CHEMICAL ENGINEERING TRANSACTIONS
VOL. 29, 2012
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at: www.aidic.it/cet
Guest Editors: Petar Sabev Varbanov, Hon Loong Lam, Jiří Jaromír Klemeš

ISBN 978-88-95608-20-4; **ISSN** 1974-9791
DOI: 10.3303/CET1229085

Green Biorefinery – Production of Amino Acids from Grass Silage Juice using an Ion Exchanger Device at Pilot Scale

Martina Schaffenberger*[a,e], Judith Ecker[b], Werner Koschuh[c], Robert Essl[c], Michael G. Mandl[a], Herbert G. Boechzelt[a], Horst Steinmueller[d], Hans Schnitzer[e]

[a]RESOURCES – Institute for Water, Energy and Sustainability, Joanneum Research Forschungsgesellschaft mbH, Elisabethstrasse 16-20, 8010 Graz, Austria
[b]Institute of Chemical Engineering, Vienna University of Technology, Getreidemarkt 9/166, 1060 Vienna, Austria
[c]Gruene-Bioraffinerie.at GmbH, Dr. Auner Strasse 2, 8074 Raaba, Austria
[d]OÖ Bioraffinerie F&E GmbH, Altenbergerstrasse 69, 4040 Linz, Austria
Institute of Process and Particle Engineering, Graz University of Technology, Inffeldgasse 21a, 8010 Graz, Austria
martina.schaffenberger@joanneum.at

The use of grass biomass in biorefineries offers sustainable routes to gain bulk and fine chemicals, such as amino acids from biobased resources. In 2008, the Green Biorefinery Upper Austria has been established to extract amino acids and lactic acid from grass silage at pilot scale. After pressing the grass and pre-treating the grass silage juice by different state of the art membrane technologies, the amino acids were purified and separated using an ion exchanger device. To guarantee higher loadings and throughputs per batch run at the ion exchanger device, a displacement chromatography was applied. Using the present device 6 to 10 kg amino acids per batch run were yielded. By monitoring the displacement step, the separation into three different amino acid product fractions was implemented. Additionally, different process parameters, such as flow rates or different amino acid concentrations in the feed were investigated to find out possible correlations and to increase the yield per batch run. Considering the investigated process conditions, it was found out that the ratio between amino acids and inorganic cations in the feed influences the yield per batch run.

1. Introduction

Grass is becoming more and more a surplus resource in Europe due to the reorganisation of the agricultural sector in many European countries (Mandl, 2010). Large areas, which were used for feeding farm animals, are unused nowadays. Therefore, concepts for the alternative use of this wide-spread resource were developed. Within these so called Green Biorefinery (GBR) concepts, whole green crops (e. g. grass, clover, lucerne or alfalfa) are used to produce value added products such as chemicals, materials, fuels or energy (biogas) by applying a comprehensive system of fractionation as summarised by Kromus et al. (2006). An Austrian GBR concept, which favoured fermented grass (silage) to ensure a decentralised and seasonally independent feedstock system, was presented by Kromus et al. (2004). Lactic acid (LA) and a mixture of amino acids (AAs), both generated during the ensiling process of grass, were seen as the key compounds within this concept. Based on this concept and preliminary findings concerning the treatment of grass at lab scale, e. g. reported by Koschuh et al.

Please cite this article as: Schaffenberger M., Ecker J., Koschuh W., Essl R., Mandl M., Boechzelt H. G., Steinmueller H. and Schnitzer H., (2012), Green biorefinery – production of amino acids from grass silage juice using an ion exchanger device at pilot scale, Chemical Engineering Transactions, 29, 505-510

(2005), a process has been developed (gruene-bioraffinerie.at 2008). In 2008, this process was implemented by the "Green Biorefinery Upper Austria F&E GmbH" by establishing a *GBR Pilot Plant* to extract LA and AAs from grass silage. More details on the performance of the *GBR Pilot Plant* are presented at Ecker et al. (2012).
AAs are extremely important products in various branches of industry especially in the food and flavour industry. Furthermore, they are applied for medical purposes (infusions or dietary food), in the cosmetic sector, for animal feed additives or as precursors in the chemical industry. Typically, AAs are produced by hydrolysing proteins, through fermentative and enzymatic processes using renewable feedstocks or artificially by chemical synthesis as summarised by Hoppe and Martens (1984). After the production process, AAs are often accumulated in mixtures, which are contaminated with by-components. For purifying and separating them at preparative scale, displacement chromatography with polymeric ion exchange resins is a well-known method due to high throughputs and loadings (Qi and Huang 2002). Therefore, this method was applied at the *Green Biorefinery Pilot Plant Upper Austria* for the final separation and purification of AAs from grass silage juice as well. In this work, results regarding the ion exchange process for the production of purified AAs mixtures using pre-treated grass silage juice at the *Pilot Plant Green Biorefinery Upper Austria* are presented. Furthermore, different experiments were performed to optimise the AA-production process. Therefore, some aspects concerning the process optimisation are presented as well.

