Biobased Industrial Chemicals from Glutamic Acid

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Biobased industrial chemicals from glutamic acid

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Thesis

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Table of Contents

Chapter 1

Introduction

1.1 Introduction

The purpose of this introductory chapter is to provide a short background on the concepts 'biobased economy' and 'biorefinery'. This is essential for understanding the broader context of the thesis, and will also show the reasons for conducting the research. The introduction will lead to the research questions that are central to this thesis, and then the outline of the thesis will follow from the research questions.

1.2 The biobased economy

The global economy is mainly based on fossil fuels. According to the US Energy Information Administration, more than 86 % of the worldwide energy consumption is fossil-based, which means that it comes from oil, gas or coal.[1] The high prices of gasoline and diesel at the petrol pump are a clear signal to the general public that oil is become more scarce. Oil prices have been increasing sharply during the last decade, leading to high petrol prices. This can be seen in figure 1.1, which shows the price of unleaded gasoline at the petrol pump in The Netherlands over the last decade.[2,3] In 2008 there was a dip in the price because of the economic crisis, but since then the price has been increasing again.

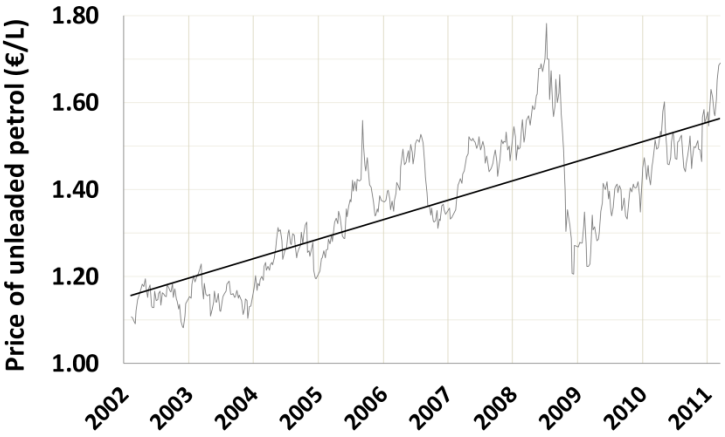

Figure 1.1. Price of gasoline at the petrol pump in The Netherlands over the last decade.

There is considerable debate on why oil is becoming more and more expensive, the theory of 'peak oil' by Hubbert being one of the most persistent. This theory describes the point at which the maximum daily extraction of petroleum from the earth is reached, after which production volumes only go down, and oil becomes more and more scarce. For some countries the peak has passed already many years ago, for example in the United States this was in the 1960's. This was accurately predicted by Hubbert in 1956, making his theory a popular one.[4-6]
Whether the world production of oil has peaked already, will peak in the (near) future, or will never peak is under debate. According to the International Energy Agency the world oil production has not peaked yet, and when it will peak depends strongly on the policies applied by governments. It could be in 2020, but also after 2035, depending on the demand for oil in the meantime.[7] In any case, the message from ExxonMobil is very clear. Already in 2005 a spokesman of that company said: "All the easy oil and gas in the world has pretty much been found. Now comes the harder work in finding and producing oil from more challenging environments and work areas."[8] Shell quantifies the "pretty much" of ExxonMobil as such: "By 2015, growth in the production of easily accessible oil and gas will not match the projected rate of demand growth."[9] This means that oil has to be extracted from less accessible places, such as from deep water wells, from the North Pole, or from Canadian tar sands. It is clear that this will lead to an increase in the price of oil, let alone the potential risks for the environment.

The story above is one of the reasons for the world to move from an economy that is based on fossil fuels to a 'biobased economy'. The biobased economy should not be looked upon as a vision of a world that does not use or need any fossil fuels, because that will not happen for a long time. Instead it is useful to define it as a change, as "the technological development that leads to a significant replacement of fossil fuels by biomass in the production of pharmaceuticals, chemicals, materials, transportation fuels, electricity and heat".[10]
Three aspects of this definition are important to point out. The first is that it not only speaks about biofuels, but about a whole range of products and energy that can be produced from biomass. This is important in relation to this PhD thesis because the focus is on the replacement of chemicals and materials, rather than on energy. The second aspect is the "significant replacement", which is not quantified

in this definition. It does not mean 100 %, nor does it mean 0.1 %. How much this will be in practice depends on many factors, such as technology development, land availability and agricultural yields that can be achieved in the future. The third aspect is the fact that it only includes biomass, and no other renewable energy sources, such as solar, wind and hydroelectric energy. In the future these energy sources are expected to play an important role, for example by the International Energy Agency,[7] but also in future energy scenarios of Shell.[11] However, when looking at the production of chemicals and materials there will always be the need for a carbon source to construct the molecules. Carbon cannot be delivered by the sun, nor by wind or water, it can only be obtained from biomass. Therefore biomass will be an essential source for renewable chemicals and materials in the future.

The future shortage and high price of fossil fuels are not the only reasons to strive for a biobased economy. Other important reasons are to decrease the dependency on unstable oil-exporting regions such as the Middle East, reducing greenhouse gas emissions because of their influence on global warming, and last but not least the economic development of rural areas.[10]
The latter can be exemplified with the case of Brazil. There bioethanol from sugarcane has been an alternative fuel for decades already. In 1919 the governor of Pernambuco ordered all official vehicles in his state to operate on alcohol, because it was a local product while gasoline was imported.[12] In the 1930's and 40's there was a great increase in ethanol production in Brazil, and after the second world war Brazil was one of the few places where blending ethanol into gasoline was continued. When the oil crisis in 1973 occurred, Brazil was the only country left with an ethanol blending program. In 1975 this led to the 'national alcohol program', of which the objective was to phase out fossil-based automotive fuels. Nowadays one third of the bioethanol that is produced worldwide is Brazilian,[1] and bioethanol provides employment to over one million people in Brazil.[13] This may in some cases go together with very poor labor conditions (especially for manual cane cutting), and even cases of slavery have been reported, but in general it does provide an income to many of the poorest people in rural areas.[13]

1.3 Biorefinery

According to the International Energy Agency, biorefinery is the "sustainable processing of biomass into a spectrum of marketable biobased products and bioenergy."[14] When you put this definition next to the one of the biobased economy that was mentioned earlier, it becomes clear immediately that biorefinery is the "technological development" that is needed for achieving the biobased economy. This is illustrated with figure 1.2.

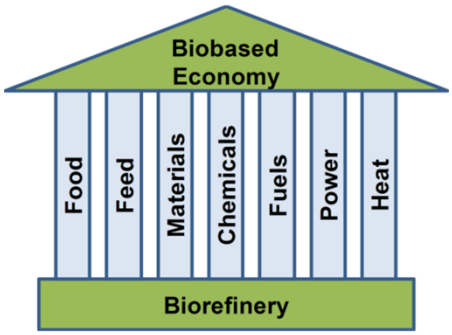

Figure 1.2. Biorefinery as the enabling technology development for achieving a biobased economy.[14]

The analogue with a petrochemical refinery is obvious. In a petrochemical refinery crude oil is separated and the different fractions are 'refined' into different products, such as fuels (e.g. gasoline, kerosene, diesel), building blocks for chemicals and materials (e.g. ethylene, propylene), and even road construction materials (bitumen). In a biorefinery biomass is the crude, which is separated into different fractions (e.g. sugar, cellulosic components, fatty acids, proteins, lignin), that are subsequently 'refined' into fuels (e.g. ethanol, biodiesel, biogas), building blocks for chemicals and materials (e.g. lactic acid, epichlorohydrin), food or feed ingredients (e.g. sugar, proteins), or that are burned for energy (lignin).
When comparing petrochemical refinery technology with biorefinery technology, it is important to note that petrochemical refineries have been optimized for over a century, which has led to very efficient processes. Much biorefinery technology on the other hand is still under development (there are of course exceptions, like fermentation of sugar to ethanol which has been around for thousands of years),

and has not been optimized very well yet. That means that there is a lot of room for improvement. An example of that is lignin, which is now generally burned for energy. There is a vast amount of research going on these days to find methods to apply lignin in a way that creates more value from it, one example being phenolic resins for binder applications.[15]

Another example where substantial improvement is possible is in the use of proteins. Currently the proteins that enter a biorefinery inside a plant leave it either in the form of fertilizer, or in the form of animal feed. Chapter 2 of this thesis will elaborate on the drawbacks of those applications of proteins, and show that it would be beneficial to further breakdown the proteins into their building blocks (amino acids). When the amino acids are separated they can then be used for different applications. Some (e.g. lysine, methionine, tryptophan) will have a value as human food or animal feed additives, and some (e.g. glutamic acid, aspartic acid) will have no value for that application and can be better used to produce chemical products instead, because that would lead to an increase in value. Another incentive to use amino acids for applications other than as food or feed could come from regulatory reasons. Consider for example byproducts of processes where genetically modified organisms (GMOs) were used. These cannot be used as food or feed. If yeast are genetically engineered to be able to produce bioethanol from cellulosic materials, the byproducts of that process can only be burned or gasified. Therefore the proteins in those byproducts could be better used to produce valuable chemical products, instead of energy.

1.4 Research questions

When stating that some amino acids can be better used for the production of chemical products, the first questions that arise are: which chemical products and how?

The choice for glutamic acid as a starting material makes sense when the above is kept in mind: it is a non-essential amino acid, so it does not have much value in food or feed applications. It does have other existing applications, the main one being a flavor enhancer (as monosodium glutamate, MSG, a.k.a. vetsin or umami[16]), but it is not directly part of the food *vs.* fuel debate. Furthermore, as

chapter 2 will show, it is in many plant proteins the most abundant amino acid, so from a resource availability point of view it is a logical choice. Currently glutamic acid is made by fermentation, which is a relatively expensive method.[17,18] If it could be isolated from inexpensive byproducts glutamic acid could become available at a lower price.

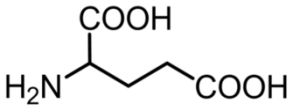

Figure 1.3 The molecular structure of glutamic acid

From the choice for glutamic acid as a starting material, the chemicals to produce follow. The key is in the functionalities that a glutamic acid molecule contains, as shown in figure 1.3. It would be best to make use of those as well as possible. Especially the nitrogen atom is valuable, as it requires a lot of energy to introduce nitrogen into a molecule. First ammonia has to be made from nitrogen gas and hydrogen (that have to be produced as well) with the Haber-Bosch process, at 500 ^{o}C and 150 bar, which requires substantial amounts of energy.[19] Then the introduction of nitrogen into the molecule requires large amounts of energy again. An example of such an energy-intensive process is the Sohio process for the manufacture of acrylonitrile from propylene and ammonia.[20] So it would be advantageous to convert glutamic acid into nitrogen containing chemicals.
The other requirement in the choice of the targets is their commercial application. There needs to be a market for the products, otherwise there would be no need to replace the petrochemical feedstock with a biobased feedstock.

Examples of molecules that can theoretically be produced from glutamic acid, and contain nitrogen, are the lactams N-methylpyrrolidone and N-vinylpyrrolidone. They both contain a four-carbon chain and a nitrogen and oxygen atom in the form of an amide. See figure 1.4. Also 1,4-diaminobutane can be related to glutamic acid, with its four-carbon chain with two amine groups, of which one is present in glutamic acid and one will have to be incorporated.

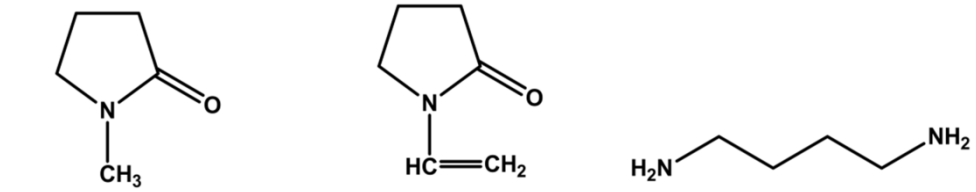

Figure 1.4. Molecular structures of target chemicals, of which the structures contain similar functionalities as glutamic acid.

N-Methylpyrrolidone (NMP) is an industrial solvent that is used in the manufacture of plastics and as an ingredient in paint removers.[21] N-vinylpyrrolidone (NVP) finds applications in the manufacturing of poly(vinylpyrrolidone) and copolymers, for example together with vinyl acetate and methyl acrylate. Further it is used as solvent in the production of inks and paints, as an additive in the cosmetics industry, and in the pharmaceutical industry for the production of a disinfectant.[22,23] 1,4-Diaminobutane is a monomer for polyamides such as nylon-4,6 or Stanyl®.[24] All these chemicals are currently made from fossil fuels commercially.

Having answered the question 'which chemicals to produce from glutamic acid?' above, the main research questions for this thesis are:

1. Where can glutamic acid be obtained from?
2. How can these chemicals be made from glutamic acid?
3. Will it be technically and economically feasible to implement these new routes?
4. How would the biobased chemicals perform environmentally, compared to their petrochemical equivalents?

1.5 Thesis layout

Chapter 2 will describe different sources that can be used now and in the future for the isolation of amino acids such as glutamic acid, and will also attempt to quantify these sources, and the amount of proteins and glutamic acid that are present in these sources. Also will it describe the technology that is currently available for the isolation of amino acids from these sources. This will answer research question 1.

Chapter 3 describes the first reaction step in the production of the pyrrolidone-derivatives NMP and NVP from glutamic acid. This comprises an enzymatic α-decarboxylation reaction of glutamic acid, forming γ-aminobutyric acid.
Chapter 4 is about the next step on the pathway from glutamic acid to NMP and NVP. It shows how NMP can be produced in a one-pot synthesis from γ-aminobutyric acid, and how the intermediate of that reaction, 2-pyrrolidone, can be turned into NVP.
Chapter 5 contains the synthetic route from glutamic acid to succinonitrile, which is the commercial precursor of 1,4-diaminobutane.
These three chapters, containing experimental work, will together answer research question 2.

Chapter 6 is a techno-economic assessment of the production of the above-mentioned chemicals (plus acrylonitrile) from glutamic acid. The goal is to determine whether it would be feasible to produce these chemicals in such a way that they can compete with their petrochemically produced equivalents. With that this chapter will answer research question 3.

Chapter 7 contains a lifecycle assessment of the biobased chemicals, which will be compared with lifecycle assessments of the petrochemical products, in order to determine which would be the best choice from an environmental perspective. This will answer research question 4.

Chapter 8 is the discussion and conclusion of this thesis. It will summarize the findings of the previous chapters, and synthesize an overall view on the production of chemicals from glutamic acid.

1.6 References

1. U.S. Energy Information Administration, **2011**, International Energy Statistics**:** 2008, http://tonto.eia.doe.gov/cfapps/ipdbproject/IEDIndex3.cfm, Accessed Mar 2011.
2. U.S. Energy Information Administration, **2011**, Weekly Retail Premium Gasoline Prices, Selected Countries, incl. taxes, www.eia.gov/emeu/international/ oilprice.html, Accessed Mar 2011.
3. OANDA, **2011**, http://www.oanda.com/currency/historical-rates, Accessed Mar 2011.
4. M. K. Hubbert, Nuclear energy and the fossil fuels, **1956**, in: Spring Meeting of the Southern District Division of Production, American Petroleum Institute.
5. J. C. Rudolf, The New York Times, 11-14-**2010**.
6. M. Inman, National Geographic News, 11-10-**2010**.
7. International Energy Agency, World Energy Outlook **2010**.
8. J. Donnelly, The Boston Globe, 12-11-**2005**.
9. Shell, Shell and the Canadian Oil Sands, 2009 Factbook, **2009**.
10. J.W.A. Langeveld, J.P.M. Sanders, in: The Biobased Economy, eds. J.W.A Langeveld, J.P.M. Sanders, M.J.G. Meeusen, Earthscan London, **2010**, vol. 1, 3-17.
11. Shell, Shell energy scenarios to 2050, **2008**.
12. W. Kovarik, Ethanol's first century, **2006,** in: International Symposium on Alcohol Fuels XVI, Rio de Janeiro, Brazil.
13. E. Smeets, M. Junginger, A. Faaij, A. Walter, P. Dolzan, Sustainability of Brazilian bio-ethanol, **2006**, Utrecht University, Copernicus Institute, Department of Science, Technology and Society.
14. R. van Ree, Integrated Biorefineries: Current Status and Developments, **2010** in: Biorefinery Training Course, Amsterdam, The Netherlands.
15. R. J. A. Gosselink, J. E. G. van Dam, E. de Jong, E. L. Scott, J. P. M. Sanders, J. Li, G. Gellerstedt, Holzforschung, **2010**, 64, 193-200.
16. Ajinomoto, **2011**, Umami global website, http://www.ajinomoto.com/features/aji-no-moto/en/umami/index.htm, Accessed Mar 2011.
17. W. Leuchtenberger, K. Huthmacher, K. Drauz, Appl. Microbiol. Biotechnol. **2005**, 1-8.
18. J.J. Bozell, G.R. Petersen, Green Chemistry **2010**, 12, 539-554.
19. M. Appl, Ammonia, In: Ullmann's Encyclopedia of Industrial Chemistry, Online edn. **2000,** Wiley InterScience.

20. F. Veatch, J.L. Callahan, J.D. Idol Jr., E.C. Milberger, Chemical Engineering Progress, **1960**, 65-67.
21. BASF, Official website of BASF chemical company, Germany, **2011**. http://www2.basf.us/diols/bcdiolsnmp.html, Accessed Mar 2011.
22. BASF, Official website of BASF chemical company, Germany, **2010**, http://world account.basf.com/wa/PublicMSDS/PDF/09007af88017cc55.pdf, Accessed Mar 2011.
23. A.L. Harreus, 2-Pyrrolidone, In: Ullmann's Encyclopedia of Industrial Chemistry, Online edn. **2000**, Wiley InterScience.
24. DSM, Official website of DSM, The Netherlands, **2011**, http://www.dsm.com/en_US/html/dep/stanyl.htm, Accessed Mar **2011**.

Chapter 2

Availability of protein-derived glutamic acid as feedstock for the production of industrial chemicals

This chapter was submitted as:
T.M. Lammens, M.C.R. Franssen, E.L. Scott and J.P.M. Sanders, "Availability of protein-derived amino acids as feedstock for the production of biobased chemicals", 2011

Abstract

Volumes at which protein sources and the amino acids therein are available were determined, and the most interesting amino acids in terms of their potential available quantity were identified. The investigated sources are maize and wheat DDGS, sugarcane vinasse and its leaves, sugarbeet vinasse and its leaves, cassava leaves, press cakes of rapeseed, sunflower, soybean, palm oil and *Jatropha*, animal slaughter waste, microalgae, macroalgae, grass and alfalfa.

It can be concluded that there are enough sources available to produce biobased chemicals such as N-methylpyrrolidone with a market sizes around 100 kton per year from glutamic acid. Bulk chemicals such as acrylonitrile can partly be replaced in the future by their biobased equivalent, depending on the amounts of biofuels that will be produced in the future. However, it is still necessary to find cost-effective methods for the isolation of amino acids from the discussed sources.

2.1 Introduction

Many crops grown around the world could be of interest for a biobased economy. Various parts of different crops will be suitable for a variety of applications. In a biorefinery the production of food, feed, fuel and chemicals will go together,[1] because some parts of plants are required for human food or animal feed production, while other parts have less potential for that purpose and are more useful for the production of fuels or chemicals. The potential of carbohydrates as sources for biofuels and biobased chemicals has been extensively reviewed, most recently by Bozell and Petersen, who identified the ten most promising carbohydrate based products for the US department of energy.[2] Proteins however have received little attention as potential sources for chemical products. In general, a protein-rich fraction of plants is more nutritious to humans or animals than a lignocellulosic carbohydrates-rich fraction for an application as food or feed. Also the economic value of proteins is generally higher than that of carbohydrates, which makes the application of proteins for the manufacture of (bulk) chemicals appear less logical. However, with increasing production of biofuels in the future the protein-containing fractions will also become more abundant, which could lead to a decrease in value. Furthermore even an already nutritious fraction could be further split into more and less nutritious fractions, for example by hydrolyzing the proteins and separating the essential (nutritious) from the non-essential (less nutritious) amino acids. This would both increase the value of the nutritious part and provide the less nutritional part for another use, namely for the production of chemicals. Practical diets of pigs for example, often contain too much crude protein, and reducing that amount does not significantly affect the growth.[3] Different studies have shown that the amount of crude protein can be reduced significantly by addition of a few essential amino acids (i.e. lysine, threonine, tryptophan, methionine) and that by applying such a diet the emissions of ammonia and nitrogen oxides are greatly reduced, which is beneficial for the environment.[4-6]

The goal of this chapter is to assess what could be interesting sources for proteins that could be used for the production of industrial chemicals in a biorefinery, at which volumes these sources are available now and in the future, and to identify the most interesting amino acids in terms of the available quantity.

There are many often complex and interrelated criteria for the choice of a certain crop for a certain application in a biorefinery. One can think of technological (i.e. availability of biomass and of technology), economic (i.e. investment and operational costs), environmental (i.e. emissions, water footprint, land use) and social (i.e. labor conditions, food vs. fuel) criteria.[7] This chapter will focus primarily on the technological aspects to assess the potential of different sources (mainly agricultural byproduct streams) for the production of chemicals.

Proteins are composed of amino acids and are a rich source of organic nitrogen in nature. For this reason, proteins would be an attractive feedstock for nitrogen containing bulk chemicals.[8] However, several factors need to be taken into account. Logically, the amount of protein in the source and the way it can be extracted is of great importance. An ideal source for nitrogen containing chemicals should contain large amounts of protein. One can then determine the relative amounts of different amino acids in those proteins to find out which amino acids are present in a significant amount for the production of certain chemicals. Finally, the question whether there will be enough of a certain source for the production of chemicals at large scale may be the most important question. In the next sections these criteria will be discussed for byproduct streams from different crops instead of the whole crops, in terms of their composition and other characteristics. In addition some non-crop sources are mentioned to broaden the thinking about potential sources now and in the future. First, the two main incentives to use byproduct streams to produce biobased bulk chemicals (economic and environmental) will be clarified with some examples.

2.2 Incentives for using byproduct streams

Some byproduct streams already have an application and therefore a commercial value. The incentive to turn (part of) them into bulk chemicals can be a value increase of part of its contents. A clear example of this is dried distiller's grains with solubles, or DDGS in short. It is a byproduct from bioethanol production from maize or grain and it is mainly used as cattle feed. The price of maize DDGS is currently around 170 €/ton.[9] Industrial intermediates such as acrylonitrile and

caprolactam have typical values between 1500 and 2000 €/ton, which provides a large price-gap between DDGS and the mentioned bulk chemicals, making their production an interesting opportunity. Products such as N-methylpyrrolidone are worth about 3000 €/ton.[8, 10]

Another example of a byproduct stream is vinasse, a byproduct from bioethanol production from sugar beets or sugarcane. For every liter of ethanol produced from sugarcane, 12 to 13 liters of vinasse are produced.[11] That means that with an annual bioethanol production of 22 billion liters,[12] Brazil produces around 275 billion liters of vinasse, which does not have any economic value. In some cases the vinasse is used as a fertilizer by returning it to the field. However, this can lead to ecological problems, such as a decrease in soil pH,[13] or contamination of ground or surface water with i.e. organic carbon and minerals, possibly leading to a break-down of flora and fauna.[11] This was clearly demonstrated in the case study of the river Ipojuca in Brazil, of which the ecological status was classified as bad, due to application of diluted vinasse for the simultaneous irrigation and fertilization of sugar cane plantations around it.[14] These ecological issues related to the use of vinasse as a fertilizer make it necessary to look for other applications. Here the incentive for change is an environmental issue related to the current application of vinasse. An alternative use is the production of energy by combustion,[15] but also the use of cane vinasse for the manufacture of bricks has been described.[16] The production of chemicals from the amino acids present in the vinasse could give rise to a new application.

What happens with the leaves of many crops, including sugarcane, is similar to the practice described with vinasse. These are left on the field for fertilization, but the nitrogen uptake efficiency of the soil is quite low, which makes it necessary to additionally apply artificial fertilizer and leads to emissions of ammonia.[17] Deepchand *et al.* suggested a method to simultaneously make electricity, ethanol and leaf protein concentrate from sugar cane leaves and tops that are normally left on the field.[18-20] Merodio *et al.* showed they could isolate the proteins from sugarbeet leaves and also make a chlorophyll-free white protein fraction,[21] whereas Dale *et al.* investigated the recovery of leaf proteins from a number of other crops, such as maize, sorghum, alfalfa and switchgrass.[22-24]

2.3 Residual protein content

In figure 2.1 the protein content of different byproduct streams is depicted, as a weight percentage of the dry matter. What this figure shows is that byproducts from first generation biofuels production, such as DDGS from maize, wheat and sorghum and vinasse from sugar beet and sugarcane typically contain between 20 % and 40 % protein. Also the leaves of a number of crops, such as alfalfa, cassava and sugar beet can contain between 20 and 40 % protein.
Looking at wheat straw and maize stover, which can be used for the production of bioethanol after the hydrolysis of the cellulosic materials, the percentage of protein is very low. However, Dale *et al.* have recently suggested an interesting model of how these proteins could still be recovered in a biorefinery, using a two-step process to extract the proteins with warm aqueous ammonia. After this the protein solution is mechanically dewatered, the ammonia recovered and the protein solution further dried. In this way they envision to recover 84% of the proteins present.[23]
Other very interesting sources of proteins are byproducts from oil or biodiesel production, such as the meals or seedcakes of rapeseed, soybean and *Jatropha*. The deviations in protein content of *Jatropha* seedcake are large and probably dependent on the method of producing the seedcake. Achten *et al.* reviewed a number of articles on this and came to an average value of 58 % of the dry matter being protein.[69] Other authors found much less crude protein present, in the order of 25 % of the dry matter.[47, 48] The meal from oil palm contains less protein, but it is still around 20 %.

Besides the proteins based on plant material, we envisage that it is also possible to use other sources for proteins. Lately (micro) algae have been receiving attention as potential sources for biodiesel that do not compete with land use for food or feed production.[70] However, it is still under debate whether this will be economically feasible. Figure 2.1 shows that microalgae can contain large amounts of protein.[26] The exact amount differs from one type of algae to another, but *Spirulina* can contain as much as 65 % protein on a dry weight basis. Coproduction of biodiesel and chemicals from both the oils and the proteins from microalgae could make both options more economically viable.

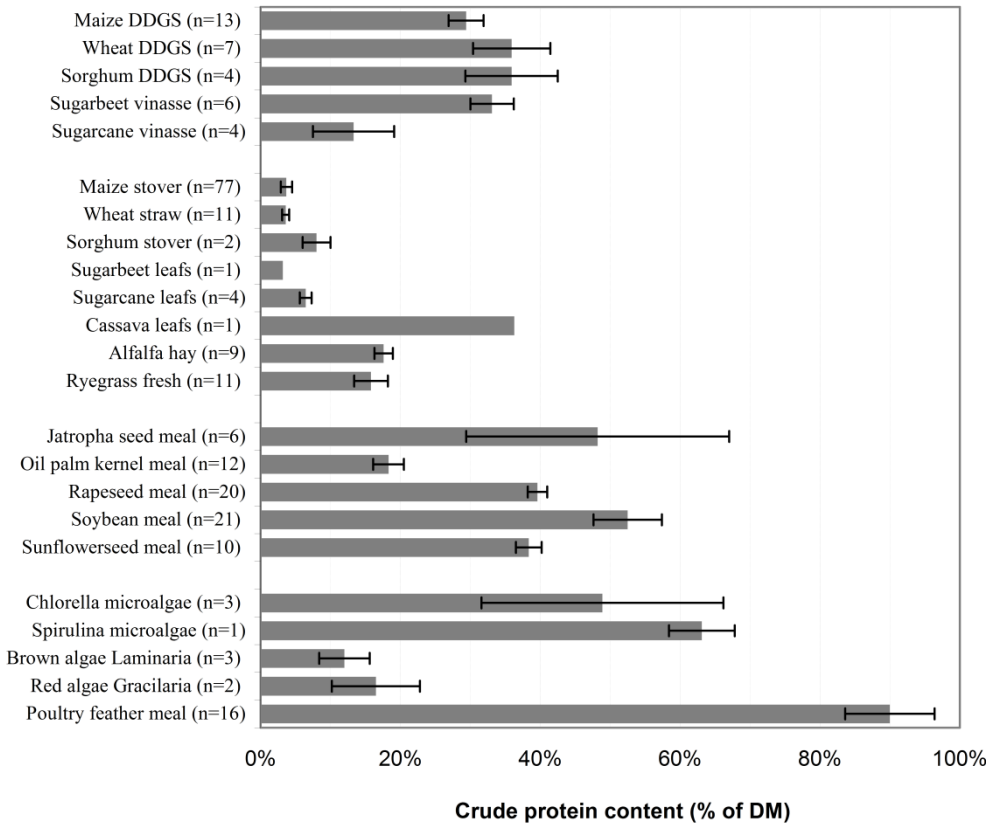

Figure 2.1. The crude protein content of a variety of agricultural byproducts, as a percentage of the dry matter of that byproduct. Error margins are standard deviation over the values in literature, whereas n is the number of values taken into account. References are given below.

Note: the composition of a certain plant can vary significantly, depending on factors like the type and contents of the fertilizer that is used, the soil composition, the local climate, etcetera. The presented data should therefore only be regarded as an estimation.

References to figure 2.1: Maize DDGS [25-27]; wheat DDGS [26, 28-30]; sorghum DDGS [31-33]; beet vinasse [26, 34-36]; cane vinasse [26, 35, 37, 38]; maize stover [39]; wheat straw [26]; sorghum stover [23, 40]; beet leaves [41]; cane leaves [19]; cassava leaves [41]; alfalfa hay [26]; ryegrass [42-44]; *Jatropha* meal [45-49]; oil palm meal [26, 50-52]; rapeseed meal [26, 53-57]; sunflower meal [26]; soybean meal [26, 55, 56, 58-60]; *Spirulina* [26, 61]; *Chlorella* [26, 62, 63]; *Laminaria* [64-66]; *Gracilaria* [67, 68]; poultry feather meal [26].

A source for proteins that received only little attention is animal slaughter waste. One typical example of animal slaughter waste is poultry feather meal, which besides fat almost exclusively contains proteins. Recently Kondamudi *et al.* showed that it is possible to extract the fat from feather meal and turn it into biodiesel.[71] Analog to the algae example above, the proteins could be simultaneously used for the production of chemicals.

2.4 Amino acid content of residual proteins

One important criterion for the assessment which amino acids have potential as building blocks for the chemical industry is the amounts at which they are present in the plant proteins. If a certain amino acid is present in significantly larger amounts than others, this amino acid may have more potential than the others as a starting material. Figure 2.2 shows the relative amount of different amino acids in the protein fraction of different byproduct streams.

Although the exact amino acid composition of the proteins will differ from one harvest or one location to another, this gives a good impression of which amino acids are present in relatively large amounts. Glutamic acid is an amino acid that can potentially be obtained in high yields from various sources. In almost all of the presented byproduct streams it is the most abundant amino acid, and in sugar beet vinasse glutamic acid was reported to be up to 63 % of the total amino acid content.[26, 34] Only in sugarcane vinasse the glutamic acid amount is surpassed by aspartic acid. Although this particular analysis of sugarcane vinasse comes from only one source,[38] a comparison with data of the amino acid content of sugarcane molasses showed that it indeed is more universally the case. Sugarcane molasses can contain up to 40 % aspartic acid and 27 % glutamic acid in its proteins,[26] which makes it likely that the same amino acids end up in the vinasse.

These data show that especially glutamic and aspartic acid have a high potential to be used as raw materials for the production of chemicals, because they are present in larger quantities than the other amino acids. Another advantage of these specific amino acids compared to others is that they are non-essential. That means that when glutamic and aspartic acid (both non-essential amino acids) are isolated from

for example DDGS without applying a chemical treatment of the DDGS, it will not lose its value as feed for animals.

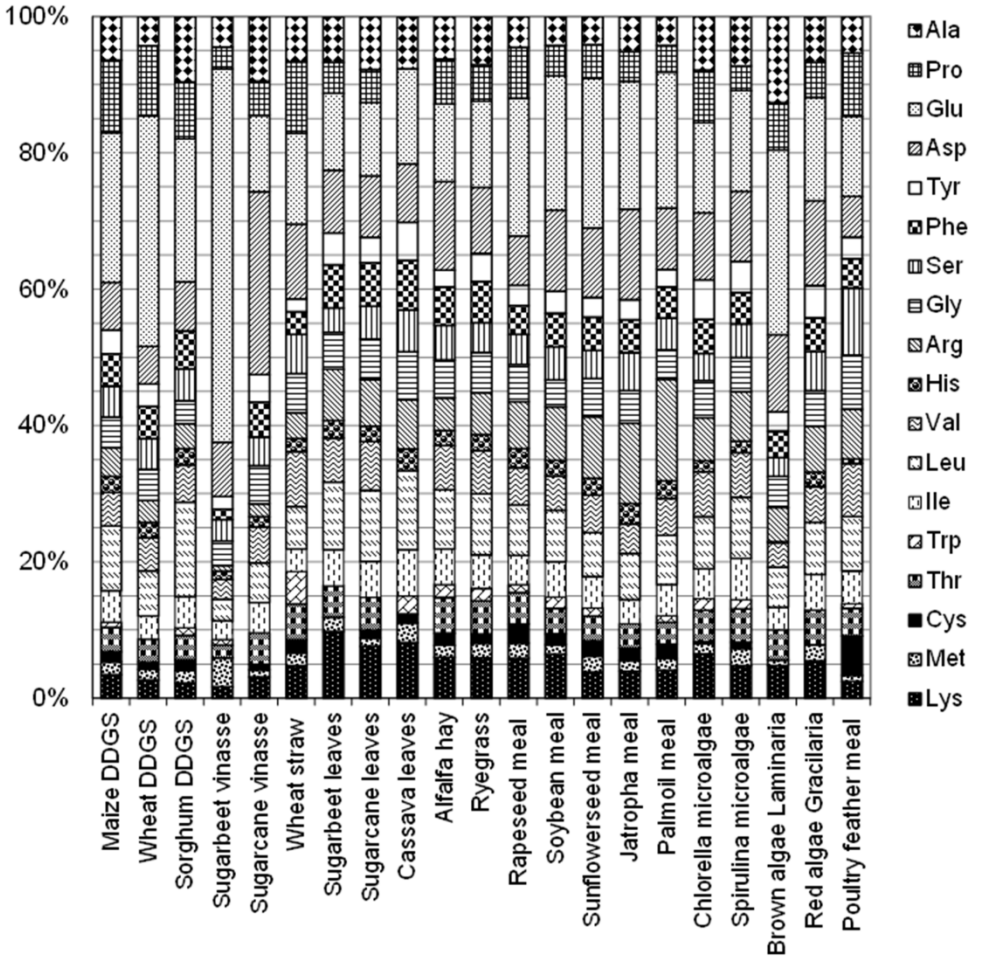

Figure 2.2. The relative presence of the different amino acids after hydrolysis of the proteins in a variety of agricultural byproducts. References are shown between brackets. Because of applied hydrolysis conditions, Glu includes glutamine and Asp includes asparagine.

References to figure 2.2: Maize DDGS [26]; wheat DDGS [26]; sorghum DDGS [32]; beet vinasse [26]; cane vinasse [38]; wheat straw [26]; sugarbeet leaves [21]; sugarcane leaves [20]; cassava leaves [72]; alfalfa hay [26]; ryegrass [73]; *Jatropha* meal [49]; oil palm meal [26]; rapeseed meal [26]; sunflower meal [26]; soybean meal [26]; *Spirulina* [26]; *Chlorella* [26]; *Laminaria* [74]; *Gracilaria* [68]; poultry feather meal [26].

2.5 Current biomass availability

This section will provide an overview of the current production of a range of protein sources, that could potentially be used for the production of chemicals. The protein sources that were chosen to assess are byproducts from bioethanol production (DDGS, leaves and vinasse of sugarcane and sugar beet, cassava leaves), byproducts from natural oil or biodiesel production (soybean, rapeseed, sunflower, oil palm, *Jatropha* meal), animal slaughter waste, some forage crops and algae. This does not pretend to be a complete overview of all the potential protein sources one could think of, but it should provide an overview of the most important ones in the world in terms of current or future (expected) production volume. For all protein sources a worldwide 'crude protein potential' will be determined. This is not done as an argument that all these proteins should be used for the production of chemicals instead of for their current use. Nor does it say anything about the economical, ecological or social viability to use these proteins for the production of chemicals. It will give an order of magnitude of the amounts of proteins that are currently present worldwide, in order to be able to determine which sources are available at sufficient volumes to provide an opportunity for the production of chemicals.

2.5.1 Maize and wheat DDGS

The total production of DDGS in the USA (by far the main producer in the world) in 2009 was 30.5 Mton, being mainly maize DDGS.[77] Assuming a crude protein content of about 27 % (see figure 2.1, compensated for 9 wt-% water content of DDGS), there is a production potential of 8 Mton crude protein from DDGS in the USA. Although the USDA expects the yearly increase of the maize ethanol production to slow down (the production in 2009 was around 38 billion liters of ethanol, which is 2 times that in 2006 and 3 times that in 2004),[78] it will continue to rise and therefore the DDGS production will continue to rise as well.

On a more local scale, a single large production plant of bioethanol from wheat, such as the one that Abengoa operates in The Netherlands, produces 500 million liters of ethanol, together with 360 kton of wheat DDGS, equivalent to 120 kton of crude protein.[79]

2.5.2 Sugarcane byproducts

As discussed above, the sugarcane vinasse production of Brazil, the main producer, is about 275 billion liters. This is non-concentrated vinasse with a dry matter content of 7-8 %.[80, 81] Assuming an average crude protein content of 13 % of the dry matter (see figure 2.1), there is a potential of 2.7 Mton residual crude protein from the vinasse of the Brazilian bioethanol industry. The error margin in this number is rather large though, because the reported compositions of sugarcane vinasse vary greatly from one source to another. On a local scale, one typical Brazilian bioethanol production plant produces 200 million liters of ethanol per year, from 2 million tons of sugarcane.[82] This yields 2.5 billion liters of vinasse, with a potential of 24 ktons of crude protein. Besides this, for every 100 tons of fresh sugarcane produced, 30 tons of leaves and tops are left on the field,[20] which means 600 kton of leaves and tops for a typical Brazilian bioethanol production plant. Assuming that 6.5 % of the dry matter of leaves and tops is protein (figure 2.1) and they contain 72 % water, the residual protein potential would be 11 kton. A practical example of the protein isolation from sugarcane leaves and tops was reported by Deepchand *et al.,* who calculated that for every ton of fresh leaves and tops, 100 kWh electricity, 8.3 litres of ethanol and 3 kg protein could be produced.[18] In their system electricity is the actual main product, and ethanol and protein are mere byproducts. Taking the numbers from their example would yield 2 kton of residual protein from 600 kton of leaves and tops, which is significantly less than the calculated potential of 11 kton. On a global scale, 1743 Mton of fresh sugarcane was produced in 2008. For this, 523 Mton of tops and leaves were left on the field. Using the same numbers, the total global potential of tops and leaves protein from sugarcane would be 1.6 Mton based on the practical example of Deepchand *et al.* and 9.6 Mton based on the total protein content of the tops and leaves.

2.5.3 Sugarbeet byproducts

In 2001, the total production of beet vinasse in ten selected countries in Europe was estimated to be 1.2 Mton.[35] These ten countries represent half of the worldwide sugarbeet production, indicating that the worldwide beet vinasse production would be about 2.4 Mton.[75] The worldwide sugarbeet production in 2008 was identical to

that in 2001 (228 Mton[75]), so one can assume that the vinasse production has remained the same as well. The global *potential* vinasse production can be estimated by comparing the concentrations of sugar beet components that should end up completely in vinasse, in between sugar beets and vinasse. Potassium is an example of this. On average, a sugar beet contains 0.24 % potassium (assuming 0.3% cations, 80% K^+ [83]). 228 Mton sugar beets then contain 0.55 Mton potassium. Beet vinasse contains on average 8 % potassium,[34, 35] resulting in a vinasse potential of 7 Mton.

Vinasse contains on average 68 % dry matter, of which 33 % is crude protein.[35] This results in a crude protein potential from *produced* beet vinasse of 0.5 Mton worldwide, and from *potentially produced* beet vinasse of 1.6 Mton. There are two main products in this 'crude protein' (calculated from the total nitrogen content, therefore containing both proteogenic and non-proteogenic amines), namely betaine and glutamic acid, and each of them can make up as much as 40% of the crude protein content.[34]

The leaves of the sugar beet represent about 38 % of the weight of the plant,[41] meaning that worldwide, the annual sugar beet leaf production is around 140 Mton. The major part of the fresh leaves consists of water, namely 86.4 %,[41] but the fresh leaves also contain 3.2 % protein. That means an annual total leaf protein potential of 4.5 Mton worldwide. On a more local scale, the bioethanol production plant of British Sugar in Wissington, UK, has a capacity for 70 million liters of bioethanol per year, thereby using 110 kton of sugar coming from 650 kton sugar beets.[84] This equals a beet leaf production of 400 kton, containing about 13 kton protein.

2.5.4 Cassava byproducts

The harvested area of cassava in 2008 was 18.7 MHa worldwide.[75] Without reducing the root yield, 12 ton/Ha of leaves can be harvested from the plants annually,[85] next to the current cassava production for starch production or other applications. This means that there is an annual potential of 224 Mton of fresh cassava leaves available. 224 Mton fresh cassava leaves contain 15.5 Mton crude protein.[41]

When cassava is used for the production of bioethanol, per ton of dried cassava roots used (containing <14% moisture), 84-89 kg of sludge is produced besides 333

liter ethanol.[86] The sludge contains 10% moisture, plus the proteins and other unconverted components. Assuming all proteins from the roots end up in the sludge and fresh cassava root contains 1-2 % protein,[41, 85] the protein content of the sludge will be about 35 %, so for every 10 L cassava-based ethanol, 1 kg protein is produced in the form of a protein-rich sludge.

2.5.5 Natural oil byproducts

Five byproduct streams from the production of vegetable oil or biodiesel are discussed. These are the meals of rapeseed, sunflower, soybean, oil palm and *Jatropha curcas*. In 2008, the total worldwide production of vegetable oil was about 132 Mton,[75] the vast majority of which being palm oil (44 Mton, including 5 Mton palm kernel oil), soybean oil (38 Mton), rapeseed oil (18 Mton) and sunflower oil (11 Mton). The total production of biodiesel was about 8.6 Mton in 2007,[87] 48 % of which came from rapeseed, 22 % from soya, 11 % from palm and about 6 % from sunflower oil.[88] As byproducts of vegetable oil production, large amounts of oil meals are produced. In 2008, Malaysia produced 2.4 Mton palm kernel meal next to 18 Mton of palm oil, so the worldwide available amount of palm kernel meal is around 5 Mton, giving a total crude protein potential (see figure 2.1) of about 1 Mton.[51, 89]The current application of palm kernel meal is as feed, but it is known to have only moderate nutritional value, making other applications interesting. Recently, Arifin *et al.* showed that they could extract over 80% of the crude protein present in palm kernel meal.[90]

Soybean meal is by far the largest available oil meal. In 2008, 158 Mton of soybean meal was produced worldwide,[91] all of it being used for feed purposes.[88] The total potential of crude protein from soybean meal would be 76 Mton. Soybean meal is very suitable for feeding purposes because of the high protein content (about 50 %) and the amino acid profile which is relatively high in essential amino acids such as lysine.

Rapeseed meal is available at lower quantities than soybean meal, but worldwide production in 2008 was 28 Mton, so there is a potential of about 10 Mton of crude protein.[92] It is almost exclusively used as feed, although it is less rich in proteins than soybean meal.

Of sunflower meal, 12 Mton was produced worldwide in 2008, with a crude protein potential of about 4 Mton.[93] Sunflower meal is also used as feed, although it has a lower feed value than soybean, because of the lower protein content.
The potential of *Jatropha* for the production of proteins is difficult to assess at this time. Recently there have been many incentives for the cultivation of *Jatropha* for biodiesel production, with varying success.[76] Because of the protein content of *Jatropha* meal, it holds a promise for the production of protein as well. There are studies on detoxifying *Jatropha* meal to make it available for feed purposes,[94-96] but producing chemicals could be a more interesting alternative.[47, 97] In 2008, the amount of seedcake that was produced was around 1.4 Mton (assuming 2 ton seedcake/Ha produced [69]), giving a crude protein potential of 0.8 Mton. When biodiesel production from *Jatropha* starts taking off, this number could increase dramatically.

2.5.6 Animal slaughter waste

For the Unites States, Kondamudi *et al.* calculated a potential of 580 million liters (153 M gallons) of biodiesel per year, produced from around 5 Mton (11300 million pounds) of poultry feather meal.[71] Besides this, assuming 85 % of protein content (a moderate estimation, given figure 2.1), there would be a potential of 4.3 Mton proteins in the United States. The worldwide poultry production in 2008 was about five times that of the USA, indicating a worldwide protein potential from poultry feather meal of about 21 Mton.[75]
There are no worldwide numbers published on different types of animal slaughter waste, but one can imagine that the slaughter waste produced from pigs and beef cattle is at least equally voluminous and contains similar amounts of fat and protein as that of poultry. In The Netherlands, the total amount of animal slaughter waste (including bones, feathers, blood, hair, etc.) that was produced in 2000, was 1.5 Mton.[98] Some of it is used in some form as animal feed, but most of it is burned for energy production because of regulatory reasons.[98] According to FAO, the total meat production in The Netherlands in 2000 was 2.9 Mton, indicating that the slaughter waste production is about half of the meat production in terms of weight. The total meat production in the world in 2008 was 280 Mton. The worldwide slaughter waste production would therefore be in the order of 140 Mton.[75] Taking

an average protein content of 50 % would mean a worldwide production of 70 Mton of proteins from animal slaughter waste in 2008, of which much is burned due to lack of possible applications. The underlying assumptions make this is a very rough estimation, but this estimation shows at least the order of magnitude of the worldwide production of animal slaughter waste and the proteins therein.

2.5.7 Microalgae

Microalgae such as *Chlorella* and *Spirulina* contain high amounts of proteins, typically in the order of 50-60 % of the dry weight. Because of the high protein content and the richness in minerals and vitamins, the current use of these types of microalgae is mainly as nutritional supplement in human diets, but they are also used as a protein supplement in poultry and lifestock feed, and as fertilizer.[61] In 2008, 62 kton of *Spirulina* (wet weight) was produced in China alone, which is about four times as much as in 2003, when 16 kton was produced.[99] There are no reliable worldwide production data of *Spirulina* available, but these FAO data show that the production numbers for *Spirulina* have been increasing rapidly over the last decade, and they do not account for at least the significant production numbers of *Spirulina* in the USA and Thailand.

When comparing these production numbers with other sources for protein that are mentioned above, it is clear that the crude protein potential from microalgae is much less than from other sources. Based on the above numbers, one can roughly estimate the current total crude protein potential from *Spirulina* on 50-100 kton. However, when the production keeps rising at it has done in the last decade, the production can become much more in the future. Recently, Wijffels *et al.* estimated that the theoretical algal biomass needed to supply 0.4 billion m^3 of biodiesel (replacing diesel for the complete European transportation market) would provide us with 300 Mton of algal protein.[70] This is a highly speculative number, but it shows that if there will be a sustainable and economically viable process for the production of microalgae in 15 years from now, the potential of proteins as co-products from algal biodiesel is very large.

