e. g. auxothropic or regulatory mutants, so called hybrid strains can be produced, which are able to overproduce AAs or other compounds. Another method within the AA biosynthesis is the amplification of speed-regulating enzymes, which leads to increasing yields. This method falls into the scope of rDNA-technology (Hoppe, Martens 1984, Crueger, Crueger 1989, Eggeling, Pfefferle et al. 2001).

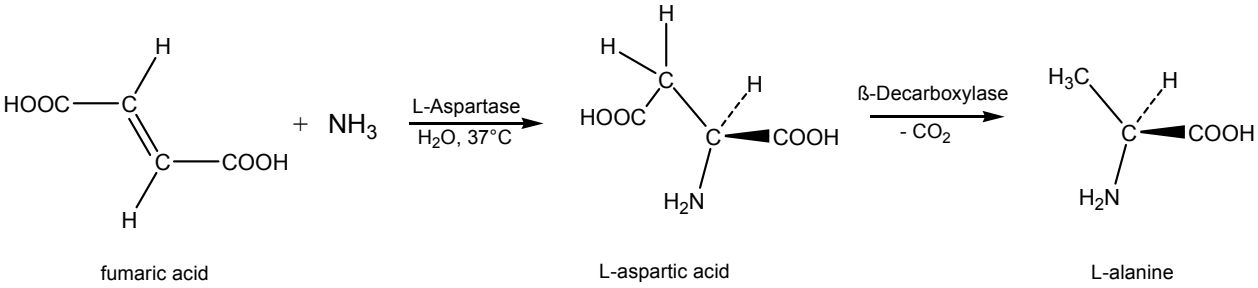
Precursor conversion using bacteria

Another method to produce AAs microbiologically is the conversion of AA precursors into the desired AA by bacteria. For this method whole cells, which contain the necessary enzyme, are used. For example, glycine is converted into L-serine by the strain *Klebsiella aerogenes*, which produces the enzyme serinhydroxymethyl-transferase (Crueger, Crueger 1989).

Enzymatic synthesis/catalysis

The production of AAs from precursors by using only enzymes is called enzymatic synthesis/catalysis. By applying this method pure L- or D-AAs can be generated, as enzymes are highly specific catalysts. Some enzymatic reactions are well established on an industrial scale. For example, from racemic mixtures of AAs (e. g. D-, L-methionine), which are derivatised first, L-AAs can be resolved by applying the acylase of the strain *Aspergillus oryzae*. To minimise the use of enzymes, this process is performed within the enzyme membrane reactor. This reactor can be run continuously, as the enzyme molecules are cut off by a membrane with hollow fibres. For instance, the Degussa AG uses this process to produce L-methionine, L-phenylalanine, L-tryptophan and L-valine (Hoppe, Martens 1984, Crueger, Crueger 1989, Leuchtenberger, Huthmacher et al. 2005, Leuchtenberger, Plöcker 1988).

L-Aspartic acid is another AA, which is preferably produced enzymatically. It is generated by an addition of ammonia to fumaric acid catalysed by the enzyme aspartase. Furthermore, the L-aspartic acid is used as precursor for the production of L-alanine. This reaction is catalysed by the aspartate β-decarboxylase (Hoppe, Martens 1984, Leuchtenberger, Huthmacher et al. 2005). The reactions are summarised in Formula 1-5.



fumaric acid L-aspartic acid L-alanine

Formula 1-5: Enzymatic synthesis of L-aspartic acid and L-alanine

1.2.6 Production of single proteinogenic amino acids

As described in the previous section, different methods for the industrial production of AAs are available. For instance, production by fermentation is the most preferable and cheapest process for the generation of bulk AAs. Depending on individual properties of AAs, different production methods are applied. Table 1-12 gives an overview on the preferred production methods for single AAs. Afterwards further details on the production and on the usage of each proteinogenic AA will be given.

Table 1-12: Preferred production methods for single AAs

Amino acid	Preferred production method
L-Alanine	enzymatic catalysis, fermentation
L-Arginine	fermentation, extraction
L-Asparagine	extraction, chemical synthesis
L-Aspartic acid	enzymatic catalysis
L-Cysteine	extraction, fermentation, enzymatic catalysis
L-Glutamic acid	fermentation
L-Glutamine	fermentation, extraction
Glycine	chemical synthesis
L-Histidine	fermentation, extraction
L-Hydroxyproline	fermentation, extraction
L-Isoleucine	fermentation, extraction
L-Leucine	fermentation, extraction
L-Lysine	fermentation
D,L-Methionine	chemical synthesis and enzymatic resolution
L-Methionine	enzymatic resolution
L-Phenylalanine	fermentation
L-Proline	fermentation, extraction
L-Serine	fermentation, extraction
L-Threonine	fermentation
L-Tryptophan	fermentation
L-Tyrosine	extraction
L-Valine	enzymatic catalysis, fermentation

1.2.6.1 L-Alanine

On an industrial scale, L-alanine is manufactured from L-aspartic acid by using immobilised *Pseudomonas dacunhae* cells (Chibata, Kakimoto et al. 1965). This reaction is catalysed by the aspartate β-decarboxylase (Hoppe, Martens 1984, Leuchtenberger, Huthmacher et al. 2005) as shown in Formula 1-5. The direct fermentation process of L-alanine is hindered as racemic mixtures of L-, D-alanine are accumulated during the fermentation process due the presence of an alanine racemase (Drauz, Grayson et al. 2005). Therefore, some mutants of

strains were developed, e.g. a D-cycloserine resistant mutant selected from *Brevibacterium lactofermentum* or an alanine racemase-deficient mutant of *Arthrobacter oxydans*, which are able to produce either D-alanine (*Brevibacterium lactofermentum) or L-alanine (Arthrobacter oxydans)* in excess (Drauz, Grayson et al. 2005, Hashimoto, Katsumata 1998). The non-essential AA L-alanine is used in infusion solutions, for flavour enhancing (sweet-sour) or for the synthesis of vitamin B_6 (Wagenknecht, Knapp 2006).

1.2.6.2 L-Arginine

The semi-essential, alkaline AA L-arginine is nowadays mainly produced by direct fermentation. Therefore, mutants from *Corneybacterium glutamicum*, *Bacillus subtilis or Serratia marcescens* are applied. These strains are able to accumulate 25-35 g/l L-arginine from glucose (*Corneybacterium glutamicum and Bacillus subtilis*) and 60-100 g/l (*Serratia marcescens)* respectively. Small amounts of L-arginine are still extracted from protein hydrolyzates (Drauz, Grayson et al. 2005). L-Arginine is mainly used for therapeutic purposes, e.g. in infusion solutions or for therapeutics (Crueger, Crueger 1989).

1.2.6.3 L-Asparagine

L-Asparagine, which is applied in therapeutic agents as well, can be extracted from potato starch or it can be synthesised from L-aspartic acid (Drauz, Grayson et al. 2005). The reaction is performed, for instance, by using the enzyme asparagin-synthetase which converts L-aspartic acid into L-asparagine while consuming adenosine-5-triphosphate (ATP) (Wagenknecht, Knapp 2006).

1.2.6.4 L-Aspartic acid

The industrial production of L-aspartic acid is done enzymatically with aspartase (L-aspartase ammonia lyase, EC 4.3.1.1). The reaction scheme is shown in Formula 1-5. Due to the enzymatic production process higher L-aspartic acid concentrations can be achieved and fewer undesired by-products are built. Afterwards, L-aspartic acid is separated from the reaction mixtures by the means of crystallization (Drauz, Grayson et al. 2005, Hoppe, Martens 1984, Leuchtenberger, Huthmacher et al. 2005). About 15000 t of L-aspartic acid are manufactured per year (Drauz, Grayson et al. 2005). High amounts of it are used for the production of the sweetener Aspartame, which is a dipeptid consisting of L-aspartic acid and L-phenylalanine. Moreover, L-aspartic acid is an ingredient of parenteral nutrition and aspartats of magnesia or potassium are used to medicate coronary heart diseases (Wagenknecht, Knapp 2006).

1.2.6.5 L-Cysteine

The production of L-cysteine on an industrial scale is done through enzymatic catalysis, by fermentation and by extraction from acid hydrolyzates, whereas the later is still an important production method for this AA. This hydrolysis process uses keratinous proteins from human hair and animal feathers (Wada, Takagi 2006). First L-cystin is isolated, which has to be reduced electrolytically to L-cysteine afterwards (Drauz, Grayson et al. 2005). Approximately 2500 t/a L-cysteine are extracted from protein hydrolyzates (Wagenknecht, Knapp 2006). Additionally, L-cysteine can be produced through a bioconversion process. This process, which has been industrialised by Ajinomoto, comprises the asymmetric hydrolysis of DL-2-amino-Δ2-thiazoline-4-carboxylic acid (DL-ATC) to L-cysteine by microorganisms (*Pseudomonas sp.*) (Wada, Takagi 2006, Ryu, Ju et al. 1997). Furthermore, fermentative

methods for the production of L-cysteine are applied as reported by Wada and Takagi. *Corynebacterium glutamicum* and *Escherichia coli* are used to synthesise L-cysteine from L-serine, whereas the direct fermentation of L-serine is a difficult process as well. As L-serine is an important intermediate and it is often converted into other substances like glycine, pyruvate and L-cysteine (Wada, Takagi 2006). Furthermore, a process was published using a modified *E. coli* strain, where L-cysteine is directly fermented from glucose (Takagi, Wada et al. 2005). Considering all available production methods about 7000 t L-cysteine are produced per year (Drauz, Grayson et al. 2005). The non-essential AA is used in cosmetics (hair), in bakery products or in pharmaceuticals. Since the thiol group can serve as a radical scavenger, it can be used, for instance, to prevent radiation damage. Furthermore, L-cysteine is applied in case of poisoning with heavy metals or in case of infectious diseases (Wagenknecht, Knapp 2006).

1.2.6.6 L-Glutamic Acid

As aforementioned (chapter 1.2.5.1), L-glutamic acid or L-glutamate, respectively, was the first AA, which was discovered to be directly excreted by wild type strains. The production of L-glutamate is still done with the *Corynebacterium glutamicum* exclusively and successfully on an industrial scale. In general, molasses (sucrose) from sugar cane or sugar beet, starch hydrolyzates (glucose) or acetic acid (especially in Japan) are used as carbon sources for this process on an industrial scale. Furthermore, ammonium sulphate, ammonia (gaseous or in water) or urea are applied as nitrogen sources. Furthermore, the process is influenced by the dissolved oxygen concentration, which has to be controlled to reduce the formation of undesired by-products, the biotin concentration and the pH (Drauz, Grayson et al. 2005, Crueger, Crueger 1989, Eggeling, Pfefferle et al. 2001). Normally, after 40-60 h the fermentation process of glutamic acid is completed and fermentation broths with more than 150 g/l glutamic acid should have been built. Yields of 60-70 % L-glutamic acid, based on the glucose input, have been reported (Eggeling, Pfefferle et al. 2001).

Within the fermentation broth, L-glutamic acid is available as ammonia glutamate. Typical downstream processes have to be applied to recover the final product. First, the fermentation broth is deactivated followed by separating the cells through ultrafiltration or centrifugation. Secondly, a basic anion exchanger is used to separate the ammonia, which does not interact with the resin's functional groups. The L-glutamate is eluted with sodium hydroxide to form monosodium glutamate and to regenerate the basic anion exchanger. From the eluates monosodium glutamate is recovered through crystallization followed by filtration and drying (Drauz, Grayson et al. 2005, Eggeling, Pfefferle et al. 2001).

As aforementioned in Table 1-11 L-glutamic acid and L-glutamat, respectively, is the most produced AA in the world (Drauz, Grayson et al. 2005). In fermentation processes with *Coryneform bacterium* approximately 1.5 million tons of L-glutamic acid are produced per year. Despite reports that monosodium glutamate could have negative impacts on human health, high amounts are still used as a taste enhancer. On average, prepared food contains 0.1-0.8 % of monosodium glutamate. In South-East Asia the supplemented amount is higher and the market is still growing by about 6 % per year. Ajinomoto, Miwon, Kyowa-Hakko and Cheil-Jedang are the major producers of monosodium glutamate (Hermann 2003).

1.2.6.7 L-Glutamine

L-Glutamine is mainly produced through fermentation by applying mutants of *Brevibacterium flavum*, *Corynebacterium glutamicum* or *Microbacterium flavum*. For the fermentation of L-glutamine the same carbon- and nitrogen-sources are usually used as for the fermentation of L-glutamic acid. For instance, it has been reported that by using the mutant *Brevibacterium*

flavum FERM BP-662 a product solution containing 39 g/l L-glutamine was produced in 48 h from 10 % glucose (Yoshinaga, Fujisawa et al. 1987). Although, L-glutamine is a non-essential AA, it is mainly applied for medical and therapeutic purposes. In particular it has been reported that L-glutamine has been used to medicate epilepsy, alcoholism and gastric disorders, such as gastric ulcers (Hermann 2003, Maison, Knapp 2007). Further applications of L-glutamine are: an ingredient of pharmaceuticals used to improve liver or brain functions, immune enhancement agents after major trauma or surgery and applications in cosmetics or as a food additive (Hermann 2003, Kusumoto 2001, Tapiero, Mathé et al. 2002). In 2001, approximately 2000 t of L-glutamine were produced for the pharmaceutical sector and as a so called health food (Kusumoto 2001).

1.2.6.8 Glycine

Glycine is an achiral AA and therefore it is possible to produce it by chemical synthesis. Details of its synthesis have already been discussed in chapter 1.2.5.2. Glycine is used in different areas, e. g. as a buffer substance, flavour enhancer or in dietary food. Furthermore, derivates of glycine are ingredients of antibiotics (ampicillin and amoxicillin) (Knapp 2004).

1.2.6.9 L-Histidine

Nowadays, L-histidine is mainly produced fermentatively, where mutants of *Corynebacterium glutamicum* or *Serratia marcescens* are used. These strains are able to produce 15 g/l and 23 g/l L-histidine, respectively. By further improvements L-histidine concentrations of 28-40 g/l have been reached (Drauz, Grayson et al. 2005). Furthermore, some L-histidine is still extracted from protein hydrolyzates. Most L-histidine is applied in therapeutic agents, which are used to medicate allergies, ateriosclerosis, anaemia or rheumatoid arthritis (Knapp 2004).

1.2.6.10 L-Hydroxyproline

Naturally, L-hydroxyproline is formed from L-proline by a posttranslational hydroxylation reaction. In particular, high amounts of L-hydroxyproline (*trans*-4-hydroxy-L-proline) are found in collagen (12.4 %) (Maison, Knapp 2007). In the past, the extraction of L-hydroxyproline from collagen or gelatine was the usual method applied (Drauz, Grayson et al. 2005). Nowadays, L-hydroxyproline is mainly manufactured enzymatically from L-proline by using modified *E. coli* strains. Furthermore, a method for the direct fermentation of L-hydroxyproline, which is an important chiral precursor for the chemical synthesis of pharmaceuticals, from glucose was established in 1997 (Ogawa, Shimizu 2002).

1.2.6.11 L-Isoleucine

L-Isoleucine is an essential AA and belongs next to L-leucine and L-valine to the branched AAs (BCAA). It is mainly used for infusions or in dietary products for special nutrition. Different production methods of L-isoleucine have been established. Firstly, some L-isoleucine is still extracted from protein hydrolyzates. Secondly, it is manufactured using microorganisms from precursors (e. g. α-ketobutyrate or ethanol). Thirdly, methods using modified classical strains, such as *Corynebacterium glutamicum*, *Serratia marcescens and Escherichia coli* starting with glucose are reported and used as well. For instance, the mutant *Escherichia coli* H-8461 accumulates 30 g/l L-isoleucine. All in all, about 500 t of L-isoleucine are produced worldwide per year (Drauz, Grayson et al. 2005, Eggeling, Morbach et al. 1997).

1.2.6.12 L-Leucine

The production of L-leucine, which is applied in the same products as L-isoleucine, is partly done by extraction from protein hydrolyzates. Hence, the fermentative production of L-leucine is becoming more considerable and the use of different mutants from the common strains *Corynebacterium glutamicum*, *Serratia marcescens or Brevibacterium flavum* has been reported. For instance, the mutant *Brevibacterium flavum* AJ 3686 is able to accumulate 19.5 g/l L-leucine (Drauz, Grayson et al. 2005, Eggeling, Morbach et al. 1997).

1.2.6.13 L-Lysine

For the production process for L-lysine, mutants from *Corynebacterium glutamicum* are applied. Raw materials and therefore carbon-sources for production are sugar cane or sugar beet molasses, high test molasses (inverted can molasses), sucrose or starch hydrolyzates. Ammonium sulphate and ammonia (gaseous or in water) are common as nitrogen sources. Additionally, based on the biosynthesis pathway of L-lysine growth factors, such as L-homoserine, L-threonine or L-methionine are necessary for the production. These species are provided directly or indirectly from plant protein hydrolyzates or cornsteep liquor. Usually the fermentation process is performed in fermenters of up to 500 m^3, which yield in L-lysine concentrations up to 170 g/l. In the fermentation broth, ammonium sulphate is used to neutralise the alkaline AA L-lysine and subsequently L-lysine is present as sulphate. Commonly, L-lysine is given as L-lysine hydrochlorid, therefore different downstream processes are known to gain the required species (Drauz, Grayson et al. 2005, Eggeling, Pfefferle et al. 2001). For example, the downstream process for L-lysine consists of:

- Removal of the bacterial cells from the fermentation broth by separation or ultrafiltration
- Adsorbing and collecting lysine in an ion exchange step
- Crystallising or spray drying of lysine as L-lysine hydrochloride

Alternative routes are also known and applied depending on the final use of the L-lysine on the market. For instance, it is possible to concentrate the fermentation broth followed by filtering the precipitated salts. This alkaline solution contains up to 50 % L-lysine and is stable enough to be sold. Moreover, the fermentation broth can be immediately evaporated, spray-dried and granulated. By applying this downstream process a feed-grade-product, which contains L-lysine sulphate, is obtained (Drauz, Grayson et al. 2005, Eggeling, Pfefferle et al. 2001). The essential AA L-lysine is mainly used as a feed additive. As aforementioned (chapter 1.2.3.2), L-lysine is the so called limiting AA for feeding animals and is often added to increase the feed quality (Hermann 2003).

1.2.6.14 D,L-Methionine and L-Methionine

As already metioned and shown in chapter 1.2.5.2, D,L-methionine is mainly produced through chemical synthesis, which is the most economic mode of production. Moreover, enzymatic production using acylase from *Aspergillus oryzae* or microbial conversion by applying the *Pseudomonas sp* strain NS671 are also known of. Methionine is a very important feed additive used on an industrial scale (Drauz, Grayson et al. 2005).

1.2.6.15 L-Phenylalanine

In the last few decades several production methods for the essential, aromatic AA L-phenylalanine comprising chemical synthesis, enzymatic methods and direct fermentation have been developed. Some years ago enzymatic methods such as the stereoselective and enantioselective addition of ammonia to *trans*-cinnamic acid by the L-phenylalanine ammonia lyase were the preferred production method for L-phenylalanine on an industrial scale. However, nowadays fermentative methods are more prevalent, due to their higher economic efficiency (Drauz, Grayson et al. 2005, Knapp 2005). These fermentations are conducted with modified strains of *Escherichia coli* or *Corynebacterium glutamicum*. Whereas, in the case of fermentations done with *E. coli*, it is important to apply an ideal sugar-feeding-strategy to avoid the formation of acetic acid. Under ideal fermentation conditions, *E. coli.* is able to produce concentrations of 50.8 g/l L-phenylalanine within 2.5 days (Eggeling, Pfefferle et al. 2001). The recovery of L-phenylalanine from the fermentation broth is done either by a two-step crystallisation or by an ion-exchange process. Firstly, the cells are separated by ultrafiltration. Subsequently, the ion exchanger resin (non-polar) is applied to generate purified L-phenylalanine (Drauz, Grayson et al. 2005). Huge amounts of L-phenylalanine are needed for the production of the sweetener Aspartame. Furthermore, it is used for parental nutrition (Knapp 2005).

1.2.6.16 L-Proline

L-Proline is still partly extracted from protein hydrolyzates, although direct fermentative production methods are becoming more considerable due to higher yields and lower costs. For this fermentation process modified strains of *Corynebacterium glutamicum*, *Serratia marcescens or Brevibacterium flavum* are applied. For instance, it is claimed that in a fermentation process using *Brevibacterium flavum AP113* up to 97.5 g/l L-proline were accumulated (Drauz, Grayson et al. 2005).

1.2.6.17 L-Serine

The microbiological or enzymatic conversion, respectively, of glycine is the most common method of producing L-serine. For this conversion *Hyphomicrobium* strains, which are able to catalyse the formation of L-serine from methanol and glycine, have been applied. The reaction is first catalysed by the methanol dehydrogenase, which converts methanol into formaldehyde. Secondly, the formaldehyde and glycine are converted into L-serine. The use of other modified strains from *Escherichia coli* or *Klebsiella aerogenes* for this reaction are reported as well (Drauz, Grayson et al. 2005, Izumi, Yoshida et al. 1993). Since L-serine is a very important intermediate in the biosynthesis of other AAs, nucleic acids and phospholipids, the direct fermentation starting with glucose was observed, but has not yet been established. L-Serine is used for pharmaceuticals (infusion), within the cosmetic industry and as educt for biochemical and chemical reactions (Peters-Wendisch, Stolz et al. 2005).

1.2.6.18 L-Threonine

Every year about 85000 t of L-threonine are manufactured, with large amounts being used as a feed additive. Furthermore, L-threonine is applied to infusion solutions and for nutrition (Drauz, Grayson et al. 2005, Knapp 2004). For production on an industrial scale fermentative processes with different microorganisms (*Escherichia Coli* or *Corynebacterium glutamicum*) have been established. However, *E. coli* mutants have shown better results. For instance, it was published that the strain *E. coli* BKIIM B-5318 is able to accumulate 70 g/l L-threonine

within 32 h of fermentation (Debabov, et al. 1992). Generally, glucose or sucrose is used as a substrate in these fermentations and some other components (e. g. yeast extract) are added. Furthermore, ammonia is supplied as a nitrogen source. Finally, these fermentation parameters result in concentrations of 85 g/l L-threonine and, depending on the carbon source, yields of up to 60 % are possible. Due to a low amount of by-products and the low solubility of L-threonine in water, the following downstream process is quite simple: Firstly, the cells are coagulated by a heat- or pH-treatment step followed by filtration. Secondly, by cooling the concentrated fermentation broth the crystallisation of L-threonine starts. Subsequently, L-threonine crystals with a purity of more than 90 % can be separated (Eggeling, Pfefferle et al. 2001).

1.2.6.19 L-Tryptophan

Different strategies are applied for the production of L-tryptophan, which is a limiting essential AAs. For instance, the enzymatic and fermentative production from various precursors and the direct fermentation from carbohydrates have been reported. Considering the enzymatic synthesis, L-tryptophan is built, for instance, from indole, pyruvate and ammonia by the enzyme tryptophanase. In particular, in so called bioreactors these production strategies of L-tryptophan were very effective and concentrations up to 200 g/l of L-tryptophan have been published. However, the enzymatic production was too expensive due to high costs for the starting materials (Drauz, Grayson et al. 2005, Knapp 2005, Hamilton, Hsiao et al. 1995). About 20 years ago, the Japanese company Showa Denko patented a process for the production of L-tryptophan with the strains *B. subtilis* and *B. amyloliquefaciens*, which were able to generate 40 g/l L-tryptophan from the precursors indol or antranilic acid. The process using antranilic acid was commercialised. Due to by-products (e.g. 1,1-ethylidene-bis-(L-tryptophan)), which were responsible for the serious disease eosinophilia-myalgia syndrome (EMS), the production was stopped (Drauz, Grayson et al. 2005, Leuchtenberger 1996, Knapp 2005, Mayeno, Gleich 1994). Therefore, direct fermentation processes with *Corynebacterium glutamicum* and *E. coli* have been favoured and improved upon. Strains have been reported which are able to accumulate 30-50 g/l L-tryptophan with yields up to 20 % depending on the carbon-source. Furthermore, some losses during the crystallisation process will occur, as L-tryptophan is not stable under oxygen (Drauz, Grayson et al. 2005, Leuchtenberger 1996). As already mentioned L-tryptophan is one of the limiting essential AAs. Therefore, it is a very important component for the breeding of pigs and poultry (Drauz, Grayson et al. 2005). Furthermore, it is used in infusion solution and for special nutrition (Knapp 2005).

1.2.6.20 L-Tyrosine

The non-essential, aromatic AA L-tyrosine is mainly extracted from protein hydrolyzates. Due to its low solubility in water, L-tyrosine can be removed from the extraction mixture quite simply. Subsequently, the downstream process is done by applying ion exchanger and/or chromatography (Drauz, Grayson et al. 2005). L-Tyrosine is an important compound for many different applications. L-Tyrosine is a common dietary supplement or it is also used to control depression and anxiety. Additionally, L-tyrosine is an important precursor for other high-value compounds such as L-Dopa, which is used to medicate Parkinson's disease (Lütke-Eversloh, Santos 2007).

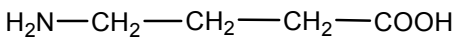
1.2.6.21 L-Valine

About 1000 t of the essential, branched AA L-Valine are produced every year. It is used, for instance, for infusion solutions or dietary food and it is partly generated through chemical

synthesis (e. g. Strecker-synthesis). Afterwards, pharmaceutical grade L-valine is produced by an enzymatic resolution of N-acetyl-D,L-valine. However, direct fermentation methods usually starting with glucose are also known and established. For instance, fermentative L-valine productions using mutants of *Brevibacterium* and *Corynebacterium glutamicum*, which accumulate up to 39 g/l or 99 g/l L-valine, have been reported (Drauz, Grayson et al. 2005, Katsurada, Uchibor et al. 1988, Knapp 2004).

1.2.6.22 γ-Aminobutyric acid

Next to the already discussed proteinogenic AAs, γ-aminobutyric acid (GABA), which is shown in Formula 1-6, was often detected in the silage juice. GABA is an inhibitory neurotransmitter, which is built from L-glutamic acid in brains (RÖMPP Online 2013). For instance, GABA has antihypertensive and anti-stress effects on human health. Moreover, GABA is widespread in nature and also found in different foods, such as tea or brown rice. Normally, it is produced fermentatively and it is mainly applied in the food sector (Iimure, Kihara et al. 2009). GABA is also an important intermediate of the promising plastic polyamide 4, which can be produced from substances out of renewable resources (Habe, Yamano et al. 2010).



Formula 1-6: γ-aminobutyric acid (GABA)

1.2.7 AA products on the market

As AAs are used in different sectors, there are many different products available. Depending on the specific market, there are differing requirements in purity grade and composition. Furthermore, based on these manifold requirements and applications for AAs, many different producers operate all over the world. For instance, the world global market leader in the production of AAs is the Japanese company Ajinomoto. As already mentioned, this company discovered the potential of monosodium glutamate very early. In general, the AA products, which are currently available on the market, can be distinguished in the following way:

1. Single AAs

   Based on further applications and on the required purity grade, this branch can be further divided into:

   - AAs for animal feed
   - AAs for food, foodstuff and functional food (flavour enhancing compounds, dietary supplements)
   - AAs for pharmaceuticals or cosmetics (Personal Care)

2. AA mixtures

   As AA mixtures are used for different applications as well, this branch can also be divided into different parts:

   - AA mixtures for fertilizers or feed stuff
   - AA mixtures as flavour enhancers or for dietary supplements
   - Mixtures in cosmetics
   - Other applications

1.2.7.1 Single AAs

Feed stuff

More and more people in emerging economies, such as China, have the opportunity of changing their nutritional behaviour and to consume larger amounts of meat. The FAO (Food and Agriculture Organization) estimates that worldwide meat consumption will grow from 37.4 kg per capita in year 2000 to 52 kg per capita in 2050. To supply the growing demand for meat, animal breeding has to be optimised. As plant protein often lacks in lysine or methionine, the use of plant protein does not support efficient animal breeding. For increasing the efficiency of breeding, some single AAs are added to conventional feed stuff like maize (Evonik Industries 2009). Some single AAs available on the market (e.g. from Evonik or Ajinomoto) used to upgrade normal feed stuff, are described in the following chapter in detail:

E v o n i k

Name **Amino acid**	**MetAmino®** (Evonik Industries 2011) **DL-Methionine, Feed Grade 99 %**
Optical properties	white to yellowish, crystalline powder
Mean particle size distribution	min. 90 %, 0-1000 µm
Solubility	app. 30 g/l in water at 20 °C
Drying loss (max.)	0.3 %
Ash (max.)	0.5 %
Methionine	99 %
Digestibility	100 %
Crude protein	58.1 % (N x 6.25)
Production	MetAmino® is synthesised from petrochemical raw materials by applying the environmentally friendly “carbonate-process”
Application	Especially suitable for feeding monogastric animals, such as poultry, to guarantee a most possible supply with sulphur containing AAs. Furthermore, a „rumen-stable“ form is available especially for ruminants

Name **Amino acid**	**TrypAmino®** (Evonik Industries 2011) **L-Tryptophan, Feed Grade 98 %**
Optical properties	white to light grey, crystalline powder
Mean particle size distribution	min. 90 %, 0-100 µm
Drying loss (max.)	1 %
Ash (max.)	1 %
Solubility	app. 11 g/l in water at 20 °C
Tryptophan	98 %
Digestibility	100 %
Crude protein	84 % (N x 6.25)
Production	by fermentation
Application	supply of the essential AA tryptophan, especially important in pig breeding

Name **Amino acid**	**ThreAmino®** (Evonik Industries 2011) **L-Threonine, Feed Grade 98.5 %**
Optical properties	beige to light brown, crystalline powder
Mean particle size distribution	min. 90 %, 0-300 µm
Drying loss (max.)	0.5 %
Ash (max.)	0.5 %
Solubility	app. 90 g/l in water at 20 °C
Threonine	98.5 %
Digestibility	100 %
Crude protein	72.4 % (N x 6.25)
Production	by fermentation
Application	supply of the essential AA threonine, especially important in pig breeding

Ajinomoto

Name	**L-Lysine HCl 99 % Feed Grade** (Ajinomoto Eurolysine S.A.S. 2011)
Optical properties	white to light yellowish, crystalline powder
Mean particle size distribution	min 95 %, 0-1250 µm
Drying loss (max.)	1.5 %
Ash (max.)	0.5 %
Solubility	642 g/l water to 20 °C
Lysine	78 %
Digestibility	100 %
Crude protein	93.4 % (N x 6.25)
Production	by fermentation with raw material from agriculture
Application	animal feed

Name **Amino acid**	**LLB 50** (Ajinomoto Eurolysine S.A.S. 2011) **Liquid Lysine Bas 50 % Feed grade**
Drying loss (max.)	53.1 %
Ash (max.)	0.25 %
Lysine	50 %
Digestibility	100 %
Crude protein	61.3 % (N x 6.25)
pH	10 to 11
Production	by fermentation with raw material from agriculture
Application	animal feed

Name	**L-Valine Feed Grade** (Ajinomoto Eurolysine S.A.S. 2011)
Optical properties	white, crystalline powder
Mean particle size distribution	1.5 %
Ash (max.)	0.5 %
Solubility	57 g/l water at 20°C
Valine	96.5 %
Digestibility	100 %
Crude protein	72.1 % (N x 6.25)
Production	by fermentation with raw material from agriculture
Application	animal feed

Name	**L-Tryptophan 98 % Feed Grade** (Ajinomoto Eurolysine S.A.S. 2011)
Optical properties	white to yellowish powder
Drying loss (max.)	1 %
Ash (max.)	1 %
Solubility	11.4 g/l water at 20 °C
Tryptophan	98 %
Digestibility	100 %
Crude protein	84 % (N x 6.25)
Production	by fermentation with raw material from agriculture
Application	animal feed

Name	**L-Threonine 98.5 % Feed Grade** (Ajinomoto Eurolysine S.A.S. 2011)
Optical properties	white to yellowish, crystalline powder
Drying loss (max.)	0.5 %
Ash (max.)	0.5 %
Solubility	97.6 g/l water at 20 °C
Threonine	98 %
Digestibility	100 %
Crude protein	72 % (N x 6.25)
Production	by fermentation with raw material from agriculture
Application	animal feed

Further sources for feed grade single AAs

Besides Evonik and Ajinomoto there are further companies, which sell feed grade AAs. Many of these companies are located in China. For example, the following product is available:

Name **Producer**	**L-Lysine Sulphate 65 % / 70 % Feed Grade** **CBH Qingdao Co., Ltd.** (CHB Qingdoa Co. Ltd., 2012)
Optical properties Lysine Impurity	brown granulate 50-55 % by-products from fermentation, other AAs
Production	by fermentation with raw materials from agriculture (maize starch)
Application	animal feed

AAs for food, foodstuff and functional food

In addition to the essential properties of AAs for human nutrition, AAs supply further properties, which are interesting for the food and foodstuff sector. For instance, AAs can be used as a flavour enhancer or sweetener:

- *Glutamate*

As aforementioned, the salts of glutamic acid (e. g. monosodium glutamate or MSG) are the most common AAs used within the nutrition sector. Due to their flavour enhancement, these salts are widespread in Asian cuisines (Drauz, Grayson et al. 2005).

- *L-Cysteine*

This AA is used as a flavour enhancer as well. Furthermore, it is used in bakeries (Drauz, Grayson et al. 2005).

- *Aspartame*

The sweetener Aspartame is a peptide made of aspartic acid and phenylalanine.

Moreover, single AAs or mixtures of AAs are applied in sports nutrition or for functional food. Subsequently, some examples for AA products on this market are presented:

Name **Producer**	**Ultimate Nutrition GABA* 90 Caps** **Ultimate Nutrition** (Body Attack Sports Nutrition, 2012)
Effects according to the producer Ingredients	GABA shortens the time of recovery and supports an effective muscle formation γ-aminobutyric acid (GABA), cellulose, dicalciumphosphate, gelantine, magnesia stearate
Information available at Price	http://www.body-attack.de/Ultimate-Nutrition-GABA.html € 24,99 per 750 mg (90 capsulas)

Name **Producer**	**Glutamine Elite Liquid – 453 ml** **EFX** (Body Attack Sports Nutrition, 2012)
Effects according to the producer Ingredients	an effective recovery of muscles L-glutamine, water, glycerine, flavour, citric acid, adhesive agent xanthum gum, colorant, potassium sorbate
Information available at Price	http://www.body-attack.de/glutamine-efx-elite-liquid.html € 22,46 for 453 ml

Name **Producer**	**Body Attack Glutamine Shock 80 Caps** **Body Attack** (Body Attack Sports Nutrition, 2012)
Effects according to the producer	accelerates the formation of muscles, improves the supply of energy, power and endurance
Ingredients	glutamine peptide, capsules are made of gelatine (cattle), L-alanine, D-aspartic acid, pyridoxine hydrochloride, separation agent: magnesia salts of fatty acids, colourants: iron oxide, titanium oxide and azorubine
Exact ingredients (for 4 capsules)	protein: 2.8 g carbohydrates:< 0.4 g fat: < 0.4 g vitamin B6: <16 mg L-alanine: 400 mg D-aspartic acid: 400 mg glutamine peptide: 2600 mg
Information available at	http://www.body-attack.de/Body-Attack-Glutamine-Shock.html
Price	€ 19,95 for 80 capsules

Name **Producer**	**Body Attack Arginine Shock - 80 capsules** **Body Attack** (Body Attack Sports Nutrition, 2012)
Effects according to the producer	arginine improves the building of NO in blood vessels cells
Ingredients	L-arginine-hydrochlorid (70.5 %), L-citrullin-DL-malate (16.8 %), capsules are made of gelatine (cattle), separation agent: magnesia salts of fatty acids, colorant: titanium oxide, iron oxide
Exact ingredients (for 4 capsules)	proteins: 2.8 g carbohydrate:< 0.4 g fat: < 0.4 g vitamin B6: <16 mg
Information available at:	http://www.body-attack.de/Body-Attack-Arginine-Shock.html
Price	€ 22,49 for 80 capsules

Application for pharmaceuticals or cosmetics

For these applications ultra-pure AAs are used, which are applied, for instance, in infusions (parenteral nutrition). Many companies specialise in this sector. Additionally, the global players Ajinomoto and Evonik industries also have affiliates, which are concentrated on this market sector.

1.2.7.2 Mixtures of amino acids

Mixtures for fertilizers or feed stuff

There are fertilizers available on the market containing mixtures of AAs. The AAs in the fertilizers, which are used for so called leaf fertilization, originate from hydrolysed vegetable or animal proteins. Subsequently, some fertilizers including AAs are presented.

Name **Producer**	**Aminosol-PS** (Lebosol-Dünger GmbH 2012) **Lebosol-Dünger GmbH**
Effects according to the producer	supports plant growth in situations of stress, improves the plants' immune system, AAs promote the cell division and the growth of roots
Ingredients	vegetable protein hydrolysate with more than 20 AAs and peptides density: 1.13 kg/l pH: 4.0 to 6.5
Application	different plants, such as strawberries, vegetables, tobacco, tree nurseries, stone fruits, wine
Information available at	http://www.lebosol.de/produkte/

Name **Producer**	**Aminosol** (Lebosol-Dünger GmbH 2012) **Lebosol-Dünger GmbH**
Effects according to the producer	supports plant growth in situations of stress, improves the plants' immune system, AAs promote the cell division and the growth of roots, AAs fend ground game
Ingredients	fertilizer solution of organic nitrogen from animal by-products (category 3) 9 % organic bound nitrogen (111 g/l N) density: 1.23 kg/l pH: 5 to 7
Application	different plants, such as strawberries, vegetables, tobacco, tree nurseries, stone fruits, wine
Information available at	http://www.lebosol.de/produkte/

Name **Producer**	**Bio-Aminosol** (Lebosol-Dünger GmbH 2012) **Lebosol-Dünger GmbH**
Effects according to the producer	supports plant growth in situations of stress, improves the plants' immune system, AAs promote the cell division and the growth of roots, AAs fend ground game
Ingredients	60 % hydrolyzed protein from slaughterhouse waste 39.8 % water 0.17 % preservative 20 different AAs and peptides pH: 5-7
Application	different plants, strawberries, vegetables, tobacco, tree nurseries, stone fruits, wine
Information available at	http://www.lebosol.de/produkte/

Name **Producer**	**Lysodin Flüssiger Spurennährstoffdünger Multimix** (Intrachem Bio Deutschland 2005) **Intrachem Bio Deutschland GmbH & Co. KG**
Effects according to the producer	reduces plant stress, immediately available for plants, supports weakened plants, tolerated by plants
Ingredients	50 % AA 0.5 % boron 0.02 % cobalt 0.5 % copper 2.0 % iron 0.5 % manganese 0.02 % molybdenum 0.5 % zinc
Application	leaf fertilizer for fruit-growing, winegrowing, arable farming, ornamental plants
Information available at	http://www.intrachem-bio.de/index.htm

Name **Producer**	**Wuxal Profi** (Syngenta Agro AG 2009) **Flüssigdünger mit Haupt- und Mikronährstoffen zur Blatt- und Wurzeldüngung** **Syngenta**
Effects according to the producer	Supports the plants' growth
Ingredients	80 g/l N total nitrogen 80 g/l P_2O_5 total phosphate 60 g/l K_2O potassium oxide 200 mg/l boron 50 mg/l copper + other mineralic compounds 0.02 % cobalt 57 g/l AAs
Application	sugar beet, potatoes, rape, vegetables, fruit-growing, wine, ornamental plants
Information available at	www.syngenta-agro.com

In addition to these liquid fertilizers with AAs, mixed AA powders are available on the market as well. These powders, which are mainly applied as feed stuff, are offered by Asian companies. For instance, the following product of AA powder is available.

Name **Producer**	**Compound Amino Acids Powder** **Wuhan Soleado Technology Co.,Ltd.** (Wuhan Soleado Technology Co. Ltd., 2012)	
Applications	feeding stuff, fertilizer or fermentation industry	
Ingredients	Compound:	Rate [%]
	L-Aspartic acid	3.85
	L-Threonine	3.82
	L-Serine	3.23
	L-Glutamic acid	8.10
	L-Proline	4.09
	Glycine	2.44
	L-Alanine	2.29
	L-Cystine	1.22
	L-Valine	3.39
	L-Methionine	0.87
	L-Isoleucine	1.70
	L-Leucine	1.76
	L-Tyrosine	0.88
	L-Phenylalanine	1.54
	L-Lysine	2.32
	L-Histidine	0.91
	L-Arginine	4.20
	L-Tryptophan	0.65
	Sum AA	47.26
	Moisture	3.21
	NH_4^+/NH_4Cl	9.7 / 43.51
	T.N.	17.12
	Heavy metals (Pb)	≤0.001
	As	≤0.0003
	Na+	0.2
	Ash	1.00
Production/Properties	1) Acidic Hydrolysis (HCl) of keratin from natural hair 2) Neutralisation and desalination 3) Spray drying 4) Properties: • yellow powder • soluble • hygroscopic • contains 18 free L-AAs	
Information available at	http://www.wuhansoleado.com/html-en/product-nTKUpfVDShnm-Compound+Amino+Acids+Powder.html	

Mixures for flavouring/cosmetics

Not only single AAs, but also mixtures of AAs are used for flavouring or as flavour enhancers. In this segment the following types of AA mixtures can be distinguished:

- *HVP (Hydrolyzed vegetable Protein)*

Hydrolysed vegetable proteins, which are used for flavouring, are called HVP. Raw materials for these AA mixtures are soybean flour, wheat or maize. The hydrolysis of these raw materials is normally done by hydrochloric acid followed by a neutralisation step with sodium hydroxide. The mixture of hydrolysed protein contains a lot of different, strong flavours originating from free AAs, low molecular peptides, salts and other volatile components. Further process steps (Maillard-reaction) enhance these flavours. HVP is used in many meals, e. g. in soups, instant meals or chips (Dall, Martens et al. 1998, Novalin-Canoy 2011).

- *HAP (Hydrolyzed Animal Protein)*

For producing these AA mixtures, animal proteins, such as feathers, fur, hair, bones, waste from leather or collagen, are treated by acidic hydrolysis (Novalin-Canoy 2011). The hydrolysed products are often used as flavour enhancers. Additionally, HAP is applied and therefore found in various cosmetic products e. g. for skin care, hair care or cosmetics. By searching on the internet for HAP products numerous offers from Asian companies can be found. For instance, the following product is offered:

Name **Producer**	**Hydrolysed animal protein with 90-98 % chicken or fish protein for cosmetic - JINJIAN** **Cangzhou City Jinjian Gelatin Co., Ltd.** (Cangzhou City Jinjian Gelatin Co. Ltd., 2012)
Application according to the producer Properties and ingredients	food and foodstuff, medicine, cosmetics, food supplements white to light yellow powder smell: hydrolysed protein <2 % ash >92 % protein <3 % moisture pH: 5-7 heavy metals (Pb): <5 ppm
Information available at	http://jinjiangelatin.en.alibaba.com/

Food supplements/products for athletes

Mixtures of AAs are also applied as food supplements particularly in products for athletes (e. g. for bodybuilding). Subsequently, some examples of products on the market are given:

- BCAAs (branched-chain amino acids)

Due to the structure of the side chain, the essential AAs L-leucine, L-isoleucine and L-valine are called branched-chain AAs. According to producers, mixtures of these AAs effectively support training and therefore the formation of muscles. However, actual effects are not proven scientifically, but BCAA products are available on the market as shown subsequently (Novalin-Canoy 2011, Negro, Giardina et al. 2008). The frequently recommended ratio for an optimal intake of L-leucine, L-isoleucine and L-valine is 2:1:1.

Name **Producer**	**Body Attack BCAA capsules** **Body Attack** (Body Attack Sports Nutrition, 2012)
Effects according to the producer	supports the formation of muscles and the regeneration; BCAAs stimulate the release of insulin, which promotes the formation of muscles; suppresses the release of serotonin, which affects exhaustion
Ingredients	L-leucine (40.3 %), L-valine (20.2 %), L-isoleucine (20.2 %), capsules are made of gelatine (cattle), separation agent: magnesia salts of fatty acids, silicon dioxide and calcium phosphate, plant oil, calcium-D-pantothenat, pyridoxine hydrochloride, D-biotin, colorant: titanium oxide
Exact ingredients (for 100g BCAA capsules):	carbohydrates: <1.0 g protein: 81 g fat: 0.0 g calcium-D-pantothenat: 54.3 mg vitamin B6 13.6 mg biotin 1.1 mg L-leucine: 40.7 g L-valine: 20.4 g L-isoleucine 20.4 g
Information available at Price	http://www.body-attack.de/body-attack-bcaa-kapseln.html € 26.95 for 180 capsules

- Hydrolysates

Beside prepared mixtures of AAs originating from single AAs, there are different protein hydrolysates available on the market, which are applied, for instance, as food supplements or protein sources for athletes. Subsequently, two different products of AA hydrolysates on the market are presented:

Name	**Body Attack Whey Amino Gold - 325 tablets**	
Producer	**Body Attack** (Body Attack Sports Nutrition, 2012)	
Effects according to the producer	contains important AAs for the formation of muscles (protein shake in tablets)	
Ingredients	89 % mixtures of whey protein (whey protein concentrate and whey protein hydrolysate), adhesive agent: Di-calcium-phosphate, separation agent: silicon dioxide and magnesia salts of fatty acids	
Exact ingredients (for 100 g):	protein: 70.2 g (with 15.8 g BCAAs) carbohydrates: 5.7 g (with 5.7 g sugars) fat: 7.7 g sodium: 0.2 g	
AA profile (per 100 g protein)	AA	mg per 100 g protein
	L-alanine	4910
	L-arginine	2500
	L-aspartic acid	10720
	L-cysteine	2350
	L-glutamic acid	16620
	L-glycine	1810
	L-histidine	1880
	L-isoleucine	6280
	L-leucine	10350
	L-lysine	8890
	L-methionine	2140
	L-phenylalanine	3110
	L-proline	5770
	L-serine	5180
	L-threonine	6760
	L-tryptophan	1720
	L-tyrosine	3120
	L-valine	5890
Production	acidic or enzymatic hydrolysis of milk- and whey proteins	
Information available at	http://www.body-attack.de/body-attack-whey-amino-gold.html	
Price	€ 24,95 for 325 tablets	

Name	**Body Attack 100% Beef Amino Liquid 1000ml**
Producer	**Body Attack** (Body Attack Sports Nutrition, 2012)
Effects according to the producer	supports formation of muscles, accelerates regeneration, supports the health of joints, protects against the degradation of muscles
Ingredients	protein hydrolysate (cattle), water, fructose, acidifier: citric acid, flavour preservative: sodium benzoate sweetener: sodium cyclamate, acesulfam-K, saccharin, vitamin B6, 550 000 mg free AAs with 35 000 mg BCAAs per bottle
Production	protein hydrolysate from cattle
Information available at	http://www.body-attack.de/Body-Attack-Beef-Amino-Liquid.html
Price	€ 26,95 for 1000 ml

Other applications

In addition, to applications in food, feed, cosmetic or for medical purposes, protein hydrolysates and therefore AAs or mixtures of AAs and peptides can be used as adhesives, surfactants, foams, for polymers, coatings, foils or for packaging materials. Furthermore, AAs can be utilized as nitrogen sources for microorganisms. For instance, a company (Animox,

Berlin) in Germany has specialised on the generation of protein hydrolysates from waste with the target to produce raw materials for applications aforementioned (ANiMOX GmbH).

1.3 Ion exchange

1.3.1 Fundamentals of ion exchange and ion exchangers

According to Helfferich (Helfferich 1995) ion exchangers (IE) are commonly defined as follows:

> *Ion exchangers are insoluble solid materials which carry exchangeable cations or anions. These ions can be exchanged for a stoichiometrically equivalent amount of other ions of the same sign when the ion exchanger is in contact with an electrolyte solution.*

This means that during an IE process, ions, which are solved in a solution, are reversibly exchanged with ions from the IE's surface. The exchanged ion can be either inorganic or organic and the process consists of two steps: First, an in-change of the solute ions into the IE followed by an ex-change of the ions out of the IE. During this process, the structure of the IE is not changed irreversibly. For the IE process, different solid and insoluble materials, such as high-molecular poly-electrolytes or inorganic materials, are used (Helfferich 1995, Dorfner 1991, Universität des Saarlandes).

Generally, two types of IEs can be distinguished (Helfferich 1995). Carriers of exchangeable cations are called cation exchangers and the reaction, where IE stands for "ion exchanger" and A and B for the exchangeable ions, proceeds as follows:

$$IE^- A^+ + B^+ \Longleftrightarrow IE^- B^+ + A^+$$

Carriers of exchangeable anions are called anion exchangers. For example, the anion process is shown by the following formula:

$$IE^+ A^- + B^- \Longleftrightarrow IE^+ B^- + A^-$$

Furthermore, a third type of IE is available, which can be used either as cation or anion exchanger. This IE type is called amphoteric IE.

In principal, the IE process shows similarities to adsorption. Indeed, in an IE process the ions are stoichiometrically exchanged between the liquid phase and the IE's surface. This means that for each equivalent of ions, which is removed from the solution by the IE, the same amount of ion equivalents is released from the IE to the solution. In an adsorption process, in turn, a charged or uncharged substance dissolved in the solution is adsorbed on the adsorbent's surface, but no substances are released by the adsorbent. Theoretically, it is quite easy to distinguish these processes, but in practice the borderlines between them are often vague. For instance, each IE process is always accompanied by an adsorption process and some adsorbents, such as activated carbon, can also be used as IE (Universität des Saarlandes).

Generally, IE is a widespread phenomenon in nature, as more ions than neutral molecules are found on earth. Therefore, it can be seen as a basic process in our environment and ion exchange processes are constantly taking place in the earth's lithosphere. From a scientific point of view, IE was first discovered in the 19th century. For instance, it was found by Thompson and Way that cultivated soil can exchange various bases, such as ammonia, calcium or magnesia. This phenomena was called base exchange. In the following decades,

researchers worked on explaining the mechanism and the kinetics of the base exchange. It was then reported in 1912, that base exchange is an ion exchange and the term ion exchange was first introduced. Indeed, exact investigations of the process were difficult, as the exchange materials used were either from a natural origin and therefore heterogeneous or poorly synthesised. For that reason, the development of adequate IE materials was also encouraged. Firstly, inorganic materials were improved by modifying them, but from 1934 organic materials, such as sulfonated coals, were also used. The next milestone within the development of IEs was the discovery of Adams and Holmes in 1935. It was the first time, the production of truly, synthetic organic IEs was implemented. Based on formaldehyde condensation products of polyhydric phenol or aromatic diamines, insoluble cation or anion IE resins were produced. These first IEs, which were obtained through polycondensation, were soon replaced by polymerisation products. For instance, after 1945 D'Alelio realised the introduction of a sulfonic acid group into a crosslinked polystyrene resin, which opened new possibilities for creating IEs with different active groups. These unifuctional addition polymer resins based on polystyrene had soon become the modern cation and also anion exchange resin, which were stable over the total pH range and at higher temperatures. Well-known, modern resins of this type are Dowex 50 from the Dow Chemical Company and Amberlite IR-120 from Rohm and Haas. In the following years and until the present day, IE resins and their fields of applications have been further developed and improved. For instance, resins with other characteristics, such as macroporous or pellicular ion exchangers for chromatographic applications, have been developed. Furthermore, new applications have been discovered and processes have been optimised (Dorfner 1991).