2. Materials and Methods

2.1 Purified grass silage juice

The ion exchanger process was performed using pre-treated grass silage juice. For this purpose silage from grassland provided by local farmers was pressed by a screw press. After pre-filtering the silage juice with a bag filter, the silage juice was purified using state of the art membrane devices at the *Pilot Plant Green Biorefinery Upper Austria*. First, the grass silage was treated by an ultrafiltration step to remove larger components and particles followed by a softening step to reduce inorganic cations, such as Ca^{2+} and Mg^{2+}. Afterwards, further separations and purifications were done by using the so called hybrid process (based on licence from gruene-bioraffinerie.at 2008), which comprised a sophisticated combination of different membrane systems, such as double-stage nanofiltration and reversed osmosis. Purified grass silage juice from different stages (ultrafiltration permeate, nanofiltration retentate) was used for experiments within the ion exchange device. Due to the use of biological resources and process variations (e.g. use of diafiltration mode or filtration mode) in the purification the composition of the ion exchange feed varied. Table 1 shows exemplary a feed composition (main ingredients, ultrafiltration permeate) for the ion exchange process.

2.2 Ion exchange device

The ion exchanger device at the *Green biorefinery Upper Austria* consisted of 5 columns and complex piping, which allowed serial or parallel operation of the columns. As shown in Figure 1, separate storage tanks for feed (purified silage juice), water, sulphuric acid and ammoniac solution where available. The pumping was done by membrane pumps (Sera, Germany). Flow rates were measured using an inline flow meter (Endress & Hauser). Other process parameters were monitored by inline measurement of conductivity and pH (both Endress & Hauser) after column 1, 2 and 5 and refractivity (K-Patents Process Instruments, Finland) after column 5.

Table 1: Exemplary chemical composition of the ion exchanger feed (pre-treated grass silage juice, ultrafiltration permeate)

Component	Concentration [g/L]	Component	Concentration [g/L]
AAs sum	17.5	Glucose	0.57
Aspartic acid	1.53	Fructose & Mannose (sum)	2.57
Leucine	1.89	Ca^{2+}	0.91
Lysine	0.79	Mg^{2+}	0.63
LA	18.6	K^{+}	8.77
Acetic acid	5.21	Cl^{-}	2.08

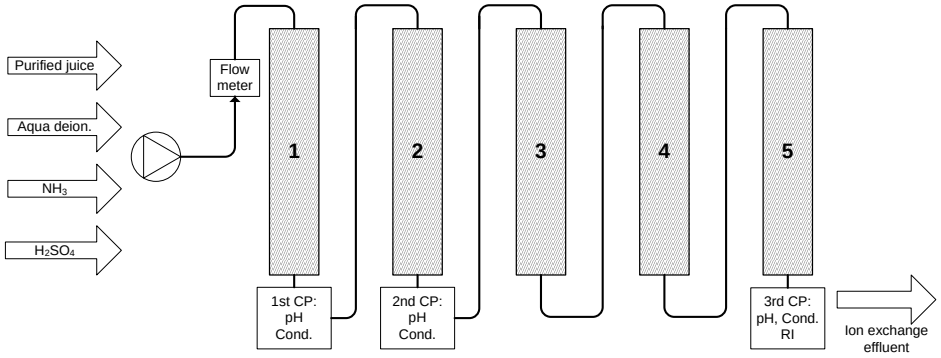

Figure 1: Simplified schema of the ion exchanger device at the Green Biorefinery Pilot Plant Upper Austria

2.3 Ion exchange resins

During the experiments two different strong cation exchanger resins were used: Dowex Marathon C (Dow, styrene-DVB functionalised with sulfonic acid, mean particle size of 600±50 µm, 1.8 eq/L, columns equipped with 116 L resin) and Dowex Monosphere C-350 (Dow, styrene-DVB matrix, functionalised with sulfonic acid, mean particle size of 350±50 µm, 2 eq/L, columns equipped with 120 L resin).