2.5.8 Macroalgae

Compared to microalgae, macroalgae or seaweeds are produced in relatively large volumes already today. The worldwide production of brown seaweed in 2004 was almost 10 Mton (dry weight), and that of red seaweed an additional 4 Mton (dry weight). [100] Macroalgae generally contain less protein than microalgae, except for *Porphyra* that is specifically used for food production (i.e. Nori in Japan) and contains over 40 % protein.[101] *Gracilaria* is a red seaweed that is produced in large quantities (890 kton per year) for the extraction of agar, used in the food industry. It also contains 15-20 % protein, which can be used for other purposes.[67, 68] Brown seaweeds such as *Laminaria* or kelp (4.5 Mton per year, containing 8-15 % protein) are mainly produced for food use, some for the production of alginate.[74]
Assuming an average protein content of 20 %, the current production of worldwide macroalgae gives a total protein potential of about 3 Mton. However, for the majority of these proteins, the use of them for chemicals would be in direct competition with food production, especially in Asia, the exception of this being the seaweeds that are used for the production of alginate and agar. Using the proteins in those seaweeds would not be directly competitive with food production.

2.5.9 Forage crops

The worldwide total area of grasslands, mostly in the form of meadows and pastures, was estimated at 3.4 billion hectares in 2008.[75] According to Duke, the most common grass, perennial ryegrass, has reported yields between 5 and 25 ton dry matter per hectare per year.[41] The yield of grass depends highly on the local climate, soil type and fertilization.[102] A conservative estimation of 5 ton dry matter per hectare per year leads to a worldwide production of grass in the order of 17 billion tons. Ryegrass dry matter contains on average 16 % protein, meaning that the total protein potential is in the order of 2.7 billion ton. This grass could of course never all be harvested and used for biorefinery purposes, but it shows the potential. In many cases, grass is used as animal fodder. However, in other cases the grass is not or cannot be used as feed, for example due to regulations (i.e. ‘contaminated’ grass that grows close to highways in western Europe). In those cases, on a local scale, the production of biofuels and chemicals could be a valuable alternative to just burning it for energy. In Germany, recently a ‘Green

Biorefinery' demonstration plant was built that uses grass and alfalfa for the production of fodder, proteins, energy and lactic acid or lysine (the latter two are made by a fermentation process).[103] The proteins (with 80 % purity, at a yield of 0.4 kton protein per 8 kton dry matter inputs) are available at a cost price of 290 euro per ton (excluding capital investment),[104] to be sold as animal feed. This shows that it is possible to extract and purify a significant amount of protein from the grass at a competing price, although it is not all of the 16 % protein that was mentioned above.

Switchgrass is also commonly regarded as being potentially interesting for bioethanol production in the future.[105] It contains similar amounts of protein as 'normal' ryegrass, and the protein fraction could be an interesting byproduct from bioethanol production, as was shown with the 'switchgrass hydrolysate', by Bals *et al.*[22]

Another forage crop that could be valuable for a biorefinery is alfalfa. The advantage of using legumes such as alfalfa is that they contain bacteria in the roots that can fix nitrogen and therefore do not require as much fertilizer as other crops.[106] The worldwide production of alfalfa in 2006 was 436 Mton.[75] Fresh alfalfa contains 5 % protein (wet weight, 76 % moisture content),[107] indicating a total protein potential of 22 Mton. Alfalfa hay contains a bit less protein, 17.6 % of the dry weight, showing that by drying the alfalfa on the field, part of the protein content is lost. However, due to its high protein content and easily digestible carbohydrates, alfalfa is considered a nutritious feed, for example for dairy cattle. Using alfalfa for the purpose of chemicals would therefore mean a competition with feed production.

Table 2.1 summarizes the estimated crude protein potential (worldwide, per annum) of the various protein sources described in section 2.5, and the amount of the most abundant amino acid, glutamic acid, that would be available in those proteins, including the major advantages and disadvantages of using the sources for the production of chemical products.

Source	Protein potential (Mton)	Glu potential (Mton)	Advantages	Disadvantages
Maize and wheat DDGS	8	1.8	Availability	Potential feed competition
Sugarcane byproducts	12.3	1.3	Other main use is inefficient fertilizer	Protein content is very dilute
Sugarbeet byproducts	6.1	1.1	Other main use is low-value additive to feed or fertilizer	
Palm kernel meal	1	0.2		Lower protein content than other meals
Soybean meal	76	15	Availability	Potential feed competition
Rapeseed meal	10	2	Availability	Potential feed competition
Sunflower seed meal	4	0.9		Potential feed competition
Jatropha seed meal[a]	~0.8	~0.15	Little other use Availability increases High protein content	Yields and future availability are uncertain
Animal slaughter waste	~70	~7	Little other use High protein content	Large variety of waste types
Cassava leaves	15	2	Availability High protein content	Potential feed competition
Grass[b]	~2700	~340	Very large volumes available	Lack of infrastructure
Alfalfa	23	2.6	High protein content	Potential feed competition
Microalgae	< 0.1	< 0.015	Very high protein content High potential availability	Potential food competition Production cost Future availability is uncertain
Macroalgae	3	~0.6	Availability	Potential food competition

[a] Based on an estimated *Jatropha* production: 721000 Ha w/w 2009[76] at 2.5 ton/Ha production[69] ~ 2 Mton. [b] Based on estimated grass production: 3.4 billion Ha pasture & meadows,[75] with average yield of 5 ton/Ha.yr dry matter and moisture content of 73.4%.[41]

Table 2.1. The current availability of crude protein and glutamic acid from a variety of sources, as estimated in section 2.5. Typical advantages and disadvantages are mentioned.

2.6 Amino acid isolation

In order to use amino acids such as glutamic acid for the production of chemical products, they will have to be isolated at some stage. For this first the protein fraction from a byproduct stream needs to be isolated, followed by hydrolysis of the protein to its individual amino acids, and then the 20 different amino acids that are obtained need to be separated. Some of these steps could perhaps be integrated.
The isolation of proteins of plant materials in a biorefinery already occurs. For example Kamm *et al.* isolated a fraction of the proteins of grass, as mentioned above.[103, 104] Another example is zein, which is a protein fraction that can be isolated from maize-bioethanol production. Studies have shown that extractive separation of zein from maize in a process with centrifugation could be economically feasible,[108, 109] and another process for this based on ultra-filtration was patented in 2006.[110]
Another method for isolation of proteins was developed by Dale *et al.*, who used warm aqueous ammonia to extract the proteins from corn stover and wheat straw.[23]
Methods for the isolation of proteins from the byproducts of natural oil or biodiesel production have also been published. For example protein from *Jatropha* meal could be isolated using hexane,[47] and protein from palm kernel meal could be isolated with a saline or an alkaline aqueous solvent.[90]
In order to keep the protein intact these procedures are often as mild as possible. However, when the next step would be a hydrolysis of the protein to amino acids, that is not necessary. Therefore chances are large that the efficiency of amino acid isolation could still increase, when the extraction of protein would be combined with the hydrolysis step.

The separation of amino acids could be done based on their charge behavior. In this way it is possible to separate protein hydrolysates into acidic, basic and neutral amino acids.[111, 112] Processes in which separation by electrodialysis are coupled to chemical or enzymatic conversion processes are currently under development as well. This has already been shown to work for the separation of arginine and lysine, which are both basic amino acids.[113]
Another promising technique to separate amino acids is reactive extraction. Phenylalanine can be reactively extracted from a fermentation broth with di-2-

ethylhexyl phosphoric acid,[114] which has also been shown to be able to selectively (dependent on pH) pertract amino acids through a membrane.[115] Tertiary amines such as tripropylamine and trioctylamine in 1-octanol/n-heptane and also alamine 336 in MIBK can extract lactic acid from an aqueous phase and could presumably do the same with certain amino acids.[116, 117]

Methods to isolate glutamic acid from beet vinasse have been patented in the past as well.[118, 119] One method consists of a reactive extraction process in which glutamate is di-esterified with n-butanol, the ester at the same time extracted from the aqueous phase to the n-butanol phase, after which it is hydrolyzed again back into another aqueous phase.[118] These methods date from before monosodium glutamate was prepared by fermentation on a large scale and have the drawback of the production of large amounts of waste salts, but they could provide inspiration for the development of modern methods to isolate amino acids from byproduct streams.

The methods mentioned above to isolate amino acids in a biorefinery process have not been applied in practice yet, and they appear to be too expensive still, but developments are going fast. Therefore they could very well become technologically and economically feasible in the (near) future.

2.7 Discussion and perspectives

The main focus of this chapter was to estimate the current protein availability from biomass, in order to obtain an order of magnitude of the amount of glutamic acid that could be available for the production of chemicals.

Glutamic acid is the most abundant building block of the proteins in all investigated byproduct streams, except for sugarcane-vinasse. Together with the fact that it is a non-essential amino acid, this makes glutamic acid an interesting starting material for chemical products. The worldwide annual production of NMP is 100-150 kton.[120, 121] This demand could be met by making it from glutamic acid. The same holds for NVP and succinonitrile, of which the annual production volume is less, but in the same order of magnitude. Recently Le Nôtre *et al.* showed that acrylonitrile can be produced from glutamic acid.[122] Acrylonitrile is a bulk chemical with an annual production volume of 4.5 Mton in 2008.[123] To

replace this market completely with glutamic acid based acrylonitrile, at least 13 Mton of glutamic acid would be necessary as starting material. Looking at table 2.1, this number seems very difficult to reach with the current biomass production, since then a large amount of all the protein-derived glutamic acid present in the world would be needed. However, if there would be an economic incentive to replace fossil based acrylonitrile with glutamic acid based acrylonitrile it could enter the market, and on the long run partly replace fossil based acrylonitrile, depending on the availability of glutamic acid in the future.

When looking at the amount of proteins or amino acids that are present in certain agricultural byproduct streams, and the amount at which those streams are currently available, some appear to have more potential than others. For example maize and wheat DDGS could provide a source for a significant amount of glutamic acid for the chemical industry. The concrete example of Abengoa that was mentioned above shows that a single bioethanol production facility could provide 120 kton of protein, or about 36 kton of glutamic acid, which is enough to produce 23 kton NMP, 15 to 20 % of the worldwide market. Byproducts of sugar beets could provide similar amounts of glutamic acid. For sugarcane the overall 'crude protein potential' is higher, but the vinasse contains much less amino acids than that of sugar beets, making it more of a technological and a logistical challenge to gather the glutamic or aspartic acid from it in order to use it as a source for chemicals. Soybean meal would be an excellent source for glutamic acid, as it could provide 15 Mton of it. However, it also has an excellent feed value, with should not be compromised by the extraction of glutamic acid. Technology to achieve this does not exist yet, but would be very valuable. Similar is the case for the byproducts of other oil crops, although the feed value is reported to be a bit less than that of soybean meal, because the amino acid composition is less optimal. The slaughter waste of animals is a source that has not received much attention, but the very rough estimation of the crude protein potential that was done above shows that it could be a very interesting source as well. The leaves of plants are often left on or plowed into the field as fertilizer, but could also be an interesting source for proteins. In that case more of another fertilizer should be applied, but this could lead to an overall more efficient nitrogen use. If this would be done for cassava, which leaves contain more protein than most plants, this could result in 2 Mton of potentially available glutamic acid. For other plants, i.e. maize, this practice could

also lead to more available amino acids. Forage crops such as grass and to a lesser extent alfalfa could be a source for enormous amounts of proteins, as is shown in table 2.1. Legumes such as alfalfa have the extra advantage that they do not require much fertilizer. The calculated number for grass-derived protein could be off by an order of magnitude, but given the fact that although grass contains much less protein, the crude protein potential is more than 30 times as much as that of soybean meal, grass has great potential as a source for chemicals. The 'Green Biorefinery' demonstration plant that was recently built in Germany shows that it is already possible to recover a significant amount of those proteins in grass and alfalfa.[103, 104]

The case for both *Jatropha* and algae as a source for chemicals is quite different. The current production volumes are low, but they do hold a promise for the future. Whether these promises, i.e. quantified by Wijffels *et al.* at 300 Mton of algal protein, will become a reality will depend on many factors, and is difficult to say at this stage.[70]

The same is true for the future size of the byproduct streams mentioned in table 2.1. It can be expected that of many crops the worldwide production will increase, together with an increasing demand for food, an increasing demand for biofuels, and an increasing agricultural yield. The green revolution dramatically increased the crop production in the last century. Langeveld calculated based on FAO data that the average worldwide production of cereals from 1961 to 1963 was 908 Mton at 643 MHa, or 1.4 ton per Ha, and from 2005 to 2007 2242 Mton at 686 MHa, or 3.3 ton per Ha.[124] In other words, while the total cereal area increased by less than 10 %, the yield more than doubled. In some areas, such as North America and Europe, the total area even decreased while the yield more than doubled. In the future the yields of crops will probably keep increasing, for example with the aid of genetic modification. However, Langeveld also showed that the potential biofuels production that is estimated for 2050 depends very much on the type of study. Looking at it from the perspective of the ecologically possible production (how much biomass can nature produce?), biofuels potentials of 360 EJ[125] and 370 to 450 EJ[126] per year have been calculated. From a market perspective (who will produce which biomass how, at what cost, and where?) the biofuels potential in 2050 was estimated at 43 EJ per year.[127] Scenario studies (i.e. how does climate change impact biomass production? Or extrapolation of trends in crop production) show

biofuels potentials in 2050 of 18 to 25 EJ[128] and 300-800 EJ[129] per year. The large differences between these examples show that it is difficult and would be arbitrary to estimate a certain amount of biomass that will be available for the production of fuels or chemicals in the future. There are simply too many factors that have an influence on this estimation. However, all studies do show an increase in biofuels potential. So one can say with a degree of certainty that the numbers shown in table 2.1 will increase in the future, and that the availability of glutamic acid for the production of chemicals will thus only increase in the future.
It needs to be emphasized that all the numbers that were discussed in this review are not absolute availability numbers, but rather the production of certain sources. The fact that a source is produced does not necessarily mean that it is available for the production of chemicals. The advantages and disadvantages of the different sources as mentioned in table 2.1 relate to that. Some potential sources have a relatively high value for another use, such as soybean meal, but other sources have much less value such as beet vinasse and the leaves of different crops, or even a negative value due to their environmental burden, such as sugar cane vinasse. Separation between essential and non-essential amino acids will certainly increase the availability of amino acids, because then the animal feeds such as soybean meal could be used to create both a higher quality feed with the right amino acids and a feedstock for the chemical industry. That would be a win-win situation. Other factors that may determine the real availability of a source is the logistical challenge, such as the example of grass clearly showed. The total worldwide area of grasslands is not a meaningful number in this case, because the grass could never all be harvested and transported for use. However, the mentioned example of the grass biorefinery by Kamm *et al. s*hows that grass does have potential as a source for chemicals as well. In all cases the availability of a protein source for the production of chemicals from the amino acids will depend largely of the added value, which will have to compete with the value of the present use, and the logistics that are related to the amounts of amino acids that are needed and the infrastructure between the place of harvest and the production site.

The isolation of amino acids from the byproduct streams still poses a large challenge. However, research is underway, and once there is an increased demand for individual amino acids, either for use in more environmentally friendly food or

feed applications, or for the production of chemical products, or for both, and once there is a sufficient supply of proteins to biorefineries, the in-between isolation processes are likely to develop rapidly.

2.8 Conclusion

The goal of this chapter was to assess the availability of glutamic acid from the proteins in different biomass byproduct streams. This showed that for the production of biobased products with a market size such as NMP, in the order of 100 kton per year, there is enough glutamic acid available to replace a significant part of the fossil based market with biobased products. This could come from byproduct streams such as DDGS or vinasse, but also from plant leaves, natural oil or biodiesel byproducts, and from slaughterhouse waste. In the future algae could be an additional source of glutamic acid.
For bulk chemicals such as acrylonitrile there is currently not enough glutamic acid available to replace a large part of the market, but this may change in the future. Although it is difficult to quantify the glutamic acid availability in for instance 2050, it is very likely that the current availability will only grow, making the use of glutamic acid as a source for bulk chemicals a possible option for the future. In this case processes for the isolation of glutamic acid and other amino acids will have to be implemented, but this research is under development already.

2.9 References

1. IEA Bioenergy, *task 42 Biorefineries*, www.ieabioenergy.com/ Task.aspx?id=42, Accessed Jan **2011**.
2. J. J. Bozell and G. R. Petersen, *Green Chemistry*, **2010**, 12, 539-554.
3. K. Tuitoek, L. G. Young, C. F. de Lange and B. J. Kerr, *Journal of Animal Science*, **1997**, 75, 1575-1583.
4. B. J. Kerr, C. J. Ziemer, S. L. Trabue, J. D. Crouse and T. B. Parkin, *Journal of Animal Science*, **2006**, 84, 1584-1592.
5. F. O. Opapeju, D. O. Krause, R. L. Payne, M. Rademacher and C. M. Nyachoti, *Journal of Animal Science*, **2009**, 87, 2635-2643.

6. W. J. Powers, S. B. Zamzow and B. J. Kerr, *Applied Engineering in Agriculture*, **2007**, 23, 539-546.
7. J.-J. Wang, Y.-Y. Jing, C.-F. Zhang and J.-H. Zhao, *Renewable and Sustainable Energy Reviews*, **2009**, 13, 2263-2278.
8. E. Scott, F. Peter and J. Sanders, *Applied Microbiology and Biotechnology*, **2007**, 75, 751-762.
9. United Stated Department of Agriculture, *National Weekly Distillers Grains Summary*, www.ams.usda.gov/mnreports/lswndgs.pdf, Accessed Jan. 2011.
10. ICIS market intelligence, www.icis.com, Accessed Jan **2011**.
11. L. A. Martinelli and S. Filoso, *Ecological Applications*, **2008**, 18, 885-898.
12. S. Matsuoka, J. Ferro and P. Arruda, *In Vitro Cellular & Developmental Biology - Plant*, **2009**, 45, 372-381.
13. A. Rosabal, E. Morillo, T. Undabeytia, C. Maqueda, A. Justo and J. F. Herencia, *Soil Science Society of America Journal*, **2007**, 71, 1292-1298.
14. G. Gunkel, J. Kosmol, M. Sobral, H. Rohn, S. Montenegro and J. Aureliano, *Water, Air, and Soil Pollution*, **2007**, 180, 261-269.
15. L. A. B. Cortez and L. E. B. Perez, *Brazilian Journal of Chemical Engineering*, **1997**, 14, 9-18.
16. W. J. Freire, M. M. Rolim, L. A. B. Cortez and A. Bauen, Use of sugarcane ethanol vinasse for brick manufacture. (Special Issue: The farm machinery industry in Japan and research activities.), AMA, Agricultural Mechanization in Asia, Africa and Latin America. 2001. 32: 1, 51-54. 13 ref., **2001**.
17. A. E. Hartemink, in *Advances in Agronomy*, **2008**, vol. 99, pp. 125-182.
18. K. Deepchand, *Solar Energy*, **1985**, 35, 477-482.
19. K. Deepchand, *Agricultural Wastes*, **1986**, 15, 139-148.
20. K. Deepchand and J. Baguant, *Biomass*, **1985**, 7, 215-223.
21. C. Merodio and B. Sabater, *Journal of the Science of Food and Agriculture*, **1988**, 44, 237-243.
22. B. Bals, L. Teachworth, B. Dale and V. Balan, *Applied Biochemistry and Biotechnology*, **2007**, 143, 187-198.
23. B. E. Dale, M. S. Allen, M. Laser and L. R. Lynd, *Biofuels, Bioproducts and Biorefining*, **2009**, 3, 219-230.
24. B. E. Dale and M. Matsuoka, *Biotechnology and Bioengineering*, **1981**, 23, 1417-1420.
25. R. L. Belyea, K. D. Rausch and M. E. Tumbleson, *Bioresource Technology*, **2004**, 94, 293-298.

26. V. Deike and J. D. Mol, *Raw Material Compendium*, Novus International Inc., Brussels **1996**.
27. M. J. Spiehs, M. H. Whitney and G. C. Shurson, *Journal of Animal Science*, **2002**, 80, 2639-2645.
28. F. M. Dong, B. A. Rasco and S. S. Gazzaz, *Cereal Chemistry*, **1987**, 64, 327-332.
29. W. G. N. Ortín and Y. Peiqiang, *Journal of the Science of Food and Agriculture*, **2009**, 89, 1754-1761.
30. G. P. Widyaratne and R. T. Zijlstra, *Canadian Journal of Animal Science*, **2007**, 87, 103-114.
31. D. Y. Corredor, S. R. Bean, T. Schober and D. Wang, *Cereal Chemistry*, **2006**, 83, 17-21.
32. P. E. Urriola, D. Hoehler, C. Pedersen, H. H. Stein and G. C. Shurson, *Journal of Animal Science*, **2009**, 87, 2574-2580.
33. Y. Wang, M. Tilley, S. Bean, X. S. Sun and D. Wang, *Journal of Agricultural and Food Chemistry*, **2009**, 57, 8366-8372.
34. A. W. M. Huijbregts, Vinasse research, in: Royal Nedalco year report **1990**.
35. W. Lewicki, *International sugar journal*, **2001**, 103, 126-128.
36. K. Stemme, B. Gerdes, A. Harms and J. Kamphues, *Journal of Animal Physiology and Animal Nutrition*, **2005**, 89, 179-183.
37. V. Parnaudeau, N. Condom, R. Oliver, P. Cazevieille and S. Recous, *Bioresource Technology*, **2008**, 99, 1553-1562.
38. K. N. Waliszewski, A. Romero and V. T. Pardio, *Animal Feed Science and Technology*, **1997**, 67, 253-258.
39. D. W. Templeton, A. D. Sluiter, T. K. Hayward, B. R. Hames and S. R. Thomas, *Cellulose*, **2009**, 16, 621-639.
40. O. N. Di Marco, M. A. Ressia, S. Arias, M. S. Aello and M. Arzadún, *Animal Feed Science and Technology*, **2009**, 153, 161-168.
41. J. A. Duke, *Handbook of Energy Crops*, www.hort.purdue.edu/newcrop/ Indices/index_ ab.html, Accessed Jan **2011**.
42. Choi G.J., Lim Y.C., Rim Y.W., Sung B.R., Kim M.J., Kim K.Y. and S. S., *Journal-Korean Society of Grassland Science*, **2006**, 26, 9-14.
43. H. H. Meissner, South African Journal of Animal Science, **1996**, 26, 37-41.
44. A. Simić, S. Vučković, M. Kresović, S. Vrbničanin and D. Božić, *Biotechnology in Animal Husbandry*, **2009**, 25, 1171-1179.
45. A. O. Aderibigbe, C. O. L. E. Johnson, H. P. S. Makkar, K. Becker and N. Foidl, *Animal Feed Science and Technology*, **1997**, 67, 223-243.

46. G. M. Gübitz, M. Mittelbach and M. Trabi, *Bioresource Technology*, **1999**, 67, 73-82.
47. D. Lestari, W. Mulder and J. Sanders, *Biochemical Engineering Journal*, **2010**, 50, 16-23.
48. H. P. S. Makkar, G. Francis and K. Becker, *Journal of the Science of Food and Agriculture*, **2008**, 88, 1542-1548.
49. J. Martínez-Herrera, P. Siddhuraju, G. Francis, G. Dávila-Ortíz and K. Becker, *Food Chemistry*, **2006**, 96, 80-89.
50. J. A. Agunbiade, J. Wiseman and D. J. A. Cole, *Animal Feed Science and Technology*, **1999**, 80, 165-181.
51. J. M. Cerveró, P. A. Skovgaard, C. Felby, H. R. Sørensen and H. Jørgensen, *Enzyme and Microbial Technology*, **2010**, 46, 177-184.
52. E. V. Ezieshi and J. M. Olomu, *African Journal of Biotechnology*, **2007**, 6, 2484-2486.
53. H. Jeroch, F. Schöne and J. Jankowski, *Archiv für Geflügelkunde*, **2008**, 72, 8-18.
54. H. Kluth, T. Engelhard and M. Rodehutscord, *Züchtungskunde*, **2005**, 77, 58-70.
55. Y.-G. Liu, A. Steg and V. A. Hindle, *Animal Feed Science and Technology*, **1994**, 45, 397-409.
56. A. R. Moss and D. I. Givens, *Animal Feed Science and Technology*, **1994**, 47, 335-351.
57. P. Shawrang, A. Nikkhah, A. Zare-Shahneh, A. A. Sadeghi, G. Raisali and M. Moradi-Shahrebabak, *Radiation Physics and Chemistry*, **2008**, 77, 918-922.
58. G. W. Barbour, M. T. Farran, N. Usayran and N. J. Daghir, *World's Poultry Science Journal*, **2008**, 64, 177-185.
59. L. K. Karr-Lilienthal, C. M. Grieshop, N. R. Merchen, D. C. Mahan and G. C. Fahey, *Journal of Agricultural and Food Chemistry*, **2004**, 52, 6193-6199.
60. D. R. Lundry, W. P. Ridley, J. J. Meyer, S. G. Riordan, M. A. Nemeth, W. A. Trujillo, M. L. Breeze and R. Sorbet, *Journal of Agricultural and Food Chemistry*, **2008**, 56, 4611-4622.
61. M. A. B. Habib, M. Parvin, T. C. Huntington and M. R. Hasan, A review on culture, production and use of spirulina as food for humans and feeds for domestic animals and fish, Report 1034, FAO, Rome, **2008**.
62. Prime Chlorella Inc., *Composition of Chlorella*, http://www.pure-chlorella.com/info.html, Accessed Jan 2011.
63. Yaeyama Shokusan Co., *Yaeyama Chlorella Vulgaris*, http://www.chlorella-europe.com/composition.html, Accessed Jan **2011**.

64. P. Gayral and J. Cosson, Exposé synoptique des données biologiques sur la laminaire digitée Laminaria digitata, Report 89, Fisheries synopsis FAO, **1973**.
65. A. Haug and A. Jensen Seasonal Variations on the Checmical Copmosition of Alaria esculenta, Laminaria saccharina, Laminaria hyperborea and Laminaria digitata from Northern Norway, Report 4, Norwegian Institute of Seaweed Research **1954**.
66. N. Jurkovic, N. Kolb and I. Colic, *Die Nahrung*, **1995**, 39, 63-66.
67. M. H. Norziah and C. Y. Ching, *Food Chemistry*, **2000**, 68, 69-76.
68. X. Wen, C. Peng, H. Zhou, Z. Lin, G. Lin, S. Chen and P. Li, *Journal of Integrative Plant Biology*, **2006**, 48, 1047-1053.
69. W. M. J. Achten, L. Verchot, Y. J. Franken, E. Mathijs, V. P. Singh, R. Aerts and B. Muys, *Biomass and Bioenergy*, **2008**, 32, 1063-1084.
70. R. H. Wijffels and M. J. Barbosa, *Science*, **2010**, 329, 796-799.
71. N. Kondamudi, J. Strull, M. Misra and S. K. Mohapatra, *Journal of Agricultural and Food Chemistry*, **2009**, 57, 6163-6166.
72. A. O. Fasuyi and V. A. Aletor, *Pakistan Journal of Nutrition*, **2005**, 4, 43-49.
73. W. H. Eppendorfer, Journal of the Science of Food and Agriculture, **1977**, 28, 607-614.
74. A. R. O. Chapman, The wild harvest and culture of Laminaria longicruris in Eastern Canada, FAO, **1987**.
75. Food and Agricultural Organization of the United Nations, *FAO STAT*, http://faostat.fao.org/, Accessed Jan 2011.
76. K. Sanderson, *Nature*, **2009**, 461, 328-329.
77. Renewable Fuels Association, *Press release: 2009 Distillers Grains Exports Shatter Previous Record*, www.ethanolrfa.org, Accessed Jan **2011**.
78. United States Department of Agriculture, *Agricultural Baseline Projections: Baseline Presentation, 2009-2018*, http://www.ers.usda.gov/Briefing/Baseline/present2009.htm#cropsbox1, Accessed Jan **2011**.
79. Abengoa Bioenergy, www.abengoabioenergy.com, Accessed Jan **2011**.
80. K. Hidalgo, *Feed Technology*, **2009**, 13, 18-20.
81. K. R. Salomon and E. E. Silva Lora, *Biomass and Bioenergy*, **2009**, 33, 1101-1107.
82. J. Goldemberg, *Biotechnology for Biofuels*, **2008**, 1, 6.
83. A. W. M. Huijbregts, Proceedings 1st joint IIRB-ASSBT Congress, San Antonio, Texas, USA, **2003**, 451-459.
84. British Sugar, www.britishsugar.co.uk/Bioethanol.aspx, Accessed Jan 2011.
85. O. O. Tewe, *Cassava for livestock feed in sub-Saharan Africa*, Food and Agricultural Organization of the United Nations, **2004**.

86. T. Nguyen, S. Gheewala and S. Bonnet, *The International Journal of Life Cycle Assessment*, **2008**, 13, 564-573.
87. B. Moser, In Vitro Cellular & Developmental Biology - Plant, **2009**, 45, 229-266.
88. The International Grains Council, *Australian grain* **2008**, March-April, 30-31.
89. Malaysian Palm Oil Board, http://econ.mpob.gov.my/economy/ EID_web.htm, Accessed Jan **2011**.
90. B. Arifin, A. Bono, Y. Y. Farm, A. L. L. Ling and S. Y. Fui, *Journal of Applied Sciences*, **2009**, 9, 2996-3004.
91. United States Department of Agriculture, in *World Agricultural Supply and Demand Estimates*, March **2010**, p. 29.
92. United States Department of Agriculture, ed. Economic Research Service Briefing Room, **2010**.
93. National Sunflower Association, www.sunflowernsa.com, Accessed Jan **2011**.
94. A. Kumar and S. Sharma, *Industrial Crops and Products*, **2008**, 28, 1-10.
95. V. Kumar, H. P. S. Makkar and K. Becker, *Aquaculture Nutrition*, **2010**, no-no.
96. P. Siddhuraju, H. P. S. Makkar and K. Becker, *Food Chemistry*, **2002**, 78, 187-205.
97. A. M. J. Kootstra, H. H. Beeftink and J. P. M. Sanders, *Industrial Crops and Products*, **2011**, In Press, Corrected Proof.
98. M. Vis, Beschikbaarheid van reststromen uit de voedings- en genotmiddelenindustrie voor energieproductie, NOVEM report, in Dutch, **2002**.
99. Food and Agricultural Organization of the United Nations Fisheries Department, Fishstat Plus: Universal software for fishery statistical time series. Aquaculture production: quantities 1950-2008, Vers. 2.30 www.fao.org/fi/statist/FISOFT/ FISHPLUS.asp, Accessed Jan **2011**.
100. G. Roesijadi, A. E. Copping, M. H. Huesemann, J. Forster and J. R. Benemann, *Techno-Economic Feasibility Analysis of Offshore Seaweed Farming for Bioenergy and Biobased Products*, Battelle Pacific Northwest Division, **2008**.
101. K. Nisizawa, H. Noda, R. Kikuchi and T. Watanabe, *Hydrobiologia*, **1987**, 151-152, 5-29.
102. J. M. Suttie, S. G. Reynolds and C. Batello, *Grasslands of the world*, Food and Agriculture Organization of the United Nations, **2005**.
103. B. Kamm, P. Schönicke and M. Kamm, *CLEAN – Soil, Air, Water*, **2009**, 37, 27-30.
104. B. Kamm, C. Hille, P. Schönicke and G. Dautzenberg, *Biofuels, Bioproducts and Biorefining*, **2010**, 4, 253-262.
105. D. R. Keshwani and J. J. Cheng, *Bioresource Technology*, **2009**, 100, 1515-1523.
106. G. Carlsson and K. Huss-Danell, *Plant and Soil*, **2003**, 253, 353-372.
107. R. Fiorentini and C. Galoppini, *Journal of Food Science*, **1981**, 46, 1514-1517.

108. L. C. Dickey, A. McAloon, J. C. Craig and N. Parris, *Industrial Crops and Products*, **1999**, 10, 137-143.
109. L. C. Dickey, N. Parris, J. C. Craig and M. J. Kurantz, *Industrial Crops and Products*, **2002**, 16, 145-154.
110. M. Cheryan, *Pat.* US7045607, **2006**.
111. Y. Hara, Bulletin of the Chemical Society of Japan, **1963**, 36, 1373-1376.
112. J. Sandeaux, R. Sandeaux, C. Gavach, H. Grib, T. Sadat, D. Belhocine and N. Mameri, *J. Chem. Technol. Biotechnol.*, **1998**, 71, 267-273.
113. Y. Teng, E. L. Scott, A. N. T. van Zeeland and J. P. M. Sanders, *Green Chemistry*, **2011**, 13, 624-630.
114. N. Rüffer, U. Heidersdorf, I. Kretzers, G. Sprenger, L. Raeven and R. Takors, *Bioprocess and Biosystems Engineering*, **2004**, 26, 239-248.
115. A.-C. Blaga, A. Galaction and D. Cascaval, *Chemical and Biochemical Engineering Quarterly*, **2008**, 22, 439-446.
116. Y. K. Hong and W. H. Hong, *Biotechnology Techniques*, **1999**, 13, 915-918.
117. K. L. Wasewar, A. B. M. Heesink, G. F. Versteeg and V. G. Pangarkar, *Journal of Biotechnology*, **2002**, 97, 59-68.
118. Ajinomoto, *Pat.* 868,580, **1961**.
119. CSM, *Pat.* NL78864, **1955**.
120. B. Flick, C. E. Talsness, R. Jäckh, R. Buesen and S. Klug, *Toxicology and Applied Pharmacology*, **2009**, 237, 154-167.
121. M. Reich, *Chemical Engineering News*, **2008**, 32.
122. J. Le Notre, E. L. Scott, M. C. R. Franssen and J. P. M. Sanders, *Green Chemistry*, **2011**, 13, 807-809
123. The Association of Petrochemicals Producers in Europe, www.petrochemistry.net/ acrylonitrile.html, Accessed Jan 2011.
124. J. W. A. Langeveld, in *The Biobased Economy*, ed. J. P. M. S. J.W.A Langeveld, M.J.G. Meeusen, Earthscan, London, **2010**, vol. 1, pp. 83-100.
125. J. Wolf, P. S. Bindraban, J. C. Luijten and L. M. Vleeshouwers, *Agricultural Systems*, **2003**, 76, 841-861.
126. G. Fischer, E. Hizsnyik, S. Prieler and H. v. Velthuizen, *Assessment of biomass potentials for biofuel feedstock production in Europe: Methodology and results*, International Institute for Applied Systems Analysis, Laxenburg, Austria, **2007**.
127. R. Doornbosch and R. Steenblik, *Biofuels: is the cure worse than the disease?*, Organisation for Economic Co-operation and Development (OECD), Paris, **2007**.
128. M. Hoogwijk, A. Faaij, B. Eickhout, B. de Vries and W. Turkenburg, *Biomass and Bioenergy*, **2005**, 29, 225-257.

129. V. Dornburg, A. Faaij, P. Verweij, M. Banse, K. v. Diepen, H. v. Keulen, H. Langeveld, M. Meeusen, G. v. d. Ven, F. Wester, G. J. v. d. Born, M. v. Oorschot, J. Ros, F. Smout, D. v. Vuuren, J. v. Vliet, H. Aiking, M. Londo, H. Mozaffarian and K. Smekens, *Biomass Assessment: Assessment of global biomass potentials and their links to food, water, biodiversity, energy demand and economy*, Netherlands Environmental Assessment Agency, Utrecht, The Netherlands, **2008**.

Chapter 3

En route to biobased N-chemicals: Enzymatic decarboxylation of glutamic acid

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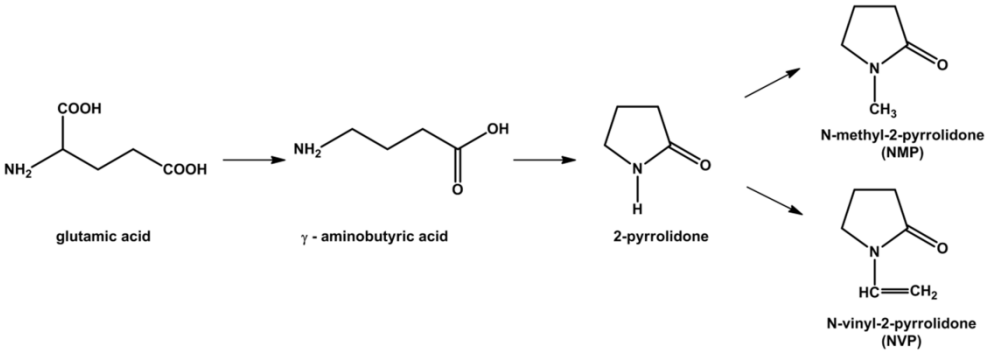
Abstract

On the pathway from glutamic acid to a range of molecules, the decarboxylation of glutamic acid to γ-aminobutyric acid (GABA) is an important reaction. This reaction, catalyzed by the enzyme glutamic acid α-decarboxylase (GAD) was studied on a gram scale. In this study, GAD was immobilized on Eupergit and in calcium alginate and its operational stability was determined in a buffer free system, using various reactor configurations. Immobilization was shown to increase the GAD stability. The conditions for the highest GABA production per gram of enzyme were determined by extrapolation of enzyme stability data. At 30 °C in a fed batch process this results in an average volumetric productivity of 35 $kg.m^{-3}.hr^{-1}$. The cost of using GAD immobilized in calcium alginate was estimated as € 5 per metric ton of product. Furthermore it was shown that the cofactor pyridoxal-5'-phosphate (PLP) could be regenerated by the addition of a small amount of α-ketoglutaric acid to the reactor. In conclusion the application of immobilized GAD in a fed batch reactor was shown to be a scalable process for the industrial production of GABA from glutamic acid.

3.1 Introduction

In the previous chapters, the potential of glutamic acid as a starting material for the production of industrial chemicals was demonstrated. This chapter will focus on the first step of the pathway towards pyrrolidone-based industrial chemicals, as can be seen in scheme 3.1. On this pathway, γ-aminobutyric acid (GABA) could be an interesting intermediate from which 2-pyrrolidone can be made by lactamization.[1] 2-Pyrrolidone itself is an industrial solvent and also the most important precursor for the monomer N-vinylpyrrolidone (NVP).[2] There are also known routes from 2-pyrrolidone to N-methylpyrrolidone (NMP), although these are not applied commercially.[3,4]



Scheme 3.1. The pathway from glutamic acid to pyrrolidone-based chemicals

The conversion of L-glutamic acid to GABA can be done enzymatically, with the enzyme glutamic acid α-decarboxylase (GAD), a pyridoxal 5'-phosphate (PLP)-dependent enzyme widely distributed among living organisms and studied extensively.[5] Recently a study of this reaction using immobilized whole cells of *Lactobacillus brevis* was published.[6] However, using whole cells for this reaction has disadvantages such as the decomposition of product by GABA transaminase in the presence of pyruvate or α-ketoglutarate,[7] or the possible decrease in activity due to lack of nutrients for the cells.[6] Until now, to the best of our knowledge, it has not been shown that this process could be profitable for the manufacture of other than food or pharmaceutical grade products.

Here an enzyme-based process for the scalable production of GABA from L-glutamic acid was developed, applicable in the chemical industry. For this, GAD

from *E. coli* was chosen as the best candidate to catalyze this reaction, because it is a very well characterized enzyme.[5,8-15] Purified GAD from *E. coli* was immobilized in two different manners (covalent binding to Eupergit and gel entrapment in calcium alginate) and the performance of the immobilized enzyme was studied in a buffer-free reaction system, to eliminate problems such as incompatibility with the co-enzyme PLP (the case with phosphate) or inhibition (the case with acetate).[9] Other advantages of a buffer-free system would be lowering process operation costs by using fewer chemicals and simplifying product recovery.

3.2 Materials and Methods

3.2.1 Materials

Sodium alginate and Eupergit 250 C were both obtained from Sigma. All other chemicals were obtained from Sigma or Fluka. Their quality was analytical grade or higher and they were used as received.

The purified GAD isoform GadB was isolated from *E. coli* and purified as published before.[8]

3.2.2 Analytical methods

For HPLC analysis, glutamic acid and GABA were derivatized with phenylisothiocyanate (PITC).[16] A 50 µl sample of reaction mixture or of a standard solution of glutamic acid or GABA was dried under vacuum. The residue was dissolved in 20 µl ethanol-water-triethylamine (2:2:1 by volume) and dried under vacuum. Then the residue was re-dissolved in 30 µl ethanol-water-triethylamine-PITC (7:1:1:1 by volume), allowed to react for 20 minutes and subsequently left overnight to dry under vacuum at room temperature. The remaining dry residue was dissolved in 0.8 ml of mobile phase, consisting of an aqueous solution of 6.57 g sodium acetate, 0.4 ml triethylamine, 0.6 ml acetic acid and 125 ml acetonitrile, made up to 1000 ml with water.[17] The final pH was 5.9.

Isocratic HPLC separation was performed on a Waters apparatus with a model 600 controller, a model 717plus autosampler with 100 µl injection loop, a model 2487 dual wavelength absorbance detector and a temperature control module. The column used was a HP Hypersil BDS C-18 (250 x 4.0 mm, 5 µm particle size). UV

detection was done at 254 nm. The flow rate was 0.6 ml/min at 30°C. Retention times of PITC-glutamic acid and PITC-GABA were 5.2 and 11.6 minutes, respectively.

3.2.3 GAD immobilization in calcium alginate

In a typical procedure, 1 mg/ml GAD solution was added to a 2% (w/v) solution of sodium alginate in sodium acetate buffer (0.1 M, pH 4.6), to give 25 μg/ml GAD. This solution was added dropwise with a syringe with a 0.4 mm (ID) needle to a continuously stirred aqueous solution of calcium chloride (0.2 M), resulting in the formation of off-white calcium alginate beads containing 0.05 mg GAD per 1 g of wet beads (GAD : alginate = 1 : 800 by weight). The beads were allowed to harden overnight at 4°C, after which they were filtrated and stored as such at 4°C. The filtrate was tested for GAD presence with an activity assay.

3.2.4 GAD immobilization on Eupergit 250 C

In a typical procedure, dry Eupergit beads (500 mg, 0.25 mm bead diameter, 100 nm pore diameter) were suspended in potassium phosphate buffer (5 ml, 1 M at pH 7.0) containing 0.1 mg/ml GAD and left overnight at room temperature on an overhead rotating disc. The suspension was filtered and the beads washed with sodium acetate (0.01 M, pH 4.6), re-suspended in sodium acetate (7 ml, 0.01 M, pH 4.6) and left for another 6 hours on the rotating disc at 4°C, to remove any non-covalently bound enzyme. Then the beads were filtered again and stored as such at 4°C, giving 0.24 mg GAD per 1 g of wet beads (theoretical value). Both filtrates were tested for GAD presence with an activity assay and a Bradford protein assay.

3.2.5 GAD activity assay

The enzyme activity assay was performed in water without any additional buffer present. The setup was a Metrohm 718 stat titrino with a titration vessel equipped with a thermostatic jacket. Titration was performed with an aqueous solution of HCl (0.1 M).

In a typical experiment, 10 ml of an aqueous solution of L-glutamic acid (0.08 M, 0.118 g) and PLP (0.5 mM, 1.33 mg) in water was brought to pH 4.6 with NaOH at 40°C. Then 0.02 mg GAD (or an equivalent of 0.02 mg GAD in the case of

immobilized GAD) was added and the titration curve recorded. GAD specific activity was determined as a function of the slope of the titration curve over 10 minutes time and is defined as $U.mg^{-1}$, equal to µmol $H^{+}_{added}.min^{-1}.mg^{-1}$.

3.2.6 GAD stability assay

The enzyme stability was determined by measuring the activity for a prolonged period of time under steady-state conditions, with a continuously operated stirred tank reactor (CSTR) setup. For this a Gilson Minipuls 2 pump with 0.25 mm (ID) PVC tubing was connected to the reaction vessel, continuously pumping a solution of L-glutamic acid and PLP (0.1 M and 0.5 mM, respectively, pH 4.6) in at 0.06 ml/min and pumping reaction mixture out at the same rate. A Biozym polyethylene filter with 16 µm pore diameter was used to keep the Eupergit beads in the reactor. Titration was done with an aqueous solution of HCl (0.1 M).

Another method for monitoring the GAD activity at a high substrate concentration for a long period of time was by titration with 0.08 M L-glutamic acid in water. In this case, GAD specific activity ($U.mg^{-1}$) was determined as µmol $L\text{-}Glu.min^{-1}.mg^{-1}$ added. When performing the reaction with GAD in calcium alginate, $CaCl_2$ (0.04 M) was added to the reaction mixture in both cases to keep the beads from dissolving in the course of time.

3.2.7 Computational fitting

Fitting of experimental data to model equations was performed with a Kevin Raner Software package called WinCurveFit, version 1.1.8, 2002.

3.3 Results and Discussion

3.3.1 GAD immobilization

Two different ways to immobilize GAD were chosen, namely gel entrapment in calcium alginate and covalent binding to Eupergit 250C epoxide beads, in order to determine whether immobilization has an influence on the enzyme performance.

GAD entrapment in calcium alginate yielded beads of approximately 1.5 mm diameter. The filtrate showed no activity for conversion of glutamic acid into GABA, so apparently the immobilization yield was 100%.

GAD binding to Eupergit was performed at pH 7. Bradford analysis of the supernatants showed no residual protein after 18 hrs of reaction and there was no activity for the conversion of L-glutamic acid (at pH 4.6), suggesting the immobilization yield was 100 %.

3.3.2 Enzyme activity assay and kinetics

In many micro-organisms, the GAD system is assumed to control the acidity of the cytosol, via the decarboxylation of glutamic acid to GABA, thereby incorporating a proton in the molecule.[10] Thus when performing this reaction without added buffer, the pH of the solution will rise in the course of the reaction. It was found that when comparing the titration curve with the GABA formation as measured by HPLC, these two graphs match (data not shown), indicating that both methods are equally suitable for determining the enzyme activity. Titration is the preferred method, because it provides a simple (online) activity assay.