1.3.1.1 Types of ion exchangers

Based on particular characteristics, materials are able to "perform" the IE process. Each IE consists of a basic structure, which is stabilised through bonds or lattice energy and is positively or negatively charged. These charges are compensated by contrarily charged ions, the so-called counter ions, which are then exchanged during the IE process (Helfferich 1995).

As aforementioned in the historical overview of IEs, different materials are equipped with these characteristics. Therefore, IEs can first be categorised into inorganic and organic solid phases.

Inorganic ion exchangers

The inorganic IE group consists of clay, clay materials, non-siliceous materials as well as zeolites. The latter are the most important IE materials of this group. For instance, cation-exchanging zeolites are, among others, analcite ($Na[Si_2AlO_6]_2 \cdot H_2O$), chabazite ($Ca, Na [Si_2AlO_6]_2 \cdot 6\ H_2O$) or heulandite ($Ca[Si_3AlO_8]_2 \cdot 5\ H_2O$). Research is still being carried out into these inorganic materials, as in some cases these materials are more selective and stable than organic IE resins. Furthermore, some attempts were made to produce synthetic, inorganic IEs (Dorfner 1991, Helfferich 1995).

Organic ion exchangers

Organic IEs comprise of the group of cellulose, dextran and agarose IEs, the group of coal based IEs and the most important group, the synthetic resin IEs. Compared to other IE materials, synthetic IE resins are more chemically and mechanically robust and provide a high capacity and a high throughput. Furthermore, by choosing the ions on the surface and the amount of crosslinking, it is possible to manufacture adequate resins for different purposes. Generally, synthetic resin IEs consists of an insoluble, polymeric matrix of crosslinked styrene-divenylbenzen copolymers and functional groups, which are incorporated

into the polymeric matrix. Depending either on the polymeric matrix or on the functional groups used, different types of synthetic resin IEs can be distinguished (Dorfner 1991, Helfferich 1995).

Based on the structure of the network of the synthetic IE resins, which is controlled by the applied production process, the following types of resins can be differed:

- gel type IE
- macroporous (macroreticular) IE
- isoporous IE

The gel type IE is an elastic material without pores, which contains solvent from the production process. The macroporous IE, in turn, consists of a porous matrix structure with large internal surfaces, which is also adequate for larger molecules. Macroporous IEs have an extremely uniform external shape and they are, in contrast to the gel type IE, opaque. Furthermore, macroporous materials are more stable regarding osmotic shock than gel type resins. The third groups are the so-called isoporous IEs. In order to obtain this kind of IE with a substantially uniform pore size, the crosslinking and the pore structure has to be modified. Isoporous IEs show some advantages compared to macroporous IEs, as they have, for instance, a little sensitivity to organic fouling, a higher capacity and regeneration efficiency (Dorfner 1991). Another type of IE is the liquid IE, which is well established in l,l-extraction. Instead of solid IE materials, liquids, which are equipped with the same IE characteristics and immiscible to the applied electrolyte solution, are used. These liquids have several advantages compared to the solid stationary phases. For instance, they are easy to manufacture and the ion-exchange is improved due to a more efficient dispersion of the organic phase into the aqueous phase. Indeed, the separation between the phases is more difficult than by using solid IEs and some liquid IE is lost during the process (Helfferich 1995).

Furthermore, depending on the incorporated functional groups different main types of IE resins can be distinguished:

1. Strong cation exchanger – sulfonic acid type
   - Functional group: $RSO_3^-H^+$
2. Weak cation exchanger – carboxylic type
   - Functional group: $RCOO^-H^+$
3. Weak base exchanger – amine type
   - Functional groups: primary ($-NH_2$), secondary (-NHR) and tertiary amines ($-NR_2$)
4. Strong base exchanger – quaternary ammonium type
   - Functional group: $NR_4^+OH^-$

Considering the types of resins, the strong cation exchanger resin is the most important type. Strong cation exchangers are able to exchange cations or split neutral salts. It can be applied over the entire range of pH. Weak cation exchangers show a high selectivity for Ca^{2+} and Mg^{2+}. Their capacity, in turn, is limited for alkali metals and they are not able to split neutral salts. They are often used for the removal of cations from alkaline solutions. Weak base exchangers sufficiently exchange anions of strong acids. Strong base exchangers show a strong affinity to silicic acid and carbonic acids during water treatment. Furthermore, these exchangers are used to remove weak acid anions (Dorfner 1991, Wheaton, Lefevre 2000). As these types are all charged either positively or negatively, these types are monopolar IE. Amphoteric IE containing cationic and anion groups, in turn, are bipolar IEs. Furthermore, an

IE consisting of a single ionogenic group is called monofunctional IE. IE with two types of iongenic groups are bifunctional IEs (Dorfner 1991).

1.3.1.2 Important parameters of ion exchange

Some parameters are important for each IE process and therefore will subsequently be discussed in more detail:

Ion exchange capacity

The capacity of an IE quantitatively describes how much counterions can be assimilated on the resin's surface. Theoretically, the capacity of an IE corresponds to the amount of fixed functional groups on the resin's surface. This theoretical available IE capacity is called theoretical capacity or total capacity. During routine work, it is not possible to use the whole theoretical capacity, as the consumption of chemicals and therefore the costs would be too high to always regenerate the IE completely. Moreover some functional groups are quickly lost irreversibly and cannot be regenerated. Therefore, in routine work the capacity available is lower than the total capacity. This available capacity is called useable capacity or operating capacity. The operating capacity during an IE process depends on the applied flow rate, the concentration and composition of the feed solution, the amount of chemicals used for regeneration, the bed height, the temperature and the pH of the feed. For instance, the operating capacity rises, when the amount of counterions with a high affinity to the resins is increased. Furthermore, higher flow rates lead to a reduction of the operation capacity, as the borderline of the exchange process becomes more imprecise and some parts of the resin remain unused. In general, capacities are always related to a respective weight (eq ions/g resin) or to a respective volume of the resin (eq ions/l resin) (Dorfner 1991, Universität des Saarlandes, Wheaton, Lefevre 2000).

Swelling

Due to hydrophilic groups within the polymeric matrix of the resins, the resins tend to swell in the presence of water. The fixed ionic groups are hydrated. The grade of swelling of the IE is defined by the amount of available water. The amount of water in the resin is then always related to the IE resin (e. g. g water/ g IE) Furthermore, the level of cross-linking at the polymeric backbone also influences the swelling behaviour, as the tendency to swell is lower for highly cross-linked resin than for weakly cross-linked resins. Moreover, the swelling is reduced, when polyvalent ions (e. g. Ca^{2+}, Fe^{3+}) are the counterions (Universität des Saarlandes, Wheaton, Lefevre 2000).

Selectivity

In IE experiments, it was observed, that some ions were preferentially adsorbed on the resin's surface than other ions. This phenomena is called selectivity of the IE for the respective counterion (Dorfner 1991). For cations the following selectivity sequences, which are true for a strong cation exchanger, have been discovered (Universität des Saarlandes):

$Ba^{2+} > Pb^{2+} > Hg^{2+} > Cu^{2+} > Sr^{2+} > Ca^{2+} > Ni^{2+} > Cd^{2+} > Co^{2+} > Zn^{2+} \sim Cs^{2+} > Rb^{2+} > Fe^{2+} > Mg^{2+} \sim K^{+} > Mn^{2+} > NH_4^{+} > Na^{+} > H^{+} > Li^{+}$

This means that barium adsorbs best on these IE resins. Furthermore, for anions applied on strong anion exchangers an analogue sequence has been defined:

Salicylate > citrate > I^- > HSO_4^- > ClO_3^- > NO_3^- > Br^- > CN^- > HSO_3^- > BrO_3^- > NO_2^- > Cl^- > HCO_3^- > IO_3^- > formate > acetate > propionate > F^- > OH^-

1.3.1.3 Ion exchange processes

Ion exchange processes are conducted in different ways. For instance, they can be performed by applying fixed beds, moving fixed beds, agitated beds or in stirred tank reactors. As fixed bed processes are the most common processes, which usually consist of four steps (loading, back-rinsing, regeneration and rinsing), this process will be subsequently discussed in detail:

Loading

During the loading the desired ion exchange process occurs. The solution, from which ions should be removed, is passed through the resin and the ions are exchanged. As soon as the defined quality of the effluent is no longer reached (e. g. the concentration of unwanted ion is getting too high), the loading process is stopped (Universität des Saarlandes).

Back-rinsing

In order to loosen the resin and to prepare it adequately for the following regeneration step, a counter-current rinsing step with purified or product water is applied (Universität des Saarlandes).

Regeneration

Afterwards the IE resin is regenerated and prepared for the next loading. In this step, ions, which were exchanged during the loading step, are replaced again and the original conditions of the resin are rebuilt. The regeneration process can be done in different ways. The simplest way is to regenerate the resin in the same direction as during the loading step (co-current regeneration). Indeed, the regeneration can also be done in the opposite direction to the loading process. This form of regeneration is called counter-current regeneration. Applying the counter-current regeneration leads to better purification performances and a higher chemical efficiency, but building such devices is more complicated and therefore more cost-intensive (Universität des Saarlandes, Wheaton, Lefevre 2000).

Rinsing after regeneration

Finally, the excess of applied regeneration solution has to be washed out of the resin. It is recommended that three bed volumes of water are used to regenerate the resin sufficiently. Mostly, the rinsing water is applied for the preparation of the next regeneration solutions (Universität des Saarlandes).

1.3.1.4 Applications of ion exchangers

According to Dorfner (Dorfner 1991), there are numerous applications of IE processes in different areas. A selection of applications is presented as follows:

- Water treatment (e. g. softening and deionisation, process water treatment, etc.)
- Hydrometallurgy (e. g. purification of plutonium, recovery and concentration of silver and gold, etc.)
- Food industry (e. g. production of sugar and sweetening agents, wine-making, fruit juices, production of amino acids, etc.)
- Dairy industry (e. g. milk whey demineralisation and deacidification, purification of lactose, etc.)
- Pharmaceuticals (e. g. separation and purification of different drugs)

- Analytical chemistry (ion exchange chromatography)
- Pollution control (e.g. removal of toxic gases, vapours and aerosols from air, treatment of waste gases and waste waters, etc.)

1.3.2 Applications of ion exchange for the isolation of amino acids

Beginning in the 1940s, the usage of IEs for the isolation and separation of AAs from protein hydrolyzates was intensively investigated. For instance, Moore and Stein (Moore, Stein 1951) reported AA separation experiments using columns filled with starch followed by similar experiments using the novel synthetic, sulfonated polystyrene resin (Dowex 50 W). Latter experiments were conducted by using sodium citrate buffers with different pH values and by applying the so-called elution chromatography. This means that in this chromatographic technique small amounts of the original AA solution are brought into the column. Subsequently, the chromatographic separation is done by eluting the AA mixture with a high amount of solvent. Based on the AAs' different affinities either to the IE resin or to the solvent, the different AAs run off consecutively and therefore they are separated.

Other researchers also applied synthetic IE resins for the separation of AAs from protein hydrolyzates, but they preferred to work with different types of resins and applied another chromatographic technique: the so-called displacement chromatography (DC). In contrast to the elution chromatography, in this technique the IE resin is loaded with the starting solution until the substances, which interact with the resin's functional groups, break through and are detected in the effluent. In other words, the complete, available resin is loaded with ions from the starting material and substances, which do not interact with the resin, are separated. The loading is stopped, when the resin is full. Afterwards, these substances are displaced by other ions, which show a greater affinity to the resin's functional groups than the desired substances. These substances will be consecutively eluted according to the strength of their affinity. In comparison to the elution chromatography, the DC has the advantage that higher throughputs are possible due to higher amounts of loading. Additionally, less dilution effects occur and the purified components can be recovered with significantly higher concentrations. Indeed, the separation between the displaced substances is worse than when applying elution chromatography. Qi et al. (Qi, Huang 2002) listed a summary of citations concerning development, advantages and disadvantages regarding the DC. In order to consider the isolation of AAs from protein hydrolysate, Winters and Kunin (Winters, Kunin 1949) were able to separate the mixed AA feed into their three charge groups (acid, neutral, alkaline) and into three single AAs (histidine, arginine and lysine). For this separation different types of IE resins were applied and it was perfomed in the DC mode. Furthermore, Buchanan (Buchanan 1957) worked in this field and tried to improve the separation between the individual AAs. Moreover, several patents dealing with the isolation of AAs from protein hydrolyzates were published between 1940 and 1960 (The Dorr Company 1948, Stevens 1952, Gerber 1942). Routes for the production on an industrial scale of at least salt-free AAs from protein hydrolyzates using DC processes and a single strong cation exchanger resin have also be reported. These production processes were performed by loading the IE resin with protein hydrolyzates followed by eluting the desired AAs with ammonia solution (Dorfner 1991).

Subsequently, both chromatographic techniques were further investigated and improved for the separation and production of AAs, although the fields of application differed. DC was mainly applied on a larger scale and the elution chromatography, in turn, was improved towards analytical applications. For instance, the composition of the buffers of the mobile phase was improved, automatic devices were developed (Dus, Lindroth et al. 1966, Blanshard, Bradford et al. 1975), the time needed for the analyses was shortened and new IE materials (pellicular IE) for a better separation were designed (Dorfner 1991). Finally,

these investigations resulted in complex and reliable instruments for routine analyses of AAs that are on the market at the present time.

The DC process, in turn, was and currently is mostly applied on a preparative scale in order to produce larger amounts of AAs. Indeed, after the boom in research into the production of AAs by using DC about 50 years ago, fewer publications on this issue were found in literature in later years. Most publications published after 1970 dealt with several improvements of the DC process, such as the development of new resins (e.g. chelate resins) (Matsuda, Yoshida 1990) or the design of new routes to obtain particular AAs from the hydrolyzates. At the beginning of the development of DC processes for the production of AAs, most AAs were obtained from protein hydrolyzates. Thus, soon the strength of biotechnology and its ability to produce individual AAs in higher concentrations were discovered. Therefore, the task of the IEs applied in DC processes was shifted from the recovery and separation of individual AAs from AA mixtures towards to the purification and generation of individual AAs from fermentation broths. As mostly only one type of AA was concentrated within the fermentation broth, the separation between individual AAs during the DC process was no longer important. Considering the downstream processing of fermentation broths of lysine, glutamic acid, tryptophan and phenylalanine IE processes are commonly used on an industrial scale (Dorfner 1991). For instance, the generation of lysine is performed by applying a strong cation exchanger resin to the filtered and acidified fermentation broth. The AAs are adsorbed on the resin and then eluted with ammonia. In order to obtain purified lysine, the IE step has to be repeated several times followed by a single crystallisation step (Hermann 2003).

Incendentially, other downstream processes for the generation and purification of AAs are, for instance, electrodialysis (Kedem 1977), ultra- and nanofiltration (Garem, Daufin et al. 1997, Timmer, Speelmans et al. 1998), countercurrent extraction (Bitar 1991) or selective precipitations and crystallisation (Hoppe, Martens 1984).

2 Starting points and objectives

Next to the exploitation and usage of alternative, renewable and CO_2-neutral energy sources, it will be also necessary to found and use alternative resources and so-called platform chemicals for the chemical industry. By using a comprehensive biorefinery concept for the exploitation of biomass both targets could be fulfilled.

In the last few decades, intensive research has been performed into biorefinery concepts and particularly into the "GBR concept" in Austria. Theoretical concepts for the comprehensive usage of grass silage and the extraction of valuable compounds and energy thereof were designed (Kromus, Wachter et al. 2004). Furthermore, the concepts that were designed were also put into practice and numerous experiments were conducted at laboratory scale. Most of these research activities were performed within the frame of the Austrian research program "Fabrik der Zunkunft"[1]. Based on positive results from generating valuable compounds from grass silage and its juice, which were obtained in experiments at laboratory scale, it was attempted to combine these findings on a larger scale. Therefore, a GBR demonstration plant for the extraction of AAs and LA from grass silage was established in Upper Austria. The overall targets of this demonstration plant, which based on the concept of the Gruene-bioraffinerie.at GmbH (Gruene-bioraffinerie.at GmbH 2008) were for instance:

- Comprehensive implementation of refining concepts and of results, which were obtained in laboratory-scaled experiments, regarding the generation of LA and AAs from grass silage on a pre-industrial scale.
- Investigations of different downstream technologies on their possible application and performance for the extraction of valuable compounds from grass silage and its juice at pre-industrial scale and in long-term tests.
- Improvements of the previously assumed process in order to optimise it for further applications.
- The generation of data and knowledge on the biorefining of grass silage juice and on the production of AAs and LA thereof, which would be needed for further plants or for a further scale up.

One part of the downstream technologies applied at the GBR demonstration plant was an ion exchange device. This ion exchange device represented the core of the generation of AAs from grass silage and grass silage juice. This PHD-thesis concentrated intensively on this part of the process of GBR demonstration plant and therefore on the generation of AAs from grass silage juice. Furthermore, the polishing and improvement of AA products obtained from the GBR demonstration plant were further investigated at laboratory scale. For that reason the following main research objectives and have been defined for the ion exchange process and were tried to be answered in this work:

- Is it feasible to generate purified AA product solutions from grass silage juice by applying the present ion exchanger device and the assumed process?
- How many AAs can be generated per batch run and what does the AA product solutions look like (appearance, AA composition, etc.)?
- Do different pre-treatment steps at the membrane devices influence the composition of the ion exchanger feed and the ion exchanger process itself?
- Is it possible to optimise the present ion exchanger process regarding AA yield or separation of AAs by varying process parameters?
- What kind and how many freights and streams would occur during the ion exchange process?

[1] more pieces of information and project reports can be found at www.nachhaltigwirtschaften.at.

- Is it feasible to polish and therefore to upgrade the AA product solutions obtained from the demonstration plant in order to enable an easier market entry for the products?

3 Materials & Methods

3.1 Experiments at pilot scale at the GBR demonstration plant Upper Austria

3.1.1 Materials

3.1.1.1 Grass silage

The experiments at the *GBR Upper Austria* were performed by using silage from permanent grassland or clover enriched grass prepared by local farmers (Utzenaich, Upper Austria). Different batches of silage, which were manufactured from May 2009 to September 2011 in four different harvests (1st, 2nd, 3rd and 4th cutting) per year, were applied. The fermentation process, which lasted about 4 to 6 weeks, was mainly done in silage bales comprising of approximately 900 kg grass per bale.

3.1.1.2 Grass silage juice

For experiments at the *GBR Upper Austria* the silage juice was obtained through a mechanical fractionation of silage, as shown in Figure 3-1. First, the silage bales were opened and the silage was cut and mixed in a feeding system (Eckhart, Germany). Subsequently, the silage was pressed in a screw press (Kufferath, Germany) with a throughput of approximately 2 t fresh silage per hour to separate the liquid phase from the feedstock. Depending on the silage quality, 300 to 400 l of silage juice was yielded per ton of fresh silage. In other words, approximately 1.2 m^3 silage juice was yielded per ton of dry substance. After the pressing process, the juice was stored to sediment for at least 12 h in a tank (volume 5 m^3) to remove sand and other soil based components from the liquid. For further processing, the silage juice was taken from the top of the tank and pre-filtered in a bag filter (mesh size 50 µm). This ensured that fibres and other materials, which could block the inlet to the first membrane filtration, were removed. In all experiments the same mechanical pre-treatment steps have been applied.

Due to the natural origin, amounts of the valuable ingredients in the silage juice such as LA and AAs varied during the operations. A generalised list of the main components and properties of the silage juice for further use in the membrane processes and IE is found in Table 3-1. The amount of LA differed from 38 g/l of high quality to 22 g/l of lower quality silage juices. AA concentrations were typically found in the range of 25 g/l for clover silage and 15 to 20 g/l for grassland silage juice. Further, the pH value gave a hint to the quality of silage, where a pH up to 4.1 presented high quality with a high level on valuable substances.

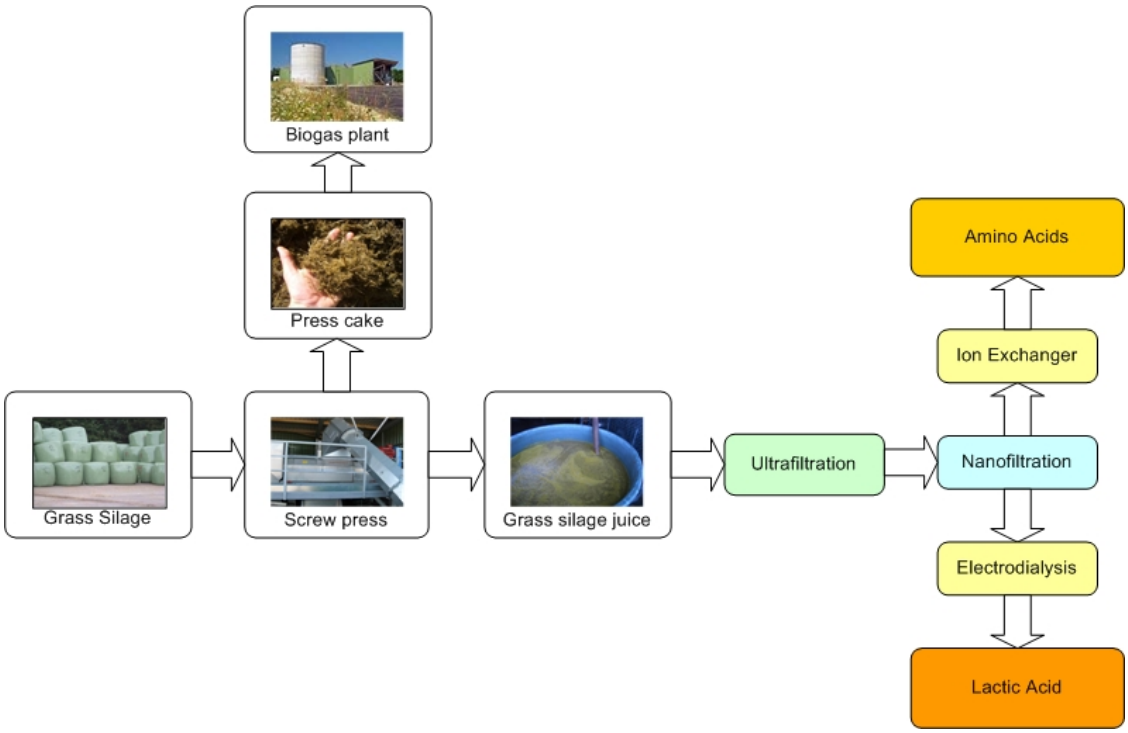


Figure 3-1: Production of grass silage juice at the *GBR Upper Austria*

Table 3-1: Typical composition and properties of grass silage juice produced at the *GBR Upper Austria*

Component	**Concentration [g/l]**
AA sum	21.4
Aspartic acid (Asp)	2.73
Glutamic acid (Glu)	1.60
Threonine (Thr)	0.86
Serine (Ser)	0.83
Proline (Pro)	1.52
Glycine (Gly)	1.05
Alanine (Ala)	2.37
Cysteine (Cys)	0.26
Valine (Val)	1.49
Methionine (Met)	0.36
Isoleucine (Ile)	1.15
Leucine (Leu)	2.03
Tyrosine (Tyr)	0.58
Phenylalanine (Phe)	0.84
Asparagine (Asn)	0.41
Tryptophan (Trp)	0.09
Lysine (Lys)	1.25
Histidine (His)	0.29
Arginine (Arg)	0.03
γ-Amino butyric acid (GABA)	1.71
LA	29.8
Acetic acid	5.38
Glucose	6.10
Fructose & Mannose (sum)	3.66
Ca^{2+}, Mg^{2+} (sum)	3.00
K^{+}	9.33
NH_4^{+}	1.14
Cl^{-}	1.40
pH [-]	4.34
Conductivity [mS/cm]	20.2
Density [kg/l]	1.04
Dry matter [%]	11.6

3.1.1.3 Feed for the IE process

Different process constellations (basic process and innovative process) have been tested at the *GBR Upper Austria*. Therefore, raw and pre-treated grass silage juices from different process stages of the *GBR Upper Austria* have been used for the IE experiments. Details of the process concepts are given in chapter 1.1.3. Subsequently, overall descriptions of pre-treatment steps for the different feed solution used at the IE device will be demonstrated.

Silage juice

In order to test the applicability of silage juice that has not had any pre-treatment at the IE device, one experiment was conducted with raw silage juice. Properties of this grass silage juice are summarised in Table 3-1.

Ultrafiltration Permeate

Experiments at the IE device based on the innovative process were done with softened UF Permeate. An overview of the pre-treatment steps for this IE feed is given in Figure 3-2. Firstly, the grass silage juice was ultrafiltered to remove suspended particles and clear the grass silage juice. Based on the results of different experiments, a ratio of 9 to 1 (permeate to retentate) was found to be ideal and therefore was applied in several UF experiments. Hence, 90 % of the valuable compounds were found in the UF permeate and 10 % were found in the retentate. Before using the ultrafiltered grass silage juice at the IE device, a softening step, which was equipped with a strong cation exchanger, was applied to reduce the amount of inorganic cations. A generalised list of the main components of the softened UF Permeate used in the IE device is presented in Table 3-2; additionally, some physical data are added.

Table 3-2: Typical composition and properties of softened UF Permeate produced at the *GBR Upper Austria*

Component	**Concentration [g/l]**
AA sum	19.0
Aspartic acid (Asp)	2.53
Glutamic acid (Glu)	1.37
Threonine (Thr)	0.78
Serine (Ser	0.75
Proline (Pro)	1.35
Glycine (Gly)	0.94
Alanine (Ala)	2.19
Cysteine (Cys)	0.23
Valine (Val)	1.36
Methionine (Met)	0.32
Isoleucine (Ile)	1.03
Leucine (Leu)	1.81
Tyrosine (Tyr)	0.50
Phenylalanine (Phe)	0.70
Asparagine (Asn)	0.44
Tryptophan (Trp)	0.06
Lysine (Lys)	1.01
Histidine (His)	0.21
Arginine (Arg)	0.03
γ-Amino butyric acid (GABA)	1.41
LA	28.0
Acetic acid	4.98
Glucose	5.27
Fructose & Mannose (sum)	3.33
Ca^{2+}, Mg^{2+} (sum)	2.11
K^{+}	5.90
NH_4^{+}	0.88
Cl^{-}	1.13

pH [-]	3.80
Conductivity [mS/cm]	16.6
Density [kg/l]	1.03
Dry matter [%]	7.2

As several experiments were done at the GBR, different modules were tested at the UF device. For instance, a ceramic (TiO_2) capillary module (19 tubes, 39 channels per tube) with a cut off of 1 kDa from TAMI industries was applied. By using this module, the experiments were operated in a pressure range of 1.5 to 5.5 bar transmembrane pressure. Other experiments were conducted using a polymer spiral wound module (GM) with a cut off of 8 kDa from GE Osmonics. As no differences were observed in the permeate quality by applying different UF modules (Ecker 2012), all UF Permeates were used at the IE device equally. Next to the interchangeable module, the UF unit was equipped with membrane housing and standard plant configurations. The devices consisted of the membrane modules, a feed tank, a pressure and booster pump and measurement equipment for pressure, flows, temperature and pH values.

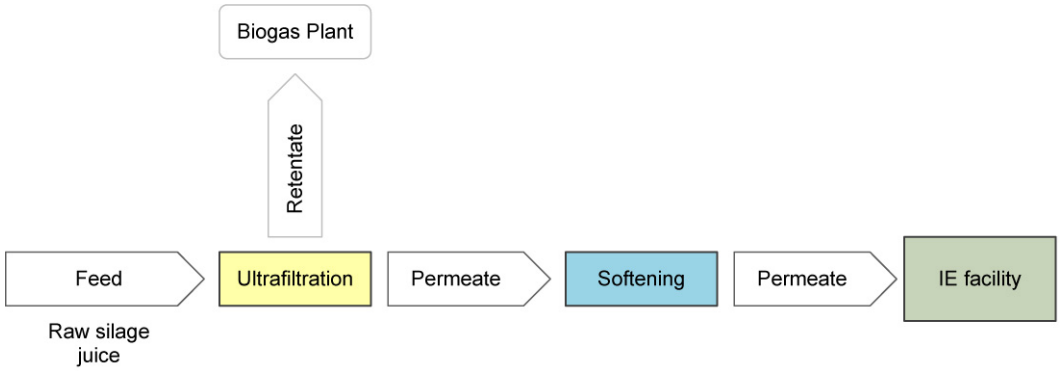


Figure 3-2: Generation of UF Permeate

Nanofiltration Retentate

As shown in chapter 1.1.3, the NF2 Retentate was used as feed for the IE step when the so called basis process (based on licence (Gruene-bioraffinerie.at GmbH 2008)) was applied. Within this basis process the softened UF Permeate, which was produced as described before, was further processed in a sophisticated hybrid system. An overview of the production of NF2 Retentate is given in Figure 3-3. First, the pre-treated silage juice was processed by a two-stage NF, which represented the key device of the *GBR Upper Austria* due to the separation of LA and AAs in this process step. Nearly all the AAs remained in the NF Retentate and large amounts of the LA were transported into the NF permeates. Therefore, the NF2 Retentate was used at the IE device and a generalised list of the main components of this medium is presented in Table 3-3.

Table 3-3: Typical composition and properties of NF2 Retentate produced at the *GBR Upper Austria*

Component	Concentration [g/l]
AA sum	26.5
Aspartic acid (Asp)	2.92
Glutamic acid (Glu)	2.58
Threonine (Thr)	1.22
Serine (Ser)	1.08
Proline (Pro)	1.56
Glycine (Gly)	1.13
Alanine (Ala)	3.23
Cysteine (Cys)	0.23
Valine (Val)	1.95
Methionine (Met)	0.58
Isoleucine (Ile)	1.53
Leucine (Leu)	2.86
Tyrosine (Tyr)	0.73
Phenylalanine (Phe)	1.26
Tryptophan (Trp)	0.19
Lysine (Lys)	1.56
Histidine (His)	0.32
Arginine (Arg)	0.08
γ-Amino butyric acid (GABA)	1.42
LA	21.2
Acetic acid	2.38
Glucose	7.02
Fructose & Mannose (sum)	9.85
Ca^{2+}, Mg^{2+} (sum)	2.27
K^{+}	3.55
NH_4^{+}	0.49
Cl^{-}	0.74
pH [-]	3.86
Conductivity [mS/cm]	13.7
Density [kg/l]	1.04
Dry matter [%]	12.4

For the NF devices spiral wound modules DL from GE Osmonics were applied, both facilities were equipped with two membrane modules and the standard unit installations (e.g. feed tank, pressure pump, booster pump and measurement equipment). The active area of the membrane was 8 m^2 per module, the cut off of this membrane type was specified to 150–300 molecular weight (membrane data sheet). At first diafiltration operation modes were applied in the NFs. The same configurations were used (concentration factor n, recirculation) for both stages, however, the requirements (slightly different feed concentrations, pressure) for the stages differed.

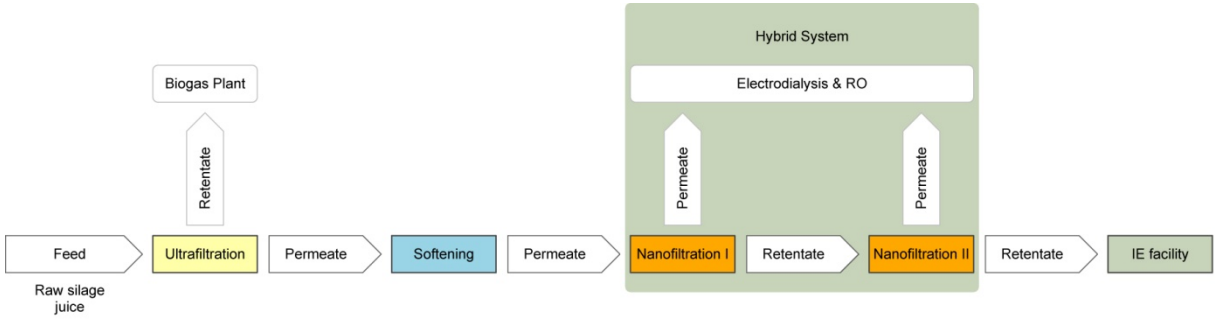


Figure 3-3: Overview on the production of NF2 Retentate

3.1.1.4 Ion exchanger equipment

The IE facility of the *GBR Upper Austria* consisted of five columns (SELGA, s.r.o, Czech Republic) and a complex connection and piping system, which allowed serial or parallel operation of the columns. Details of the connection and piping system are found in the appendix; a simplified overview is given in Figure 3-4. Additionally, some pictures of the columns are shown (Figure 3-5 and Figure 3-6). The IE system was automatically controlled via pressure-driven valves, which were operated through a control panel on a PC. Furthermore, before starting the experiments manually controlled valves had to be set directly on the device. The columns 1 to 4 were made of stainless steel and column 5 was made of glass for monitoring purposes. More specifications for the columns are presented in Table 3-4.

Table 3-4: Specifications of the IE columns at the *GBR Upper Austria*

	Height [cm]	Inner diameter [cm]	Volume [l]	Max. pressure [bar]
Steel column (1-4)	150	16.8	31	4
Glass column (5)	150	15	26	1.5

As shown in Figure 3-4, separate storage tanks for feed, water, sulphuric acid and ammonia solution were used. The tank for the IE feed had a capacity of 400 l and the tanks for water, sulphuric acid and ammonia solution 100 l respectively. The pumping was done by two equal piston diaphragm pumps (Typ: R-411.1-400 ml, SERA, Germany). The pumping of IE feed, water and ammonia solution was done by one pump and the pumping of sulphuric acid was done by the second. Flow rates were measured using an inline flow meter (PROMAG-H, Endress&Hauser). Other process parameters were monitored by inline measurement of conductivity (Liquiline M CM 42, Endress&Hauser) and pH (Liquiline M CM 42, Endress&Hauser) after column 1, 2 and 5 and the refractive index (Process Refractometer PR-23, K-Patents Process Instruments, Finland) after column 5. These measurement points were important checkpoints (CP) within the batch experiments and were called CP 1, 2 and 3. Additionally, each column was equipped with a pressure sensor (PMC 131, Endress&Hauser) to monitor the pressure in the columns. Data from the measurement points was collected and stored automatically.

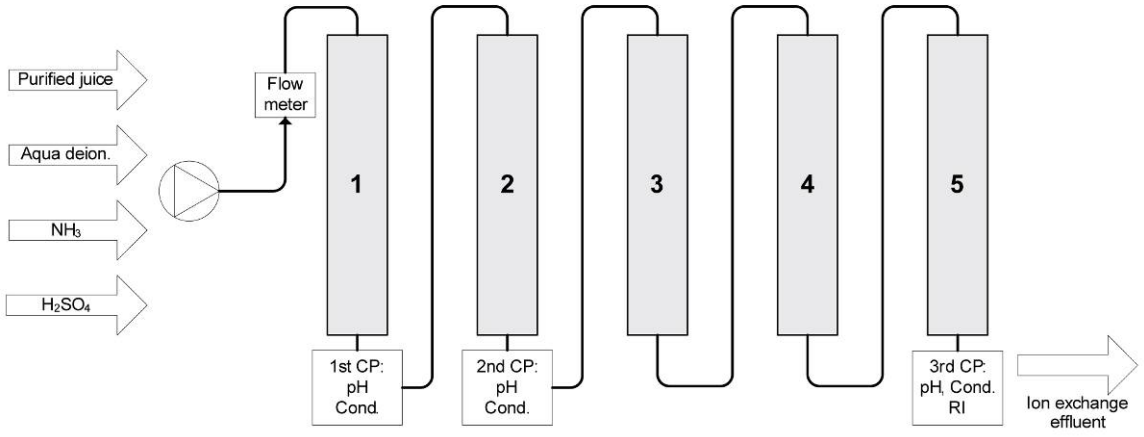


Figure 3-4: Schema of the IE facility at the *GBR Upper Austria* (simplified)

Figure 3-5: IE device at the *GBR Upper Austria*: column 1-4 made of stainless-steel (left)
Figure 3-6: Column 5 made of glass (right)

3.1.1.5 Ion exchange resins

During the operation of the pilot plant, the performance of two different strong cation exchanger resins was tested. First, the columns were filled with 116 l of Dowex Marathon C (wet resin). Secondly, experiments with 120 l of Dowex Monosphere C-350 (wet resin) were conducted. Both resins are usually applied for demineralisation and softening in industry and households. In Table 3-5 the properties of both resins are shown. In order to protect the resin bed during service, each resin bed was covered with 1 l of a floating inert resin (Dowex™ IF-59, polypropylene matrix).

Table 3-5: Strong cation exchange resins – properties and characteristics (according to data sheets of Dow (The Dow Chemical Company, The Dow Chemical Company))

	Dowex Marathon C	**Dowex Monosphere C-350**
	Resin I	**Resin II**
Manufacturer	Dow	Dow
Type	Strong acid cation	Strong acid cation
Matrix	Styrene-DVB, gel	Polystyrene-DVB, gel
Functional group	Sulphonic acid	Sulphonic acid
Total exchange capacity (H^+) [eq/l]	1.8	2.0
Sum exchange capacity IE device [eq]	208.8	240
Mean particle size [µm]	600±50	350±50

3.1.1.6 Chemicals

All solutions, which were used for experiments at the IE device, were prepared immediately before use. The preparation was done automatically by mixing the chemicals with reversed osmosis water in mixing tanks.

Sulphuric acid (H_2SO_4)

Technical grade sulphuric acid (96 %, Brenntag) was used to prepare 0.25 M, 0.5 M or 1 M sulphuric acid for the preparation and regeneration of the resins.

Ammonia (NH_3)

For eluting the AAs 1 M ammonia solution was used, which was prepared from a technical grade ammonia solution (18 %, Brenntag). Before using the ammonia solution for elution, it was heated up to 50 to 60 °C to avoid precipitates of amino acids within the columns.

Water from Reversed Osmosis

In order to dilute the concentrated chemicals and to rinse the columns, either fresh water or recycled process water was used. In both cases the water was purified by reversed osmosis (RO) before use.

3.1.2 Methods

3.1.2.1 Filling of the columns

Firstly, the columns were filled with the respective resin. For this purpose water was added and mixed with an adequate amount of the new resin per column to prepare a fluid, which was able to be filled into the columns. In the columns the resin was deposited and the resin bed was built. Surplus water was removed through opening the bottom of the column, whereas the discharge of the resin was prevented by a fine sieve. Afterwards, 1 l of the inert resin was put on the top of the resin bed. Finally, the whole IE system was completely filled with water to remove any air from the resin and pipes.

3.1.2.2 Displacement chromatography

Due to higher outputs and product solutions with higher concentrations, the IE process was operated as DC. Therefore, every cycle consisted of three steps:

- Loading
- Displacement
- Regeneration

Each IE cycle was performed in batch mode. Subsequently, each process step is described in detail.

Preparation (first cycle)

Before starting any experiments, the resin within the columns was converted into the H^+-form by rinsing it with 1 M sulphuric acid. Afterwards, the IE was rinsed with a sufficient amount of water to remove any remaining sulphuric acid.

Loading

Within the loading step, the IE resin was loaded serially with IE feed. Positively charged components, such as inorganic cations and AAs, interacted with the functional groups on the resin's surface. Other components, such as organic acids, sugars or inorganic anions were passed through and were found in the IE effluent. After the loading had begun, the first 90 to 100 l of the effluent (void volume of the columns), which only consisted of traces of substances, was always discarded. As soon as the valuable compounds (e. g. LA, other organic acids or sugars) broke through, the collection of the effluent was started if necessary (innovative process). The loading was always done at an ambient temperature and at flow

rates of between 2.7 to 10.8 m/h. In addition, a single experiment was done with flow rates up to 16.2 m/h. Generally, flow rates were set manually at the respective pump by adjusting the length of stroke through a hand-wheel. The breakthrough of inorganic cations from column 2 to column 3 (monitored at CP 2) indicated the end point of the loading. Before displacing the AAs the IE system was rinsed with water to remove any contamination to the AA production (organic acids, sugars) from the void volume and to transfer them into the IE effluent as well. In order to balance the process, proportional samples from the IE feed and from the IE overflow were taken and analysed.

Displacement

After loading the columns and rinsing them with water, each column was eluted with 1 M ammonia successively and the amino acids were displaced. During the elution step flow rates of between 5.4 and 10.8 m/h were applied. Flow rates of up to 16.2 m/h were tested in a single experiment. The process was observed by monitoring refractivity, pH and conductivity at CP 3. In order to balance each batch experiment, proportional samples were taken from each pre-product, product and post-product fraction. After eluting, the columns were rinsed with water again.

Regeneration

After each loading and displacement process, the IE resin had to be regenerated. Due to high amounts of inorganic cations, column 1 and 2 were regenerated in a double-stage process. First, these columns were rinsed with 0.25 M sulphuric acid, followed by rinsing them with 1 M sulphuric acid at ambient temperature. Column 3, 4 and 5 were only rinsed with 1 M sulphuric acid at ambient temperature.

3.2 Laboratory-scale amino acid product polishing

3.2.1 Materials

3.2.1.1 Amino acid product raw solutions

For the fractionation experiments raw AA product solutions from the *GBR Upper Austria,* which are consequently called Product 1A and 1B, were used. The spray drying experiments were done with another product solution obtained at the *GBR Upper Austria*: an average Product 2 solution generated in campaign 26 and 31. Some characteristics of the solutions used are shown in Table 3-6 and Table 3-7. The AA product solutions were stored at -18 °C until usage.

Table 3-6 AA composition and characteristics of Product 1A and 1B

Product	**1A**	**1B**
from campaign	26 (November 2010)	38 (November 2011)
Amino acids [mg/l]		
Aspartic acid	10.06	3.42
Threonine	2.62	3.32
Serine	1.88	1.89
Glutamic acid	6.16	2.97
Asparagine	n.a.	n.a.
Proline	3.46	3.53
Glycine	1.26	7.39
Alanine	5.19	12.33
Cysteine	0.93	0.53
Valine	2.58	5.86
Methionine	0.51	1.50
Isoleucine	1.07	4.07
Leucine	1.78	7.10
Tyrosine	0.27	0.90
Phenylalanine	0.57	2.96
γ-Amino butyric acid	0.08	0.15
Ammonia	0.15	0.39
Lysine	n.a.	0.05
Histidine	n.a.	n.a.
Tryptophan	n.a.	0.27
Arginine	n.a.	n.a.
Sum	38.41	58.25
pH [-]	3.34	3.98
Dry matter [%]	4.47	8.11

Table 3-7: Characteristics of the solutions of Product 2 used for spray drying

	pH [-]	**Dry matter [%]**	**NH_3 [g/l]**
Product 2	9.74	2.86	7.75
Product 2 w/o NH_3	7.76	3.89	1.12
Product 2 w/o NH_3 and concentrated	8.00	5.41	1.44

3.2.1.2 Ion exchange resin

In order to fractionate Product 1A a strong anion ion exchanger resin (characteristics are shown in Table 3-8) was used.

Table 3-8: Characteristics of Lewatit S6368 A (Lewatit 2011)

Name	Lewatit S 6368 A
Description	strong alkaline, macroporous anion exchanger (type I); applicable for food and beverage
Functional group	quaternary amines
Matrix	polystyrene
Structure	macroporous
Capacity	1.0 eq/l

3.2.1.3 Spray drying equipment

For the spray drying experiments with Product 2 a Mini Spray Dryer B-191 (Büchi, Switzerland) at the institute for Chemical Engineering at the Vienna University of Technology was used (Figure 3-7). The Mini Spray Dryer was equipped with an injector system consisting of a peristaltic pump and a spray nozzle, a heated drying chamber and a cyclone for separating the powder and transferring it into the product vessel. The spray flow, the inlet temperature of the airstream and the pump flow were adjustable.

Figure 3-7: Mini Spray Dryer B-191 (Büchi, Switzerland)

3.2.1.4 Chemicals

Decolourisation was done by using activated carbon powder (Roth, Germany). The strong anion exchanger resin was prepared and regenerated with 0.5 M sodium hydroxide, which was prepared by dissolving solid sodium hydroxide (Roth, Germany) in deionised water. For the elution step 1 M acetic acid prepared from pure acetic acid (Roth, Germany) was used. Recrystallisation was done in solutions of ethanol (95 %) and deionised water.

3.2.2 Methods

3.2.2.1 Decolourisation (Product 1A and Product 1B)

Based on results from pre-experimental testing of different adsorbing materials, the decolourisation of Product 1A and 1B was done with activated carbon (67 g/l product). Therefore, activated carbon was added to the product solutions and stirred for 30 min at room temperature. Afterwards, the activated carbon was removed by filtering the cleared liquid.

3.2.2.2 Fractionation of Product 1A

The fractionation of Product 1A contained two main steps, as shown in Figure 3-8. Firstly, Product 1A was separated by applying a DC step with a strong anion exchange resin. For this step the resin (100 ml wet resin in an open glass column) was prepared with 300 ml of 0.5 M sodium hydroxide followed by rinsing with deionised water. Subsequently, the resin was loaded with Product 1A (coloured or decoloured) and fractions of 20 ml each were collected until the breakthrough of AAs in the effluent was detected by thin layer chromatography (TLC). At this point the loading was stopped and the column was rinsed with water again. All fractions from the loading and rinsing, which contained AAs, were combined and named “loading 1”. Afterwards, the acidic AAs were desorbed from the resin by using 1 M acetic acid. Again fractions of 20 ml each were collected. According to the TLC results the fractions were combined and therefore two solutions (“elution 2A” and “elution 2B) were obtained. Secondly, all fractions obtained in the chromatographic process were further treated with a vacuum evaporator (Laborota 4000 efficient, Heidolph), where the water bath temperature was 45-55 °C and a vacuum of approximately 55 mbar was applied. The precipitates and solutions that were obtained, were separated through filtration. Subsequently, precipitates were dried (drying cabinet, 75 °C) and weighed (analytical balance PRJ 620-3M, Kern). All fractions were investigated analytically on the AA analyser.

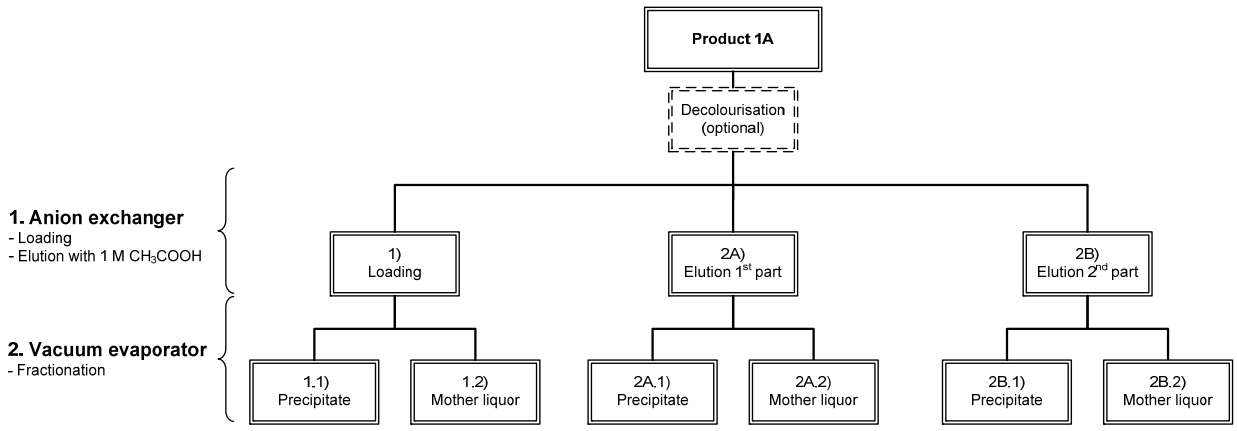


Figure 3-8: Overall schema of the fractionation of Product 1A

3.2.2.3 Fractionation of Product 1B

The overall schema of the fractionation of Product 1B is shown in Figure 3-9.

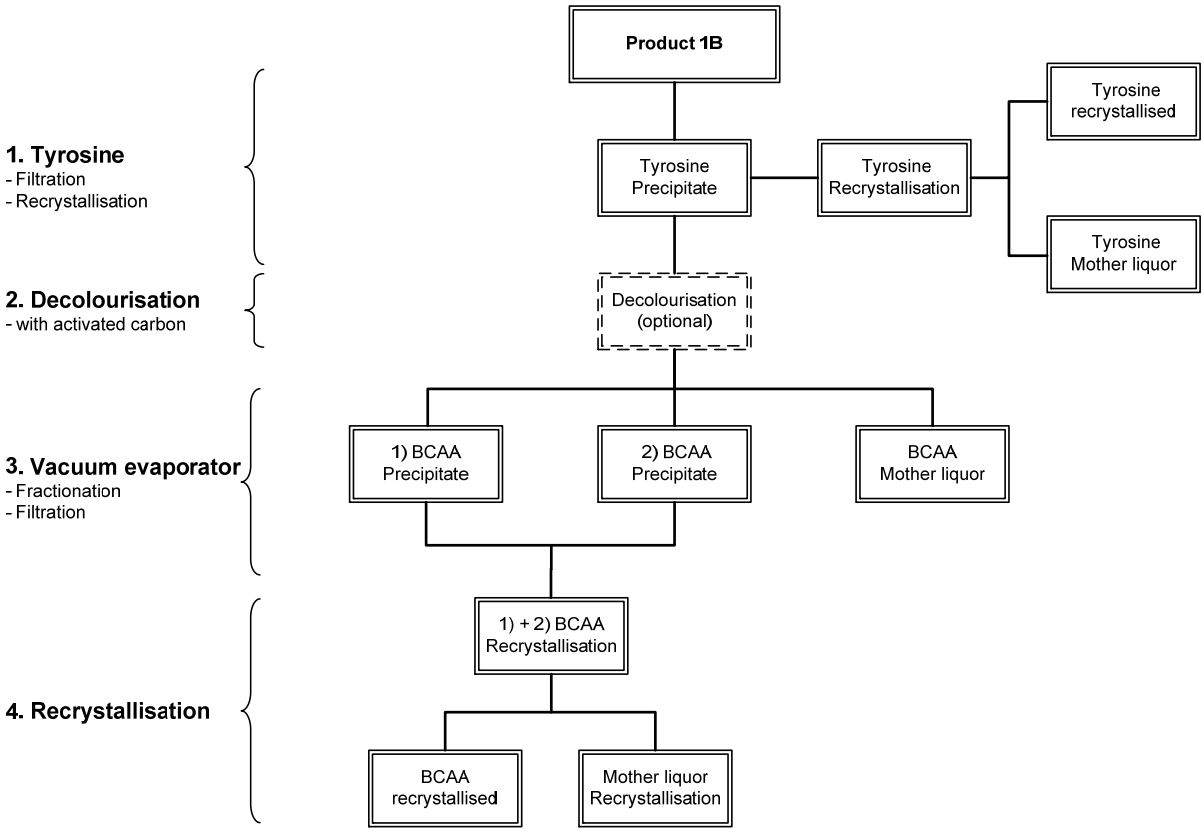


Figure 3-9: Overall schema of the fractionation of Product 1B

Since solubility of L-tyrosine in aqueous solutions is limited compared to other AAs, a precipitate mainly consisting of L-tyrosine was always found in Product 1B after cooling the eluted product solution. Therefore, the removal of L-tyrosine from the starting solution was done first using a simple filtration step. The separated precipitate was dried in a drying cabinet at 75 °C and quantified afterwards. In order to improve the purity of this precipitate, it was dissolved in ethanol/water and heated. Subsequently, the solution cooled slowly and some precipitate was built again, which was removed, dried and analysed.

