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1A limited LCA of Bio-Adipic Acid:

2manufacturing the nylon-6,6 precursor adipic acid using the

3benzoic acid degradation pathway from different feedstocks

4

5Limited LCA of Bio-Adipic Acid

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7J.B.J.H. van Duuren^{1,2,3,5}, B. Brehmer^{4**}, A.E. Mars^{1,5}, G. Eggink^{1,2,5}, V.A.P. Martins dos Santos^{3,5***}, J.P.M. Sanders⁴

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9¹Wageningen UR Food & Biobased Research, P.O. Box 17, 6700AA Wageningen, The Netherlands

10²Wageningen University, Department Bioprocess Engineering, Bomenweg 2, 6781 EV Wageningen, The Netherlands

11³Helmholtz Centre for Infection Research, Systems and Synthetic Biology, Inhoffenstraße 7, 38124 Braunschweig, Germany

12⁴Wageningen University, Valorization of Plant Production Chains, Bornsesteeg 59, 6708PD Wageningen, The Netherlands

13⁵Kluyver Centre for Genomics of Industrial Fermentation, P.O. Box 5057, 2600 GA Delft, The Netherlands

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2*Corresponding author. mailing address: Helmholtz Centre for Infection Research, Inhoffenstrasse 7, 38124 Braunschweig, Germany. Phone +491 315775682698. Fax +49-53164049. E-mail: joost.vanduuren@helmholtz-hzi.de

4**Present address: Evonik Degussa GmbH, Creavis Technologies & Innovation, Paul-Baumann-Strasse 1, 45772 Marl, Germany

5***Present address: Wageningen University, Laboratory of Systems and Synthetic Biology, Dreijenplein 10, 6703 HB Wageningen, The Netherlands

2Abstract

3A limited life cycle assessment (LCA) was performed on a combined biological and chemical
4process for the production of adipic acid, which was compared to the traditional
5petrochemical process. The LCA comprises the biological conversion of the aromatic
6feedstocks benzoic acid, impure aromatics, toluene or phenol from lignin to *cis*, *cis*-muconic
7acid, which is subsequently converted to adipic acid through hydrogenation. Apart from the
8impact of usage of petrochemical and biomass-based feedstocks, the environmental impact of
9the final concentration of *cis*, *cis*-muconic acid in the fermentation broth was studied using
101.85% and 4.26% *cis*, *cis*-muconic acid. The LCA focused on the cumulative energy demand
11(CED), cumulative exergy demand (CExD) and the CO₂ equivalent (CO₂eq) emission, with
12CO₂ and N₂O measured separately. The highest calculated reduction potential of CED and
13CExD were achieved using phenol, which reduced the CED by 29% and 57% with 1.85% and
144.26% *cis*, *cis*-muconic acid, respectively. A decrease in the CO₂eq emission was especially
15achieved when the N₂O emission in the combined biological and chemical process was
16restricted. At 4.26% *cis*, *cis*-muconic acid, the different carbon backbone feedstocks
17contributed to an optimized reduction of CO₂eq emissions ranging from 14.0 to 17.4ton
18CO₂eq/ton adipic acid. The bulk of the bioprocessing energy intensity is attributed to the
19hydrogenation reactor, which has a high environmental impact and a direct relationship with
20the product concentration in the broth.

21

22Keywords

23Adipic acid, Biotechnology, *cis*, *cis*-Muconic acid, Energy, Exergy, LCA

2Introduction

3Ever since its discovery in the late 1930's, nylon-6,6 has been intrinsically linked to the
4production of adipic acid, being the main intermediate reactant. Adipic acid is a high volume
5bulk petrochemical commodity as is reflected in its low selling price, 1315 – 1385\$/ton
6(2008), 1120 – 1190\$/ton (2009) and high consumption volumes, 2.6 Mton worldwide (2006)
7(ICIS Pricing and ICIS News). Adipic acid has several commercial production route options,
8all of which originate from fossil fuels. The most common is the nitric acid oxidation of a
9cyclohexanol/cyclohexanone mixture derived from cyclohexane. Cyclohexane itself
10originates from naphtha benzene and nitric acid originates from ammonia, which in turn is
11most commonly produced from natural gas. N₂O is a byproduct in the process of nitric acid
12oxidation (Shimizu et al., 2000). Adipic acid is thus associated with a high fossil fuel energy
13demand and a high level of N₂O greenhouse gas emission. The cumulative energy demand
14(CED), which includes the total fossil fuel feedstock cost and all subsequent process energy
15inputs, has been reported to be 104.2GJ/ton (Patel, 2003). As a consequence of modern
16catalytic decomposition techniques and tail gas treatment, on average the current state-of-the-
17art will reduce the 301kg/ton N₂O by 80%, which will be selected for comparison in this study
18(EcoInvent Databank V2.1).

19An alternative production route for adipic acid could occur via biological conversion of
20benzoic acid or other aromatics to *cis*, *cis*-muconic acid, which can be easily converted to
21adipic acid by hydrogenation under slightly elevated pressure (3.5bar) and electric potential
22for 3 hours (Draths and Frost, 1993). Several bacteria have been described that convert
23benzoic acid to *cis*, *cis*-muconic acid (Bang and Choi, 1995; Mizuno et al., 1988; Schmidt and