With this assay Michaelis-Menten kinetics of the native and immobilized enzyme were studied. For this, the initial enzyme activity was measured as a function of the glutamic acid concentration and the data points were fitted to the Michaelis-Menten equation. The obtained values for the apparent K_m in the case of native GAD, GAD in calcium alginate and GAD on Eupergit were 2.0, 6.3 and 1.6 mM, respectively. So immobilization of GAD in calcium alginate increases the apparent K_m value. An explanation for this difference could be diffusion limitation. Since the alginate beads are much larger in size, glutamic acid needs to diffuse further to reach the GAD located in the center of the beads, a process which is favored by a higher concentration of glutamic acid. The obtained apparent K_m value of native GAD in water is a little higher than the K_m found by Shukuya in pyridine buffer (0.8 mM)[9] and lower than the K_m found in acetate buffer (6.7 mM).[11] The reason for a decrease in K_m compared to the one found in acetate buffer is thought to be the partial displacement of substrate from the enzyme surface by acetic acid.[9]

3.3.3 Enzyme stability

The GAD activity as a function of time was determined under semi steady-state conditions, keeping the substrate concentration well above K_m. This was done in order to eliminate any concentration effects from the measured enzyme activity and

thus to determine the stability in time under turnover conditions. Two different ways to do this were chosen. The first was a CSTR setup, which is a convenient way to compare the stability of GAD on the two different carriers. However, it is unsuitable to determine the stability of native enzyme, because the enzyme will not be retained by the polyethylene filter. The activity of GAD on Eupergit and alginate was measured for 24 hours continuously. HPLC analysis of reaction samples showed that in the CSTR setup at 40 ^{o}C and pH 4.6, a steady state with a constant concentration level of both glutamic acid and GABA was reached after four hours. The activity measured as a function of time was for both GAD in calcium alginate and GAD on Eupergit the same as determined with a fed batch setup, which was chosen as the preferred method to assess the stability of the native GAD and compare it with immobilized GAD. In this case, titration was done with 80 mM glutamic acid in water instead of with hydrochloric acid, in order to replenish the substrate and in doing so keeping the substrate concentration well above K_m. A comparison of the enzyme stability of native GAD, GAD in calcium alginate and GAD on Eupergit during 24 hours of turnover conditions is shown in figure 3.1. This shows that immobilizing GAD significantly improves the enzyme stability. Similar curves were obtained at 40 ^{o}C and 50 ^{o}C (data not shown).

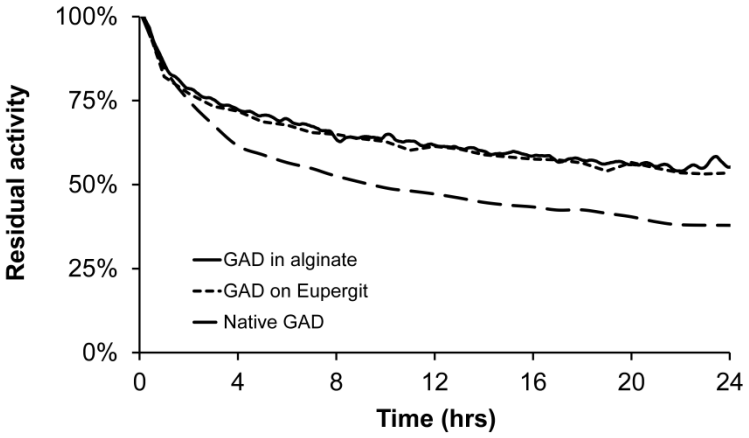


Figure 3.1. The influence of immobilization on GAD stability in a fed batch setup. Individual titration curves with native GAD, GAD in calcium alginate and GAD on Eupergit were recorded for 24 hours at pH 4.6 and 30 ^{o}C. The residual activity is defined as the specific activity at a given time divided by the initial specific activity.

3.3.4 Reaction conditions

The effect of pH on the enzyme activity was determined for the immobilized as well as the native GAD. The native enzyme showed a strong decrease of the activity above pH 5, which is similar to data reported before.[9] The immobilized enzyme showed in both cases this profile too (data not shown), indicating that the immobilization had no influence on the pH dependency of the enzyme activity. The pH dependency of the enzyme activity is known to be associated to a protein conformational change,[12] so the immobilization had no effect on the conformational mobility of the enzyme. 24 Hours fed batch experiments were also performed at different pHs in the range of 3.6 to 4.6 (data not shown). No differences were found in the enzyme stability.

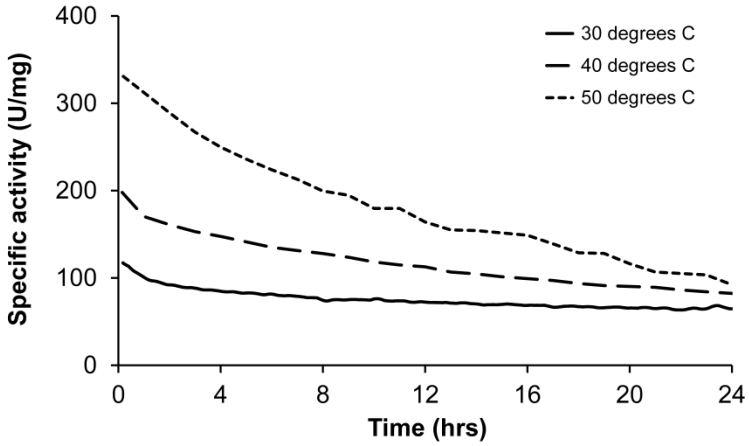


Figure 3.2. The influence of temperature on the long-term GAD activity in a fed batch setup. Individual titration curves with GAD in calcium alginate were recorded at 30, 40 and 50 ^{o}C for 24 hours at pH 4.6.

The influence of temperature on the enzyme performance (activity and stability) was also studied. A higher temperature gives a higher enzyme activity, but also a faster decrease of the activity, as can be seen from figure 3.2, which shows the specific enzyme activity as a function of time with GAD immobilized in calcium alginate. Similar curves were obtained with native GAD and GAD on Eupergit (data not shown).

3.3.5 GAD deactivation

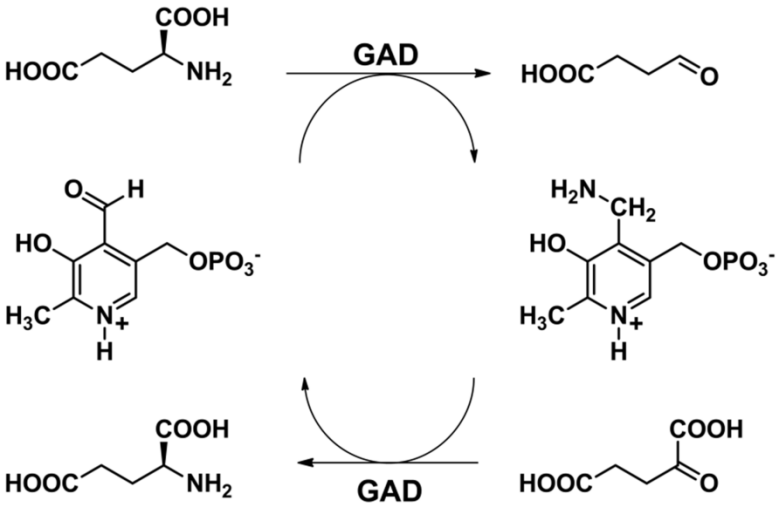
The cause of the decrease of GAD activity with time is known to be related to a side reaction that takes place once every 300,000 turnovers, which is an abortive transamination reaction.[13,14] In this case, GAD replaces a carbonyl group of PLP with an amine group from a GABA molecule, thereby producing pyridoxamine-5-phosphate (PMP) and succinic semialdehyde. PMP dissociates from the active site, leaving GAD in its apo-form, which is conformationally less stable.[15] Meeley and Martin showed that the inactivation of GAD from hog brain is not first order, but is best described as the sum of two exponential decay processes.[18] The reason for this biphasic inactivation could be in the multiple PLP binding sites that GAD contains, which might inactivate at different rates. In the case of GAD from *E. coli* the measured deactivation curves of GAD show a good fit to this model, at different temperatures and for both the immobilized and the native GAD. The results for the fit of the curves in figure 3.2 are shown in table 3.1. The rate constant of the first term (a) shows a clear temperature dependency, with an increasing rate of deactivation at increasing temperature. The second rate constant (b) shows no clear temperature dependency; however, the errors in the determined values for b are high, making it difficult to draw any conclusions from the second term of deactivation.

	Coefficient (% of initial rate)		Rate constant (hr^{-1})	
T	A_0	B_0	a	b
30 °C	71 ± 0.2	36 ± 6.5	0.014 ± 0.002	0.74 ± 0.19
40 °C	85 ± 3.8	19 ± 1.7	0.037 ± 0.008	1.13 ± 0.45
50 °C	84 ± 0.6	20 ± 1.2	0.058 ± 0.016	0.40 ± 0.01

Table 3.1. The determined coefficients and rate constants for a fit of the obtained data for GAD in calcium alginate with a double exponential decay function: residual activity = $A_0 e^{-at} + B_0 e^{-bt}$. Given errors are the deviations between two measurements.

3.3.6 Cofactor regeneration

It was shown before that the stability of GAD can be greatly increased by adding PLP to the reaction mixture.[11,14] Another approach can be the regeneration of PLP from PMP by GAD transaminase activity, which is known from human GAD.[19] This is shown in scheme 3.2. When a small amount of α-ketoglutaric acid is added to the system, GAD catalyzes the transamination of α-ketoglutaric acid to glutamic acid, thereby converting PMP into PLP.



Scheme 3.2. The regeneration of PLP with α-ketoglutaric acid

Figure 3.3 shows that if neither PLP nor α-ketoglutaric acid is added, that leads to a fast deactivation of GAD. If no PLP is added, it will only be present in a stoichiometric amount, for in active GAD from *E. coli* PLP is bound covalently to the active site. This stoichiometric amount of PLP will be transaminated over time, leading to a fast decrease of the enzyme activity. The addition of 5 mM α-ketoglutaric acid, however, has the same influence on the enzyme activity as the addition of extra PLP to the system, probably because the PLP is now regenerated. Addition of less (0.5 mM) and more (50 mM) α-ketoglutaric acid was also tested. The first showed no effect, which is likely due to unfavorable kinetics, and the second showed a stable but lower GAD activity, probably due to competitive inhibition of glutamic acid by α-ketoglutaric acid. The fact that replacing PLP with

α-ketoglutaric acid is a possibility for this process is especially interesting from an economic point-of-view, because α-ketoglutaric acid is much less expensive than PLP. Moreover, the transamination reaction regenerates glutamic acid which can then be recycled.

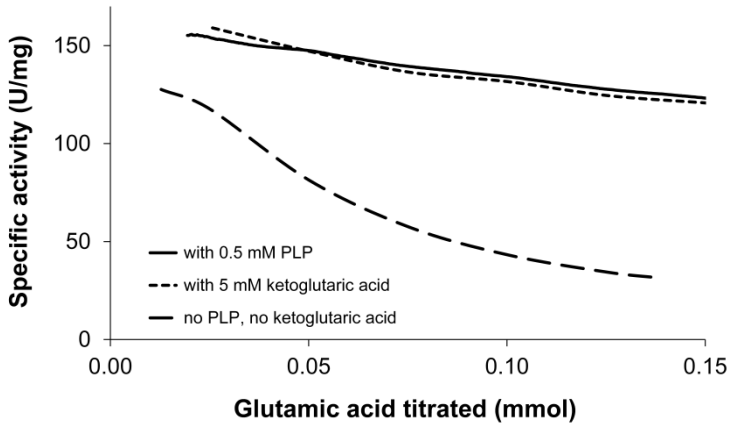


Figure 3.3. The influence of α-ketoglutaric acid on the GAD stability. Individual titration curves were recorded with native GAD at pH 4.6 and 40 °C, in the presence of 0.5 mM PLP, in the presence of 5 mM α-ketoglutaric acid and in the presence of neither PLP, nor α-ketoglutaric acid.

3.3.7 Reactor design and productivity

The long-term experiments with immobilized GAD described in this chapter have been performed in two different ways of operation, in a CSTR and in a fed batch reactor. From the obtained data it is possible to derive what would be the best mode of operation and to calculate if this process would be economically viable in an industrial application. In order to do this, a mass balance of the reactor was made and from this the amount of GABA that can be produced per gram of GAD was calculated, as was the volumetric productivity of the reactor in that case.[20] GAD immobilized in calcium alginate was chosen as the model catalyst, as it has been shown to perform better than GAD immobilized on Eupergit. Alginate is also less expensive than Eupergit. The chosen operating conditions for these calculations were calcium alginate at pH 4.6 and 30 °C. These conditions were chosen because an extrapolation of the reactor productivity based on the derived inactivation data

(Table 3.1) shows that at 30 degrees the total expected GABA yield per gram GAD is the highest, although it takes more time (Figure 3.4).

This reaction can conveniently be performed in a fed batch process, as was shown above. In order to keep the volume of the reactor constant, the reaction could be performed by the addition of solid glutamic acid instead of an aqueous solution of glutamic acid. In this way, the substrate concentration would remain constant as the product concentration increases, resulting in an accumulation of product. Therefore, it is important that product inhibition of GAD by GABA does not take place. This was examined up to a GABA concentration of 5 mol/L. No decrease in enzyme activity was detected, as was expected because the presence of two free carboxylic acid groups in a molecule is required for effective inhibition of GAD.[21]

Figure 3.4 showed the expected productivity of the reactor with GAD in calcium alginate at 30 ^{o}C. After eight days only 5 % of the initial GAD activity remains. After this time 1 gram of GAD has formed 34 kg GABA. For a production of 1000 kg GABA in these eight days, 29.4 gram GAD of high purity would be needed. The activity of the purified GAD is 5.2 times higher than that of the crude extract as reported by De Biase et al.[8] Should a crude extract of GAD be used (which is likely to be used in industry), 150 gram would be needed.

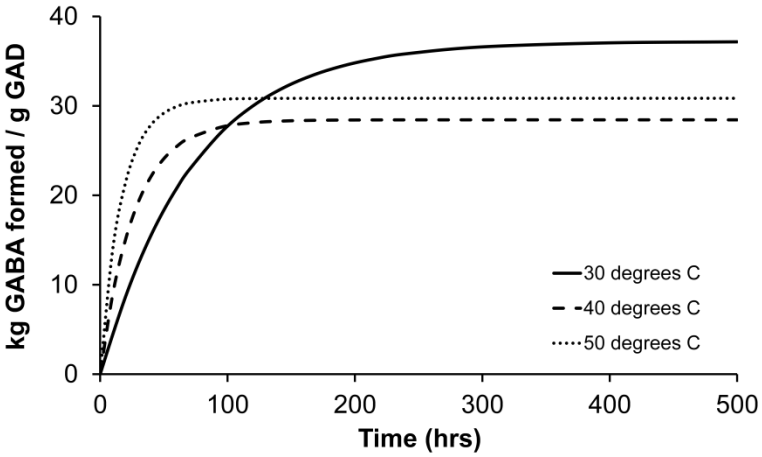


Figure 3.4. The expected productivity of GAD in calcium alginate at different temperatures, based on the inactivation data shown in table 3.1.

For the immobilization of an enzyme in calcium alginate, an enzyme to alginate weight ratio of 0.5 is achievable with lipase.[22] Assuming that would also be the case with GAD, 300 gram alginate is needed for the immobilization of 150 gram GAD. The alginate concentration is 2 % (w/v), so 300 gram alginate is equal to 15 L of beads. If the catalyst loading is 10 %, then the total reactor volume would be 0.15 m^3. The average volumetric productivity of this reactor over eight days based on the above assumptions would then be 35 kg GABA. $m^{-3}.hr^{-1}$.
The same calculation can be done for performing the process at 50 ^oC. Then the reaction time to 5% remaining enzyme activity is 50 hours and by then 1 gram GAD has formed 29 kg GABA. The average volumetric productivity would then become as high as 111 kg GABA.$m^{-3}.hr^{-1}$.
A cascade of three CSTR's which each 80% conversion (overall giving 99% conversion) showed to be less effective than a fed batch process. This is because of the low glutamic acid concentration in the second and third reactor (4 and 1 mM, respectively). These concentrations are below the determined apparent K_m of the enzyme in calcium alginate and therefore the reaction rates are relatively low.

3.3.8 Cost analysis

To estimate the price of the needed enzyme per metric ton of produced GABA, one can assume that a typical price for a crude enzyme applied in the chemical industry would be € 100 per kg of pure enzyme equivalent.[23] Then the cost of enzyme would be € 3 per ton GABA. Using crude enzyme and immobilizing that with alginate would cost 0.3 kg alginate at € 6 per kg is € 1.80 per ton GABA extra. The cost of using immobilized GAD in this process would therefore be around € 5 euro per ton of produced GABA. That means the price will be in the same range as for example amylase (used for the hydrolysis of starch), which costs € 2-3 per ton of product.[24]
Furthermore, because of the high volumetric productivity of this bioreactor (which is related to the high enzyme activity), the operational costs are not expected to form an obstacle. Also the costs of PLP and α-ketoglutaric acid are not expected to pose a problem, because the amounts used in the process will be very limited and most of it can be recycled.

The major obstacle for the application of this process is the current cost of glutamic acid, which is too high because it is still produced by a fermentative process.[25] The isolation of glutamic acid from protein rest streams is essential to make this process cost-effective, and is therefore a challenge that is under current investigation.

3.4 Conclusion

Here we have shown that it is possible to develop a practical and economically feasible process for the industrial bulk production of GABA, based on glutamic acid. GABA can then be a precursor for different nitrogen-containing materials in such a way that it can be implemented in the current infrastructure of the chemical industry, thereby reducing the dependency on fossil fuels.
By way of immobilization, the enzyme (GAD) was made more stable and easily separable from the reaction mixture. Furthermore, a reactor setup with a pH stat was tested on a gram scale with the immobilized enzyme, showing that it is possible to perform the reaction without any buffer present. The subsequent reactor design, based on the acquired results and assuming no major scale-up problems, showed that a fed batch process would be the preferred method. A major advantage of this is that the process can be run up to a high product concentration, making the product recovery easier. Finally, and important for an industrial application, it was shown that the enzyme cost will not be an obstacle for this process.

Acknowledgements

We wish to thank professor Kenji Soda (Kansai University, Japan) for his fruitful suggestions concerning the reactivation of apo-GAD and Kees van Kekem (WUR, The Netherlands) for providing a HPLC apparatus and assistance. Further we are grateful to NWO-Aspect for funding of this work.

3.5 References

1. A. Blade-Font*, Tetrahedron Letters*, **1980**, 21, 2443-2446.
2. A.L. Harreus, 2-Pyrrolidone, In: Ullmann's Encyclopedia of Industrial Chemistry, Online edn., **2000**, Wiley InterScience.
3. T. Setoyama, T. Ogoshi, JP2001288164A, **2001.**
4. T. Setoyama, T. Ogoshi, JP2001288165A, **2001**.
5. H. Ueno, Journal of Molecular Catalysis B: Enzymatic, **2000**, 10, 67-79.
6. J. Huang, L. Mei, H. Wu, D. Lin, *World Journal of Microbiology and Biotechnology*, **2007**, 23, 865-871.
7. B.J. Shelp, A.W. Bown, M.D. McLean, *Trends in Plant Science*, **1999**, 4, 446-452.
8. D. De Biase, A. Tramonti, R.A. John, F. Bossa, *Protein Expression and Purification*, **1996**, 8, 430-438.
9. R. Shukuya, G.W. Schwert, *Journal of Biological Chemistry*, **1960**, 235, 1649-1652.
10. G. Capitani, D. De Biase, C. Aurizi, H. Gut, F. Bossa, M.G. Grütter, *EMBO Journal*, **2003**, 22, 4027-4037.

A. Tramonti, D. De Biase, A. Giartosio, F. Bossa, R.A. John, *Journal of Biological Chemistry*, **1998**, 273, 1939-1945.

11. H. Gut, E. Pennacchietti, R.A. John, F. Bossa, G. Capitani, D. De Biase, M.G. Grütter*, EMBO Journal*, **2006**, 25, 2643-2651.
12. T.G. Porter, D.C. Spink, S.B. Martin, D.L. Martin, *Biochemical Journal*, **1985**, 231, 705-712.
13. V.P. Almazov, I.V. Morozov, F.A. Savin, B.S. Sukhareva, *Journal of Molecular Biology U.S.S.R.*, **1985**, 19, 359-370.
14. P.L. Grant, J.M. Basford, R.A. John, *Biochemical Journal*, **1987**, 241, 699-704
15. S. Gunawan, N.Y. Walton, D.M. Treiman, *Journal of Chromatography A*, **1990**, 503, 177-187.
16. V. Rossetti, A. Lombard, *Journal of Chromatography B*, **1996**, 681, 63-67.
17. M.P. Meeley, D.L. Martin, *Cellular and Molecular Neurobiology*, **1983**, 3, 39-54.
18. T.G. Porter, S.B. Martin, D.L. Martin, *Journal of Neurochemistry*, **1986**, 47, 468-471.
19. See appendix for calculations.
20. M.L. Fonda, *Biochemistry*, **1972**, 11, 1304-1309.
21. K. Won, S. Kim, K. Kim, H.W. Park, S. Moon, *Process Biochemistry*, **2005**, 40, 2149-2154.
22. Estimation by J.P.M. Sanders, based on industrial expertise.

23. P.S.J. Cheetham, in *Applied Biocatalysis*, ed. J.M.S. Cabral, D. Best, L. Boross, J. Tramper, Harwood Academic Publishers, Chur, 1st edn., **1994**, ch. 11, pp. 431-469.
24. T. Kawakita, C. Sano, S. Shioya, M. Takehara, S. Yamaguchi, *Monosodium Glutamate,* In: *Ullmann's Encyclopedia of Industrial Chemistry,* Online edn., **2000**, Wiley InterScience.

Chapter 4

Synthesis of biobased pyrrolidones from γ-aminobutyric acid

This chapter was published (in adapted form) as:
T.M. Lammens, M.C.R. Franssen, E.L. Scott and J.P.M. Sanders, "Synthesis of biobased N-methylpyrrolidone by one-pot cyclization and methylation of γ-aminobutyric acid", *Green Chemistry* 2010, 12, p. 1430–1436.

Abstract

N-methylpyrrolidone (NMP) is an industrial solvent that is currently based on fossil resources. In order to prepare it in a biobased way, the possibility to synthesize NMP from γ-aminobutyric acid (GABA) was investigated, since GABA can be obtained from glutamic acid, an amino acid that is present in many plant proteins. Cyclization of GABA to 2-pyrrolidone and subsequent methylation of 2-pyrrolidone to NMP was achieved in a one-pot procedure, using methanol as the methylating agent and a halogen salt (i.e. ammonium bromide) as a catalyst. A selectivity above 90 % was achieved, as well as a high conversion. Methylation of 2-pyrrolidone could also be done with dimethyl carbonate, but then the selectivity for NMP was less (67 %). When part of the 2-pyrrolidone is separated from the produced NMP, it could be used as well for the production of N-vinylpyrrolidone.

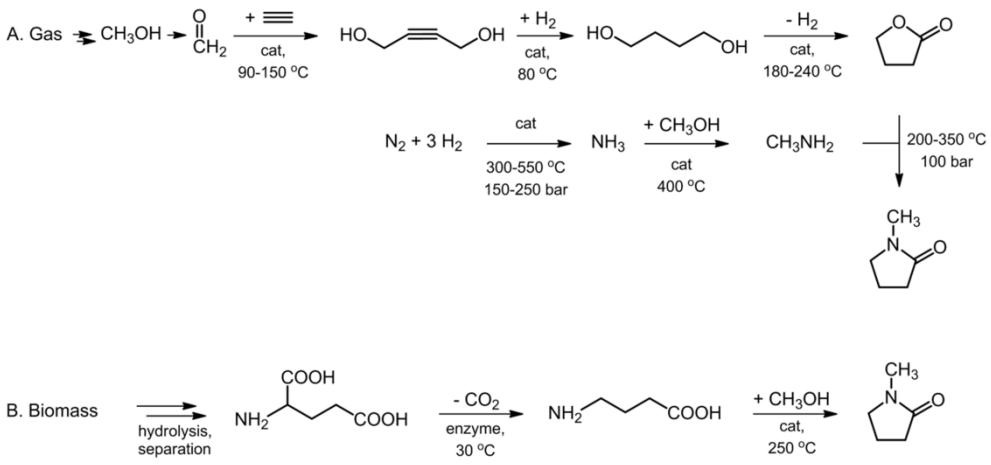
4.1 Introduction

Chapter 3 discussed the production of GABA from glutamic acid. This chapter will focus on the next steps that were shown in scheme 3.1, namely the production of 2-pyrrolidone, N-methylpyrrolidone (NMP) and N-vinylpyrrolidone (NVP).
NMP is an example of an industrial solvent that is currently produced from fossil resources. NMP has high chemical and thermal stability making it suitable for a range of applications including the use as a solvent for plastics and as an ingredient in paint removers.[1] It has been reported that NMP is safe and without any acute harmful effects,[2] although there have been other reports of toxicity developing upon inhalation or oral exposure.[3] The global annual production of NMP is estimated to be 100-150 kton.[3,4] From a commercial point of view, this amount can be interesting to be produced from products from a biorefinery instead of from conventional petrochemical products, because the production volume is large enough to be worth investing in a new process. In the previous chapter we showed that glutamic acid, which can be derived from waste streams from biofuels production, can be enzymatically decarboxylated to form γ-aminobutyric acid (GABA), in a process that we expect to be both technically and economically feasible.[5] GABA could then be an intermediate for the synthesis of a variety of nitrogen containing industrial chemicals, such as NMP.
NVP finds applications in the manufacturing of poly-vinylpyrrolidone and copolymers, for example together with vinyl acetate and methyl acrylate. Further it is used as solvent in the production of inks and paints, as an additive in the cosmetics industry, and in the pharmaceutical industry for the production of a disinfectant.[2]

Taking the large bioethanol production plant that the company Abengoa recently built in the Port of Rotterdam in the Netherlands as an example, this plant will annually produce around 500 million liters of grain-based ethanol, resulting in 360 kton of the byproduct dried distiller's grains with solubles (DDGS).[6] Wheat DDGS can contain as much as 10 wt-% glutamic acid (see chapter 2),[7] resulting in a potential glutamic acid stream of 36 kton. If this would all be converted into NMP, it would result in an annual NMP production of 23 kton, a significant part of the world market. Once glutamic acid becomes available in large volumes from

byproduct streams such as DDGS, it will become less expensive than when it is produced by the current fermentative process.[8] Then the production of NMP from glutamic acid could become an interesting process for chemical companies, if the process can be competitive with current production technology.

Scheme 4.1 shows the current and the proposed processes for the production of NMP. Currently NMP is made by reacting butyrolactone with methylamine, at high temperature (200-350 ^{o}C) and pressure (100 bar). Methylamine is a corrosive and highly flammable gas.[10] If NMP could be made by methylation of 2-pyrrolidone, which is readily formed by the cyclization of GABA,[11] the NMP would become largely biomass based instead of fossil based, and its production process could become more energy-efficient, safer and dependent on fewer reagents. If the methylating agent would be biobased as well, NMP would become fully biobased.



Scheme 4.1. Simplified overview of all process steps towards NMP. A: Fossil based;[9] B: Biomass based.

Traditional methylating agents, such as dimethyl sulfate and methyl iodide, are toxic and not environmentally friendly, because in their use stoichiometric amounts of waste salts are produced. In search of a better methylating agent, dimethyl carbonate (DMC, produced from methanol, oxygen and carbon monoxide) was shown to be capable of methylating various molecules and forming only methanol

and CO_2 as side products.[12,13] In a reaction catalyzed by zeolites, DMC can methylate different phenols and amines.[14] Ben Taleb *et al.* showed that it is possible to use DMC to methylate amides, such as acetamide and several lactams (e.g. 2-pyrrolidone), with the quaternary ammonium salt cetyl trimethylammonium bromide (CTAB) as a catalyst.[15] Unfortunately, methanol did not show any methylating activity under these conditions (220 ^{o}C in an autoclave). To perform a methylation reaction with just methanol would represent a big advantage as only water is generated as a co-product and the atom efficiency would also be better than with DMC. Oku *et al.* showed that N-methylation of several amines with methanol is possible under supercritical conditions with a bifunctional acid-base catalyst.[16] A prerequisite for good conversion with their catalyst is an 'anchoring group' on the amine, such as an alcohol or another amine that can form a bond with the surface of the catalyst. Therefore the catalyst worked well for 2-aminoethanol, but resulted in a poor conversion with molecules such as aniline and 2-pyrrolidone. Other methylation reactions of lactams such as 2-pyrrolidone with methanol that are reported in literature were performed in the gas phase, at temperatures that are typically as high as 400 ^{o}C.[17]

The goal of this chapter is to investigate the possibility to synthesize NMP from GABA, in order to obtain biobased NMP. We will show that it is possible to perform the cyclization of GABA and the subsequent catalytic methylation of 2-pyrrolidone with methanol in a one-pot procedure, under conditions that are milder than those of the current process for the production of NMP from butyrolactone, with a catalytic amount of an ammonium salt such as CTAB or ammonium bromide. We also investigated the possibility to use DMC in combination with a NaY zeolite catalyst to perform the methylation of 2-pyrrolidone. This will be compared with the use of methanol as a methylating agent.

4.2 Materials and Methods

4.2.1 Materials and equipment

γ-Aminobutyric acid (Fisher, >99%), 2-pyrrolidone (Sigma, >99%), anhydrous methanol (Sigma, >99.8%), ammonium bromide (Sigma, >99%), ammonium

chloride (Sigma, >99.5%), ammonium iodide (Fisher, >99%), cetyltrimethylammonium bromide (CTAB, Fisher, >99%), cesium bromide (Sigma, >99.5%), sodium bromide (Sigma, >99%), 1-butyl-3-methylimidazolium bromide (BMIM, Sigma, >98.5%), and methyltriphenylphosphonium bromide (MTPB, Sigma, >98%) were all used as received. Dimethyl carbonate (DMC, Sigma, >99%) was dried by refluxing over sodium sulfate and distilled under N_2 prior to use. NaY (CBV100, Zeolyst) was activated overnight at 70 ^{o}C under vacuum prior to use.

All reactions were performed in a Parr Series 5000 Multiple Reactor System, with six stainless steel autoclaves of 75 mL internal volume used in parallel.

High resolution MS spectra were recorded on an Exactive apparatus from Thermo Scientific, equipped with an ESI probe. Spectra were recorded both in positive and negative mode. M/z ratios were detected from 50 to 500.

GC/MS was performed with a Finnigan GC8000top apparatus connected to a Finnigan Automass II quadrupole EI-MS system. The used column was a BPX5 from SGE, 30 m x 0.25 mm x 0.25 μm. Helium carrier gas was applied at 100 kPa, the temperature program 50-300 ^{o}C at 10 ^{o}C/min. M/z ratios were detected from 35 to 500.

4.2.2 Methylation procedure with methanol

In a typical experiment, a glass liner was charged with ammonium bromide (59 mg, 0.60 mmol), 2-pyrrolidone (0.90 mL, 12 mmol) and methanol (3.0 mL, 74 mmol), and placed in an autoclave. Before reaction, the atmosphere in the autoclave was replaced with nitrogen by applying five vacuum-nitrogen cycles. Each reactor was heated to 250 ^{o}C in 20 minutes and left at 250 ^{o}C for five hours (the pressure in the reactor is then approximately 5 bar), after which heating was stopped and the reactor cooled to 100 ^{o}C in one hour and down to room temperature in five hours at which point the reactor was opened and the contents removed. Methanol was evaporated under reduced pressure and a crude sample was taken for determining the conversion and selectivity by 1H-NMR (400 MHz). GC/MS and high resolution ESI-MS were further used to identify the formed products. No sampling was performed during the reaction, in order to avoid interference with the experiment. For purification, column chromatography was

used with a 4:1 (vol.) mixture of chloroform and ether as the mobile phase. On TLC, the R_f values of NMP and 2-pyrrolidone with the same mobile phase were 0.2 and 0.1, respectively.

4.2.3 Methylation procedure with DMC

In a typical experiment, a glass liner was charged with NaY zeolite (0.50 g), 2-pyrrolidone (0.50 mL, 6.6 mmol), DMC (8 mL, 0.1 mol) and diglyme (0.20 ml, 1.4 mmol, internal standard) and placed in an autoclave. Before reaction, the atmosphere in the autoclave was replaced with nitrogen by applying five vacuum-nitrogen cycles. The reactors were heated to the required temperature and kept at this temperature for the set amount of time, after which heating was stopped and the reactor cooled down to room temperature in about five hours at which point the reactor was opened and the contents removed. DMC was then evaporated under reduced pressure and a crude sample was taken for determining the conversion and selectivity by ^{1}H-NMR (400 MHz). Yield, conversion and selectivity were determined by comparison of the ^{1}H-NMR signal of diglyme with those of 2-pyrrolidone and NMP. GC/MS was further used to identify the formed products. No sampling was performed during the reaction, in order to avoid interference with the experiment.

4.3 Results and Discussion

4.3.1 Catalyst screening

Ben Taleb *et al.* showed that CTAB can be used for the methylation of 2-pyrrolidone with DMC.[15] Here the use of different halogen salts as catalysts for the methylation of 2-pyrrolidone with methanol is investigated. This was done in order to determine the possibility to use CTAB as a catalyst, to investigate the possibility to use other halogen salts, and to obtain mechanistic information.

As can be seen in figure 4.1, bromide containing salts such as cetyl trimethylammonium bromide (CTAB), 1-butyl-3-methylimidazolium bromide ([Bmim]Br), methyltriphenyl-phosphonium bromide (MTPB) and ammonium bromide show an excellent ability to catalyze the methylation with methanol at 250 ^{o}C. Similar results were also achieved with ammonium bromide and ammonium

iodide. Ammonium bromide was chosen as the preferred catalyst for further studies. Although it does not provide the highest conversion, it was chosen because it has the same high selectivity and is a readily available and inexpensive salt.

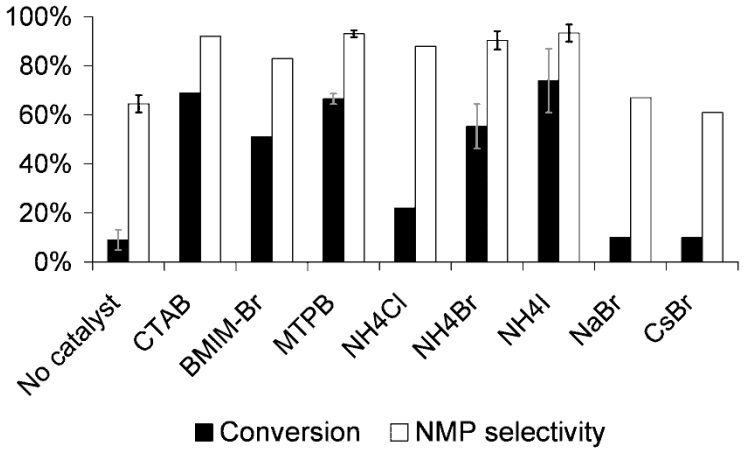
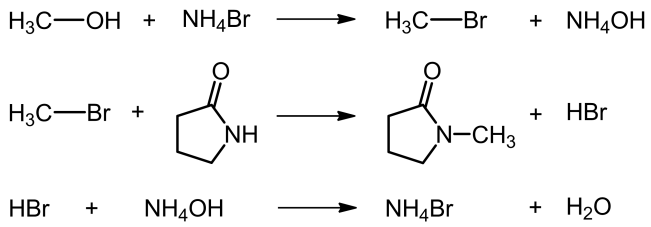


Figure 4.1. Conversion and selectivity determined for the methylation of 2-pyrrolidone with methanol in the presence of different catalysts. Experiments were carried out with 0.6 mmol catalyst, 1.0 g (12 mmol) 2-pyrrolidone and 3.0 mL (74 mmol) methanol, at 250 ^{o}C, for 5 hours.

4.3.2 Reaction mechanism

The fact that ammonium chloride is less active than ammonium bromide which in turn is less active than ammonium iodide (figure 4.1), leads us to believe that a halomethane is involved in the reaction sequence. In such a mechanism, the hydroxy group of methanol is replaced by a halide ion in an S_N2 reaction, which is easiest for a good nucleophile like iodide.[18] The proposed reaction sequence, shown in scheme 4.2, is analogous to the one proposed for the methylation with DMC or methyl formate.[15]



Scheme 4.2. Proposed reaction sequence for the methylation of pyrrolidone. In this scheme, NH_4 can be substituted by any of the bromide salts.

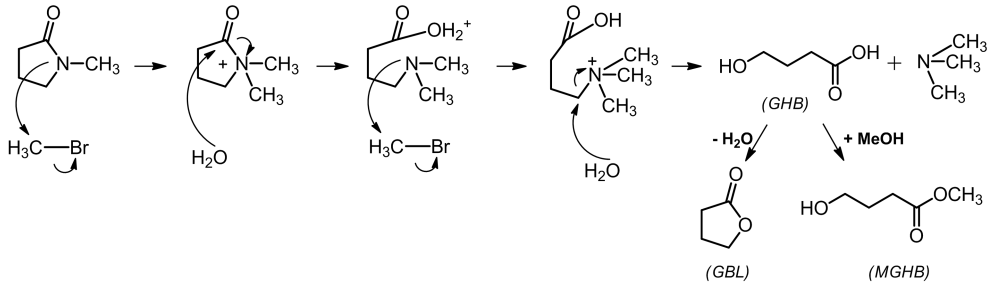
As the selectivity is not 100 %, the formation of side products was investigated. Five side products were identified by high resolution MS (table 4.1): γ-hydroxybutyric acid (GHB), γ-hydroxybutyric acid methyl ester (MGHB), γ-butyrolactone (GBL), trimethylamine and the tetramethylammonium ion. The formation of these compounds was unexpected, as it means that the nitrogen atom from the lactam, which is a poor leaving group, has been replaced by oxygen.

	M / Z	Molecular formula (theoretical mass)	Derived compound
Positive mode	58.0656	C_3H_8N (58.0651)	Trimethylamine (-H)
	60.0812	$C_3H_{10}N$ (60.0808)	Trimethylamine (+H)
	74.0967	$C_4H_{12}N$ (74.0964)	Tetramethylammonium
	86.0602	C_4H_8ON (86.0600)	2-Pyrrolidone (+H)
	100.0759	$C_5H_{10}ON$ (100.0757)	N-Methylpyrrolidone (+H)
Negative mode	78.9178 80.9157	Br (78.9178)	Bromide (2 isotopes)
	84.0444	C_4H_6ON (84.0444)	2-Pyrrolidone (-H)
	85.0284	$C_4H_5O_2$ (85.0284)	γ-Butyrolactone (-H)
	103.0390	$C_4H_7O_3$ (103.0390)	γ-Hydroxybutyric acid (-H)
	117.0574	$C_5H_9O_3$ (117.0546)	γ-Hydroxybutyric acid methyl ester (-H)
	126.9040	I (126.9039)	Iodide

Table 4.1. Measured m/z ratios with the molecular formula and the anticipated products.

Scheme 4.3 shows a suggested reaction mechanism for the formation of these side products. Methylation of NMP would turn the nitrogen atom into a good leaving group, making the amide susceptible to a nucleophilic ring opening. Further methylation then yields a quaternary

nitrogen that is eventually substituted by water, giving GHB and trimethylamine as products. GHB can cyclize to GBL, or react with methanol to MGHB. Trimethylamine can be methylated to form a tetramethylammonium salt.
Strengthening the postulated reaction mechanism is that both the presence of GHB, MGHB and GBL were detected (by H-NMR and ESI-MS), and also the presence of trimethylamine and the tetramethylammonium salt (by ESI-MS). The latter two molecules could have been formed by methylation of ammonium bromide, but they were also detected when MTPB was used as catalyst. As MTPB contains no nitrogen, the methylated amines must originate from 2-pyrrolidone and are likely to be side products from the formation of GHB.



Scheme 4.3. Suggested reaction mechanism for the formation of the side-product GHB from methylated 2-pyrrolidone. GHB may then react with methanol to form MGHB or cyclize to GBL.

4.3.3 Ammonium bromide catalyzed methylation

The influence of pressure and temperature on the conversion and selectivity of the reaction were studied with ammonium bromide as a catalyst, to determine the optimal reaction conditions. Our results showed that an increased pressure does not lead to a significant change in conversion, but does lead to a decrease in NMP selectivity (from > 90 % to 70 %), due to the formation of more of the side products GHB, MGHB and GBL. The best pressure to perform this reaction was found to be 5 bar (obtained by starting with atmospheric pressure and heating the closed autoclave).
Figure 4.2 shows that there is a temperature threshold value between 200 and 225 ^{o}C, from which the reaction rate increased dramatically. At very high temperatures the selectivity of the reaction decreases due to the formation of a black tar.

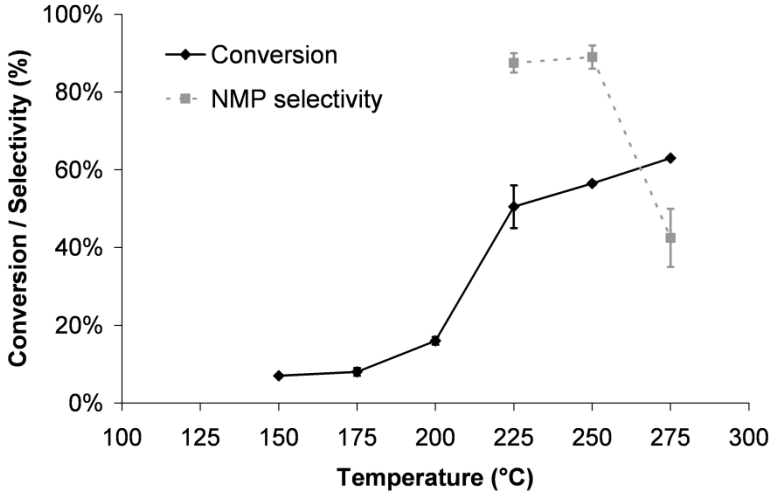


Figure 4.2. Conversion and selectivity as a function of the reaction temperature. Experiments were carried out with 59 mg NH_4Br, 1.0 g 2-pyrrolidone and 3.0 mL methanol, for 5 hours. Error margins indicate the differences between two duplicate experiments. Selectivity at low temperature could not be accurately determined because of the low conversion.

A study of conversion and selectivity in time (figure 4.3) shows that next to a high selectivity it is also possible to achieve high conversions, by allowing the reaction to proceed for a longer period of time. After nine hours, the conversion achieved is 88 ± 6 % and the corresponding selectivity 87 ± 4 %. While it is possible to obtain high conversions with this reaction, the employed reaction time in our studies is five hours, as it offers a high selectivity in combination with a medium conversion, making it possible to study how other factors such as the reaction temperature can influence the conversion.

An isolated yield of 57 mol-% NMP was obtained after purification of the product of a five-hour experiment at 250 ^{o}C by column chromatography, which is consistent with the data shown in figure 4.3.

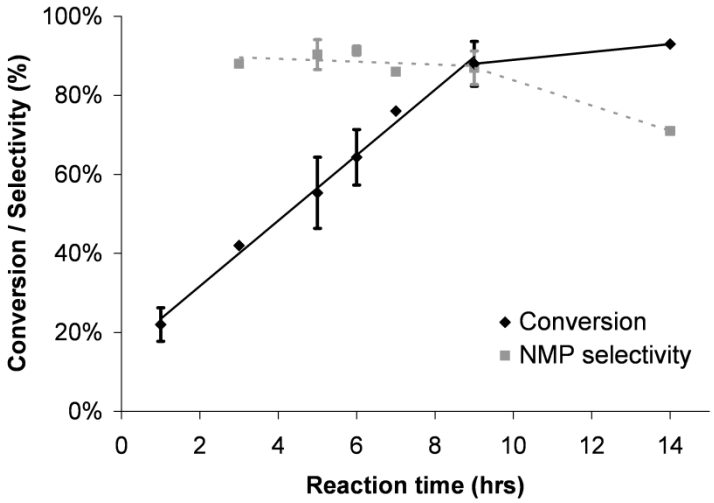


Figure 4.3. Conversion and selectivity as a function of the reaction time. Experiments were carried out with 59 mg NH_4Br, 1.0 g 2-pyrrolidone and 3.0 mL methanol, at 250 ^oC. Error margins indicate the standard deviation between two or three identical experiments. Fitting the data points from 1 to 9 hours to a linear function gives an R^2 value of 0.99.

4.3.4 Scope of the alkylation

To investigate the scope of the alkylation reaction, it was also performed with other, more sterically hindered alcohols. For this were chosen: ethanol, n-propanol, n-butanol, 2-butanol, t-butanol and benzyl alcohol. The results are shown in table 4.2. For the sake of comparison, the ratios (determined by ^{1}H-NMR without evaporating the alcohol) between 2-pyrrolidone and the N-alkylated pyrrolidone (NAP) are shown, which are a good indication of the reaction progress after five hours.

These results show that methanol is more reactive than ethanol, n-propanol and n-butanol. This is probably related to the initial substitution of the alcohol with bromide, which proceeds more readily when it is less sterically hindered. In between ethanol, n-propanol and n-butanol, no trend could be observed. 2- And t-butanol yielded no alkylated product. In their case, elimination of the alcohol probably took place instead of bromination, leading to the formation of gaseous (iso)butene, because an extra increase of pressure (from 3 to 7 bar) was observed during the reaction. Another indication for the formation of butenes was that after

the reaction, 2- and t-butanol had been partially consumed in the reaction mixture. In the case of benzyl alcohol, N-benzylpyrrolidone was formed at a similar rate as N-methylpyrrolidone. In this case the mechanism can be S_N2 or S_N1, because it is well known that adjacent π-bonds enhance both mechanisms.

NAP / Pyrrolidone	(mol / mol)
Methanol	1.2 ± 0.3
Ethanol	0.15 ± 0.03
n-Propanol	0.07 ± 0.01
n-Butanol	0.16 ± 0.02
2-Butanol	0
t-Butanol	0
Benzyl alcohol	1.4 ± 0.1

Table 4.2. N-alkylation of 2-pyrrolidone, with different alcohols. Shown here is the ratio between 2-pyrrolidone and N-alkylated pyrrolidone (NAP) in the crude reaction mixture, after 5 hours reaction time. Experiments were carried out with 59 mg NH_4Br, 1.0 g 2-pyrrolidone and 72 mmol alcohol, at 250 ^oC. Error margins indicate the variation between two identical experiments.

4.3.5 One-pot cyclization and methylation of GABA

From literature it is known that GABA can cyclize rather easily, for example in boiling toluene, catalyzed by silica using a soxhlet set-up for water removal.[11] Therefore our hypothesis was that it should be possible to cyclize and subsequently methylate GABA in a simple one-pot procedure, in order to obtain biobased NMP. Figure 4.4 shows that upon heating GABA in methanol without added catalyst, 2-pyrrolidone is indeed obtained in a high yield, even without water removal. When GABA was heated in methanol with ammonium bromide present, there was only 2 mol-% GABA left after five hours at 250 ^oC and the subsequent conversion of 2-pyrrolidone was 42 mol-%, with a selectivity for NMP of 92 %. This means that it is actually quite straightforward to convert GABA to NMP in a one-pot procedure, with methanol and a catalytic amount (5 mol-%) of ammonium bromide. Figure 4.4 also shows that when starting with NMP, 99 % of it was recovered, indicating that NMP is stable under the applied conditions. However, when the reaction was performed at an increased pressure, NMP became unstable with ammonium

bromide present and formed 17 mol-% of the GHB-related side products (data not shown), which is in accordance with the postulated mechanism in scheme 4.3.