2.4 Experimental

The ion exchanger process was operated as displacement chromatography. Therefore, experiments were performed in batch mode and every batch experiment consisted of three steps, namely loading, displacement and regeneration. First, the strong cation exchanger resins were loaded with the purified grass silage juice. Due to positive charging, inorganic cations and AAs interacted with the negatively charged functional groups and stuck to the ion exchanger resin. Negatively charged or neutral components, such as organic acids and sugars, did not interact with the resin's functional groups and were distributed through the ion exchanger bed. Every loading step was stopped when inorganic ions were detected after column two by measuring the conductivity. This was possible as AAs caused a low conductivity firstly and the break through of inorganic ions caused an increase in conductivity afterwards. After loading, the resin was rinsed with deionised water. Followed by displacing the AAs with 1 M NH_3 (technical grade, Fa. Brenntag). Finally, the resin was converted into to H^+-form and therefore regenerated by treating it with 0.5 M and 1 M H_2SO_4 (technical grade, Fa. Brenntag). Every process step was monitored inline by measuring the conductivity, the pH and by using a refractometer.
For optimising the yield at AA production process, different process parameters, such as flow rates (between 2.9 to 11.5 m/h) or input AA concentration (10 to 25 g/L) varied. By using different silages, which are biological products, the ratio between AAs to inorganic cations varied as well. Furthermore, purified grass silage juice from different stages (ultrafiltration permeate, nanofiltation retentate) was tested.

2.5 Analyses

Organic acids and sugars were quantified by HPLC (Dionex Ultimate 3000), using the Aminex HPX-87H column (Bio-Rad Co) with pre column, mobile phase H_2SO_4 5 mM, 65 °C. An UV detection system (Dionex Ultimate 3000 RS) for organic acids (210 nm) and a RI detection system (Shodex) for sugars were applied. The concentrations of AAs were chromatographically determined by a BioChrom 30 apparatus (Biochrom), using an ion exchanger column (particle size 8 µm) and a hydrolysate program with different lithium-citrate buffers. After derivatisation with ninhydrin, AAs were detected photochemically at 570 nm and 440 nm (proline). After treating the samples by acidic pulping (Multiwave 3000, Anton Paar), inorganic cations were analysed by ICP-OES (Spectro Arcos, Spectro,

Kleve). For analysing inorganic anions, an ion chromatography (Dionex ICS 90, eluent $NaHCO_3/Na_2CO_3$-Buffer, column AG14A with pre-column) was applied.

2.6 Calculations

Solutions of AAs were generated through displacing them with NH_3-solutions from the ion exchanger resin. To allow adequate comparisons between the ion exchange resins yields were referred to amount of used resin within the experiments:

$$Yield\ AA\,[eq/L] = \frac{displaced\ AA\,[eq]}{amount\ ion\ exchanger\ [L]} \quad (1)$$

The total amount of displaced AAs per batch run was calculated by using the following equitation first, followed by summing up the calculated data for whole experiment. Whereas, for alkaline AAs (lysine, histidine and argine) z = 2.

$$Displaced\ AA\ per\ \ fraction\ [eq] = \frac{c_{aa} \cdot volume\ of\ \ fraction}{M \cdot z} \quad (2)$$

3. Results

Considering experiments with different process parameters at the ion exchanger device, it was possible to produce 6 to 10 kg AAs per batch run from purified grass silage juice. Referred to the applied amount of ion exchange resin, in average 0.45 eq AAs (considering both resins) were gained per litre of resin. Although, the resin Monosphere C-350 contained a higher capacity, no major differences on AA yields per batch run were observed.

Due to different properties, AAs were not distributed equally within the ion exchanger bed, as shown in Figure 2a. Acidic AAs with negatively charged side chains, such as aspartic acid or glutamic acid, interacted less with the resin's functionalised groups. Therefore, they were mainly found on column 4 and 5. Alkaline AAs, such as lysine, with positively charged side chains did not cover long distances within the resin, due to stronger interactions. Neutral AAs without charged side chains, such as leucine or alanine, remained in between. As shown in Figure 2a, most AAs were found on the columns 3 to 5. Inorganic cations, like K^+, Mg^{2+} or Ca^{2+}, which were not removed in the process before and also interacted with the cation exchanger resin, influenced the distribution of the AAs on the columns. Therefore, less AAs were eluted from column 1 and 2, because these columns were mainly occupied with inorganic cations and the AAs were displaced by them. Nevertheless, approximately 10 % of the yielded AAs, basically alkaline AAs, remained on column 1 and 2. Considering the data for column 3 to 5 in Figure 2a, the amount of eluted AAs increased permanently. About 20 % of all AAs were obtained from column 3. Due to the present ratio of AAs and cations in the feed, some inorganic ions were on column 3, too. However, 70 % of all AAs were obtained from column 4 and 5. In particular, neutral AAs were most prevalent on these columns followed by acidic AAs. Based on the variable distribution behaviour of the AA groups (acidic, neutral and alkaline) on the ion exchanger, it was possible to produce three different AA fractions comprising mainly acidic, neutral or alkaline AAs.