After removing the L-tyrosine precipitate further fractionations of Product 1B (coloured or decoloured) were done in a vacuum evaporator (Laborota 4000 efficient, Heidolph), where the water bath was held at 45-55 °C and a vacuum of approximately 55 mbar was applied. The product solution was concentrated continuously and a precipitate was built. At a certain amount of precipitate the concentration was stopped as bumping occurred. In order to support the formation of crystals the solution was stored at 4 °C for some hours. Afterwards, the precipitate (BCAA-1) was removed by filtration, dried in a drying cabinet (75 °C, 24 h) and quantified. The remaining solution was further concentrated in the vacuum evaporator until enough precipitate was built again. The second precipitate (BCAA-2) was separated, dried and quantified equally to BCAA-1. The remaining mother liquor was not treated further, but analysed as well.

As shown in previous results, the composition of BCAA-1 and BCAA-2 are quite similar. Therefore, for the recrystallisation step these precipitates were combined and homogenised.

For the recrystallisation the precipitate was suspended in ethanol, which was heated. By adding deionised water when the suspension started to boil the precipitate was dissolved completely. Afterwards, the solution was cooled slowly to room temperature followed by further cooling in a fridge (4 °C, 12 h). The refined crystals were separated by filtration and dried in a drying cabinet (75 °C, 24 h).

3.2.2.4 Upgrading Product 2

Removal of ammonia ("stripping")

In order to remove ammonia from Product 2, the product solutions were heated (80 °C) and air was injected (4 l/min) for two hours. These parameters were chosen based on the results of pre-experiments. In Figure 3-10 the apparatus used for the removal of ammonia is shown.

Figure 3-10: Apparatus used for the removal of ammonia from Product 2

Some of the "stripped" Product 2 was concentrated by using a vacuum evaporator (Laborota 4000 efficient, Heidolph) at a water bath temperature of 50 °C and a vacuum of 30-50 mbar.

Spray drying

The spray drying experiments using a Mini Spray Dryer B-191 (Büchi, Switzerland) were conducted with untreated Product 2 adulterated with ammonia, stripped Product 2 without ammonia and stripped and concentrated Product 2. However, all spray drying experiments were started with deionised water to reach a constant system status first. Subsequently, the weighed sample was introduced into the spray dryer and the parameters for operation were adjusted to avoid any condensate formation within the spraying chamber. In particular, the pump flow was varied to avoid condensate formation combined with sufficient feeding. Exact operation data for the spray drying experiments can be found in the appendix.

3.3 Analyses

AAs, organic acids and sugars were analysed in house. Depending on the concentrations, inorganic components were analysed either by the Laboratory Centre for Isotope Hydrology and Environmental Analytics (Institute Resources, Joanneum Research Forschungsgesellschaft mbH) or by Bioenergy2020+ (Graz) with different methods. If an immediate analyses of the samples was impossible, the samples were stored at 4 °C (short term) or at -18 °C (long term).

3.3.1 Amino acids

In order to prepare the samples for the analyses of AAs, liquid samples were filtered (0.2 µm, Minisart RC, Sartorius) and then diluted with a lithium-citrate loading buffer (pH=2.2, Biochrom). Solid samples (e. g. AA powders) were solved in the same loading buffer, filtered (0.2 µm, Minisart RC, Sartorius) and diluted if necessary.

The concentration of AAs was chromatographically determined by a BioChrom 30 apparatus (Biochrom), which is built especially to determine AAs. The complex apparatus consisted of an automatic injection system and a HPLC system. Within the HPLC system the separation of AAs was done on an ion exchanger column (particle size 8 µm) by applying a gradient system consisting of lithium citrate buffers with different pH values (from Biochrom) as mobile phase and a temperature gradient under nitrogen atmosphere. The AAs were separated by using a program, which is used to detect about 20 proteinogenic AAs. Details of the program are shown in Table 3-9. In order to calibrate the system, adequate standards from Biochrom were used. After post-column derivatisation at 120 °C with ninhydrin, which was prepared immediately before starting the analyses by mixing a solvent buffer (Ultrasolve Plus, Biochrom) with the ninhydrin reagent (Ultra Ninhydrin Solution, Biochrom), AAs were detected photochemically at 570 nm and 440 nm (proline). Evaluations were done by applying the program EZ Chrom SI.

Table 3-9: Details of the applied program at the Biochrom 30 Apparatus

Duration of the program [min]	105
Injection volume [µl]	30
Flow lithium citrate buffers [ml/h]	20
Flow ninhydrin reagent [ml/h]	30
Temperature gradient [°C]	35-80

Applied lithium reagents as mobile phases (from Biochrom):
Buffer B pH 3.00, 0.20mol/l
Buffer C pH 3.15, 0.50mol/l
Buffer D pH 3.50, 0.90mol/l
Buffer E pH 3.55, 1.65mol/l
Lithium hydroxide 0.30mol/l

3.3.2 Peptide content

In order to identify the possible amount of peptides within the AA powders a hydrolysing step according to the “Commission Directive 98/64/EC of 3 September 1998 establishing Community methods of analysis for the determination of amino-acids, crude oils and fats, and olaquindox in feedingstuffs” (Europäische Gemeinschaften 1998) was applied. Indeed, as the analytical method used for AAs runs with lithium buffers, the hydrolysis was done with lithium buffers instead of sodium buffers. For the hydrolysis step about 0.8 g of the respective

AA powder was put into a 250 ml lidded glass bottle. 25 ml hydrolysis mixture, which was prepared of 0.5 g phenol (p.a., Roth) in 246 ml hydrochloric acid (37 %, Roth) and diluted with deionised water to 500 ml, were added to the powder. Furthermore, 2 ml of a norleucine-solution (2.653 g/l; D,L-Norleucine from Sigma Aldrich) as an internal standard was added. Subsequently, the hydrolysis was conducted in a drying cabinet (110 °C, 24 h). After 24 h the solutions was cooled down using an ice bath and neutralised by adding some lithium-hydroxide solution (10 g $LiOH \cdot H_2O$ (>55 % LiOH, Roth) in 100 ml ultrapure water) until a pH of 2.2 was reached. Further dilutions were done with the lithium-citrate loading buffer (pH=2.2, Biochrom). Analyses were done according to chapter 3.3.1.

Additionally, the possible peptide content was investigated by an external laboratory (C.A.T. GmbH & Co, Chromatographie und Analysentechnik KG, Germany) through gas chromatography. AAs were quantified through enantiomer-labelling, whereas D-enantiomers of AAs were used as standards. Quantifications of the AA powders were done before and after a hydrolysis step (24 h, 110 °C, vacuum).

3.3.3 Thin layer chromatography (TLC)

For monitoring experiments with AAs continuously at lab scale a TLC-method was applied. Silica gel plates (SilG, Macherey&Nagel) were used as a stationary phase. The mobile phase consisted of n-butanol: ethyl acetate :water (5:2:3). The AAs were detected by using a ninhydrin reagent for derivatisation.

3.3.4 Organic acids and sugars

In order to prepare the samples for the HPLC, liquid samples were filtered (0.2 µm, Minisart RC, Sartorius) and diluted with the mobile phase (5 mM sulphuric acid). The mobile phase was produced by diluting a 0.05 M sulphuric acid (Roth) with ultrapure water. Depending on the concentrations of LA, which vary in a wide range within the process chain, the samples were diluted differently.

The system was calibrated by using single analytical grade chemicals (D-L-Na-lactate (>99 %, Sigma-Aldrich), acetic acid (100 %, Roth), butyric acid (>99 %, Roth), propionic acid (>99,5 %, Roth), isobutyric acid (>99 %, Sigma), formic acid (p.a., Roth)) and analytical standard mixtures (Wine analysis: stock solution I (Fluka), Carbohydrate Standard (Bio-Rad)).

Organic acids and sugars were quantified by HPLC (Dionex Ultimate 3000), using the Aminex HPX-87H column (Bio-Rad Co) equipped with a pre-column; mobile phase 5 mM sulphuric acid, 65 °C. Samples were injected automatically (10 µl per run). An UV detection system (Dionex Ultimate 3000 RS) for organic acids (210 nm) and a RI detection system (Shodex) for sugars were applied. Evaluations were done by using the program Chromeleon.

3.3.5 Inorganic ions

Analyses of inorganic ions done by the Laboratory Centre for Isotope Hydrology and Environmental Analytics were carried out with a Dionex ICS-3000 at 30 °C oven temperature. Cations were quantified by using the Dionex IonPac CS12A column with the pre-column Dionex GC12A, mobile phase methane sulphonic acid 20 mM, 1 ml/min and 25 µl injection volume. Anions were quantified by using the Dionex AS9HC column with the pre-column Dionex AG9HC, mobile phase sodium carbonate 12 mM, 1 ml/min and 100 µl injection volume. Detections were done by applying a conductivity detector.

For analyses done by Bioenergy2020+, the samples were treated by acidic pulping (Multiwave 3000, Anton Paar) first. Subsequently, inorganic cations were analysed by ICP-OES (Spectro Arcos, Spectro, Kleve). For analysing the inorganic anions, an ion chromatography (Dionex ICS 90, eluent $NaHCO_3/Na_2CO_3$-Buffer, column AG14A with pre-column) was applied.

3.3.6 Dry matter and organic dry matter

The dry matter content of liquids, grass silage or solids (e. g. AA powders) was determined by drying the sample in a drying balance at 105 °C (MA 35, Sartorius).

Furthermore, investigations were done by using a drying cabinet (Heraeus, 110 °C for 48 h or 75 °C for 96 h) followed by combusting the dried samples at 610 °C for 6 h (oven: Thermolyne Type 48000 Furnance). Before and after each step, the samples were weighed (PRJ 620 – 3M, Kern).

3.3.7 Determination of pH, conductivity and density

pH and conductivity were measured by using a pH meter (pH 3110 Set 2, WTW or 340i/Set, WTW) and a conductivity meter (LF 340 - B / Set, WTW). Density was determined by using a pycnometer and a balance (ABJ 220 - 4M, Kern).

3.3.8 Determination of ammonium (Method of Kjehldahl)

Ammonium was quantified by applying the method of Kjehldahl without pre-treatment (no digestion). Firstly, some sodium hydroxide was added to the sample. Subsequently, the sample was flushed with water steam to extract the ammonia, which was collected in 50 ml boric acid 2 % (from Boric acid, ≥ 99,8 % p.a. ACS). By applying an indicator (Tashiro) the amount of ammonium was quantified through titration with 0.1 N sulphuric acid.

3.3.9 Determination of proteins according to Biurett

Proteins and peptides are forming complexes with copper-ions in alkaline solutions. Free AAs do not react in this way. Therefore, this method was applied to determine the peptide content within the AA powders. Firstly, a calibration curve using the protein bovine serum albumin with data points at 1, 2.5, 5 and 10 mg/ml and solutions of the AA powders were made. The solutions were mixed with the Biurett-reagent and after a short time of incubation (30 min) the determination was done using a photometer (Shimadzu UV-1601, UV-VIS Spectrometer) at 578 nm.

3.3.10 Determination of the water content (Karl-Fischer-Titration)

The determination of the water content of the AA powder was done by an external laboratory (Institute of Chemistry, University of Graz) according to ISO 12937. Every sample was measured three times.

3.4 Calculations and evaluations

All calculations were done by using Microsoft Excel. The AA concentrations of AAs were resulted from the AA analyzer and the data of organic acid and sugars were obtained from the HPLC device. As numerous analytical samples occurred during monitoring the processes at the *GBR Upper Austria* and the analyses needed much time, each sample was measured once. Therefore, in the evaluations it was mostly tried to use analytical data from several experiments using the same process constellations to calculate average values to get overall trends of the processes. This was also done for compensating possible fluctuations caused by the changing quality of the raw material or by the process itself. During the process, with large amounts of liquid were manipulated, samples were snap-shots.

3.4.1 Pre-treamtment steps of the ion exchanger feed

The efficiency per AA of each pre-treatment step (UF, NF) for preparing the silage juice for ion exchanger was calculated as follows:

$$Efficiency\ [\%] = \frac{AA\ in\ the\ outputstream\ [g]}{AA\ in\ the\ feed\ [g]} * 100$$

Formula 3-1: Calculation of the efficiency of the transfer of individual AAs at the membrane devices

3.4.2 Balances and AA yields at the IE device

The total amount of yielded AAs per batch run was calculated from the eluted amount per product [l] and the analysed concentrations of AAs [g/l] thereof. These calculations were done per product and then summarised for each column. Finally, the total AA yield per batch run was then calculated as follows:

$$AA\ yield\ \ per\ batch\ run\ [kg] = \sum Displaced\ \ AAs\ \ from\ column\ 1-5$$

Formula 3-2: Calculation of the AA yield per batch run at the IE device

AA-solutions were generated through displacing the AAs with NH_3-solution from the IE resin. Therefore, the displaced AAs in the product solutions were referred to the AAs in the feed at the loading step to calculate the recovery rate for AAs per batch run:

$$\text{Recovery rate } AAs\ [\%] = \frac{AA\ in\ the\ \ products\ [kg]}{AA\ in\ the\ outputstream\ [kg]} * 100$$

Formula 3-3: Calculation of the AA recovery per batch run

Additionally, to allow adequate comparisons between the resins, some operating figures were calculated by referring the displaced AAs to the amount of used resin and to the available theoretical capacity:

$$Efficiency\ AA\ adsorption\left[eq*l^{-1}\right]=\frac{AAs\ in\ the\ products\left[eq\right]}{used\ resin\left[l\right]}$$

Formula 3-4: Calculation of the AA adsorption efficiency of the resin per litre resin

$$Efficiency\ AA\ adsorption\left[eq*eq^{-1}\right]=\frac{AAs\ in\ the\ products\left[eq\right]}{theoretical\ exchange\ capacity\left[eq\right]}$$

Formula 3-5: Calculation of the AA adsorption efficiency of the resin referred to the theoretical capacity

Moreover, the behaviour of non-AA components was investigated and the rate of transfer to the IE effluent was calculated as follows:

$$Transfer\ rate\ non-AAs\left[\%\right]=\frac{non-AAs\ in\ the\ effluent\left[kg\right]}{non-AAs\ in\ the\ IE\ \ feed\left[kg\right]}*100$$

Formula 3-6: Calculation of the transfer rate on non-AAs at the IE device

3.4.3 Further scenarios for the ion exchange process

For the assumed scenarios of the IE process, the energy needed for heating and pumping was calculated:

$$Heating\ energy\left[kW*h^{-1}\right]=\frac{liquid\left[kg\right]*\Delta T\left[K\right]*Cp\ H_2O\left[kJ*kg^{-1}\ *K\right]}{3600}$$

Formula 3-7: Calculation of energy needed for heating

$$Energy\ \ for\ \ pumping\left[kW*h^{-1}\right]=\frac{volume\left[m^3\ *\sec^{-1}\right]*\ pressure\ drop\left[Pa\right]}{efficiency\left[-\right]}$$

Formula 3-8: Calculation of energy needed for pumping

3.4.4 Removal of ammonia

The efficiency of the ammonia removal during the “ammonia stripping” was calculated as follows:

$$Efficiency\,NH_3 - removal\,[\%] = \frac{NH_3\ in\ the\ sample\,[g]}{NH_3\ in\ the\ starting\ solution\,[g]} * 100$$

Formula 3-9: Calculation of the efficiency of the NH_3-removal from Product 2

4 Results and Discussion

4.1 Pre-treatment steps of the silage juice used at the ion exchange process

For the IE process the silage juice was prepared by applying different state of the art membrane technologies. Findings from the processing of grass silage juice using these membrane technologies are not part of this work. Detailed pieces of information on the behaviour, process optimisations and total yields of AAs and LA at the membrane facilities are found in Ecker (Ecker 2012) and Ecker et al (Ecker, Schaffenberger et al. 2012). Indeed, a detailed examination of individual potentials of single AAs in the silage juice has not been presented so far. Therefore, the distribution of single AAs starting with raw silage juice and the yields per process step have been investigated more closely in this work. Additionally, overall balances for both processes (basic and innovative process) have been made. Data from different campaigns at the *GBR Upper Austria* (2010 and 2011) have been used for these calculations.

4.1.1 Amino acids in the raw silage juice

As already discussed in chapter 1.1.2, the ensiling process is a biological process, which is not easy to control. Therefore, the concentrations of AAs in the grass silage and in grass silage juice are varied. In Figure 4-1 an overview is given on the measured, total AA concentrations in different silages, which were locally produced at the *GBR Upper Austria* in 2010 and 2011.

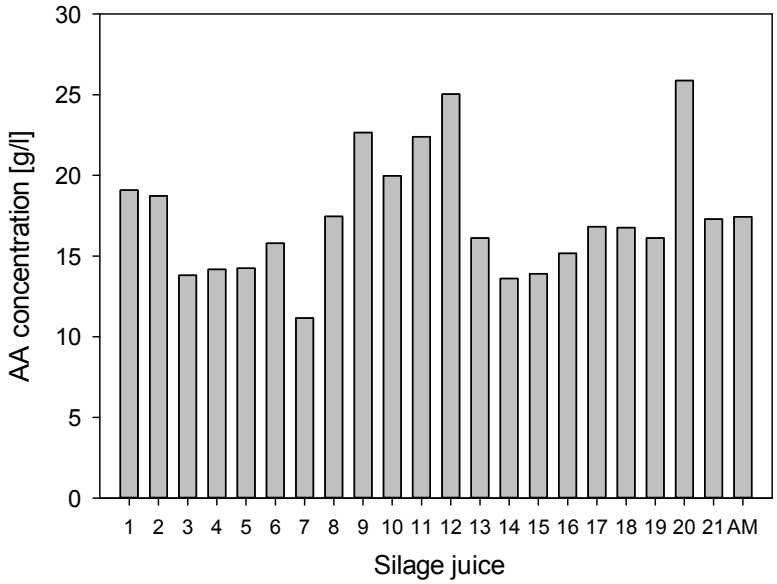


Figure 4-1: Comparison of total AA concentrations in grass silage juice from the *GBR Upper Austria* (n=21)

The total AA concentration in the grass silage juice varied from 11 g/l to 25 g/l. Generally, higher amounts of AAs were found in grass silage juice from silage harvested in May (1st cutting). Based on the data produced, an average AA concentration of **17.4 ± 3.9 g/l** in the raw silage juice was determined. The average dry substance of these juices was **10.3 ± 2.1 %.**

Further information on the distribution of single AAs and their average concentrations are found in Table 4-1, Figure 4-2 and Figure 4-3.

Table 4-1: Mean concentration of single AAs in the grass silage juice and mean percentage of single AAs on the total AA concentration

AA	Average concentration	SD	Average percentage of total AA concentration	SD	Ranking
	[g/l]	[g/l]	[%]	[%]	[-]
Asp	1.47	0.39	8.50	1.62	4
Glu	1.24	0.75	6.98	2.84	5
Thr	0.88	0.28	5.02	1.15	8
Ser	0.72	0.27	4.08	1.30	13
Pro	0.94	0.35	5.41	1.79	9
Gly	0.92	0.24	5.22	0.45	10
Ala	1.96	0.66	11.2	2.66	1
Cys	0.27	0.39	1.68	2.46	17
Val	1.22	0.40	6.98	1.92	5
Met	0.41	0.18	2.35	0.99	15
Ile	0.87	0.24	4.99	0.99	12
Leu	1.65	0.56	9.41	2.37	2
Tyr	0.41	0.32	2.32	1.85	16
Phe	0.89	0.33	5.09	1.56	11
Asn	0.77	0.47	3.32	3.14	14
Trp	0.25	0.29	1.51	2.02	18
GABA	1.46	0.52	8.68	3.75	3
Amm	0.89	0.27	-	-	-
Lys	0.99	0.43	5.58	1.93	7
His	0.23	0.08	1.30	0.43	19
Arg	0.06	0.05	0.35	0.27	20
SUM AA	17.4	3.90	100		

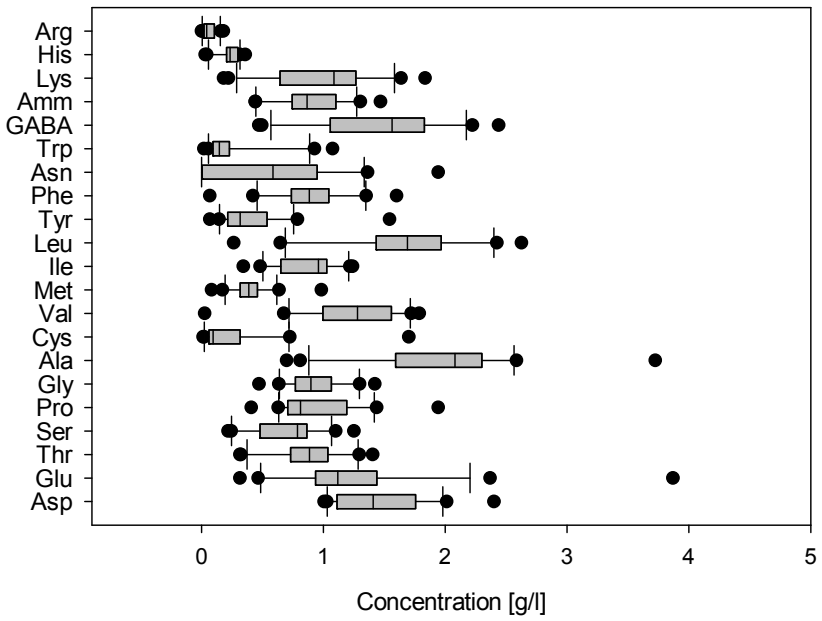


Figure 4-2: Distribution of analysed AA concentrations (single AAs)

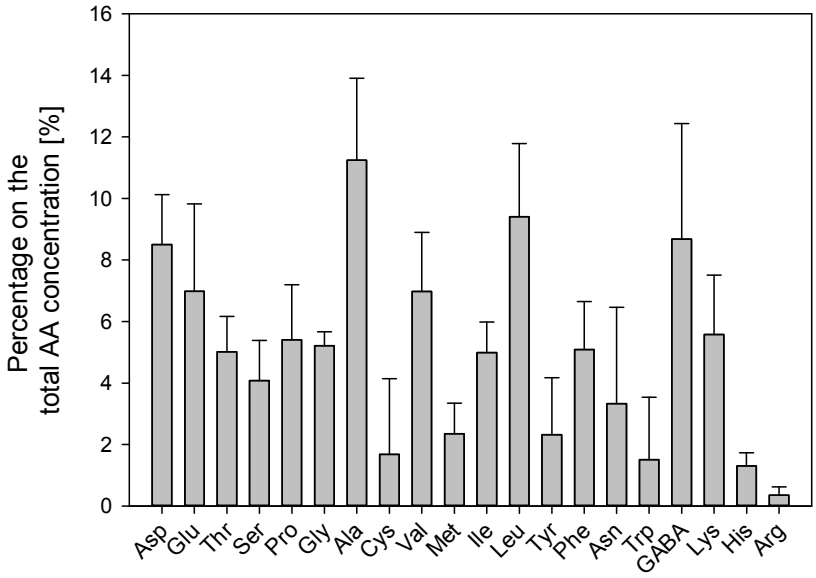


Figure 4-3: Percentage of single AAs on the total concentration of AAs in the silage juice

Based on the varying concentration of the single AAs (shown in Figure 4-3), the individual percentages of the total AA concentrations were inhomogeneous. On average, alanine (11.2 ± 2.66 %), leucine (9.41 ± 2.37 %), γ-amino butyric acid (8.68 ± 3.75 %) and aspartic acid (8.50 ± 1.62 %) with average concentrations up to 2 g/l were found most frequently in the grass silage juice. The average concentrations of arginine, histidine and tryptophan were always quite low with concentrations below 0.5 g/l.

4.1.2 AAs in the pre-treatment steps

As two different downstream processes for the treatment of grass silage juice have been applied at the *GBR Upper Austria*, the behaviour of single AAs in both processes is investigated in detail in the following chapter. Possible losses from the silage juice to the feed for the IE process have been estimated. Finally, the results for each process have been summarised in balances for each AA.

4.1.2.1 Basic process

In the basic process (Gruene-bioraffinerie.at GmbH 2008) the pre-treatment for the IE feed comprised of an UF step, a softening step and a two-stage NF. The behaviour of single AAs within these processes has been investigated. For each process step the efficiency for each AA has been calculated by referring the amount of the respective AA in the output to the introduced amount of this AA. The data on the behaviour for the cumulated AAs was based on findings of Ecker (Ecker 2012). The overall findings are summarised in Table 4-2. Subsequently, details of the findings per process step are added and discussed.

Table 4-2: Estimated efficiency per process step for each AA and AAs in total within the basic process

Amino acid	Efficiency Ultrafiltration	Efficiency Softening	Efficiency Nanofiltration
	[%]	[%]	[%]
Asp	90	>99	>95
Glu		>99	>95
Thr		>99	>95
Ser		>99	>90
Pro		>99	>90
Gly		>99	>65
Ala		>99	>85
Cys		>97	>90
Val		>99	>95
Met		>99	>95
Ile		>97	>95
Leu		>95	>95
Tyr		>95	>95
Phe		>90	>95
Asn		>99	>95
Trp		>80	>95
GABA		>75	>75
Lys		>65	>95
His		>55	>95
Arg		>50	>95
SUM AA	90*	95**	83.4*

* based on results found at Ecker (Ecker 2012)
** based on the total AA losses on the 1st column at the ion exchanger device

Ultrafiltration

Considering the individual distribution behaviour of each AA within the UF device, no specific differences in the separation per AA have been observed. The efficiency for each AA at the UF device was 90 %. In other words, about 90 % of each AA were found in the UF Permeate and 10 % were found in the UF Retentate, which was discarded and used energetically in a biogas plant nearby. Therefore, it was found that the losses within this process step were only dependent on the chosen process constellation for the UF. Normally, a concentration ratio between 9:1 (UF Permeate to UF Retentate) was applied and resulted in the above mentioned transfer of 10 % of AAs to the UF Retentate. This ratio was chosen as it was found to be the best compromise between a stable operation and minimal losses of AAs.

Softening

As the softening device at the *GBR Upper Austria* was a closed and automatically operated system, an accurate sampling of this device was impossible. Therefore, the estimation of the efficiency of this step including specific losses was done by using data and results from the first column of the IE step. This was feasible, as the softening device and the IE were equipped with the same resin (strong cation exchanger) and the same amount of resin. Furthermore, the first column of the IE was used to remove inorganic cations from IE feed as well.

Considering the efficiency of the softening step, the results summarised in Table 4-2 show that alkaline AAs, which are positively charged, interacted more strongly with the negatively

charged functional groups of the resin than other AAs. Therefore, about 35-50 % of arginine, histidine and lysine were lost during the softening step. The efficiency of the softening step for aromatic AAs (tryptophan and phenylalanine) and for γ-aminobutyric acid was lower than for other AAs as well. On average, 20 % of tryptophan, 10 % of phenylalanine and 25 % of γ-aminobutyric acid were adsorbed by the resin and were therefore lost for the subsequent processes. Other AAs did not show strong interactions with the resin and therefore efficiencies over 95 % were observed.

Nanofiltration

The efficiency of the double-stage NF device was calculated by referring the amount of AAs in the NF Retentate (2nd NF-stage) to the amount of AAs in the NF feed (1st NF-stage).

Considering the behaviour for single AAs within the double-stage NF, the yield for AAs with lower molecular weights was lower than for AAs with higher molecular weights. In other words, higher amounts of AAs with low molecular weights, such as glycine, alanine or γ-aminobutyric acid, were transported to the NF Permeates. Therefore, 15-35 % of these AAs were lost for the subsequent IE process. On the contrary, the yields and therefore the efficiency for other AAs with higher molecular weights were quite high. Most of these AAs, 95 %, were transported to the NF Retentate.

Balance

Based on the estimated efficiencies per process step for each AA in the basic process, mass balances starting with 1000 l raw silage juice were calculated. Incidentally, for the production of 1000 l raw silage juice 2.6 t silage (fresh) were needed on average. The results are summarised in Table 4-3.

Table 4-3: Mass balances for single AAs - raw silage juice to IE feed (basic process)

Amino acid	average AA-concentration (juice)	AAs from 1000l juice	Yield AA UF permeate	Yield AA Softening	Yield AA NF Retentate
	[g/l]	[kg]	[kg]	[kg]	[kg]
Asp	1.47	1.47	1.33	1.33	1.26
Glu	1.24	1.24	1.12	1.12	1.06
Thr	0.88	0.88	0.79	0.79	0.75
Ser	0.72	0.72	0.65	0.65	0.58
Pro	0.94	0.94	0.85	0.85	0.76
Gly	0.92	0.92	0.82	0.82	0.54
Ala	1.96	1.96	1.77	1.77	1.50
Cys	0.27	0.27	0.25	0.24	0.21
Val	1.22	1.22	1.09	1.09	1.04
Met	0.41	0.41	0.37	0.37	0.35
Ile	0.87	0.87	0.78	0.76	0.72
Leu	1.65	1.65	1.48	1.41	1.34
Tyr	0.41	0.41	0.37	0.35	0.33
Phe	0.89	0.89	0.80	0.72	0.69
Asn	0.77	0.77	0.70	0.70	0.66
Trp	0.25	0.25	0.22	0.18	0.17
GABA	1.46	1.46	1.32	0.99	0.74
Lys	0.99	0.99	0.89	0.58	0.55
His	0.23	0.23	0.21	0.11	0.11
Arg	0.06	0.06	0.06	0.03	0.03
SUM AA	17.4	17.4	15.7	14.9	13.4

Summing up, the yield of each AA and calculating the total value, a total isolation efficiency of 77 % for the pre-treatment steps for AAs within the basic process can be calculated. That means, in total 77 % of the AAs introduced to the process were transferred to the starting material for the IE process. For instance, based on the amount of AAs in 1000 l silage juice (17.4 kg) 13.4 kg of AAs were transported to the feed for the IE process. Comparing this rate, which was based on the behaviour of each single AA, with the efficiencies for the total amount of AAs per process step, slightly higher efficiencies were calculated here. For example, Ecker (Ecker 2012) observed a total efficiency for the AAs in the UF, the NF and for the 1st column of the IE facility of 71 %. It is assumed that these deviations resulted mainly from the NF process, as it was very difficult to calculate consistent mass balances for this double-stage process.

4.1.2.2 Innovative process

Within the innovative process, the softened UF Permeate was used directly in the IE facility. In contrary to the basic process, the double-stage NF step was skipped. The efficiencies for the UF and softening step have been similarly calculated to the basic process. The data is summarised in Table 4-4.

Table 4-4: Estimated efficiency per process step for each AA and AA sum within the innovative process

Amino acid	Efficiency Ultrafiltration	Efficiency Softening
	[%]	[%]
Asp	90	>99
Glu		>99
Thr		>99
Ser		>99
Pro		>99
Gly		>99
Ala		>99
Cys		>97
Val		>99
Met		>99
Ile		>97
Leu		>95
Tyr		>95
Phe		>90
Asn		>99
Trp		>80
GABA		>75
Lys		>65
His		>55
Arg		>50
SUM AA	90	95

Balance

Based on the estimated efficiencies for each AA per process step in the innovative process, mass balances based on 1000 l raw silage juice were calculated. For the production of 1000 l raw silage juice 2.6 t silage (fresh) were needed in average. The results are summarised in Table 4-5.

Table 4-5: Mass balances for single AAs - raw silage juice to IE feed (innovative process)

Amino acid	average AA-concentration (juice)	AAs from 1000l juice	Yield AA UF permeate	Yield AA Softening
	[g/l]	[kg]	[kg]	[kg]
Asp	1.47	1.47	1.33	1.33
Glu	1.24	1.24	1.12	1.12
Thr	0.88	0.88	0.79	0.79
Ser	0.72	0.72	0.65	0.65
Pro	0.94	0.94	0.85	0.85
Gly	0.92	0.92	0.82	0.82
Ala	1.96	1.96	1.77	1.77
Cys	0.27	0.27	0.25	0.24
Val	1.22	1.22	1.09	1.09
Met	0.41	0.41	0.37	0.37
Ile	0.87	0.87	0.78	0.76
Leu	1.65	1.65	1.48	1.41
Tyr	0.41	0.41	0.37	0.35
Phe	0.89	0.89	0.80	0.72
Asn	0.77	0.77	0.70	0.70
Trp	0.25	0.25	0.22	0.18
GABA	1.46	1.46	1.32	0.99
Lys	0.99	0.99	0.89	0.58
His	0.23	0.23	0.21	0.11
Arg	0.06	0.06	0.06	0.03
SUM AAs	17.4	17.4	15.7	14.9

Summing up the yield of each AA, a total efficiency of 86 % for the pre-treatment steps within the innovative process was observed. In other words, based on 17.4 kg of AAs from 1000 l raw silage juice, it was feasible to transfer 14.9 kg to the IE feed. That means, by skipping the NF step, the transfer of AAs from raw silage juice to the IE feed was increased by about 9 %.

4.1.3 Starting materials for the IE process

Since two different process assemblies were used to produce the starting materials for the IE process, the AA composition and AA concentration in the starting material were investigated and compared as well.

4.1.3.1 Basic process

As aforementioned, in the basic process (Gruene-bioraffinerie.at GmbH 2008) the NF Retentate was used as IE starting material. Due to the optimisation of the NF process different operation modes were tested. For instance, different degrees of dilution were applied in the first and second stage of the NF device. The first stage was run in diafiltration mode (adding additional, deionised water) and the second stage was run in filtration mode (without adding water). Based on the varying operation modes, the AA concentrations in the retentate differed widely. AA concentrations between 15-35 g/l were observed and more data on the varying concentrations are presented in Figure 4-4.

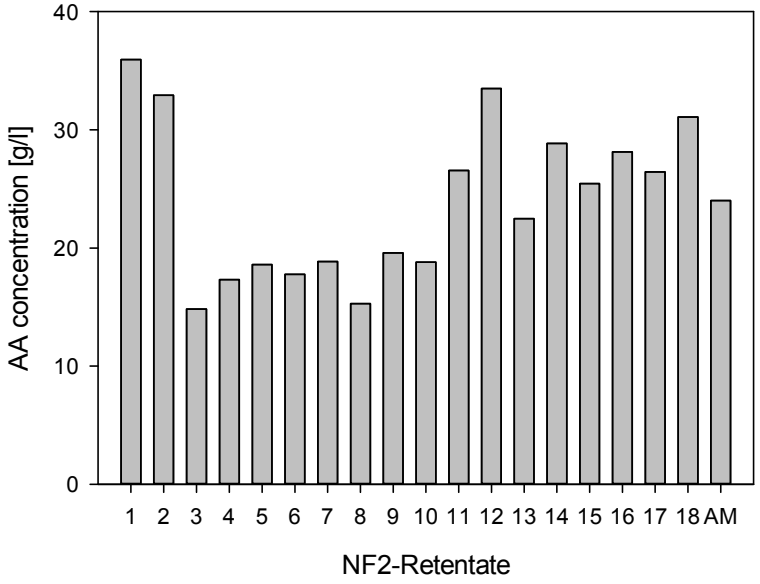


Figure 4-4: Comparison of the AA concentrations in NF2-Retentates from different experiments (n=18)

Furthermore, in Table 4-6 an average composition of the NF2 Retentate and the percentage per AA on the total AA concentration is shown. As during the NF step the retentate is normally concentrated, higher AA concentrations (in average 24 g/l) were detected in this process stream than in the silage juice (in average 17 g/l) or in the UF Permeate. However, considering the concentration and rates of the total AA amount per individual AA, the highest concentration and therefore the highest rates were again observed for alanine, leucine, aspartic acid and γ-aminobutyric acid.

Table 4-6: Average composition of NF2 Retentate and rates per AA on the total AA concentration

Amino acid	Average concentration	SD	Average percentage on total AA concentration	SD
	[g/l]	[g/l]	[%]	[%]
Asp	2.33	0.73	9.73	1.35
Glu	1.24	0.61	5.27	2.24
Thr	1.08	0.54	4.25	1.16
Ser	0.82	0.44	3.21	1.12
Pro	1.25	0.36	5.24	0.69
Gly	1.08	0.32	4.49	0.55
Ala	2.98	0.76	12.7	1.88
Cys	0.39	0.14	1.71	0.93
Val	1.92	0.48	8.07	0.69
Met	0.54	0.14	2.26	0.13
Ile	1.45	0.40	6.06	0.49
Leu	2.62	0.75	10.9	0.40
Tyr	0.49	0.17	2.03	0.31
Phe	1.22	0.38	5.02	0.29
Asn	1.38	1.11	2.81	3.72
Trp	0.16	0.05	0.61	0.28
GABA	2.08	0.76	8.93	2.96
Lys	1.29	0.55	5.28	1.18
His	0.32	0.10	1.31	0.12
Arg	0.05	0.04	0.18	0.14
SUM AA	24.0	6.7	100	

4.1.3.2 Innovative process

UF Permeate was used as a starting material for the IE process in the innovative process. On average, the UF Permeate contained lower AA concentration (11-20 g/l) than the silage juice at the process start. The decreased AA concentration in the UF permeate was caused by some losses in the UF device and in the softening step. Indeed, the process steps did not strongly influence the AA concentration. First and foremost, the AA concentration depended on the AA concentration in the silage juice. The following figure (Figure 4-5) shows examples for AA concentration in the UF Permeate. Furthermore, Table 4-7 shows the average AA composition and the rates of individual AAs of the total AA concentration. The most frequent AAs were again alanine, leucine, aspartic acid and γ-aminobutyric acid.

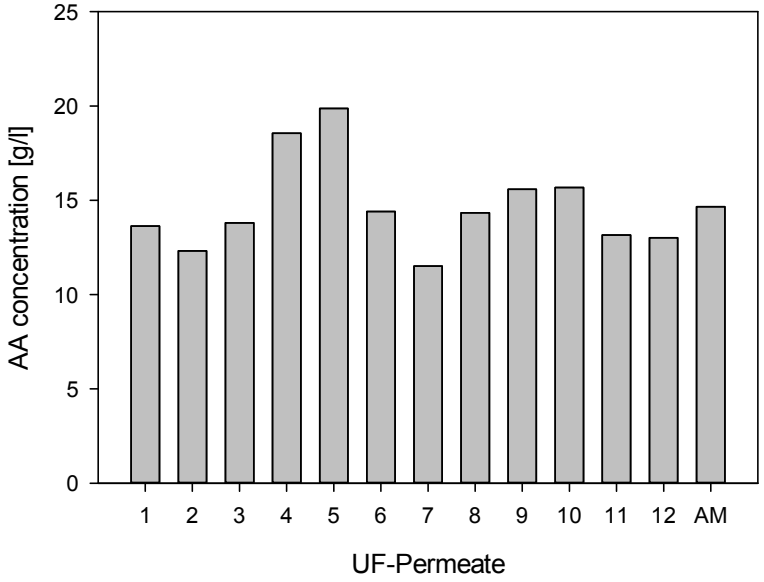


Figure 4-5: Comparison of the AA concentrations in UF Permeate from different experiments (n=12)

Table 4-7: Mean composition of UF Permeate and percentage per AA

Amino acid	Average concentration	SD	Average percentage on total AA concentration	SD
	[g/l]	[g/l]	[%]	[%]
Asp	1.21	0.20	8.30	1.14
Glu	1.11	0.47	7.44	2.51
Thr	0.79	0.24	5.35	1.11
Ser	0.67	0.25	4.54	1.31
Pro	0.71	0.15	4.85	0.52
Gly	0.77	0.15	5.28	0.35
Ala	1.74	0.27	12.1	1.86
Cys	0.10	0.12	0.66	0.87
Val	1.04	0.21	7.13	1.15
Met	0.33	0.08	2.24	0.31
Ile	0.74	0.16	5.08	0.91
Leu	1.54	0.25	10.5	0.31
Tyr	0.22	0.10	1.50	0.52
Phe	0.75	0.17	5.09	0.44
Asn	0.82	0.54	4.03	3.46
Trp	0.09	0.06	0.41	0.36
GABA	1.17	0.44	8.16	3.27
Amm	0.62	0.27	4.37	1.99
Lys	0.89	0.30	6.04	1.57
His	0.16	0.02	1.11	0.13
Arg	0.04	0.02	0.22	0.13
SUM AA	14.7	2.50	100	

In order to compare the AA pattern of the IE feeds, only some fluctuations were observed, as some AAs were enriched and some were depleted during the NF process. Nevertheless, the overall AA pattern was not strongly influenced at all.

4.2 Ion exchange experiments at the *GBR Upper Austria* demonstration plant

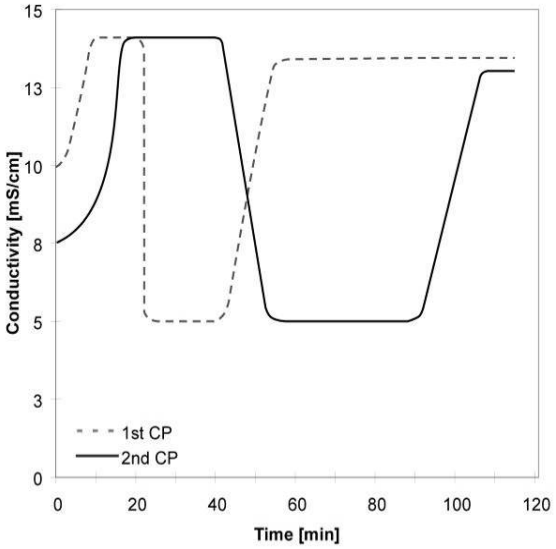
4.2.1 Process observations at the ion exchange facility at pilot scale

Grass silage juice is an unusual starting material for a DC process at pilot scale and so far only some results and findings using this material have been published. Therefore, an overview of findings and characteristics of all process steps during the DC process conducted with grass silage juice will be shown first. During the operation of the demonstration plant, different feeds and two IE resins were tested, but in overall the findings presented in the following chapter were true for all process constellations.

4.2.1.1 Loading

Each DC experiment started with loading the cation exchange resin with purified silage juice. Based on preliminary IE experiments at laboratory scale and former analyses of the grass silage juice, the IE process assembly and the process control for the IE device at the *GBR Upper Austria* were selected as shown simplified in Figure 3-4. Former analyses of the grass silage juice showed a ratio of 2:3 (eq:eq) of inorganic cations to AAs within the juice. Therefore, five columns were installed to provide two columns to fix inorganic cations and three columns to generate AAs.

Generally, AAs and inorganic cations show strong differences in conductivity: AAs do not influence the conductivity strongly and inorganic cations cause higher values of conductivity. Hence, it was assumed, that these differences in conductivity combined with the pH were ideal parameters for monitoring and controlling the loading process. Check points for measuring the data needed were installed after the first (CP 1) and second column (CP 2). By monitoring the data at these CPs the loading process could be controlled and stopped. For instance, Figure 4-6 and Figure 4-7 show the assumed course of conductivity and pH at CP 1 and CP 2. The figures show that after a short start-up period, the conductivity should remain at a high level (approx. 14 mS/cm) and the pH at a low level (approx. 2). This behaviour would be caused by H^+-ions, which are displaced from inorganic cations and AAs from the resin's surface. This section would be followed by a strong decline of the conductivity initialised by the breakthrough of AAs. After this area of low conductivity, it should rise quickly again which is caused by the breakthrough of inorganic cations combined with a rise in the pH. Generally, all these developments should be first observed at CP 1 and then time delayed at CP 2. Based on this data the appropriate point to stop the loading process has been assumed: the breakthrough of inorganic cations at CP 2. Stopping the loading process at this point should result in high yields of AAs on column 3, 4 and 5 combined with a low risk of losing them after column 5.



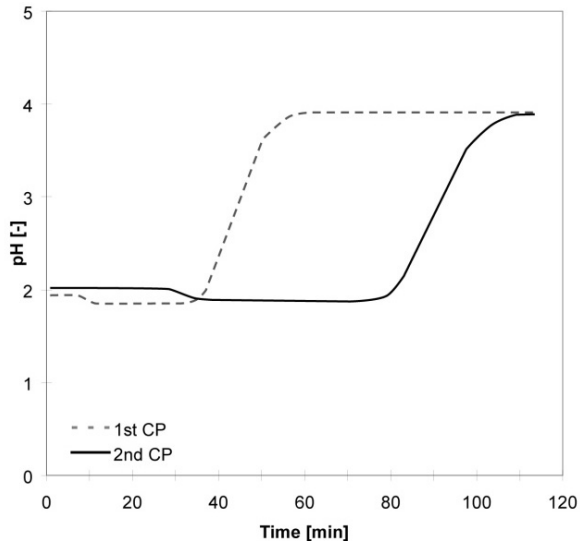


Figure 4-6: Preliminarily assumed trend of conductivity the loading process (left)
Figure 4-7: Preliminarily assumed trend of pH during the loading process (right)

In experiments at the IE device at the *GBR Upper Austria* the aforementioned assumptions were put into practice. For example, Figure 4-8 and Figure 4-9 show the data observed during a real experiment at the IE using Resin II. Interpreting the data of CP 1 first (Figure 4-8 and Figure 4-9): after a short initial phase the conductivity value in this experiment amounted to 10 to 14 mS/cm combined with a low pH (<2). As assumed before, H^+-ions were displaced from AAs and inorganic ions from the resin's surface causing the high conductivity and the low pH in the first section of the graph. After 20 minutes, the conductivity declined sharply to 5-6 mS/cm and the pH started to rise. This area indicated the passage of AAs at CP 1, which caused an area of lower conductivity than the H^+-ions before and forced the pH to rise. Afterwards, the conductivity increased again and finally reached a plateau at about 13 mS/cm. In this area the pH rose continuously and reached a stable level as well. All these rises were generated by the passage of inorganic cations. Finally, the conductivity dropped quickly and the pH started to rise again. These developments were caused by the breakthrough of water used for rinsing the resin. Looking at the courses of CP 2 (Figure 4-8 and Figure 4-9), all trends of the graph from CP 1 were repeated time-delayed, as predicted theoretically. Additionally, the area where AAs are present and which was indicated through low conductivity, was widened at CP 2, due to the higher amount of AAs within the system.

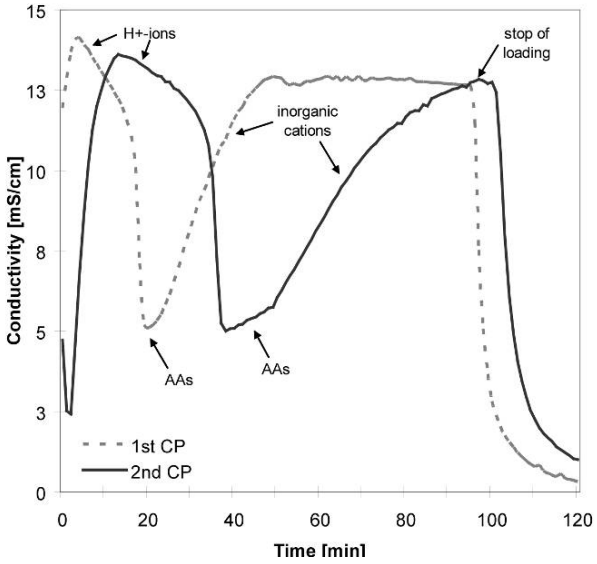


Figure 4-8: Development of conductivity during the loading (Resin II, 8.1m/h flow rate), measurements at 1st and 2nd CP

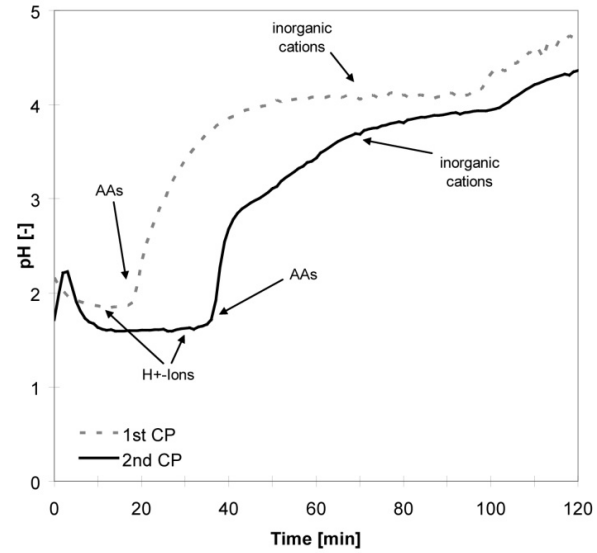


Figure 4-9: Development of pH during the loading (Resin II, 8.1 m/h flow rate), measurements at 1st and 2nd CP

On comparing the data and observations from the real experiment with the theoretical assumptions shown in Figure 4-6 and Figure 4-7, the overall trends of the graphs were related. Nevertheless, some differences appeared and it was not possible to implement the theoretical assumptions completely. Firstly, considering the development of the conductivity, the area with low values indicating the breakthrough of AAs was smaller and less exact. Furthermore, the breakthrough of inorganic cations did not force the graph to rise as sharply as assumed. This observation also indicated that there was no sharp borderline between AAs and inorganic cations at CP 2, which was confirmed in further investigations of the loading process discussed in detail in chapter 4.2.3.1. Indeed, based on this fact that the breakthrough of inorganic ions was less exact and sharp, it was more difficult to find the ideal point to stop the loading as assumed before. Secondly, it was also found that the content of inorganic ions in the grass silage juice used in experiments at the *GBR Upper Austria* was higher than in previous experiments. That means that the ratio between AAs and inorganic cations was shifted to the inorganic substances and consequently led to lower yields of AAs

by using the assumed point to stop the loading (breakthrough of inorganic cations at CP 2). To increase the yield of AAs per batch run it was necessary to prolong the loading process. Therefore, the stopping point was shifted and the loading was stopped when the conductivity measured at CP 2 reached the same plateau as the conductivity measured at CP 1 (see Figure 4-8). Indeed, by adapting the process in this way higher yields on column 4 and column 5 were observed and no ammonia was wasted by eluting them, but it led to the presence of inorganic cations and lower AA yields on column 3 as well.