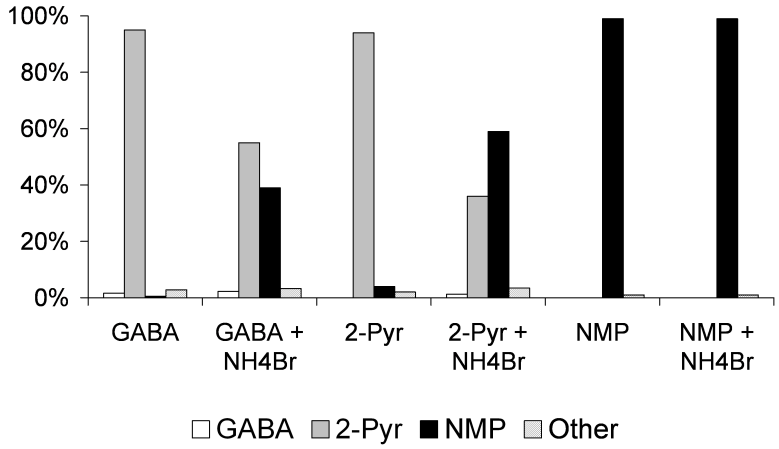


Figure 4.4. Molar ratio of the products present in the reaction mixture after 5 hours at 250 ^{o}C, when starting with different materials, in the presence and absence of NH_4Br catalyst. Determined by ^{1}H-NMR.

4.3.6 Process design

In an industrial process design, the removal of water from the reactor could be incorporated in a continuous process, together with continuous product removal by vacuum distillation. 2-Pyrrolidone and NMP are easily separable by distillation (their boiling points at 1 atm. are 245 and 202 ^{o}C, respectively), so one could think of a product stream continuously leaving the reactor, passing through a water absorber (for example a zeolite drier) into a first distillation column for the removal of the excess of methanol and then into a second distillation column, where the top fraction would be NMP and the bottom fraction, 2-pyrrolidone, could be either isolated as a co-product, or could be recycled to the reactor. Schematically this process is shown in figure 4.5. What should be considered is the fate of the catalyst. As the salt is a homogeneous catalyst, part of it will be removed from the reactor with the product removal and will end up in the rest of the process. A part will probably stay in the zeolite dryer where it may be recovered and the rest will end up in the 2-pyrrolidone stream and can directly be recycled to the reactor.

When warm 2-pyrrolidone is recycled, ammonium bromide will stay in solution, but it could also be cooled to room temperature at which point the ammonium bromide is sparingly soluble in 2-pyrrolidone and could be filtered off to a large extent.

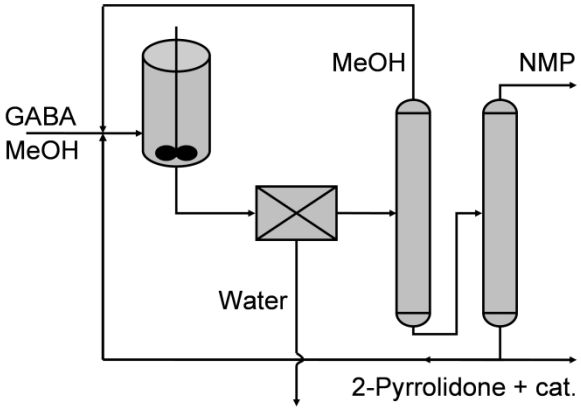


Figure 4.5. Process scheme for the continuous production of NMP from GABA and methanol.

4.3.7 Comparison with DMC as methylating agent

Although the atom efficiency of the above described process is relatively high (73 %), the use of a homogeneous halogen-based catalyst may be perceived as a drawback in a commercial application, for there is the risk of product contamination with traces of bromide. Oku *et al.* reported an acid-base bifunctional zeolite that catalyzes methylation reactions with supercritical methanol, but for 2-pyrrolidone the conversion was quite poor.[16] As was discussed in the introduction, DMC is a versatile methylation agent, also in combination with heterogeneous catalysts such as NaY zeolite.[12-14] However, to our knowledge there is no report of the use of DMC for the methylation of a (cyclic) amide, in combination with a heterogeneous catalyst. Therefore we attempted the methylation of 2-pyrrolidone with this reagent, in the presence of NaY zeolite. The results, shown in table 3, indicate that it is also possible to methylate 2-pyrrolidone with DMC. At a temperature of 250 ^{o}C, a maximum NMP yield of 57 mol-% was obtained after 30-60 minutes of reaction. The results show that a high temperature is needed for the N-methylation of 2-pyrrolidone with DMC, because at 180 ^{o}C the NMP yield goes

down while the conversion of 2-pyrrolidone remains the same, indicating that the selectivity towards NMP goes down, and at 130 °C 2-pyrrolidone is hardly converted. At lower temperatures (130 and 180 °C), we found that 2-pyrrolidone is partly N-methoxycarbonylated instead of N-methylated. This is in line with previous reports by Selva *et al.*, who indicated that at a lower temperature DMC acts as a methoxycarbonylating agent and at higher temperatures as a methylating agent, although there is not always a clear cut-off at which temperature which reactions occurs.[12]

Both at 180 and 250 °C, the results indicate that NaY has a directive effect towards the methylation reaction, because the selectivity towards NMP formation increases with the zeolite present, although this effect is not large.

Temp (°C)	Catalyst	Time (min)	2-Pyrrolidone Conversion (mol-%)	NMP Selectivity (mol-%)	NMP Yield (mol-%)
130	-	60	8	0	0
130	NaY	120	7	0	0
130	NaY	240	5	0	0
180	-	60	76	4	3
180	NaY	60	81	17	14
180	NaY	240	84	23	19
250	-	60	90	48	43
250	NaY	30	86	66	57
250	NaY	60	85	67	57

Table 4.3. Results of N-methylation procedure with DMC, in the presence and absence of NaY.

When comparing these results with the results that were obtained with the methylation procedure with methanol, they seem rather poor. Although the DMC reaction is quicker, both the atom efficiency (51 %) and the achieved selectivity (67 %) with DMC are less than with methanol, which leads to the conclusion that the methanol procedure is preferable over the DMC procedure.

4.3.8 Simultaneous NVP production

Besides for the production of NMP, 2-pyrrolidone could also be used for the production of NVP. In the current commercial processes for the manufacturing of NVP, 2-pyrrolidone is reacted with acetylene in high-pressure autoclaves at 130-160 ^{o}C and a pressure up to 26 bar, in the presence of a potassium or sodium catalyst in the form of i.e. sodium tert-butanoate.[2,19] Then, NVP can be purified by distillation.

Figure 4.3 shows that when the methylation of 2-pyrrolidone does not proceed to full conversion, the selectivity remains higher, which is due to less formation of side products. When the conversion would be allowed to proceed to about 50 % and then be stopped, the selectivity of the methylation reaction is above 90 %. The 2-pyrrolidone that remains present in the reaction mixture can then be separated easily from the NMP by distillation, as indicated before, and could be used for the production of NVP, which could probably be done in the current industrial infrastructure. The NVP would not be completely biobased, as acetylene is derived from oil. However, on a weight basis, it would still be 77 % biobased.

4.4 Conclusion

The goal of this chapter was to investigate the possibility to synthesize pyrrolidones from GABA, that can be obtained by the α-decarboxylation of glutamic acid.[5] In this way NMP could be made largely biobased, and with the use of a biobased methylating agent (for example biomethanol[20]) NMP would become fully biobased.

The synthesis of NMP from GABA was done in two steps, the first being the cyclization of GABA to form 2-pyrrolidone, and the second the N-methylation of 2-pyrrolidone to form NMP. We found that this is possible in a one-pot procedure, where the methylation reaction can be performed with methanol as the methylating agent, catalyzed by a halogen salt such as ammonium bromide, ammonium iodide or CTAB. The selectivity that was achieved for this reaction is greater than 90 %, although we found that at an increased pressure the selectivity towards NMP goes down.

The methylation of 2-pyrrolidone can also be done with DMC in the presence of NaY zeolite. However, the selectivity that was found for this methylation procedure (67 %) was significantly less than that of the methylation with methanol and a halogen salt catalyst. Combining this with the lower atom efficiency in the use of DMC, the preferred method of methylation would be the procedure with methanol.
This chapter shows that there is now a straightforward route to synthesize biobased NMP, based on glutamic acid. This can be done by combining the enzymatic decarboxylation of glutamic acid to form GABA with the one-pot cyclization and methylation of GABA to form NMP. By not fully converting 2-pyrrolidone into NMP, part of it could also be used for the simultaneous production of NVP.

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4.5 References

1. BASF, http://www2.basf.us/diols/bcdiolsnmp.html *Official website of BASF chemical company, Germany* (accessed Mar 30, **2010**).
2. (a) BASF, http://worldaccount.basf.com/wa/PublicMSDS/PDF/09007af88017cc55.pdf *Official website of BASF chemical company, Germany* (accessed Mar 30, **2010**). (b) A.L. Harreus, 2-Pyrrolidone, In: *Ullmann's Encyclopedia of Industrial Chemistry*, Online edn., **2000**, Wiley InterScience.
3. B. Flick, C. E. Talsness, R. Jäckh, R. Buesen, S. Klug, *Toxicol. Appl. Pharmacol.* **2009**, 237, 154-167.
4. M. Reich, *Chem. Eng. News*, July 21, **2008**, 32.
5. T. M. Lammens, D. De Biase, M. C. R. Franssen, E. L. Scott, J. P. M. Sanders, *Green Chem.* **2009**, 11, 1562-1567.
6. Abengoa, http://www.abengoabioenergy.com, *Official website of Abengoa Bioenergy, Spain* (accessed Mar 30, **2010**).

7. V. Deike, J. De Mol, *Wheat distillers grains and solubles*, in: *Raw Material Compendium*, 2nd edition reprint, **1996**, p. 117, Novus International Inc. Brussels.
8. T. Kawakita1, C. Sano, S. Shioya, M. Takehara, S. Yamaguchi, *Monosodium Glutamate,* In: *Ullmann's Encyclopedia of Industrial Chemistry*, Online edn., **2000**, Wiley InterScience.
9. W. Schwarz, J. Schossig, R. Rossbacher, H. Höke, *Butyrolactone*, In: *Ullmann's Encyclopedia of Industrial Chemistry*, Online edn., **2000**, Wiley InterScience.
10. Praxair, http://www.praxair.com/praxair.nsf/AllContent/25C6766DFFF78DC785256A860081A221/$File/p4626d.pdf, *Official website of Praxair* (accessed Mar 30, **2010**).
11. Blade-Font, *Tetrahedron Lett.* **1980**, 21, 2443.
12. P. Tundo, M. Selva, *Acc. Chem. Res.* **2002**, 35, 706-716.
13. M. Selva, A. Perosa, *Green Chem.* **2008**, 10, 457-464.
14. (a) M. Selva, E. Militello, M. Fabris*, Green Chem.* **2008**, 10, 73-79. (b) M. Selva, A. Perosa, M. Fabris, *Green Chem.* **2008**, 10, 1068-1077.
15. Ben Taleb, G. Jenner, *J. Mol. Catal.,* **1993**, 84, L131-L136.
16. T. Oku, Y. Arita, H. Tsuneki, T. Ikariya, *J. Am. Chem. Soc.*, **2004**, 126, 7368-7377.
17. (a) T. Setoyama, T. Ogoshi, JP2001288164A, **2001**. (b) T. Setoyama, T. Ogoshi, JP2001288165A, **2001.**
18. P.Y. Bruice, *Substitution reactions of alkyl halides*, In: *Organic Chemistry*, 2nd edition **1998**, Prentice-Hall, Inc. New Jersey
19. (a) K.-C. Liu, P.D. Taylor, GAF, US4873336, **1989**. (b) W. Staffel, S. Käshammer, R. Kessinger, R. Vogelsang, A. Paul, L. Tuttelberg, BASF, WO2009/071479 A1, **2009**.
20. http://www.biomcn.eu *Official website of BioMCN, The Netherlands* (accessed Mar 30, **2010**) (b) A. Demirbas, *Energy Sources Part A* **2008**, 30, 565 – 572.

Chapter 5

Synthesis of biobased succinonitrile from glutamic acid and glutamine

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Abstract

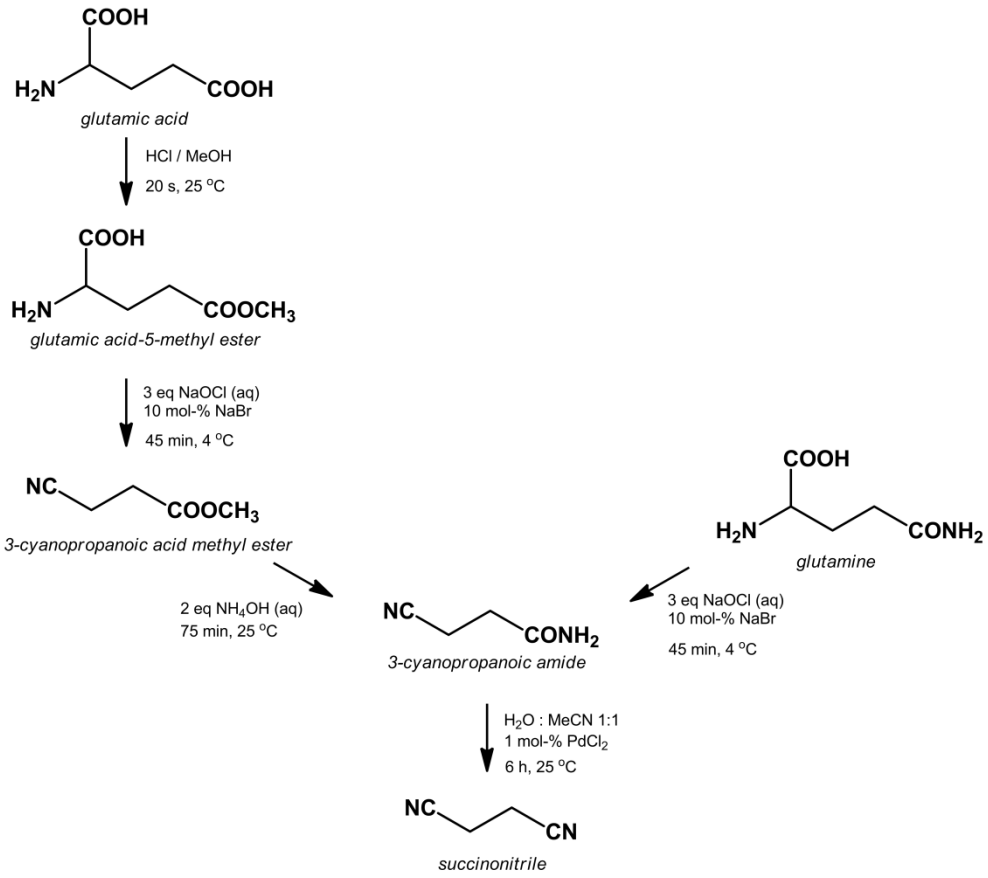
Succinonitrile is the precursor for 1,4-diaminobutane, which is used for the industrial production of polyamides. This chapter describes the synthesis of biobased succinonitrile from glutamic acid and glutamine, amino acids that are abundantly present in many plant proteins. Synthesis of the intermediate 3-cyanopropanoic amide was achieved from glutamic acid 5-methyl ester in 86 mol-% yield and from glutamine in 56 mol-% yield. 3-Cyanopropanoic acid can be converted into succinonitrile with a selectivity close to 100 % and 62 % conversion using a palladium(II) catalyzed equilibrium reaction with acetonitrile. With this, a new route to produce biobased 1,4-diaminobutane has been discovered.

5.1 Introduction

The chemical industry is in transition from a petrochemical based towards a more biobased industry. Large scale production of biobased chemicals is becoming a reality, as is shown by Solvay's plant for the production of epichlorohydrin from glycerol, which is in operation since 2007.[1] Apart from this example, from the recent review by Bozell and Petersen can be concluded that the current worldwide research effort in the search for biobased chemicals is enormous.[2] Most of this research focuses on the use of carbohydrates, but the proteins that are left in byproduct streams from e.g. bioethanol and biodiesel production could also be valuable building blocks for chemicals. Especially the non-essential amino acids in these proteins, which have no significant value for food or feed, can be interesting starting materials for a variety of nitrogen containing products.[3] The most abundant (non-essential) amino acid in many plant proteins is glutamic acid.[4] In previous chapters we have shown the possible production of γ-aminobutyric acid,[5] N-methylpyrrolidone and N-vinylpyrrolidone[6] from glutamic acid. Very recently also a route from glutamic acid to acrylonitrile was published by our group.[7]
The current chapter describes the synthesis of succinonitrile from glutamic acid. Succinonitrile, produced commercially from acrylonitrile and hydrogen cyanide,[8] is the precursor of 1,4-diaminobutane (DAB, putrescine) in the industrial production of the polyamides Stanyl and EcoPaXX.[9] The volume of DAB that is produced per year in Europe is estimated at about 10,000 metric tonnes.[10]

Recently, the fermentative and enzymatic production of DAB have been described.[11] However, DAB is very soluble in water and therefore difficult to isolate from a fermentation broth. The production of biobased succinonitrile can be an advantageous route towards the production of biobased nylon as it can be readily isolated by extraction with organic solvents. An option for the production of biobased succinonitrile would be to produce it from sugar-derived succinic acid, which has been receiving increased interest as a versatile biobased building block.[12] This has been reported recently by a reaction with ammonia and $Si_3(PO_4)_4$ at 420 ^{o}C, with a yield of 70 %.[13] Although this is a promising route to biobased succinonitrile, the harsh conditions could pose a drawback for implementation, for example by leading to high capital investments. Other reports for converting

carboxylic acids into nitriles do exist, for example by Telvekar *et al.*, who discovered a one-pot procedure for this reaction.[14] However, this procedure has not been shown to work for small polar molecules like succinic and glutamic acid, and it has drawbacks such as the stoichiometric use of the hazardous reagent diphosphorus tetraiodide. Since glutamic acid and glutamine are abundantly present in low-value byproducts of biofuels production such as vinasse, distillers grains with solubles, and natural oil meals (see chapter 2),[4] our approach to the synthesis of biobased succinonitrile is to start from glutamic acid or glutamine and to go via the intermediate 3-cyanopropanoic amide. This is shown in Scheme 5.1.



Scheme 5.1. A new route to biobased succinonitrile, from glutamic acid or glutamine.

In chapter 4 was shown that γ-aminobutyric acid (GABA) readily cyclizes, thereby forming the very stable lactam 2-pyrrolidone.[6] 3-Cyanopropanoic amide cannot cyclize to a lactam, and is therefore a more promising intermediate than GABA for the synthesis of linear molecules such as succinonitrile.

The goal of this chapter was to investigate the synthetic route to biobased succinonitrile, from either glutamine or glutamic acid. The first part describes the synthesis of 3-cyanopropanoic amide from glutamine and glutamic acid whereas the second part describes the synthesis of succinonitrile from 3-cyanopropanoic amide. Once succinonitrile is formed, hydrogenation to form DAB may be carried out. This step is already a commercial process applied by DSM.[15]

5.2 Materials and Methods

5.2.1 Materials and equipment

L-Glutamic acid (>99.5 %), L-glutamic acid 5-methyl ester (Glu-Me, 99 %), L-glutamine (99 %), sodium bromide (>99 %), palladium chloride (99.99 %), palladium acetate (99.99 %), palladium hydroxide (20 wt % / C), triphenylphosphine sulfonate (>90 %), zinc chloride (98 %), paraformaldehyde (>95 %), formic acid (>98 %), polyacrylonitrile (M_w 150,000), perrhenic acid (99.99 %, 65-70 wt-% / H_2O), sodium hypochlorite (R.G., 10-15 wt-% / H_2O) and ammonium hydroxide (puriss., 30-33 wt-% NH_3 / H_2O) were all purchased from Sigma-Aldrich. The organic solvents that were used were all analytical grade and used as received.

High resolution MS spectra were recorded on an Exactive apparatus from Thermo Scientific, equipped with a DART probe. Spectra were recorded in positive mode. M/z ratios were detected from 50 to 500.

5.2.2 Oxidative decarboxylation of Glu-Me to CPA-Me

In a typical experiment, a round bottomed flask was charged with Glu-Me (6.0 g, 37 mmol) and NaBr (0.38 g, 3.7 mmol), in 75 mL water. This was placed in an ice-bath and 45 mL NaOCl (aqueous, 15 wt-%, 111 mmol) was added slowly, with

vigourous stirring. After 30 minutes, the ice-bath was removed and after a further 15 minutes the reaction was quenched with $Na_2S_2O_3$. The solution was then saturated with NaCl and extracted with four portions of diethyl ether (4x125 mL). The organic fractions were combined and dried with $MgSO_4$. After filtration, the solvent was removed under reduced pressure, yielding 3-cyanopropanoic acid methyl ester (CPA-Me) as a yellowish liquid (3.81 g), which was not purified. The product was analyzed with ^1H and ^{13}C NMR (400 MHz) to determine the crude CPA-Me yield (>84 mol-%).

CPA-Me: ^1H NMR (DMSO-d6): δ = 3.64 (s, 3H), 2.69 (m, 4H) ppm; ^{13}C NMR (DMSO-d6): δ = 171.1, 119.8, 51.7, 29.0, 12.4 ppm.

5.2.3 Oxidative decarboxylation of glutamine

In a typical experiment, a round bottomed flask was charged with L-glutamine (146 mg, 1 mmol), NaBr (10.3 mg, 0.1 mmol) and diglyme (I.S., 33.6 mg, 0.25 mmol), in 1.75 mL D_2O. This was placed in an ice-bath and 1.25 mL NaOCl (aqueous, 15 wt-%, 3 mmol) was added slowly with vigorous stirring. After 30 minutes, the ice-bath was removed and after another 15 minutes the reaction was quenched with $Na_2S_2O_3$. A ^1H NMR spectrum of the reaction mixture was recorded and the relative amount of 3-cyanopropanoic amide was determined using the internal standard.

3-cyanopropanoic amide: ^1H NMR (D_2O): δ = 3.04 (m, 2H), 2.99 (m, 2H) ppm;
Diglyme: ^1H NMR (D_2O): δ = 3.95 (m, 4H), 3.90 (m, 4H), 3.66 (s, 6H) ppm.

5.2.4 Amidation of CPA-Me to CPAm

Caution: This experiment should be performed in a well-ventilated fumehood, as there is a risk of hydrogen cyanide formation.

In a typical experiment, a round bottomed flask was charged with unpurified CPA-Me (3.81 g, obtained from the oxidative decarboxylation of Glu-Me), to which 10 mL of NH_4OH (30-33 % in water, ~75 mmol) was slowly added whilst stirring. After addition, the flask was closed and left at room temperature for 75 minutes, after which the reaction mixture was evaporated to dryness at 65 ^oC under reduced pressure, yielding an orange solid, which according to ^1H NMR was >95 % pure 3-cyanopropanoic amide (3.33 g, >86 mol-% yield from Glu-Me). Recrystallization

of crude 3-cyanopropanoic amide from ethyl acetate[32] afforded analytically ($^{1}H/^{13}C$ NMR) pure 3-cyanopropanoic amide (2.86 g, 29 mmol, 78 mol-% yield from Glu-Me), as a white powder. For further experiments purified 3-cyanopropanoic amide was used.

3-cyanopropanoic amide: ^{1}H NMR (DMSO-d6): δ = 7.43 (s, 1H), 6.98 (s, 1H), 2.60 (t, 2H), 2.40 (t, 2H) ppm; ^{13}C NMR (DMSO-d6): δ = 171.1, 120.3, 30.2, 12.4 ppm.

HRMS for 3-cyanopropanoic amide + NH_4^+ ($C_4H_{10}ON_3$): theoretical m/z: 116.08239, found m/z: 116.08182.

5.2.5 Perrhenic acid-catalyzed dehydration of CPAm to succinonitrile

A round bottomed flask was charged with 3-cyanopropanoic amide (98.1 mg, 1.0 mmol), 1,4-dioxane (10 mL) and $(HO)ReO_3$ (120 mg, 0.3 mmol). The flask was equipped with a Soxhlet apparatus filled with molecular sieves (3Å, activated overnight at 220 ^{o}C) and refluxed under a nitrogen atmosphere for 22 hours, after which the solution was cooled to room temperature, the solvent removed under reduced pressure and the crude product analyzed with ^{1}H and ^{13}C NMR (400 MHz) to determine the crude succinonitrile yield.

5.2.6 $PdCl_2$-catalyzed dehydration of CPAm to succinonitrile

In a typical experiment, a round bottomed flask was charged with $PdCl_2$ (17.7 mg, 0.10 mmol), 6 mL of a 1:1 (vol.) mixture of water and acetonitrile, and 3-cyanopropanoic amide (98.1 mg, 1.0 mmol). The reaction mixture was stirred for 6 hours under a nitrogen atmosphere. Subsequently the reaction mixture was extracted with three portions of dichloromethane or diethyl ether (3x6 mL), the organic fractions combined, dried over $MgSO_4$, filtered and the solvent evaporated under reduced pressure. The crude product was analyzed with ^{1}H and ^{13}C NMR (400 MHz) to determine the crude succinonitrile yield (typically 50-60 mol-%). The crude product can be purified by column chromatography with diethyl ether as mobile phase. Retention factors for 3-cyanopropanoic amide and succinonitrile on TLC with diethyl ether are 0.1 and 0.7, respectively.

For the experiment with poly(acrylonitrile), a round bottomed flask was charged with $PdCl_2$ (17.7 mg, 0.10 mmol), polyacrylonitrile (0.50 g, 10 mmol CN), water (3

mL), and 3-cyanopropanoic amide (98.1 mg, 1.0 mmol). The reaction mixture was stirred for 18 hours, after which the reaction mixture was extracted with diethyl ether (3x6 mL), the rest of the procedure being identical to the one directly above. Polyacrylonitrile could be separated from the aqueous phase by filtration through a 0.2 μm filter.

Succinonitrile: ^{1}H NMR ($CDCl_3$): δ = 2.79 (s, 4H) ppm; ^{13}C NMR ($CDCl_3$): δ = 116.5, 14.7 ppm.

HRMS for succinonitrile + NH_4^+ ($C_4H_8N_3$): theoretical m/z: 98.07182, found m/z: 98.07149.

5.2.7 $ZnCl_2$-catalyzed dehydration of CPAm to succinonitrile

An Ace pressure tube was charged with $ZnCl_2$ (0.136 g, 1.0 mmol), 1 mL of a 1:1 (vol.) mixture of water and acetonitrile, and 3-cyanopropanoic amide (98.1 mg, 1.0 mmol). The mixture was irradiated for 2 minutes at 450 W in a microwave oven and then cooled to room temperature. The mixture was extracted with two portions of dichloromethane (2x5 mL) and the organic fractions combined, dried over $MgSO_4$, filtered, and the solvent evaporated under reduced pressure. The crude product was analyzed with ^{1}H and ^{13}C NMR (400 MHz) to determine the crude succinonitrile yield.

5.3 Results and Discussion

5.3.1 Synthesis of 3-cyanopropanoic amide

Le Nôtre *et al.* previously showed that glutamic acid can be oxidatively decarboxylated to 3-cyanopropanoic acid with sodium hypochlorite in the presence of a catalytic amount of sodium bromide.[7] Starting from glutamine, 3-cyanopropanoic amide could be prepared directly by performing the same reaction. Starting from glutamic acid, 3-cyanopropanoic amide could be prepared in three steps, as is shown in Scheme 5.1: a) esterification of the γ-carboxylic acid group with methanol to form glutamic acid 5-methyl ester (Glu-Me);[16,17] b) oxidative decarboxylation of the α-carboxylic acid group to form 3-cyanopropanoic acid methyl ester (CPA-Me); c) amidation of the ester group with aqueous ammonia to form 3-cyanopropanoic amide. As the first step was described in literature as being

a facile procedure with a yield of 95 %,[17] only the second and third steps were investigated for this chapter, while starting from commercially available glutamic acid 5-methyl ester.

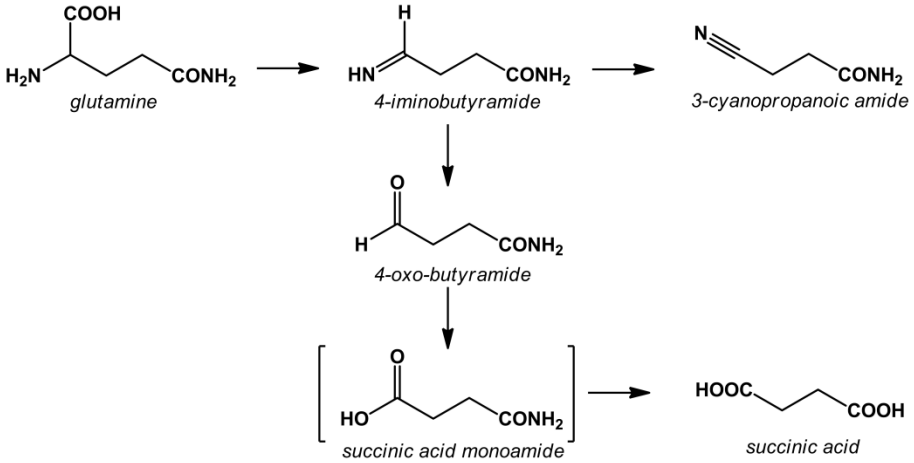
As can be seen in Table 5.1, 3-cyanopropanoic amide could be prepared from both glutamine and glutamic acid 5-methyl ester. The yield that was obtained by starting from glutamine (56 mol-%) was significantly lower than when starting from glutamic acid 5-methyl ester (86 mol-%). Moreover, 3-cyanopropanoic amide is difficult to extract from the reaction mixture, so an isolated and purified yield could not be obtained with glutamine as the starting material.

Starting material	Reaction conditions	Crude CPAm yield	Purified CPAm yield
Glutamine	0.57 M in D_2O, 3 eq NaOCl, 10 mol-% NaBr, 4 ^oC, 45 min.	56 mol-%	n.d.
Glu-Me	A) 0.50 M in H_2O, 3 eq NaOCl, 10 mol-% NaBr, 4 ^oC, 45 min. B) 2 eq NH_4OH, 25 ^oC, 75 min.	86 mol-%	78 mol-%

Table 5.1. Yields of 3-Cyanopropanoic amide, starting from glutamine and glutamic acid 5-methyl ester, respectively.

To be able to determine a crude yield of the reaction with glutamine, the reaction was performed in D_2O in the presence of an internal standard. Then the reaction progress was monitored by ^{1}H-NMR. This showed that after 45 minutes glutamine was fully converted into three products. The main product (56 mol-%) was the desired 3-cyanopropanoic amide, the two side products were 4-oxo-butyramide and succinic acid. Scheme 5.2 shows the probable route of formation of the side products. The formation of 4-oxo-butyramide is the result of the hydrolysis of the imine that is formed as an intermediate in the oxidative decarboxylation of glutamine. Presumably the carboxamide group in the glutamine derivative assists in the hydrolysis of the imine, because when the reaction was performed with glutamic acid 5-methyl ester instead of with glutamine, this compound was

observed in much smaller amounts. Oxidation of 4-oxobutyramide would result in succinic acid monoamide, but this compound could not be detected in the reaction mixture. Apparently the hydrolysis of succinic acid monoamide to succinic acid occurs readily under the applied conditions. Decreasing the reaction time was found to have a positive influence on the 3-cyanopropanoic amide yield, by reducing the formation of side products. However, 56 mol-% was the highest yield that was obtained.



Scheme 5.2. Byproducts formation in the oxidative decarboxylation reaction of glutamine.

Starting from glutamic acid 5-methyl ester, 3-cyanopropanoic acid methyl ester was obtained in a crude yield of 86 mol-%, with 5 mol-% of 4-oxo-butanoic acid methyl ester present as a side product. Addition of aqueous ammonia to this crude product gave quantitative formation of 3-cyanopropanoic amide, which could be purified by recrystallization. These results show that the most preferable way to synthesize 3-cyanopropanoic amide in the laboratory is by starting with the 5-methyl ester of glutamic acid and not with glutamine. This allows the intermediate 3-cyanopropanoic acid methyl ester to be easily extracted from the aqueous solution. This is not the case for 3-cyanopropanoic amide, which is highly soluble in water. Isolation of 3-cyanopropanoic amide from the aqueous ammonia solution can be achieved by simply evaporating the solution to dryness. In an industrial setting, the added reaction steps and unit operations that are needed when starting

from glutamic acid may be a drawback. Then glutamine could be a more suitable starting material than glutamic acid. However, in that case the isolation of 3-cyanopropanoic amide from the aqueous chloride solution as well as the selectivity will have to be improved. Also the isolation of glutamine from plant proteins is more difficult, because typically applied conditions for protein hydrolysis (heating in the presence of a strong acid) will also hydrolyze glutamine to glutamic acid.

We recognize that the use of stoichiometric amounts of sodium hypochlorite for this reaction makes it difficult to qualify this part of the procedure as sustainable. Considering however the effort that is currently put in the development of more suitable oxidants, such as oxygen, we believe that further improvement of this part of the procedure will be very well possible, for example with haloperoxidase enzymes or mimics thereof.[18] This will be the subject of further research.

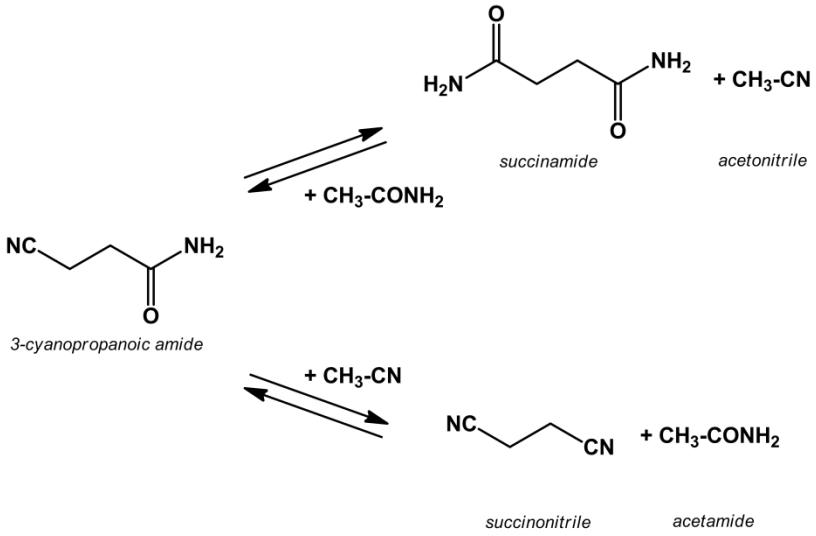
5.3.2 Succinonitrile synthesis by dehydration

The dehydration of 3-cyanopropanoic amide to form succinonitrile was investigated under different conditions. The most preferable way to achieve this would be a catalytic system as opposed to traditional dehydrating agents such as phosphorous pentoxide and thionyl chloride[19] that generate stoichiometric amounts of waste salts. A method for the dehydration of primary amides (both aromatic and aliphatic) was reported by Ishihara *et al.*[20] It is based on rhenium(VII) oxo complexes, i.e. perrhenic acid, that catalyze the dehydration of primary amides in refluxing toluene with simultaneous removal of water in a Dean-Stark apparatus. In this case no byproduct is formed.

The solubility of 3-cyanopropanoic amide proved to be a serious issue in applying this system. 3-cyanopropanoic amide has poor solubility in apolar solvents such as toluene and mesitylene, and is only partly soluble in the more polar dioxane. However, Ishihara *et al.* showed that in polar solvents such as dioxane, the dehydration reaction catalyzed by perrhenic acid proceeds much less, if at all. Different solvents were tested: toluene, anisole, dioxane, acetonitrile, and N-methylpyrrolidone. These solvents provided a yield in the range of 0 to 6 %. Dioxane provided the best balance between 3-cyanopropanoic amide solubility and catalyst activity, while yielding only 6 mol-% succinonitrile.

5.3.3 Succinonitrile synthesis by water transfer

Different reports show that primary amides can also be catalytically dehydrated in the presence of acetonitrile and water. Water is then transferred from the amide to acetonitrile, thereby forming acetamide and the corresponding nitrile.[21-23] With this reaction stoichiometric amounts of the byproduct acetamide are produced. However, this does not pose a drawback, as a commercial method of producing acetamide is also through the hydrolysis of acetonitrile.[24] So under these reaction conditions two chemical products are simultaneously produced, as shown in Scheme 5.3. Separation of the two products in an industrial setting would not be very difficult, considering the large difference in polarity. Succinonitrile is very well soluble in an organic solvent like ethyl acetate and can be extracted from an aqueous solution, while acetamide cannot be extracted. After extraction of succinonitrile, acetamide could be isolated from the aqueous phase by crystallization.



Scheme 5.3. Water transfer reaction with 3-cyanopropanoic amide and acetonitrile. Reaction of 3-cyanopropanoic amide with acetonitrile leads to the desired succinonitrile (bottom). Reaction of 3-cyanopropanoic amide with acetamide leads to undesired succinamide (top).

Catalyst	Reaction conditions	Succinonitrile Yield (mol-%)
$PdCl_2$	25 ^oC, 18 hr, MeCN:H_2O (1:1)	59
$Pd(OAc)_2$	25 ^oC, 18 hr, MeCN:H_2O (1:1)	48
$Pd(OH)_2/C$	25 ^oC, 18 hr, MeCN:H_2O (1:1)	3
$Pd(OH)_2/C$	Reflux, 18 hr, MeCN:H_2O (1:1)	9
$ZnCl_2$	25 ^oC, 18 hr, MeCN:H_2O (1:1)	0
$ZnCl_2$	Reflux, 18 hr, MeCN:H_2O (1:1)	0
$ZnCl_2$	MW 450W, 120 s, MeCN:H_2O (1:1)	0

Table 5.2. Activity of different catalysts for the water transfer reaction of 3-cyanopropanoic amide with acetonitrile.

The results are summarized in Table 5.2. From the results it becomes apparent that palladium(II) catalyzes the dehydration of 3-cyanopropanoic amide to succinonitrile, and that although zinc(II) and formaldehyde were reported to catalyze this reaction with other substrates,[22-23] this does not occur with 3-cyano-propanoic amide. Immobilized palladium(II) in the form of palladium(II) hydroxide on carbon catalyzes this reaction too, but the succinonitrile yield is much lower. Mass transfer limitations could play a role here, since the yield increases threefold under reflux conditions, compared to the reaction at room temperature. Palladium chloride affords the highest succinonitrile yield (59 mol-%).

Figure 5.1 shows the palladium chloride catalyzed conversion of 3-cyanopropanoic amide to succinonitrile in time, followed by ^{1}H-NMR in a 1:1 (vol.) mixture of deuterated acetonitrile and deuterated water, in comparison with the isolated yields of succinonitrile and 3-cyanopropanoic amide. It shows that all the succinonitrile could be extracted from the aqueous phase, but not all the 3-cyanopropanoic amide. This was expected because 3-cyanopropanoic amide is much more polar than succinonitrile.
What Figure 5.1 further shows is that the equilibrium between 3-cyanopropanoic amide and succinonitrile is reached after about 5 hours, at 38 mol-% 3-cyanopropanoic amide and 62 mol-% succinonitrile. Besides 3-cyanopropanoic

amide and succinonitrile, acetamide (mostly present as deuterated acetamide) and traces of succinamide (<1 mol-%) were detected as the only other compounds present in the reaction mixture, indicating that no other byproducts were formed. When this reaction was scaled up to gram scale and the crude products purified by column chromatography, an isolated yield of 41 mol-% pure succinonitrile was obtained. This seems a rather poor yield, however a high selectivity is achieved here. The poor yield is therefore attributed to the equilibrium, and not due to the formation of other products than succinonitrile. According to a thermodynamic calculation at the B3LYP/6-311++G(d,p) level, this system has a slightly positive Gibbs free energy of reaction, leading to an unfavourable reaction equilibrium for the formation of succinonitrile. Increasing the amount of acetonitrile would lead to a higher yield of the desired product, but it would also dilute the reaction mixture, so presumably it is more economical to separate the product from the reaction mixture and recycle the remaining substrate, than to increase the amount of acetonitrile.

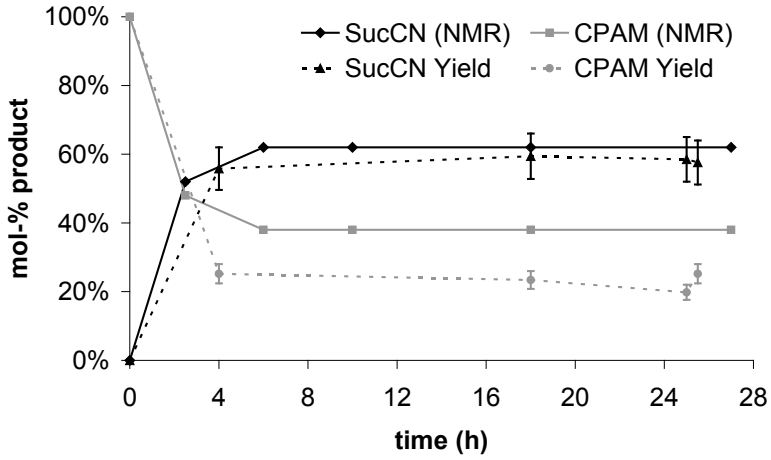


Figure 5.1. Relative amounts of succinonitrile and 3-cyanopropanoic amide in the reaction mixture in time, as detected by direct ^{1}H-NMR of the reaction mixture (straight lines) and the crude yields obtained by extraction of the products from the reaction mixture (dotted lines).

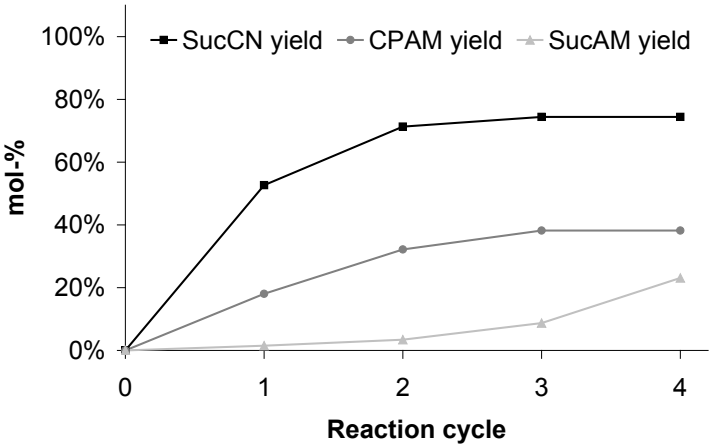


Figure 5.2. Cumulative amounts of succinonitrile, 3-cyanopropanoic amide and succinamide that were isolated after four reaction cycles. Note: Some fresh 3-cyanopropanoic amide was added to the reaction mixture after each cycle, to compensate for the loss of 3-cyanopropanoic amide due to its partial extraction together with succinonitrile. Therefore the sum of the cumulative amounts exceeds 100%.

The difference in polarity could provide a means of shifting the equilibrium to the formation of succinonitrile. Extraction of succinonitrile in a biphasic system with diethyl ether as one phase and the water-acetonitrile mixture as the other could lead to a higher conversion of 3-cyanopropanoic amide. Figure 5.2 shows the cumulative amounts of succinonitrile, 3-cyanopropanoic amide and succinamide, over four reaction cycles. The yield of succinonitrile after the first cycle was 53 mol-%, which is the same as in a single phase system. This indicates that the equilibrium is not shifted towards succinonitrile formation in the biphasic system, but remains constant. After the second and third reaction cycles the cumulative succinonitrile yield increased to a final value of 74 mol-%, after which no further increase was observed. However, the succinamide yield increased significantly. This is probably due to an accumulation of acetamide in the aqueous phase (acetamide will not be extracted from an aqueous phase by diethyl ether), which shifts the equilibrium shown in scheme 5.3 further to the left and even leads to the uptake of one molecule of water by the starting material 3-cyanopropanoic amide, yielding succinamide.

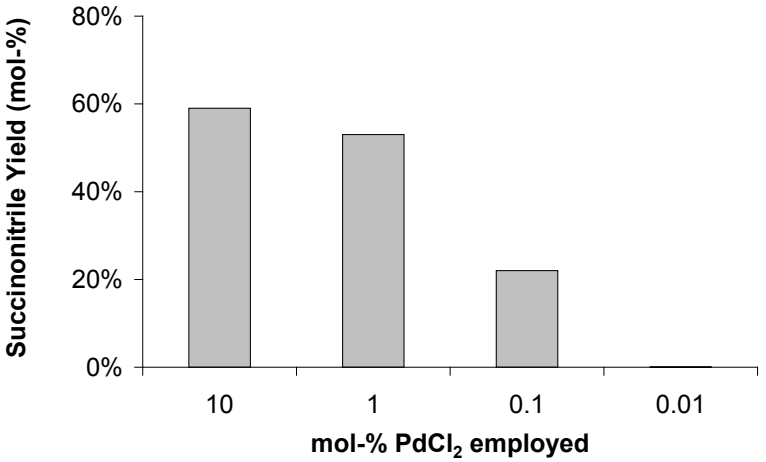


Figure 5.3. Crude yield of succinonitrile as a function of the amount of catalyst that was employed. (conditions: 18 h, 25 ^{o}C).

To improve the economic feasibility of palladium based catalysts for application in an industrial process for the production of succinonitrile, the amount used should be less than the 10 mol-% that was reported in literature[21] and that was applied in the experiments above. The current market price of palladium is about 19,000 €/kg,[25] while the market price of succinonitrile would probably be similar to that of caprolactam, which is 2 €/kg.[26] Since palladium is about ten thousand times as expensive as succinonitrile, the palladium loading of the reactor should either be very low, or the palladium should be recycled in the process. Figure 5.3 shows the succinonitrile yield using various amounts of palladium chloride. The succinonitrile yield that was obtained with 0.01 mol-% palladium chloride was less than 1 mol-%, which makes it apparent that catalyst recycling will be required for a commercial application of this process. There are a number of reports in literature about recycling or immobilizing a homogeneous catalyst based on palladium(II), for example on silica,[27] polymeric resins,[28] polyethylene glycol,[29] or on paramagnetic nanoparticles.[30] In most cases it is based on palladium-phosphine interactions, such as the immobilization on silica by Van Koten *et al.*[27] Although the exact mechanism of the current reaction has not been fully investigated,[21] it is likely that coordination of acetonitrile to palladium(II) plays a crucial role, as the Lewis acidity of zinc chloride alone is not sufficient to promote reaction (Table

5.2). This was supported by our observation where the addition of two equivalents of a triphenylphosphine sulfonate (TPPS) ligand to the palladium chloride solution (before adding the substrate) resulted in no succinonitrile formation. TTPS ligands are known to interact strongly with transition metals such as palladium,[31] and in this case they probably displace the coordinated acetonitrile from the palladium forming an inactive species. This eliminates the possibility to use a palladium-phosphine interaction for the immobilization of the catalyst to a support or for entrapment in the aqueous phase. Therefore a supported palladium(II) species to which acetonitrile can still coordinate would be the preferred catalyst for an industrial application of this reaction.
Another approach to the recycling of the catalyst would be the use of another water acceptor than acetonitrile. An experiment with a suspension of poly(acrylonitrile) in water instead of a 1:1 mixture of acetonitrile and water was performed, in which succinonitrile was also found as the sole product. Under the same conditions as with the acetonitrile system, the succinonitrile yield that was achieved in this case was 11 mol-%. No palladium(II) was found in the organic phase after extraction, meaning that the catalyst could be recycled efficiently. A reason for the lower yield could be the less favourable reaction conditions, by using a heterogeneous system instead of a homogeneous one. When acetonitrile would become the water acceptor of choice, it would be best if it would be biobased as well. This is expected to be possible by the oxidative decarboxylation of the amino acid alanine, which will be the subject of further research. In that case this route would not only yield biobased succinonitrile, but also biobased acetamide.