During the experiments on the ion exchanger, the influence of different process parameters on the AA yield per batch run was investigated. For instance, experiments with different flow rates (2.9 to 11.5 m/h) for loading were performed to notice possible connections between the time of exposure and yields. Results for the investigated flow rates did not show correlations between yield and flow rates. Furthermore, the influence of AA concentrations (c_{aa}) in the raw material for ion exchanger on the AA yield was observed. Figure 2b shows AA concentrations in the raw material referred to the yielded AAs per batch run.

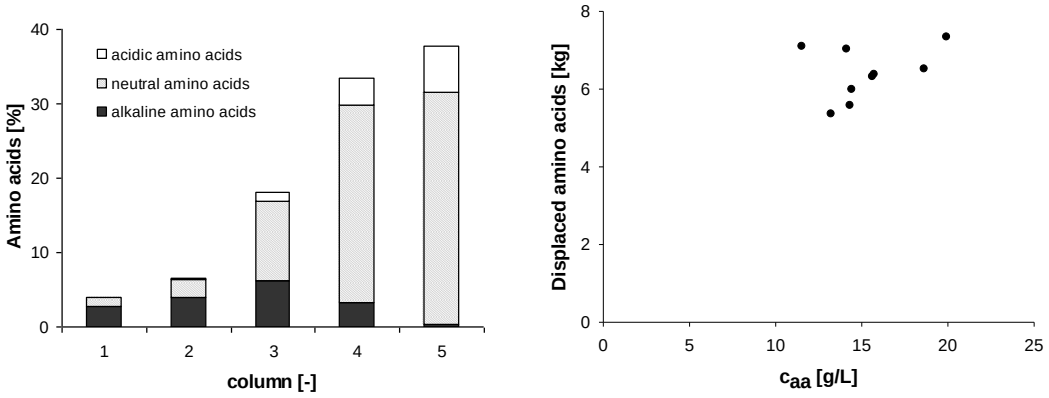

Figure 2a and 2b: Distribution of AAs per column (left); Comparison of AA concentration (sum) in the ion exchanger feed (ultrafiltration permeate) vs. the displaced AAs per batch run (right)

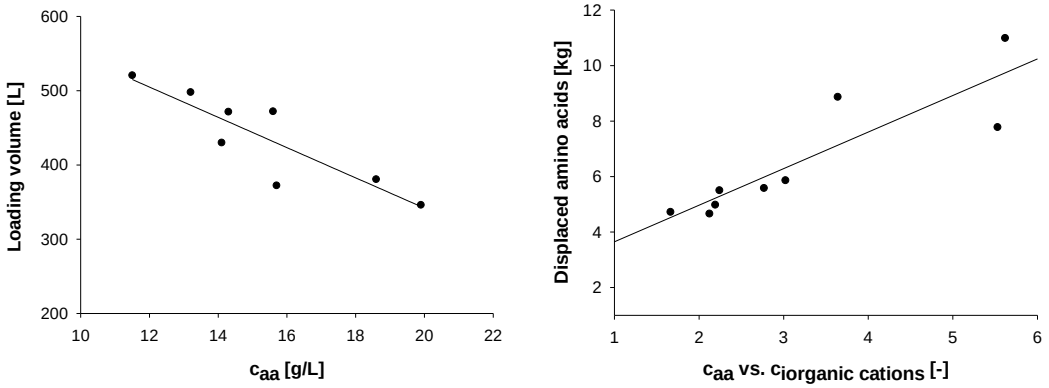

Figure 3a and 3b: AA concentration (sum) in the ion exchanger feed (ultrafiltration permeate) compared to the loading volume per experiment (left); Comparison of the ratio between the concentration of AAs (sum) and the concentration of inorganic cations (K^+, Na^+, Ca^{2+}, Mg^{2+}) in the feed vs. the displaced AAs per batch run (right)