4.2.1.2 Displacement

The displacement step contained the generation and collection of purified AA solutions. Each column was treated with 1 M ammonia separately. Consequently, the AAs were displaced and found in the eluate. The elution process of every column was monitored and controlled by observing the conductivity, pH and refractive index, whereas the latter was applied to see the start and the end of the AA elution. Conductivity and pH, in turn, were monitored, to separate the AAs into different fractions.

In order to demonstrate this process in more detail, the process data of a displacement process conducted on column 4 are shown as an example in Figure 4-10 and Figure 4-11. Figure 4-10 displays the course of the refractive index (in % Brix) and the pH; Figure 4-11 shows the data of the refractive index (in % Brix) and conductivity. Generally, it was possible to split the displacement process of column 4 into five parts. In the first section of the process called phase 1, the void volume was displaced from the column. Due to some solved components in the void volume the refractive index rose temporarily in this phase, followed by a strong decrease. As soon as the refractive index went up strongly once more initiated by the breakthrough of displaced AAs, the second phase (phase 2) started and the first AA-product (Product 1A) was eluted and its collection started. Product 1A contained high amounts of acidic AAs, which are negatively charged. Therefore, an increase of the conductivity was observed during this phase. In addition, during the elution of the acidic AAs, the refractive index went up considerably as the concentration of the displaced AAs in the eluate increased. As soon as most acidic AAs were eluted, the conductivity slightly declined again, which indicated the elution of neutral AAs and consequently the collection of the second product (Product 1B) started (=phase 3). Phase 3 and therefore the collection of Product 1B ended as soon as the pH, which permanently increased during phase 2 and 3, the conductivity and the refractive index suddenly rose caused by incoming ammonia. This breakthrough of ammonia indicated the next phase of the displacement process (=phase 4). During this phase the third product (Product 2) was collected. This product contained alkaline AAs, neutral AAs and ammonia. In phase 4 pH, conductivity and refractive index again declined very quickly. Once the refractive index was lower than 0.3 due to low AA concentrations the collection of Product 2 was stopped and phase 5 started. In phase 5, which was called post-product phase, only some alkaline AAs were still eluted and as soon as the refractive index was 0.0, the displacement process was stopped.

Typically, lower yields of AAs were observed on column 1, 2 and 3. Therefore, slight differences in the course of the displacement process were observed on these columns. For instance, it was impossible to separate the first product into Product 1A and 1B, as the amounts of acidic AAs that were found on them were too low.

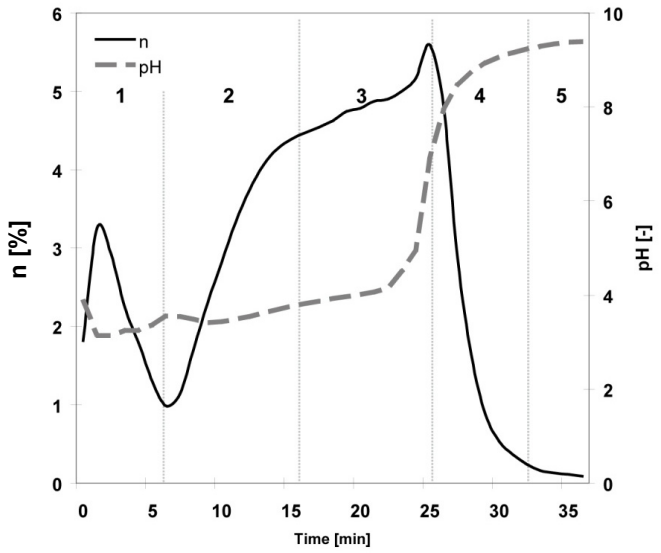


Figure 4-10: Representative displacement process and product fractionation (data of refractive index (n) and pH, displacement medium 1 M NH_3, flow rate 8.1 m/h, Resin II)

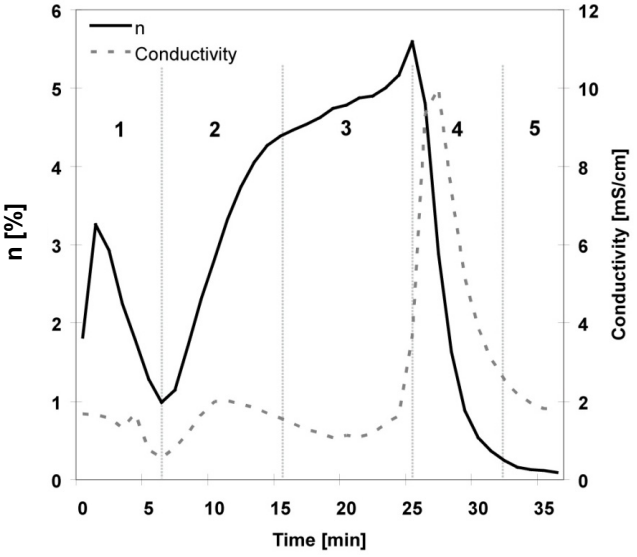


Figure 4-11: Displacement process and product fractionation (data of refractive index (n) and conductivity, displacement medium 1M NH_3, flow rate 8.1 m/h, Resin II)

4.2.1.3 Regeneration

The regeneration was adapted to each column after considering the components, which are fixed to the resin. Due to high concentrations of calcium in column 1 and 2 and its propensity to build insoluble precipitates with sulphate, these columns were regenerated using a double-stage process. This regeneration process consisted of a first step using 0.25 M sulphuric acid followed by a regeneration step with 1 M sulphuric acid. With this procedure, it was possible to regenerate both ion exchange resins sufficiently and without any problems. Furthermore, it was found out that heating up the sulphuric acid before regeneration is not necessary, as the

solubility of calcium sulphate (gypsum) is higher at lower temperatures (Stark, Stürmer 1996). For that reason the energy used for the heating up was saved.

4.2.2 Balances and yields of the ion exchange facility at pilot scale

Considering the economic feasibility of the present IE process, the most important factor to be investigated was the AA yield per batch run. As different starting materials for the IE process and two IE resins were tested, average yields of AAs per batch run were calculated for all process assemblies. Furthermore, the behaviour of "non-AA" components such as sugars, organic acids and inorganic ions on the IE was investigated. In the following chapter the results are shown and discussed.

4.2.2.1 Feed comparison in the ion exchange process regarding yields of amino acids

Depending on the applied process assembly at the demonstration plant, either UF Permeate (innovative process) or NF2 Retentate (basic process) were used as a starting material for the IE process. Furthermore, in one experiment the direct usage of untreated silage juice was tested at the IE facility. Subsequently, the average results using different starting materials on the AA yield per batch run, the AA yield per product fraction and per column, as well as the recovery rate are presented.

Comparison of the basic and innovative process

In order to be able to make a decision regarding the final process assembly of the *GBR Upper Austria* the performances of the two feeds NF2 Retentate and UF Permeate at the IE device were investigated and compared. To guarantee comparability between the feeds, only experiments conducted on Resin II were taken into account for this comparison. Overall results considering yields, efficiency and the relative separation into product fractions for both feeds are summarised in the following table (Table 4-8). Furthermore, Figure 4-12 gives an overview of the yields per column.

Table 4-8: Comparison of average results of experiments using UF Permeate (innovative process) or NF2 Retentate (basic process) on Resin II

	Basic process		**Innovative process**	
	[kg]	[eq]	[kg]	[eq]
Yield AAs per batch run (all columns)	7.76	64.7	6.33	56.9
Theoretical capacity occupied with AAs [%]	26.9		23.7	
Recovery rate of AAs per batch run [%]	>95		>95	
Efficiency AA adsorption [eq/l resin]	0.54		0.47	
Efficiency AA adsorption [eq/eq resin]	0.27		0.24	
Percentage per AA products [%]				
Pre- and post product phase	11		8	
Product 1A	18		21	
Product 1B	46		42	
Product 2	25		29	

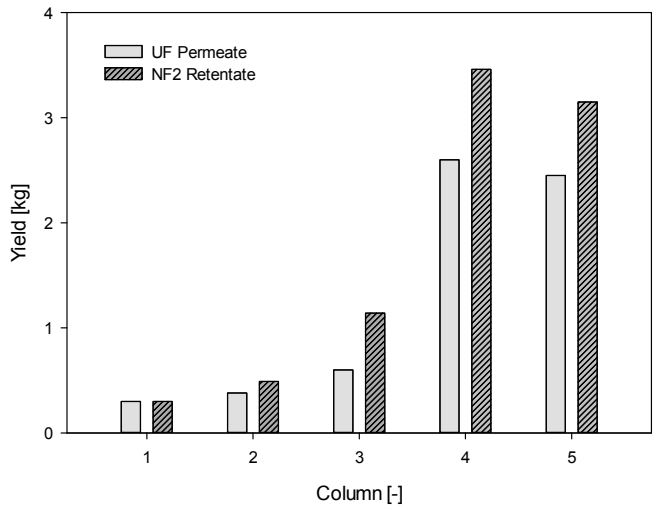


Figure 4-12: Comparison of average, absolute AA yield per column using UF Permeate and NF2 Retentate (experiments with Resin II)

The data (see Table 4-8) shows that experiments conducted with NF2 Retentate led to higher AA yields per batch run over all. On average 18 % more AAs were achieved in these experiments than in experiments with UF Permeate. Therefore, in these experiments the efficiency in adsorbing AAs was higher than in experiments done with UF Permeate. That means, in experiments with NF2 Retentate more AAs per litre and per available space in the resin were adsorbed. Furthermore, as shown in Figure 4-12, the absolute yield of AAs per column was also increased. Only the yield of column 1 remained stable. On the contrary, similar trends for both starting materials were observed regarding the percentage per product fraction.

It was assumed that the different yields were based on the more favourable ratio between AAs and inorganic cations in the NF2 Retentate, which led to the possibility of loading the resin with a higher amount of feed than under the present circumstances for loading. The shift of the ratio between AAs and inorganic cations in favour of the AAs was caused by the NF step. In this step, AAs were concentrated and remained in the retentate. Monovalent inorganic cations (e. g. potassium or sodium), in turn, were partially transferred to the permeate and therefore their concentration in the retentate was reduced. In order to demonstrate these differences more precisely, Table 4-9 shows data from two experiments in the IE device using UF Permeate or NF2 Retentate, which were made from the same raw grass silage juice. For instance, in this case the ratio was shifted from 1:0.50 to 1:0.40 (g/g) or from 1:1.8 to 1:1.7 (eq/eq) in favour of the AAs.

Table 4-9: Comparison of the ratio between AAs and inorganic cations in UF Permeate or NF2 Retentate; concentration factors in the NF step

	UF Permeate		NF2 Retentate	
	Concentration [g/l]	Molar Concentration [eq/l]	Concentration [g/l]	Molar Concentration [eq/l]
Amino acids	13.0	0.118	31.1	0.286
Inorganic cations	6.47	0.214	12.4	0.482
Ratio AAs to inorganic cations [g/g]	1 : 0.5		1: 0.4	
Ratio AAs to inorganic cations [eq/eq]		1 : 1.8		1 : 1.7
Factor of concentration during the NF step				
AAs	2.4			
Potassium	1.5			
Sodium	1.3			
Ammonium	2.3			
Calcium	5.4			
Magnesium	3.8			

Furthermore, as assumed, the different concentration factors obtained during the NF step demonstrated the different behaviour of AAs and inorganic cations. Monovalent inorganic cations were concentrated the least followed by the AAs, as some smaller AAs were transferred to the permeate as well. Bivalent cations, like calcium and magnesia, remained in the retentate and therefore were concentrated most of all. Indeed, the reduction of monovalent cations was enough to shift the ratio in favour of AAs.

Untreated silage juice

In order to investigate the direct applicability of untreated grass silage juice in the IE device, one experiment was conducted using this feed. In the following table (Table 4-10) the data, which originates from this single experiment conducted on Resin II are presented. Next to overall balances and the distribution of the AAs, this experiment with silage juice focussed on the overall feasibility and quality of the products obtained.

Table 4-10: Yields and rates using raw silage juice (data from a single experiment)

	Raw silage juice	
	[kg]	[eq]
Yield AAs per batch run (all columns)	5.14	49.7
Theoretical capacity occupied with AAs [%]	20.7	
Recovery rate of AAs per batch run [%]	>99	
Efficiency AA adsorption [eq/l resin]	0.41	
Efficiency AA adsorption [eq/eq resin]	0.21	
Percentage per AA products [%]		
Pre- and post product phase	6	
Product 1A	0	
Product 1B	23	
Product 2	71	

Using raw grass silage juice in the IE device led to a considerably lower AA yield compared to the yields observed in experiments done with NF2 Retentate or UF Permeate. Thereby, less of the theoretical capacity was occupied with AAs and a lower adsorption efficiency was also noticed. Further investigation showed that the ratio between AAs and inorganic cations in the grass silage juice laid at the inorganic cations more heavily, as no softening step was

applied before. Therefore, lower yields were obtained using untreated silage juice. A too brief time of loading, in turn, can be excluded as the main amount of AAs was found in column 4 and 5 (see Figure 4-13).

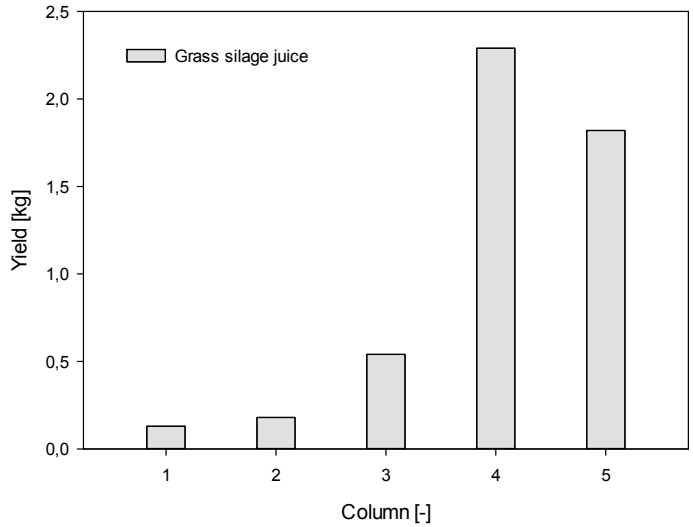


Figure 4-13: Comparison of average, absolute AA yields per column using raw grass silage juice

Besides the low yields, it was noted that there were differences in the percentage per product fraction. For instance, by using raw silage juice it was not feasible to separate any Product 1A at column 4 or 5, although the elution process was monitored as usual and the percentage per AA group (acidic, alkaline and neutral) in the product obtained was quite similar to the results from experiments with NF2 Retentate or UF Permeate. Furthermore, the percentage of AAs which were eluted at the same time as ammonia, and therefore the percentage of Product 2, increased to over 70 % (cf. UF Permeate: 29 %; NF2 Retentate: 25 %). The percentage of Product 1B, in turn, was decreased.

Moreover, the optical properties of the gained products differed from the yellow coloured, but not cloudy products produced in experiments with UF Permeate or NF2 Retentate. The AA product solutions that were obtained from silage juice were contaminated with particles and therefore cloudy. At first sight, the quality of these products was lower than the quality of AA products from UF Permeate or NF2 Retentate. Indeed, it was not possible to confirm these visual observations through measuring any increased dry matter in these products compared to dry matter from products of UF Permeate or NF2 Retentate. Nevertheless, it was assumed that further usage of the products from raw silage juice would be more difficult. Considering, on the other hand, the application of grass silage juice on the IE device on a basic level, the usage was feasible. For instance, pressure limits during the loading step were not exceeded, but after a longer period of operation it was not possible to keep the flow constant. Possibly, small particles led to blockages within the columns or within the pump and therefore to a constant flow decrease. Furthermore, the usage of grass silage juice for loading increased the amount of rinsing water needed, as it was more difficult to remove the higher amounts of dyes and particles put onto the IE columns. So summing up, the application of grass silage juice was possible, but was associated with more effort and resulted in low-quality products.

4.2.2.2 Ion exchange resins

Two strong cation exchange resins with different properties were tested at the demonstration plant. Based on data from several experiments using either Resin I or Resin II, average

results of the AA yield per batch run and other results were observed and calculated. The data, which is summarised in Table 4-11, based on experiments conducted with NF2 Retentate. Moreover, the amount of inorganic cations (Na^+, K^+, NH_4^+, Mg^{2+}, Ca^{2+}) put on the resin during an average loading step, is added, as these cations are the main competitors of the AAs for the functionalised groups on the resin's surface.

Table 4-11: Average yields and rates per IE batch run comparing Resin I and Resin II

	Resin I		**Resin II**	
	[kg]	[eq]	[kg]	[eq]
Yield AAs per batch run (all columns)	7.90	65	8.30	70
Loading with inorganic cations	2.14	90	2.09	86
Theoretical capacity used in total [%]		74.2		65.0
Theoretical capacity occupied with AAs [%]		31.1		29.2
Theoretical capacity occupied with inorganic cations [%]		43.1		35.8
Used capacity [eq]		155		156
Rate of AA used capacity [%]		41.9		44.9
Rate of inorganic ions used capacity [%]		58.1		55.1
Recovery rate of AAs per batch run [%]		>95		>95
Efficiency AA adsorption [eq/l resin]		0.56		0.58
Efficiency AA adsorption [eq/eq resin]		0.31		0.29
Efficiency adsorption of inorganic cations [eq/l resin]		0.78		0.72
Efficiency adsorption of inorganic cations [eq/eq resin]		0.43		0.36

Generally, as shown in Table 4-11, the results of experiments on both resins point out that the recovery rate of the AAs per batch run was high. Over 95 % of the introduced AAs were found in the displaced AA solutions again. That means that the AAs were desorbed by the ammonia sufficiently.

Although, differences between the resins were observed regarding the AA yield per batch run. On average, 7.90 kg or 65 eq AAs were yielded in experiments using Resin I. With an average yield of 8.30 kg or 70 eq the output per batch run of Resin II was slightly higher. In both cases, the theoretical capacity of the resins that was occupied with AAs, was about 30 %. Considering the amount of inorganic cations, mean results showed that Resin I was loaded with 2.14 kg or 90 eq of inorganic ions per batch run. That means that 43.1 % of the theoretically available capacity was loaded with inorganic ions in this case. Resin II, in turn, was loaded with 2.09 kg or 86 eq of inorganic cations on average, which means that 35.8 % of the theoretical capacity was occupied by them. Therefore, in total 74.2 % of the capacity of Resin I and 65.0 % of the capacity of Resin II was used either by AAs or inorganic cations. On the other hand, 25.8 % on Resin I and 35 % Resin II of the theoretical capacity were neither occupied by AAs nor by inorganic cations. There may be some probable reasons for the unoccupied functional groups. Firstly, as known, it is impossible to regenerate the resins completely after the first usage and some theoretical capacity is lost irreversibly. Secondly, due to steric problems between the resin and AAs it was possible that some parts of the resins remained unoccupied (Jones, Carta 1993). However, in total the relative used theoretical capacity of Resin I was higher than of Resin II. Calculations showed that the effective capacity used was 155 eq for Resin I and 156 eq for Resin II, which is nearly equal, although the theoretical capacity of Resin II was 31 eq higher.

In order to assess the performances of both resins in more detail and to provide comparable data, some operating figures were calculated using Formula 3-4 and Formula 3-5. Results for the inorganic cations were equally calculated with corresponding data. The results showed that Resin I was able to adsorb 0.56 eq per litre of this resin or 0.31 eq AAs per available mol equivalent. The efficiency of Resin II was slightly higher per litre of resin (0.58 eq AA/l resin),

but per available mol equivalent the efficiency was lower (0.29 eq AA/eq resin). Examining the efficiency of adsorbing inorganic cations, in turn, more of them were adsorbed in Resin I per litre and per available mol equivalents as well.

Based on this data it can be summarised that Resin II is slightly more adequate for adsorbing AAs than Resin I, as the yielded AAs per litre of Resin II were higher, but the theoretical available capacity of Resin I was used more efficiently, as its AA output per mol equivalent was higher than that of Resin II. However, results on the adsorption efficiency of inorganic cations showed that Resin I is more capable of removing them from an aqueous solution than Resin II. Consequently, the breakthrough of the ions at CP 2 was delayed in Resin I, as more inorganic cations were adsorbed per litre of resin. Therefore, the loading was prolonged as the breakthrough of inorganic cations was delayed and it was possible to load the IE with more feed and thereby more AAs. For this reason, quite similar AA yields were observed in experiments either using Resin I or using Resin II, although the theoretical capacity of Resin II was 15 % higher.

4.2.2.3 Further investigated parameters in order to optimise the yield per ion exchange run

Since only slight differences were observed in the yields of AAs by using the investigated starting materials and resins, some more process parameters (e. g. flow rates) were varied to potentially stimulate the output. Furthermore, other factors that could possibly influence the process, but which were given through the input material or through the pre-treatment steps, were examined more precisely.

Flow rates

In order to get higher AA yields different flow rates were applied during the loading step. It was tested, if an extended residence time of the components, caused by lower flow rates, would lead to more efficient interactions between the AAs and the resin's functional groups and therefore to higher yields. Possible connections between flow rates during the loading step and the yields of AAs were studied by comparing results of experiments applying different flow rates between 2.7 to 10.8 m/h. All these experiments were done on Resin II and using UF Permeate gained from silage juice, which originated from silage made during a single harvest (first harvest of 2011, May 2011). Indeed, as shown in Figure 4-14, no direct impact of the flow rates on the AA yield per batch run was observed within the investigated range of flow rates. For this reason the conclusion can be drawn that the AA yield is independent of the applied flow rates.

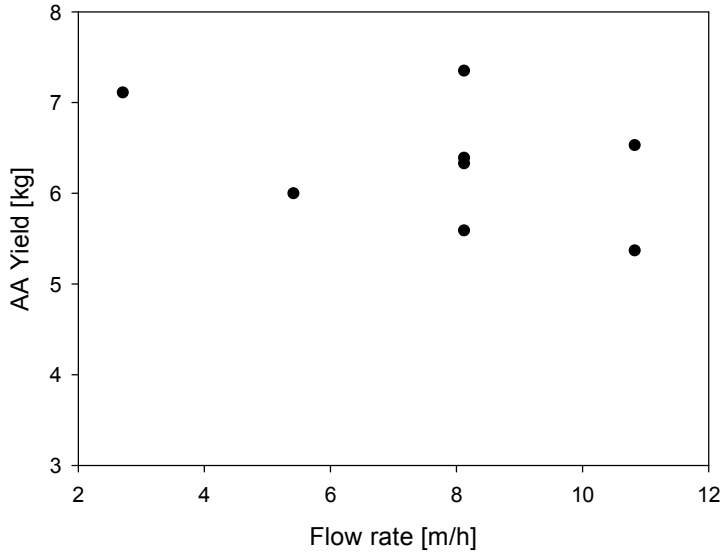


Figure 4-14: AA yields per batch run at flow rates between 2.7 to 10.8 m/h (experiments using UF Permeate at Resin II)

Furthermore, possible impacts of the applied flow rates on the amount of gained product fractions were examined. In particular, the rate of Product 2 was studied, which was contaminated with ammonia and therefore more difficult to process afterwards. Hence, the same experiments were investigated regarding their rate of AAs in Product 2, dependent on the applied flow rate during the elution step. Though, no considerable influence on the rate of Product 2 was observed. In order to demonstrate this visually, results of the elution experiments are shown in Figure 4-15.

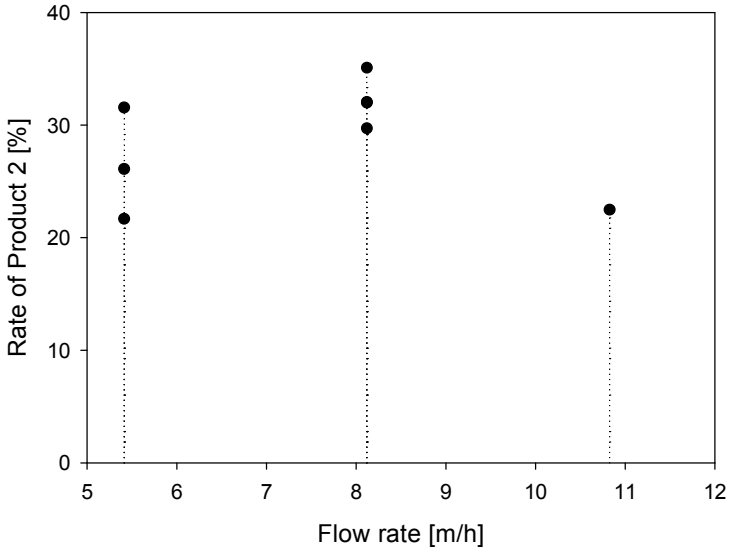


Figure 4-15: Rate of Product 2 on the total amount of eluted AAs, which was dependent on the applied flow rate for elution (investigated flow rates: 5.4-10.8 m/h in experiments using UF Permeate at Resin II)

Summing up the results of the investigated flow rates neither direct impacts on the AA yields per batch run nor improvements regarding the distribution of product fractions were observed. However, due to economical reasons the feasibility of an increase of flow rates was also considered. Enhanced flow rates would lead to enhanced throughputs and therefore to higher yields in shorter periods of time. In this respect, it has to be noted that higher flow rates can be applied for the tested resins according to their fact sheets. For

instance, it is feasible to use Resin I in the range of 5-60 m/h during the service mode (Dow Chemical Company) and Resin II in the range of 5-200 m/h (Dow Chemical Company). Hence, due to plant-specific reasons comparatively low flow rates were applied using the present IE device. Experiments have shown that flow rates of 10.8 m/h led to backpressures around 3.5 bar on column 1 and the columns made of steel were constructed according to the fact-sheet for a maximum pressure of 4 bar. Nevertheless, in a single experiment higher flow rates of up to 18.9 m/h during the loading and the elution were planned, as in previous tests with water it was feasible to reach this flow rate using the present pump. Nevertheless, during the loading with UF Permeate it was not able to reach flow rates of 18.9 m/h even though the effective stroke length of the pump was set to its maximum. In these experiments flow rates of 14.3 m/h were reached on average leading to backpressures of 4.5 to 5.5 bar on column 1. As the elution was done, in turn to the loading process, per column and not serially with all five columns an increased flow rate of 16.2 m/h was observed. Generally, by applying enhanced flow rates comparable results to former experiments were achieved, as seen by the data summarised in Table 4-12.

Table 4-12: Results of a single experiment applying flow rates up to 16.2m/h (UF Permeate, Resin II)

	Raw silage juice	
	[kg]	[eq]
Yield AAs per batch run (all columns)	4.74	42.5
Percentage per AA products [%]		
Pre- and post product phase	7	
Product 1A	17	
Product 1B	41	
Product 2	35	

Basically, it can be concluded that it is feasible to perform the loading as well as the elution at increased flow rates, as both steps can be monitored and controlled by using the usual parameters. Considering the output of the experiment shown in Table 4-12, below average AA yields were observed. Indeed, it is assumed that the low yield was caused by the feed used, as the yield observed in a reference experiment done with the same feed (flow rate: 8.7 m/h) was low as well (4.55 kg AAs). Furthermore, a too short duration of the loading can also be excluded, as a high amount of AAs was eluted from column 5. Quite similar trends regarding the distribution of the AAs in the product fractions were observed compared to experiments done with the usual flow rates. Only an increased percentage of Product 2 was noticed in the present experiment. Summing up it can be concluded that experiments done with increased flow rates lead to the same outcomes as experiments done with lower flow rates. An adaptation of the IE device to apply increased flow rates during routine work or the consideration of this fact in a case of rebuilding the plant, respectively, seems useful. Indeed, detailed data and appropriate operating parameters have to be achieved at increased flow rates once more.

Further parameters

Next to the flow rates further process parameters, which possibly influence the IE process and therefore the yield per batch run, were investigated in detail. An additional target of these analyses was also the identification of easy measureable process parameters, which allow, for instance, easy estimating of the expected output. Firstly, it was examined whether there is a direct correlation between the amount of feed used in a batch run under normal loading conditions and the AA yield per batch run. Considering this question, Figure 4-16 shows data from different experiments using NF2 Retentate or UF Permeate on Resin II.

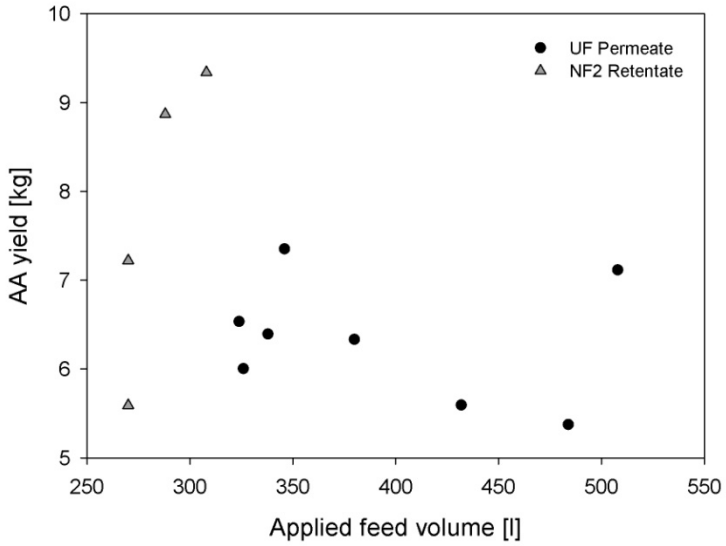


Figure 4-16: Analysis of AA yields per batch run and the applied feed per batch run (experiments on Resin II using UF Permeate or NF2 Retentate)

Based on the data shown in Figure 4-16 no correlations between the volume, which was necessary for loading the IE device, and the generated AAs were observed. Indeed, based on this data it can be concluded that in experiments using NF2 Retentate with higher AA concentrations less feed is used per batch run. Subsequently, less time for pumping is needed in this case, which further leads to cost reductions.

Secondly, possible correlations between the concentration of AAs in the feed and the yield of AAs were investigated. Considering the data shown in Figure 4-17, again no considerable correlations between these parameters were observed. Although a slight tendency was recognised as the highest yields were gained in experiments with higher concentrations of AAs in the feed. In particular, higher concentrations were detected in NF2 Retentate and increased yields were often observed in these experiments, which was already discussed and shown in chapter 4.2.2.1.

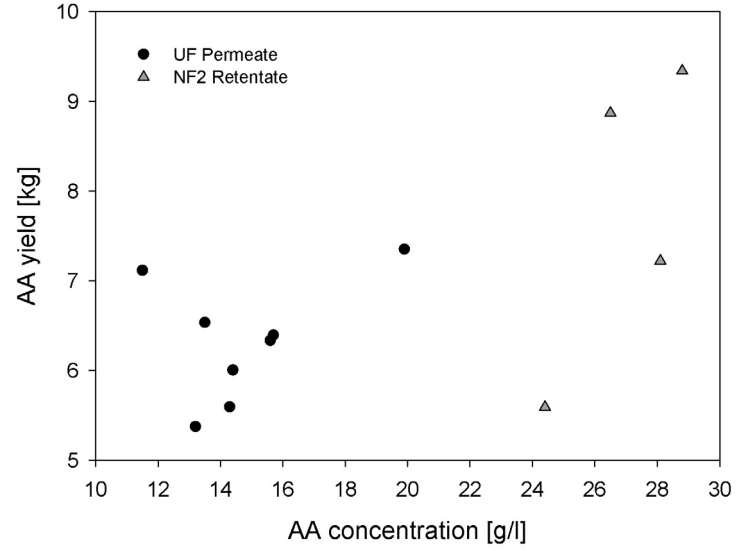


Figure 4-17: Analysis of AA yields per batch run and the concentrations of AAs in the feed (experiments on Resin II using UF Permeate or NF2 Retentate)

Furthermore, other parameters given by the feed were investigated. For example, a possible influence of the pH in the feed on the AA yields was examined. The results are shown in Figure 4-18. Within the investigated range of pH, which was given by the raw silage juice and the pre-treatment steps, no influences on the yields of AAs were observed. Indeed, it is not excluded that a lowering or an enhancement of the pH (Dorfner 1991) would stimulate the interactions between AAs and the resins and consequently the yields of AAs.

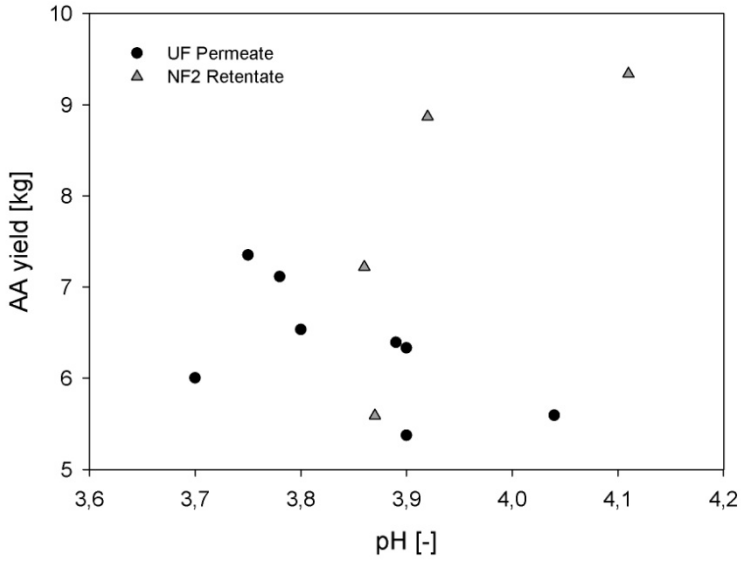


Figure 4-18: Analysis of AA yields per batch run and pH in the feed (experiments on Resin II using UF Permeate or NF2 Retentate)

Next to the pH the possible correlation of the measured conductivity and yields of AAs were investigated as well. For instance, in Figure 4-19 the yield of AAs combined with the measured conductivity in the feed were analysed. Indeed, considering the results, previous assumptions that higher values of conductivity were caused by higher amounts of inorganic ions within the feed and would therefore lead to predictions of lower yields per batch run, were not fulfilled. No correlations between these parameters were observed. Probably, too many charged components were in the feeds with interacting ions (Atkins 2001) and therefore no direct correlations to concentrations of solved ions were possible at all.

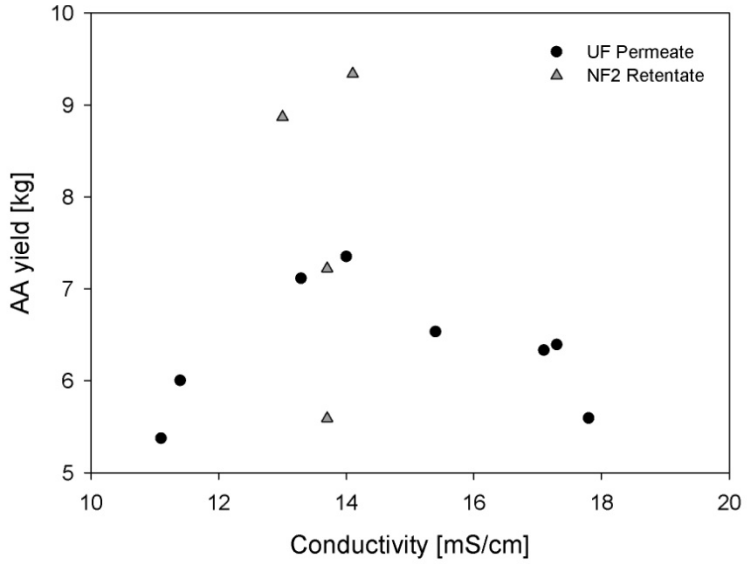


Figure 4-19: Analysis of AA yields per batch run and the measured conductivity in the feed (experiments on Resin II using UF Permeate or NF2 Retentate)

Moreover, a ratio between the measured conductivity and the concentration of AAs in the feed was calculated and compared with the yield of AAs obtained in the respective experiments. It was assumed that the height of the measured conductivity mainly depended on the amount of solved inorganic cations in the feed. Therefore it was thought that calculating this ratio between the measured conductivity and AA concentration would give hints to possible yields of AAs. However, investigation of this data, shown in Figure 4-20, indicated only slight tendencies that feeds with a higher ratio led to higher output. That means that higher outputs were observed in the case of higher AA concentrations combined with a lower conductivity. Nevertheless, based on this data no exact predictions on the yields would be feasible.

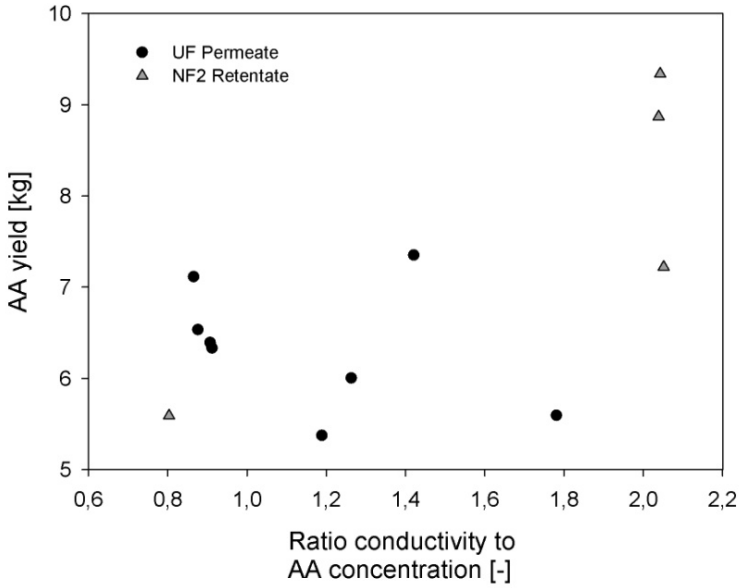


Figure 4-20: AA yields per batch run compared to the calculated ratio between the measured conductivity and the analysed AA concentration in the feed (experiments on Resin II using UF Permeate or NF2 Retentate)

More correlations were observed, in turn, by plotting the yields of AAs per batch together with the calculated ratios of concentrations of AAs and inorganic cations, which is shown in Figure

4-21. Indeed, the measuring of inorganic cations was done externally and fewer analyses were available. Therefore, the data shown in Figure 4-21originated from experiments using either Resin I or Resin II. Nevertheless, it was added to this work as it once more demonstrates the findings already discussed in chapter 4.2.2.1.

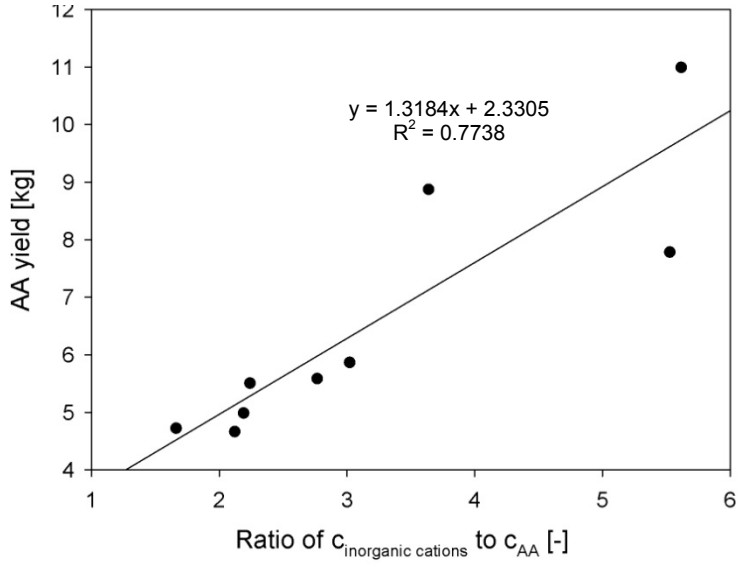


Figure 4-21: AA yields per batch run compared to the calculated ratio between the analysed concentration of inorganic cations and the AA concentration in the feed

4.2.2.4 Non-AA components

Due to the complex feed composition, the behaviour of “non-AA”-components at the IE process was observed in detail as well. Average results of both IE resins regarding the distribution and output of LA, acetic acid, sugars and some inorganic ions during the loading step are shown in Table 4-13. The calculations were done using Formula 3-6.

Table 4-13: Behaviour and distribution of “non-AA”-components during the loading step (IE effluent referring to IE feed)

Component	**Transport rate to IE effluent [%]**	
	Resin I	**Resin II**
LA	> 90	> 90
Acetic acid	> 90	> 90
Glucose	> 90	> 90
Sum Fructose&Mannose	> 90	> 90
Na^+	< 1	< 1
K^+	< 1	< 1
Ca^{2+}	< 1	< 1
Mg^{2+}	< 1	< 1
Cl^-	> 90	> 90

Organic acids and sugars are either neutral or partly negatively charged depending on their degree of dissociation. For instance, about 50 % LA (pK_a=3.86) are found deprotonated in the feed solution (pH=3.9). Therefore, these components and inorganic anions did not interact with the negatively charged functionalised groups on the resin’s surface or were repelled by them. Hence, most of these components were found, as expected, in the IE effluent. Marginal parts of LA, acetic acid, sugars and inorganic anions remained in the column’s void volume. Therefore, a sufficient rinsing with deionised water after the loading

step was necessary to sufficiently remove these components from the void volume and to avoid subsequent contamination of the AA products. Comparing the results of Resin I and Resin II, over 90 % of the organic acids, sugars and inorganic cations (e. g. chloride) were found in the IE effluent and separated from the AA products satisfactorily in both cases. As already mentioned almost all inorganic cations, such as Na^+, K^+, Mg^{2+} and Ca^{2+}, interacted strongly with the sulphonic acid groups on the resin's surface and were mainly found on column 1 and 2. Only marginal amounts of these species are found in the IE effluent.

On average, the IE effluent consisted of LA (15-30 g/l), acetic acid (2-10 g/l), sugars (2-20 g/l) and inorganic anions (e. g. 1-2 g/l Cl^-, 2-3 g/l PO_4^{3-}). The total amount of these components depended on their concentrations within the IE feed and on the volume of feed used for the loading process, which depended on the concentration of inorganic cations within the feed as described in chapter 4.2.1.1. The pH of the IE effluent was quite low (1.8 to 2), as during the loading process H^+-ions were displaced by AAs and inorganic cations from the resins. Within the basic process, the IE effluent was discarded and used energetically in a biogas plant. Within the innovative process, in turn, the IE process was brought forward in the process chain. Therefore, the IE effluent was further processed to gain the second valuable compound lactic acid.

4.2.3 Separation behaviour

Next to the yield per batch run, the separation behaviour between AAs and inorganic cations and between the AAs themselves into product fractions on the IE device was investigated in detail. In particular, the separation performances of both resins were compared.

4.2.3.1 Separation between inorganic cations and AAs

As already discussed in chapter 4.2.1.1, the breakthrough of inorganic cations, which is combined with an increase in conductivity, displayed the point for stopping the loading. However, previous assumptions that a sharp borderline between inorganic cations and AAs would exist, were not fulfilled. On the contrary, it was found that a mixing zone was prevalent. For instance, Figure 4-22 shows details of the development of the concentrations of AAs and inorganic cations along with the measured conductivity in an experiment using Resin I during the loading step. As soon as the conductivity declined, the concentration of AAs increased. As expected, the lowest values of conductivity occurred simultaneously to the highest AA concentrations. The breakthrough of inorganic cations, in turn, caused an increase in conductivity. Indeed, as shown in Figure 4-22, the detected concentrations of inorganic cations only rose slowly and no sudden increase was observed. The concentrations of AAs, in turn, did not decrease very fast. Therefore, the loading process was not as easy to handle as assumed previously and a sharp separation of AAs and inorganic cations was not feasible.

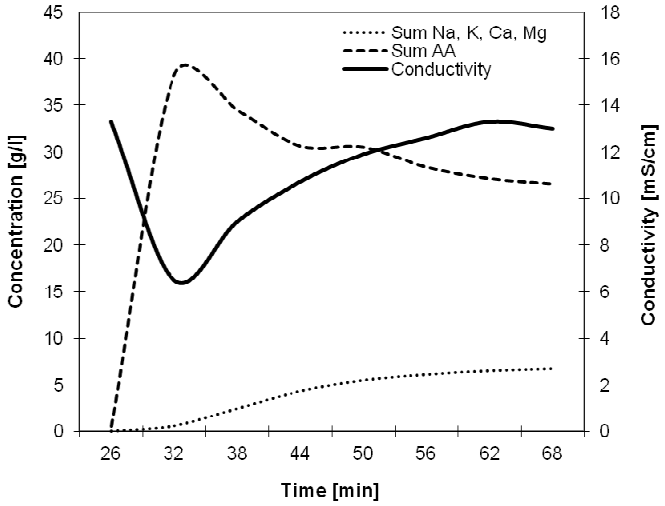


Figure 4-22: Development of concentrations (AAs and inorganic cations) and conductivity during a loading step (part), samples taken after CP 2; flow 13.0 m/h, experiment done with Resin I

Among other measures Resin II with a smaller mean particle size was applied in order to improve the separation between inorganic cations and AAs. Indeed, results of experiments with this resin did not show improvements. For instance, a slow increase of the conductivity after the breakthrough of inorganic cations was still observed. Moreover, an immediate stop after the breakthrough of inorganic cations led to low AA yields, as the loading was stopped too early. Furthermore, the low amounts of AAs, which were yielded from column 3, showed that AAs and inorganic cations were found there. More details on the distribution of the AAs are discussed in the following chapter.

4.2.3.2 Overall distribution of AAs on the IE device

The overall distribution of the AAs within the IE device was investigated. On the one hand, the output per column was observed to guarantee an efficient displacing. That means that only columns with high yields on AAs should be treated with ammonia due to economic considerations. On the other hand, the distribution of acidic, neutral and alkaline AAs was studied. Average results of experiments using Resin I or Resin II considering the distribution of AAs are summarised in Figure 4-23.

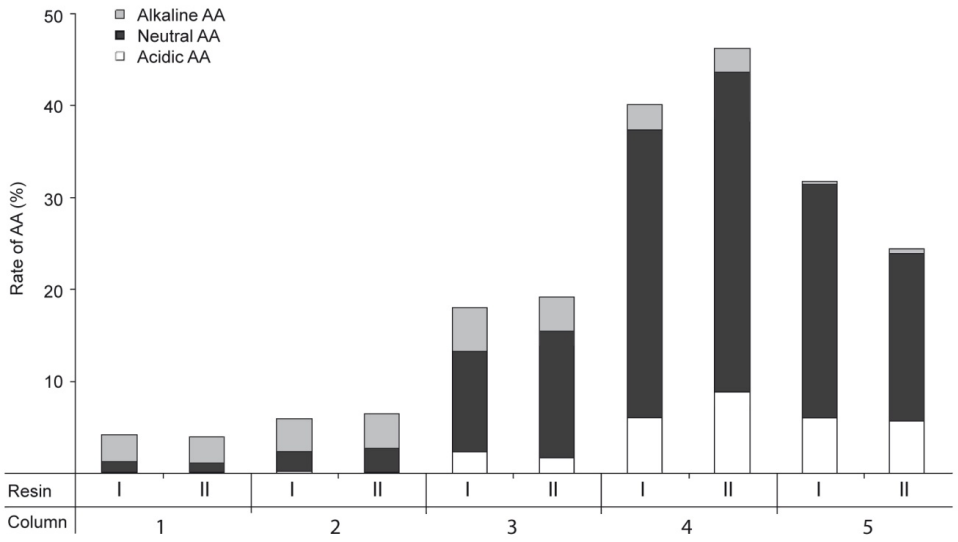


Figure 4-23: Distributions of AAs per column comparing Resin I and Resin II (data based on experiments with NF2 retentate as starting material)

The average results in Figure 4-23 show that very few AAs were eluted from column 1 and column 2. About 20 % of the total yield of AAs was found on column 3, but most AAs were yielded from column 5 and in particular from column 4. The distribution behaviour of AAs in the present IE process was strongly influenced by the inorganic cations, as the inorganic species showed a stronger affinity to the sulphonic acid groups on the resin's surface than the AAs. As expected, most AAs were displaced by the inorganic cations and were mainly shifted from column 1 and 2 to column 3, 4 and 5. Nevertheless, some AAs, mostly alkaline ones, showed a strong affinity to the resin's functional groups as well and remained on column 1 and 2. Therefore, no sharp separation between AAs and inorganic cations was feasible and mixing zones were prevalent, as already discussed before. In the case of economic considerations column 1 and 2 would not be eluted with ammonia during routine work, due to too low yields. In routine AA production processes they would be lost. Higher amounts of AAs, in turn, were yielded from column 3, although these yields were still lower than the yields of column 4 and 5. Furthermore, an overload of column 5, which could lead to a loss of AAs during the loading process, was carefully avoided. For that reason, the yield from column 5 was lower than the amount of AAs eluted from column 4. Moreover, the lower amount of resin in column 5 (glass column) is responsible for the reduced yield from this column as well.

Comparing the results of Resin I and Resin II, it was observed that about 10 % of the total AA amount remained in column 1 or column 2 using either Resin I (9.9 %) or Resin II (10.4 %). In other words, by using these resins 0.3 kg AAs were yielded from column 1 and 0.5 kg AAs were yielded from column 2 on average. Moreover, quite similar results were

observed comparing the relative yields of AAs gained from column 3. By using Resin I 18 % or 1.4 kg AAs of the average, total AA yield (7.9 kg) were eluted from this column and 19 % (1.6 kg) of the AAs were eluted from column 3 using Resin II. Most AAs were yielded from column 4 in both cases, indeed the output of Resin II was slightly higher (Resin I: 40 % 3.2 kg; Resin II: 46 %, 3.8 kg). Whereas considering the AA yield in column 5, more AAs were produced from this column in experiments with Resin I (32 % or 2.5 kg). In experiments with Resin II 24 % or 2.0 kg AAs were yielded from column 5 on average. The reduced yields of column 5 in experiments with Resin II probably resulted from the higher capacity of Resin II in almost unchanged process conditions (e. g. the loading process stopped at the same point). That means more AAs were fixed in column 4 and less were transported to column 5. Overall, 67 % of the eluted AAs were found in column 4 and 5 when using Resin I and 70 % were found in these columns filled with Resin II.