5.4 Conclusion

The goal of this chapter was to investigate the possibility to synthesize succinonitrile from glutamic acid, in order to be able to produce biobased 1,4-diaminobutane. This conversion was shown to be feasible. The first step is either an oxidative decarboxylation of the 5-methyl ester of glutamic acid, followed by an amidation of the crude product, or an oxidative decarboxylation of glutamine. In both cases the product is 3-cyanopropanoic amide. From glutamic acid 5-methyl ester, 3-cyanopropanoic amide was obtained in a crude yield above 86 mol-%. In

the second step, 3-cyanopropanoic amide can be dehydrated to succinonitrile. The most effective method to achieve this is a palladium(II) catalyzed water transfer reaction with acetonitrile as the water acceptor. Under the employed conditions, no significant amounts of side products were found, indicating that although the conversion of 3-cyanopropanoic amide to succinonitrile is only 62 %, the selectivity of this reaction is close to 100 %.
There is still the need to establish an effective method to recycle the palladium, to find a greener oxidant than hypochlorite, and to design a process for the isolation of glutamic acid or glutamine from agricultural byproduct streams, but when these requirements are met this biobased route will be a good alternative to the fossil based route for the production of succinonitrile.

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5.5 References

1. Solvay **2010**, www.solvaychlorinatedorganics.com, accessed Dec 2010.
2. J. J. Bozell, G. R. Petersen, *Green Chem.* **2010**, 12, 539-554.
3. E. Scott, F. Peter, J. Sanders, *Appl. Microbiol. Biotechnol.* **2007**, 75, 751-762.
4. V. Deike, J. De Mol, *Raw Material Compendium*, 2nd ed., Novus International Inc. Brussels **1996**.
5. T. M. Lammens, D. De Biase, M. C. R. Franssen, E. L. Scott, J. P. M. Sanders, *Green Chem.* **2009**, 11, 1562-1567.
6. T. M. Lammens, M. C. R. Franssen, E. L. Scott, J. P. M. Sanders, *Green Chem.* **2010**, 12, 1430-1436.
7. J. Le Nôtre, E. L. Scott, M. C. R. Franssen, J. P. M. Sanders, *Green Chem.* **2011**, 13, 807-809.
8. P. Pollak, G. Romeder, F. Hagedorn, H. Gelbke, *Nitriles*, in Ullmann's Encyclopedia of Industrial Chemistry, Wiley-VCH, Weinheim, **2002**.

9. a) DSM **2011**, www.dsm.com/en_US/html/dep/stanyl.htm, b) DSM **2011** www.dsm.com/nl_NL/html/dep/news_items/ecopaxx_pr.htm; accessed Feb 2011.
10. E. Scott, F. Peter, J. Sanders, *Appl. Microbiol. Biotechnol.*, **2007**, 75, 751-762.
11. a) Z.-G. Qian, X.-X. Xia, S. Y. Lee, *Biotechnol. Bioeng.* **2009**, 104, 651-662. b) K. Eppelmann, P. M. M. Nossin, S. M. Kremer, M. G. Wubbolts, US Patent US2009/0275093A1, **2009**. c) P. M. Könst, M. C. R. Franssen, E. L. Scott, J. P. M. Sanders, *Green Chem.*, **2011**, *Advance article*, DOI: 10.1039/C0GC00564A,
12. I. Bechthold, K. Bretz, S. Kabasci, R. Kopitzky, A. Springer, *Chem. Eng. Technol.* **2008**, 31, 647-654.
13. R. Jacquot, P. Marion, World Patent WO2010/063632A1, **2010.**
14. V. N. Telvekar, R. A. Rane, *Tet. Lett.* **2007**, 48, 6051-6053.
15. F. H. A. M. Vandenbooren, H. J. M. Bosman, A. V. Peters, M. J. G. Van Den Boer, US Patent US2006/0122433A1, **2006.**
16. D. Coleman, *J. Chem. Soc. (C)* **1951**, 2294.
17. J. E. Baldwin, M. North, A. Flinn, M. G. Moloney, *Tetrahedron* **1989**, 45, 1453-1464.
18. A. Podgorsek, M. Zupan, J. Iskra, *Ang. Chem. Int. Ed.*, **2009**, 48, 8424-8450.
19. M. B. Smith, J. March, *March's advanced organic chemistry*, 6th ed.; John Wiley & Sons: Hoboken, New Jersey, **2007**, p 1549.
20. K. Ishihara, Y. Furuya, H. Yamamoto, *Angew. Chem. Int. Ed.* **2002**, 41, 2983-2986.
21. S. I. Maffioli, E. Marzorati, A. Marazzi, *Org. Lett.* **2005**, 7, 5237-5239.
22. K. Manjula, M. A. Pasha, *Syn. Comm.* **2007**, 37, 1545-1550.
23. M. P. Heck, A. Wagner, C. Mioskowski, *J. Org. Chem.* **1996**, 61, 6486-6487.
24. H. Cheung, R. S. Tanke, G. P. Torrence, *Acetic Acid*, in Ullmann's Encyclopedia of Industrial Chemistry, Wiley-VCH, Weinheim, **2002**.
25. Johnson Matthey **2010**, www.platinum.matthey.com, accessed Dec 2010.
26. ICIS **2010**, www.icis.com, accessed Dec 2010.
27. N. C. Mehendale, J. R. A. Sietsma, K. P. de Jong, C. A. van Walree, R. J. M. Klein Gebbink, G. van Koten, *Adv. Syn. Catal.* **2007**, 349, 2619-2630.
28. N. T. S. Phan, D. H. Brown, P. Styring, *Tetrahedron Lett.* **2004**, 45, 7915-7919.
29. W. Mai, L. Gao, *Synlett* **2006**, 2553-2558.
30. P. D. Stevens, G. Li, J. Fan, M. Yen, Y. Gao, *Chem. Commun.* **2005**, 4435-4437.
31. G. Van Koten, P. W. N. M. Van Leeuwen, *Catalysis: an integrated approach*, Chapter 6, 2nd ed.; Elsevier, Amsterdam, **2000**, p 294.
32. A. Kleemann, W. Leuchtenberger, J. Martens, H. Weigel, *Angew. Chem. Int. Ed.* **1980**, 19, 627-627.

Chapter 6

Techno-economic assessment of the production of biobased chemicals from glutamic acid

This chapter was submitted as:
T.M. Lammens, S. Gangarapu, M.C.R. Franssen, E.L. Scott, J.P.M. Sanders, "Techno-economic assessment of the production of biobased chemicals from glutamic acid", 2011

Abstract

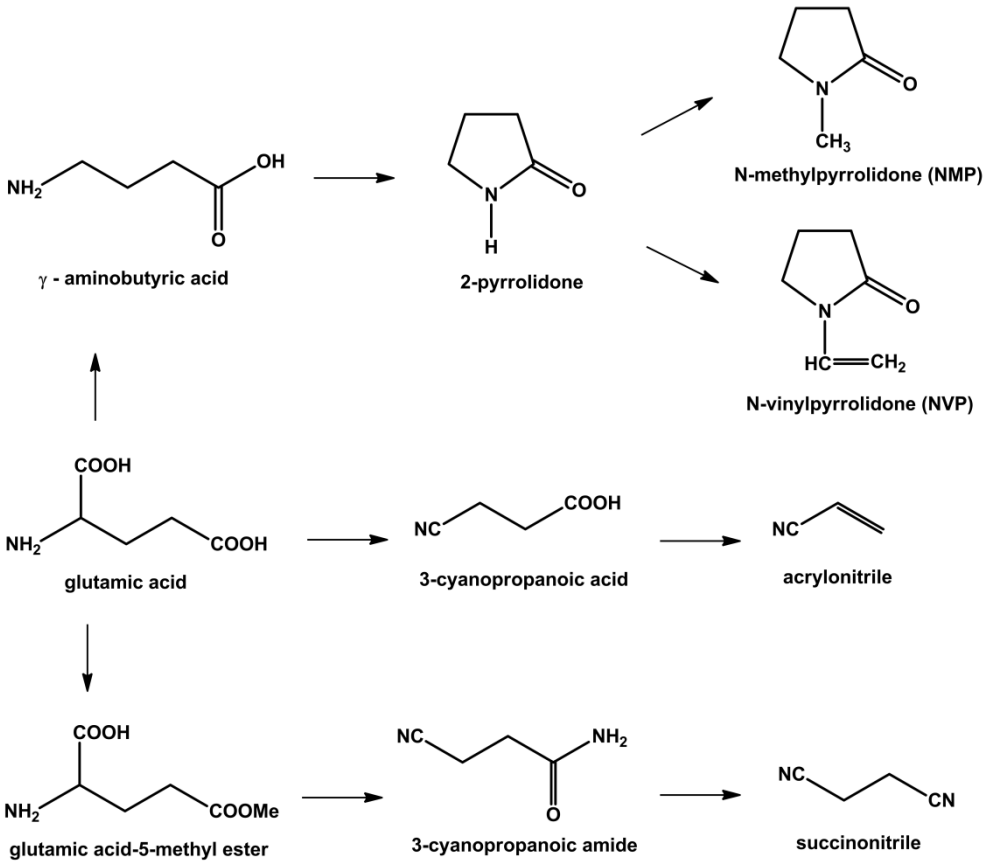
In this chapter possible process steps for the production of biobased industrial chemicals from glutamic acid are described, including a techno-economic assessment of all processes. The products under investigation were those that were shown to be synthesized from glutamic acid on lab-scale, namely N-methylpyrrolidone (NMP), N-vinylpyrrolidone (NVP), succinonitrile, and acrylonitrile. The techno-economic assessment leads to the conclusion that the production of NMP and NVP is feasible both in terms of technology and economy. Although some assumptions were rough, an estimated cost-benefit margin of about 60 % shows that there is sufficient room to maneuver within the process. Biobased acrylonitrile and succinonitrile do not seem very profitable under the current process configurations. Especially the acrylonitrile process shows very high costs in relation to the possible gains. Further optimization is necessary, but a clear direction where the optimization should be aimed at was provided in the discussion of the processes. The main point to optimize was the reaction of glutamic acid with sodium hypochlorite, a bottleneck in both the acrylonitrile and the succinonitrile process.

6.1 Introduction

In previous research the possible production of biobased γ-aminobutyric acid (chapter 3),[1] 2-pyrrolidone, N-methylpyrrolidone (chapter 4),[2] acrylonitrile[3] and succinonitrile (chapter 5)[4] from glutamic acid was demonstrated. The goal of this chapter is to assess the technological and the economic feasibility of industrial processes based on the chemistry that was described in the above-mentioned papers. In order to do that, the different process steps for the production of biobased N-methylpyrrolidone (NMP), N-vinylpyrrolidone (NVP), succinonitrile, and acrylonitrile, all from glutamic acid, will be described.
The isolation of glutamic acid from byproduct streams has not been achieved yet and is the subject of further investigation. Here it will be assumed that glutamic acid can be isolated from byproduct streams of bioethanol production, such as distiller's grains with solubles or sugar beet vinasse.
Scheme 6.1 shows the routes from glutamic acid to these four chemicals. Based on the knowledge acquired in the syntheses of these products, the processes will be defined at a level of moderate detail, which is sufficient to determine mass and energy balances for the production of the different chemicals. The goal of this paper is not to provide process details at the level of industrial implementation, but to provide an indication of potential strengths and weaknesses in the processes, a guideline to where further improvement should be directed at, and an idea of potential feasibility of these processes at industrial scale.

The first process is the simultaneous production of NMP and NVP from glutamic acid. Synthesis of NMP from glutamic acid was achieved in the lab by enzymatic decarboxylation of L-glutamic acid to γ-aminobutyric acid (GABA),[1] followed by cyclization of GABA to 2-pyrrolidone and subsequent catalytic methylation of 2-pyrrolidone with methanol to form NMP.[2] The latter two steps can be performed in a one-pot procedure. The conversion of GABA to 2-pyrrolidone proceeds readily and completely under the conditions applied, but the catalytic methylation of pyrrolidone to NMP requires more effort and, depending on the reaction time applied, leads to the formation of byproducts.[2] Therefore a process is anticipated where GABA is fully converted to 2-pyrrolidone, but where 2-pyrrolidone only undergoes 50 % conversion to NMP. 2-Pyrrolidone can then be separated from the

formed NMP, and further processed in a reaction with acetylene to form NVP. The reaction of 2-pyrrolidone with acetylene is a well-known and commercially applied procedure. The two main global producers of NVP applying this procedure are BASF and GAF. Data from a GAF patent will be used as input for this part of the process.[5]



Scheme 6.1. The routes from glutamic acid to the four biobased industrial products

The second process is the production of succinonitrile from glutamic acid. The first step is the mono-esterification of glutamic acid with methanol to form glutamic acid 5-methyl ester.[6,7] This subsequently undergoes oxidative decarboxylation to 3-cyanopropanoic acid methyl ester, which is amidated using ammonia to form 3-cyanopropanoic amide. The last step in the process is a catalytic dehydration of 3-cyanopropanoic amide to succinonitrile.[3]

The third process to be described in this chapter is the production of acrylonitrile from glutamic acid. The first step in this case is the oxidative decarboxylation of glutamic acid to 3-cyanopropanoic acid followed by a catalytic carbonylation-elimination reaction to give acrylonitrile.[4]

6.2 Process descriptions

6.2.1 NMP and NVP production

Figure 6.1 shows a simplified diagram of the main unit operations that are required to produce NMP and NVP from glutamic acid, with the flows that enter and leave the different operations. The overall mass balance of this process, including a list of the assumptions that were made, can be found in appendix B.

The process consists of first a fed batch reactor with glutamic acid α-decarboxylase (GAD) as catalyst that converts glutamic acid into GABA and CO_2. GAD is immobilized in calcium alginate. The reaction is performed in water, at a constant pH of 4.6 and can be maintained at 4.6 by the addition of solid glutamic acid, or by adding a strong acid such a hydrochloric acid, which can be recycled after GABA crystallization. For the crystallization of GABA, which is very soluble in water, a large volume of water will have to be evaporated. For the calculation of the required energy the assumption will be a worst case scenario, in which all the water is evaporated. Here improvements are still possible. Mutants of the enzyme have been reported that can perform this reaction at a higher pH, which means that glutamic acid solubility increases and GABA solubility decreases, because the pH will become closer to the isoelectric point of GABA and further from that of glutamic acid.[12] This will facilitate faster crystallization of GABA.
The next step is a reactor in which GABA is reacted with methanol and a bromide or iodide catalyst, first to 2-pyrrolidone and then to NMP. For every mole of pyrrolidone and for every mole of NMP that is formed, one mole of water is coproduced. This reaction takes place at 250 ^{o}C.[2] After 50 % conversion of pyrrolidone to NMP, the products are sent to a distillation section, where first methanol and water are removed, which are subsequently separated further by distillation. NMP is separated from pyrrolidone by distillation as well.

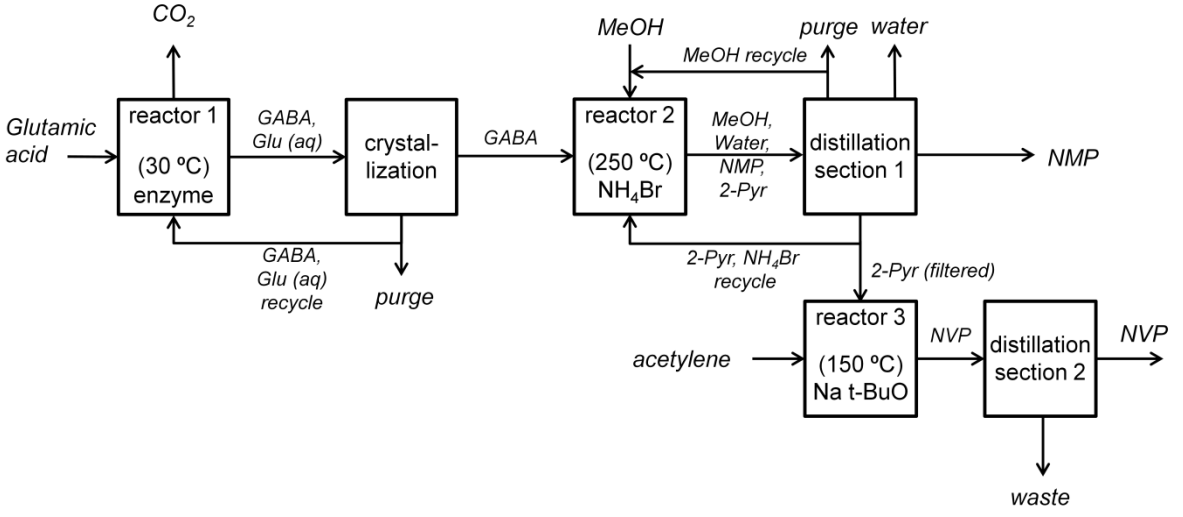


Figure 6.1. Process flow diagram for biobased NMP and NVP production.
Abbreviations: 2-Pyr = 2-pyrrolidone, GABA = γ-aminobutyric acid, Glu = glutamic acid, MeOH = methanol, Na t-BuO = sodium t-butoxide, NH_4Br = ammonium bromide, NMP = N-methylpyrrolidone, NVP = N-vinylpyrrolidone.

Pyrrolidone is fed to the next reactor after filtration of the remaining catalyst. There pyrrolidone reacts with acetylene in the presence of sodium or potassium *tert*-butoxide to form NVP with a selectivity of 90 %.[5] NVP is isolated from the product mixture by distillation.
Instead of simultaneous production of NMP and NVP, one of the two could also be produced. When only NMP would be produced, a recycle of pyrrolidone from the distillation section to reactor 2 would be necessary, as increasing the conversion of pyrrolidone to NMP in reactor 2 would decrease the selectivity of that reaction. The energy requirement per ton of product would increase due to this recycle. When only NVP would be produced, the harsh methylation conditions (250 ^{o}C) would not be necessary, nor the presence of a bromide or iodide catalyst. The reason why coproduction was assumed in this case is that the volume of NMP that is produced worldwide is higher than that of NVP, so NMP is likely to be the driver for installing such a process. Coproduction of NVP could then make the process more efficient.

The energy that is required to operate this process was estimated based on the above data and computationally calculated enthalpies and entropies of the reactions taking place.[9] For every block in the flow diagram in figure 6.1, the use or production of energy was calculated. Calculations can be found in appendix B.
The energy requirement for heating was transferred into an amount of steam needed to provide that energy, with a conversion factor of 3 MJ/kg.[10] Table 6.1 shows the main inputs of the process in terms of the stoichiometric reagents and the steam for the energy requirement, including the price that was used for the economic assessment.

	Amount (kg):	Price (€/kg):
Valuable products:		
NMP	477	3.07[a]
NVP	523	3.07[b]
Main process input:		
Glutamic acid	1538	0.50[c]
Methanol	162	0.37[a]
Acetylene	136	1.03[a]
Steam for heating	3100	0.015[d]

Table 6.1. Products and main input of the simultaneous production process of NMP and NVP. a) Price obtained from ICIS.[11] b) the price of NVP at bulk scale could not be obtained, but is higher than NMP.[12] This is a conservative estimation. c) Estimated below current market price, as obtained from byproducts. d) Personal communication of DSM.

6.2.2 Acrylonitrile production

Figure 6.2 shows a simplified diagram of the process steps needed to produce acrylonitrile from glutamic acid. For the mass balance and a list of assumption, see appendix B.

The first reactor could be a continuously stirred tank reactor (CSTR) or a tubular plug flow reactor (PFR). In this reactor glutamic acid undergoes oxidative decarboxylation to cyanopropionic acid (CPA), CO_2 and water. For this, an oxidant is needed for the formation of Br^+. This can be derived from sodium hypochlorite in combination with a catalytic amount of sodium bromide. In order to avoid byproduct formation, the reactor needs to be cooled to 4 ^oC. In the lab, this reaction was only carried out using sodium hypochlorite, but due to the large amount of waste salt (NaCl) that is produced, a reaction with oxygen, hydrogen peroxide, an enzyme, or an enzyme mimic would be more preferable.[4] This will be the subject of future investigation. For this process, a system with two equivalents of sodium hypochlorite per glutamic acid molecule will be assumed, in the presence of a catalytic amount (10 %) of sodium bromide. The assumed conversion is 100 mol-%, with 90 mol-% selectivity for CPA. For a CSTR such a high conversion is not possible, but for a PFR it is. The stream leaving the reactor should then consist of

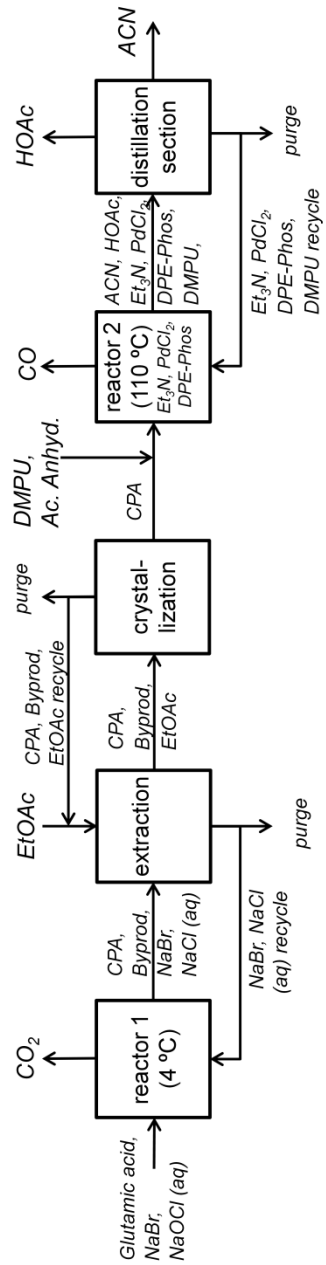


Figure 6.2. Process flow diagram for biobased acrylonitrile production.
Abbreviations: Ac. Anhyd. = acetic anhydride, ACN = acrylonitrile, Byprod = byproducts, CPA = cyanopropanoic acid, DMPU = 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone, DPE-Phos = bis(2-diphenylphosphinophenyl) ether, Et_3N = triethylamine, EtOAc = ethyl acetate, HOAc = acetic acid, NaOCl = sodium hypochlorite, NaBr = sodium bromide, NaCl = sodium chloride, $PdCl_2$ = palladium chloride.

CPA, byproducts, NaCl and NaBr in water. This stream can partly be recycled, in order to partly reuse NaBr.
CPA can be extracted from the aqueous solution with ethyl acetate. That can be done in a standard (i.e. continuous counter flow) extraction column. Based on laboratory data, the ethyl acetate volume that is needed is assumed to be twice that of the aqueous solution.[4] This is a worst case scenario, because in a countercurrent setup the required volume of ethyl acetate will be less. The solubility of ethyl acetate in water at 25 ^{o}C is 8 wt-%,[13] so in order to reduce the loss of ethyl acetate it will have to be recovered from the aqueous effluent stream, i.e. by evaporation. CPA can then be crystallized from the ethyl acetate solution, which should be cooled.
The second reactor could also be a CSTR or a PFR. Here, CPA in N,N'-dimethyl propyleneurea (DMPU) reacts with acetic anhydride, in the presence of palladium chloride, bis(2-diphenylphosphinophenyl)ether (DPE-Phos) and triethylamine, to form acrylonitrile, acetic acid and carbon monoxide. The reaction is performed at 110 ^{o}C. At labscale, an acrylonitrile yield of 17 % was obtained, because of product degradation. This yield will need to be improved drastically to make the process feasible. For now, a yield of 90 % will be assumed. This means the overall yield would become 81 %, similar to that of an efficiently operated SOHIO process.[14] Finally, acrylonitrile can be isolated by distillation. The rest of the reaction mixture can then be recycled back to the reactor.

For every block in the flow diagram in figure 6.2, the energy use or production was calculated. Calculations can be found in appendix B, results in table 6.2.
The reaction in reactor 1 is exothermic but because the reactor is operated at a low temperature this will require extra energy for cooling rather than delivering energy to the rest of the process. It will be assumed that the cooling of reactor 1 will be powered with electricity and will have an efficiency of 67 %. In other words, it will require 1.5 MJ of electric energy to compensate for 1 MJ of heat that is produced in the reactor. The required energy as calculated for the extraction and crystallization is the energy that is needed to cool down ethyl acetate from 25 ^{o}C to 4 ^{o}C, which is needed to crystallize the cyanopropanoic acid. For the distillation of acrylonitrile additional heating energy should be supplied, some of which could be supplied

from the heat released by the exothermic reaction in reactor 2. The calculations and the results per unit operation can be found in appendix B.
Table 6.2 shows the main process inputs with the values that were used for the economic assessment.

	Amount (kg):	Price (€/kg):
Valuable products:		
Acrylonitrile	1000	1.37[a]
Acetic acid	2515	0.65[a]
Main process input:		
Glutamic acid	3423	0.50[b]
Sodium hypochlorite	3464	1.01[a]
Acetic anhydride	2375	0.81[a]
Steam for heating	421	0.015[c]
Electricity for cooling (kWh)	7962	0.055[c]

Table 6.2. Products and main input of the production process of acrylonitrile. a) Price obtained from ICIS.[11] b) Estimated below current market price, as obtained from byproducts. c) Personal communication of DSM.

6.2.3 Succinonitrile production

Figure 6.3 shows a simplified diagram of the process steps needed to produce succinonitrile from glutamic acid. Appendix B contains the mass balance and assumptions of this process.

In the first reactor, glutamic acid undergoes mono-esterification to glutamic acid-5-methyl ester. From literature, this reaction was reported with a selectivity of 98 % for the mono-ester and 2 % for the di-ester.[6] Therefore, a yield of 98 % is assumed for this step. In the second reactor the same oxidative decarboxylation reaction takes place as described earlier in the acrylonitrile process, so the assumptions here are the same. In the third reactor ammonia is added, thereby forming cyanopropanoic amide and methanol.
Excess ammonia and methanol are subsequently removed by distillation and can be reused in reactors 3 and 1, respectively. Intermediate purification of cyanopropan-

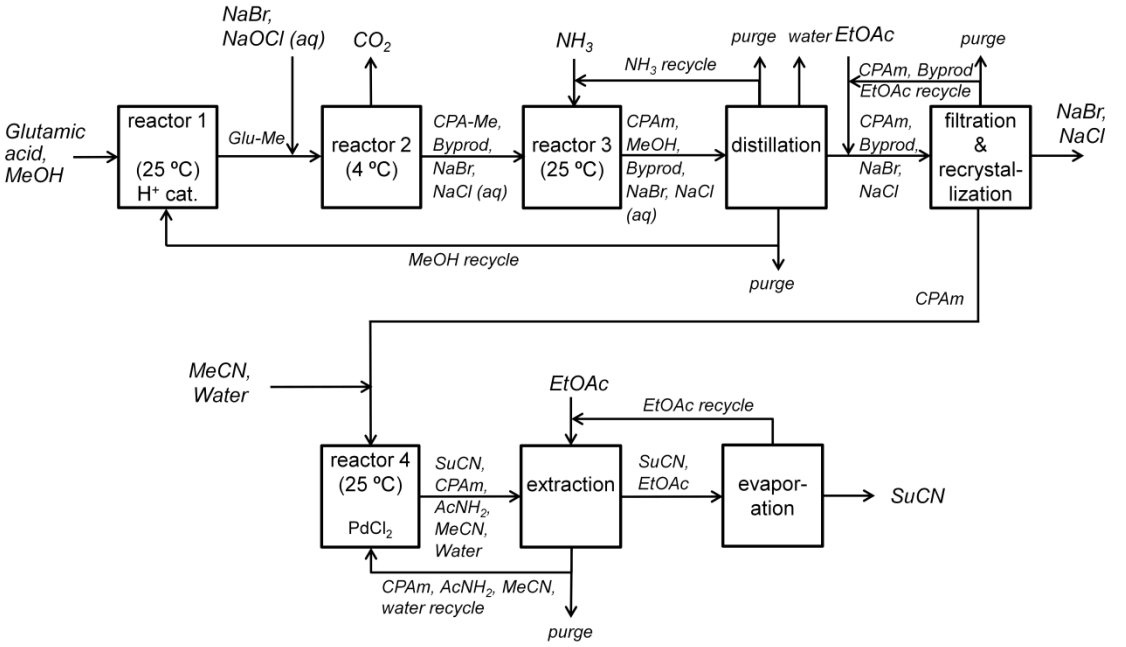


Figure 6.3. Process flow diagram for biobased succinonitrile production.

Abbreviations: $AcNH_2$ = acetamide, Byprod = byproducts, CPA-Me = cyanopropanoic acid methyl ester, CPAm = cyanopropanoic amide, EtOAc = ethyl acetate, Glu-Me = glutamic acid-5-methyl ester, MeCN = acetonitrile, MeOH = methanol, NaOCl = sodium hypochlorite, NaBr = sodium bromide, NaCl = sodium chloride, $PdCl_2$ = palladium chloride, SuCN = succinonitrile.

oic amide is assumed, therefore the water will be evaporated, and the cyanopropanoic amide recrystallized from ethyl acetate. It is assumed that the amount of ethyl acetate that is needed for this is twice the mass of cyanopropanoic amide. In this step, sodium bromide and chloride can be removed from the warm cyanopropanoic amide solution by filtration, and can be recycled to reactor 2.

In reactor 4, a water-transfer reaction takes place, converting cyanopropanoic amide and acetonitrile into succinonitrile and acetamide. This reaction is catalyzed by palladium (II) (i.e. $PdCl_2$). The reaction takes place at room temperature and is an equilibrium reaction, with the position of the equilibrium resulting in 60 mol-% succinonitrile. Since no byproducts are formed, it is assumed that by recycling the starting material, a final yield of 95 mol-% succinonitrile will be obtained. Succinonitrile can be easily extracted from the reaction mixture with ethyl acetate. The catalyst will have to be retained in the aqueous phase, which has been achieved by using poly(acrylonitrile) instead of acetonitrile as water acceptor, however the yield was then lower.[3] This system will require further optimization, but currently it is assumed that the palladium loss is only 0.01 mol-%, as in the case of the acrylonitrile process.

The energy calculations can be found in appendix B, the results are shown in table 6.3.
The reaction in reactor 2 is very exothermic and therefore produces energy. However, because the reactor is operated at a low temperature this will require extra energy for cooling rather than delivering energy to the rest of the process. A required energy input that is equal to the produced energy is assumed here. The reaction in reactor 1 is exothermic as well, whereas the reactions in reactor 3 and 4 are endothermic and therefore require some energy input. It was assumed that the energy released in reactor 1 could be used to power reactors 3 and 4, by choosing the right reaction temperatures. For the cooling of reactor 2, the same assumptions will be made as for reactor 1 in the acrylonitrile process.

	Amount (kg):	Price (€/kg):
Valuable products:		
Succinonitrile	1000	3.07[a]
Acetamide	738	3.07[a]
Main process input:		
Glutamic acid	2193	0.50[b]
Sodium hypochlorite	2175	1.01[c]
Ammonia	224	0.81[c]
Acetonitrile	540	1.13[c]
Steam for heating	2001	0.015[d]
Electricity for cooling (kWh)	4663	0.055[d]

Table 6.3. Products and main input of the production process of succinonitrile. a) Price at bulk scale could not be obtained, therefore same price as NMP was assumed. b) Estimated below current market price, as obtained from byproducts. c) Price obtained from ICIS.[11] d) Personal communication of DSM.

6.3 Discussion and economic assessment

Table 6.4 shows the results of the economic assessment for all processes described above. All calculations and assumptions are available in appendix B. In this paragraph the results will be discussed per process. The economic assessment and the discussion of the processes will show a number of weaknesses of the processes, and suggestions how to improve the processes will be given.

	NMP & NVP	acrylonitrile	succinonitrile
Variable costs (k€ / ton)	1.2	6.5	4.5
Goods sold (k€ / ton)	3.1	3.2	5.5
Margin (k€ / ton)	1.9	- 3.3	1.0
Margin (% of output)	61 %	- 103 %	18 %

Table 6.4. Estimated margins between variable input and the output of biobased chemicals from glutamic acid with the current process configuration. Calculations can be found in appendix B. Fixed costs could not be determined due to roughness of process design.

6.3.1 NMP & NVP

According to the derived mass balance for this process, for each ton of combined NMP and NVP (477 and 523 kg, respectively), 1.5 ton of glutamic acid is needed as input.

The main weight loss when converting glutamic acid to NMP and NVP is simply due to the changing molecular weights, which are lower for the products than for glutamic acid. This weight is lost in the form of water and CO_2 that are produced as byproducts. About 0.1 ton of waste products per ton of valuable products are anticipated in this process, when CO_2 and water are not counted as waste. This could mainly be reduced with an increased selectivity of the reactions of 2-pyrrolidone to NMP and NVP.

The energy necessary for this process is 9.3 GJ per ton of products. More than 50 % of the energy requirement is due to the very high solubility of GABA in water, which means large amounts of water have to be evaporated for the isolation of GABA. This could be improved by using an enzyme mutant that is active at a higher pH which may facilitate the precipitation of GABA.[8]

The results of the economic assessment are shown in table 6.4. The variable costs were calculated based on the in- and outputs of the process in terms of materials and energy. As a result of the moderate level of process detail, it was not feasible to estimate the capital investments for these processes. The calculations show that there is a positive margin on this process of 1900 €/ton. This number should not be taken as absolute for two reasons: firstly crude assumptions had to be made; secondly the calculations do not include the fixed costs of the processes. A margin of more than 60 % however provides room for additional fixed costs. Therefore the calculations point towards the conclusion that this process could be economically viable when applied industrially.

Furthermore these calculations show important cost drivers. The most important driver (more than 50 %) for the variable costs of this process is the price of glutamic acid. This price was assumed to be 500 €/ton, which is under the reported market price of 800-1000 €/ton for monosodium glutamate (MSG).[18] The reason for this assumption was described in the introduction, namely that glutamic acid is expected to be obtained from low-value byproduct streams of biofuels production.

The current market price of MSG is based on a fermentation process that provides a food-grade quality product, which will not be necessary for an industrial process. Although not quantified, it can be assumed that a large part of the capital investment of this process will go into the reactor where the catalytic methylation takes place. This is due to the corrosive properties of bromide or iodide salts at 250 ^{o}C, which means that an expensive steel alloy will have to be used for this reactor. It would be advisable to compare the financial implications of a reactor that can cope with these conditions with those of a reactor that needs to withstand the conditions of other catalytic methylation reactions with methanol. An example is by using a zeolite catalyst in a gas-phase reaction above 400 ^{o}C.[15,16]

6.3.2 Acrylonitrile

For every ton of acrylonitrile, 3.4 tons of glutamic acid are necessary as feedstock. The reason for that is mainly the difference in mass between the two compounds as a result of the reaction. The molar mass of glutamic acid is almost three times as much as that of acrylonitrile. Much of that mass is lost in the process as CO_2, CO and water. The amount of waste products is 0.7 ton per ton of acrylonitrile.

From an energy perspective, the main energy need comes from the cooling of the oxidative decarboxylation reaction. This accounts for 85 % of the total energy requirement of 20 GJ per ton of product. As a result of the products formed, this reaction will always be exothermic, also when it would be done with hydrogen peroxide or oxygen as the oxidant. The best way to improve this process would be to perform this reaction at a higher temperature, so that the produced energy could be used via heat integration. In that case this process could become a net energy producer. In the present case the energy needed for the cooling is expensive, because cooling below room temperature must be powered by electricity. However, with the present reagents and conditions increasing the reaction temperature would lead to a lower selectivity, so further optimization is needed.

Table 6.4 also shows the results of the economic calculations that were performed for acrylonitrile. This shows that there is a negative profit margin for the

production of acrylonitrile from glutamic acid of 3300 € per ton of acrylonitrile. In other words, the inputs for this process are far more costly than the outputs, even without including capital investments and other fixed costs. The glutamic acid is more expensive than the produced acrylonitrile, which is due to the large mass losses when converting glutamic acid to acrylonitrile. If glutamic acid could become available at a lower price, such as 200-300 €/ton, this would help, but still there would be no margin because the cost of the sodium hypochlorite is a limiting factor as well. Further research should therefore be aimed firstly at replacing hypochlorite with a less expensive oxidant, for example oxygen. Acetic anhydride is also a major cost factor, but if the acetic acid that is formed from the acetic anhydride as byproducts can be recovered and sold (which was in fact assumed in the calculations), that would be no problem. A way to remove the use of acetic anhydride could be by reacting cyanopropanoic acid with itself to form an anhydride, rather than with acetic anhydride. This will be the subject of future research as well.

These results show that it will be very difficult to produce biobased acrylonitrile in a cost-effective way using the envisaged route from glutamic acid. However, by replacing some of the reactants the process could become more viable, especially when acrylonitrile becomes more expensive in the future due to an increasing oil price.

6.3.3 Succinonitrile

The mass balance of succinonitrile is better than that of acrylonitrile. For every ton of succinonitrile, 2.2 ton of glutamic acid is required, and 0.45 ton of waste products are anticipated.

This process requires the most energy per ton of products, requiring 17 GJ per ton of succinonitrile. Again, the oxidative decarboxylation at 4 ^{o}C requires by far the largest part of the energy in the process (65 %). This will influence the economic performance of this process in two ways: firstly the capital investment will be larger because of the cooling installation and secondly the variable costs for utilities will be high because this cooling will have to be powered by electricity. To improve this, the reaction should be performed at an elevated temperature,

following the same line of argumentation as with the acrylonitrile process. If this would prove to be possible, and the energy could be used in the rest of the process, overall the process could actually produce energy, instead of using 15 GJ.

As can be seen from table 6.4, the outcome of the economic analysis is a positive margin of 1000 € per ton of produced succinonitrile. When fixed costs would be added to the costs, chances are large that the positive margin would change into a negative margin. For example if the fixed costs would be 30 % of the total costs, the positive margin would change into a negative margin of 900 € per ton succinonitrile. However, because market data for succinonitrile and acetamide could not be found (succinonitrile is not sold on a bulk scale, but used immediately for the production of polyamides, which are much more expensive), the price of both components was estimated to be similar to that of NMP, which is a conservative estimation. If the real prices would be higher, that would have a large effect on the margin of this process. A succinonitrile value of 5 €/kg instead of 3, for example, would turn the loss of 900 €/ton that was mentioned above around into a profit of 1000 €/ton. This shows that the calculation is very sensitive to the price at which the product can be sold, which means that the techno-economic assessment in this case does not say much about whether this process will be viable or not. However it does usefully point out the main cost drivers of the process, where improvements will be necessary to make this process more economically robust.
The main cost driver in this case is the sodium hypochlorite, which counts for more than 2000 € per ton of succinonitrile. If this could be replaced by a less expensive oxidizing agent the margin could easily become positive. The isolation of acetamide in this process was not taken into account. This means that in practice probably more energy is needed to make it crystallize from the aqueous solution. However, when looking at the cost drivers, that is not expected to be a limiting factor. What could prove to be a limiting factor is the possibility to recycle the palladium(II) in this process. This catalyst is so expensive that it will be essential for the economic feasibility to design a recycle that can achieve or at least get close to the assumed palladium recovery of 99.99%.

For the manufacture of succinonitrile from glutamic acid the economic assessment showed that it does not seem to be economically feasible to perform this process under the assumed conditions. However, there are some important uncertainties in prices that should be investigated further performing studies on the scaling up of this process. Replacing hypochlorite with another oxidizing agent will be a prerequisite for applying this process.

6.4 Conclusions

The discussion above leads to the conclusion that the production of NMP and NVP can be a feasible process, in terms of technology and economy. The assumptions that were done are rough, but an estimated margin of about 60 % shows that there is some room to maneuver within the process. The main matter of concern is the material that will be needed for the methylation reactor, because of the highly corrosive conditions, which may lead to high capital investment and maintenance costs. This should be investigated before studying the scale-up of this process.

Biobased acrylonitrile and succinonitrile do not seem (very) profitable under the current process configurations. Especially acrylonitrile shows very high costs in relation to the possible gains. It will be necessary to obtain glutamic acid much less expensive than estimated to make this process profitable, which poses constraints on the method of isolation. Relatively expensive methods such as electrodialysis or reactive extraction will then probably not be viable.[17-19] Instead, less expensive methods such as amino acid crystallization should be investigated for the isolation of glutamic acid from byproduct streams.[20,21]

Moreover, an alternative to the oxidative decarboxylation reaction with hypochlorite will have to be found, that uses less expensive reagents (i.e. oxygen) and can be operated at a higher temperature so that the produced energy can be used elsewhere in the process. For the succinonitrile process the oxidative decarboxylation reaction is also the main cost driver. The margin of succinonitrile is much better than that of acrylonitrile. The uncertainties in this estimation are large, but finding an alternative for sodium hypochlorite will probably lead to an economically viable process.

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6.5 References

1. T. M. Lammens, D. De Biase, M. C. R. Franssen, E. L. Scott and J. P. M. Sanders, *Green Chem.* **2009**, 11, 1562-1567.
2. T. M. Lammens, M. C. R. Franssen, E. L. Scott and J. P. M. Sanders, *Green Chem.*, **2010**, 12, 1430-1436.
3. T. M. Lammens, J. Le Nôtre, M.C.R. Franssen, E.L. Scott and J. P. M. Sanders, *ChemSusChem* **2011**, 4, p. 785–791.
4. J. Le Notre, E. L. Scott, M. C. R. Franssen and J. P. M. Sanders, *Green Chem.* **2011**, 13, 807-809.
5. K.-C. Liu, P.D. Taylor, US4873336, **1989**.
6. D. Coleman, *J. Chem. Soc. (C)*, **1951**, 2294-2295.
7. J. E. Baldwin, M. North, A. Flinn and M. G. Moloney, *Tetrahedron* **1989**, 45, 1453-1464.
8. E. Pennacchietti, T. M. Lammens, G. Capitani, M. C. R. Franssen, R. A. John, F. Bossa and D. De Biase, *J. Biol. Chem.* **2009**, 284, 31587-31596.
9. J.W. Ochterski, www.gaussian.com/g_whitepap/thermo/thermo.pdf, **2000**, accessed May 2011.
10. DSM, *Personal communication* **2011**.
11. ICIS market intelligence, www.icis.com, **2011**, accessed Jan 2011.
12. BASF, *Personal communication* **2011**.
13. A. P. Altshuller and H. E. Everson, *J. Am. Chem. Soc.* **1953**, 75, 1727-1727.
14. J. Halliday, www.foodnavigator.com, **2008**, accessed Jan 2011.
15. T. Setoyama, T. Ogoshi, JP2001288164A, **2001**.
16. T. Setoyama, T. Ogoshi, JP2001288165A, **2001**.
17. Y. Teng, E. L. Scott, A. N. T. van Zeeland and J. P. M. Sanders, *Green Chem.* **2011**, 13, 624-630.
18. J. Sandeaux, R. Sandeaux, C. Gavach, H. Grib, T. Sadat, D. Belhocine and N. Mameri, *J. Chem. Technol. Biotechnol.* **1998**, 71, 267-273.

19. A.-C. Blaga, A.-I. Galaction and D. Cascaval, *Chem. Biochem. Eng. Q.* **2008**, 22, 439-446.
20. C. J. Orella and D. J. Kirwan, *Biotechnol. Progress* **1989**, 5, 89-91.
21. L. A. Rodrigues da Fonseca Ferreira, *PhD thesis*, University of Porto, **2008**.

Cited in Appendix B:

22. National Insititute of Standards and Technology, *Chemistry webbook,* **2011**.
23. R. C. Weast, *CRC Handbook of Chemistry and Physics*, CRC Press, Inc., Boca Raton, Florida, **1983**.
24. Johnson-Matthey, www.platinum.matthey.com, **2010**, accessed Dec 2010.

Chapter 7

Environmental comparison of biobased chemicals from glutamic acid with their petrochemical equivalents

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Abstract

Glutamic acid is an important constituent of waste streams from biofuels production. It is an interesting starting material for the synthesis of biobased chemicals, thereby decreasing the dependency on fossil fuels. The objective of this chapter was to compare the environmental impact of the potential industrial production of four biobased chemicals from glutamic acid with their petrochemical equivalents, i.e. N-methylpyrrolidone (NMP), N-vinylpyrrolidone (NVP), acrylonitrile (ACN), and succinonitrile (SCN). A consequential life cycle assessment was performed, wherein glutamic acid was obtained from sugarbeet vinasse. The removed glutamic acid was substituted with cane molasses and urea. The comparison between the four biobased and petrochemical products showed that for NMP and NVP the biobased version had less impact on the environment, while for ACN and SCN the petrochemical version had less impact on the environment. For the latter two an optimized scenario was computed, which showed that the process for SCN can be improved to a level at which it can compete with the petrochemical process. For biobased ACN large improvements are required to make it competitive with its petrochemical equivalent.

The results of this LCA and the research preceding it also show that glutamic acid can be a building block for a variety of molecules that are currently produced from petrochemical resources. Currently, most methods to produce biobased products are biotechnological processes based on sugar, but this chapter demonstrates that the use of amino acids from low-value byproducts can certainly be a method as well.

7.1 Introduction

The chemical industry is in transition from petrochemical-based towards biobased production. A practical example is Solvay's facility for large scale production of epichlorohydrin from biobased glycerol, operational since 2007.[1] Most research on biobased chemicals focuses on the use of carbohydrates as feedstock.[2] Proteins in by-products from bioethanol and biodiesel production can be another valuable feedstock. Examples of protein-containing by-products are vinasse (from sugar beet or cane), distiller's grains with solubles (DGS, from wheat or maize), press cakes (from oil seeds like palm, rapeseed and *Jatropha*), and leaves of crop residues. Many of these by-products contain significant amounts of proteins or amino acids.[3] Especially non-essential amino acids, with no significant value for food or feed, can be interesting starting-materials for a variety of nitrogen containing products.[4] The most abundant (non-essential) amino acid after the hydrolysis of many plant proteins is glutamic acid.[5]
In previous chapters we demonstrated the possibility of producing biobased γ-aminobutyric acid,[6] N-vinylpyrrolidone (NVP), N-methylpyrrolidone (NMP),[7] acrylonitrile (ACN),[8] and succinonitrile (SCN)[9] from glutamic acid. We showed the technological and economic feasibility of producing biobased NMP and NVP. Before biobased SCN and ACN can be a future option, however, fundamental research is needed to improve their production processes.[10]

Introducing a new process requires assessment of its environmental impact as well. Life cycle assessment (LCA) is a standardized method for environmental evaluation of a product along its life cycle.[11] So far, LCAs of biobased chemicals are scarce. Hermann *et al.* recently published an LCA comparing a number of biobased chemicals with their petrochemical equivalents.[12] Other LCAs of biobased products report mostly about bioethanol[13-15] or biopolymers and their building blocks (i.e. polyhydroxyalkanoates[16], polylactic acid[17], 1,3-propanediol[18]). This chapter contributes to the still small LCA database of biobased products.
The objective of this research was to compare the environmental impact of four biobased chemicals with their petrochemical equivalents (i.e. NMP, NVP, SCN, ACN). In combination with the techno-economic assessment, this environmental assessment may help chemical industry to consider replacing petrochemical

products with biobased ones. Furthermore it will indicate weak points that either industry or academia need to further investigate in order to improve them.