The shown data was generated in experiments using ultrafiltration permeate on the resin Monosphere C-350. In the present range of concentration (10 to 20 g/L) no correlations to the yield per batch run were observed. The variable AA concentrations within the feed resulted from differences in the grass silage and depended on the settings of pre-treatment. However, higher AA concentrations did not lead to higher yields and lower AA concentrations did not result in lower yields. Therefore, it was assumed that within the present process configuration lower AA concentrations were compensated by a higher usage of feed. That means that in experiments, which were performed with lower concentrated feed, larger amounts of feed were used per batch run. Within the presented range of concentration (10 to 20 g/L), this assumption was confirmed, as shown in Figure 3a. In this figure, the data sets from Figure 2b were referred to the appropriate used volume of feed for loading. The data shows that experiments with lower AA concentrations lasted longer, because more feed volume was needed to provide an adequate amount of AAs or inorganic cations to fill the ion exchanger completely. Hence, lower AA concentrations can be compensated by a higher amount of feed, but result in higher costs for pumping. For example, within an experiment, which was performed with a feed with 20 g/L AAs, 346 L feed were

used. In contrary, 520 L feed were transported through the ion exchanger with a starting concentration of 11.5 g/L until the loading was stopped.
Additionally, the impact of the ratio between inorganic cations and AAs in the feed on the yields of AA was investigated more closely. Figure 3b shows some corresponding data to this question gained from different experiments using either ultrafiltration permeate or nanofiltration retentate. Firstly, the ratio between total concentration of Na^+, K^+, Ca^{2+} and Mg^{2+} in the feed to the AA concentration in the feed was calculated. Higher ratios mean that less inorganic cations per sum AAs were measured. Subsequently, these results were referred to the AA yields. As shown in Figure 3b, a trend was observed that higher ratios lead to higher AA yields. The ratio's impact on the AA yield was expected, as inorganic cations and AAs were competitors for the ion exchanger's functional groups. Secondly, the concentration of inorganic cations affected the amount of needed feed per loading directly, as the break trough of inorganic cations between column 2 and 3 indicated the stop of loading. Therefore, a ratio in favour of AAs caused higher yields.

4. Conclusion

Strong cation exchange resins can be applied to extract three fractions of purified AA solutions from pre-treated grass silage juice. In average 6 to 10 kg AAs per batch run were yielded. Based on different charged side chains, acidic, neutral or alkaline AAs show differences in their distribution behaviour. Therefore, it was possible to gain three different AA product fractions. Considering the impact of some process parameters on the yield per batch run, it was observed that variations in the time of exposure or different AA concentrations in the feed within the presented range did not influence the yield per batch run. Results showed that lower AA concentrations in the feed were compensated by increased use of feed. As expected, it was found out that the ratio of AAs to inorganic cations within the feed for ion exchange influenced the yield per batch run in the present process assembly.

Acknowledgments

This project was funded by Linz AG, RAG, Ferngas OÖ, Land Oberösterreich, OÖ Energie AG, Kommunal-Credit and the research framework program Fabrik der Zukunft founded by bmvit.

References

Ecker J., Schaffenberger M., Koschuh W., Mandl M., Böchzelt H.G., Schnitzer H., Harasek M., Steinmüller H., 2012, Green Biorefinery Upper Austria-Pilot Plant Operation. Separation and Purification Technology, 96 237-247.

Gruene-bioraffinerie.at GmbH, 2008, A method of treating a material flow (in German), AT000000504206: Austria.

Hoppe B., Martens J., 1984, Amino acids - preparation and recovery (in German). Chemie in unserer Zeit, 18(3), 73-86.

Koschuh W., Thang V. H., Kravesta S., Novalin S., Kulbe K. D., 2005, Flux and retention behavior of nanofiltration and fine ultrafiltration membranes in filtrating juice from a green biorefinery: A membrane screening. Journal of Membrane Science, 261, 121-128.

Kromus S., Wachter B., Koschuh W., Mandl M., Krotscheck C., Narodoslawsky M., 2004, The Green Biorefinery Austria - Development of an integrated system for green biomass utilization. Chem. Biochem. Eng. Q.,18(1), 7-12.

Kromus S., Kamm B., Kamm M., Fowler P., Narodoslawsky M., 2006, The Green Biorefinery Concept - Fundamentals and Potential, in: Biorefineries - industrial processes and products (status quo and future directions), B. Kamm, Gruber P. R., Kamm M. (Editor), Wiley-VHC: Weinheim, Germany

Mandl M.G., 2010, Status of green biorefining in Europe. Biofuels Bioproducts & Biorefining, 4, 268-274.

Qi Y., Huang J., 2002, Displacement chromatography of isomers and therapeutic compounds. Journal of Chromatography A, 959. 85-93.