Furthermore, the distribution of the AA groups (acidic, neutral and alkaline) is also shown in Figure 4-23. The AAs were distributed between the columns differently, based on their charged or uncharged side chains. Generally, at the present pH (3.86) alkaline AAs (e.g. Lys) did not cover long distances, due to stronger interactions with the resin's functionalised, negatively charged sulphonic acid groups. Acidic AAs (e.g. Asp) were transported through the resin, due to their negatively charged side chains and therefore mostly found in column 4 and 5. Neutral AAs (e.g. alanine, leucine) remained in between. Comparing the distribution behaviour of AA groups in Resin I and Resin II, similar results were observed for both resins. Most alkaline AAs were eluted from column 1, 2 and 3. As expected, nearly no alkaline AAs were found in column 5. Whereas the rate of alkaline AAs was lower in this column when using Resin II. Neutral AAs were found in every column. In particular, high amounts of neutral AAs were yielded from column 4. Acidic AAs were found mainly in column 4 and 5. Applying Resin I, the rate of AAs in column 4 and 5 were nearly identical. Using Resin II, more acidic species were found in column 4 than on column 5. Nevertheless, the AA groups showed some differences in their distribution behaviour, it was not possible to separate them clearly by applying the presented IE process. For instance acidic AAs were not only found on column 5. On the contrary, mixing zones of the different AA groups were prevalent on every column and no improvements were observed by using Resin II with smaller particles.

In order to understand the process more explicitly, it was investigated whether there is a correlation between the degree of dissociation (α) of the alkaline AAs and their amount in column 1 and column 2. Therefore, the extent of each species per AA, which are charged differently, at the present pH (3.86) was calculated and compared with results from real experiments. The theoretical values and the average results for alkaline AAs in column 1 and column 2 are summarised in Table 4-14.

Table 4-14: Degree of dissociation at pH=3.86 of lysine, histidine, arginine and GABA; average percentage of these AAs on column 1 and column 2 (feed: NF2 retentate)

	Degree of dissociation (α) [%]			
Species/Charge	**Lysine**	**Histidine**	**Arginine**	**GABA**
2 +	2.00	0.80	1.39	-
1 +	98.0	98.6	98.6	70.1
0	0.00	0.57	0.00	29.9
1 -	0.00	0.00	0.00	0.00
2 -	0.00	0.00	0.00	-
Amount on column 1 and column 2 [%]	68	82	95	37

As shown in Table 4-14, at the present pH these AAs mainly existed as single charged cations and only low amounts of double charged cations occurred. GABA, which has no

charged side chain, was an exception as nearly 30 % of this AA was available in the neutral form. Differences were observed when comparing the calculated data with the amount of AAs eluted from column 1 and 2 in the real experiments. The figures show, that there was no direct connection between the theoretical amount of charged, alkaline AAs and the effective eluted amount of alkaline AAs from column 1 and 2. Although the AAs were positively charged, some of them were displaced from column 1 and 2 by inorganic cations. Only arginine was mainly found in column 1 and 2. Indeed, considering the data of GABA it can be shown that a reduced amount of the charged species also leads to a reduced amount of this AA eluted from column 1 and 2. In other words, less GABA is eluted from column 1, as less GABA is charged at this pH. Regarding this data reducing the pH and therefore increasing the percentage of the double charged cations could improve the separation of alkaline AAs within this process. Indeed, high amount of chemicals would be necessary for lowering the pH, as the different components in the starting material work as buffer system.

4.2.3.3 Separation of amino acids during the elution

Furthermore, the time-dependent separation behaviour between the AA groups during the elution was observed in detail for both resins, as more distinct separations of the AA fractions would lead to more specific and defined products. For example, in Figure 4-24 and Figure 4-25 the course of concentrations of selected AAs, which represent the acidic (glutamic acid and aspartic acid), neutral (alanine and leucine) and alkaline fraction (lysine), on three columns (column 2, 3 and 4) for Resin I and Resin II are shown.

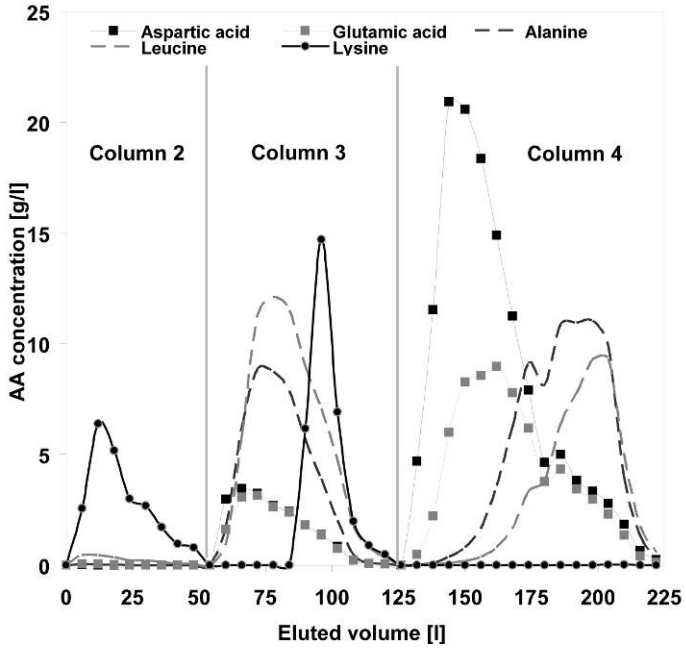


Figure 4-24: Resin I - separation performance of selected AAs on column 2 to 4 (flow rate 8.1 m/h)

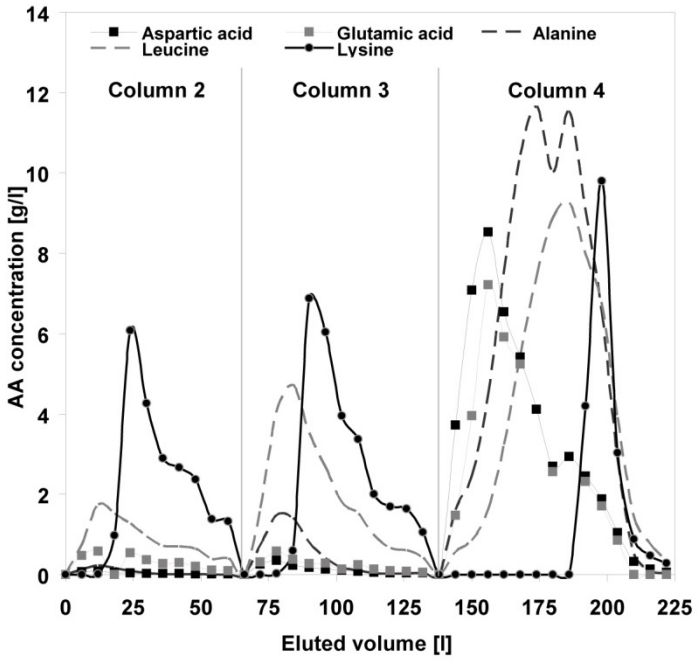


Figure 4-25: Resin II – separation performance of selected AAs on column 2 to 4 (flow rate 8.1 m/h)

Considering the separation performance of Resin I (Figure 4-24), alkaline AAs were the main fraction in column 2 with maximum concentrations of 6 g/l (lysine). Only very low amounts of neutral AAs and nearly no acidic AAs were found on this column. In the eluate of column 3, AAs of the acidic, neutral and alkaline species occurred. First, acidic and neutral AAs were eluted at the same time, whereas the maxima of the species eluted slightly differently. First, acidic AAs eluted with maximum concentrations of 3.5 g/l for aspartic acid and 3.0 g/l for glutamic acid respectively followed by leucine (12 g/l) and alanine (8 g/l). The highest concentrations of alkaline AAs (lysine: 14.7 g/l) were detected later. In column 4, mainly acidic AAs, which were eluted first in high concentrations (aspartic acid: 21 g/l, glutamic acid: 9 g/l), and later neutral AAs (alanine: 11 g/l, leucine: 9.3 g/l) were found.

Secondly, the separation performance of Resin II is shown in Figure 4-25. The following AA distributions were observed: On column 2, mainly alkaline AAs (maximum concentration of lysine 6.1 g/l) and only a small amount of neutral AAs (leucine 1.8 g/l) emerged. Nearly the same constellation occurred on column 3, whereas a higher amount of neutral AAs (leucine 4.7 g/l) was noticed. In the eluate from column 4 all groups of AAs were detected. First high concentrations of acidic AAs (aspartic acid 8.5 g/l, glutamic acid 7.2 g/l) were eluted, followed by the neutral components (leucine 9.3 g/l, alanine 11.7 g/l). Finally, alkaline AAs were eluted (lysine 9.8 g/l) as well.

In order to compare the results of Resin I and Resin II regarding the AA separation performance applying the same flow rates, only small differences occurred. The experiments showed, that an exact separation of the AAs into fractions is not feasible by using either Resin I or Resin II. By monitoring the refractive index, pH and conductivity, as described in chapter 4.2, product fractions, which contained high concentrations of a certain AA group (acidic, neutral or alkaline), can be generated with both resins. The prior expected improvements in the separation performance by applying Resin II, due to its smaller mean particle size, were not observed (Hamilton 1958). On the contrary, by using Resin II in some areas more overlays of the AA groups were experienced than by using Resin I.

Furthermore, it was investigated whether an extension of the resin volume available for the separation of AAs would positively support the separation of AAs. Therefore, in a first step

the IE feed (NF2 Retentate) was desalinated using all columns. Secondly, all IE columns were loaded with the desalinated medium, which mainly contained AAs. Finally, the columns were eluted and the results per column are presented combined in Figure 4-26.

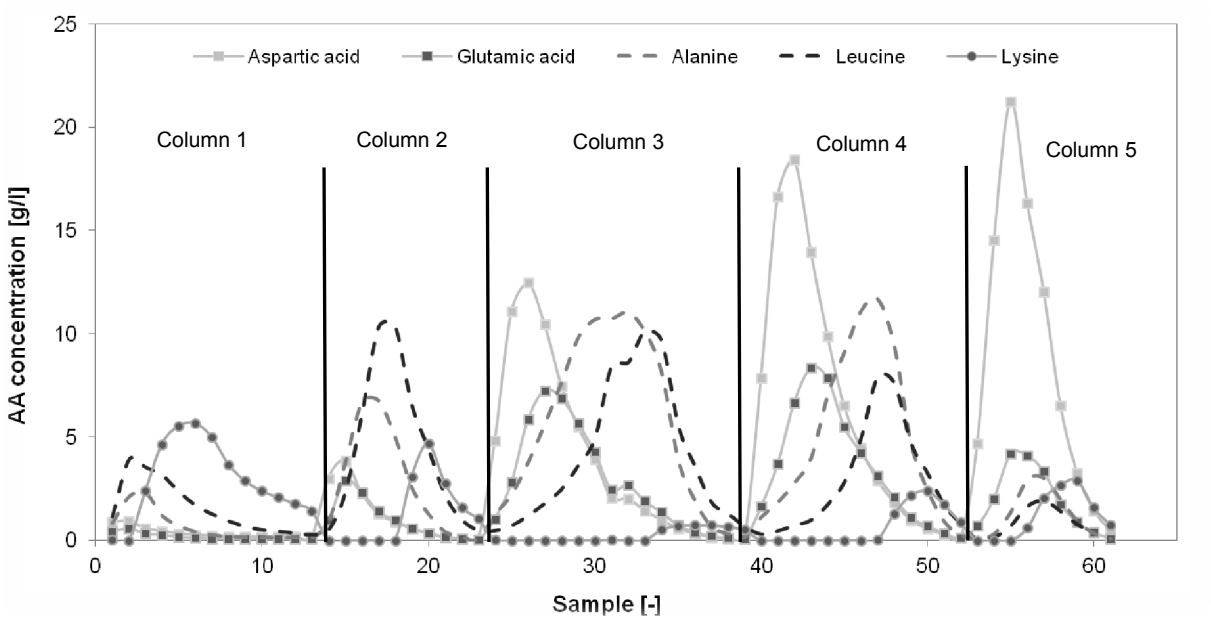


Figure 4-26: Time-dependent elution of AAs per column (overall view): Resin I with desalinated IE feed; separation on 5 columns (flow rate: 8.1 m/h)

With the help of some selected AAs, which represent the AA groups (acidic, neutral and alkaline), the separation performance for AAs using an increased resin bed volume is shown in Figure 4-26. Using lysine as an example, it was observed that alkaline AAs were mainly eluted from column 1 and 2. In particular, it was possible to gain an amount of nearly pure alkaline product from column 1. Considering column 2, in turn, first mainly neutral AAs were eluted followed by alkaline ones. Indeed, areas where the groups of AAs overlap were prevalent in this column. Starting with column 3, a greater amount of acidic AAs was eluted. The concentrations of acidic AAs, in particular of aspartic acid, increased from column 3 to column 5 and the main AA eluted from column 5 was aspartic acid. However, from column 3 and column 4 acidic and neutral AAs were eluted. The AA groups also overlapped on these columns, but a rough separation would be feasible. In general, comparing these results with the separation performance of AAs in experiments using the bed volume of three columns an improvement was observed. Nevertheless, overlaps of AA groups were still investigated and a sharp separation between these groups was not feasible.

4.2.4 Amino acid products

4.2.4.1 Liquid amino acid products

As already mentioned, it was feasible to gain three different, purified AA product fractions from grass silage juice using the presented DC process. Based on the main ingredients the products were named acidic (Product 1A), neutral (Product 1B) or alkaline (Product 2) product, whereas the latter contained ammonia. Example pictures of the liquid products are shown in Figure 4-27 and average compositions gained during an experiment from column 4 using UF Permeate as feed are summarised in Table 4-15.

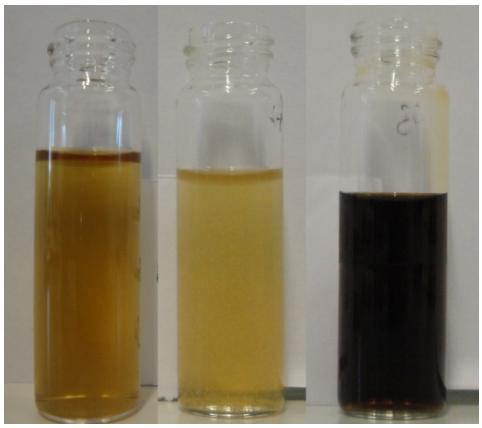


Figure 4-27: Products gained from column 4 using UF Permeate and Resin II: Product 1A (left), Product 1B (centre) and Product 2 (right)

Table 4-15: Average percentage of main ingredients of the AA product fractions obtained from column 4 (experiments using UF Permeate, Resin II)

Product 1A (acidic)		**Product 1B (neutral)**		**Product 2 (alkaline)**	
AA	[%]	AA	[%]	AA	[%]
Aspartic acid	16±6	Alanine	15±3	γ-Aminobutyric acid	21±9
Glutamic acid	14±7	Leucine	15±4	Leucine	15±3
Alanine	11±3	Valine	10±1	Lysine	13±10
Threonine	10±2	Glycine	8±3	Alanine	9±3
Asparagine	10±6	Glutamic acid	7±3	Valine	7±1

Product 1A, which was eluted from column 4 first, consisted mainly of aspartic acid, glutamic acid and alanine. The neutral Product 1B, in turn, was enriched with alanine, leucine and valine and Product 2 with γ-aminobutyric acid, leucine and the essential AA lysine. In general, a broad distribution considering the AA rates within the products was observed, but clear tendencies on the main ingredients, which are also reflected by the data in Table 4-15, were found. It is assumed that the broad distribution of the percentages of the AAs was caused by some differences in the raw material, on the one hand, but also due to the applied monitoring process for the loading and the separation of the product fractions during the displacement.

In order to recognise differences in the product fractions that were caused by using different raw materials or resins, the composition of the product fractions per column was investigated in detail and compared. The results showed that independent from the raw material and the resin used similar trends in the product composition were observed. Therefore, the compositions per column were not shown separately, but combined in Figure 4-28, which gives an overview on the overall AA compositions (mean values from all investigated experiments) of the gained product fractions per column.

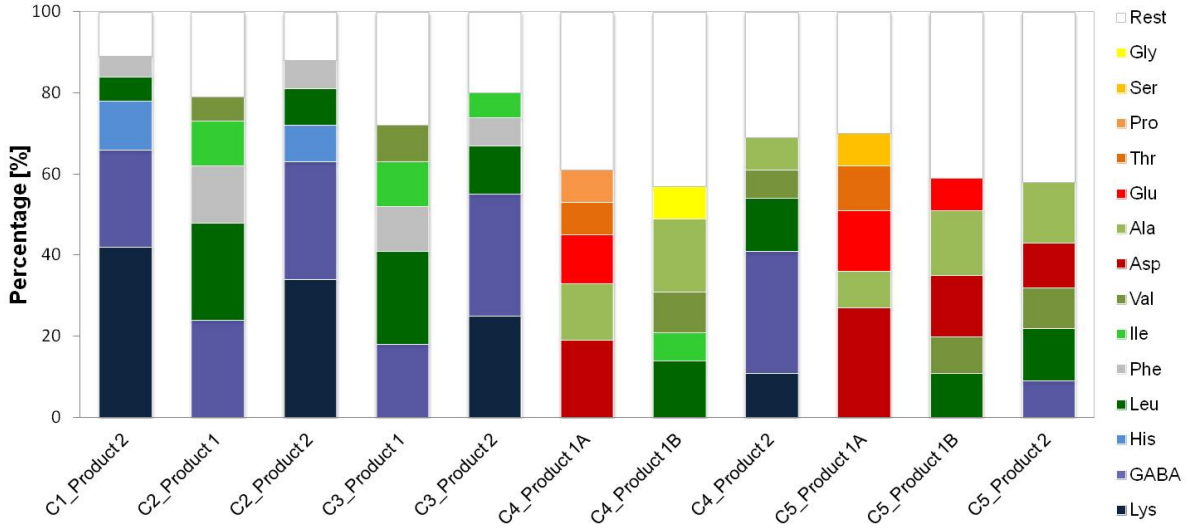


Figure 4-28: Percentage on AAs per column and possible products per column

Column 1

As only a small amount of AAs were found in column 1, it was only feasible to produce one product fraction from this column. The product was eluted at the same time as ammonia and therefore called Product 2. Independent from the starting material and the resin used similar AA patterns were observed in this product. It mostly contained alkaline AAs such as lysine (42 %) and histidine (12 %) as well as γ-aminobutyric acid (24 %). Furthermore, on average 6 % leucine and 5 % phenylalanine were detected. Only 11 % were distributed among other proteinogenic AAs in this product, whereas it was seen that in experiments using Resin II a broader range of proteinogenic AAs was detected in this product. In experiments using Resin I, in turn, no aspartic acid, glutamic acid, threonine, serine, proline and glycine were detected.

Column 2

From column 2, on the other hand, it was feasible to separate the AA solution into two different fractions. The average AA pattern of Product 1, which was eluted first, consisted of leucine (24 %), γ-aminobutyric acid (24 %), phenylalanine (14 %), isoleucine (11 %), valine (6 %) and other proteinogenic AAs (21 %). The composition of Product 2 eluted from this column is comparable to the second product from column 1. Comparing the composition of this product per resin, a smaller range of other AAs was again observed in experiments using Resin I. Furthermore, in experiments conducted on Resin I and using UF Permeate higher amounts of γ-aminobutyric acid (up to 40 %) and lower amounts of lysine (about 20 %) were detected than in other experiments. These high percentages of γ-aminobutyric acid could be explained by the fact that these experiments were done with silage from the third harvest, which was of lower quality on average and obviously led to an increased amount of γ-aminobutyric acid in this product.

Column 3

Two product fractions were gained from column 3 as well, whereas Product 1 consisted of leucine (23 %), γ-aminobutyric acid (18 %), phenylalanine (11 %), isoleucine (11 %), valine (9 %) and other proteinogenic AAs (28 %). Despite the high amount of other proteinogenic AAs detected in this fraction no alkaline AAs were found. Generally, the composition is comparable to the AA pattern of Product 1 from column 2. The composition of Product 2, in turn, was quite similar to the AA pattern of Product 2 from column 1 and column 2. Product 2 from column 3 was also a fraction rich in γ-aminobutyric acid (30 %), lysine (25 %), leucine (12 %), phenylalanine (7 %) and isoleucine (6 %). In comparison with column 1 and 2 the amount of alkaline AAs (lysine and histidine) decreased and more neutral AAs were

detected. Even small amounts of acidic amino acids, independent from the used resin, were found within this Product 2.

Column 4
The AA pattern of Product 1A eluted from column 4 was considerably different to the fractions discussed so far. Independent from the starting materials and the resin used it consisted mainly of aspartic acid (19 %), alanine (14 %), glutamic acid (12 %), threonine (8 %) and proline (8 %). The remaining part contained all other neutral proteinogenic AAs, but no alkaline species. The second product from this column, Product 1B consisted of alanine (18 %), leucine (14 %), valine (10 %), glycine (8 %) and isoleucine (7 %). The remaining 40 % comprised of other neutral AAs, acidic AAs, about 5 % γ-aminobutyric acid and a very small percentage of alkaline AAs. All in all, considering the composition of this product that was gained in different experiments, some slight fluctuations were observed, whereas differences in the loading and elution process were seen as reasons for that. The third product from column 4, Product 2, showed a similar AA pattern to the second products from column 1 to 3, whereas the amount of alkaline AAs and γ-aminobutyric acid was further decreased and the amount of neutral AAs was increased.

Column 5
Considering the AA composition of Product 1A from this column a higher amount of acidic AAs was observed in this fraction than in Product 1A from column 4. That means that more acidic AAs (27 % aspartic acid, 15 % glutamic acid) were accumulated in column 5 due to their distribution behaviour on the IE based on their negatively charged side chains. Furthermore, some trends were observed in that Product 1A from column 5, which was prepared with Resin I, showed an increased amount of aspartic acid. Next to the acidic AAs different neutral AAs, but no alkaline AAs were found in this fraction. The composition of Product 1B from column 5 differed from the Product 1B gained at column 4, as the amount of acidic AAs was higher. On average, the following AAs were found mostly in this product: alanine (16 %), aspartic acid (15 %), leucine (11 %), valine (9 %) and glutamic acid (8 %). Furthermore, the AA composition of Product 2 from column 5 showed considerable differences when compared to the other products containing ammonia, as it only contained a small amount of alkaline AAs and γ-aminobutyric acid (9 %). The majority in this product consisted of alanine (15 %), leucine (13 %), aspartic acid (11 %), valine (10 %) and other neutral AAs.

Nevertheless, the product fractions per column showed differences in their compositions for further processing (e.g. for producing AA powders) the same products from different columns were combined. For instance, based on economical and practical considerations (e.g. low output per column) Product 1A from column 4 was combined with Product 1A from column 5. A separate treatment of the fractions per column would be inefficient. Based on the same economic reasons and despite their quite homogenous AA products, column 1 and 2 would never be eluted as the yields of AAs were too low compared to very high amounts of ammonia needed. In order to show this fact in detail, which was already discussed in chapter 4.2.3.2 as well, further impressions on the average AA concentrations per liquid product fraction from all columns, are summarised in the following figure (Figure 4-29). Depending on the yield per column different AA concentrations were observed. In products from column 1 to 3 AA concentrations around 6 to 11 g/l were observed. Highest yields, in turn, and therefore higher AA concentrations were analysed in product fractions from column 4 and 5. For instance, in Product 1A mean AA concentrations of 29 g/l (column 4) or 37 g/l (column 5) were observed. Nearly the same AA concentrations for Product 1B (approx. 44 g/l) and Product 2 (approx. 22 g/l) were measured on average on both columns.

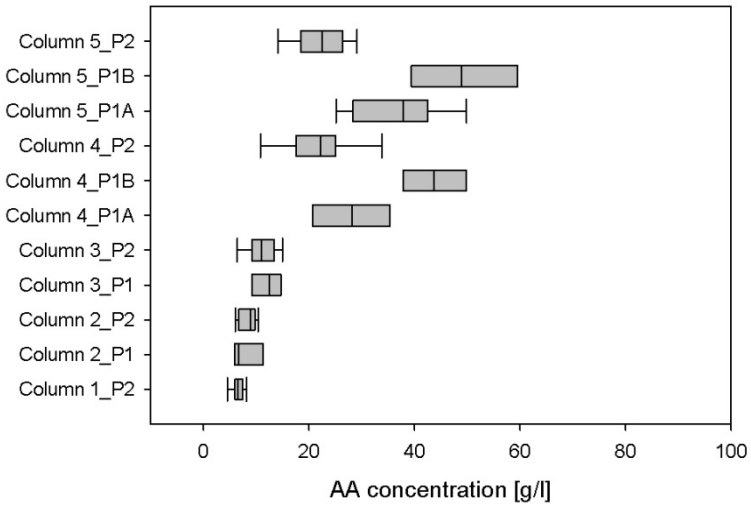


Figure 4-29: AA concentrations in different product fractions gained from column 1 to 5 (UF Permeate, Resin II); P=Product

4.2.4.2 Amino acid powders

In order to study the production of AA powders, which might be easier to pack and to transport, drying the AA product solutions was tested by applying spray-drying (spray-drying device of the company Prolactal, Hartberg, Austria). For these post-treatments the respective AA product solutions from the DC process were combined, concentrated by applying a vacuum evaporator and finally dried in a spraying tower at pilot scale. It was feasible to successfully produce AA powder from Product 1A and 1B and a product based on a mixture of these fractions. Due to the increased solubility of the AA product solutions at higher temperatures (up to 60 °C) it was possible to use AA solutions with a dry matter of up to 45 % for the spray-drying process. For instance, in Figure 4-30 an AA powder gained through spray drying is shown. All powders had a strong smell and were yellow, whereas the powder made of Product 1A was slightly darker. Due to its contamination with ammonia the spray-drying of Product 2, in turn, was not tested. However, in the following figure (Figure 4-31) a comparison of the compositions of the powders gained will be shown.

Figure 4-30: AA powder produced by spray-drying

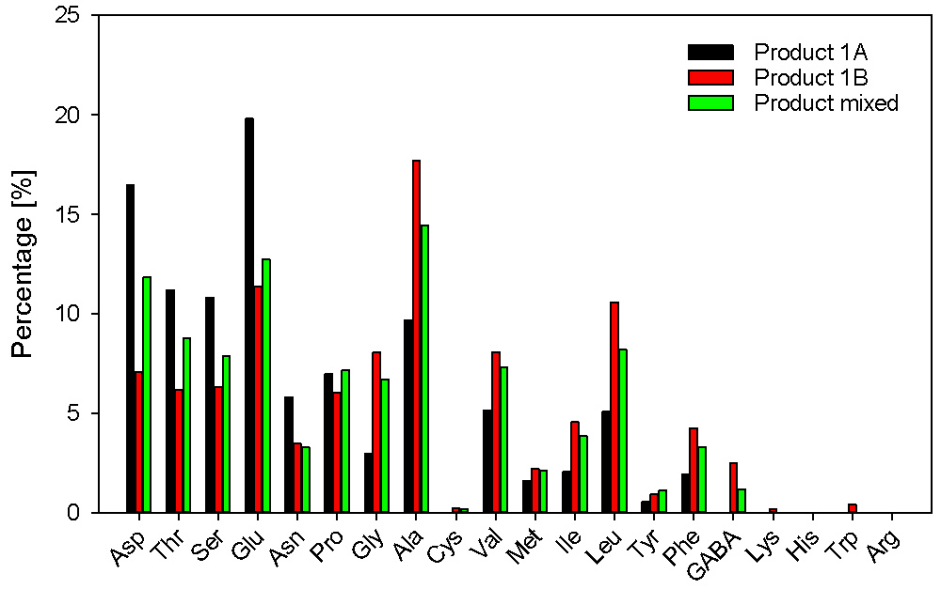


Figure 4-31: Comparison of the AA compositions of the powder made from Product 1A, Product 1B and a mixed fraction (all made from UF Permeate)

Figure 4-31 shows differences regarding the AA compositions of the product powders. For instance, the powder made from Product 1A has high percentages of aspartic acid, glutamic acid, threonine, serine and alanine. Percentages of these AAs (except alanine) were considerably lower in Product 1B. Higher percentages, in turn, of valine, leucine and isoleucine were observed. Percentages of the mixed fraction lay in between.

Through analysing the AAs it was possible to identify 75 to 80 % of the powders' ingredients. For example, it was assumed that through an incomplete degradation of the proteins in silage, peptides could occur in the product. For further characterisations different analyses were used. Based on the results it was finally possible to summarise mass balances for the powders. By taking into consideration measurement uncertainties, which mainly resulted from the inhomogeneity of the powders and the low amounts, which were used for the respective analyses, it was possible to identify up to 95 % of the powders' ingredients (Table 4-16).

Table 4-16: Mass balance of AA powders from the *GBR Upper Austria*

Compound	**Product 1A [%]**	**Product 1B [%]**	**Product mixed [%]**
Amino acids	74.6	74.4	81.3
Amino acids as peptides	8.5	11.8	4.4
Organic acids	1.6	0.5	0.4
Sugars	-	-	-
Inorganic compounds	<1	<1	<1
Water content	7.1	7.2	1.3
Summe	**92.8**	**94.9**	**88.4**

The amount of peptides added in Table 4-16 was analysed by an external laboratory by gas chromatography before and after a hydrolysing step. These results globally confirmed findings of intern analyses, where a peptide rate of about 10 % was found out. Furthermore, on average a 15 % rate of proteins was analysed by using the protein test according to Biurett. Due to the cut off of 1 kD at the UF step the content of proteins should be equated

with the content of peptides. Due to strong matrix effects a specific determination of inorganic compounds was impossible. Therefore, the whole amount of the inorganic components was analysed by combusting the dried AA powder. The water content was determined by using the Karl-Fischer-Titration.

4.2.4.3 Summary amino acid products

Summing up the experiments it was possible to produce different AA fractions from pre-treated grass silage juice of the *GBR Upper Austria*. At this point it was necessary to determine possible markets for the products. In order to obtain a rough estimation regarding this question the AA patterns of the generated products (only the AA powders) were compared with AA patterns of common protein sources such as soy flour, wheat, dried egg and whey powder. Results of these comparisons are shown in Figure 4-32. Incidentally, the respective AA patterns of the common protein sources were calculated by using the data given in Table 1-9. The AA composition of the GBR products was based on analysed powders.

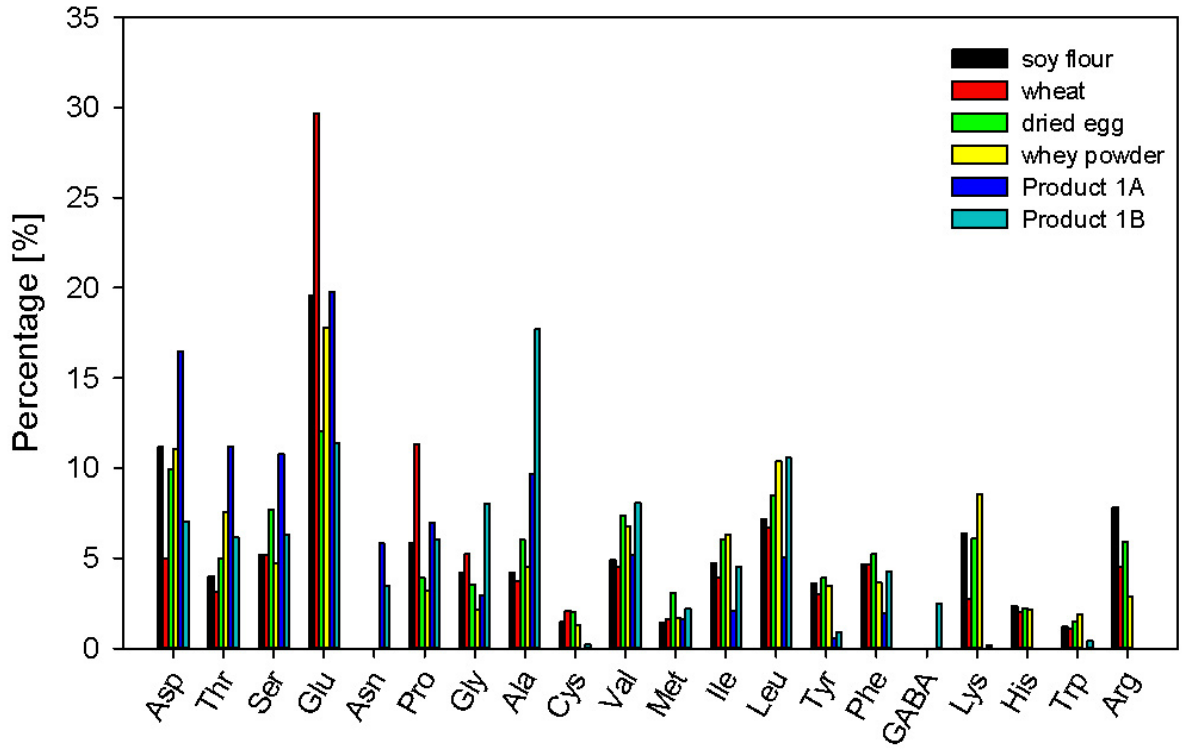


Figure 4-32: Comparison of different AA patterns of common protein sources and AA powders from the *GBR Upper Austria*

Based on the presented data the AA products can only be evaluated roughly and exact assessments can only be done by experts in nutrition or animal breeding. Nevertheless, on the first view it is noted that unlike to the common protein sources some important AAs, such as L-lysine, L-histidine, L-tryptophan and L-arginine, are almost missing in the products from the grass silage juice. In particular, the low percentage of the essential AAs lysine and tryptophan, which play an important role in feeding and breeding animals, lowers the quality of the products and limits their applicability. Additionally, this effect is increased through the low percentages of arginine and histidine, which are essential for some animals as well. Comparing the amount of the remaining AAs with the common protein sources, mostly comparable percentages were reached. In some cases (e.g. alanine or aspartic acid) even the highest percentages were observed in the GBR products. Indeed, in order to compensate

for stronger fluctuations either of Product 1A (e.g. very low percentage of isoleucine and leucine) or of Product 1B compared to the AA patterns of the common protein sources, the production of a mixed product would be advisable.
Based on these AA patterns a direct introduction of the GBR products on the market would be difficult, as no other products with similar characteristics have already been sold. As all GBR products are mixtures of AAs, the huge market for only single AAs can be excluded a priori. Furthermore, it is difficult to use the GBR products for existing applications (e.g. as fertilizer), as products that are already sold always contain the whole spectrum of proteinogenic AAs at a minimum. However, apart from the AA patterns the product powders from GBR show comparable characteristics to the AA powder sold by the Chinese company Wuhan Soleado Technology Co.,Ltd. (see chapter 1.2.7), which is applied as feeding stuff, fertilizer or for the fermentation industry.

The content of peptides, which was discussed in chapter 4.2.4.2, should not decrease the quality of the GBR products, as peptides originating from an incomplete hydrolysis are often found in protein hydolysates. For instance, Chiang et al (Chiang, Shih et al. 1999) indicated that an enzymatic hydrolysis of soy protein followed by an ultrafiltration step resulted in a percentage of 12.1 % free AAs. Furthermore, some of the products shown in chapter 1.2.7 also contain peptides next to free AAs.

Product 2, in turn, which contained a high percentage of the essential AA lysine, was not treated further until now, due to its contamination with ammonia. For instance, the production of AA powders through spray drying was not tested with this fraction yet. However, based on its important ingredients and economical considerations further polishing of this fraction would be desirable.

4.2.5 Use of chemicals and water at the displacement chromatography process

Next to the AA yields the usage of chemicals and water during the DC process was investigated. Subsequently, the amounts of ammonia, sulphuric acid and deionised water needed to perform the IE process are given for each process step. Furthermore, problems and possible reductions of the amount of chemicals are discussed. Finally, optimised amounts of chemicals, which will be used for calculating further scenarios for the IE device are summarised in Table 4-20. The data presented in this chapter was observed in experiments using UF Permeate at Resin II, but based on further process observations overall tendencies were true for both resins and both IE feeds.

4.2.5.1 Loading

During the loading step the respective feed for the process is applied onto the IE resins. After the signal for stopping the loading was reached, the columns were rinsed with 120 l of deionised water to transport remaining organic acids and sugars into the IE effluent.

4.2.5.2 Displacement with ammonia

During different experiments with UF Permeate on Resin II, the following amounts of 1 M ammonia were consumed during the displacement of column 3, 4 and 5 on average (Table 4-17). The added amounts represent the amounts of eluted solution obtained per product during the application of ammonia solution in the respective column. By summing up the amounts per solution, the total amount of ammonia solution needed to displace all AAs from one column was calculated. As already mentioned in routine work column 1 and 2 would not

be eluted, as the yields would be too low. Therefore, the amounts of ammonia needed for these columns were not added.

Table 4-17: Liquid fraction per product and average consumption of 1 M ammonia solution during the displacement (experiments done with UF Permeate on Resin II)

Column	Pre-product [l]	Product 1 [l]	Product 2 [l]	Sum [l]
3	31	8	39	78
4	31	33	29	93
5	33	50	18	101

After starting the displacement step by adding 1 M ammonia solution into the respective column, about 30 l were needed until the first AAs (Product 1) were eluted. Subsequently, the AAs of Product 1 (Product 1A and Product 1B) and Product 2 were eluted, whereas the amount of the ammonia solution needed per product depended on the AA composition per column.

However, considering the real consumption of the ammonia solution, as shown in Table 4-17, the theoretical capacity per column (50 eq) was considerably exceeded on every column. Indeed, during the experiments some reasons for the exceeding have been identified. Firstly, it was observed that the elution of AAs was not suddenly stopped when the ammonia broke through. On contrast, high amounts of AAs eluted at the breakthrough of the ammonia and then simultaneously with the ammonia in Product 2. Therefore, a slight excess of ammonia to elute all AAs would be necessary anyway. Nevertheless, it is assumed that the high amounts of ammonia needed were partly system-based. That means that some of ammonia disappeared from the solution through the applied mixing system and heating, which was also confirmed by analyses of the ammonia solution where mostly concentrations lower than 17 g/l were observed. Therefore, to optimise the elution process and to decrease the amount of ammonia needed some improvements in the chemical mixing system would be necessary. For instance, an on-site system for monitoring the concentration of the ammonia solution would be helpful.

Indeed, considering economic reasons a reduction of the ammonia needed per elution step would be desirable. Therefore, an optimised amount of ammonia, which would be needed for ideal process conditions, was estimated and is summarised in Table 4-18. Firstly, the observed amount of 1 M ammonia solution was corrected by the factor 0.82. It was calculated by comparing the analysed ammonia concentrations (approx. 14 g/l) with the originally applied ammonia concentrations (17 g/l). Secondly, the amount was further reduced by 10 l, as during routine work the rinsing step with deionised water could already be started during the elution of Product 2. Finally, the amount of ammonia was rounded up and some more litres were added for contingency purposes. The optimised ammonia consumption was used for further calculations done in chapter 4.3 as well.

Table 4-18: Estimated consumption of 1 M ammonia solution at optimised process conditions

	Column 3 [l]	Column 4 [l]	Column 5 [l]
Observed consumption of 1M ammonia on average	78	93	101
Reduction by correcting the concentration of ammonia	14	17	19
Reduction by applying ideal conditions	10	10	10
Corrected amount	54	66	72
Estimated amount of ammonia (rounded up + safety factor)	**60**	**80**	**80**

4.2.5.3 Regeneration

Generally, column 1 and 2 were regenerated in a two step process due to the amount of inorganic cations fixed in these columns. In order to avoid precipitates of gypsum a 0.25 M solution of sulphuric was used first, followed by the final regeneration with 1 M sulphuric acid. The other columns, in turn, were regenerated by applying 1 M sulphuric acid directly. The sulphuric acid was diluted with deionised water within a 100 l tank. Unfortunately, no stirrer was installed within the tank to provide a sufficient mixing of water and acid. Therefore, to guarantee an adequate amount of acid for regenerating the resin nearly the whole tank was used and the columns where always regenerated with an excess of sulphuric acid. Nevertheless, through some small adaptations at the chemical mixing device the amount of sulphuric acid needed could be reduced as well. For this reason, optimised amounts of 1M and 0.25 M sulphuric acid are added in Table 4-19, which should be adequate to regenerate the resins. These optimised amounts were determined in experiments using a lower amount of sulphuric acid, on the one hand, but also by studying instructions from literature (Mitsubishi Chemical Company 1997).

Table 4-19: Estimated consumption of 0.25 M and 1 M sulphuric acid for regeneration at optimised

Column	1st regeneration step 0.25M sulphuric acid [l]	2nd regeneration step 1M sulphuric acid [l]
1	50	50
2	50	50
3	-	60
4	-	60
5	-	60

4.2.5.4 Summary of chemicals and water consumption

Finally, an overview of the chemicals and deionised water needed for preparing the dilutions of ammonia and sulphuric acid, on the one hand, but also for the rinsing steps, on the other hand, at optimised process conditions are given in Table 4-20.

Table 4-20: Overview on the consumption of water and chemicals per batch run

Process step		Deionised water	Ammonia (technical grade, 18 %)	Sulphuric acid (technical grade, 96 %)	
				1st Regeneration	2nd Regeneration
		[l]	[l]	[l]	[l]
Loading	Loading with feed	-	-	-	-
	Rinsing	120	-	-	-
Elution	Column 3	54	6	-	-
	Column 4	72	8	-	-
	Column 5	72	8	-	-
	Rinsing each column	150	-	-	-
Regeneration	Column 1	97	-	0.7	2.6
	Column 2	97	-	0.7	2.6
	Column 3	57	-	-	3.1
	Column 4	57	-	-	3.1
	Column 5	57	-	-	3.1
	Rinsing each column	250	-	-	-
Sum		**1083**	**22**	**15.9**	

4.2.6 Summary and discussion

Summing up the findings of the DC process at *GBR Upper Austria* it can be noted that it is feasible by applying the presented process constellation to produce different purified AA products from pre-treated grass silage juice. It was feasible to generate a product enriched with acidic AAs (Product 1A), a product enriched with neutral AAs (Product 1B) and a third product enriched with alkaline AAs and γ-aminobutyric acid (Product 2). Indeed, not all results have met the assumed expectations. As already mentioned an improvement of the AA separation would be desirable, but first and foremost, the AA yields per batch run were lower than theoretically expected.

In particular, considering the overall batch process it was found that the loading process mostly influences the AA yields. As shown in chapter 4.2.1.1, by applying the presented loading process, indications were given for monitoring a process, which is difficult to monitor in principal. Especially, by applying this way of monitoring and stopping the loading process after the breakthrough of inorganic ions it was feasible to compensate fluctuations in concentrations and compositions within the feed material. Indeed, based on this monitoring the ratio of AAs and inorganic cations determined the output, as shown in chapter 4.2.2.3. Furthermore, the broad mixing zone between AAs and inorganic cations also hindered a clear monitoring of the process and led to low yields either in column 5, when the loading was stopped too early, or in column 3. By taking these findings into consideration some strategies for improving the loading process would come into question. Firstly, the reduction of the amount of inorganic cations and therefore a shift of the ratio between them and the AAs would lead to higher yields from the existing device. Indeed, for the removal of inorganic cations an efficient method where a lower amount of chemicals is needed should be applied. Secondly, the existing device could be used more efficiently by installing further CPs. For instance, a CP between column 4 and 5 to observe the breakthrough of the AAs would avoid the elution of this column in case of a too short loading. Furthermore, it would be feasible as well to adapt the loading process slightly and to stop the process so that most inorganic cations remain in column 1 and 2 and most AAs remain in column 3 and 4. Column 5 would serve as a kind of backup system and would be eluted after some batch runs, if an adequate yield would be guaranteed. Therefore, the elution of columns that are not fully occupied could be avoided.

Furthermore, based on the different ratios between AAs and inorganic cations in the starting materials, differences in the average AA yields were observed. Higher average AA yields were gained by applying NF2 Retentate on IE, as the amount of inorganic cations was reduced by the NF step before. Nevertheless, both feeds were adequate for the presented IE process and led to products with the same quality. However, considering the whole process at the *GBR Upper Austria*, on the other hand, the usage of UF Permeate at the IE device led to a more efficient separation of the AAs at an earlier stage within the process chain than the NF step. Furthermore, in the so called innovative process the effluent from the IE device, which contained organic acids and sugars, was further processed and not only used energetically as in the basic process. Therefore, considering the overall context the application of UF Permeate on the IE process was preferred. Detailed results and comparisons of the basic and innovative process combined with investigations of the behaviour of grass silage juice on different membrane systems are discussed by Ecker (Ecker 2012).

In further experiments, the performances of the two strong cation exchanger resins were compared. Again differences concerning the average yield per batch run and per litre resin were noted, whereas slightly higher yields were always observed in experiments with Resin II. Nevertheless, less considerable differences between the resins were observed than expected. The increased theoretical capacity of Resin II did not stimulate the yield very strongly. Therefore, based on the data of the adsorption of inorganic cations it was assumed

that Resin I was more adequate to adsorb them under the given conditions, which leads to a prolonged loading process and to a higher amount of AAs, which were brought on the columns. Next to the yields the separation performances of the resins were compared as well. Again the expected improvements considering the separation between AAs and inorganic cations as well as the separations between the AAs themselves were not fulfilled by applying Resin II. On the contrary, similar separation performances were observed in both cases.

Beside the resins and different starting materials the impact of other parameters on the AA yield per batch run was investigated. Indeed, no correlations were observed between different flow rates, the applied volume starting material, AA concentrations, pH, conductivity of the starting material and the AA yield per batch. Only in the case of comparing the ratio between AAs and inorganic cations with the output per batch run, a trend was observed and previous assumptions were confirmed.

Generally, in the previous chapters different aspects concerning the IE process at the *GBR Upper Austria* used for the generation of purified AA solutions from silage juice have been shown. Based on the different findings at the IE device, but also based on findings concerning the overall process of the *GBR Upper Austria* (further discussed by Ecker (Ecker 2012)) the starting material UF Permeate used on Resin II will be the preferred constellations for further consideration. Therefore, the findings of this process are summed up in Figure 4-33.

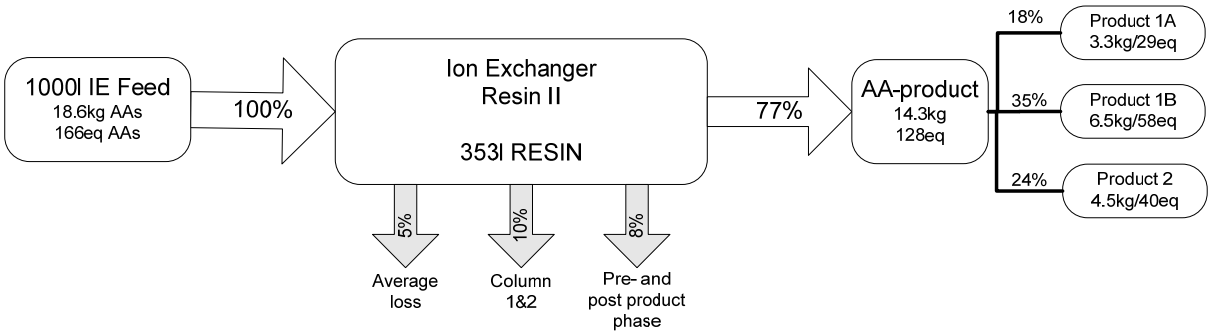


Figure 4-33: Summarised IE process used in the innovative process (UF Permeate, Resin II)

Based on the data obtained by studying the presented IE process 353 l strong cation exchanger resin would be necessary for processing 1 m^3 UF Permeate consisting of 18.6 kg or 166 eq AAs. That means that about three experiments have to be done at the existing IE device at the demonstration plant. On the IE device a recovery rate of 95 % for AAs was observed on average. Therefore, 5 % of the introduced AAs will be lost. Furthermore, in routine work AAs in column 1 and 2 will also count as losses, as these columns would not be eluted due to their low yields. Moreover, some AAs were always found in the pre- or post product phase and therefore lost as well. All in all, it would be possible to transfer 77 % or 14.3 kg of the introduced AAs into the product fractions, whereas 3.3 kg Product 1A, 6.5 kg Product 1B and 4.5 kg Product 2 would be yielded.

4.3 Further process evaluations and scenarios for the ion exchanger process

Based on the results of the IE process, which were presented earlier, further evaluations were done and scenarios for the IE process and the production of AAs have been developed. Although, IE experiments conducted with NF Retentate (basic process) resulted in higher yields, overall results of the *GBR Upper Austria* showed that the "innovative process" was more successful due to higher outputs and lower energy consumption (Ecker 2012). Therefore, the calculations presented are based on results gained in IE experiments conducted with UF Permeate, which was used as the raw material in the so called "innovative process". The installation of a permanent production side for LA and AAs from grass silage could be a possible option as a prospective use of the *GBR Upper Austria*. Due to this fact some scenarios based on the current capacity of the IE upgraded with minimal reconstructions and improvements have been developed. In the following chapter these scenarios will be shown and discussed in detail.

In order to optimise the existing IE facility for routine work some adaptations would be necessary. In the calculations and descriptions subsequently presented these adaptations have already been taken into account. For instance, an enlargement of the chemical tanks and the installation of a stirrer to guarantee the supply of chemicals in a timely manner and at a constant quality for increasing the throughput and minimising dead times would be important. Furthermore, the existing glass column (column 5), which is smaller and more unstable than the columns made of steel, would be replaced by a steel column. Therefore, the amount of useable resin would increase from 120 l to 125 l in total. That means in total 250 eq would be available when using Resin II. As two pumps are installed at the IE facility, which can be operated in parallel, a reduced pumping time per batch run has also been assumed. Therefore, within the results gross pumping times and net pumping times, where operations are considered in parallel, are presented. Moreover, an upgrade in the performance of the existing pumps would be performed in order to reach a flow rate of 21.7 m/h (8 l/min). These minimal improvements and a rational process operation of the IE process have been considered in the following calculations.