7.2 Methods

7.2.1 Product system boundaries and general approach

LCA relates the environmental impact of a product to a functional unit that expresses its main function in quantitative terms. The functional unit in this study was 1 kg of produced chemical leaving the factory gate. Such "cradle to factory gate" LCA covers the environmental impact for all processes up to the moment that chemicals leave the factory gate. Biobased and petrochemical products were assumed to be functionally equivalent because they can be used in exactly the same way for production of end-products, such as plastics. Further production of end-products was therefore excluded from the analysis.

Figure 7.1 schematically shows the biobased production system (left) and petrochemical production system (right). A detailed overview of today's petrochemical production systems for these chemicals can be found in appendix C. The cradle for all petrochemical products was oil or natural gas extraction. Their biobased alternatives were produced from glutamic acid that can be obtained from different byproducts of sugar and biofuel production (notably in vinasse from sugar beet processing). The cradle for all biobased chemicals was assumed to be sugarbeet vinasse, because of its high content of glutamic acid compared to other sources.[3]

The parts of the production systems in bold in figure 7.1 were compared in this LCA. This simplified diagram shows the main products from sugar beets, namely sugar, ethanol and vinasse. Vinasse provides the glutamic acid needed for the production of biobased chemicals. Glutamic acid is worth about twice as much as sugar, but sugar beet contains approximately hundred times more sugar than glutamic acid, so the value of the sugar fraction is 50 times higher than the value of the glutamic acid fraction.[10,19,20] We assumed glutamic acid will never drive the processing of sugar beets because of the difference in relative value between the sugar and glutamic acid fractions. The implication of this assumption is that with

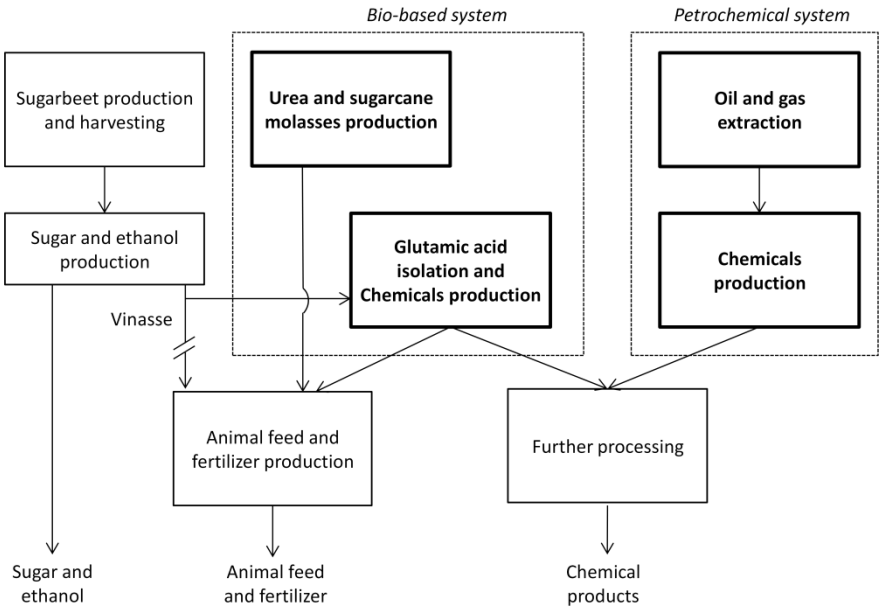

Figure 7.1. Systems for the chemicals production from either glutamic acid or petrochemical resources. This LCA compared the processes in bold within the dotted lines. Glutamic acid was isolated from vinasse. The remaining material was enriched with sugarcane molasses or urea for its regular use in the production of respectively feed or fertilizer (as indicated by the broken arrow).

an estimated worldwide beet vinasse production volume of 2.5 Mton[3] and glutamic acid not being the driver of sugar beet production, complete replacement of petrochemical production of NVP (10-20 kton[4]) and SCN (10 kton[4]) with vinasse-derived glutamic acid would be possible. However replacement of petrochemical NMP (100-150 kton[7]) and ACN (4.5 Mton[21]) from vinasse-derived glutamic acid could in practice be only partial. We excluded the impact related to sugar beet production, because of the assumption that glutamic acid would not drive sugar beet production. However, we included the impact related to producing the substitutes for glutamic acid in vinasse. These substitutes are necessary to fulfill the two main applications of vinasse, namely feed additive and organic fertilizer. Both applications were assumed equally important.[22] For feed application, the glutamic acid removed from beet vinasse was assumed to be replaced by an identical amount of cane molasses. That has a similar influence on the physical

structure of feed, while it has a higher caloric value than beet vinasse.[23] For fertilizer application, glutamic acid in beet vinasse was assumed to be replaced by the same amount of nitrogen in another form, namely urea. Complete LCIs for production of urea and sugarcane molasses were included in the biobased system. In figure 7.1 the schematic representation of these LCIs was simplified to a single box for the purpose of clarity. This system expansion follows ISO 14044 guidelines to avoid allocation for multi-output processes in consequential LCA.[11]

Detail on inventory analysis is provided in section 7.2.2 for production of petrochemical products, and in section 7.2.3 for biobased chemicals. It needs to be emphasized here, however, that the petrochemical processes are currently applied in the chemical industry on a multi-ton scale, whereas the alternative biobased processes are yet only designed on paper and demonstrated in lab scale experiments. This means that many assumptions were made for the biobased processes about, for example, conversion rates and emissions. A main uncertainty in this LCA was anticipated in the inventory data for the production of the glutamic acid from vinasse. For this a sensitivity analysis was performed (see section 7.2.3).

The environmental impact was assessed for each chemical from the inventory data according to the CML 2001 methodology (midpoint approach).[24] Eleven environmental impact categories are considered:[24] impact categories related to the use of resources such as abiotic depletion (kg Sb eq.) and land use (m^2 per year), and impact categories related to the emission of pollutants, i.e. acidification (kg SO_2 eq.), eutrophication (kg PO_4 eq.), climate change (kg CO_2 eq. for 100 years), ozone layer depletion (kg CFC-11 eq.), human toxicity (kg 1,4-dichlorobenzene (1,4-DB) eq.), fresh water aquatic ecotoxicity (kg 1,4-DB eq.), marine aquatic ecotoxicity (kg 1,4-DB eq.), terrestrial ecotoxicity (kg 1,4-DB eq.), and photochemical oxidation (kg C_2H_4 eq.).
Land use is actually not an impact category, but an inventory item. We added it to the impact assessment, however, as a proxy for the biodiversity impact from the use of agricultural land in the production of the biobased chemicals. Biodiversity is an important category when comparing biobased with petrochemical products, but its assessment methodology is still immature.[25] Furthermore, the use of agricultural land for the production of feedstock for chemicals production could lead to

competition with land claims for other purposes, meaning that agricultural land could become a scarce resource in the future.[25]

For each of the four chemicals separately, section 7.3 interprets the impact assessment results for the biobased and petrochemical route. For each chemical, the eleven impact categories were compared, and the main impact contributors identified and discussed for their robustness. We will also discuss the potential for impact reductions in the production of the biobased chemicals.
Modeling and analysis were done with the software package SimaPro 7.

7.2.2 Life cycle inventory for petrochemical products

The eco-invent database contains complete life cycle inventories for NMP and ACN, which were used in this study.[26] Inventory data for the petrochemical production of NVP were obtained from patents of GAF[27] BASF[28] and Ullmann's encyclopedia of chemical technology.[29] Inventory data for the petrochemical production of SCN were obtained from a recent patent of DSM.[30] The commercial processes were assumed to be similar to the ones described in the patents. Appendix C contains a complete general overview of the petrochemical processes, as well as all the inventory data and the modeled processes.

7.2.3 Life cycle inventory for biobased chemicals

Glutamic acid is currently already produced on large scale in the form of monosodium glutamate (MSG), a flavour enhancer. This is done by fermentation of carbohydrates with a nitrogen source. For economically feasible production of chemicals from glutamic acid, however, the production costs for glutamic acid need to be drastically reduced.[2,10] The LCA here is moreover part of a larger study[6,7,9,10] focusing on the valorisation of the large quantities of by-products like vinasse from sugar beet production.
Glutamic acid is present in concentrated vinasse at a high concentration (reported up to 10 % by weight[31]). Its isolation is not yet a commercial process, but amino acids may be isolated by reactive extraction, dialysis, crystallization or similar techniques.[32-34] We assumed energy and material requirements for isolation of

glutamic acid from vinasse as proportional to that for isolation of sugar from sugar beets by crystallization in eco-invent.
We assumed cane molasses and urea to replace glutamic acid in vinasse for upgrading it again for its regular applications as feed and fertilizer. Inventory data for cane molasses were taken from Renouf *et al.,*[35] whereas inventory data for urea were obtained from the eco-invent database.[26]
Biobased production does not yet occur in practice. Technical data required to define biobased production of these chemicals were derived from basic mass and energy balances determined in a previous study.[10] Energy inputs were estimated for the different unit operations (reaction, purification, etc.), to arrive at the cumulative energy use for obtaining the final products. Raw material inputs were computed stoichiometrically on the basis of experimentally obtained yields and on estimated losses of catalysts and solvents. Inventory data for raw materials of biobased processes were taken from the eco-invent database if available. For some raw materials eco-invent data were not available. In this case we modeled the production processes of these raw materials with input and efficiency data from literature. All production processes for raw materials were modeled to a level where eco-invent data for their inputs were available. Modeling details and data sources, either the eco-invent database or additional literature where needed, can be found in the supplementary material.

Many assumptions had to be made about the emissions from biobased production, since data from the industry were not available. Identical electricity requirements for general plant operation were assumed for the biobased and petrochemical production processes. Although the configuration and the raw material inputs of the biobased and the petrochemical production processes are not the same, they are related, and many of the intermediates and reagents are similar. Therefore emissions of organic matter to surface water (BOD, COD, DOC, TOC) were assumed to be the same between biobased and petrochemical production processes. Air emissions of very volatile and/or harmful components used or produced in biobased processes were estimated based on the amounts that were present in the process. For example, ammonium bromide that is used for the production of biobased NMP (and not for petrochemical NMP) can lead to the formation of methyl bromide, a strongly ozone-depleting gas. Small amounts of atmospheric gas

releases to the environment were assumed in addition to other process emission such as methylamine and methanol. Although the exact amounts of the emission were uncertain, the assumptions provide an indication of which components could have an impact on which categories and in which order of magnitude. This enables us to point out potential weaknesses in the processes.
Glutamic acid was assumed to be isolated from vinasse at the site of a sugar-bioethanol production facility in Western Europe. Transportation distances within Western Europe by railway and road transport were assumed to be the same for the biobased and the petrochemical processes.
Appendix C specifies all the inventory data and the modeled processes.

7.2.4 Sensitivity analysis

Sensitivity of the LCA for data and model uncertainty was assessed where relevant. A major uncertainty exists for isolation of glutamic acid from vinasse. The energy requirement for this process was tripled to evaluate the influence of its uncertainty on the overall impact of the biobased chemicals (see 7.3.1). Also the influence of using system expansion versus economic allocation for multi-output processes was evaluated by applying both in modeling the environmental impact of glutamic acid (see 7.3.5). The economic allocation assumed sugar beet cultivation and refinery for glutamic acid production. One ton of fresh sugar beets was taken to yield 1.7 kg and 1.70 € glutamic acid compared to 105 € for seven other marketable products (i.e. sugar, beet pulp, beet tails, chalk, ethanol, yeast, and vinasse)[20,31] This results in economic allocation of 1.6 % of the environmental burden of sugar beet cultivation and refinery to glutamic acid. More detailed information can be found in the supporting information.

7.3 Results and discussion

7.3.1 N-Methylpyrrolidone and glutamic acid

Figure 7.2 shows the relative impacts of biobased and petrochemical NMP. Seven out of eleven categories had a lower environmental impact for biobased NMP than for petrochemical NMP. Exceptions were ozone layer depletion, terrestrial ecotoxicity, photochemical oxidation and land use. The higher contribution from

biobased NMP to these impact categories was mainly due to processes related to glutamic acid, which will be discussed below. Glutamic acid dominated the contribution of biobased NMP to all impact categories, except for ozone layer depletion, ranging from 30 % to 90 % of the total impact.

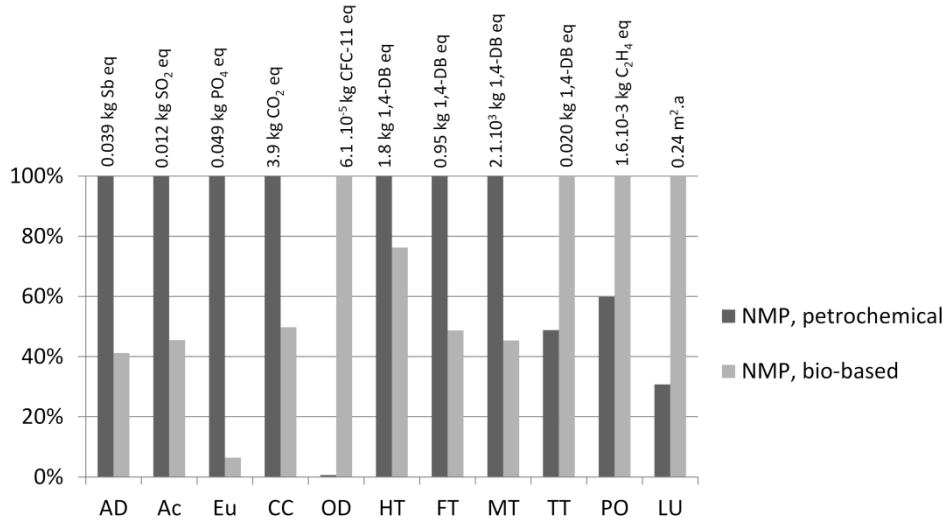

Figure 7.2. Impact of petrochemical and biobased NMP and the impact corresponding to 100 % for each category. AD = abiotic depletion, Ac = acidification, Eu = eutrophication, CC = climate change, OD = ozone layer depletion, HT = human toxicity, FT = fresh water aquatic ecotoxicity, MT = marine aquatic ecotoxicity, TT = terrestrial ecotoxicity, PO = photochemical oxidation, LU = land use.

The impact of biobased NMP on ozone layer depletion was fully due to methyl bromide, formed from the catalyst ammonium bromide, that was assumed to be emitted from the process. The calculated ozone layer depletion per kg biobased NMP was 61 mg CFC-11 eq., while that of petrochemical NMP was 0.37 mg CFC-11 eq., about a factor 160 less. Industry data for biobased NMP were not available, and the roughly estimated methyl bromide emission can deviate with a factor 10 or more. Even with a factor 10 less emissions, however, the contribution of this process to ozone layer will still be considerably more than from petrochemical NMP. It is therefore advisable to search for another catalyst than ammonium

bromide for this process in order to prevent formation of methyl bromide. Other catalysts for this type of reaction were reported.[36]

The contribution to terrestrial ecotoxicity and photochemical oxidation of biobased NMP was mainly due to substitution of glutamic acid with sugarcane molasses in feed applications. Most sugarcane is currently burned prior to harvesting. The resulting carbon monoxide emission was responsible for 85 % of the photochemical oxidation. However, Renouf *et al.* indicate that emissions related to sugarcane harvesting fluctuate greatly with the applied practice.[37] Manufacture and use of machineries, fertilizer and agrochemicals leads to emissions of heavy metals such as arsenic to the soil, which is for 87 % responsible for terrestrial ecotoxicity.[37] Consequential LCA does not include manufacture of already existing machineries, but by using an attributional LCI for sugarcane molasses these items also entered this LCA. The contribution to terrestrial ecotoxicity and photochemical oxidation contains altogether a high uncertainty and may change dramatically with another replacement than cane molasses for feed application (e.g. beet molasses instead of cane molasses leads to an almost tenfold decrease in terrestrial ecotoxicity and a fivefold decrease in photochemical oxidation).

Land use for production of biobased NMP was for 90 % required for sugar cane cultivation that provided molasses replacing glutamic acid in vinasse for feed application. Land use for biobased chemicals is expected to be almost always higher than that for petrochemical products. Biobased NMP produced from glutamic acid from byproducts is likely to use less land, however, than biobased NMP produced from sugar by fermentation. Biobased NMP production from vinasse-derived glutamic acid requires 0.2 m^2/kg, whereas more than 3 m^2/kg is required for fermentative production of caprolactam (a lactam, like NMP and NVP) from cane sugar.[12]

Process energy (heat and electricity combined) for isolation of glutamic acid from vinasses was another large contributor to all impact categories for biobased NMP. The large uncertainties in the process requirements for this process raised the question about its influence on the LCA result. Tripling the energy requirements for the isolation of glutamic acid from vinasse increased the impact of biobased

NMP with more than 15 % in abiotic depletion (from 0.016 to 0.019 kg Sb eq.) and global warming potential (from 2.0 to 2.4 kg CO_2 eq.). However, the impact of biobased NMP in these categories was still clearly less than that of petrochemical NMP. Only a tenfold increase of the energy requirement for the isolation of glutamic acid from vinasse to above 20 MJ/kg, which is unlikely, brought the global warming potential of biobased NMP above that of petrochemical NMP. The other categories then still had better scores for biobased NMP than for its petrochemical equivalent.

The assumptions about the process emissions from the biobased processes did not have a large influence on the overall impact as in all categories their accumulated emissions contributed to less than 10 % of the overall impact. The assumption that the emissions of the biobased and the petrochemical processes are similar therefore did not significantly influence the outcome of the impact assessment. The only exception to this was the emission of methyl bromide and its influence on the ozone layer, which was discussed above.

Despite some major uncertainties in the inventory for biobased NMP, its environmental impact was convincingly less than that of petrochemical NMP in seven of the eleven assessed categories. The results for these categories were hardly sensitive to changes in inventory items with a high uncertainty. The impacts on terrestrial ecotoxicity and photochemical oxidation of biobased NMP were higher than those of petrochemical NMP. These results represent a worst case. They are not reliable, however, as current practises of sugarcane production are known to vary.[37] Improving global practises of sugarcane production would lead to a lower impact of biobased NMP. An alternative catalyst in the production of biobased NMP can greatly reduce ozone layer depletion.

7.3.2 N-Vinylpyrrolidone

The relative impacts of biobased and petrochemical NVP are shown in figure 7.3. Biobased NVP had a lower impact than petrochemical NVP, except for terrestrial ecotoxicity, photochemical oxidation and land use. Differences between biobased and petrochemical NVP were largely similar to those for NMP, except for ozone

layer depletion and eutrophication. In contrast to biobased NMP, biobased NVP used no bromides and no ozone-depleting gases were formed. The absolute contribution of biobased NVP and NMP to eutrophication was similar, but petrochemical NVP contributed almost ten times less than petrochemical NMP. Petrochemical NMP required production of dimethylamine, which emissions were largely responsible for the higher impact on eutrophication. The higher impacts on terrestrial ecotoxicity, photochemical oxidation and land use were all due to sugarcane production and harvesting (that contain for NVP the same uncertainties as for NMP).

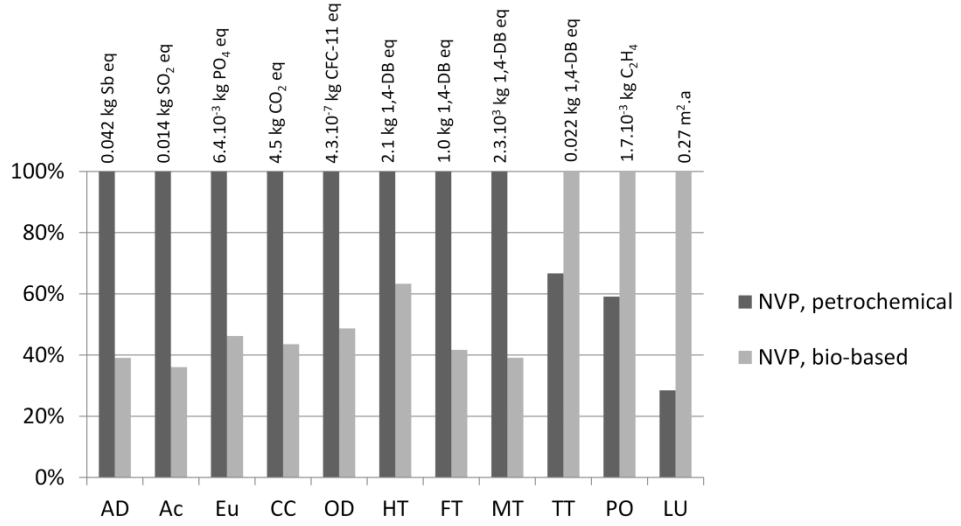

Figure 7.3. Impact of petrochemical and biobased NVP and the impact corresponding to 100 % for each category. AD = abiotic depletion, Ac = acidification, Eu = eutrophication, CC = climate change, OD = ozone layer depletion, HT = human toxicity, FT = fresh water aquatic ecotoxicity, MT = marine aquatic ecotoxicity, TT = terrestrial ecotoxicity, PO = photochemical oxidation, LU = land use.

The main inventory items responsible for the total impact of the production of biobased NVP were processes related to glutamic acid (45 – 60 %) and the energy use (20 – 50 %), like in the NMP process. Therefore the results of the sensitivity analysis for NMP were applicable to NVP as well. The result of the impact assessment of the biobased NVP process can be considered as robust, except for the impact on terrestrial ecotoxicity and photochemical oxidation. It can be

concluded that biobased NVP has convincingly less environmental impact than petrochemical NVP in most of the assessed categories.

7.3.3 Acrylonitrile

The comparison of biobased and petrochemical ACN gave a different result than NMP and NVP. Figure 7.4 shows that the environmental impact of petrochemical ACN was considerably lower than that of biobased ACN in all impact categories.

In contrast with NMP and NVP, processes related to glutamic acid did not have the largest impact in biobased ACN production (although it contributed significantly). The three main contributions came from sodium hypochlorite (on average 25 %), acetic anhydride (19 %) and the use of electricity (22 %). The high electricity requirement was mainly related to the use of sodium hypochlorite. The reaction of glutamic acid with sodium hypochlorite is very exothermic and needs to be cooled below room temperature. This requires electricity rather than standard cooling water from e.g. a river. The use of palladium chloride as a catalyst had a significant impact on acidification, which could be traced back to the extraction of palladium from ores. The comparison of biobased and petrochemical ACN was not sensitive to model assumptions made, because differences in impact between both were so large.

Substantial improvement in the chemistry of the biobased process may make it competing with petrochemical ACN. First, the oxidation reaction with sodium hypochlorite may be improved. It currently requires 3.5 kg sodium hypochlorite per kg ACN, and a lot of electricity for cooling the reactor. A reaction with oxygen (i.e. air) as an oxidant and at an elevated temperature would greatly reduce the impact of the biobased process. Heat produced by this improved reaction may also substitute the production of heat for another part of the process, for example distillation. Second, the use of acetic anhydride may be avoided. The intermediate product of the process may be able to react with itself instead of with acetic anhydride to form another intermediate that could be converted to ACN.[10] However, this still has to be demonstrated even at lab scale.

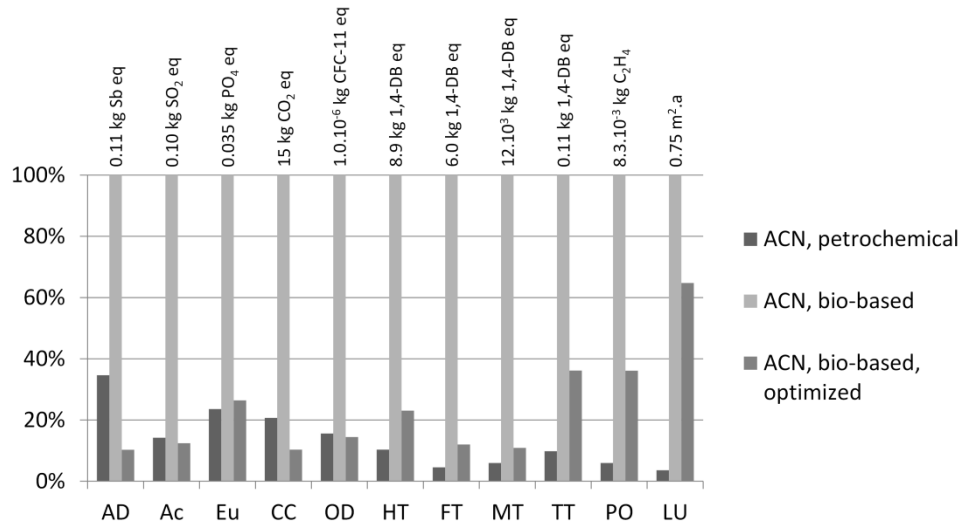

Figure 7.4. Impact of petrochemical and biobased ACN and the impact corresponding to 100 % for each category. AD = abiotic depletion, Ac = acidification, Eu = eutrophication, CC = climate change, OD = ozone layer depletion, HT = human toxicity, FT = fresh water aquatic ecotoxicity, MT = marine aquatic ecotoxicity, TT = terrestrial ecotoxicity, PO = photochemical oxidation, LU = land use.

Based on these improvements, a strongly improved biobased ACN case was compared with petrochemical ACN. The inventory for the improved biobased process did not contain sodium hypochlorite, reduced electricity requirement from 6.3 to 0.2 kWh, produced instead of required heat (8.9 MJ or 50 % of the produced reactor-heat), used no more acetic anhydride and ethyl acetate, decreased palladium chloride requirement by a tenfold (by more efficient recycling), and treated waste water and exhaust gases in order to mitigate emissions to surface water and air. Figure 7.4 shows that a strongly improved biobased ACN could compete with petrochemical ACN, especially in terms of global warming potential and abiotic depletion. However, the uncertainties in this scenario were large. The main impact contributor of the best case scenario was glutamic acid, which indicated not much more potential for process improvement.

Summarizing, production of biobased acrylonitrile from glutamic acid was unable to compete with petrochemical acrylonitrile under current process configurations.

However, considerable improvements in the process could make it environmentally performing similar to petrochemical acrylonitrile. It is advisable to further investigate the reaction steps with sodium hypochlorite and acetic anhydride, as most improvements in the process can be achieved with those steps.
Another biobased route to acrylonitrile has been published recently,[38] so it would also be interesting to determine the environmental impact of that route, and compare the results with this study.

7.3.4 Succinonitrile

Results of the impact assessment for SCN are depicted in figure 7.5. The environmental impact of petrochemical SCN was lower than that of biobased SCN for all assessed categories (See figure 7.5). The differences for SCN were smaller than for ACN, but the major contributions to the impact of biobased SCN similarly come from glutamic acid (on average 23 %), sodium hypochlorite (26 %) and the use of electricity (27 %). The use of sodium hypochlorite and electricity were also here interrelated. Therefore a best case scenario was designed like for the acrylonitrile process, in which no sodium hypochlorite was used, the heat produced in the reactor was used elsewhere, use of ethyl acetate was avoided, palladium chloride recycling efficiency was increased tenfold, and wastewater and gas exhaust treatment were installed to avoid emissions.
The best case resulted in a lower impact than the initial biobased scenario (figure 7.5). The impacts on abiotic depletion, acidification and global warming potential of the optimized biobased production were less than 50 % of those of the petrochemical production. Terrestrial ecotoxicity, photochemical oxidation potential and land use, however, were still higher for biobased SCN than for petrochemical SCN. This again related to the production and harvest of sugarcane.
The two items with a major contribution to the impact of biobased SCN in the best case scenario were glutamic acid and ammonia. Tripling the energy requirement for the isolation of glutamic acid from the vinasse (the main uncertainty) increased the impact of biobased SCN by a maximum of 30 %, but did change the conclusions. The results were not sensitive to the assumptions made for the isolation of glutamic acid from vinasse.

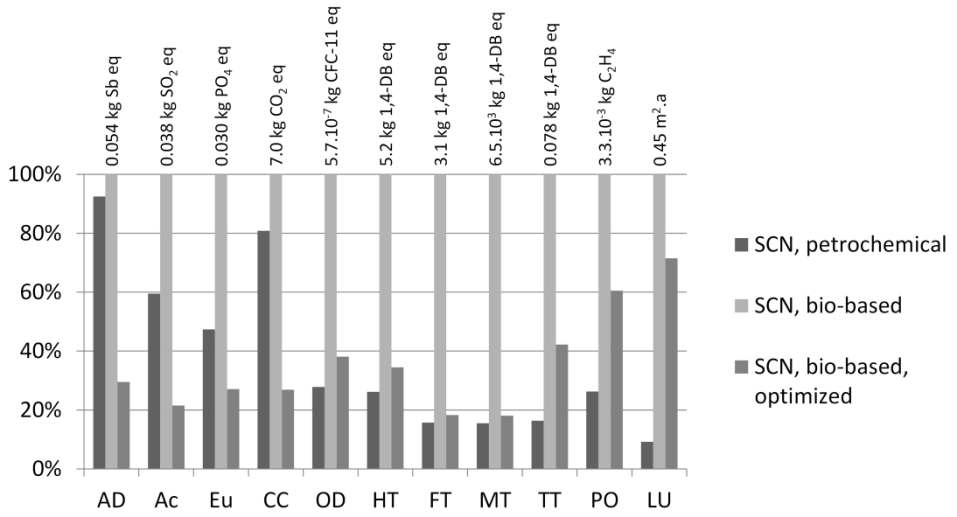

Figure 7.5. Impact of petrochemical and biobased SCN and the impact corresponding to 100 % for each category. AD = abiotic depletion, Ac = acidification, Eu = eutrophication, CC = climate change, OD = ozone layer depletion, HT = human toxicity, FT = fresh water aquatic ecotoxicity, MT = marine aquatic ecotoxicity, TT = terrestrial ecotoxicity, PO = photochemical oxidation, LU = land use.

In summary biobased SCN production with the current process configuration would have a higher environmental impact than petrochemical SCN, but with an optimized process configuration that could change. Therefore it would be advisable to further investigate the reaction with sodium hypochlorite, because that step showed to be the turning point from a worse process to a better process, when comparing the impacts of biobased with petrochemical SCN.

7.3.5 System expansion vs. economic allocation

For each of the four chemicals above, the environmental burden of glutamic acid was also calculated using economic allocation (i.e. attributional LCA) instead of system expansion (i.e. consequential LCA). The environmental burden of the multi-output sugar beet refinery process was allocated for 1.6 % to glutamic acid (see supporting info for details).

The impact of glutamic acid itself was significantly different when the attributional and consequential analyses were compared. Seven of eleven categories changed by

a factor 2 or higher. However, in the LCAs of the four chemicals these changes did not lead to changes in the overall outcome of most impact categories, except for terrestrial ecotoxicity, photochemical oxidation and land use. The first two were already discussed above as being a worst case in the consequential analysis, due to the variety in sugar cane production processes. Using economic allocation decreased these impacts for biobased NMP and NVP to a value even below the impacts by petrochemical production. Economic allocation increased land use by a factor 12, to 2.6, 2.9, 5.6 and 3.5 m^2 per year per kilo of NMP, NVP, ACN and SCN, respectively. This is in the same order as biobased chemicals produced by fermentation.[12]

The comparison of the attributional and consequential approach shows that, despite significant changes in impact assessment results, there is no significant change in overall conclusions on the comparison of bio- and the petrochemical products, except for land use.

7.4 Conclusions

The results of the LCA comparison between the four biobased and petrochemical products showed generally less environmental impact for the biobased version of NMP and NVP, while for acrylonitrile and succinonitrile the petrochemical version has less impact on the environment.

Most results were shown to be robust, except for the impacts on terrestrial ecotoxicity and photochemical oxidation due to varying practices around sugarcane production. Also system expansion versus economic allocation showed, despite significant results change for some impact categories, that overall conclusions remained the same.

For the production of biobased NMP, ACN and SCN, the LCA clearly pointed to parts of the processes for which the environmental performance can be improved. An alternative catalyst in the production of biobased NMP can greatly reduce ozone layer depletion. In the production of biobased ACN and SCN, the use of sodium hypochlorite as an oxidant should be avoided by finding an alternative. Using oxygen instead of sodium hypochlorite would make biobased SCN environmentally better than petrochemical SCN. In the process of biobased ACN

more changes are necessary to make the environmental impact comparable to petrochemical ACN. Avoidance of the use of acetic anhydride should be the next main objective for the improvement of this process. When these improvements would be achieved, biobased acrylonitrile could be and biobased SCN would be more environmentally friendly than their petrochemical equivalents.
The results of this LCA and the research preceding it also show that glutamic acid can be a building block for a variety of molecules that are currently produced from petrochemical resources. Currently, most methods to produce biobased products are biotechnological processes based on sugar, but the use of amino acids from low-value byproducts can certainly be a method as well, as was demonstrated in this chapter.

Acknowledgement

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7.5 References

1. Solvay, www.solvaychlorinatedorganics.com. May **2011**.
2. Bozell, J. J.; Petersen, G. R., Technology development for the production of biobased products from biorefinery carbohydrates-the US Department of Energy's "Top 10" revisited. *Green Chemistry* **2010,** *12*, (4), 539-554.
3. Lammens, T. M.; Franssen, M. C. R.; Scott, E. L.; Sanders, J. P. M., Availability of protein-derived amino acids as feedstock for the production of biobased chemicals. *submitted to Biomass and Bioenergy* **2011**.
4. Scott, E.; Peter, F.; Sanders, J., Biomass in the manufacture of industrial products - the use of proteins and amino acids. *Applied Microbiology and Biotechnology* **2007,** *75*, (4), 751-762.
5. Deike, V.; Mol, J. D., *Raw Material Compendium*. 2 ed.; Novus International Inc. : Brussels **1996**.

6. Lammens, T. M.; De Biase, D.; Franssen, M. C. R.; Scott, E. L.; Sanders, J. P. M., Application of glutamic acid decarboxylase for the valorization of glutamic acid. *Green Chemistry* **2009,** *11*, (10), 1562-1567.
7. Lammens, T. M.; Franssen, M. C. R.; Scott, E. L.; Sanders, J. P. M., Synthesis of biobased N-methylpyrrolidone by one-pot cyclization and methylation of γ-aminobutyric acid. *Green Chemistry* **2010,** *12*, (8), 1430-1436.
8. Le Notre, J.; Scott, E. L.; Franssen, M. C. R.; Sanders, J. P. M., Biobased synthesis of acrylonitrile from glutamic acid. *Green Chemistry* **2011,** *13*, 807-809.
9. Lammens, T. M.; J. Le Nôtre; M.C.R. Franssen; E.L. Scott; Sanders, J. P. M., Synthesis of biobased succinonitrile from glutamic acid and glutamine. *ChemSusChem* **2011,** *4,* 785-791.
10. Lammens, T. M.; Gangarapu, S.; Franssen, M. C. R.; Scott, E. L.; Sanders, J. P. M., Techno-economic assessment of the production of biobased chemicals from glutamic acid. *submitted to Biofuels, Bioproducts and Biorefineries* **2011**.
11. International Organization for Standardization, *Environmental management, Life cycle assessment, Requirements and guidelines. ISO 14044:2006*; Geneva, **2006**.
12. Hermann, B. G.; Blok, K.; Patel, M. K., Producing Biobased Bulk Chemicals Using Industrial Biotechnology Saves Energy and Combats Climate Change. *Environmental Science & Technology* **2007,** *41*, (22), 7915-7921.
13. Blottnitz, H. v.; Curran, M. A., A review of assessments conducted on bio-ethanol as a transportation fuel from a net energy, greenhouse gas, and environmental life cycle perspective. *Journal of Cleaner Production* **2007,** *15*, (7), 607-619.
14. Kim, S.; Dale, B., Life Cycle Assessment Study of Biopolymers (Polyhydroxyalkanoates) - Derived from No-Tilled Corn. *The International Journal of Life Cycle Assessment* **2005,** *10*, (3), 200-210.
15. Kim, S.; Dale, B. E., Life cycle assessment of fuel ethanol derived from corn grain via dry milling. *Bioresource Technology* **2008,** *99*, (12), 5250-5260.
16. Akiyama, M.; Tsuge, T.; Doi, Y., Environmental life cycle comparison of polyhydroxyalkanoates produced from renewable carbon resources by bacterial fermentation. *Polymer Degradation and Stability* **2002,** *80*, 183-194.
17. Gironi, F.; Piemonte, V., Life cycle assessment of polylactic acid and polyethylene terephthalate bottles for drinking water. *Environmental Progress in Sustainable Energy* **2010**, 1-20.
18. Urban, R. A.; Bakshi, B. R., 1,3-Propanediol from Fossils versus Biomass: A Life Cycle Evaluation of Emissions and Ecological Resources. *Industrial & Engineering Chemistry Research* **2009,** *48*, (17), 8068-8082.

19. Sugar Engineers, Daily sugar prices. http://sugartech.co.za/sugarprice/index.php. May **2011**.
20. Swaaij, A. C. P. M. v.; Maassen, J. *IRS Bietenstatistiek 2004 (Beet statistics)*; IRS Foundation: Bergen op Zoom, The Netherlands, **2005**.
21. The Association of Petrochemicals Producers in Europe, Acrylonitrile. www.petrochemistry.net/acrylonitrile.html, Jan **2011**.
22. Lewicki, W., An introduction to vinasse from beet and cane molasses fermentation. *International sugar journal* **2001,** *103*, (1227), 126-128.
23. Duinkerken, G. v., Personal communication. In **2011**.
24. Guinée, J. B.; Gorrée, M.; Heijungs, R.; Huppes, G.; Kleijn, R.; Koning, A. d.; Oers, L. v.; Wegener Sleeswijk, A.; Suh, S.; Udo de Haes, H. A.; Bruijn, H. d.; Duin, R. v.; Huijbregts, M. A. J., *Handbook on life cycle assessment. Operational guide to the ISO standards.* Kluwer Academic Publishers: Dordrecht, **2002**; p 692.
25. Dornburg, V.; Lewandowski, I.; Patel, M., Comparing the Land Requirements, Energy Savings, and Greenhouse Gas Emissions Reduction of Biobased Polymers and Bioenergy. *Journal of Industrial Ecology* **2003,** *7*, (3-4), 93-116.
26. Ecoinvent, Swiss Centre for Life Cycle Inventories. In **2010**.
27. Liu, K.-C.; Taylor, P. D. Process for the preparation of N-vinyl lactams. US4873336, **1989**.
28. Rudloff, M.; Stops, P.; Henkes, E.; Schmidtke, H.; Fischer, R.-H.; Julius, M.; Lebkücher, R.; Ross, K.-H. Method for the production of 2-pyrrolidone. US7164031B2, **2007**.
29. Harreus, A. L., 2-Pyrrolidone. In *Ullmann's Encyclopedia of Industrial Chemistry*, Wiley InterScience: **2000**.
30. Oevering, H.; Vandenbooren, F. H. A. M.; Poorter, O. Process for preparing succinonitrile and use of succinonitrile. US7371884B2, **2008**.
31. Huijbregts, A. W. M. Vinasse research. In *Royal Nedalco year report* **1990**; pp 64-65.
32. Blaga, A.-C.; Galaction, A.-I.; Cascaval., D., Separation of Amino Acids from Their Mixture by Facilitated Pertractionwith D2EHPA. *Chemical and Biochemical Engineering Quarterly* **2008,** *22*, (4), 439-446.
33. Rodrigues da Fonseca Ferreira, L. A., Separation and purification of amino acids - PhD thesis. University of Porto, Porto, Portugal, **2008**.
34. Teng, Y.; Scott, E. L.; van Zeeland, A. N. T.; Sanders, J. P. M., The use of l-lysine decarboxylase as a means to separate amino acids by electrodialysis. *Green Chemistry* **2011,** *13*, (3), 624-630.

35. Renouf, M.; Pagan, R.; Wegener, M., Life cycle assessment of Australian sugarcane products with a focus on cane processing. *The International Journal of Life Cycle Assessment* **2011,** *16*, (2), 125-137.
36. Setoyama, T.; Ogoshi, T. Method for producing N-substituted amide compound. JP2001288164A, **2001**.
37. Renouf, M.; Wegener, M.; Pagan, R., Life cycle assessment of Australian sugarcane production with a focus on sugarcane growing. *The International Journal of Life Cycle Assessment* **2010,** *15*, (9), 927-937.
38. Calvino-Casilda, V.; Guerrero-Pérez, M. O.; Bañares, M. A., Microwave-activated direct synthesis of acrylonitrile from glycerol under mild conditions: Effect of niobium as dopant of the V-Sb oxide catalytic system. *Applied Catalysis B: Environmental* **2010,** *95*, (3-4), 192-196.

Chapter 8

Discussion and Conclusions

8.1 Research objectives

Being part of the quest for the replacement of petrochemical products with biobased ones, the goal of this thesis was to find new routes for the production of biobased chemicals from glutamic acid. In the introduction of this thesis, four main research questions were articulated:

1. Where can glutamic acid be obtained from?
2. How can the target chemicals (N-methylpyrrolidone, N-vinylpyrrolidone and 1,4-diaminobutane) be made from glutamic acid?
3. Will it be technologically and economically feasible to implement these new routes?
4. How would the biobased chemicals perform environmentally, compared to their petrochemical equivalents?

The purpose of this chapter is to provide an overview of the results that were reported in chapters 2 to 7, and to integrate that overview with a discussion of these results. In paragraph 8.2.2 the results of the techno-economic assessment and the life cycle assessment (LCA) are integrated with the experimental results, in order to put them into a practical perspective. From this conclusions can be drawn and recommendations made.

8.2 Thesis overview and discussion

8.2.1 Glutamic acid: how to obtain it?

In chapter 2, a range of different sources that could be used to obtain glutamic acid were discussed. Besides perennial crops like grass and alfalfa, the byproducts from bioethanol and biodiesel production were identified as potentially available sources. For example the byproduct from bioethanol production from maize or wheat, dried distiller's grains with solubles (DDGS), contains about ten percent glutamic acid. The potential availability of glutamic acid from DDGS was estimated at about 2 Mton (see table 2.1). This could grow in the future, with an increasing bioethanol production. Vinasse from bioethanol production from

sugarcane and sugarbeet was also identified as a potential source for glutamic acid. The amounts of glutamic acid in sugarcane vinasse are relatively low, however sugarbeet vinasse contains high amounts of glutamic acid, and could be a source for 1 Mton (see table 2.1) of glutamic acid with the current production volumes. Byproducts from natural oil or biodiesel production, i.e. the press cakes of palm kernels, soybean seeds, rapeseeds, sunflower seeds and *Jatropha* seeds, can also yield glutamic acid. Especially soybean showed to have a high potential for obtaining glutamic acid (potentially 15 Mton, table 2.1), because the seeds contain high amounts of protein that could yield substantial amounts of glutamic acid after hydrolysis.

The example of soybean triggers the question about the *real* availability of amino acids for the production of chemicals. The real availability of glutamic acid depends on a multitude of factors. First of all, it depends on the technology that is available for the isolation of glutamic acid or other amino acids from these kinds of byproducts. Paragraph 2.6 described that there are methods available for extracting proteins from these byproducts. The isolation of amino acids from these proteins, however, has received little attention yet. Currently there is no technology available to cost-effectively isolate amino acids from these kinds of waste streams, although technologies like electrodialysis,[1] reactive extraction,[2] and crystallization[3] are all under investigation. This is related to the second factor of importance, the current global market. There is an existing market for products like oil seed meals and DDGS, being mainly the animal feed industry. Furthermore, most of the individual amino acids are currently produced by fermentation (e.g. lysine, glutamic acid) or chemical synthesis (e.g. methionine).[4] Amino acid production can be done against a price that the market is willing to pay, because amino acids are generally considered as high value additives for food or feed. Therefore there have not been incentives to radically change the production systems for amino acids, by going from fermentation to large scale isolation from low-value byproducts. Moreover, it is uncertain whether the isolation of amino acids from the above-mentioned sources would be able to supply the complete market demand. For example the current market demand for food-grade monosodium glutamate (MSG, the sodium salt of glutamic acid) is over 1.5 Mton.[5] Although there is a fierce competition in the world of MSG producers, which can lead to a decrease in price,[6]

creating an extra demand for glutamic acid such as the production of chemicals could potentially lead to an increase in the price of MSG.
However, there are arguments against this line of reasoning. One is the purity of the amino acids. When glutamic acid would be used for the production of chemicals, it does not need to be as pure as for food applications. Therefore the investments in downstream processing would be less, making industry-grade glutamic acid less expensive than food-grade glutamic acid. An argument against the notion that the demand for MSG limits the production of chemicals from glutamic acid, is the fact that the supply of low-value byproducts such as DDGS has been increasing rapidly over the last decade, and is expected to increase further with an increasing production of biofuels. The reason that there is no technology available to isolate amino acids from these byproducts could simply be due to the fact that these streams have not yet existed on a large scale for a long time, so not many people have recognized it as an opportunity yet. Interestingly, Petersen *et al.* included glutamic acid in 2004 in their "US Department of Energy (DoE) top chemical opportunities from carbohydrates" list. That is, the fermentative production of glutamic acid.[7] In 2010 the DoE published a revision of this list where glutamic acid had been removed, because "glutamic acid has remained a terminal product of the chemical industry and research activity in either glutamic acid production or its use as a platform was minimal".[8] In other words, besides the use of glutamic acid as a platform chemical, to their opinion research to improve the fermentative production of glutamic acid would be needed in order to make glutamic acid an opportunity again. This shows that the isolation of glutamic acid from inexpensive agricultural byproducts is generally not considered as an opportunity, but a further increase in the amounts of these byproducts could make it one.

The third factor that influences the *real* availability of amino acids from byproducts is a more societal one, because it is related to the "food *versus* fuel" debate. Proteins are an essential part of the diets of both humans and animals. Therefore the use of soybean meal for the production of chemicals instead of as animal feed appears to go right against the common notion that we should not use precious food or feed ingredients to create fuels or chemicals. However, as was explained in paragraph 2.1, glutamic acid is a non-essential amino acid, and removing it from

the diet of e.g. pigs would actually increase the quality of their diet and would decrease emissions of ammonia and nitrogen oxides, creating a win-win situation. Going against a common notion means that implementing technologies to isolate amino acids from what is currently considered as high quality animal feed, will encounter public objections. These objections could influence the possibility of implementing technology to use a part of what is now considered as food or feed for the production of chemicals. In other words, amino acids would not be available as a feedstock for chemicals production because the general public does not want it. To be backed by public opinion is very important when one wants to implement a new technology, as was demonstrated recently in the Netherlands with CO_2 capture and storage in empty gas fields underneath Barendrecht.[9] Therefore it would be advisable to first demonstrate amino acid isolation technology that does not influence the nutritious value of food or feed, before starting to produce chemicals from amino acids such as glutamic acid.