Figures of four different scenarios, which are called 4l-A, 4l-B, 8l-A and 8l-B, have been calculated. On the one hand, they differ in the applied speed for loading, elution and regeneration. Firstly, calculations applying flow rates of 4 l/min (10.8 m/h) have been done, as this flow rate represents a common flow rate in experiments already implemented at the GBR *Upper Austria*. Secondly, calculations for flow rates of 8l/min (21.7 m/h) have been done, as these flow rates can be realised by slightly adapting the current equipment. Furthermore, the impact of different scenarios during the elution step was tested. In the scenarios 4l-A and 8l-A columns 3, 4 and 5 were eluted. In the scenarios 4l-B and 8l-B, in turn, only columns 4 and 5, due to their higher yields of AAs, were eluted. As very low amounts of AAs were obtained from columns 1 and 2, these columns were never eluted in the current scenarios.

4.3.1 Starting material and factors used to calculate the scenarios

The calculations of the scenarios were done with the UF Permeate presented in chapter 3.1.1 (Materials and Methods). The most important ingredients and their concentrations in this feed are presented in Table 4-21 once more.

Table 4-21: Composition of the input material for future scenarios at the IE facility

Main ingredients	Concentration [g/l]	Mole [mol/l]	Mole equivalent [eq/l]
Sum AAs	19.01	0.161	0.169
Sum inorganic cations	8.10	0.218	0.281
Ca^{2+}	1.46	0.036	0.072
Mg^{2+}	0.65	0.027	0.053
K^{+}	5.90	0.151	0.151
Na^{+}	0.09	0.004	0.004

	Concentration [g/l]
LA	28.0
Acetic Acid	4.98
Sum sugars (glucose, fructose, mannose)	8.60
Sum inorganic anions (Cl^{-}, PO_4^{3-}, SO_4^{2-}, NO_3^{-})	5.42

Based on this starting material and on average results gained during the IE process, factors for loading, elution and regeneration used in the scenarios were calculated. These factors are summarised in Table 4-22, and Table 4-23. The respective calculations will be discussed subsequently.

Table 4-22: Summarised factors for calculating future scenarios at the existing IE device of the *GBR Upper Austria* - Loading

Total, theoretical capacity of Resin II [eq]		250*
Theoretical capacity available for inorganic cations [eq]		125
Efficiency of adsorption of inorganic cations [eq/l]		0.72
Assumed effective capacity adsorbed with inorganic cations [eq]		**90**
Feed for loading [l]		**320**
with:	Inorganic cations [kg]	2.59
	AAs [kg]	6.08
	LA [kg]	8.96
	Acetic acid [kg]	1.59
	Sugars [kg]	2.75
	Inorganic anions [kg]	1.73

* Sum of columns 1, 2, 3, 4 and 5

As already discussed, it was observed that no sharp separation between inorganic cations and AAs occurs. Therefore, by applying the current method for loading, inorganic cations are found in column 3 as well. Hence, for calculating the scenarios it was assumed that half of the third column was occupied with inorganic cations until the loading was stopped. That means that theoretically 125 eq of the resin could be occupied with inorganic cations. Indeed, further investigations of Resin II showed that on average 0.72 eq of inorganic cations were fixed on the resin's surface per litre. Therefore, an occupation of effectively 72 % or 90 eq for inorganic cations in column 1, 2 and 3 was assumed. Based on the available space for inorganic cations and the concentrations in the feed, the amount of UF Permeate needed for the loading as well as the amount of the compounds brought onto the IE facility were determined as shown in Table 4-22.

Table 4-23: Summarised factors for calculating future scenarios at the existing IE device of the *GBR Upper Austria* - Elution

		Amino acids		
		[%]	**[kg]**	**[eq]**
Elution of column 3 to 5	Input AA at the loading	100	6.08	54
	Overall loss per run on average	5	304	
	Remaining AAs	95	5.78	51
	Loss in column 1&2	10	0.58	
	Loss to the pre- and post product phase	8	0.46	
	Effective yield of Product 1&2	**78**	**4.74**	**42**
Elution of column 4 to 5	Input AA at the loading	100	6.08	54
	Overall loss per run on average	5	304	
	Remaining AAs	95	5.78	51
	Loss in column 1&2	10	0.58	5
	Loss in column 3	10	0.58	5
	Loss in the pre- and post product phase	6	0.30	3
	Effective yield of Product 1&2	**70**	**4.28**	**38**

During the routine production of AAs not all columns would be eluted and the AAs found in the pre- and post product phase will be discarded due to economical reasons. Therefore, the relative amounts of AAs in the product fractions that were obtained from IE process and were ultimately used, were determined and were consequently used for calculating the scenarios as shown in Table 4-23.

Finally the consumption of water and chemicals according to chapter 4.2.5 and applied for calculating the scenarios were summarised in Table 4-24. In contrast to the amounts shown in chapter 4.2.5, the regeneration was extended by one step, as column 3 would be regenerated first with 0.25 M sulphuric acid to avoid any precipitates of gypsum in this column as well. Moreover, it was assumed that the ammonia solution was heated to 45 °C, as this temperature represents a compromise between losses of ammonia caused by higher temperatures and an increase of solubility of AAs at higher temperatures.

Table 4-24: Overview on the assumed consumptions of water, 0.25 M H_2SO_4, 1 M H_2SO_4 and 1 M NH_3 per batch run

Column	**1**	**2**	**3**	**4**	**5**
Process step	**Volume [l]**				
Rinsing after loading			120		
Elution with 1M NH_3	-	-	60	80	80
Rinsing after elution	-	-	50	50	50
Regeneration with 0.25M H_2SO_4	50	50	50	-	-
Regeneration with 1M H_2SO_4	50	50	50	60	60
Rinsing after Regeneration	50	50	50	50	50

4.3.2 Scenario calculation

Scenario 4l-A

The following key points have been assumed for Scenario 4l_A:

- Flows:
  - Loading 4 l/min
  - Elution 4 l/min
  - Regeneration 4 l/min

- Elution of:
  - Column 3
  - Column 4
  - Column 5

- Useable AA-product from column 3,4 and 5:
  - 4.74 kg

Results:

Calculated time needed per batch run

Process step	Column [-]	Volume [l]	Pumping gross time [min]	Pumping net time [min]
Loading	1-5	320	80	80
Rinsing after loading	1-5	120	30	30
Elution with 1 M NH_3	3-5	220	55	55
Pre-product & Rinsing	3-5	150	38	38
Regeneration with 0.25 M H_2SO_4	1-2	100	25	-
Regeneration with 1 M H_2SO_4	1-2	100	25	-
Rinsing after regeneration	1-2	100	25	-
Regeneration with 0.25 M H_2SO_4	3	50	13	13
Regeneration with 1 M H_2SO_4	3	50	13	13
Rinsing after regeneration	3	50	13	-
Regeneration with 1 M H_2SO_4	4-5	120	30	30
Rinsing after regeneration	4-5	100	25	25
Total		**1480**	**370**	**283**

Time per batch run [h]	**4.7**

Calculated consumption of water and chemicals needed per batch run

Process step	Column [-]	Volume [l]	RO-Water [l]	H_2SO_4 conc. [l]	NH_3 18% [l]
Loading	1-5	320	-	-	-
Rinsing after loading	1-5	120	120	-	-
Elution with 1 M NH_3	3-5	220	198	-	22
Pre-product & Rinsing	3-5	150	150	-	-
Regeneration with 0.25 M H_2SO_4	1-2	100	99	1.4	-
Regeneration with 1 M H_2SO_4	1-2	100	95	5.2	-
Rinsing after regeneration	1-2	100	100	-	-
Regeneration with 0.25 M H_2SO_4	3	50	49	0.7	-
Regeneration with 1 M H_2SO_4	3	50	47	2.6	-
Rinsing after regeneration	3	50	50	-	-
Regeneration with 1 M H_2SO_4	4-5	120	114	6.2	-
Rinsing after regeneration	4-5	100	100	-	-
Total			**1120**	**16.1**	**22.0**

RO-Water per batch run [l]	**1122**
H_2SO_4 conc. per batch run [l]	**16.1**
NH_3 18 % per batch run [l]	**22.0**

Calculated energy consumption per batch run						
Process step	**Column [-]**	**Volume [l]**	**Δ T [K]**	**cP H2O [kJ/(kg*K)]**	**Heat [kJ]**	**Energy [kWh]**
Heating of 1 M NH_3 (to 45 °C)	3-5	220	25	4	22000	6.11

Process step	**Column [-]**	**Volume [l]**	**Volume [m3/sec]**	**Pressure drop [Pa]**	**Efficiency [-]**	**Energy [kWh]**
Pumping	1-5	1480	$6.67*10^{-5}$	300000	1	0.01

Energy per batch run [kWh]	**6.12**

Scenario 4l-B

The following key points have been assumed for Scenario 4l_B:

- Flows:
  - Loading 4 l/min
  - Elution 4 l/min
  - Regeneration 4 l/min
- Elution of:
  - Column 4
  - Column 5
- Useable AA-product from column 4 and 5:
  - 4.28 kg

Results:

Calculated time needed per batch run				
Process step	**Column [-]**	**Volume [l]**	**Pumping gross time [min]**	**Pumping net time [min]**
Loading	1-5	320	80	80
Rinsing after loading	1-5	120	30	30
Elution with 1 M NH_3	4-5	160	40	40
Pre-product & Rinsing	4-5	100	25	25
Regeneration with 0.25 M H_2SO_4	1-2	100	25	-
Regeneration with 1 M H_2SO_4	1-2	100	25	-
Rinsing after regeneration	1-2	100	25	25
Regeneration with 0.25 M H_2SO_4	3	50	13	-
Regeneration with 1 M H_2SO_4	3	50	13	-
Rinsing after regeneration	3	50	13	13
Regeneration with 1 M H_2SO_4	4-5	120	30	15
Rinsing after regeneration	4-5	100	25	25
Total		**1370**	**343**	**253**

Time per batch run [h]	**4.2**

Calculated consumption of water and chemicals needed per batch run					
Process step	**Column**	**Volume**	**RO-Water**	**H_2SO_4 conc.**	**NH_3 18%**
	[-]	**[l]**	**[l]**	**[l]**	**[l]**
Loading	1-5	320	-	-	-
Rinsing after loading	1-5	120	120	-	-
Elution with 1 M NH_3	4-5	160	144	-	16
Pre-product & Rinsing	4-5	100	100	-	-
Regeneration with 0.25 M H_2SO_4	1-2	100	97	1.4	-
Regeneration with 1 M H_2SO_4	1-2	100	95	5.2	-
Rinsing after regeneration	1-2	100	100	-	-
Regeneration with 0.25 M H_2SO_4	3	50	49	0.7	-
Regeneration with 1 M H_2SO_4	3	50	47	2.6	-
Rinsing after regeneration	3	50	50	-	-
Regeneration with 1 M H_2SO_4	4-5	120	114	6.2	-
Rinsing after regeneration	4-5	100	100	-	-
Total			**1017**	**16.1**	**16.0**

RO-Water per batch run [l]	**1017**
H_2SO_4 conc. per batch run [l]	**16.1**
NH_3 18 % per batch run [l]	**16.0**

Calculated energy consumption per batch run						
Process step	**Column**	**Volume**	**Δ T**	**cP H2O**	**Heat**	**Energy**
	[-]	**[l]**	**[K]**	**[kJ/(kg*K)]**	**[kJ]**	**[kWh]**
Heating of 1 M NH_3 (to 45 °C)	4-5	160	25	4	16000	4.44

Process step	**Column**	**Volume**	**Volume**	**Pressure drop**	**Efficiency**	**Energy**
	[-]	**[l]**	**[m3/sec]**	**[Pa]**	**[-]**	**[kWh]**
Pumping	1-5	1370	$6.67*10^{-5}$	300000	0.70	0.01

Energy per batch run [kWh]	**4.45**

Scenario 8l-A

The following key points have been assumed for Scenario 8l_A:

- Flows:
  - Loading 8 l/min
  - Elution 8 l/min
  - Regeneration 8 l/min

- Elution of:
  - Column 3
  - Column 4
  - Column 5

- Useable AA-product from column 3,4 and 5:
  - 4.74 kg

Results:

Calculated time needed per batch run

Process step	Column	Volume	Pumping gross time	Pumping net time
	[-]	[l]	[min]	[min]
Loading	1-5	320	40	40
Rinsing after loading	1-5	120	15	15
Elution with 1 M NH_3	3-5	220	28	28
Pre-product & Rinsing	3-5	150	19	19
Regeneration with 0.25 M H_2SO_4	1-2	100	13	-
Regeneration with 1 M H_2SO_4	1-2	100	13	-
Rinsing after regeneration	1-2	100	13	-
Regeneration with 0.25 M H_2SO_4	3	50	6	6
Regeneration with 1 M H_2SO_4	3	50	6	6
Rinsing after regeneration	3	50	6	-
Regeneration with 1 M H_2SO_4	4-5	120	15	15
Rinsing after regeneration	4-5	100	13	13
Total		**1480**	**185**	**141**

Time per batch run [h]	**2.4**

Calculated consumption of water and chemicals needed per batch run

Process step	Column	Volume	RO-Water	H_2SO_4 conc.	NH_3 18%
	[-]	[l]	[l]	[l]	[l]
Loading	1-5	320	-	-	-
Rinsing after loading	1-5	120	120	-	-
Elution with 1 M NH_3	3-5	220	198	-	22
Pre-product & Rinsing	3-5	150	150	-	-
Regeneration with 0.25 M H_2SO_4	1-2	100	99	1.4	-
Regeneration with 1 M H_2SO_4	1-2	100	95	5.2	-
Rinsing after regeneration	1-2	100	100	-	-
Regeneration with 0.25 M H_2SO_4	3	50	49	0.7	-
Regeneration with 1 M H_2SO_4	3	50	47	2.6	-
Rinsing after regeneration	3	50	50	-	-
Regeneration with 1 M H_2SO_4	4-5	120	114	6.2	-
Rinsing after regeneration	4-5	100	100	-	-
Total			**1122**	**16.1**	**22.0**

RO-Water per batch run [l]	**1122**
H_2SO_4 conc. per batch run [l]	**16.1**
NH_3 18 % per batch run [l]	**22.0**

Calculated energy consumption per batch run						
Process step	**Column [-]**	**Volume [l]**	**Δ T [K]**	**cP H2O [kJ/(kg*K)]**	**Heat [kJ]**	**Energy [kWh]**
Heating of 1 M NH_3 (to 45 °C)	3-5	220	25	4	22000	6.11

Process step	**Column [-]**	**Volume [l]**	**Volume [m3/sec]**	**Pressure drop [Pa]**	**Efficiency [-]**	**Energy [kWh]**
Pumping	1-5	1480	$1.33*10^{-4}$	300000	0.70	0.02

Energy per batch run [kWh]	**6.13**

Scenario 8l-B

The following key points have been assumed for Scenario 8l_A:

- Flows:
  - Loading 8 l/min
  - Elution 8 l/min
  - Regeneration 8 l/min
- Elution of:
  - Column 3
  - Column 4
- Useable AA-product from column 4 and 5:
  - 4.28 kg

Results:

Calculated time needed per batch run				
Process step	**Column [-]**	**Volume [l]**	**Pumping gross time [min]**	**Pumping net time [min]**
Loading	1-5	320	40	40
Rinsing after loading	1-5	120	15	15
Elution with 1 M NH_3	4-5	160	20	20
Pre-product & Rinsing	4-5	100	13	13
Regeneration with 0.25 M H_2SO_4	1-2	100	13	-
Regeneration with 1 M H_2SO_4	1-2	100	13	-
Rinsing after regeneration	1-2	100	13	13
Regeneration with 0.25 M H_2SO_4	3	50	6	-
Regeneration with 1 M H_2SO_4	3	50	6	-
Rinsing after regeneration	3	50	6	6
Regeneration with 1 M H_2SO_4	4-5	120	15	15
Rinsing after regeneration	4-5	100	13	13
Total		**1370**	**171**	**128**

Time per batch run [h]	**2.1**

Calculated consumption of water and chemicals needed per batch run

Process step	Column [-]	Volume [l]	RO-Water [l]	H_2SO_4 conc. [l]	NH_3 18% [l]
Loading	1-5	320	-	-	-
Rinsing after loading	1-5	120	120	-	-
Elution with 1 M NH_3	4-5	160	144	-	16
Pre-product & Rinsing	4-5	100	100	-	-
Regeneration with 0.25 M H_2SO_4	1-2	100	99	1.4	-
Regeneration with 1 M H_2SO_4	1-2	100	95	5.2	-
Rinsing after regeneration	1-2	100	100	-	-
Regeneration with 0.25 M H_2SO_4	3	50	49	0.7	-
Regeneration with 1 M H_2SO_4	3	50	47	2.6	-
Rinsing after regeneration	3	50	50	-	-
Regeneration with 1 M H_2SO_4	4-5	120	114	6.2	-
Rinsing after regeneration	4-5	100	100	-	-
Total			**1017**	**16.1**	**16.0**

RO-Water per batch run [l]	**1017**
H_2SO_4 conc. per batch run [l]	**16.1**
NH_3 18 % per batch run [l]	**16.0**

Calculated energy consumption per batch run

Process step	Column [-]	Volume [l]	Δ T [K]	cP H2O [kJ/(kg*K)]	Heat [kJ]	Energy [kWh]
Heating of 1 M NH_3 (to 45 °C)	4-5	160	25	4	16000	4.44

Process step	Column [-]	Volume [l]	Volume [m3/sec]	Pressure drop [Pa]	Efficiency [-]	Energy [kWh]
Pumping	1-5	1370	$1.33*10^{-4}$	300000	0.70	0.02

Energy per batch run [kWh]	**4.46**

4.3.3 Overview on the results of the calculated scenarios

Finally, the results of the calculated scenarios were compared. As shown in Table 4-25, it is possible to produce 1 kg AAs per hour when columns 3, 4 and 5 were eluted. Under these conditions one batch run would ideally last 4.7 hours. Consequently, by applying a continuous operation (24 h) the production of 24.2 kg AAs per day or 169 kg AAs per week would be feasible. The production of approximately the same amounts on AAs per day and per week would also be possible in scenario 4l-B, although the yield per batch run is lower in this case than in scenario 4l-A. Indeed, by skipping the displacement step of column 3 it was feasible to save time. Therefore, more batch runs per day could be conducted and would lead to the same output of AAs per day and per week as in scenario 4l-A. Doubling the pumped volume per hour resulted, as expected, in a doubled output of AAs per hour. Therefore, by applying flow rates of 8 l/min or 21.7 h/m respectively, it would be possible to produce 48.3 kg AAs per hour or 338 kg AAs per week. Again the same final results were obtained in scenario 8l-A and 8l-B, as the time saved in scenario 8l-B compensated for the decreased AA yield per batch run. So at a first glance, upgrading the pumps to reduce the

time required per batch run would be desirable. Indeed, to conduct 10.2 batch runs per day under the assumed conditions nearly 3.3 m^3 of UF Permeate would be necessary to run the IE experiments. In order to produce this amount of UF Permeate about 3.7 m^3 raw silage juice from 9 t silage (fresh mass) have to be ultrafiltered per day. By using the currently installed UF device at the GBR Upper Austria it is possible to produce about 2 m^3 UF Permeate per day. Therefore, in order to double the flow rate at the IE device further investments in the pre-treatment would be necessary as well, if a continuous operation of the IE device is desired.

Table 4-25: Comparison of the calculated scenarios regarding the yields of AAs per time

	Scenario			
	4l-A	**4l-B**	**8l-A**	**8l-B**
AA product [kg]	4.74	4.28	4.74	4.28
Required time [h]	4.7	4.2	2.4	2.1
AA/h [kg]	1.01	1.02	2.01	2.01
Batch runs per day [-]	5.1	5.7	10.2	11.3
AA product per day [kg]	24.2	24.4	48.3	48.3
AA product per week [kg]	169	171	338	338

In addition to the yields of AAs per duration of time, the consumption of chemicals, water and energy per batch run was also investigated and compared. The results calculated for the four scenarios are summarised in Table 4-26.

Table 4-26: Comparison of the calculated scenarios regarding the consumption of water, chemicals and energy

	Scenario			
	4l-A	**4l-B**	**8l-A**	**8l-B**
RO-water per batch run [l]	1122	1017	1122	1017
RO-water per kg AA [l/kg]	237	238	237	238
H_2SO_4 conc. per batch run [l]	16.1	16.1	16.1	16.1
H_2SO_4 conc. per kg AA [l/kg]	3.40	3.76	3.40	3.76
NH_3 per batch run [l]	22.0	16.0	22.0	16.0
NH_3 per kg AA [l/kg]	4.64	3.74	4.64	3.74
Energy per batch run [kWh]	6.12	4.45	6.13	4.46
Energy per kg AA [kWh/kg]	1.29	1.04	1.29	1.04

Generally, the results regarding the consumption of water, chemicals and energy per yielded AAs were not influenced by the applied flow rate. Only differences between the A- and B-scenarios were found, as in scenario B column 3 was not treated with ammonia. For instance, by applying scenario 4l-B or 8l-B it would be possible to reduce the overall water supply needed per batch run by almost 10 % compared to scenario 4l-A or 8l-A. Indeed, as the yield of AAs in the B-scenarios would be lower as well, only slight differences in the RO-water amount needed per kilogram AAs would be observed. The same amount of sulphuric acid would be needed in all scenarios per batch run, as the same regenerations steps would be necessary. Indeed, per kilogram of yielded AAs 11 % more sulphuric acid would have been applied in the B-scenarios B than in the A-scenarios. On the other hand, in the B-scenarios the ammonia consumption would be lowered in total by 27 % per batch run and by 19 % per yielded kilogram of AAs. Based on this reduced amount of ammonia needed for the elution step less energy for heating the ammonia solution would also be necessary in the B-scenarios. So in these scenarios 19 % of energy would be saved per kilogram AAs.

Summing up the findings of the scenarios it has to be noted that a decision between the applied flow rate (4 or 8 l/min) would not only depend on the IE process itself, but also on the capacity at the pre-treatment steps to supply an adequate amount of IE feed. A decision between scenario A and B at both flow rates would be more complex and depends on priorities set during the production process. Considering the costs for the chemicals needed in the IE process only, the B-scenarios would be preferential. Comparisons of the costs showed that the reduced amount of ammonia needed in the B-scenarios would compensate the costs for the higher amount of sulphuric acid per kilogram of eluted AAs. Calculations based on data from a chemical supplier[2] showed that in the A-scenarios the chemicals needed per 1kg AA would cost € 3.07 and in the B-scenarios it would cost € 2.97. Additionally, less energy per kg AA would be needed in the latter scenarios as less ammonia solution would be heated. Nevertheless, skipping the elution would also reduce the available amount of Product 2, which is enriched with lysine and γ-amino butyric acid especially in column 3. Therefore, in order to make a final decision the potential of this product has to be investigated in detail as well.

4.3.4 Overview of streams and mass flow in the scenarios

Flow diagrams have been created to provide a more comprehensive overview of the IE process and its in- and output streams. Next to the AA yields, estimations regarding the freights and compositions of any output streams during the IE process were added and subsequently discussed. The results shown in the following flow diagrams (Figure 4-34 to Figure 4-38) are based on calculated data either from scenario 4I-A and 4I-B.

4.3.4.1 Loading

The first figure (
Figure 4-34) presents an overview of the streams and mass flows during the loading process. As an equal loading process was applied for both scenarios, the overview of this process step was only added once. Within the presented scenarios 320 l of UF Permeate containing 6.08 kg AAs and other components were serially brought into the columns. The AAs and inorganic cations interacted with the strong cation exchanger and were fixed on the resin. The remaining components were transferred through the columns without any interaction and were found in the IE effluent. After stopping the loading, the columns were rinsed with 120 l of deionised water to remove organic acids, sugars or inorganic anions from the resin's void volume. In total 440 l of liquid were introduced onto the resin during each loading step. 90 l of liquid which was run off from the IE device first correlated to the columns' void volume and was always discarded, as only small amounts of valuable components were found in this stream. In order to reduce the water consumption per batch run this stream could be easily recycled. For instance, it could be used in the rinsing step after the elution, as completely clean water would not be necessary in this case. The IE effluent (350 l), in turn, would be further processed using the concept of the innovative process in order to obtain a stream enriched with organic acids and a stream enriched with sugars.

[2] Fa. Brenntag (Austria)

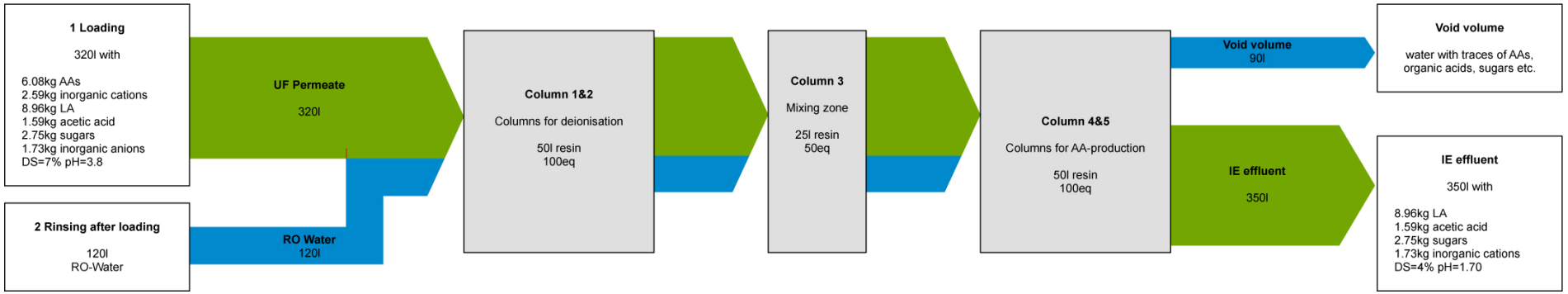


Figure 4-34: Streams and mass flow during the loading process (scenario 4l-A and 4l-B)

4.3.4.2 Elution

In the elution step, in turn, different streams and mass flows were observed in the two scenarios: in scenario 4I-A columns 3,4 and 5 and in scenario 4I-B only columns 4 and 5 were eluted. Therefore, the results are presented separately in Figure 4-35 for scenario 4I-A and in Figure 4-36 for scenario 4I-B. Subsequently, the streams introduced and the possibilities of recycling will be discussed.

For the DC step at column 3, 60 l of 1M ammonia or 1.02 kg of ammonia were used. As higher yields of AAs from columns 4 and 5 were common, these columns were each eluted with 80 l or 2.72 kg of ammonia. The introduced ammonia solution was divided into different fractions after the respective column. Firstly, the pre-product was eluted. This fraction contained low amounts of AAs, but there were also some remaining organic acids or sugars, which were not removed during the rinsing step. The recycling of these pre-product streams within the IE process should be easily performed, as it could be used for rinsing steps where absolute clear deionised water (e. g. for rinsing after the elution) would not be necessary. Secondly, the different AA product fractions were eluted. Considering scenario 4I-A and the elution of column 3 only small amounts of Product 1 (6 l, 0.12 kg AA) without ammonia were observed. Most AAs from column 3 were eluted within Product 2 (30 l, 0. 35kg). The elution of column 4 and 5 done in both scenarios, on the other hand, led to a high amount of Product 1 (69 l, 3.47 kg AAs) and less Product 2 (39 l, 0.87 kg AAs). Finally, some AAs and the remaining ammonia were found in the so called post-product phase. The recycling of this last-mentioned stream would be more difficult than the recycling of the pre-product fraction, as it would be contaminated with ammonia. In this case a purification of the water combined with concentrating the ammonia, by applying a RO-step, would be considerable. The cleaned water could then be used again within the process chain. The ammonia could be recycled more efficiently from the concentrated solution by applying, for instance, a stripping and resolving process.

Considering the ammonia consumption during the elution step more exactly, it has to be noted that the ammonia input was based on adapted experimental observations. The ammonia consumption, in turn, was based on theoretical assumptions. For instance, it was assumed that 18 eq of the resin in column 3 would be occupied with inorganic cations and 32 eq would be available for ammonia. Therefore, 0.54 kg of the introduced ammonia (1.02 kg) would be fixed on the resin and the rest would be found in Product 2 (0.36 kg) and in the post-product fraction (0.12 kg). For column 4 and 5 it was assumed that the available resin would be completely occupied with ammonia (100 eq or 1.70 kg ammonia). The remaining 60 eq or 1.02 kg ammonia would be split into Product 2 (0.55 kg) and into the post-product fraction (0.48 kg) again. The relative distribution of the surplus ammonia into Product 2 or into the post-product fraction was based on the experimentally observed ammonia concentrations in these fractions. Indeed, the experimentally observed ammonia concentrations in Product 2 and in the post-product fraction were lower than the theoretically estimated concentrations. These observations led again to the assumptions and problems of the ammonia supply and consumption, which have already been discussed in chapter 4.2.5. Nevertheless, the theoretically calculated ammonia freights shown in Figure 4-35 and Figure 4-36 could be helpful for further estimations and process concepts regarding the treatment of these streams.

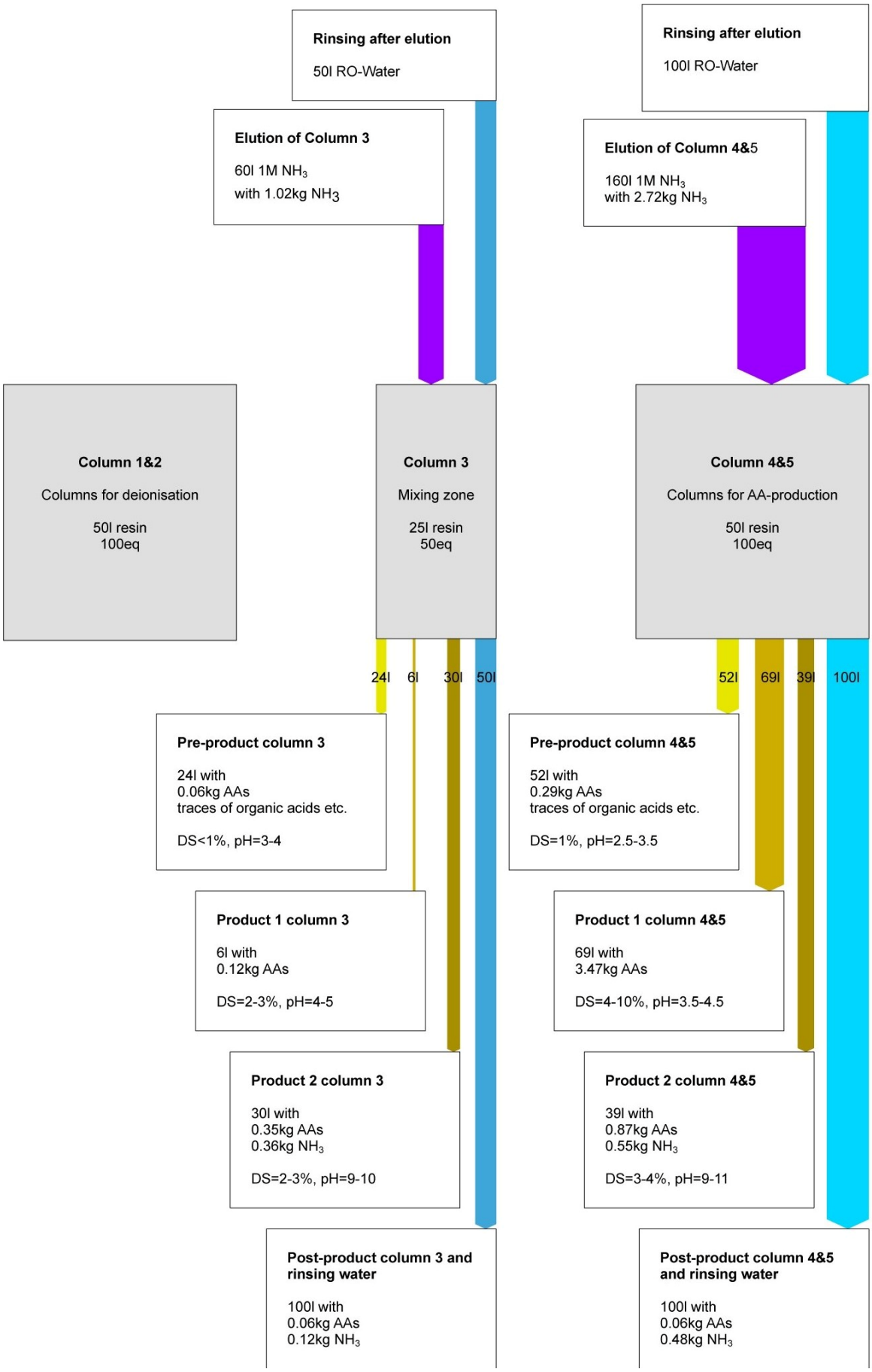


Figure 4-35: Streams and mass flow during the displacement process (scenario 4l-A)

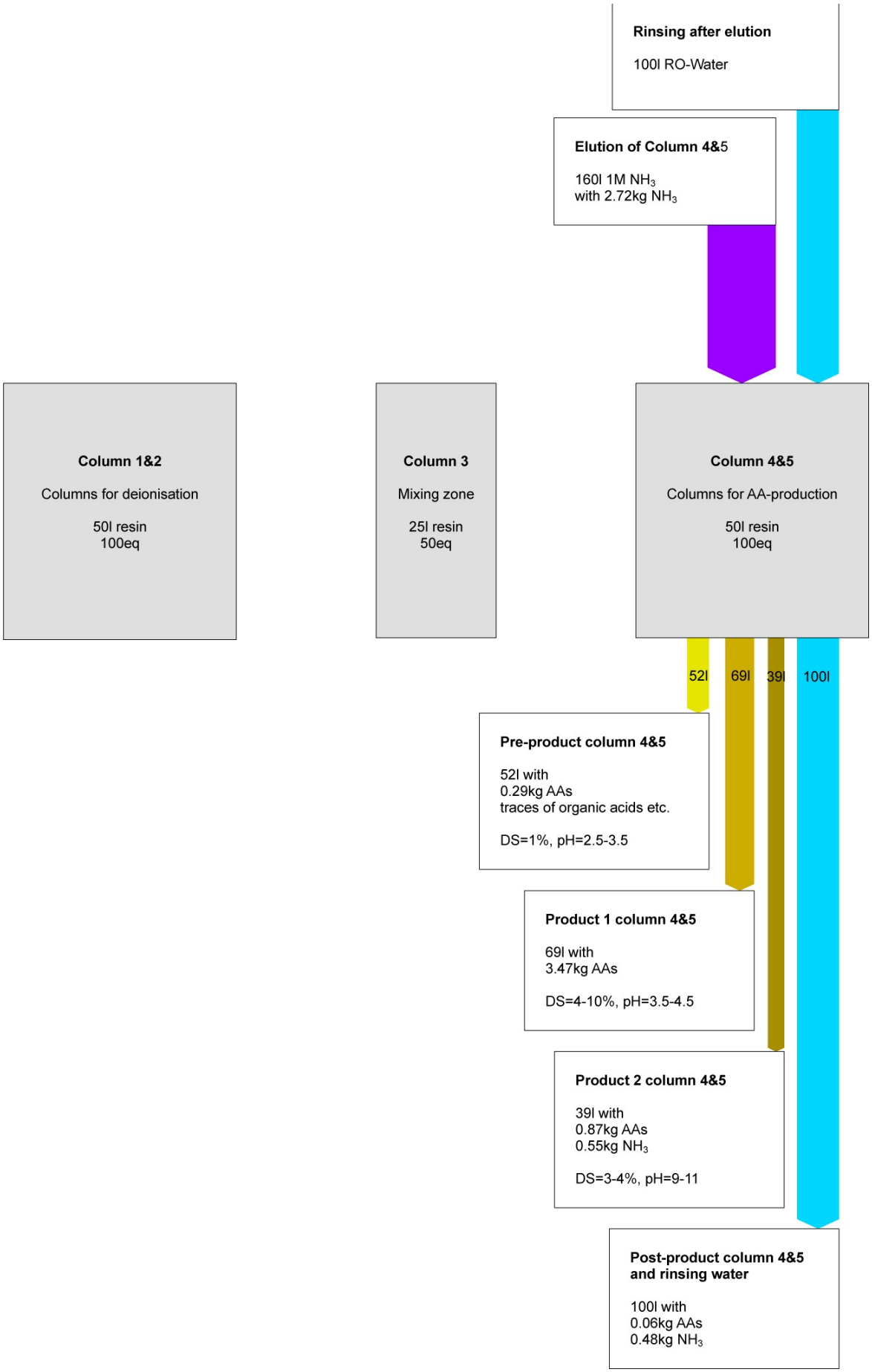


Figure 4-36: Streams and mass flow during the displacement process (scenario 4l-B)

4.3.4.3 Regeneration

Finally, streams and mass flows of the regeneration step were observed in detail. The amounts of sulphuric acid introduced were based on adapted experimental observations. By combining the amount of sulphuric acid introduced with the assumed amount of displaced AAs and inorganic cations, the freights eluted during the regeneration processes were calculated.

Figure 4-37 gives an overview of the streams and freights occurring in scenario 4l-A, where columns 3,4 and 5 were treated with ammonia. In total approximately 30 kg of concentrated sulphuric acid were introduced to the IE device in order to regenerate the strong cation exchanger efficiently (Mitsubishi Chemical Company 1997). By introducing this amount of sulphuric acid it was calculated that 1.58 kg of calcium sulphate (gypsum), 4.23 kg of potassium sulphate, 1.03 kg of magnesia sulphate and 0.06 kg of sodium sulphate would theoretically run off from columns 1, 2 and 3 in scenario 4l-A. Additionally, the ammonia introduced during the elution step would be displaced by H^+-ions. Therefore, 2.11 kg of ammonia sulphate would be existent in the regeneration effluent as well. Furthermore, some AAs, which were found on columns 1 and 2, would be displaced by the acid. In contrast to columns 1, 2 and 3, which were occupied by inorganic cations and AAs, columns 4 and 5 were only occupied by AAs. Therefore, ammonia sulphate (6.60 kg) would be the only salt which would be eluted from these columns during the regeneration. However, a surplus of acid was necessary to regenerate the resin efficiently in all columns and therefore a huge amount of unmodified sulphuric acid would still be found in the effluents as well. Finally, each column was rinsed with 50 l of deionised water to remove any acid residues. Regarding the second scenario (4l-B) quite similar freights and streams were observed in the regeneration step compared with scenario 4l-A. From columns 1, 2 and 3 the same amounts of calcium sulphate, potassium sulphate, magnesia sulphate and sodium sulphate would be eluted. Furthermore, the same freights from columns 4 and 5 would be noted. Nevertheless, some differences would be observed. For instance, as no AAs would be eluted from column 3 during the DC with ammonia, no ammonia sulphate would be obtained from this column. This would lead, in turn, to a higher amount of AAs (1.16 kg) being eluted during the regeneration step.

All in all high concentrations of calcium sulphate (5.3 g/l) were observed within the regeneration solutions, but no gypsum precipitates were noticed when applying the two-step regeneration procedure. Normally, the solubility of calcium sulphate is only 2.7 g/l at 18 °C (Sitzmann 2013) but in the presence of other charged species the solubility of sulphates can be increased (Nießner 2010). Furthermore, relatively high concentrations of sulphuric acid and therefore a low pH can lead to a better solubility of sulphates as HSO_4^- is the prevalent species at this low pH. These facts could also be responsible for the increased solubility of gypsum in the present regeneration solutions.

The recycling of the effluent from the regeneration step would be more difficult, as the composition is complex. Due to the high content on inorganic salts a direct reuse during the IE process would be impossible. Furthermore, a direct disposal would not be feasible, as the solutions are too acidic. In order to dispose these solutions a neutralisation step with a low-cost base would be necessary at least. For instance, by using lime the remaining sulphuric acid could be transferred into gypsum as well. Further processes for treating the effluent found in literature are membrane processes including membrane distillation and thermal methods (Gryta, Karakulski et al. 2005). The reuse, in turn, of the rising water after the regeneration would be much easier, as it should only contain traces of sulphuric acid. Therefore, the rinsing water could be directly used for the preparation of new regeneration solutions.

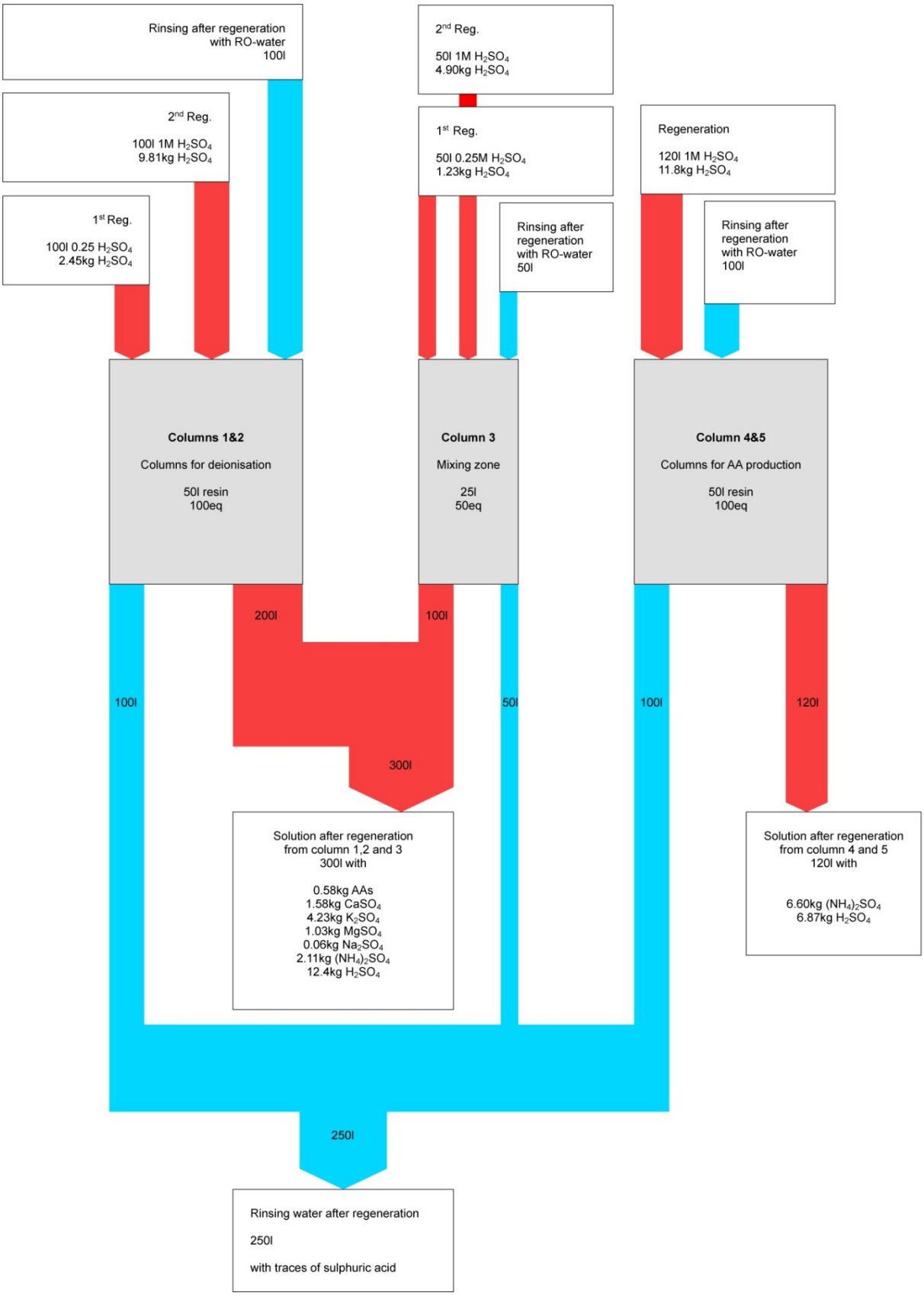


Figure 4-37: Streams and mass flow during the regeneration process (scenario 4l-A)

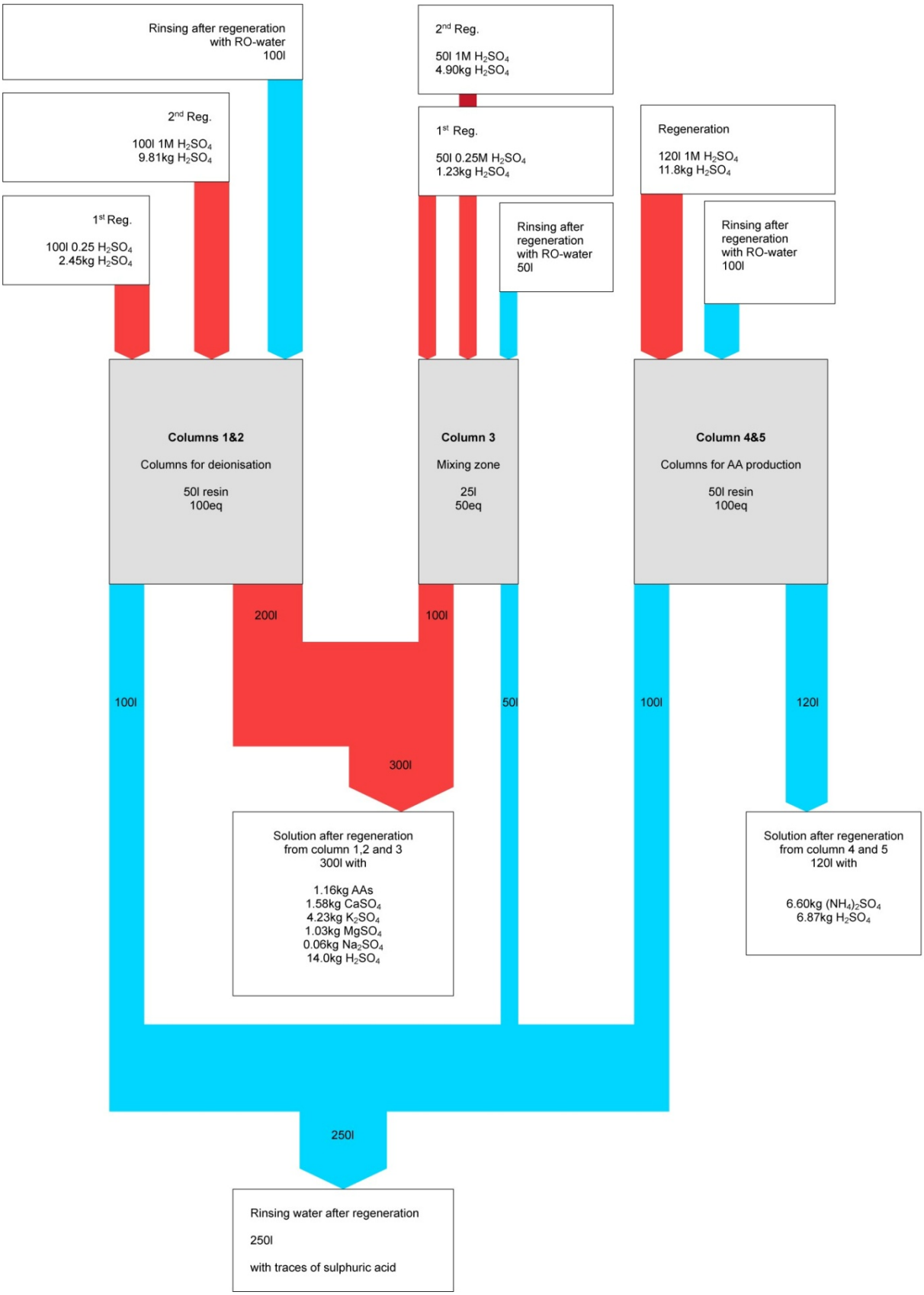


Figure 4-38: Streams and mass flow during the regeneration process (scenario 4l-B)

4.4 Amino acid product polishing

At the *GBR Upper Austria* the production of three different AA product solutions from grass silage juice was implemented successfully, as already shown and discussed in detail in chapter 4.2.4. Indeed, these product fractions are mixtures of AAs, which contain particular AAs in higher concentrations (e. g. acidic AAs in Product 1A), but the production of more defined products or pure substances was not fulfilled until now. Moreover, the AA powders of Product 1A and 1B made by spray drying have colour and scent. Furthermore, the alkaline AA product (Product 2) still contains ammonia caused by the displacement process. Therefore, the further manufacture and usage of Product 2 was omitted so far. However, the manufacture of more defined AA products from grass silage could increase the attractiveness of these products to potential markets. For this reason AA polishing experiments at lab scale were done to reach more defined and more appealing AA products from grass silage juice.

In pre-experiments different methods were tested and identified to fractionate and improve the raw products, where simple methods with low chemical inputs were preferred. Based on the results of pre-experiments, extensive fractionation experiments for Product 1A and Product 1B were performed and balanced. Furthermore, Product 2 was upgraded by removing the ammonia and producing an AA powder. Subsequently, the results of these fractionation experiments are shown and discussed.

4.4.1 Acidic AA-Product (Product 1A)

Generally, the fractionation of the acidic Product 1A contained two steps. Firstly, it was possible to separate the raw material into three different fractions by applying a strong anion exchange resin. Secondly, the fractions gained from the IE process were further separated by concentrating these solutions in a vacuum evaporator and removing the precipitates that had built up from the mother liquor. These fractionation steps were done with a coloured (untreated) and a decoloured starting material. An overview of the results and the AA composition of the products obtained for the coloured and decoloured starting materials is shown in Figure 4-39 and Figure 4-40. Within these figures the percentages in the bottom left-hand corner in each box represent the total AA yield for this step with regard to the introduced starting material. The percentages over the boxes, in turn, reflect the distribution of the AAs per experimental step. Summing up these percentages per step implies the total recovery rate for the respective step. As in the first part, the yield referred to the starting material equals the AA distribution in this step, the latter was not headed. The pie charts in each box show the AA composition of each fraction. Furthermore, the effective amount of the AAs transferred to the respective step was added as well as the percentage of AAs on the detected dry substance. Subsequently, these results are discussed in detail.

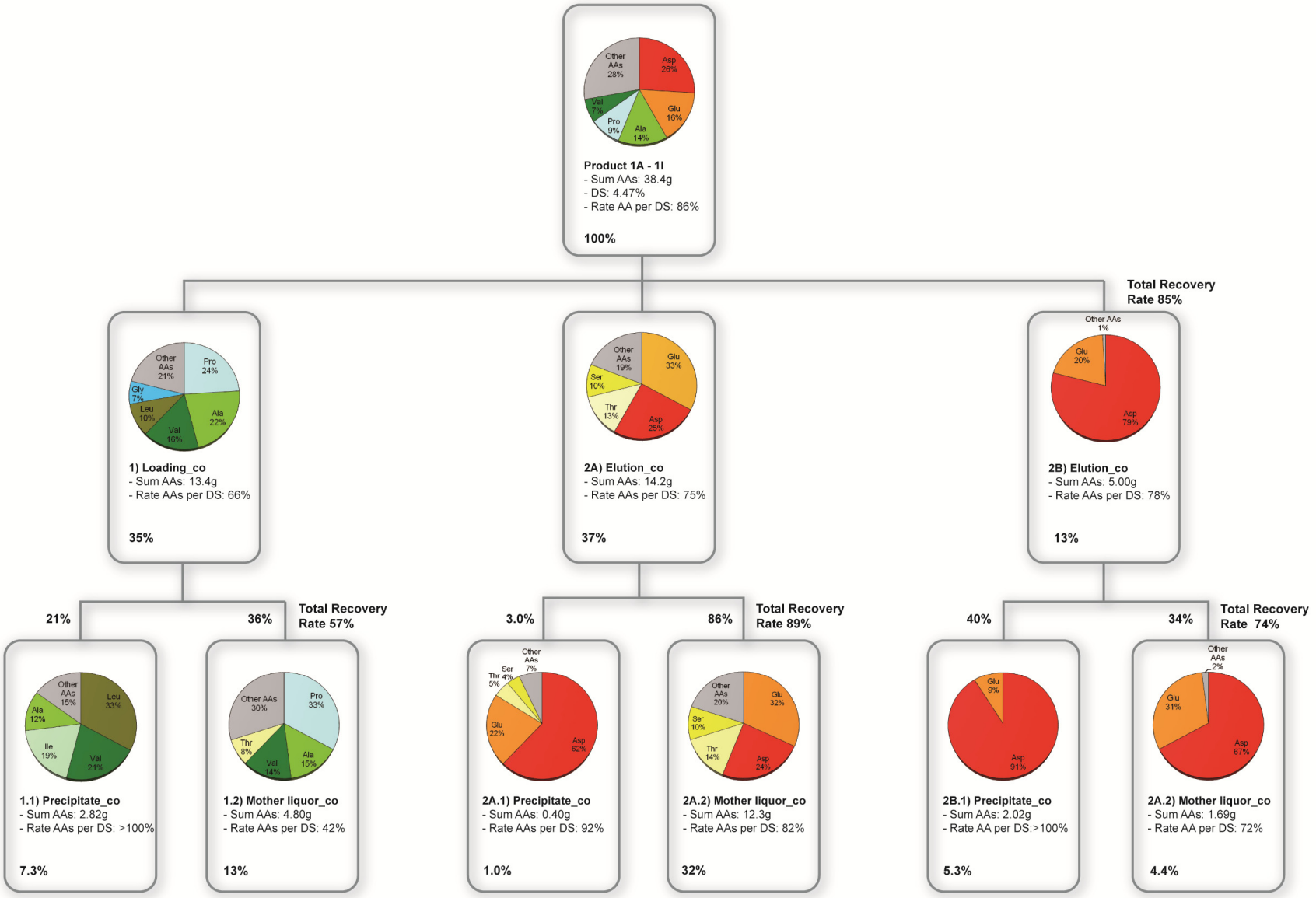


Figure 4-39: Overview on the fractionation of Product 1A without decolourisation step

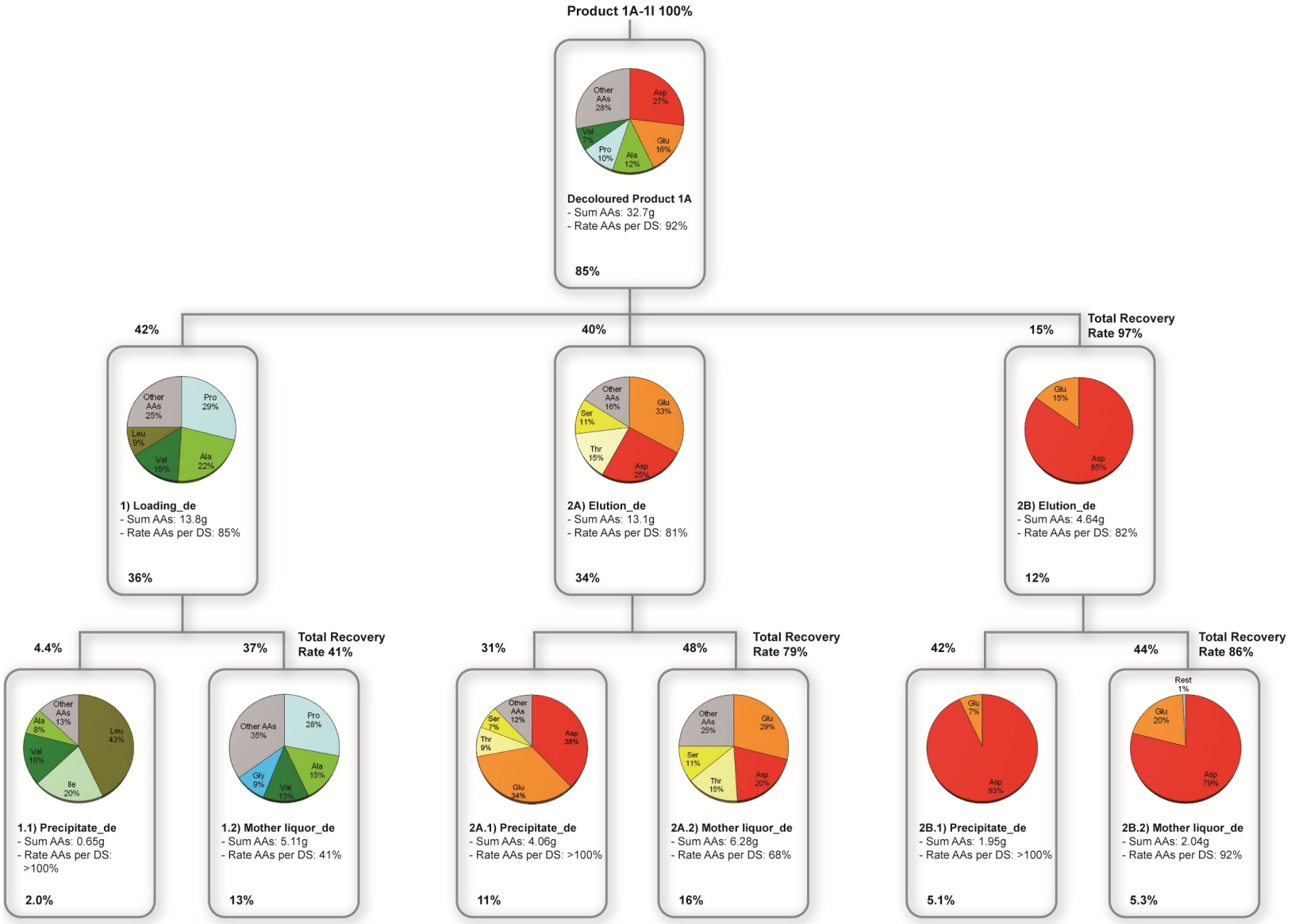


Figure 4-40: Overview on the fractionation of Product 1A with decolourisation step

4.4.1.1 Decolourisation

Pre-experiments with different adsorption materials (molecular sieve, natural zeolites and activated carbon) were performed (Steinbrecher 2012). The results showed that among these tested materials activated carbon is most suitable for removing the colouring from the GBR AA solutions. Therefore, some amount of the starting material (Product 1A) was treated with activated carbon. Consequently, the products obtained from experiments with decoloured starting material were compared with products from untreated starting material. However, by using activated carbon it was possible to remove the brown colour completely from the product solution, as shown in Figure 4-41.

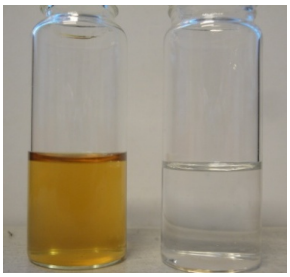


Figure 4-41: Product 1A before (left) and after (right) treating with activated carbon

Next to the coloured particles some AAs - in total 15 % from the starting material - were adsorbed by activated carbon as well. From most AAs 10-20 % of the amount found in the starting material was adsorbed, whereas different AAs showed differences in their adsorption behaviour. For instance, the aromatic AAs L-phenylalanine and L-tyrosine were adsorbed more strongly, as demonstrated in Figure 4-42. More than 70 % of these AAs were removed from the starting material during the decolourisation step. This behaviour was expected, as activated carbon is a well known adsorption material for aromatic AAs (Lopes, Delvivo et al. 2005, Clark, Alves et al. 2012). Therefore, next to the decolourisation this step could be used for the selective adsorption and production of aromatic AAs. Moreover, the amount of diluted ammonia in the product solution was reduced. L-asparagine, L-lysine, L-histidine, L-tryptophan and L-arginine were not found in the starting material and therefore no adsorption was possible at all.

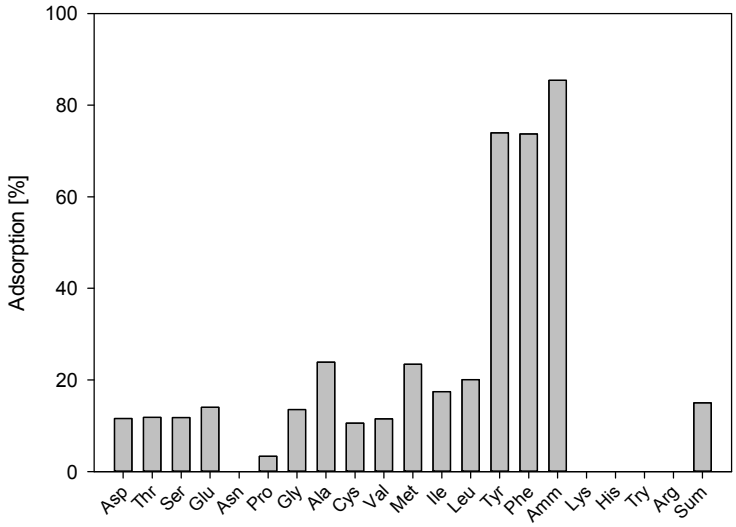


Figure 4-42: Relative adsorption of AAs by activated carbon using Product 1A (single AAs and AAs in sum)

In Figure 4-40, which shows an overview on the whole fractionation experiment for the decoloured Product 1A, no distinctive differences considering the AA composition before and after the treatment with activated carbon have been observed. The rate of the main components aspartic acid, glutamic acid, alanine, proline and valine has not been affected considerably. Moreover, through the treatment with activated carbon the AA percentage per amount of dry substance was increased from 86 % to 92 %. Indeed, if the AAs, which are fixed on the activated carbon, are not recovered anymore, the cleaning with activated carbon would lead to a loss of 15 % of AAs per fractionation cycle.

4.4.1.2 Ion-exchange process

The starting material Product 1A (coloured or decoloured) was separated into three different liquid fractions by applying a strong anion exchanger resin. An overview on the AA composition of these fractions is given in Figure 4-39 (coloured) and Figure 4-40 (decoloured). The first fraction, which was gained during the loading step and the rinsing step with deionised water, mainly consisted of the neutral AAs proline, alanine, valine and leucine (1-Loading). As these neutral AAs have no charged side chains, they did not interact with the resin's functionalised groups at all and were eluted immediately. During the elution step – using 1 M acetic acid – two different fractions (2A-Elution, 2B-Elution) were produced. First a solution consisting mainly of glutamic acid, aspartic acid, threonine and serine was eluted. Secondly, a solution consisting only of aspartic acid and glutamic acid was obtained. Considering these fractions in detail, serine and threonine, which are polar, uncharged AAs, interacted with the resin as well, but less strongly than the AAs with charged side chains aspartic acid and glutamic acid. Therefore, serine and threonine were displaced by the acetate ions earlier and found in the first elution fraction (2A-Elution) only. Moreover, both elution fractions contained acetic acid, as this acid was used for displacing.

Comparing the AA composition of the fractions gained from the decoloured starting material with the fractions based on untreated starting material, only few differences were observed. For instance, the rate of proline in the loading fraction (1-Loading) is slightly higher using the decoloured starting material (29 %) than using the untreated one (24 %). Moreover, by using the decoloured starting material the rate of aspartic acid in the second elution fraction (2A-Elution) increased to 85 % (cf. 79 % untreated starting material) and no other AAs were found in this fraction at all. In addition, the treatment with activated carbon led to an increase of the rate of AAs per amount of detected dry substance. Nevertheless, the dry substance rate was lower in the fractions after the IE process than in the starting material, as by the IE process substances like acetic acid were introduced to the AA fractions. The rate of AAs per amount dry substance was >80 % for all fractions in this experiment. The optical properties of these product fractions were compared as well. The colourisation of every fraction was, as expected, decreased by treating the starting material with activated carbon. The yellow colour of the eluted fractions was minimised.

Next to qualitative aspects, the AA yields for the IE step were investigated as well. For instance, referred to the introduced AAs 35 % AAs were transferred to the loading-fraction (1-Loading_co), 37 % to the first elution-fraction (2A-Elution_co) and 13 % to the second elution-fraction (2B-Elution_co) in the experiment with untreated starting material. Therefore, a total recovery rate of 85 % was observed. By using the decoloured starting material the total recovery rate was increased to 97 %. In this case 42 % were found in the loading-fraction (1-Loading_de), 40 % in the first elution-fraction (2A-Elution_de) and 15 % of the AAs were found within the second elution-fraction (2B-Elution_de). Although, including the losses of the decolourisation step about the same amount of the introduced AAs were transferred to the fractions of the IE process in both experiments.

4.4.1.3 Fractionation in the vacuum evaporator

Secondly, a further separation of each liquid fraction obtained from the IE process was done by concentrating them by the means of a vacuum evaporator. Based on the different solubility a separation between AAs was reached. The overall results of these fractionations are shown in Figure 4-39 and Figure 4-40.

By concentrating the effluent from the loading process a precipitate (1.1-Precipitate) consisting mainly of leucine, valine, isoleucine and alanine was separated. Due to the comparatively low solubility of leucine, isoleucine and valine it was possible to produce this precipitate with a high rate in BCAAs. AAs with a higher solubility in aqueous solutions such as proline and alanine remained solved and were mainly found in the mother liquor (1.2-Mother liquor). By using the decoloured starting material, it was possible to gain a higher rate of BCAAs within the precipitate than in the experiment with coloured feed. In total, 79 % of all AAs in this precipitate consisted of leucine (43 %), isoleucine (20 %) and valine (16 %). Indeed, the overall yield of AAs transferred into this precipitate (1.1-Precipitate_de) referred to the starting material was low with 2 %. 13 % of the AAs were found in the mother liquor (1.2-Mother liquor_de). Thus, the efficiency of this step was 41 % in total. The precipitate, on the other hand, obtained from the untreated starting material contained 73 % BCAAs with 33 % leucine, 21 % valine and 19 % isoleucine. Generally, higher yields were gained in this experiment. In total 7.3 % AAs were transferred to the precipitate (1.1-Precipitate_co) and 12.5 % were found in the mother liquor (1.2-Mother liquor_co). The efficiency of this fractionation step was 57 %. However, it was feasible to produce a white precipitate from both starting materials.

Generally, separating any solid from the fraction 2A-Elution was difficult. The solution had to be greatly concentrated to force the generation of any precipitate. Therefore, the amount of AAs transferred to the built precipitate (2A.1-Precipitate_co) using coloured feed was low (1 %). In the second experiment it was feasible to transfer 11 % of the introduced AAs to this precipitate (2A.1-Precipitate_de). As aspartic acid and glutamic acid are less soluble than other AAs, these AAs were finally accumulated there. In particular, a high rate of aspartic acid and glutamic acid (sum 84 %) was found in the precipitate, which was obtained during the experiment with the untreated starting material. Both remaining mother liquors (2A.2-Mother liquor), which mainly consisted of aspartic acid, glutamic acid, threonine and serine, could be further used to produce these AAs. 32 % of the introduced AAs were found in 2A.2-Mother liquor_co and 16 % in 2A.2_Mother liquor_de. Considering the efficiency of this fractionation step in the vacuum evaporator, the recovery rate starting with the fraction 2A-Elution_co was 89 % and with 2A-Elution_de was 79 %. Both precipitates were light brown and smelled very intense, therefore the pre-treating with activated carbon did not lead to any qualitative improvement in this case.

The solution, which was produced in the second part of the elution process (2B-Elution), was already quite pure and only consisted of glutamic acid and aspartic acid. Nevertheless, by concentrating the solution it was possible to separate a solid phase (2B.1-Precipitate), which consisted of over 90 % of aspartic acid and an increased AA rate per dry substance (>100 %). This further separation was possible as aspartic acid is slightly less soluble in aqueous solutions than glutamic acid. The production of a precipitate with a high rate of aspartic acid was implemented with both starting materials and quite similar results were observed. Moreover, independent from the starting material white precipitates were generated.

Considering the rate of AAs per dry substance, it can be noticed that higher AA rates were observed in all precipitates compared to the respective input material in the vacuum evaporator. Especially, in the experiment with the decoloured starting material precipitates with high rates on AAs were produced. Lower AA rates, in turn, were observed in the mother liquors, which leads to the assumption that most contaminates remained solved.

4.4.2 Neutral AA-Product (Product 1B)

The fractionation of the neutral AA-Product 1B comprised of four steps. Firstly, a precipitate, which was already found in the starting material, was removed by a simple filtration. Secondly, an optional decolourisation step using activated carbon was done. Thirdly, the coloured or decoloured starting material was fractionated in a vacuum evaporator. By concentrating the starting material a precipitate was built and subsequently separated. Finally, the purity of the precipitates that were obtained was improved through recrystallisation. An overview on the results and the AA-composition of the products that were attained using either the coloured or the decoloured starting material is shown in Figure 4-43, Figure 4-44 and Figure 4-45. The quoted data was calculated in a similar manner to the data quoted in the illustrations from Product 1A.

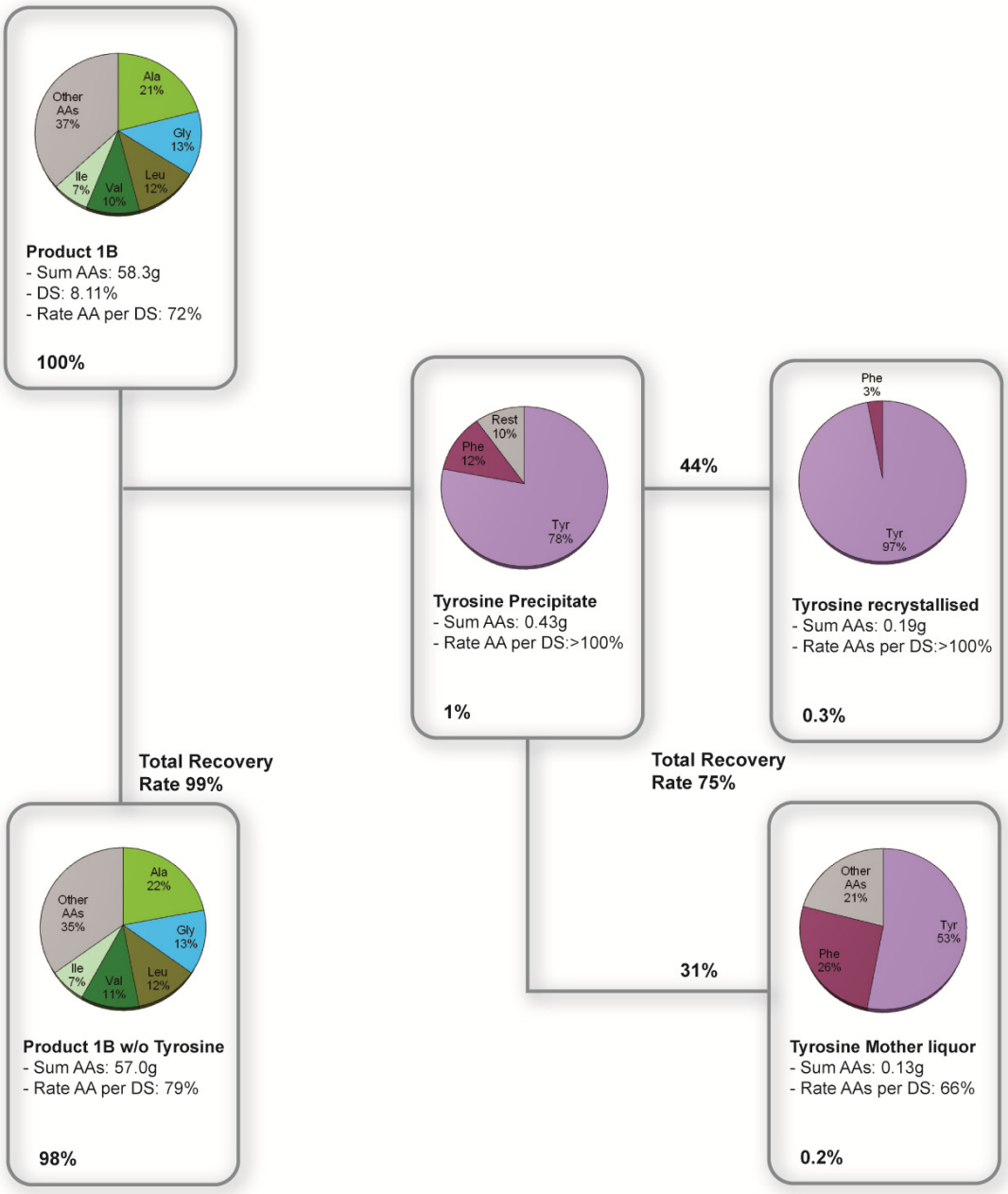


Figure 4-43: 1st part of the fractionation of Product 1B – removal of L-tyrosine

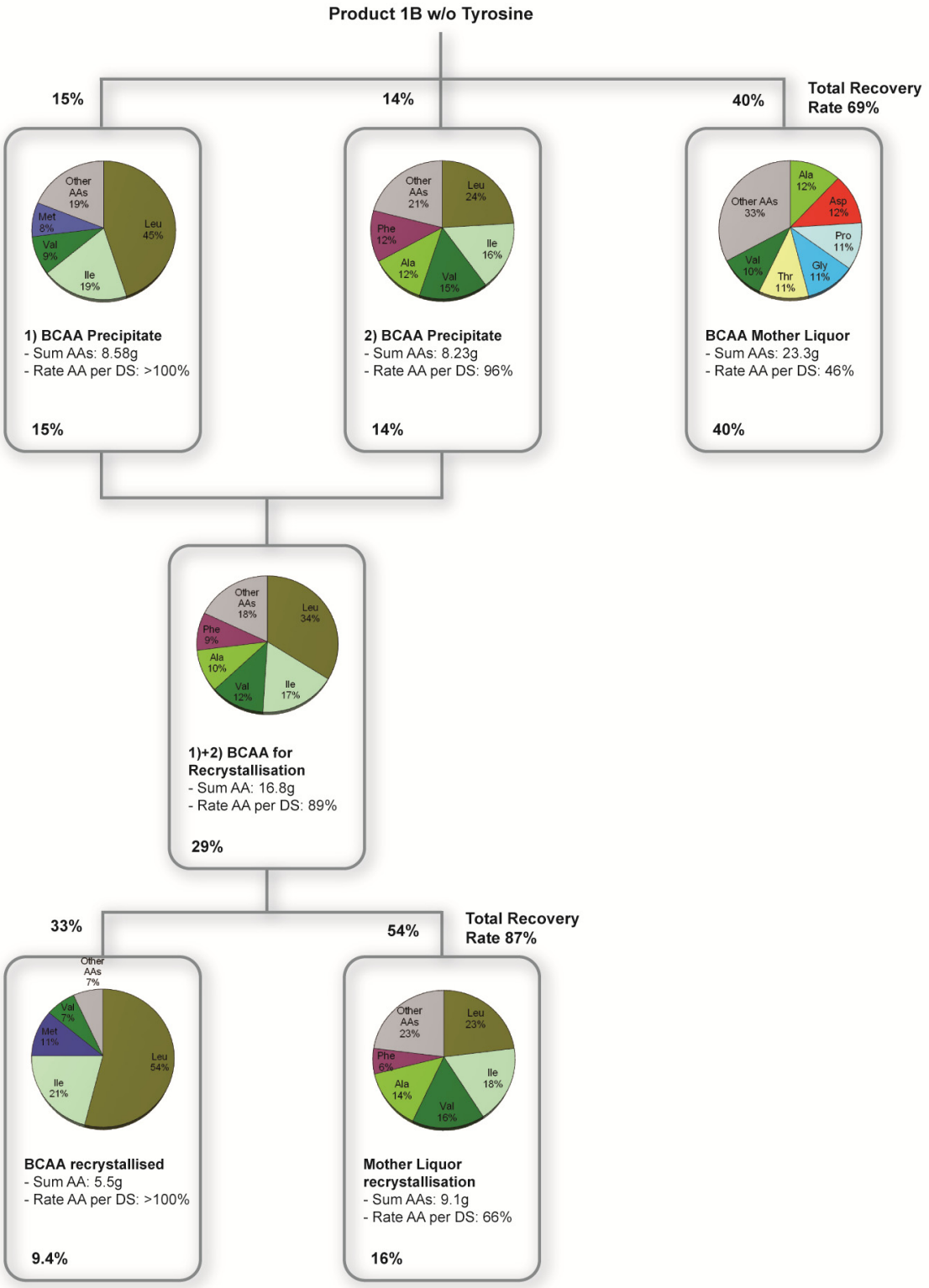


Figure 4-44: Fractionation of Product 1B without decolourisation step

Figure 4-45: Fractionation of Product 1B with decolourisation step

4.4.2.1 Precipitate with L-tyrosine

As soon as the aqueous solution of Product 1B was cooled down after the elution process with heated ammonia solution, a precipitate was observed within the solution. As the solubility of L-tyrosine in water is quite low (0.45 g/l), former analyses showed that the spontaneously built precipitate mainly consisted of this AA. Within the experiments that are presented a solid phase (beige powder) consisting of AAs with 78 % L-tyrosine, 12 % L-phenylalanine and 10 % other AAs was separated. By recrystallising these solid fractions the purity was improved and analyses showed that the cleaned precipitate (dark brown granulate) consisted of 97 % L-tyrosine referred to the sum of analysed AAs. Within the remaining mother liquor after the recrystallisation step the L-phenylalanine was accumulated. As shown in Figure 4-43, 1 % of the total introduced AAs were transferred to the tyrosine precipitate and 98 % remained in solution for further fractionation. After the recrystallisation step 0.3 % of the total AAs, but 20 % of the tyrosine from the feed was found in the precipitate. In total, the recovery rate of the recrystallisation step was 74 %. As the decolourisation was done after the removal of the tyrosine precipitate, these results were true for both experiments.

4.4.2.2 Decolourisation

As already discussed in chapter 4.4.1.1 activated carbon was identified to be the most suitable adsorption material for removing unwanted colour from the product solution. Therefore, it was applied to clear the liquid Product 1B successfully as well. Consequently, the results gained in experiments with decoloured starting material were compared with results from experiments done with the coloured starting material.

The relative adsorbed amount per AA from the starting material on activated carbon is shown in Figure 4-46. Comparing the adsorption of Product 1A and Product 1B quite similar results were observed. In total, 16.5 % of the AAs solved in Product 1B were absorbed by the activated carbon (cf. Product 1A on average 15 %). Considering the adsorption behaviour per AA, mostly 10 to 20 % of the AA amount in the starting material was adsorbed. The aromatic AAs L-phenylalanine and L-tyrosine were an exception again, where about 50 % of L-phenylalanine and 63 % of L-tyrosine were fixed on the activated carbon. Thus from Product 1B less aromatic AAs have been removed than from Product 1A. Possible reasons for these differences could be the different pH of the starting solutions and the different concentrations of aromatic AAs therein. No adsorption of L-glutamic acid was observed although this AA was in the starting solution. On the other hand, L-asparagine, L-lysine, L-histidine, L-tryptophan and L-arginine have not been analysed in the starting material, therefore no adsorption was possible. However, the overall AA composition of Product 1B before and after the treatment with activated carbon and the rate of the main ingredients alanine, glycine, leucine, valine and isoleucine remained stable. Referring the available AAs after the decolourisation step to the starting material, 83 % of the introduced AAs are available for further fractionation after this step.

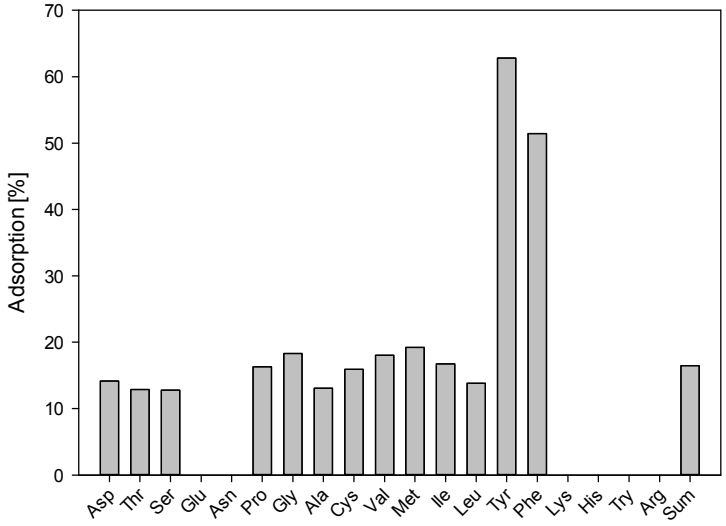


Figure 4-46: Relative adsorption of AAs by activated carbon from Product 1B (single AAs and AAs in sum)

4.4.2.3 Fractionation in the vacuum evaporator

The decoloured and coloured Product 1B without the tyrosine precipitate was further fractionated in a vacuum evaporator, where the different solubility characteristics of AAs were utilised. The overall results of these fractionations are shown in Figure 4-44 (coloured) and Figure 4-45 (decoloured).

Based on a starting material consisting among other AAs of 22 % alanine, 13 % glycine, 12 % leucine, 11 % valine and 7 % isoleucine (referred to the total amount of analysed AAs), precipitates rich in leucine, isleucine and valine were separated by continuously concentrating the product solution.

The separation of the solid phase from the liquid starting material was divided into two steps, as a further concentration of the solution was impossible when a high amount of precipitate had already built up (permanent delay in boiling). Consequently, the concentration was interrupted, the precipitate was removed by filtration, followed by restarting the vacuum evaporator and separating another precipitate. Therefore, two precipitates with quite similar compositions were produced per run. The AA compositions of these precipitates are shown in Figure 4-44 and Figure 4-45. As the solubility of L-leucine is quite low a higher amount of this AA (>40 %) was always found in the first precipitate. Within the second precipitate, in turn, less leucine, but more isoleucine, valine and alanine were detected. However, overall BCAA-rates of 55-73 % (referred to the total amounts of AAs) were reached.

Moreover, differences in the AA composition have not only been observed between the first and second precipitate, but also between the experiment with coloured and decoloured starting material. By treating the starting material with activated carbon L-phenylalanine is partly removed from the starting material as well. As a consequence no accumulation of L-phenylalanine was observed in the second precipitate in the experiment using the decoloured starting material. Untreated starting material, in turn, led to a rate of 12 % phenylalanine within the second precipitate. Comparing the remaining mother liquors, which are mixtures of a multiple number of AAs, quite equal AA compositions were observed using the decoloured or the coloured starting material. In the experiment without any treatment with activated carbon 29 % of AAs from the input material were transferred to the BCAA-precipitate. This yield was reduced to 19 % in the second experiment including the

decolourisation step. In both cases, the recovery rate for AAs within this fractionation step was about 70 %. The treatment with activated carbon also led to higher rates of AAs per dry substance. Furthermore, from the optical point of view more attractive precipitates were produced from the decoloured starting material as very attractive, white powders and a nearly clear mother liquor were generated. The products using the coloured starting material were light yellow and the mother liquor was golden brown.

4.4.2.4 Recrystallisation

In order to purify the precipitates a recrystallisation step was applied. For this process the first and second precipitate from the fractionation in the vacuum evaporator were combined, as the AA composition was quite equal. In the experiment with untreated starting material, it was feasible to further purify the precipitate and to produce a white to yellowish recrystallised precipitate consisting of 54 % leucine, 21 % isoleucine, 11 % methionine, 7 % valine and 7 % other AAs. Thus the rate of BCAAs referred to total amount of AAs within the precipitate was increased from 63 % to 82 %. Referring the yielded AAs within precipitate to the starting material, it was possible to transfer 9.4 % of the AAs to the recrystallised precipitate. Furthermore, a high rate of AAs was reached, as impurities were removed by recrystallisation. In the second experiment (decoloured starting material) a white, crystalline solid containing 59 % leucine, 21 % isoleucin and 6 % valine was produced (see Figure 4-47). 7.5 % of the AAs, which were introduced into this experiment, were found in this precipitate. In both experiments the recrystallisation step was quite effective, as over 85 % of the AAs were recovered.

Figure 4-47: Recrystallised BCAA-precipitate from the decoloured Product 1B

4.4.3 Alkaline AA-Product (Product 2)

Due to its contamination with ammonia the alkaline Product 2 from the *GBR Upper Austria* remained, in contrary to Product 1A and 1B, unused until now. For instance, AA powders generated by a spray drying process were successfully produced from Product 1A and 1B. The production of AA powder using Product 2, in turn, had not yet been tested. Indeed, the usage of the AAs from Product 2 (mainly γ-aminobutyric acid and lysine) would be desirable as the product portfolio of *GBR Upper Austria* could be extended. Furthermore, the usable AA output per batch run on the IE would be increased. For this reason processes to upgrade this product were tested at lab scale. The results are shown in the following chapter.

4.4.3.1 Removal of ammonia (“stripping”)

Based on findings from literature (Quan, Wang et al. 2009, Walker, Iyer et al. 2011, Zhang, Lee et al. 2011) a method for removing ammonia from Product 2 was tested and optimised. The tests at laboratory scale comprised the injection of compressed air into the heated product solution. Results of pre-experiments pointed out that higher temperatures and higher injection rates stimulate the removal of ammonia from the aqueous solution of Product 2. For instance, in Figure 4-48 the efficiency of the NH_3-removal depending on the applied temperature is shown. The graphs show that higher temperatures led to a faster removal of ammonia. Furthermore, it was found out that higher flow rates support a fast removal of ammonia additionally, as the data shown Figure 4-49 point out. All in all, by “stripping” Product 2 in this way it was feasible to reduce the NH_3-concentration of Product 2 to 0.1 g/l (cf. concentration at the beginning 10 g/l). Consequently, the unpleasant odour was reduced as well and usage of this product would be possible. Furthermore, analyses of the AA concentrations before and after the stripping process showed only low impacts on the AA concentration (Steinbrecher 2012).

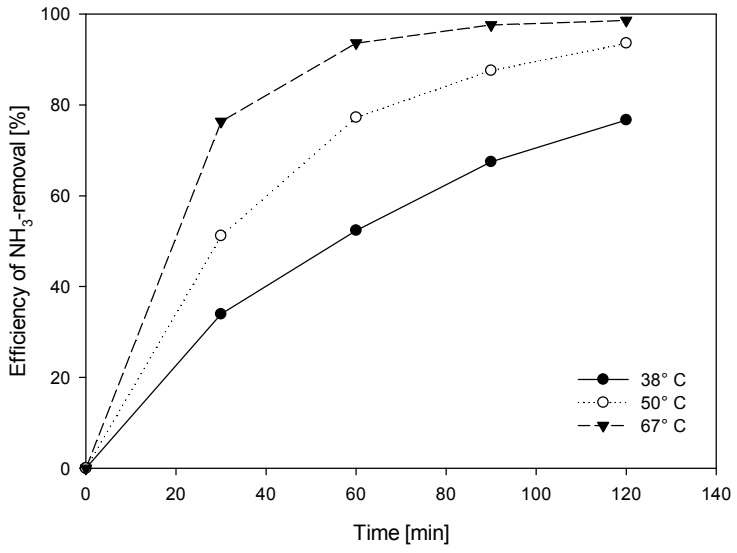


Figure 4-48: Efficiency of the NH_3-removal from Product 2 depending on the temperature (flow rate of compressed air 2 l/min) (Steinbrecher 2012)

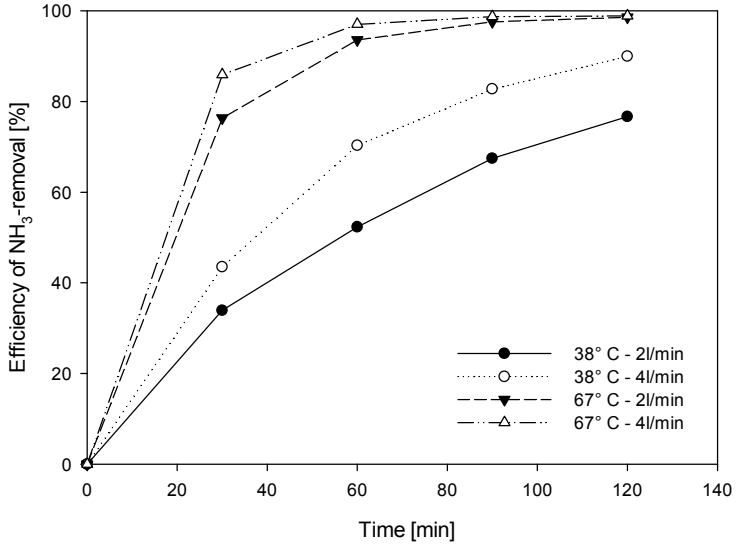


Figure 4-49: Efficiency of the NH_3-removal from Product 2 depending on the temperature and flow rate of compressed air (Steinbrecher 2012)

Hence, for preparing Product 2 for the spray drying test the removal of ammonia was done by heating up the solution to 66 °C and injecting compressed air at a flow rate of 4 l/min.

4.4.3.2 Spray drying

On the one hand, the spray drying experiments were done with stripped Product 2 (dry substance 3.89 %) and stripped Product 2 with an increased dry substance (dry substance 5.41 %). The stripped starting materials contained NH_3 only in low concentrations. On the other hand, one experiment was done using untreated Product 2. That means this starting material still contained ammonia. In all three cases the spray drying test was successful and it was feasible to produce AA powder at lab scale. No problems such as a blockage of the nozzle were observed during the spray drying. Indeed, the starting solutions with low dry substances were used, as the equipment at lab scale is normally applied for drying milk or coffee. At larger scale the use of product solutions containing higher dry substances would be thinkable and due to economic considerations necessary as well. Based on these successful pre-tests at lab scale it can be assumed that the production of AA powder from Product 2 with higher dry substances (40-50 %) analogue to the powder production from Product 1A and 1B, which was demonstrated at the spray drying tower of Prolactal (Hartberg, Austria), would be also feasible.

All AA powders that were produced by these experiments were beige. One powder is shown as an example in Figure 4-50 and some properties of the AA powder are summarised in Table 4-27.

Figure 4-50: AA-powder produced through spray drying of Product 2 from the GRB Upper Austria

Table 4-27: Properties of AA-powders from Product 2

Starting material	Dry substance [%]	Rate of NH_3 [%]
1) Product 2 w/o NH_3	72.6	0.37
2) Product 2 w/o NH_3 and increased dry substance	73.8	0.33
3) Product 2 with NH_3	71.0	0.44

AA composition	1) Product 2 w/o NH_3	2) Product 2 w/o NH_3 (increased DS)	3) Product with NH_3
		AA composition [%]	
Asp	5.89	5.40	5.49
Glu	5.35	5.07	5.58
Thr	2.24	2.09	2.22
Ser	1.46	1.41	1.47
Pro	4.38	3.98	3.94
Gly	3.41	3.29	3.45
Ala	11.8	11.9	12.3
Cys	1.77	2.68	1.88
Val	8.29	10.4	8.29
Met	1,13	2.63	1.32
Ile	6.82	6.46	6.53
Leu	11.7	11.0	11.8
Tyr	1.44	1.38	1.53
Phe	4.15	3.69	4.27
Asn	0.54	0.63	0.65
Trp	n.a.	n.a.	n.a.
GABA	25.0	23.4	24.5
Lys	4.66	4.59	4.76
His	n.a.	n.a.	n.a.
Arg	n.a.	n.a.	n.a.

Independent from the starting material for the spray drying, AA powders with similar optical and sensory properties were obtained. The texture of these AA powders was comparable to the AA powders generated from Product 1A and 1B produced at spray drying tower of Prolactal (Hartberg, Austria). All powders are hygroscopic, but smell more intensive than the powders made from Product 1A and 1B. Ammonia odour was no longer noticed, as confirmed by the results from NH_3-analyses as well. In Table 4-27 it is shown that through the spray drying process the NH_3-content can be minimised (<1 %) independent from the NH_3-concentration in the starting material. Therefore, it is feasible to remove ammonia directly through the spray drying process without any pre-treatment. Indeed, for up-scaling

safety-related questions and further processing of the exhaust air would have to be cleared-up.

Considering the AA composition of the powders obtained, in all three experiments quite similar results were observed. The most frequent AA was always γ-amino butyric acid (rate of about 25 %) followed alanine, leucine, isoleucine and valine. In contrast to the powders made from Product 1A and 1B lysine was also detected, which would display a benefit for applications as feed stuff.

Next to the optical and sensory properties of the AA powders yields were taken into account. Results concerning the yield per input and per amount of time are summarised in Table 4-28. As discovered by other experiments at the spray drying facility, about 50 % of the produced powder is transferred to the collection vessel. The remaining 50 % of the powder is lost in other parts of the drying system. Therefore, corrected results are shown in Table 4-28 as well.

Table 4-28: Spray drying experiments with Product 2 - yields per starting material and per time

Starting material	**1**	**2**	**3**
Collected powder [g]	26.0	15.8	6.74
Sum of used liquid starting material [g]	1373.3	649.9	374.7
Dry substance starting material [%]	3.89	5.41	2.86
Dry substance starting material per kg [g/kg]	38.9	54.1	28.6
Dry substance powder [%]	72.6	73.8	71.0
Duration of the experiment [h]	3.5	2.5	2.0
Yield per kg starting material [g/kg]	18.9	24.2	18.0
Yield per kg starting material incl. losses in the system [g/kg]*	37.9	48.5	36.0
Corr. yield per kg starting material w/o moisture [g/kg]	27.5	35.8	25.5
Corr. yield referred to input dry substance [%]	70.7	66.1	89.3
Yield per h [g/h]	7.43	6.30	3.31
Yield per h incl. losses in the system [g/h]*	14.9	12.6	6.63
Corr. yield per h w/o moisture [g/h]	10.8	9.3	4.7
Input starting material per h [g/h]	392.4	256.0	184.6
Input starting material per min [g/min]	6.50	4.33	3.08

***** corrected yield (factor 2)

Quite similar yields (corr.) per kilogram of starting material were observed by using the first (37.9 g/kg) and third (36.0 g/kg) starting material. Due to the higher dry substance in the starting material the yield using starting material 2 (48.5 g/kg) was higher and this experiment was most effective. However, referring the yielded dry substance per run to the introduced dry substance the best results were observed with the third starting material. In this experiment about 90 % of the introduced dry substance was recovered in the powder.

Furthermore, the yield per time was investigated as well. These results mainly depended on a compromise between the introduction of a sufficient amount of input material and avoiding any condensate formation within the spraying chamber. Using the first starting material it was possible to apply a flow rate of 6.5 g/min without condensate formation, which led to the highest amount of input material per hour. In the second experiment, in turn, a decreased yield per hour was observed as the formation of condensate already occurred at lower flow rates. The lowest flow rates had to be applied in the experiment using the third starting material contaminated with ammonia. In this case a condensate formation was observed much earlier at nearly constant ambient conditions. It can be assumed that these differences are based on the different properties of the input materials. For example, Saß (Saß 2010)

showed that different solvent compositions and solid concentrations lead to different flow rates until condensations in the spray drying chamber occur.

Summing up, it can be said that the production of an AA-powder from Product 2 at lab-scale is possible. Beige powders with a low content of ammonia can be produced either from pre-treated Product 2 without ammonia or from untreated Product 2 with ammonia. Some preliminary data concerning the yields was collected. For instance, based on these results it can be assumed that higher dry substances per litre of input material lead to higher outputs. The output per time, in turn, mainly depends on the affinity of the input material to form condensate.

4.4.4 Discussion

By evaluating the results of the fractionation experiments it can be concluded that better defined and more attractive AA products from purified grass silage juice were produced. Indeed, some problems occurred during the fractionation experiments. For example, the recovery rate of some steps was low. Next to some usual losses during the experiments themselves, the inhomogeneity of the powder resulted in analytical deviations and therefore in losses too. In addition, it has to be considered that the AA composition of the starting material always depends on the quality of the silage juice and consequently natural variations in the fractions can occur. Nevertheless, the results obtained in the presented experiments concerning achievable product quantities and qualities represent good approximate values. Moreover, the amount of analysed AAs in the fractions was often higher than the analysed dry substance. This led to AA rates per dry substance >100 %. An explanation could be that substances (e.g. not identified dyes) are already volatile at 105 °C and therefore falsify the rate of dry substance. A decomposition of AAs can be excluded, as most AAs decompose at temperatures over 200 °C. In addition, parts of these inconsistencies were based on measurement errors at analytics and sample preparation (±5 %).

Generally, the following improvements and products have been realised through the presented fractionation experiments:

- Decolourisation – production of attractive AA solutions
- Product rich in BCAAs from Product 1A
- Product rich in aspartic acid/glutamic acid from Product 1A
- Product rich in aspartic acid from Product 1A
- Tyrosine precipitate
- Product rich in BCAAs from Product 1B
- Production of an AA powder from Product 2

In the following section the product fractions, the AA composition and their possible applications are discussed:

4.4.4.1 Decolourisation

By applying activated carbon, dyes in the starting material were completely removed and a clear solution was manufactured (see Figure 4-41). Therefore, a more attractive AA solution for further applications was produced. For instance, it would be possible to produce a white AA powder from this solution through spray drying, as white precipitates were already separated from the decoloured solutions in some experiments. This AA powder would still contain different AAs, but by decolouring a more attractive product with a wider scope of application would be generated (e. g. the usage in cosmetics would be easier).

4.4.4.2 BCAA-Product from Product 1A

Table 4-29 shows the AA-composition and some properties of the BCAA-products generated from Product 1A.

Table 4-29: Properties and AA-composition of BCAA-products from Product 1A

Properties/Composition	Product 1A_coloured [%]	Product 1A_decoloured [%]
Yield per litre Product 1A	7.3	2.0
Amino acids		
Leu	33	43
Val	21	16
Ile	19	20
Ala	12	8.0
Cys	5.0	4.0
Pro	5.0	2.0
Met	3.0	4.0
Gly	<2	<1
Thr	<1	-
Ser	<1	<1
Asp	-	<1
Rate AAs per dry substance	>100	>100
Colour	white to yellowish	white

As already mentioned in chapter 4.4.1, similar BCAA-precipitates were produced in both experiments using Product 1A. From 1 l untreated Product 1A (total AA-concentration 38.4 g/l) it was possible to produce 2.82 g of the precipitate. Including an additional decolourisation step the yield per litre decreased to 2 % and therefore 0.65 g of the BCAA-product were yielded per litre. Both precipitates mainly consisted of neutral AAs. The percentage of essential AAs was 77 % (coloured) or 83 % for the decoloured starting material. The prevalent essential AAs in these precipitates were leucine, isoleucine and valine. That means, 73 % or 79 % of all AAs in these precipitates belong to BCAAs. According to other producers of BCAA-products the recommended ratio for an optimal intake of L-leucine, L-isoleucine and L-valine is 2:1:1. Within the presented BCAA-precipitates, BCAA-ratios of 1.7:1.1:1.0 (untreated starting material) and 2.7:1.3:1.0 (decoloured starting material) were reached. Consequently, the BCAA-ratios that were reached differed from the usual ratio. Nevertheless, these precipitates, which consisted 3 or 4 % of the essential AA methionine too, could represent an interesting product. Especially, if the natural source of the product is emphasised. Generally, the production quantity (tons/year) for the BCAA is low (500-1100 t/year), therefore higher prices would be achievable (Drauz, Grayson et al. 2005, Crueger, Crueger 1989).

4.4.4.3 Aspartic acid/glutamic acid-product from Product 1A

Table 4-30 shows the AA-composition and some properties of the aspartic acid/glutamic acid-product generated from Product 1A.

Table 4-30: Properties and AA-composition of aspartic acid/glutamic acid-products from Product 1A

Properties/Composition	Product 1A_coloured [%]	Product 1A_decoloured [%]
Yield per litre Product 1A	1.0	11
Amino acids		
Asp	62	38
Glu	22	34
Thr	5.0	9.0
Ser	4.0	7.0
Val	<2	<3
Cys	<2	<1
Phe	<2	<1
Leu	<2	<2
Ala	<2	3.0
Gly	<1	<2
Met	-	<2
Ile	-	<1
Rate AAs per dry substance	92	>100
Colour	light brown	light brown

The precipitates generated from the first elution fraction contained high amounts of the acidic AAs aspartic acid and glutamic acid (84 % coloured starting material, 72 % decoloured starting material). Besides, the precipitates included some amounts of threonine and serine. Glutamic acid or glutamate, which is used particularly as a flavour enhancer, is one of the most produced AAs in the world. Moreover, the world production of aspartic acid is high as well, as aspartic acid is used for the production of Aspartame, a popular sweetener. Taking into account these facts and the different AAs, which are still in the precipitate, combined with low yields, it would not make sense to gain these aspartic acid/glutamic acid-precipitates. An application of the starting material containing high amounts of glutamic acid and aspartic acid would be more effective.

4.4.4.4 Aspartic acid product from Product 1A

Table 4-31 shows the AA-composition and some properties of the Asp-product generated from Product 1A.

Table 4-31: Properties and AA-composition of Asp-products from Product 1A

Properties/Composition	Product 1A_coloured [%]	Product 1A_decoloured [%]
Yield per litre Product 1A	5.3	5.1
Amino acids		
Asp	91	93
Glu	9.0	7.0
Rate AAs per dry substance	>100	>100
Colour	white to yellowish	white

By concentrating the second elution fraction, precipitates with a high proportion of aspartic acid (<90%) were generated. In contrary to the precipitates obtained from the first elution fraction, a precipitate with a quite high level of aspartic acid was produced through simple process steps from a renewable resource. After a marginal cleaning process applications in cosmetics or for functional food could be possible.

4.4.4.5 Tyrosine from Product 1B

Table 4-32 shows the AA-composition and some properties of the tyrosine precipitate generated from Product 1B.