8.2.2 How to produce chemicals from glutamic acid?

Glutamic acid to γ-aminobutyric acid

The first step on the pathway from glutamic acid to pyrrolidones, the α-decarboxylation of glutamic acid to γ-aminobutyric acid (GABA) and CO_2, was described in chapter 3. The enzyme glutamic acid α-decarboxylase (GAD) from *E. coli* was the catalyst of choice for this reaction. GAD is a pyridoxal 5'-phosphate (PLP)-dependent enzyme that is distributed widely among living organisms and has been studied extensively in the past.[10] Native GAD from *E. coli* has the highest specific activity at a pH below 5, being about 200 U/mg at pH 4.6 and 40 ^{o}C, when purified.[11] The purified enzyme was immobilized by entrapment in calcium alginate and by covalent binding to Eupergit, which led to an increased stability compared to the native enzyme. Despite of the increased stability the enzyme still deactivated at a significant rate. In order to determine whether an industrial process would be feasible, the inactivation rate was mathematically modeled and extrapolated from the experimental data. In this way a reactor productivity could be

estimated, which led to the conclusion that this process would be economically feasible in an industrial setting.
It was further found that the deactivation of the cofactor PLP by a side reaction that forms pyridoxamine 5'-phosphate (PMP) could be avoided by addition of α-ketoglutaric acid. That was then transaminated by GAD to form glutamic acid, and at the same time PMP reacts back to PLP.

The most efficient way to perform this reaction was in a fed batch setup, where a solution of glutamic acid was added to keep the pH constant. In this way GABA could be accumulated in the solution. In theory the reaction can proceed until GABA is present at such a concentration that it starts to precipitate, because product inhibition does not take place. However, glutamic acid is a weak acid and the solubility of GABA at a pH below 5 is much higher than that of glutamic acid. Therefore addition of a solution of glutamic acid is not sufficient to keep the pH below 5 above a certain concentration of GABA, at which point inactivation of the enzyme occurs. To avoid this, a strong acid such as hydrochloric acid can be added together with glutamic acid to maintain a constant pH above a certain conversion. However, this may be a disadvantage at industrial scale because hydrochloric acid is corrosive and therefore higher capital investment would be required.
A way to avoid the need for a strong acid to keep the pH below 5 would be by engineering an enzyme that is active at a pH higher than 5, so that it remains active until GABA starts to precipitate at around pH 6. An additional advantage would then be that less water would have to be evaporated for the isolation of GABA therefore potentially reducing energy requirements. Calculations in chapter 6 showed that half of the energy requirement in the production process of pyrrolidones from glutamic acid would be the evaporation of water to isolate GABA. Avoiding that would mean large energy savings, which would be good for both the economy and the environmental performance of the process.
Two mutants of GAD, both active above pH 5,[12] were immobilized and tested at pHs of 5.7 and 6. Indeed the immobilized mutants were also able to convert glutamic acid to GABA at these pHs, but they were found to be less stable than the wild type enzyme under storage and turnover conditions.[13] Further research to stabilize the mutants is necessary to be able to perform the enzymatic α-decarboxylation of glutamic acid at an increased pH.

γ-Aminobutyric acid to pyrrolidones

GABA can be cyclized easily, thereby forming 2-pyrrolidone.[14] Chapter 4 described the N-methylation of 2-pyrrolidone with methanol and dimethyl carbonate (DMC) to form N-methylpyrrolidone (NMP), and the one-pot synthesis of NMP from GABA and methanol.

Methylation of 2-pyrrolidone with methanol was catalyzed by the addition of a chloride, bromide or iodide salt to the reaction mixture and heating it to 250 ^{o}C in an autoclave. Ammonium iodide was found to be more active than ammonium bromide, which was found to be more active than ammonium chloride. Therefore a reaction sequence was proposed that proceeds through an intermediate halomethane that is formed by an S_N2 substitution of the hydroxyl group of methanol with a halide ion. Other bromide salts such as cetyltrimethylammonium bromide and methyltriphenylphosphonium bromide were also able to catalyze this reaction. A further study of the reaction conditions with ammonium bromide as catalyst showed that the reaction only proceeds readily above a temperature of 225 ^{o}C (Figure 4.2). The highest yield (77 mol-%) was obtained after nine hours at 250 ^{o}C, with a 2-pyrrolidone conversion of 88 ± 6 mol-% and a selectivity for NMP of 87 ± 4 mol-%.

Methylation of 2-pyrrolidone could also be achieved with DMC. The selectivity then, however, was lower than with the halide-catalyzed methylation with methanol. The highest NMP selectivity that was found (67 mol-%) was achieved with a NaY zeolite as the catalyst Table 4.3). Other zeolites, with a higher degree of acidity for example, may improve the selectivity of the methylation reaction with DMC. This could be the subject of further research.

The cyclization of GABA to 2-pyrrolidone and the subsequent methylation with methanol was also carried out as a one-pot procedure. The conversion of GABA was almost quantitative after five hours at 250 ^{o}C, and the subsequent conversion of 2-pyrrolidone to NMP was 42 mol-%, with a selectivity for NMP of 92 mol-%.

Two important drawbacks of the use of bromides to catalyze this reaction were addressed in both chapters 6 and 7. The first is the corrosive properties of methyl bromide or methyl iodide under the applied conditions. This would require the use of expensive steel alloys for the manufacture of the reactor for this process, leading to high capital investments. Another drawback was found in LCA. This is the

potential impact on ozone layer depletion by methyl bromide emissions. The process has not been applied commercially yet and therefore it was difficult to estimate these emissions. It may be possible to completely contain all the gases in the process, but if a purge of gases would be required this would have an impact. The best solution to both these drawbacks would be to search for another catalyst for the methylation with methanol. There are reports describing a gas phase methylation reaction with a heterogeneous catalyst at 400 ^{o}C.[15] Although these are quite harsh conditions, it might be better for both the economics and the environmental performance of the process. This arises due to the exothermic nature of both the cyclization and the methylation reaction (see appendix B). Therefore operation at 400 ^{o}C could be done with heat integration to produce high pressure steam, which would be an additional benefit because it could be used elsewhere in the process. In this case however, the cyclization and the methylation reaction cannot be done in a one-pot reaction. Therefore the installation of two reactors (operated under different conditions) would be required, which would also lead to higher capital investments.

N-vinylpyrrolidone (NVP) is produced commercially from 2-pyrrolidone, amongst others by BASF. Therefore NVP production from 2-pyrrolidone was not further investigated in this thesis. Although NVP that is produced from biobased 2-pyrrolidone and acetylene would not be 100 % biobased, reports exist that show the possibility to produce NVP by catalytic dehydration of N-hydroxyethylpyrrolidone (NHP).[16-19] When NHP could be made from pyrrolidone and ethylene glycol (the catalytic alkylation with ammonium bromide described in chapter 4 was not tested for ethylene glycol), there would be a route towards completely biobased NVP, because ethylene glycol can be obtained from glycerol, a byproduct of biodiesel production from natural oils such as palm and rapeseed oil.[20] This would be an interesting topic for further research.

Glutamic acid to nitriles

Chapter 5 contains the complete route from glutamic acid to succinonitrile, the commercial precursor of 1,4-diaminobutane. The main intermediate in this route was 3-cyanopropanoic amide (CPAm), which could be dehydrated to form succinonitrile. The cyano group in CPAm was obtained by an oxidative

decarboxylation reaction using sodium hypochlorite, in the presence of a catalytic amount of sodium bromide. The amide group could not be formed directly from the carboxylic acid group of glutamic acid. Therefore glutamic acid first needed to be mono-esterified, after which the amide could be obtained readily by reacting the methyl ester of glutamic acid with aqueous ammonia. 3-Cyanopropanoic acid (obtained by oxidative decarboxylation of glutamic acid) is much more soluble in water than its methyl ester. This means that the use of glutamic acid 5-methyl ester (Glu-Me) instead of glutamic acid in the oxidative decarboxylation reaction provided the advantage of an easy extraction from the aqueous reaction mixture. The optimal reaction sequence from glutamic acid to succinonitrile was therefore: 1. Mono-esterification; 2. Oxidative decarboxylation; 3. Amidation; 4. Dehydration.
Glu-Me was obtained commercially, steps 2 and 3 were achieved with a final yield of 86 mol-% CPAm. The best way to perform step 4, the dehydration of CPAm to succinonitrile, was by a catalytic water transfer reaction with acetonitrile. In this reaction, catalyzed by palladium(II), water was transferred from CPAm to acetonitrile, thereby forming succinonitrile and acetamide. This reaction is an equilibrium reaction, the highest yield that was achieved was 59 mol-%. The selectivity, however, was 100 %, probably because of the very mild reaction conditions.

Although the yields obtained in this procedure were acceptable, there are three main drawbacks. The first is the oxidative decarboxylation reaction, which was performed with hypochlorite. This is corrosive and therefore not a preferred oxidant in terms of safety and investments. Also the need to operate the reactor at a temperature well below 20 ^{o}C, when the reaction taking place is quite exothermic, is far from desirable, because of the high energy requirement. This requires large investments in cooling equipment and leads to a high energy bill (see chapter 6) and a poor environmental performance (see chapter 7). Moreover, the reaction generates stoichiometric amounts of waste salts, which cannot be considered environmentally friendly, nor efficient. The paper industry, for example, has been moving away from the use of hypochlorite as bleaching agent for this reason. Therefore it would be desirable to use another oxidant than hypochlorite for this reaction. The two main candidates would be molecular oxygen and hydrogen

peroxide. Recently it was shown in our lab that phenylalanine can be oxidatively decarboxylated at room temperature with the aid of an enzyme and hydrogen peroxide.[21] If that were also possible for glutamic acid, it would already be a great improvement of the process.
The second issue with this procedure is the use of palladium(II) as a catalyst for the water transfer reaction. In literature the reaction was reported with 10 mol-% catalyst loading, which was already reduced to 1 mol-% by us (see chapter 5), but if the palladium could not be recovered that would still be too expensive. A rough estimation in chapter 5 showed that the financial break-even point would be around 0.01 mol-% palladium loading without recovery, but at that concentration limited conversion was observed (Figure 5.3). Therefore a method for the immobilization and re-use of palladium would be essential to make this process work in an industrial setting. This was achieved by using poly(acrylonitrile) as the water acceptor instead of acetonitrile. Palladium then coordinated to the polymer and remained in the solution. The yield of succinonitrile in this case was low (11 mol-%), but this was due to a low conversion rather than a low selectivity. Furthermore, no optimization was done so there is probably room for improvement. That would be an interesting subject of further research, also because the use of acetonitrile would then be avoided, which would simplify the process. To regenerate the poly(acrylonitrile) it would then have to be dehydrated, in order to convert the formed amide groups back into nitriles.
When these two issues are overcome, the production of succinonitrile from glutamic acid could very well be a viable industrial process.

Developing a synthetic route from glutamic acid to acrylonitrile was not a part of the research for this thesis, but it was recently shown to be possible.[22] Although this route has the advantage that no ammonia needs to be introduced into the molecule, chapters 6 and 7 showed that this route is still far away from commercial feasibility. It cannot compete with petrochemical acrylonitrile with the current process configuration. An alternative route to biobased acrylonitrile is currently under development by Bañares *et al.*[23, 24] In that route glycerol from biodiesel production is the main feedstock, and heterogeneous catalysts such as alumina-supported mixed oxides are being developed to catalyze the reaction of glycerol with ammonia, under microwave irradiation. Recently they were able to

show a selectivity over 80 % at a glycerol conversion of 50 %, with a vanadium-antimony mixed oxide supported on alumina.
This route to biobased acrylonitrile would be the main competitor of the glutamic acid-based route, and it would be interesting to compare both their economics and environmental performance. A rough calculation can already provide some insight in the economic comparison of the two biobased procedures. For the production of 1 ton of acrylonitrile from glutamic acid, 3.4 ton of glutamic acid would be required, while the other procedure would require more or less 2 ton of glycerol per ton of acrylonitrile, plus 0.4 ton of ammonia. Given that the prices for crude glycerol and ammonia are currently in the order of 200 and 300 euro per ton, respectively,[25] and the price of glutamic acid anticipated at 500 euro per ton (see chapter 6), the price difference in feedstock would be between 520 and 1700 euro per ton for the glycerol and the glutamic acid-based processes, respectively. A market price of 1400 euro per ton for acrylonitrile (see chapter 6) immediately shows what is (glycerol) and what is not (glutamic acid) economically feasible. The price of glutamic acid would have to drop to a level of 150 euro per ton in order to obtain the same total feedstock price as with the glycerol process. That seems unlikely.
Comparing the environmental performance of both processes would require more insight in the glycerol-based process, because for that process it would largely depend on the type and robustness of the catalyst (given that toxic metals such as antimony are required), and the amount of energy that is needed to power the microwave reactor.

8.3 Conclusions and recommendations

This thesis demonstrated the feasibility of producing a number of industrial chemicals from glutamic acid. Especially products in the group of the pyrrolidones (i.e. NMP, NVP) have potential to be made from glutamic acid. It was shown that this can be done efficiently at lab scale, that it would be economically feasible to do it at an industrial scale, and that producing these compounds from glutamic acid rather than from fossil resources provides environmental benefits.

The main recommendations for research to further improve these processes are:

1. The development of more stable mutants of GAD, that can be used at a pH around 6, could lead to an enzymatic process with less required investments and less energy use.
2. Finding an alternative catalyst for the methylation of 2-pyrrolidone with methanol in the production of NMP would be beneficial, because it would lead to lower investment costs and less environmental impact.
3. Developing a route to NVP from glutamic acid and glycerol instead of from glutamic acid and acetylene would enable the production of completely biobased NVP and further reduce the need for fossil resources.

The production of nitriles (succinonitrile and acrylonitrile) from glutamic acid was achieved at lab scale, but appeared to be less feasible at an industrial scale, at least with the current process configurations. Both processes cannot currently compete with petrochemical production at this stage, from an economic and an environmental point-of-view.

In general it can be concluded that the closer the functionalities in the final product are to glutamic acid, the easier it is to design a process that is technologically, economically and environmentally feasible. Epichlorohydrin was one of the first biobased products made from glycerol industrially,[26] which shows that a low-value byproduct with certain functionalities can be the right feedstock for making industrial chemicals with similar functionalities. With glutamic acid as a feedstock that is mostly the case for the pyrrolidones, less for succinonitrile and even less for acrylonitrile. For producing all of these molecules it is an advantage that glutamic acid already contains a nitrogen atom, but for producing succinonitrile and acrylonitrile the rest of the modifications that are needed still provide a challenge.
For acrylonitrile, designing a competitive process from glutamic acid will be especially challenging, and glycerol may prove to be a more suitable feedstock for its biobased production, despite the fact that it may require more energy for the introduction of nitrogen into the molecule. Biobased succinonitrile is expected to be able to compete with its petrochemical equivalent both economically and environmentally, once some improvements have been achieved.

The main recommendations for research to further improve these processes are:

1. The most important aspect to improve is the oxidative decarboxylation reaction with sodium hypochlorite. The use of hypochlorite results in high investments, high energy use and a high environmental impact. Using hydrogen peroxide instead of hypochlorite would greatly improve these processes, using oxygen would be even better. The reaction should also be operated at a temperature as high as possible, so that the energy produced by the reactor can be used as efficiently as possible in other parts of the process.
2. For the succinonitrile process, finding a way to immobilize palladium(II) in order to be able to reuse it is essential as well. The use of poly(acrylonitrile) would be a method to achieve this, and it would also avoid the need for using acetonitrile in the process.

Besides further improving the processes that were developed in this thesis, finding a cost-effective way to isolate glutamic acid (and other amino acids) from such sources as were discussed in chapter 2 is of equal or even higher importance. One of the basic assumptions in the economic analysis was the availability of glutamic acid for a price of about 500 euro per ton. An inexpensive isolation method for glutamic acid will therefore be a prerequisite for its application as a feedstock for producing industrial (bulk) chemicals. When that has been developed, it may also pave the way to the use of other amino acids than glutamic acid as feedstock for the production of industrial chemicals.

8.4 references

1. Y. Teng, E. L. Scott, A. N. T. van Zeeland and J. P. M. Sanders, *Green Chemistry*, **2011**, 13, 624-630.
2. A.-C. Blaga, A. Galaction and D. Cascaval, *Chemical and Biochemical Engineering Quarterly*, **2008**, 22, 439-446.
3. L. Alexandre Rodrigues da Fonseca Ferreira, PhD Thesis, University of Porto, **2008**.
4. W. Leuchtenberger, in *Biotechnology 2nd edn, vol 6. Products of primary metabolism*, eds. Rehm H-J., Reed G., Pühler A. and S. P., VCH, Weinheim, **1996**, pp. 465-502.

5. W. Leuchtenberger, K. Huthmacher and K. Drauz, *Applied Microbiology and Biotechnology*, **2005**, 69, 1-8.
6. J. Halliday, **2008**, *Chinese MSG exporters to EU to pay stiff anti-dumping tariffs*, www.foodnavigator.com, accessed May 2011.
7. T. Werpy and G. Petersen, *Top Value Added Chemicals From Biomass, Volume I: Results of Screening for Potential Candidates from Sugars and Synthesis Gas*, U.S. Department of Energy, **2004**.
8. J. J. Bozell and G. R. Petersen, *Green Chemistry*, **2010**, 12, 539-554.
9. Stichting CO2isNee, http://www.co2isnee.nl/, **2011**.
10. H. Ueno, *Journal of Molecular Catalysis B: Enzymatic*, **2000**, 10, 67.
11. D. De Biase, A. Tramonti, R. A. John and F. Bossa, *Protein Expression and Purification*, **1996**, 8, 430.
12. E. Pennacchietti, T. M. Lammens, G. Capitani, M. C. R. Franssen, R. A. John, F. Bossa and D. De Biase, *Journal of Biological Chemistry*, **2009**, 284, 31587-31596.
13. J. Resende Ortigueira, MSc Thesis, Wageningen University, **2009**.
14. (a) T. M. Lammens, **2007-2011**, *Lots of unpublished data*; (b) A. Blade-Font, *Tetrahedron Letters*, **1980**, 21, 2443-2446.
15. T. Setoyama and T. Ogoshi, Pat. JP2001288164A, **2001**.
16. A. W. Schnizer, Pat. US2669570 (A), **1954**.
17. J. Kanetaka, T. Shimodiara and K. Hayashi, Pat. US3821245 (A), **1974**.
18. K. Pine-Sci;, C. S.-J. D., Chu-Chang;, H. Hsi-Yen;, L. Ching-Tang; and L. Yi-Yun, Pat. US 5569770 (A), **1996**.
19. A. P. Kahn, Pat. US 2004063970 (A1), **2004**.
20. A.-Y. Yin, X.-Y. Guo, W.-L. Dai and K.-N. Fan, *Green Chemistry*, **2009**, 11, 1514-1516.
21. A. But and J. L. Nôtre, **2011**, *personal communication*.
22. J. Le Notre, E. L. Scott, M. C. R. Franssen and J. P. M. Sanders, *Green Chemistry*, **2011**, 13, 807-809.
23. V. Calvino-Casilda, M. O. Guerrero-Pérez and M. A. Bañares, *Applied Catalysis B: Environmental*, **2010**, 95, 192-196.
24. M. O. Guerrero-Pérez and M. A. Bañares, *ChemSusChem*, **2008**, 1, 511-513.
25. ICIS market intelligence, www.icis.com, accessed May **2011**.
26. Solvay, www.solvaychlorinatedorganics.com, accessed May **2011**.

Appendix A

Supplementary information to chapter 3

Fed batch mass balance

$$Accumulation = in - out + production$$

Mass balance on GABA: nothing is going in or out, except that solid glutamic acid is going in. So the accumulation of GABA is equal to the production of it:

$$V_{reactor}\frac{dC}{dt} = r_{GABA} \cdot m_{GAD} \tag{1}$$

$$\int_{C_{GABA}^{t=0}}^{C_{GABA}^{t=t}} dC = \int_{t_0}^{t_t} \frac{r_{GABA} \cdot m_{GAD}}{V_{reactor}} dt \tag{2}$$

The enzyme activity (r_{GABA}) can be described with the empirically determined equation for inactivation:

$$r_{GABA}(t) = r_{GABA}^{init} \cdot (0.71 \cdot e^{-0.014t} + 0.36 \cdot e^{-0.74t}) \tag{3}$$

C_{Glu}= 100 mM. This concentration is kept constant by the continuous addition of solid glutamic acid to the reactor. That means that the initial reaction rate is equal to v_{max}, which is 117 U/mg at 30 ^{o}C and pH 4.6, equal to 7.02 mol/g.hr.

$$\int_{C_{GABA}^{t=0}}^{C_{GABA}^{t=t}} dC = \frac{r_{GABA}^{init} \cdot m_{GAD}}{V_{reactor}} \int_{t_0}^{t_t} (0.71 \cdot e^{-0.014t} + 0.36 \cdot e^{-0.74t}) dt \tag{4}$$

Because glutamic acid is added in solid form, also $V_{reactor}$ is assumed to be independent of time, so it can be removed from both sides of equation 4:

$$\int_{n_{GABA}^{t=0}}^{n_{GABA}^{t=t}} dn = r_{GABA}^{init} \cdot m_{GAD} \int_{t_0}^{t_t} (0.71 \cdot e^{-0.014t} + 0.36 \cdot e^{-0.74t}) dt \tag{5}$$

The reaction will be stopped when there is only 5% enzyme activity left, which is after 192 hours:

$$n_{GABA} = 7.02 \cdot 1 \cdot \left[\frac{0.71}{-0.014} \cdot e^{-0.014t} + \frac{0.36}{-0.74} \cdot e^{-0.74t} \right]_{192}^{0} \quad (6)$$

n_{GABA} = 335 mol/g, so the overall productivity of this bioreactor loaded with 1 g enzyme is 34 kg GABA in eight days.

Appendix B

Supplementary information to chapter 6

The data that were used as input for the calculations are shown in the table below, n.a. stands for not applicable.
All computational calculations for the determination of the Gibbs free energy of the various reactions were done with the Møller–Plesset perturbation theory method (second order),[13] with the basis set 6-311+G(2d,2p), using the software package Gaussian 09.

Compound	Mol. wt. (g/mol)	ΔH_{vap} (kJ/kg)	C_p (kJ/kg.K)	Source
Glutamic acid	147.13	n.a.	n.a.	[26]
γ-Aminobutyric acid	103.12	n.a.	n.a.	[26]
Carbon dioxide	44.01	n.a.	n.a.	[26]
Water	18.02	2256	4.2	[26]
2-Pyrrolidone	85.11	807	2.0	[26]
N-Methylpyrrolidone	99.13	553	3.1	[26]
Methanol	32.04	1155	2.5	[26]
N-Vinylpyrrolidone	111.14	446	1.8	[26]
Sodium hypochlorite	74.44	n.a.	n.a.	[27]
Sodium chloride	58.44	n.a.	n.a.	[26]
3-Cyanopropanoic acid	99.09	n.a.	n.a.	n.a.
Acetic anhydride	102.09	n.a.	n.a.	[26]
Acetic acid	60.05	n.a.	n.a.	[26]
Acrylonitrile	53.06	615	2.1	[26]
Carbon monoxide	28.01	n.a.	n.a.	[26]
Ethyl acetate	88.11	397	1.9	[26]
Glutamic acid 5-methyl ester[a]	161.16	n.a.	n.a.	n.a.
Methyl-3-cyanopropanoate	113.12	n.a.	n.a.	n.a.
3-Cyanopropanoic amide	98.10	n.a.	n.a.	n.a.
Acetonitrile	41.05	725	2.2	[26]
Succinonitrile	80.09	n.a.	n.a.	[26]
Acetamide	59.07	n.a.	n.a.	[26]

a) IUPAC name: 2-amino-5-methoxy-5-oxopentanoic acid

B.1 NMP & NVP, techno-economic data

B.1.1 Mass balance

The overall mass balance for the production of 1 ton of products:

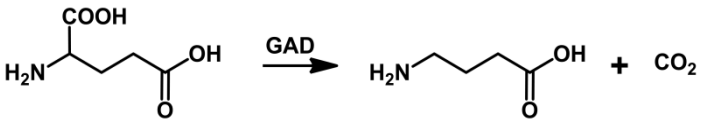
	IN (kg)	OUT (kg)
starting materials		
Glutamic acid	1538	0
Methanol	162	0
Acetylene	136	0
catalysts		
Calcium alginate	0.3	0
Enzyme (GAD)	0.2	0
Ammonium bromide	15	0
Sodium tert-butoxide	4.2	0
valuable products		
N-methylpyrrolidone	0	477
N-vinylpyrrolidone	0	523
non-valuable products		
Carbon dioxide	0	460
Water	0	283
Inactive enzyme + carrier	0	0.5
Waste products	0	113
Total	1857	1857

Assumptions:

- Glutamic acid to GABA selectivity: 100 mol-%, conversion: 98 mol-%;
- Enzyme deactivated after use;
- GABA to pyrrolidone: selectivity and conversion 100 mol-%;
- Pyrrolidone to NMP selectivity: 92 mol-%, conversion: 50 mol-%;
- Recovery of excess methanol: 95 wt-%;
- Recovery of ammonium bromide from pyrrolidone stream: 70 wt-%;
- Pyrrolidone to NVP selectivity: 90 mol-%, conversion: 100 mol-%;
- Distillation reflux ratios: 2.

B.1.2 Process energy requirements

Reactor 1



The Gibbs free energy of the reaction (ΔG) was calculated from ΔH and TΔS of the reaction, which were determined computationally:
ΔH = -30.29 kJ/mol.
TΔS = 43.47 kJ/mol.
ΔG = ΔH - TΔS = -73.76 kJ/mol.

This means that the reaction is exothermic, so the reactor needs to be cooled. The energy requirement for the cooling associated with the conversion of 1000 kg glutamic acid (6.8 kmol) into 687 kg GABA (6.66 kmol) is 73.76 x 6800 = 501 MJ.

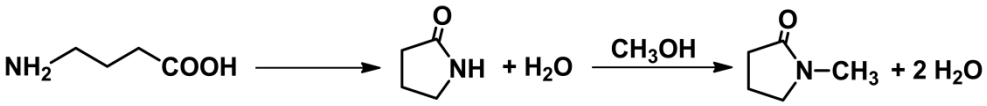
Crystallizer

The assumption was made that all the water needs to be evaporated to isolate the well-soluble GABA. At a final concentration of 5 mol/L GABA, 687 kg is dissolved in 1330 kg water.
To calculate the energy requirement for this step, the energy required to heat water to its boiling point was added to the amount of energy required to evaporate water. Therefore the amount of water was multiplied with its heat capacity and the temperature change, and added to that was the energy of vaporization of water multiplied by the amount.

	water	T_0 (^oC)	T_1 (^oC)
C_p (kJ/kgK)	4.2		
ΔH_{vap} (kJ/kg)	2256		
Amount (kg)	1330	30	100

The energy requirement for the water evaporation is 3392 MJ. This is a worst case scenario, in which all the water needs to be evaporated. In practice the required energy would be less, because not all the water needs to be evaporated and part of the condensation heat of the water can be recovered by heat integration.

Reactor 2



The Gibbs free energy of the reaction (ΔG) was calculated from ΔH and TΔS of the reaction, which were determined computationally:
For the first reaction step (GABA to pyrrolidone):
ΔH = -7.87 kJ/mol.
TΔS = 35.69 kJ/mol.
ΔG = ΔH - TΔS = -43.56 kJ/mol.
For the second reaction step (pyrrolidone to NMP):
ΔH = -61.88 kJ/mol.
TΔS = 4.98 kJ/mol.
ΔG = ΔH - TΔS = -56.90 kJ/mol.

Both reactions are exothermic, so the reactor needs to be cooled. The energy release with the production of 310 kg NMP and 284 kg pyrrolidone from 687 kg GABA (6.66 kmol) is 6660 x 43.56 + 3330 x 56.90 = 480 MJ.
This energy release occurs at 250 ^{o}C, which makes it useful energy for the distillation of products. It can therefore be subtracted from the energy use of the rest of the process.

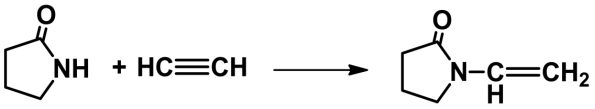
Distillation section 1

A distillation in three parts was assumed, the first for the removal of methanol, the second to remove water and the third to remove NMP.
To calculate the energy requirement, the energy required to heat the mixtures to the boiling point of the component going off the top was added to the amount of energy required to evaporate the boiling component. This means multiplying all the masses of the individual components with their heat capacity and the temperature change, and adding the energy of vaporization multiplied by the mass of the boiling component to that.
A reflux ratio of 2 was assumed, which means that the products at the top will be evaporated and condensed twice, in order to obtain sufficient separation.

		methanol	water	NMP	Pyr.	T_0 (^oC)	T_1 (^oC)
	C_p (kJ/kg.K)	2.5	4.2	3.1	2.0		
	ΔH_{vap} (kJ/kg)	1155	2256	553	807		
1	Amount (kg)	657.6	360.2	310	283.5	25	65
2	Amount (kg)		360.2	310x2	283.5	65	100
3	Amount (kg)			310x2	283.5x2	100	200

The energy requirement for the methanol distillation is: 947 MJ
The energy requirement for the water distillation is: 953 MJ
The energy requirement for the NMP distillation is: 648 MJ
The total energy requirement for the distillation section is: 2547 MJ

Reactor 3



The Gibbs free energy of the reaction (ΔG) was calculated from ΔH and TΔS of the reaction, which were determined computationally:
ΔH = -141.84 kJ/mol.
TΔS = -45.77 kJ/mol.
ΔG = ΔH - TΔS = -96.06 kJ/mol.

The energy release is 96.06 x 3330 = 320 MJ per 340 kg NVP formed from 284 kg of pyrrolidone and 88.5 kg acetylene.
This energy release occurs at 150 ^oC, which makes it useful energy for the distillation of products. It can therefore be subtracted from the energy use of the rest of the process.

Distillation section 2

Here the NVP is purified by distillation. This is assumed to happen at atmospheric pressure, at the boiling point of NVP, 90 ^oC. Again a reflux ratio of 2 is assumed.

	NVP	T_0 (°C)	T_1 (°C)
C_p (kJ/kg.K)	1.8		
ΔH_{vap} (kJ/kg)	446		
Amount (kg)	340x2	25	90

The energy requirement for the NVP distillation is 384 MJ.

Total process energy

	Per ton starting material	Per ton combined products
In:	1000 kg glutamic acid	1538 kg glutamic acid
Out:	310 kg NMP, 340 kg NVP	477 kg NMP, 523 kg NVP
Reactor 1	501 MJ	771 MJ
Crystallizer	3392 MJ	5217 MJ
Reactor 2	-480 MJ	-738 MJ
Distillation 1	2547 MJ	3919 MJ
Reactor 3	-320 MJ	-492 MJ
Distillation 2	384 MJ	591 MJ
TOTAL	**6.0 GJ**	**9.3 GJ**

B.1.3 Economic assessment

	IN (kg)	OUT (kg)	Price (€/kg)	Value (€/ton NMP+NVP)
starting materials				
Glutamic acid	1538	0	0.50[a]	769
Methanol	161.9	0	0.37[b]	59
Acetylene	136.1	0	1.03[b]	141
catalysts				
Calcium alginate	0.31	0	8.88[c]	2.80
Enzyme (GAD)	0.16	0	100[d]	16
Ammonium bromide	15.4	0	1.94[b]	30
Sodium *tert*-butoxide	4.2	0	24.90[e]	106
non-valuable products				
Carbon dioxide	0	299.0		0
Water	0	183.8		0
Enzyme waste (biodegr.)	0	0.3		0
Waste	0	133.3		0 [f]
utilities				
Steam	3100	0	0.015[g]	47
Variable costs				1171
valuable products				
N-Methylpyrrolidone	0	476.9	3.07[b]	1463
N-Vinylpyrrolidone	0	523.1	3.07[i]	1605
Margin				**1897**

a) Price was estimated as described in the discussion above.
b) Price was obtained from ICIS pricing and was an average bulk price of 2008.[15]
c) Cost of calcium chloride neglected compared to sodium alginate.
d) Potential commercial price was estimated by coauthor (Sanders), based on own industrial experience.
e) Price at bulk scale could not be obtained. Therefore the price was estimated by dividing the Sigma-Aldrich catalogue price by a factor 10.
f) The byproducts are assumed to be burned for energy by an external party at zero cost.

g) Personal communication by DSM, 2011. Steam depicted in €/kg. Steam has an assumed energy density of 3 MJ/kg.
h) Electricity use is neglected for this case.
i) Price at bulk scale could not be obtained, but is higher than the price of NMP.[16] This is therefore a conservative value.

B.2 Acrylonitrile, techno-economic data

B.2.1 Mass balance

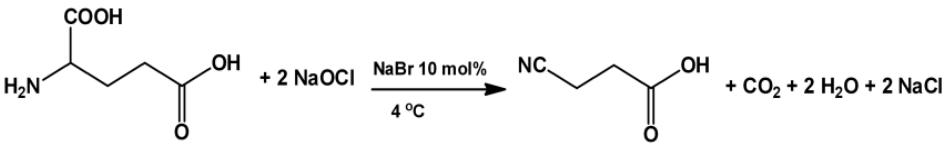
The overall mass balance for the production of 1 ton of acrylonitrile:

	IN (kg)	OUT (kg)
starting materials		
Glutamic acid	3423	0
Sodium hypochlorite	3464	0
Acetic anhydride	2375	0
catalysts & solvents		
Sodium bromide	60	0
Ethyl acetate	350	0
DMPU	2.7	0
Triethylamine	2.1	0
Palladium chloride	0.01	0
DPE-Phos	0.10	0
valuable products		
Acrylonitrile	0	1000
Acetic acid	0	2515
Sodium chloride-bromide	0	2779
non-valuable products		
Water	0	839
Carbon dioxide	0	922
Carbon monoxide	0	528
Waste products	0	1095
Total	9678	9678

Assumptions:
- Glutamic acid to CPA selectivity: mol-90 %, conversion: 100 mol-%;
- CPA extraction and crystallization yield: 100 mol-%;
- Ethyl acetate loss: 1 wt-%;
- NaBr recycle loss: 25 wt-%;
- DMPU and Et_3N recycle-loss: 0.1 wt-% each;
- $PdCl_2$ and DPE-Phos recycle loss: 0.01 wt-%;
- Distillation reflux ratio: 2.

B.2.2 Process energy requirements

Reactor 1



The Gibbs free energy of the reaction (ΔG) was calculated from ΔH and TΔS of the reaction, which were determined computationally:
ΔH = -640.53 kJ/mol.
TΔS = 120.62 kJ/mol.
ΔG = ΔH - TΔS = -761.15 kJ/mol.

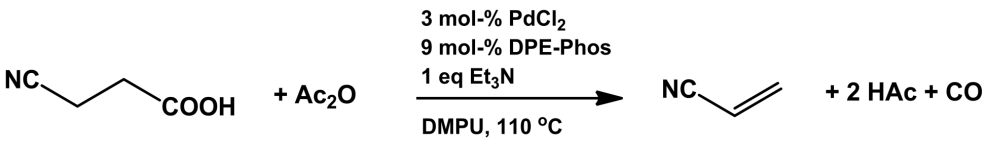
The reaction is strongly exothermic. The energy requirement for the cooling associated with the conversion of 1000 kg glutamic acid (6.8 kmol) into 606 kg CPA (6.1 kmol) is 761 x 6800 = 5173 MJ.
Because cooling below room temperature is required, the energy for this cooling will have to be supplied as electricity. It will be assumed that due to losses, 1.5 times the required cooling energy is required as electricity input.

Extraction & crystallization

In order to estimate the energy required for extracting and crystallizing the CPA from the aqueous reaction mixture, the assumption was made that the needed volume of ethyl acetate is twice that of water in the reaction mixture. Then it is assumed that the ethyl acetate

solution is cooled down from 25 to 4 °C, to aid the crystallization of CPA. Other energy consumption in this step is neglected.
Cooling down 10200 kg of ethyl acetate (Cp = 1.9 kJ/mol.K) from 25 to 4 °C, for the crystallization of 606 kg CPA, requires 408 MJ.
Because cooling below room temperature is required, the energy for this cooling will have to be supplied as electricity. It will be assumed that due to losses, 1.5 times the required cooling energy is required as electricity input.

Reactor 2



The Gibbs free energy of the reaction (ΔG) was calculated from ΔH and TΔS of the reaction, which were determined computationally:
ΔH = 91.59 kJ/mol.
TΔS = 100.42 kJ/mol.
ΔG = ΔH - TΔS = -8.83 kJ/mol.

This means that the reaction is slightly exothermic. The released energy is 8.83 x 6100 = 54 MJ per 292 kg acrylonitrile.
This energy release occurs at 110 °C, which makes it useful energy for the distillation of products. It can therefore be subtracted from the energy use of the rest of the process.

Distillation section

Acrylonitrile can be isolated by distillation. This would normally take place under reduced pressure, to decrease the applied temperature. In this case an atmospheric distillation was assumed for the energy calculations, with a reflux ratio of 2.

	ACN	T_0 (°C)	T_1 (°C)
C_p (kJ/kgK)	2.09		
ΔH_{vap} (kJ/kg)	615	25	77
Amount (kg)	292x2		

The energy requirement for this distillation is 423 MJ per 292 kg acrylonitrile.

Total process energy

	Per ton starting material	Per ton product
In:	1000 kg glutamic acid	3423 kg glutamic acid
Out:	292 kg ACN	1000 kg ACN
Reactor 1	5173 MJ	17711 MJ
Extract. & Cryst.	408 MJ	1396 MJ
Reactor 2	-54 MJ	-185 MJ
Distillation	423 MJ	1447 MJ
TOTAL	**5.9 GJ**	**20 GJ**

B.2.3 Economic assessment

	IN (kg)	OUT (kg)	Price (€/kg)	Value (€/ton acrylonitrile)
starting materials				
Glutamic acid	3423	0	0.50[a]	1712
Sodium hypochlorite	3464	0	1.01[b]	3486
Acetic anhydride	2375	0	0.81[b]	1918
catalysts & solvents				
Sodium bromide	60	0	1.13[b]	68
Ethyl acetate	350	0	0.81[b]	282
DMPU	2.7	0	28.21[c]	76
Triethylamine	2.1	0	2.44[c]	5
Palladium chloride	0.010	0	19000[d]	195
DPE-Phos	0.10	0	630[c]	65
non-valuable products				
Water	0	839		0
Carbon dioxide	0	922		0
Carbon monoxide	0	528		0
Waste	0	1095		0[e]
energy input				
Steam	421	0	0.015[f]	6
Electricity (kWh)	7962	0	0.055[f]	438
Variable costs				6539
valuable products				
Acrylonitrile	0	1000	1.37[b]	1373
Acetic acid	0	2515	0.65[b]	1625
Sodium chloride-bromide	0	2780	0.08[g]	224
Margin				**-3317**

a) Price was estimated as described in the discussion above.
b) Price at bulk scale was obtained from ICIS pricing.[15]
c) Price at bulk scale could not be obtained. Therefore the price was estimated by dividing the Sigma-Aldrich catalogue price by a factor 10.
d) Price of palladium in 2010 according to Johnson-Matthey.[28]

e) The byproducts are assumed to be burned for energy by an external party at zero cost.
f) Personal communication by DSM. Steam depicted in €/kg and electricity in €/kWh. Steam has an assumed energy density of 3 MJ/kg. 1 MJ equals 0.2778 kWh electricity.
g) An aqueous stream of NaCl with little NaBr is expected to be possibly sold at an estimated price of 10 % of NaCl equivalent.

B.3 Succinonitrile, techno-economic data

B.3.1 Mass balance

The overall mass balance for the production of 1 ton of succinonitrile:

	IN (kg)	**OUT (kg)**
starting materials		
Glutamic acid	2193	0
Methanol	56	0
Sodium hypochlorite	2175	0
Ammonia	224	0
Acetonitrile	540	0
catalysts & solvents		
Sodium bromide	15	0
Palladium chloride	0.002	0
Ethyl acetate	26	0
valuable products		
Succinonitrile	0	1000
Acetamide	0	738
Sodium chloride-bromide	0	1723
non-valuable products		
Water	0	737
Carbon dioxide	0	579
Waste products	0	452
total	5229	5229

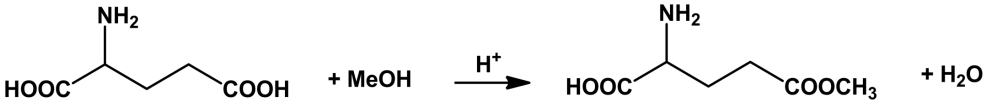
Assumptions:

- Glutamic acid mono-esterification: selectivity 98 mol-%, conversion 100 mol-%;

- Esterification catalyst is fixed acid bed, no loss.
- Glutamic acid 5-methyl ester decarboxylation: selectivity: 90 mol-%, conversion: 100 mol-%;
- Amidation yield: 100 mol-%;
- NaBr recycle-loss: 25 wt-%;
- Methanol loss due to byproducts formation in reactor 1 and 2: 12 mol-%;
- $PdCl_2$ loss: 0.01 wt-%.
- Ethyl acetate loss: 1 wt-%;
- Distillation reflux ratio: 2.

B.3.2 Process energy requirements

Reactor 1



The Gibbs free energy of the reaction (ΔG) was calculated from ΔH and TΔS of the reaction, which were determined computationally:
ΔH = -23.60 kJ/mol.
TΔS = -4.69 kJ/mol.
ΔG = ΔH - TΔS = -18.91 kJ/mol.

The reaction is exothermic. The energy release associated with the conversion of 1000 kg glutamic acid (6.8 kmol) into 1073 kg glutamic acid 5-methyl ester (6.66 kmol) is 18.91 x 6800 mol = 129 MJ.

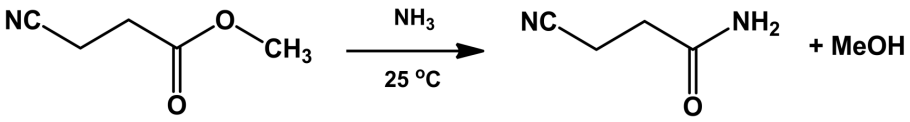
Reactor 2

The Gibbs free energy of the reaction (ΔG) was calculated from ΔH and TΔS of the reaction, which were determined computationally:
ΔH = -640.53 kJ/mol.

TΔS = 120.62 kJ/mol.
ΔG = ΔH - TΔS = -761.15 kJ/mol.

The reaction is strongly exothermic. The energy requirement for the cooling associated with the conversion of 1073 kg glutamic acid 5-methyl ester (6.66 kmol) into 678 kg cyanopropanoic acid methyl ester (5.99 kmol) is 761.15 x 6660 mol = 5070 MJ. Because cooling below room temperature is required, the energy for this cooling will have to be supplied as electricity. It will be assumed that due to losses, 1.5 times the required cooling energy is required as electricity input.

Reactor 3



The Gibbs free energy of the reaction (ΔG) was calculated from ΔH and TΔS of the reaction, which were determined computationally:
ΔH = 17.36 kJ/mol.
TΔS = 8.16 kJ/mol.
ΔG = ΔH - TΔS = 9.20 kJ/mol.

This reaction is endothermic. The energy requirement for the heating associated with the conversion of 678 kg cyanopropanoic acid methyl ester (5.99 kmol) into 588 kg cyanopropanoic amide (5.99 kmol) is 9.2 kJ/mol x 5990 mol = 55 MJ.

Distillation

It was assumed that cyanopropanoic amide needs to be purified, so both the methanol and the water are evaporated. Methanol and water are both heated to 65 ^{o}C, and the methanol evaporated. Then the water is further heated to 100 ^{o}C and then evaporated. A reflux ratio of 2 was assumed.

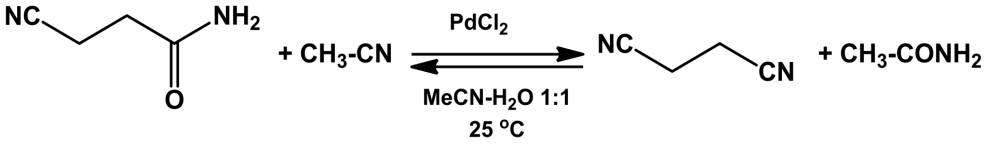
	methanol	water	T_0 (°C)	T_1 (°C)
C_p (kJ/kgK)	2.5	4.2		
ΔH_{vap} (kJ/kg)	1155	2256		
Amount	384	877	25	65
Amount		877	65	100

The energy requirement per 588 kg cyanopropanoic amide is 2716 MJ.

Recrystallization

The energy input of the recrystallization step was neglected when compared to the energy required for the removal of water.

Reactor 4



The Gibbs free energy of the reaction (ΔG) was calculated from ΔH and TΔS of the reaction, which were determined computationally:
ΔH = 13.47 kJ/mol.
TΔS = 7.07 kJ/mol.
ΔG = ΔH - TΔS = 6.4 kJ/mol.

The reaction is endothermic. The energy requirement for the heating associated with the conversion of 588 kg cyanopropanoic amide (5.99 kmol) into 456 kg succinonitrile (5.69 kmol) is 6.4 kJ/mol x 5990 mol = 38 MJ.
It was assumed that by slightly modifying the reaction temperatures of reactors 1, 3 and 4, in order to create a temperature gradient, the reaction energy created in reactor 1 can be used for heating the endothermic reactions in reactor 3 and 4, so it can be subtracted from their energy use.

Extraction and evaporation

The solubility of succinonitrile in ethyl acetate is 4 kg/L at 25 ^{o}C (own determination). Therefore, it is assumed that per kg succinonitrile, 0.25 kg of ethyl acetate is needed for the extraction. It will be assumed that all ethyl acetate needs to be evaporated to obtain pure succinonitrile.

	Ethyl acetate	T_0 (^{o}C)	T_1 (^{o}C)
C_p (kJ/kgK)	1.9		
ΔH_{vap} (kJ/kg)	363		
Amount	114	25	77

This will require 57 MJ for the ethyl acetate evaporation.

Total process energy

	Per ton starting material	Per ton products
In:	1000 kg glutamic acid	2193 kg glutamic acid
Out:	456 kg succinonitrile	1000 kg succinonitrile
Reactor 1	-129 MJ	-282 MJ
Reactor 2	5070 MJ	11118 MJ
Reactor 3	55 MJ	121 MJ
Distillation	2716 MJ	5957 MJ
Reactor 4	38 MJ	84 MJ
Extraction	57 MJ	124 MJ
TOTAL	**7.8 GJ**	**17 GJ**

B.3.3 Economic assessment

	IN (kg)	OUT (kg)	Price (€/kg)	Value (€/ton succinonitrile)
starting materials				
Glutamic acid	2193	0	0.50[a]	1096
Methanol	56	0	0.37[b]	21
Sodium hypochlorite	2175	0	1.01[b]	2188
Ammonia	224	0	0.81[b]	181
Acetonitrile	540	0	1.13[b]	610
catalysts & solvents				
Sodium bromide	15	0	1.13[b]	17
Ethyl acetate	26	0	0.81[b]	21
Palladium chloride	0.002	0	19000[c]	42
non-valuable products				
Water	0	737		0
Carbon dioxide	0	579		0
Waste	0	452		0[d]
utilities				
Steam	2001	0	0.015[e]	30
Electricity (kWh)	4633	0	0.055[e]	255
Variable costs				4461
valuable products				
Succinonitrile	0	1000	3.07[f]	3071
Acetamide	0	738	3.07[f]	2264
Sodium chloride-bromide	0	1723	0.08[g]	139
Margin				**1013**

a) Price was estimated as described in the discussion above.
b) Price at bulk scale was obtained from ICIS pricing.[15]
c) Price of palladium in 2010 according to Johnson-Matthey.[28]
d) The byproducts were assumed to be burned for energy by an external party at zero cost.

e) Personal communication by DSM. Steam depicted in €/kg and electricity in €/kWh. Steam has an assumed energy density of 3 MJ/kg. 1 MJ equals 0.2778 kWh electricity.

f) Prices of succinonitrile and acetamide at bulk scale were unknown. A conservative estimation was done by taking the same price as NMP.

g) An aqueous stream of NaCl with little NaBr was expected to be sold at an estimated price of 10 % of NaCl equivalent.