Table 4-32: Properties and AA-composition of tyrosine from Product 1B

Properties/Composition	Product 1B [%]
Yield per litre Product 1A	0.3
Amino acids	
Tyr	97
Phe	3.0
Rate AAs per dry substance	>100
Colour	dark grey

By a simple filtration and recrystallisation step a precipitate consisting of 97 % tyrosine and 3.0 % phenylalanine was generated. Indeed, the yield per litre Product 1B of the non-essential, aromatic AA tyrosine was quite low. Generally, only a small amount of tyrosine, which is mainly used for pharmaceuticals, is produced on the world market. Hence, tyrosine belongs to more expensive AAs. Due to the higher prices for this AA and the simple generation step from Product 1B, the production of tyrosine from Product 1B could be, despite the low yield, an interesting procedure.

4.4.4.6 BCAA-Product from Product 1B

Table 4-33 shows the AA-composition and some properties of the BCAA-products generated from Product 1B.

Table 4-33: Properties and AA-composition of BCAA-products (recrystallised) from Product 1B

Properties/Composition	Product 1B_coloured [%]	Product 1B_decoloured [%]
Yield per litre Product 1A	9.4	7.7
Amino acids		
Leu	54	59
Ile	21	21
Met	11	11
Val	7.0	6.0
Ala	3.0	-
Phe	3.0	<2
Glu	<1	<2
Cys	<1	<1
Thr	-	<1
Rate AAs per dry substance	>100	>100
Colour	white to yellowish	white

In both experiments using Product 1B BCAA-products with a similar AA composition were generated. From 1 l untreated Product 1B (total AA-concentration 58.3 g/l) it was possible to produce 5.50 g of the precipitate. Including an additional decolourisation step the yield per litre decreased to 7.7 % and therefore 4.5 g were yielded. Both precipitates mainly consisted of neutral AAs. The percentage of essential AAs was 96 % (coloured) and 99 %, respectively, for the decoloured starting material. In contrary to the BCAA-product obtained from Product 1A, the prevalent AAs in these precipitates are leucine, isoleucine and methionine. The amount of valine is lower than in the other BCAA-product. Consequently, other ratios, which

differ more strongly from the ideal ratio of leucine : isoleucine : valine were found: 7.7:3.0:1.0 in the experiment using untreated starting material and 9.8:3.5:1.0 for the decoloured material. In addition to leucine, isoleucine and valine both precipitates consisted of a high amount of the essential AA methionine. However, the present precipitates would be, despite differing ratios, an interesting product for numerous applications.

4.4.4.7 Production of an AA powder from Product 2

By removing the ammonia on the one hand and by producing an AA powder from the product solution, on the other hand, it was feasible to produce a further usable product from the *GBR Upper Austria*. Due to the proportion of the essential AA L-lysine, a possible area of application for this AA powder would be the feed stuff field. Additionally, the application of further cleaning processes, e.g. the decolourisation through activated carbon or recrystallisation steps, could be considered to increase the attractiveness of the product and to extend the field of application for this fraction from the *GBR Upper Austria*.

5 Conclusion and outlook

Summing up the investigations at the *GBR Upper Austria*, it can be noted that it was feasible to produce purified AA product solutions from grass silage juice by applying the assumed IE process on a pilot scale. In particular, it was possible to produce three different AA product solutions:

- Product 1A: enriched with acidic AAs
- Product 1B: enriched with neutral AAs
- Product 2: enriched with neutral AAs, alkaline AAs and GABA, but contaminated with ammonia

On average, 7.76 kg in total or 0.54 eq of AAs per litre of resin were yielded in IE experiments using NF2 Retentate (basic process). 6.33 kg in total or 0.47 eq of AAs per litre of resin were yielded, in turn, in IE experiments when using UF Permeate (innovative process). In general, it was calculated that the usage of 77 % of the AAs, which were yielded per batch run in total, would be economically worthwhile. The remaining 33 % of AAs would be lost during the process, as some AAs were found, for instance, in the pre- and post product fractions or on column 1 and 2. Due to low yields, the elution of these columns and the extraction of the AAs would be too costly and therefore would be skipped.

Furthermore, the results of the experiments showed that by applying the current method of loading, the yield per batch run depended mostly on the ratio between inorganic cations and AAs in the feed. The breakthrough of inorganic cations was used as an indicator for stopping the loading and the amount of AAs, which was brought onto the columns, was a result thereof. For that reason, higher amounts of AAs in the feed referred to the amount of inorganic cations in the feed, led to higher AA yields per batch run. Based on these findings, it was also possible to explain the different yields observed by applying either UF Permeate or NF2 Retentate. Due to different pre-treatment steps, the ratio of inorganic cations and AAs in NF2 Retentate was shifted to the AAs. On average, this fact resulted in higher AA yields per batch run when using NF2 Retentate. Nevertheless, considering the overall results and observation of the whole process applied at the *GBR Upper Austria* the innovative process was preferred, as this process was simpler and fewer losses, purer products and a lowered energy consumption were achieved (Ecker 2012).

In order to compare the AA pattern of the starting materials UF Permeate and NF2 Retentate, only some fluctuations were observed. Some AAs were enriched and some were depleted during the NF process, but the overall AA pattern remained stable. However, more AAs were lost in the pre-treatment steps when applying the basic process. In total 77 % were transported from the raw silage juice to the IE feed in this case. By applying the innovative process, in turn, it was possible to transfer 86 % to the feed for the IE process.

Furthermore, the products obtained in the IE process were compared regarding the starting material that was applied. Only slight differences in the products were observed when using either UF Permeate or NF2 Retentate. In general, quite similar distributions of the AAs on the columns and similar AA compositions in the product fractions of were observed. Indeed, the direct application of untreated, raw silage juice in the IE device is limited, as the products obtained in this case were cloudy and therefore less attractive. Furthermore, the effort of rinsing was considerably increased when using raw silage juice.

Next to the effects of different starting materials, possible impacts of other parameters, such as different flow rates, the pH of the feed and the AA concentrations of the feed, were also investigated. Thus, it can be summarised that these parameters did not affect the present IE process at all. Moreover, during the experiments two strong cation exchange resins with different particle sizes were tested. The mean particle size of Resin I was 650 µm and Resin

II consisted of particles with a mean size of 350 µm. Before the application of Resin II, it was assumed that the separation between inorganic cations and AAs as well as the separation between individual AAs and AA groups would be improved by using this resin. Indeed, no considerable improvements were observed regarding the separation of substances when applying Resin II. Considering, in turn, the yield per batch run, the amount of AAs yielded when using Resin II was slightly higher on average (8.30 kg) within the observed experiments than in experiments using Resin I (7.90 kg). This observation was also confirmed by calculating the AA adsorption efficiency per litre of resin: Resin I adsorbed 0.56 eq AAs per litre of resin and Resin II adsorbed 0.58 eq AAs per litre. Therefore, summing up these findings it can be said that the usage of both resins would be feasible for the AA generation from pre-treated grass silage juice, but the usage of Resin II would be slightly more effective. Thus, Resin II did not fulfil previous assumptions regarding an improvement of the separation between the substances and overlapping was still oberserved. Furthermore, despite a considerably increased capacity per litre of resin, yields increased only slightly.

Calculations of future scenarios of the *GBR Upper Austria* showed that it would possible, to produce 24 kg of AAs each day by applying flow rates of 4 l/min. As expected, the yield was doubled when applying flow rates of 8 l/min. Indeed, for these outputs some adaptions of the current IE would be necessary and the whole process flow would also needed to be optimised. Next to the investigations into the impact of the applied flow rates, the effects of the elution of column 3 on the water and chemical consumption as well as on the duration of each batch run were studied. By skipping the elution of column 3, it would be possible to reduce the overall water supply needed per batch run by almost 10 %. Indeed, only slight differences in the RO-water amount needed per kilogram of AAs would be observed, as the AA yield would be lower as well. Furthermore, the consumption of ammonia would be lowered by 27 % in total per batch run and by 19 % per yielded kilogram of AAs. Therefore, the scenario where column 3 is not eluted would be preferential. Nevertheless, skipping the elution of column 3 would also reduce the available amount of Product 2, which is enriched with lysine and γ-amino butyric acid. Therefore, in order to make a final decision the potential of this product has to be investigated in detail as well. In order to provide an overall picture of freights and streams occurring in the IE process, a detailed overview of all output streams eluted from the IE during loading, elution and regeneration was summarised. Furthermore, further uses or treatments needed for the individual streams were discussed. For instance, some of the streams that are currently discarded could be recycled and therefore lead to considerably reduced water consumption per batch run.

Next to experiments on a pilot scale, investigations regarding the polishing of the AA products obtained at the *GBR Upper Austria* were performed. Generally, it was possible to produce AA powders from Product 1A and 1B by applying a spray drying device. However, a preliminary review of the potential markets showed that a polishing of the AA products that were obtained would be desirable in order to gain more defined products or even pure substances. Therefore, the polishing of the AA products was tested at laboratory scale. For instance, it was found that the colouration of the products can be removed by applying powdered, activated coal. Furthermore, fractionation routes for Product 1A and Product 1B were developed. Resulting, among others, in an aspartic acid powder, a tyrosine precipitate and powders enriched with BCAAs. Next to Product 1A and 1B also Product 2 was improved, as the removal of ammonia was implemented through a so-called stripping process. Moreover, it was also feasible to produce an AA powder from Product 2 at laboratory scale.

Summing up the research performed, it can be declared that new findings regarding the downstreaming of grass silage juice and, in particular, regarding the production of AAs on a pilot scale were achieved. Nevertheless, further improvements would be desirable for economical reasons. For instance, futher investigation should be done regarding the choice of the resin, as the yield per litre of resin and the routine operation capacity was quite low. Possibly, more adequate resins are available on the market for use at the *GBR Upper*

Austria or the combination of different types of resins would be more efficient (e. g. a weak acid cation exchanger for removing calcium and magnesia). Additionally, a more efficient removal of inorganic cations before the feeds are applied at the IE would also be helpful. Indeed, some precipitate experiments in order to reduce the amount of calcium in the raw silage juice had been already tested, but the experiments were not successful. Furthermore, the polishing of the AA products could be further improved. For instance, it would be necessary to upscale the experiments, which were implemented at laboratory scale, in order to provide a comprehensive route from the raw material grass silage juice to the final, high-quality amino acid product.

6 References

AJINOMOTO EUROLYSINE S.A.S., 30.09.2011, 2011-last update, Technical data Sheet of L-Tryptophane, L-Threonine, L-Valine, L-Lysine HCl, LLB 50 (Feed Grade). Available: http://ajinomoto-eurolysine.com/ [19.07.2012, 2012].

ANDERSEN, M. and KIEL, P., 2000. Integrated utilisation of green biomass in the green biorefinery. *Industrial Crops and Products,* **11**, pp. 129-137.

ANIMOX GMBH, Available: http://www.animox.de/index.php?menuid=1 [30.07.2012, 2012].

ATKINS, P.W., 2001. *Physikalische Chemie.* 3 edn. Weinheim: WILEY-VCH Verlag GmbH.

BITAR, M.C., 1991. *Selective extraction of amino acids.* U.S.A.: US 5049279.

BLANSHARD, K.C., BRADFORD, H.F., DODD, P.R. and THOMAS, A.J., 1975. A simple semi-automatic apparatus for the purification of amino acids from extracts of tissues and body fluids. *Analytical Biochemistry,* **67**, pp. 233-244.

BODY ATTACK SPORTS NUTRITION GmbH & Co KG, Available: www.body-attack.de [30.07.2012, 2012]

BOISEN, S., HVELPLUND, T. and WEISBJERG, M.R., 2000. Ideal amino acid profiles as a basis for feed protein evaluation. *Livestock production science,* **64**, pp. 239-251.

BREGENDAHL, K., 2008. Amino acid nutrition of modern laying hens, N. ZIMMERMANN, ed. In: *6th Mid-Atlantic Nutrition Conference, Timonium Maryland*, 26.-27.03.2008 2008.

BUCHANAN, D.L., 1957. Preparative isolation of amino acids by carrier displacement chromatography on ion exchange resins. *Journal of Biological Chemistry,* **229**, pp. 211-229.

BUCHGRABNER, K., 1998. *Nutzung und Konservierung des Grünlandfutters im österreichischen Alpenraum.* 31. Irdning: Bundesanstalt für alpenländische Landwirtschaft Gumpenstein.

CANGZHOU CITY JINJIAN GELATIN Co Ltd., Available: http://jinjiangelatin.en.alibaba.com [30.07.2012, 2012]

CBH QINGDAO CO Ltd, Available: http://cbhcn.en.alibaba.com/product/314690749-218673194/Lysine_Sulphate_65_70_Feed_Grade_.html [30.07.2012, 2012]

CHERUBINI, F., 2010. The biorefinery concept: Using biomass instead of oil for producing energy or chemicals. *Energy Conversion and Management,* **51**, pp. 1412-1421.

CHIANG, W., SHIH, C. and CHU, Y., 1999. Functional properties of soy protein hydrolysates produced from a continous membrane reactor system. *Food Chemistry,* **65**(2), pp. 189-194.

CHIBATA, I., KAKIMOTO, T. and KATO, J., 1965. Enzymatic production of L-alanine by *Pseudomona dacunhae*. *Applied microbiology,,* pp. 638-645.

CLARK, H.M., ALVES, C.C.C., FRANCA, A.S. and OLIVEIRA, L.S., 2012. Evaluation of the performance of an agricultural residue-based activated carbon aiming a removal of

phenylalanine from aqueous solutions. *LWT - Food Science and Technology,* **49**, pp. 155-161.

CRUEGER, W. and CRUEGER, A., 1989. *Biotechnologie - Lehrbuch der angewandten Mikrobiologie.* 3. edn. München: R. Oldenbourg Verlag GmbH.

DALL, M., MARTENS, M., POLL, L., NIELSEN, P.M., FLYGE, H. and LARSEN, L.M., 1998. Chemical and Sensory Characterization of Hydrolyzed Vegetable Protein, a Savory Flavoring. *Journal of Agricultural and Food Chemistry,* **46**(2), pp. 481-489.

DANNER, H., MADZINGAIDZO, L., HOLZER, M., MAYRHUBER, L. and BRAUN, R., 2000. Extraction and purification of lactic acid from silages. *Bioresource Technology,* **75**, pp. 181-187.

DATTA, R. and HENRY, M., 2006. Lactic acid: recent advances in products, processes and technologies - a review. *Journal Chem Technol Biotechnol,* **81**, pp. 1119-1129.

DEBABOV, V.G. and ET AL., 1994. *Novel L-threonine-producing microbacteria and a method for the production of L-threonine. Europe: EP-A 593 792*

DORFNER, K., ed, 1991. *Ion Exchangers.* Berlin: Walter De Gruyter.

DOW CHEMICAL COMPANY, Dowex Marathon C Resin - Product Data Sheet [Homepage of Dow Chemical Company]. Available: http://www.dowwaterandprocess.com/products/ix/dx_mar_c.htm [08/01, 2012].

DOW CHEMICAL COMPANY, Dowex Monosphere C-350 - Product data sheet [Homepage of Dow Chemical Company]. Available: http://www.dowwaterandprocess.com/products/ix/dx_mn_c350.htm [08/01, 2012].

DRAUZ, K., GRAYSON, I., KLEEMANN, A., KRIMMER, H., LEUCHTENBERGER, W. and WECKBECKER, C., 2005. Amino Acids. *Ullmann's Encyclopedia of industrial chemistry.* 7th edn. Weinheim: Wiley-VCH Verlag GmbH & Co. KGaA, .

DUS, K., LINDROTH, S., PABST, R. and SMITH, R.M., 1966. Continous amino acid analysis: Elution programming and automatic column selction by means of a rotating valve. *Analytical Biochemistry,* **14**, pp. 41-52.

ECKER, J., SCHAFFENBERGER, M., KOSCHUH, W., MANDL, M., BÖCHZELT, H.G., SCHNITZER, H., HARASEK, M. and STEINMÜLLER, H., 2012. Green Biorefinery Upper Austria - Pilot Plant operation. *Separation and Purification Technology,* **96**, pp. 237-247.

ECKER, J., 2012. *Membrantrennverfahren zur Gewinnung von Wertstofflösungen fermentativen Ursprungs im Pilotmaßstab*, Dissertation, Institut für Verfahrenstechnik, Umwelttechnik und Technische Biowissenschaften, University of Technology Vienna.

EGGELING, L., PFEFFERLE, W. and SAHM, H., 2001. Amino Acids. In: CAMBRIDGE UNIVERSITY PRESS, ed, *Basic Biotechnology.* Cambridge: pp. 201-303.

EGGELING, L., MORBACH, S. and SAHM, H., 1997. The fruits of molecular physiology: engineering the L-isoleucine biosynthesis pathway in Corynebacterium glutamicum. *Journal of Biotechnology, **56** (3)* , pp. 167-182.

EUROPÄISCHE GEMEINSCHAFTEN, 1998. *Richtlinie 98/64/EG der Kommission zur Festlegung gemeinschaftlicher Analysemethoden für die Bestimmung von Aminosäuren, Rohfetten und Olaquindoxin in Futtermitteln.* Richtlinie edn. Europäische Gemeinschaften.

EVONIK INDUSTRIES, 09/2011, 2011-last update, Product information TrypAMINO, ThreAMINO, MetAMINO. Available: http://feed-additives.evonik.com/product/feed-additives/en/about/downloads/product-information/pages/default.aspx [21.07.2012, 2012].

EVONIK INDUSTRIES, 2009. *Und was essen wir morgen...?* Evonik Industries. Available: http://corporate.evonik.de/de/content/product-stories/Pages/tierernaehrung.aspx [21.07.2012, 2012]

FLATNITZER, F., 2009. *Gute Silagen sind kein Zufall, Schlechte Silagen sind kein Naturgesetz - Ein Leitfaden für die Praxis.* Bonn, Deutschland: ADCON Europe.

GAREM, A., DAUFIN, G., MAUBOIS, J.L. and LÉONIL, J., 1997. Selective separation of amino acids with a charged inorganic nanofiltration membrane: effect of physiochemical parameters on selectivity. *Biotechnology and Bioengineering,* **54**(4), pp. 292-302.

GERBER, L., 1942. *Amino acid separation from proteins.* Canada: CA 405274

GRUENE-BIORAFFINERIE.AT GMBH, 2008. *Verfahren zur Behandlung eines Stoffstroms.* Austria: AT 504 206.

GRYTA, M., KARAKULSKI, K., TOMASZEWSKA, M. and MORAWSKI, A., 2005. Treatment of effluents from the regeneration of ion exchangers using the MD process. *Desalination,* **180**, pp. 173-180.

HABE, H., YAMANO, N., TAKEDA, S., KATAOKA, S. and NAKAYAMA, A., 2010. Use of electrodialysis to separate and concentrate GABA. *Desalination,* **253**, pp. 101-105.

HAMILTON, B.K., HSIAO, H., SWANN, W.E., ANDERSON, D.M. and DELENTE, J.J., 1985. Manufacture of L-amino acids with bioreactors. *Trends in Biotechnology, **3** (3), pp. 186-189*.

HAMILTON, P.B., 1958. Ion exchange chromatography of amino acids. *Anal. Chem.,* **30**(5), pp. 914.

HARTWICH, T.S., 2008. *Untersuchungen zur biotechnischen Methionin-Produktion in Corynebacterium glutamicum ATCC 13022: Entwicklung einer Screening-Strategie*, Dissertation, Fakultät für Lebenswissenschaften der Technischen Universität Carolo-Wilhemina zu Braunschweig.

HASHIMOTO, S. and KATSUMATA, R., 1998. L-Alanine Fermentation by an Alanine Racemase-Deficient Mutant of the DL-Alanine Hyperproducing Bacterium Arthrobacter oxydans HAP-1. *Journal of Fermentation and Bioengineering, **86** (4)* , pp. 385-390.

HELFFERICH, F.G., 1995. *Ion exchange.* New York: Dover Publications.

HERMANN, T., 2003. Industrial production of amino acids by coryneform bacteria. *Journal of Biotechnology,* **104**, pp. 155-172.

HOPPE, B. and MARTENS, J., 1984. Aminosäuren - Herstellung und Gewinnung. *Chemie in unserer Zeit,* **18**(3), pp. 73-86.

IEA BIOENERGY, Task 42 Biorefinery. Available: http://www.iea-bioenergy.task42-biorefineries.com/activities/classification/ [02/21, 2013].

IIMURE, T., KIHARA, M., HIROTA, N., ZHOU, T., HAYASHI, K. and ITO, K., 2009. A method of production of gamma-amino butyric acid (GABA) using barley bran supplemented with glutamate. *Food Research International,* **42**, pp. 319-323.

INTRACHEM BIO DEUTSCHLAND, 2005-last update, Product data sheet - Lysodin Spurennährstoffdünger. Available: http://www.intrachem-bio.de/index.htm [27.07.2012, 2012].

IZUMI, Y., YOSHIDA, T., MIYAZAKI, S.S., MITSUNAGA, T., OHSHIRO, T., SHIMAO, M., MIYATA, A. and TANABE, T., 1993. L-Serine production by a methylotroph and its related enzymes. *Applied Microbiological Biotechnology,* ***39***, pp. 427-432.

JAKUBKE, H. Aminosäuren Peptide Proteine. 3. ed. Berlin: Akademie-Verlag; 1982.

JONES, I.L. and CARTA, G., 1993. Ion exchange of amino acids and dipeptides on cation resins with varying degree of cross linking - 2. Intraparticle transport. *Ind. Eng. Chem.,* **32**(1), pp. 117-125.

KAMM, B., 2008. *Biomasse-Wirtschaft und Bioraffinerie-Systeme.*

KAMM, B., GRUBER, P., R. and KAMM, M., eds, 2006. *Biorefineries - Industrial Processes and Products: Status Quo and Future Directions, Volume 1.* 1 edn. Weinheim, Germany: WILEY-VCH Verlag GmbH & Co KGaA.

KAMM, B., HILLE, C. and SCHÖNICKE, P., 2010. Green biorefinery demonstration plant in Havelland (Germany). *Biofuels, Bioproducts and Biorefining,* **4**, pp. 253-262.

KAMM, B. and KAMM, M., 2004. Principles of biorefineries. *Applied Microbiological Biotechnology,* **64**, pp. 137-145.

KAMM, B. and KAMM, M., 1999. The green biorefinery - Principles, Technologies and Products, 2nd International Symposium Green Biorefinery, SUSTAIN, VEREIN ZUR KOORDINATION VON FORSCHUNG ÜBER NACHHALTIGKEIT, October, 13-14 1999, pp. 46-69.

KATSURADA, N., UCHIBOR, H. and TSUCHIDA, T., 1988. *L-Valine producing microorganisms and a process for producing L-valine by fermentation.* Europe: EP 278 123.

KEDEM, O., 1977. *Electrodialysis process for the separation of non-essential amino acids from derivatives thereof.* Israel: IL 52687.

KNAPP, H., 2005. *L-Phenylalanin. from RÖMPP-ONLINE, Stuttgart: Georg-Thieme Verlag KG*

KNAPP, H., 2005. *L-Tryptophan. from RÖMPP-ONLINE, Stuttgart: Georg-Thieme Verlag KG*

KNAPP, H., 2004. *Glycin. from RÖMPP-ONLINE, Stuttgart: Georg-Thieme Verlag KG*

KNAPP, H., 2004. *Histidin. from RÖMPP-ONLINE, Stuttgart: Georg-Thieme Verlag KG*

KNAPP, H., 2004. *L-Threonin. from RÖMPP-ONLINE, Stuttgart: Georg-Thieme Verlag KG*

KNAPP, H., 2004. *Methionin. from RÖMPP-ONLINE, Stuttgart: Georg-Thieme Verlag KG*

KNAPP, H., 2004. *Valin. from RÖMPP-ONLINE, Stuttgart: Georg-Thieme Verlag KG*

KOSCHUH, W., THANG, V.H., KRASTEVA, S., NOVALIN, S. and KULBE, K.D., 2005. Flux and retention behaviour of nanofiltration and fine ultrafiltration membranes in filtrating juice from a green biorefinery: A membrane screening. *Journal of Membrane Science,* **261**, pp. 121-128.

KROMUS, S., NARODOSLAWSKY, M. and KROTSCHECK, C., 2002. *Grüne Bioraffinerie - Integrierte Grasnutzung als Eckstein einer nachhaltigen Kulturlandschaftsnutzung. Berichte aus Energie- und Umweltforschung,* Vienna: Federal Ministry for Transport, Innovation and Technology, Austria, 18/2002.

KROMUS, S., WACHTER, B., KOSCHUH, W., MANDL, M., KROTSCHECK, C. and NARODOSLAWSKY, M., 2004. The Green Biorefinery Austria - Development of an Integrated System for Green Biomass Utilization. *Chem. Biochem. Eng. Q.,* **18**(1), pp. 7-12.

KUMAR, D. and GOMES, J., 2005. Methionine production by fermentation. *Biotechnology Advances,* **23**, pp. 41-61.

KUSUMOTO, I., 2001. Industrial Production of L-Glutamin. *Journal of Nutrition, **131** (9), pp. 25525-25555*.

KYTE, J. and DOOLITTLE, R.F., 1982. A simple method for displaying the hydrophathic character of a protein. *Journal of Molecular Biology,* **157**, pp. 105-132.

LEBOSOL-DÜNGER GMBH, 24.07.2012, 2012-last update, Product data sheets of Aminosol-PS, Aminosol und Bio-Aminosol. Available: http://www.lebosol.de/index.php [27.07.2012, 2012].

LEISS, S., VENUS, J. and KAMM, B., 2010. Fermentative Production of L-Lysine-L-lactate with Fractionated Press Juices from the Green Biorefinery. *Chem. Eng. Technol.,* **33**(12), pp. 2102-2105.

LEUCHTENBERGER, W., 1996. Amino Acids - Technical Production and Use. In: H. REHM and G. REED, eds, *Biotechnology.* 2. edn. Weinheim: VCH Verlagsgesellschaft mbH, pp. 465-502.

LEUCHTENBERGER, W., HUTHMACHER, K. and DRAUZ, K., 2005. Biotechnological production of amino acids and derivatives: current status and prospects. *Applied Microbiological Biotechnology,* **69**, pp. 1-8.

LEUCHTENBERGER, W. and PLÖCKER, U., 1988. Herstellung von Aminosäuren mit Hilfe biotechnologischer Methoden. *Chem.-Ing.-Tech.,* **60**(1), pp. 16-23.

LEWATIT, 13/10/2011, 2011-last update, Product information Lewatit S6368 A [Homepage of Lewatit]. Available: http://www.lenntech.com/Data-sheets/Lewatit-S-6368-A-L.pdf [08/01, 2012].

LINDORFER, J., 2009. Was ist eine Bioraffinerie? *Energie Info - Energie Institut an der Johannes Kepler Universität Linz,* **2**.

LOPES, D.C.F., DELVIVO, F.M. and SILVESTRE, M.P.C., 2005. Use of activated carbon for removing phenylalanine from reconstituted skim milk powder hydrolysates. *LWT,* **38**, pp. 447-453.

LÜTKE-EVERSLOH, T. and SANTOS, N.S., 2007. Perspectives of biotechnological production of L-tyrosine and its applications. *Applied Microbiological Biotechnology,* ***77***, pp. 751-762.

MAISON, W. and KNAPP, H., 2007. *4-Hydroxyprolin. from RÖMPP-ONLINE, Stuttgart: Georg-Thieme Verlag KG .*

MAISON, W. and KNAPP, H., 2007. *L-Glutamin. from RÖMPP-ONLINE, Stuttgart: Georg-Thieme Verlag KG*

MANDL, M., 2010. Status of green biorefining in Europe. *Biofuels, Bioeproducts & Biorefining,* **4**, pp. 268-274.

MATSUDA, M. and YOSHIDA, Y., 1990. *Process for refining amino acids,* U.S. A.: US 4927541.

MAYENO, A.N. and GLEICH, G.J., 1994. Eosinophilia-myalgia syndroma and tryptophan production: a cautionary tale. *Trends in Biotechnology,* ***12*** *(9)* , pp. 346-352.

MITSUBISHI CHEMICAL COMPANY, 1997. *Relite - Diaion ion exchange and adsorbent resins; Column test procedures.*

MOORE, S. and STEIN, W.H., 1951. Chromatography of amino acids on sulfonated polystyrene resins. *Journal of Biological Chemistry,* **192**, pp. 663-681.

NEGRO, M., GIARDINA, S., MARZANI, B. and MARZATICO, F., 2008. Branched-chain amino acid supplementation does not enhance athletic performance but affects muscle recovery and the immune system. *Journal of sports medicine and physical fitness,* **48**(3), pp. 347-351.

NIESSNER, R., ed, 2010. *Höllwasser - Nutzung im Kreislauf Hygiene, Analyse und Bewertung.* 9 edn. Berlin/New York: Walter de Gruyter & Co.

NOVALIN-CANOY, P., 2011. *Grüne Bioraffinerie Phase III - Marktstudie zum Thema Aminosäuren und deren Anwendungen.* Vienna: Federal Ministry for Transport, Innovation and Technology.

OGAWA, J. and SHIMIZU, S., 2002. Industrial microbial enzymes: their discovery by screening and use in large-scale production of usefuel chemical in Japan. *Current Opinion in Biotechnology,* , pp. 367-375.

O'KEEFFE, S., SCHULTE, R.P.O., LALOR, S.T.J., O'KIELY, P.O. and STRUIK, P.C., 2011. Green biorefinery (GBR) scenarios for a two-cut silage system: Investigating the impacts of sward botanical composition, N fertilisation rate and biomass availability on GBR profitability and price offered to farmers. *Biomass and Bioenergy,* **35**, pp. 4699-4711.

PETERS-WENDISCH, P., STOLZ, M., ETTERICH, H., KENNERKNECHT, N., SAHM, H. and EGGELING, L., 2005. Metabolic Engineering of Corynebacterium glutamicum for L-Serine Production. *Applied and Environmental Microbiology,* ***71*** *(11)* , pp. 7139-7144.

PROJEKT GRASSA!, , Grassa! Meer waarde uit gras. Available: http://www.grassanederland.nl/ [02/22, 2013].

QI, Y. and HUANG, J., 2002. Displacement chromatography of isomers and therapeutic compounds. *Journal of Chromatography A,* **959**, pp. 85-93.

QUAN, X., WANG, F., ZHAO, Q., ZHAO, T. and XIANG, J., 2009. Air stripping of ammonia in a water-sparged aeorcyclone reactor. *Journal of Hazardous Materials,* **170**, pp. 983-988.

RÖMPP ONLINE, 2013. *Aminobuttersäuren.* 3.29 edn. Stuttgart: Georg Thieme Verlag KG.

RYU, O.H., JU, J.Y. and SHIN, C.S., 1997. Continuous L-cysteine production using immobilized cell reactors and product extractors. *Process Biochemistry, **32***, pp. 201-209.

SASS, A., 2010. *Spray drying of peptides from aqueous organic liquid feeds*, Dissertation, Naturwissenschaftlichen Fakultät der Friedrich-Alexander-Universität Erlangen-Nürnberg.

SHARMA, H.S.S., CARMICHAEL, E., MUHAMAD, M., MCCALL, D., ANDREWS, F., LYONS, G., MCROBERTS, W.C. and HORNSBY, P.R., 2012. Biorefining of perennial ryegrass for the production of nanofibrillated cellulose. *RSC Advances,* **2**, pp. 6424-6437.

SIEKER, T., NEUNER, A., DIMITROVA, D., TIPPKÖTTER, N., BART, H., HEINZLE, E. and ULBER, R., 2010. Grassilage als Rohstoff für die chemische Industrie. *Chemie Ingenieur Technik,* **82**(8), pp. 1153-1159.

SITZMANN, H., 2013. *RÖMPP Online - Calciumsulfate.* 3.30 edn. Stuttgart: Georg-Thieme Verlag KG.

SOUCI, S., FACHMANN, W. and KRAUT, H., eds, 2008. *Die Zusammensetzung der Lebensmittel: Nährwerttabellen.* 7. edn. Medpharm.

STARK, J. and STÜRMER, S., 1996. *Bauschädliche Salze.* Weimar: Bauhaus-Universität.

STEINBRECHER, V., 2012. *Reinigung von Aminosäureprodukten aus einer Grünen Bioraffinerie-Demonstrationsanlage (Bachelorarbeit)*, Fachhochschul-Bachelorstudiengang Biotechnische Verfahren.

STEVENS, A.H., 1952. *Improvements in or relating to a process for manufactur of amino acids.* Great Britain: GB 671042 .

SYNGENTA AGRO AG, 03/2009, 2009-last update, Product data sheet Wuxal Profi. Available: www.syngenta-agro.ch [27.07.2012, 2012].

TAKAGI, H., WADA, M. and NAKAMORI, S., 2005. *L-Cysteine producing bacterium and method for producing L-cysteine.U.S.A.: US 6 946 268 B2*

TAPIERO, H., MATHÉ, G., COUVREUR, P. and TEW, K.D., 2002. II. Glutamine and glutamate. *Biomed Pharmacother, **56**,* pp. 446-457.

THANG, V.H., KOSCHUH, W., KULBE, K.D. and NOVALIN, S., 2005. Detailed investigation of an electrodialytic process during the separation of lactic acid from a complex mixture. *Jounal of Membrane Science,* **249**, pp. 173-182.

THE DORR COMPANY, 1948. *Fractionation of amino acid mixtures.* Great Britain: GB 682762 .

THE DOW CHEMICAL COMPANY, Produkt Information: DOWEX™ MARATHON™ C. Available: http://www.dowwaterandprocess.com/products/ix/dx_mar_c.htm [12.11.2012, 2012].

THE DOW CHEMICAL COMPANY, Produkt Information: DOWEX™ MONOSPHERE™ C-350. Available: http://www.dowwaterandprocess.com/products/ix/dx_mn_c350.htm [11.12.2012, 2012].

THIMANN, K.V., ed, 1980. *Senescence in Plants.* Florida: CRC Press.

THOMSEN, M.H., BECH, D. and KIEL, P., 2004. Manufacturing of Stabilised Brown Juice for L-lysine production - from University Lab Scale over Pilot Scale to Industrial Production. *Chem. Biochem. Eng. Q.,* **18**(1), pp. 37-46.

TIMMER, J.M.K., SPEELMANS, M.P.J. and VAN DER HORST, H. C., 1998. Separation of amino acids by nanofiltration and ultrafiltration membranes. *Separation and Purification Technology,* **14**, pp. 133-144.

U. S. DEPARTMENT OF ENERGY, 1997-last update, Energy, Environmental, and Economics (E3) Handbook - a resource tool to aid the office of industrial technologies. Available: http://infohouse.p2ric.org/ref/36/e3/www.oit.doe.gov/e3handbook/b.shtml.htm [02/21, 2013].

UNIVERSITÄT DES SAARLANDES, , Skript zur Vorlesung Technische Chemie 1 - Ionenaustausch. Available: http://www.uni-saarland.de/fak8/heinzle/de/teaching/Bioverfahrenstechnik_P/TC1_Ionenaustausch.pdf [06/12, 2012].

VOET, D., VOET, J.G. and PRATT, C.W., 2002. *Lehrbuch der Biochemie.* 1 edn. Weinheim: WILEY-VCH Verlag GmbH & Co. KGaA.

WADA, M. and TAKAGI, H., 2006. Metabolic pathways and biotechnological production of L-cysteine. *Applied Microbiologica Biotechnology, **73***, pp. 48-54.

WAGENKNECHT, H. and KNAPP, H., 2006. *Alanin. from RÖMPP-ONLINE, Stuttgart: Georg-Thieme Verlag KG*

WAGENKNECHT, H. and KNAPP, H., 2006. *L-Asparagin. from RÖMPP-ONLINE, Stuttgart: Georg-Thieme Verlag KG*

WAGENKNECHT, H. and KNAPP, H., 2006. *L-Asparaginsäure. from RÖMPP-ONLINE, Stuttgart: Georg-Thieme Verlag KG*

WAGENKNECHT, H. and KNAPP, H., 2006. *L-Cystein. from RÖMPP-ONLINE, Stuttgart: Georg-Thieme Verlag KG*

WALKER, M., IYER, K., HEAVEN, S. and BANKS, C.J., 2011. Ammonia removal in anaerobic digestion by biogas stripping: An evaluation of process alternatives using a first order rate model based on experimental findings. *Chemical Engineering Journal,* **178**, pp. 138-145.

WHEATON, R.M. and LEFEVRE, L.J., 2000. *Dowex Ion Exchange Resins - Fundamentals of Ion Exchange.* Dow Liquid Separations.

WHO, FAO and UNU, 2007. *Protein and Amino acid requirements in human nutrition.* Genenva: World Health Organization.

WHO, FAO and UNU, 1973. *Energy and protein requirements.* World Health Organization.

WILHELM, H. and WURM, K., 1999. *Futterkonservierung und -qualität.* Graz-Stuttgart: Leopold Stocker Verlag.

WINTERS, J.C. and KUNIN, R., 1949. Ion exchange in the pharmaceutical field. *Ind. Eng. Chem.,* **41**, pp. 460.

WUHAN SOLEADO TECHNOLOGY Co Ltd., Available: http://www.wuhansoleado.com [07/2012, 2012]

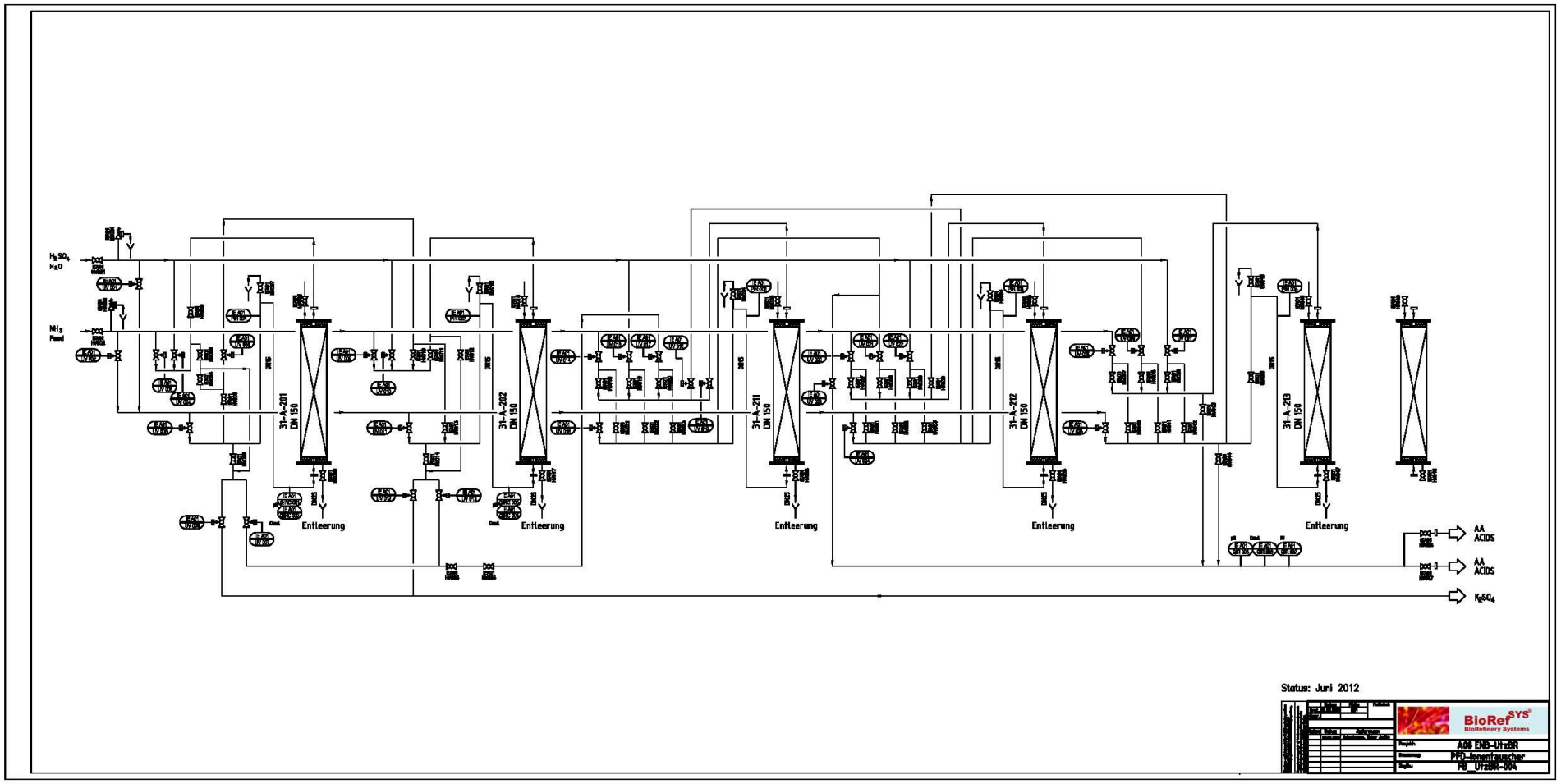
YOSHINAGA, F., FUJISAWA, K., TSUCHIDA, T., KIKUCHI, K., KAWASAKE, K. and OKUMURA, S., 1987. *Verfahren zur Gewinnung von L-Glutamin.* Germany: DE 2 362 288 C2.

ZHANG, L., LEE, Y. and JAHNG, D., 2011. Ammonia stripping for enhanced biomethanization of piggery wastewater. *Journal of Hazardous Materials,* **199-200**, pp. 36-42.

7 List of figures

8 List of tables

9 Appendix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NH_3
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DN 150
31-A-213
DN 150
Entleerung
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K_2SO_4
Status: Juni 2012
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BioRefinery Systems
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FB_UT28R-004

9.1 Spray drying experiments at labaratory scale

9.1.1 1st experiment

Starting material: Product 2 w/o ammonia
Date of experiment: 22.08.2012 from 12:15 to 15:45

Operation parameter (at the beginning)

- Aspirator flow: 100 %
- Inlet temperature: 125 °C
- Spray flow: 450 l/h
- Pump flow: 40 %

Ambient parameters:

- Ambient moisture (at the beginning): 49.1 % rH at 27 °C and 991 mbar air-pressure
- Ambient moisture (at the end): 40.9 % rH at 29.4 °C and 989 mbar air-pressure

Journal 1st experiment

Time [hh:mm]	T_{in} real [°C]	T_{out} [°C]	$T_{exhaust}$ [°C]	exhaust moisture [%]	Comment
12:15	124	74	50.4	26.9	
12:16					Pump flow (PF) to 42 %
12:25					PF to 43 %
12:26					PF to 44 % → condensate
12:27					PF to 43 %
12:30	125	72	51.6	26.9	
12:36					
12:45	124	71	52.1	26.6	
12:57					
13:00	125	74	52.3	25.7	
13:00					PF to 40 %
13:03					
13:15	124	69	52.3	27.1	
13:30	125	69	52.0	27.7	
13:45	125	66	51.8	28.2	
14:00	124	66	51.8	28.0	
14:15	125	66	51.5	28.5	
14:30	125	65	51.7	28.4	
14:45	125	64	51.6	28.6	
15:00	125	64	51.6	29.1	
15:15	125	63	51.7	28.6	
15:30	126	69	52.0	25.8	
15:40					PF to 36 % → condensate
15:45	124	68	52.2	27.9	

9.1.2 2nd experiment

Starting material: Product 2 w/o ammonia and concentrated
Date of experiment: 23.08.2012 from 08:30 to 11:00

Operation parameter (at the beginning)

- Aspirator flow: 100 %
- Inlet temperature: 125 °C
- Spray flow: 400 l/h
- Pump flow: 28 %

Ambient parameters:

- Ambient moisture (at the beginning): 48 % rH at 27.6 °C and 991 mbar air-pressure
- Ambient moisture (at the end): 44.9 % rH at 28.7 °C and 992 mbar air-pressure

Journal 2nd experiment

Time [hh:mm]	T_{in} real [°C]	T_{out} [°]	$T_{exhaust}$ [°C]	Exhaust moisture [%]	Comment
08:30	124	76	50.5	22.2	
08:35					Condensate → PF to 20 %
08:45	125	79	52.2	19.6	
08:49					PF slowly increased to 28 % → ok
09:00	126	76	51.6	21.9	
09:15	125	75	51.2	22.3	
09:30	126	77	51.5	21.5	
10:00	125	74	51.2	22.4	
10:18	126	74	51.2	22.7	
10:30	125	74	51.2	22.5	
10:45	126	73	51.2	22.7	
11:00	126	73	51.0	22.4	

9.1.3 3rd experiment

Starting material: Product 2 untreated
Date of experiment: 23.08.2012 from 12:13 to 14:15

Operation parameter (at the beginning)
- Aspirator flow: 100 %
- Inlet temperature: 125 °C
- Spray flow: 400 l/h
- Pump flow: 23 %

Ambient parameters:
- Ambient moisture (at the beginning): 45.3 % rH at 28.7 °C and 991 mbar air-pressure
- Ambient moisture (at the end): 40.8 % rH at 29.4 °C and 990 mbar air-pressure

Journal 3rd experiment

Time	T_{in} real [°C]	T_{out} [°C]	$T_{exhaust}$ [°C]	Exhaust moisture [%]	Comment
12:13	124	74			
12:32	126	75	51.3	21.1	
12:32					PF to 21 %
12:42					PF to 20 %
12:45	124	75	51.8	20.2	
13:00	124	75	51.9	19.9	
13:15	124	75	51.9	19.7	
13:30	125	76	51.9	19.1	
13:47	124	75	51.9	19.2	
14:02	125	75	52.0	19.6	
14:15	125	75	52.0	19.6	

10 Curriculum Vitae

Personal information	
Surname/ First names	SCHAFFENBERGER, Martina Simone
Date of Birth	17.02.1983
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Nationality	Austrian
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Education	
2009-2013	PHD-programme: Doctoral school of Chemistry Graz University of Technology, Rechbauerstraße 12, A-8010 Graz, Austria
2008-2009	Master thesis: “Balancing of a biogas fermentation involving syngas-passing” conducted at: JOANNEUM RESEARCH Forschungsgesellschaft mbH, Institute for Sustainable Systems and Techniques, Elisabethstraße 16-18, A-8010 Graz, Austria supervised by: Univ.-Prof. Dipl.-Ing. Dr. Hans Schnitzer (Institute for Process and Particle Engineering, Graz University of Technology)
2007-2009	Master’s programme: Environmental System Sciences with focus on Chemistry University of Graz, Universitätsplatz 3, A-8010 Graz, Austria
2003-2007	Bachelor’s programme: Environmental System Sciences with focus on Chemistry University of Graz, Universitätsplatz 3, A-8010 Graz, Austria
1998-2003	Höhere Bundeslehranstalt für wirtschaftliche Berufe with focus on language and economy Kerpelystraße 17, A-8700 Leoben, Austria

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09/2009-until now	PHD-student and Junior Scientist at JOANNEUM RESEARCH Forschungsgesellschaft mbH, RESOURCES-Institute for Water, Energy and Sustainability Elisabethstraße 16-20, A-8010 Graz, Austria
04/2008-09/2009	Assistant for Product Management (part-time) at AVL Emission Test Systems, Hans-List-Platz 1, A-8010 Graz, Austria
08/2007	Student worker, Institute for Organic Chemistry, University of Graz, A-8010 Graz, Austria
10/2006-07/2007	Student worker, Institute for Physical Chemistry, University of Graz, A-8010 Graz, Austria
09/2006 and 09/2005	Student worker, Institute for Organic Chemistry, University of Graz, A-8010 Graz, Austria
08/2005	Student worker, Labor Dr. Ehrenstorfer-Schäfers, Bgm.-Schlosser-Straße 6 A, D-86199 Augsburg, Germany

Personal skills and competences	
Mother tongue	German
Other languages	English (European level C1), Spanish (European level A2)
Computer skills	good command of Microsoft Office tools (Word, Excel and Power Point)
Driving licence	Category B
Other competences	Organisational skills, accounting and economical knowledge
Interests and hobbies	Travelling, playing in a wind orchestra (clarinet), history, reading historical novels, sports (skiing, jogging, badminton, hiking)

Scientific publications and lectures

2010

Ecker .J., Schaffenberger M. , Harasek H.M.
Grüne Bioraffinerie Oberösterreich - Membran-basierte Produktion von Aminosäuren und Milchsäure. DOI: 10.1002/cite.201050406, Chemie Ingenieur Technik, 82 (9), 1565 - 1566, 2010

Ecker J., Schaffenberger M.
Grüne Bioraffinerie Oberösterreich - Membran-basierte Produktion von Aminosäuren und Milchsäure ,ProcessNet-Jahrestagung 2010, Aachen, 2010 (LECTURE)

2011

Schaffenberger M., Green biorefinery – production of amino acids from grass silage juice, Sustainable Development 2011 – 1st Annual European Postgraduate Symposium, Dublin Ireland, 23-24 February, 2011 (LECTURE)

Schaffenberger M., Green biorefinery – production of amino acids from silage juice, Vienna Workshop on Sustainable Development, Vienna, Austria, November 23-24, 2011 (LECTURE)

2012

Ecker J., Schaffenberger M., Koschuh W., Mandl M., Boechzelt H.G., Schnitzer H., Harasek M., Steinmüller H.
Green Biorefinery Upper Austria-Pilot Plant Operation, Separation and Purification Technology, Vol. 96, pp 237-247, 2012

Schaffenberger M.
Production of amino acids from grass silage juice using an ion exchange device at pilot scale. Book of Abstracts zum 8. Minisymposium der Verfahrenstechnik (1. Auflage 2012, Verlag der Johannes Kepler Universität Linz, ISBN-Nr. 978-3-200-02647-6), Linz, 2012

Schaffenberger M.
Production of amino acids from grass silage juice using an ion exchange device at pilot scale. Minisymposium der Verfahrenstechnik Linz, 2012 (LECTURE)

Schaffenberger M., Ecker J., Koschuh W., Essl R., Mandl M., Boechzelt H., Steinmueller H., Schnitzer H.
Green Biorefinery - Production of Amino Acids from Grass Silage Juice using an Ion Exchanger Device at Pilot Scale. Chemical Engineering Transactions, Vol. 29, 2012 Part 1, 505-511; PRES

2012, 15th Conference on Process Integration, Modelling and Optimisation of Energy Saving and Pollution Reduction, Prag, 2012

Schaffenberger M.
Green Biorefinery - Production of Amino Acids from Grass Silage Juice using an Ion Exchanger Device at Pilot Scale, PRES 2012, 15th Conference on Process Integration, Modelling and Optimisation of Energy Saving and Pollution Reduction, Prag, 2012 (LECTURE)

Ecker J., Schaffenberger M., Essl R., Raab T., Harasek M.
Comparison of nanofiltration and ion exchange downstream technology for the usage at the green biorefinery upper Austria, Procedia Engineering, Vol. 44, 1340-1341, Euromembrane Conference 2012, London