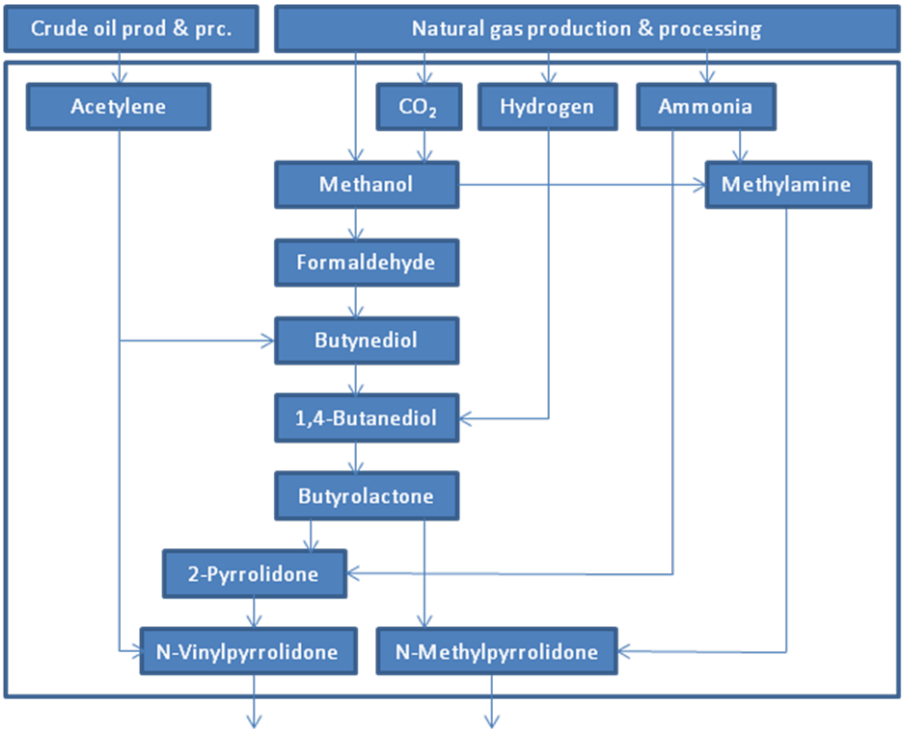
Appendix C

Supplementary information to chapter 7

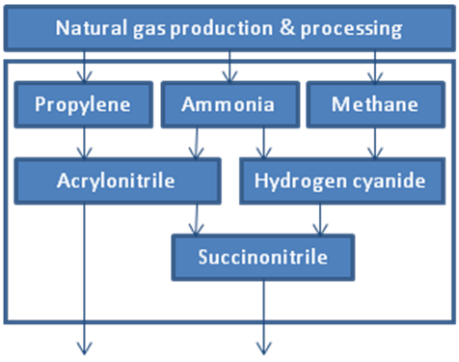
This supporting information provides full life cycle inventories as they were entered in Simapro 7. Inventory items marked with an asterisk (*) were modelled and can be found in this document. All other inventory items were taken directly from the Eco-invent database.

Petrochemical production routes

NMP and NVP:



ACN and SCN



N-Methylpyrrolidone, biobased

Output		
N-methylpyrrolidone, biobased	1	kg
Input from technosphere		
Glutamic acid from vinasse*	1.65	kg
Glutamic Acid Decarboxylase (enzyme)*	0.17	g
Calcium alginate*	0.34	g
Methanol	0.359	kg
Ammonium bromide*	0.0165	kg
Heat, from industrial furnace	11	MJ
Electricity, medium voltage	0.333	kWh
Transport, road	0.1244	tkm
Transport, rail	0.74641	tkm
Chemical plant	4E-10	p
Emissions to air (stratosphere + troposphere)		
Waste heat	4	MJ
Carbon dioxide, biogenic	0.49	kg
Methyl amine	0.66	g
Bromomethane	0.165	g
Methanol	0.2	g
Emissions to water (river)		
Ammonium	0.01	g
Bromide	0.01	g
COD	0.0135	kg
DOC	0.00356	kg
TOC	0.00356	kg
BOD5	0.0135	kg

Remarks

Input of chemicals and heat was derived from Lammens *et al*.[10]

COD, DOC, TOC, BOD5 were assumed identical to petrochemical NMP.

* Modelled process, inventory is present below.

N-Vinylpyrrolidone, biobased

Output		
2-Pyrrolidone, biobased	1	kg
Input from technosphere		
Glutamic acid from vinasse*	1.88	kg
Glutamic Acid Decarboxylase (enzyme)*	0.19	g
Calcium alginate*	0.38	g
Methanol	0.02	kg
Heat, from industrial furnace	11	MJ
Electricity, medium voltage	0.333	kWh
Transport, road	0.1244	tkm
Transport, rail	0.74641	tkm
Chemical plant	4E-10	p
Emissions to air (stratosphere + troposphere)		
Waste heat	3	MJ
Carbon dioxide, biogenic	0.49	kg
Methanol	0.2	g
Emissions to water (river)		
COD	0.0135	kg
DOC	0.00356	kg
TOC	0.00356	kg
BOD5	0.0135	kg

Remarks

The functional unit for NVP is 2-pyrrolidone, for reason of comparison between petrochemical and biobased NVP.

Input of chemicals and heat were derived from Lammens *et al.*[10]

Input of electricity and transport was assumed identical to petrochemical NMP.

Methanol was used as solvent. 1% Methanol loss to the air was estimated. This was assumed to be burned for energy in an industrial furnace.

COD, DOC, TOC, BOD5 were assumed identical to petrochemical NMP.

A chemical plant use of 4.10^{-10} p equals a land use of 0.00112 m^2. This was assumed the same as for the NMP process.

* Modelled process, inventory present below.

Succinonitrile, biobased

Output

Succinonitrile, biobased	1	kg

Input from technosphere

Glutamic acid from vinasse*	2.193	kg
Methanol	56	g
Sodium hypochlorite	2.175	kg
Sodium bromide*	15	g
Ammonia	0.224	kg
Palladium(II)chloride*	2	mg
Ethyl acetate	26	g
Heat, from industrial furnace	6	MJ
Electricity, medium voltage	4.7	kWh
Transport, road	0.134	tkm
Transport, rail	0.805	tkm
Chemical plant	4E-10	p

Emissions to air (stratosphere + troposphere)

Waste heat	2	MJ
Carbon dioxide, biogenic	0.591	kg
Triethylamine	0.02	g

Emissions to water (river)

Triethylamine	0.02	g
COD	0.04	kg
DOC	0.012	kg
TOC	0.012	kg
BOD5	0.04	kg

Remarks

Input of chemicals and energy was derived from Lammens *et al.*[10]

Input of transport was assumed identical to petrochemical acrylonitrile.

COD, DOC, TOC, BOD5 were assumed identical to petrochemical acrylonitrile.

A chemical plant use of 4.10^{-10} p equals a land use of 0.00112 m^2. This is assumed the same as for the ACN process.

* Modelled process, inventory present below.

Succinonitrile, biobased, optimized case

Output		
Succinonitrile, biobased, optimized	1	kg
Input from technosphere		
Glutamic acid from vinasse*	2.193	kg
Methanol	56	g
Ammonia	0.224	kg
Palladium(II)chloride*	0.2	mg
Electricity, medium voltage	0.209	kWh
Transport, road	0.134	tkm
Transport, rail	0.805	tkm
Chemical plant	4E-10	p
Emissions to air (stratosphere + troposphere)		
Waste heat	1	MJ
Carbon dioxide, biogenic	0.591	kg
Waste treatment		
Wastewater treatment	0.4	m3

Remarks

Same improvement assumptions as in the optimized biobased acrylonitrile process.
* modelled process, inventory present below

Acrylonitrile, biobased

Output		
Acrylonitrile, biobased	1	kg
Output - Avoided product		
Acetic acid	2.515	kg
Input from technosphere		
Glutamic acid from vinasse*	3.42	kg
Sodium hypochlorite	3.464	kg
Sodium bromide*	60	g
Acetic anhydride	2.375	kg
Ethyl acetate	0.35	kg
Triethylamine*	2.1	g
N,N'-Dimethylpropyleneurea*	2.7	g
Palladium(II)chloride*	10.3	mg
Heat, from industrial furnace	1.3	MJ
Electricity, medium voltage	8	kWh
Transport, road	0.134	tkm
Transport, rail	0.805	tkm
Chemical plant	4E-10	p
Emissions to air (stratosphere + troposphere)		
Waste heat	1	MJ
Carbon dioxide, biogenic	1.84	kg
Triethylamine	0.02	g
Emissions to water (river)		
Triethylamine	0.02	g
COD	0.04	kg
DOC	0.012	kg
TOC	0.012	kg
BOD5	0.04	kg

Remarks

Input of chemicals and energy were derived from Lammens *et al.*[10]

Input of DPE-Phos was neglected because it was only 0.1 g of organic compound.

Input of transport was assumed identical to petrochemical acrylonitrile.

Carbon monoxide was assumed to be burned to carbon dioxide before release to the air.

COD, DOC, TOC, BOD5 are assumed identical to petrochemical acrylonitrile.
A chemical plant use of 4.10^{-10} p equals a land use of 0.00112 m^2. This is assumed the same as for the petrochemical ACN process.
* Modelled process, inventory present below.

Acrylonitrile, biobased, optimized case

Output		
Acrylonitrile, biobased, optimized	1	kg
Output - Avoided product		
Heat, from industrial furnace	7.2	MJ
Input from technosphere		
Glutamic acid from vinasse	3.42	kg
Triethylamine	2.1	g
N,N'-Dimethylpropyleneurea	2.7	g
Palladium(II)chloride	1.03	mg
Electricity, medium voltage	0.3	kWh
Transport, road	0.134	tkm
Transport, rail	0.805	tkm
Chemical plant	4E-10	p
Emissions to air (stratosphere + troposphere)		
Waste heat	1	MJ
Carbon dioxide, biogenic	1.84	kg
Waste treatment		
Wastewater treatment	0.4	m3

Remarks

Input of chemicals and energy was derived from the biobased model.
Changes: It was assumed that oxygen can be used instead of sodium hypochlorite for the oxidation reaction, and that the reaction produced heat instead of needing to be cooled below room temperature. That will be enough for all process heat.
It was assumed that 50% of the heat (8.5 MJ) that is produced in the reactor can be used to replace otherwise produced heat.
The decarbonylation was assumed to be done via an internal anhydride reaction, of the intermediate product 3-cyanopropanoic acid with itself, instead of with acetic anhydride.

Palladium recycle efficiency was improved by a factor 10.

Wastewater treatment was assumed identical to petrochemical succinonitrile.
A chemical plant use of 4.10^{-10} p equals a land use of 0.00112 m^2. This is assumed the same as for the petrochemical ACN process.
* Modelled process, inventory present below.

Glutamic acid from vinasse, replaced by cane molasses

Inventory of normal scenario

Output		
Glutamic acid from vinasse	1	kg
Input		
Molasses, from sugar cane*	0.5	kg
Urea (N)	0.0476	kg
Heat, from industrial furnace	2	MJ
Electricity, medium voltage	0.2	kWh
Transport, road	0.1244	tkm
Transport, rail	0.74641	tkm
Sugar refinery	8.82E-11	p
Emissions		
Waste heat to air	0.8	MJ
Waste treatment		
Wastewater treatment	0.01	m3

Remarks

Glutamic acid was not produced from sugarcane molasses, but from vinasse, in a process that requires heat and electricity, and leads to waste heat and waste water that needs to be treated in a wastewater treatment.

50% Molasses was taken as input because it was assumed to replace the glutamic acid that was taken from the vinasse 1 to 1 on a mass basis for feed purposes.

50% Urea (N-basis) was taken as input because it was assumed to replace the glutamic acid that was taken from the vinasse 1 to 1 on a mass basis for fertilizer purposes.

The amounts of heat, electricity and wastewater treatment were assumed identical for those of a sugar production process, as was the size of the plant.

A sugar refinery use of 1 p equals a land use (occupation) of 2291660 m^2 per annum.

A sugar refinery use of $8.82.10^{-11}$ p equals a land use of 0.000202 m^2.

* Modelled process, inventory present below.

Glutamic acid from vinasse, replaced by cane molasses

Inventory of high energy scenario

Output		
Glutamic acid from vinasse, HE	1	kg
Input from technosphere		
Molasses, from sugar cane*	0.5	kg
Urea (N)	0.0476	kg
Heat, from industrial furnace	6	MJ
Electricity, medium voltage	0.2	kWh
Transport, road	0.1244	tkm
Transport, rail	0.74641	tkm
Sugar refinery	8.82E-11	p
Emissions		
Waste heat to air	2.4	MJ
Waste treatment		
Wastewater treatment	0.01	m3

Remarks

The amounts of heat and waste heat were tripled compared to the 'normal scenario'.
* Modelled process, inventory present below.

Multi-output sugar beet biorefinery

Output			**Allocation**
Sugar	1	kg	75.41%
Beet pulp	356	g	12.90%
Ethanol	51.8	g	6.93%
Glutamic acid	10.6	g	1.60%
Vinasse	109	g	1.42%
Beet tails	93.8	g	1.34%
Chalk	206	g	0.31%
Yeast	2.19	g	0.09%
Input from technosphere			
Sugar beets	6.374989	kg	
Sulphuric acid	0.002373	kg	
Soda	0.002289	kg	
Ammonium sulphate	0.000261	kg	
Diammonium phosphate	0.000391	kg	
Chemicals organic	0.000615	kg	
Chemicals inorganic	0.000886	kg	
Limestone	0.192345	kg	
Tap water	7.444529	kg	
Transport, road	0.01217	tkm	
Transport, rail	0.53541	tkm	
Transport, tractor and trailer	0.06375	tkm	
Heat	2.12344	MJ	
Electricity	0.222256	kWh	
Hard coal coke	0.016635	MJ	
Sugar refinery	9.72E-11	p	
Ethanol fermentation plant	1.1E-11	p	
Emissions to air (stratosphere + troposphere)			
Waste heat	0.8906	MJ	
Carbon dioxide, biogenic	0.5504	kg	

Waste to treatment

Disposal of limestone residue	0.23325	kg
Wastewater treatment	0.011515	m3

Remarks

The process (sugar refinery, molasses fermentation and distillation, and glutamic acid isolation) was treated as a single process with a multi-output of products.

The individual contributions of inventory items were added together and the total inventory was allocated according to the economic value (output x price) of the different products.

Example: the environmental impact of 1 kg of glutamic acid is (1000 g / 10,6 g) x (0,016 x total process impact).

A sugar refinery use of $9.72.10^{-11}$ p equals a land use of 0.000223 m^2.

An ethanol fermentation plant use of 1 p equals a land use of 937500 m^2, $1.1.10^{-11}$ equals 0.0000103 m^2.

Determination of allocation factors

Products *1000 kg beet*	Amount *kg*	Price *€ / kg*	Value € /ton beet	Allocated impact (% of total value)
Refined sugar	160	0.5	80	75.41%
Dried pulp	57	0.24	13.68	12.90%
Ethanol (in liters)	10.5	0.7	7.35	6.93%
Vinasse	15.8	0.095	1.501	1.41%
Beet tails	15	0.095	1.425	1.34%
Chalk	33	0.01	0.33	0.31%
Yeast	0.35	0.28	0.098	0.09%
Glutamic acid	1.7	1	1.7	1.60%

Summary

The chemical industry is in transition from a petrochemical based towards a more biobased industry. The current worldwide research effort in the search for biobased chemicals is enormous. Most of this research focuses on the use of carbohydrates, but the proteins that are left in the byproducts from bioethanol and biodiesel production could also be valuable building blocks for chemicals. Especially the non-essential amino acids in these proteins, which have no significant value for food or feed, can be interesting starting materials for a variety of nitrogen containing products. The most abundant (and non-essential) amino acid in many plant proteins is glutamic acid (chapter 2).

This thesis focused on new, catalytic and sustainable routes from glutamic acid to industrial chemicals. The goal was to create the possibility to replace oil by glutamic acid as the feedstock for a range of chemicals. The application of glutamic acid as a feedstock is especially valuable for chemicals that contain nitrogen because the introduction of nitrogen into molecules is very energy-intensive due to the production of ammonia, while glutamic acid already contains nitrogen, therefore eliminating the need for ammonia production.
The target industrial chemicals were chosen because they are nitrogen-functionalized like glutamic acid and are currently produced from petrochemical resources. The chosen targets for this thesis were N-methylpyrrolidone (NMP), N-vinylpyrrolidone (NVP) and 1,4-diaminobutane. NMP is a common industrial solvent, NVP is the precursor for poly(vinylpyrrolidone), which has numerous applications in glues, and 1,4-diaminobutane in the production of polyamides such as nylon-4,6. Another target in the research project of which this thesis was a part was acrylonitrile, a precursor for many polymers and rubbers. The synthetic route from glutamic acid to acrylonitrile was developed by a postdoctoral researcher, Dr. J. Le Nôtre. That route was therefore not reported in this thesis. Figure S1 summarizes all the routes described in this thesis, including the one to acrylonitrile.

In chapter 3 the α-decarboxylation of glutamic acid to form γ-aminobutyric acid (GABA) was described. This was carried out enzymatically using glutamic acid α-decarboxylase (GAD). To study the applicability of this reaction in an industrial setting, the operational stability of GAD was determined. Immobilization increased the GAD stability. The conditions for the highest GABA production per gram of

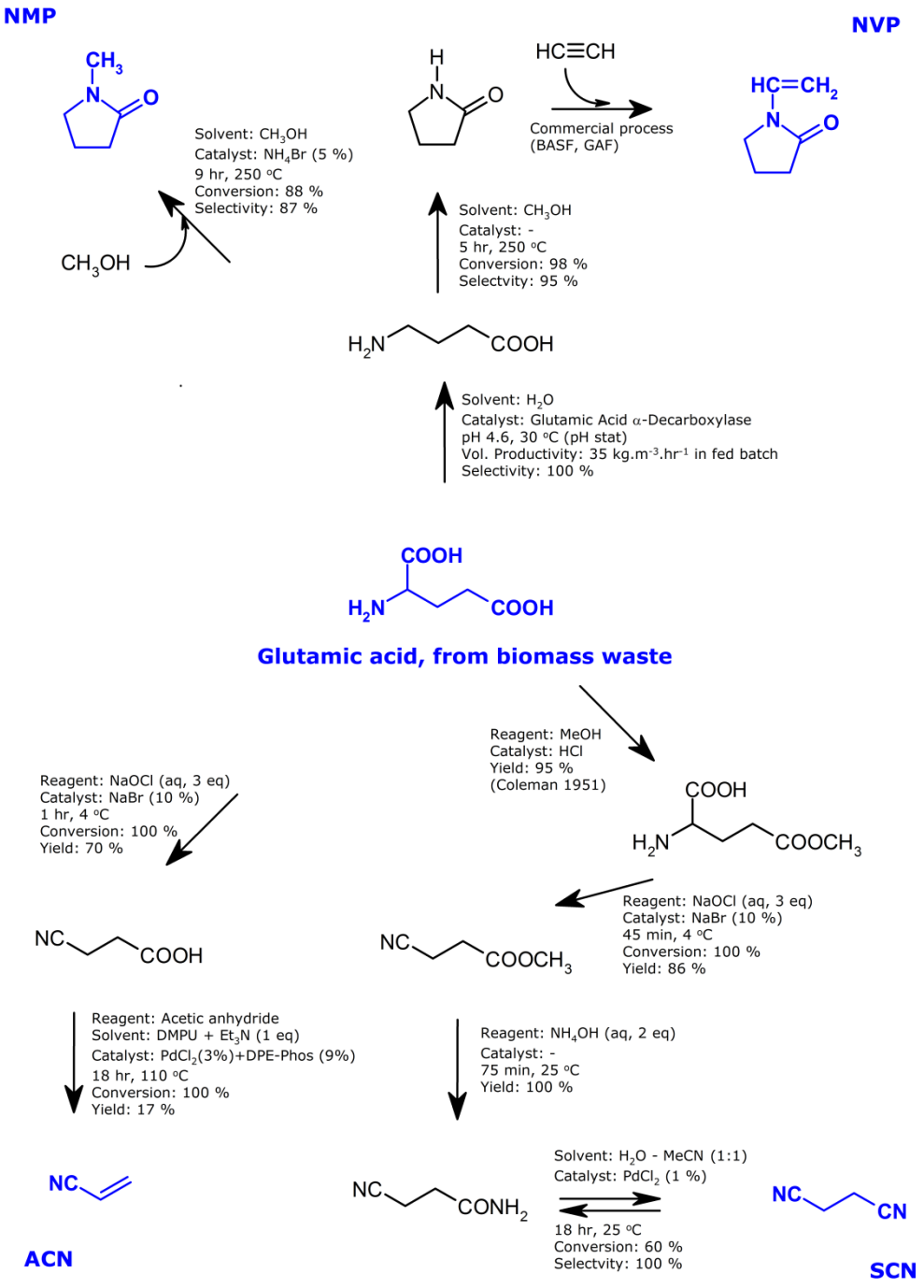


Figure S1. All the chemical routes from glutamic acid towards the target products.

enzyme were determined by extrapolation of enzyme stability data. At 30 ^{o}C in a fed batch process, this results in an average volumetric productivity of 35 kg $m^{-3}h^{-1}$. The cost of using GAD immobilized in calcium alginate at 30 ^{o}C was estimated to be €5 per metric ton of produced GABA.
In conclusion the application of immobilized GAD in a fed batch reactor was shown to be a scalable process for the industrial production of GABA from glutamic acid. And there is still room for improvement. Experiments performed with mutants of GAD showed that it was possible to perform this conversion at an increased pH, at which glutamic acid has a higher solubility. This could lead to an even higher volumetric productivity of the reactor.

The next step, described in chapter 4, was to cyclize GABA to form 2-pyrrolidone, which was subsequently methylated to form NMP. It was shown that it is possible to perform the cyclization of GABA and the subsequent catalytic methylation of 2-pyrrolidone with methanol in a one-pot procedure. This was done with a catalytic amount of a halogen-containing salt such as ammonium bromide. The advantage over conventional methylation procedures with methyl iodide or dimethyl sulfate is that with methanol the only byproduct that is stoichiometrically formed is water, which makes the process more sustainable. A high conversion and a high selectivity (90 %) were achieved for this reaction. Thus a straight-forward route to synthesize biobased NMP from glutamic acid was developed.
When the one-pot reaction of GABA to NMP was stopped halfway during the process (after 5 hours), the 2-pyrrolidone and NMP formed could be readily separated by distillation. Since 2-pyrrolidone is the commercial precursor for NVP (a.o. in a BASF process), this means that there is now also a straight-forward route to synthesize biobased NVP.

Chapter 5 described the synthesis of succinonitrile from either glutamine or glutamic acid, via the intermediate 3-cyanopropanoic amide (CPAm). From glutamine, CPAm could be prepared directly by an oxidative decarboxylation reaction using sodium hypochlorite in the presence of a catalytic amount of sodium bromide. From glutamic acid, CPAm could be prepared in three steps: 1) esterification with methanol to form glutamic acid 5-methyl ester (Glu-Me); 2) oxidative decarboxylation of the α-carboxylic acid group to form 3-cyanopropanoic

acid methyl ester (CPA-Me); 3) amidation of the ester group with aqueous ammonia to form CPAm. As the first step was described in literature as being a facile procedure with a yield of 95 %, only the second and third step were investigated. The crude CPAm yield that was achieved with the oxidative decarboxylation of Glu-Me was significantly higher (86 %) than the one achieved starting from glutamine (56 %).
The dehydration of CPAm to form succinonitrile was investigated under different conditions. The most effective system that was found was a palladium(II) catalyzed water transfer reaction with acetonitrile as the water acceptor. Under the conditions employed, no significant side products were formed, indicating that although the conversion of CPAm to succinonitrile is only 62 %, the selectivity of this reaction is close to 100 %.
This could be an interesting alternative to the current fossil based route for the production of 1,4-diaminobutane. The main advantage over biotechnological alternatives to produce this compound is that succinonitrile is much less soluble in water than 1,4-diaminobutane, so it can easily be extracted from an aqueous solution with an organic solvent like ethyl acetate. This would greatly reduce the energy required for the work-up of the product.

In chapter 6 the synthetic routes from glutamic acid to NMP, NVP, succinonitrile and acrylonitrile were translated into designs for chemical processes. Based on the knowledge acquired in the syntheses of these products, the processes were defined at a level of moderate detail, sufficient to determine mass and energy balances for the production of the different chemicals, and for an estimation of the process costs. The goal of this was not to provide process details at the level of industrial implementation, but to provide an indication of potential strengths and weaknesses in the processes, a guideline to where further improvement should be directed at, and an idea of potential feasibility of these processes at an industrial scale.
The conclusion was that the production of NMP and NVP from glutamic acid was expected to be feasible both in terms of technology and economy. Although some assumptions were rough, an estimated cost-benefit margin of about 50 % shows that there is sufficient room to maneuver within the process.
Biobased acrylonitrile and succinonitrile did not seem very profitable under the current process configurations. Especially the acrylonitrile process showed very

high costs in relation to the possible gains. It would be necessary to obtain glutamic acid much less expensive than estimated to make this process profitable, which poses constraints on the method of isolation. Moreover, an alternative to the oxidative decarboxylation reaction that was performed with hypochlorite will have to be found, using less expensive oxidizing agents such as hydrogen peroxide or oxygen. Also operation at a higher temperature would be advantageous, in order to benefit from the energy created by the exothermic reaction taking place. For the succinonitrile process, the oxidative decarboxylation reaction appeared to be the main cost driver. Finding an alternative oxidant for sodium hypochlorite would lead to an economically viable process.

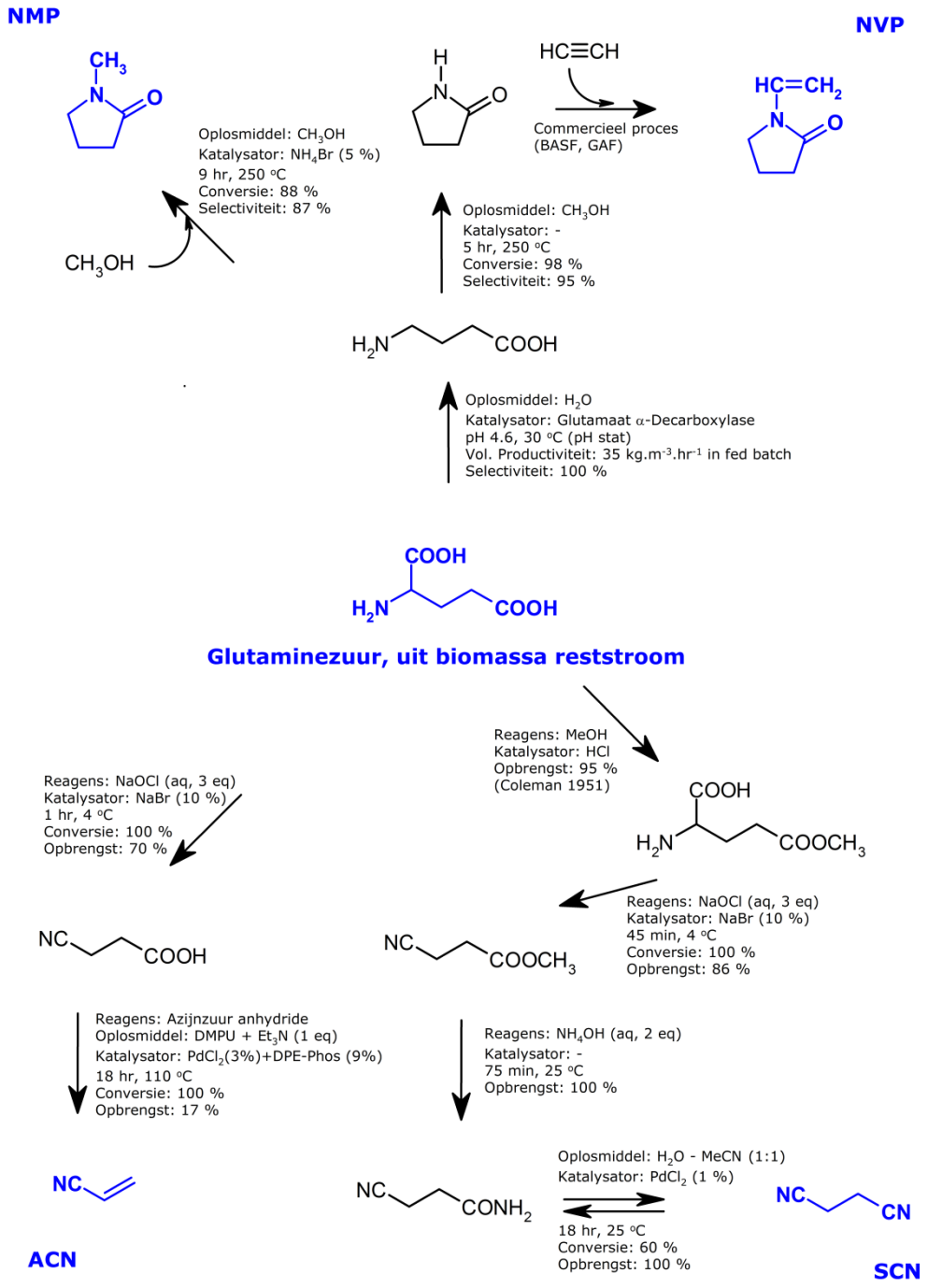
A consequential LCA case study was prepared for chapter 7, wherein glutamic acid was assumed to be obtained from sugarbeet vinasse. The removed glutamic acid was substituted with cane molasses and ureum. The comparison between the four biobased and petrochemical products showed that for NMP and NVP the biobased version had less impact on the environment, while for acrylonitrile and succinonitrile the petrochemical version had less impact on the environment. For the latter two an optimized scenario was computed, which showed that the process for succinonitrile can be improved to a level at which it can compete with the petrochemical process. For acrylonitrile this is more difficult.

In the general discussion (chapter 8) was concluded that the closer the functionalities in the final product are to glutamic acid, the easier it is to design a process that is technologically, economically and environmentally feasible. That is mostly the case for the pyrrolidones, less for succinonitrile and even less for acrylonitrile. For producing all of these molecules it is an advantage that glutamic acid already contains a nitrogen atom, but for producing succinonitrile and acrylonitrile the rest of the modifications that are needed still provide a challenge. Recommendations were given where to aim further research at, in order to further improve the processes that were developed in this thesis. Furthermore it was concluded that finding a cost-effective way to isolate glutamic acid from sources like those discussed in chapter 2 (e.g. vinasse) is of equal or even higher importance.

Samenvatting

De chemische industrie bevindt zich in een overgangsfase van petrochemie naar een meer op biomassa gebaseerde industrie. De hoeveelheid onderzoek die er op dit moment naar de productie van “biobased chemicals” (chemicaliën uit biomassa) gedaan wordt is dan ook enorm. Het grootste deel van dat onderzoek richt zich op koolhydraten, maar de eiwitten die aanwezig zijn in reststromen van bijvoorbeeld de productie van biobrandstoffen kunnen ook waardevolle bouwstenen zijn voor de chemische industrie. Met name de niet-essentiële aminozuren in die eiwitten, die weinig waarde hebben in voedsel voor mens of dier, kunnen interessante bouwstenen zijn voor diverse stikstofhoudende chemicaliën. Uit hoofdstuk 2 van dit proefschrift blijkt dat glutaminezuur (een niet-essentieel aminozuur) in allerlei planteneiwitten het meest voorkomende aminozuur is.

Dit proefschrift richt zich op het vinden van nieuwe, katalytische, en duurzame routes van glutaminezuur naar bulkchemicaliën. Het doel van dit onderzoek was de mogelijkheid te creëren om bij de productie van bepaalde chemicaliën fossiele grondstoffen (steenkool, aardolie, aardgas) te vervangen door glutaminezuur. De toepassing van glutaminezuur als grondstof is vooral waardevol voor chemicaliën die stikstof bevatten, omdat de introductie van stikstof in moleculen zeer veel energie kost vanwege de productie van het benodigde ammoniak. Doordat glutaminezuur de benodigde stikstof al bevat is geen ammoniak meer nodig, wat flinke energiewinst betekent.
De chemicaliën die we voor dit onderzoek ten doel gesteld hebben zijn dan ook stikstofhoudende chemicaliën die in de huidige industriële processen uit fossiele grondstoffen gemaakt worden. Deze stoffen zijn: N-methylpyrrolidon (NMP), N-vinylpyrrolidon (NVP), en 1,4-diaminobutaan (DAB). NMP is een veelgebruikt oplosmiddel en NVP is de bouwsteen voor het polymeer poly(vinylpyrrolidon), dat bijv. toepassingen heeft in lijmen. DAB wordt gebruikt voor de productie van polyamides zoals nylon-4,6. Een ander doel in dit onderzoeksproject was acrylonitril, een bouwsteen voor veel polymeren en rubbers. De synthetische route van glutaminezuur naar acrylonitril is ontwikkeld door Dr. J. Le Nôtre en is daarom niet meegenomen in dit proefschrift.
Figuur S1 vat alle synthetische routes in dit proefschrift samen.



Figuur S1. De chemie van glutaminezuur naar de ten doel gestelde stoffen.

In hoofdstuk 3 wordt de α-decarboxylering van glutaminezuur beschreven, waarbij γ-aminoboterzuur (γ-aminobutyric acid, GABA) gevormd wordt. Deze reactie is uitgevoerd met behulp van het enzym glutaminezuur α-decarboxylase (GAD). Om een inschatting te kunnen maken van de industriële toepasbaarheid van dit enzym is de operationele stabiliteit bepaald. Door het enzym te immobiliseren is de stabiliteit verhoogd. De condities met de hoogste GABA opbrengst per gram enzym zijn bepaald door extrapolatie van de gevonden waarden voor de stabiliteit van GAD. Een "fed batch" proces op 30 ^{o}C levert de hoogste opbrengst, bij een gemiddelde volumetrische productiviteit van 35 kg $m^{-3}h^{-1}$. De kosten voor het gebruik van GAD geïmmobiliseerd in calcium alginaat onder die omstandigheden is geschat op 5 euro per ton geproduceerd GABA.
De conclusie is dat de toepassing van geïmmobiliseerd GAD voor de industriële productie in een fed batch reactor met op labschaal verzamelde data haalbaar is, en dat de volgende stap opschaling is. Er is nog ruimte voor verbetering. Experimenten die uitgevoerd zijn met mutanten van GAD lieten de mogelijkheid zien om deze reactie bij een hogere pH uit te voeren, waarbij glutaminezuur beter oplosbaar is. Dat zou kunnen leiden tot een nog hogere volumetrische productiviteit.

De volgende stap, beschreven in hoofdstuk 4, was de ringsluitingsreactie van GABA, waarbij 2-pyrrolidon gevormd wordt. Pyrrolidon kan daarna gemethyleerd worden tot NMP. Dit hoofdstuk laat zien dat het mogelijk is om de ringsluiting en de methylering met methanol samen uit te voeren in één reactor. Dit is gedaan met een katalytische hoeveelheid van een halogeenhoudend zout zoals ammoniumbromide. Het voordeel hiervan boven conventionele methyleringsprocedures zoals die met methyljodide of dimethylsulfaat is dat door het gebruik van methanol water het enige bijproduct is dat stoichiometrisch gevormd wordt. Dat maakt het proces milieuvriendelijker.
Er is zowel een hoge conversie (87 %) als een hoge selectiviteit (88 %) behaald voor deze reactie, waarmee er een directe route van glutaminezuur naar NMP ontwikkeld is.
Indien de reactie van GABA naar NMP halverwege gestopt wordt, kunnen het tussenproduct 2-pyrrolidon en het eindproduct NMP eenvoudig gescheiden worden door middel van vacuümdestillatie. Aangezien NVP door BASF en anderen al uit

2-pyrrolidon wordt gemaakt, betekent dit dat er nu ook een directe route is naar biobased NVP.

Hoofdstuk 5 beschrijft de synthese van succinonitril uit glutamine en glutaminezuur, via het tussenproduct 3-cyanopropionamide (CPAm). CPAm kan direct uit glutamine gemaakt worden via een oxidatieve decarboxyleringsreactie met natriumhypochloriet, in de aanwezigheid van een katalytische hoeveelheid natriumbromide. CPAm kan in drie stappen uit glutaminezuur gemaakt worden: 1) mono-esterificatie met methanol tot glutaminezuur-5-methylester (Glu-Me); 2) oxidatieve decarboxylering met natriumhypochloriet en een katalytische hoeveelheid natriumbromide tot 3-cyanopropionzuurmethylester (CPA-Me); 3) amidering van de estergroep met ammoniak tot CPAm.
Aangezien de eerste stap in de literatuur beschreven staat als een simpele procedure met een 95 % opbrengst zijn hier alleen de tweede en derde stap onderzocht. De opbrengst van ruwe CPAm vanuit glutaminezuur was duidelijk hoger (86 %) dan die vanuit glutamine (56%).
Dehydratatie van CPAm, waarbij succoninitril gevormd wordt, is onder verschillende omstandigheden onderzocht. Het meest effectieve systeem was een door palladium(II) gekatalyseerde wateroverdrachtsreactie, met acetonitril als het molecuul dat het water opneemt. Onder de geteste omstandigheden werden er geen bijproducten gevonden, wat erop wijst dat de selectiviteit van de reactie dicht tegen de 100 % ligt. Doordat het een evenwichtsreactie is was de conversie maar 62 %.
Dit kan een interessant alternatief zijn voor de fossiele productieroute van 1,4-diaminobutaan (DAB), die ook succinonitril als tussenproduct heeft. Het grote voordeel van deze route boven biotechnologische alternatieven die ook biobased zijn, is dat succinonitril veel minder oplosbaar is in water dan DAB. Het kan daardoor op eenvoudige wijze uit water geëxtraheerd worden met een organisch oplosmiddel zoals ethylacetaat. Dit kan de hoeveelheid energie die nodig is voor het opzuiveren van het product drastisch verlagen.

In hoofdstuk 6 worden de synthetische routes van glutaminezuur naar NMP, NVP, succinonitril en acrylonitril vertaald naar ruwe procesontwerpen. Gebaseerd op de opgebouwde kennis in de synthese van deze producten, zijn de processen gedefinieerd op een detailniveau dat voldoende was om massa- en energiebalansen

op te stellen voor de productie van de verschillende chemicaliën. Hiermee kon een schatting van de proceskosten gemaakt worden. Het doel hiervan was niet om een procesontwerp te maken op het niveau van industriële implementatie, maar een uitgangspunt te creëren van waaruit sterktes en zwakheden van de processen bepaald konden worden, zodat duidelijk zou worden waar verbeteringen op gericht moeten zijn en of de processen haalbaar zouden zijn op industriële schaal.
De conclusie is dat de productie van NMP en NVP uit glutaminezuur zowel qua technologie als qua economie mogelijk moet zijn. Ook al zijn sommige van de aannames vrij ruw, binnen de marge tussen kosten en opbrengst van 50 % is er voldoende ruimte voor de onzekerheden in het proces.
Biobased acrylonitril en succinonitril lijken niet erg winstgevend te zijn onder de huidige procesconfiguraties. Vooral in het acrylonitrilproces zijn de verwachte kosten een stuk hoger dan de opbrengst. Onder andere het glutaminezuur zou goedkoper geproduceerd moeten worden om dit proces rendabel te maken. Verder moet er een voordeliger alternatief voor de reactie met hypochloriet gevonden worden, en zou het beter zijn als de oxidatieve decarboxylering op een hoge temperatuur uitgevoerd zou kunnen worden, zodat de exotherme reactie energiewinst oplevert. Voor het succinonitrilproces is de reactie met hypochloriet de grootste kostenpost, en een reductie van de kosten daar zou leiden tot een rendabel proces.

Van het scenario waarin glutaminezuur eerst uit suikerbietenvinasse gehaald word en er vervolgens chemicaliën van worden geproduceerd, is een levenscyclusanalyse (LCA) gemaakt die staat beschreven in hoofdstuk 7. In deze consequentiële LCA wordt er vanuit gegaan dat het uit de vinasse verwijderde glutaminezuur gesubstitueerd wordt met rietmelasse en urea.
De vergelijking tussen de vier biobased chemicaliën en hun petrochemische equivalenten liet zien dat voor NMP en NVP de biobased versie de minste impact heeft op het milieu, terwijl voor acrylonitril en succinonitril de petrochemische versie minder impact heeft. Voor de laatste twee is ook een geoptimaliseerd scenario doorgerekend, dat liet zien dat het proces voor biobased succinonitril verbeterd kan worden tot een niveau waar het beter is dan zijn petrochemische equivalent. Voor acrylonitril is dat moeilijker.

In de algemene discussie (hoofdstuk 8) wordt geconcludeerd dat hoe dichter de functionaliteiten in het eindproduct bij die van glutaminezuur liggen, hoe gemakkelijker het is een proces te ontwerpen dat technologisch haalbaar is, en bovendien economisch rendabel en beter voor het milieu. Dat is vooral het geval voor de pyrrolidonderivaten, in wat mindere mate voor succinonitril en in nog mindere mate voor acrylonitril. Voor al deze eindproducten heeft glutaminezuur als grondstof het voordeel dat het al een stikstofatoom bevat, maar voor succinonitril en acrylonitril zijn de rest van de modificaties die nodig zijn nog een uitdaging.
In hoofdstuk 8 worden aanbevelingen gedaan waar vervolgonderzoek op gericht zou moeten zijn om deze processen verder te verbeteren. Verder wordt er geconcludeerd dat een kosteneffectieve methode om glutaminezuur uit reststromen te winnen essentieel is om dit alles mogelijk te maken.

Acknowledgments

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Dankwoord

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About the author

Curriculum Vitae

Tijs Merijn Lammens was born in Amersfoort on August 12, 1981, and grew up in Apeldoorn. There he finished secondary school (Stedelijk Gymnasium Apeldoorn), after which he moved to Enschede in 1999, to study chemical engineering at Twente University.

To get his MSc. degree with a major in organic chemistry, he did a research project about mannose-functionalized polymer brushes for biosensors in the polymer chemistry group of prof. Wilhelm Huck at Cambridge University in the UK, and wrote a MSc. thesis about poly(amino ester)s for gene delivery in the biomedical chemistry group of prof. Johan Engbersen at Twente University.

After Tijs graduated he went to Brazil to do a traineeship in the R&D department of Prati, Donaduzzi, a pharmaceutical company in Toledo, Paraná. Back in the Netherlands he started the research for this thesis in February 2007 at Wageningen University, in the Valorisation of Plant Production Chains group of prof. Johan Sanders, in collaboration with the Laboratory of Organic Chemistry. Afterwards he spent a few months in Nigeria, working as a volunteer for DADTCO, for which he set up a quality assurance laboratory for tapioca flour in Jalingo, Taraba state.

Publications

T.M. Lammens, D. De Biase, M.C.R. Franssen, E.L. Scott, J.P.M. Sanders, 'The application of glutamic acid α-decarboxylase for the valorization of glutamic acid', *Green Chemistry* **2009**, 11, p. 1562-1567.

E. Pennacchietti, T.M. Lammens, G. Capitani, M.C.R. Franssen, R.A. John, F. Bossa, D. De Biase, 'Mutation of His465 alters the pH-dependent spectroscopic properties of *Escherichia coli* glutamate decarboxylase and broadens the range of its activity toward more alkaline pH', *Journal of Biological Chemistry* **2009**, 284, p. 31587–31596.

T.M. Lammens, M.C.R. Franssen, E.L. Scott, J.P.M. Sanders, 'Synthesis of biobased N-methylpyrrolidone by one-pot cyclization and methylation of γ-aminobutyric acid', *Green Chemistry* **2010**, 12, p. 1430–1436.

T.M. Lammens, J. Le Nôtre, M.C.R. Franssen, E.L. Scott, J.P.M. Sanders, 'Synthesis of biobased succinonitrile from glutamic acid and glutamine', *ChemSusChem* **2011**, 4, p. 785–791.

T.M. Lammens, J. Potting, J.P.M. Sanders, and I.J.M. De Boer, "Environmental comparison of biobased chemicals from glutamic acid with their petrochemical equivalents", *Environmental Science & Technology* **2011**, 45, p. 8521–8528.

T.M. Lammens, M.C.R. Franssen, E.L. Scott, J.P.M. Sanders, 'Availability of protein-derived glutamic acid as a source for the production of bulk chemicals', *submitted* **2011**.

T.M. Lammens, S. Gangarapu, M.C.R. Franssen, E.L. Scott, J.P.M. Sanders, 'Techno-economic assessment for the production of four biobased chemicals from glutamic acid', *submitted* **2011**.

T.M. Lammens, D. De Biase, M.C.R. Franssen, E.L. Scott, J.P.M. Sanders, 'Enzymatic α-decarboxylation of L-glutamic acid in the production of biobased chemicals', In: Practical Methods in Biocatalysis and Biotransformations, ed. John Whitall, *in press* **2011**.

Overview of completed training activities

Discipline-specific courses

- Socrates Intensive Program on Renewable Resources, INP-Ensiacet, Toulouse, 2007;
- Catalysis, an integrated approach, NIOK, Schiermonnikoog, 2007;
- Renewable resources in the bulk chemical industry, VPP, Wageningen, 2009;
- Advanced organic chemistry, ORC, Wageningen, 2010.

Discipline-specific meetings

- NWO-ACTS-Aspect meetings, Netherlands, 2007-2011;
- NWO organic chemistry meetings, Lunteren, 2009, 2010;
- Netherlands' Catalysis & Chemistry Conferences, Noordwijkerhout, 2008, 2011;
- Netherlands' Process Technology Symposia, Veldhoven, 2008, 2009;
- Biotrans symposia, Oviedo 2007, Bern 2009;
- Strategic Initiative COST workshop, Oostende, 2010;
- Renewable Resources and Biorefineries conferences, Rotterdam 2008, Düsseldorf 2010.

General courses

- PhD competence assessment, WGS, Wageningen, 2007;
- Information literacy, WGS, Wageningen, 2007;
- Afstudeervak begeleiden, WGS, Wageningen, 2007;
- Scientific writing, WGS, Wageningen, 2007;
- Philosophy and ethics of food science, VLAG, Wageningen, 2010;
- Retorica, Studium Generale Twente, Enschede, 2010;
- Entrepreneurial Bootcamp, University of Wisconsin & Dafne, Madison & Wageningen, 2010.

Optional activities

- Biofuels Discussion Group, Wageningen, 2008-2011;
- Preparation of a research proposal, WGS, Wageningen, 2007;
- Biotechnology study trip, Biotechnology/VPP, France, 2008;
- PhD study trip, ORC, China, 2009.

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