Knackmuss, 1984). Recently a derivative of *P. putida* KT2440 (*P. putida* KT2440-JD1), which can no longer use benzoic acid as a carbon source, but still co-metabolizes benzoic acid to *cis, cis*-muconic acid when grown on glucose was isolated (van Duuren(1) et al., submitted). *P. putida* KT2440 is a soil bacterium with a versatile metabolism. It is able to convert several aromatic compounds, such as derivatives of lignin proceeding from the recycling of plant derived material (Jiménez et al., 2002). Furthermore, the genome has been sequenced, the strain is genetically accessible, and it is the first Gram-negative soil bacterium that was certified as a safety strain by the Recombinant DNA Advisory Committee (Federal register. Certified host-vector systems; Nelson et al., 2002; Ramos et al., 1987). In this respect, it has significant potential for biotechnological applications, particularly those involving bioremediation and biotransformation (Wackett, 2003). One of the most influential parameters in the life cycle performance of chemical production systems is the feedstock (Hatti-Kaul et al., 2006). Various *P. putida* species have been described that can metabolize other aromatic compounds (Feist and Hegeman, 1969; Panke et al., 1998; Tao et al., 2004; Wackett, 2003). Since *P. putida* is genetically accessible, it is likely that *P. putida* KT2440-JD1 can be modified in such a way that it can convert the petrochemical feedstocks benzoic acid, toluene, and impure aromatics (e.g. collected from waste of the benzene, toluene, xylene (BTX) process), as well as phenol from the biomass-based feedstock lignin to *cis, cis*-muconic acid (Haigler et al., 1992).

P. putida KT2440-JD1 has the highest measured specific production rate of *cis, cis*-muconic acid (van Duuren(1) et al., submitted). The highest final concentration of *cis, cis*-muconic acid that has been achieved was 4.4% in a bioprocess with a fed-batch process using *Arthrobacter* sp. (Mizuno et al., 1988). A final concentration of *cis, cis*-muconic acid of 1.85% with *P. putida* KT2440-JD1 during a small-scale pH-stat fed-batch process was

1reached. At this concentration, benzoic acid was only converted to the intermediate catechol;
 2known to be toxic to cells (van Duuren(2) et al., submitted). It may be possible to optimize
 3this conversion leading to a higher final concentration of *cis*, *cis*-muconic acid, because
 4benzoic acid can be converted by *P. putida* KT2440-JD1 in the presence of up to 4.26% of
 5*cis*, *cis*-muconic acid (vanDuuren(2) et al., submitted).

6In this study, the overall goal was to assess the possibility to reduce the environmental impact
 7of the current petroleum-based adipic acid production by comparing different carbon
 8backbone feedstocks (petrochemical and biomass-based) for the combined biological and
 9chemical production of adipic acid using a limited life cycle assessment (LCA). Moreover, the
 10environmental impact of an increase in the final concentration of *cis*, *cis*-muconic acid was
 11studied at two final fermentation broth concentrations of 1.85% (option 1) and 4.26% (option
 122). At 104.2GJ/ton, 60.2kgN₂O/ton and 5.1tonCO₂/ton there is a large potential to reduce the
 13environmental impact of adipic acid production by employing bio-processing techniques
 14based on aqueous solutions.

15

16**Methodology**

17*Biological production of cis, cis-Muconic Acid*

18*cis*, *cis*-Muconic acid has been produced by *P. putida* KT2440-JD1 in a small-scale pH-stat
 19fed-batch process using benzoic acid as a feedstock (van Duuren(2) et al, submitted). The data
 20rendered from this study were used in the limited LCA as a model process regarding the
 21assessment of conditions of biological production systems.

22*Release of N₂O in the Petrochemical Industry*

Catalytic techniques to decompose N_2O to elemental nitrogen and oxygen (an exothermic reaction) operate at elevated temperatures and can effectively reduce the overall N_2O emission and if the decomposition of the tail gas is performed under adiabatic conditions the heat of the reaction will continue the decomposition to a total emissions reduction of 99.9% (Shimizu et al., 2000). In these recently outfitted production plants the resulting emission of N_2O can be as low as 0.3kg per ton of adipic acid (EcoInvent Databank V2.1). On a worldwide scale, only an average of 80% N_2O emission reduction is actually realized with these tail-gas abatement technologies. In this respect, the production of adipic acid is still associated with for more than 44.8Mton CO_2 equivalent (CO_2eq) or about 60.2kg N_2O emission per ton of adipic acid. This average will be used to denote the current state-of-the-art reduction potential in this study.

LCA Goal and Scope

The standard methodology of LCA based on the ISO14040 series for environmental management systems was followed to a great extent with the exception that only the CED and cumulative exergy demand (CExD) were documented with the CO_2eq emission (CO_2 and N_2O measured separately) as the only environmental considerations (International Organization for Standardization, 2006). This specific setup of LCA was performed due to the interest of the study. The apparent environmental impact, namely the reduction of fossil fuel and greenhouse gas emissions of the compared production routes was to be assessed. Exergy, the quality of energy or maximum obtainable work, was mainly included to provide further depth in the energy efficiency of the processes and as an indication of future energy efficiency improvements. The cradle was set at the point of base fossil fuel delivery (naphtha, diesel, natural gas, etc.) including their respective extraction values. The grave was set at the factory gate for nylon-6,6 production, essentially the delivery point of a pure adipic acid. The

boundary was effectively confined to the process. This particular LCA technique is called an exergetic life cycle assessment (or E-LCA), but is in essence a limited exergetic cradle-to-factory gate assessment.

Data and System

GaBi and its associated datasets were used to accumulate life cycle inventory (LCI) data (PE International, GaBi 4). It is vital to set realistic and comparable factory gates in the LCI. The carbon contained in feedstocks of naphtha products from the petrochemical route is not yet released into the environment but is contained in the chemical end product. With this perspective, the analysis was partially taken beyond the factory gate and to the grave, when decades or centuries from now the nylon is presumably disintegrated or incinerated. Only at this stage will the petrochemical material feedstocks receive an extra emission charge, which was already incorporated according to common LCA practice. As the final products are identical, any other emissions associated with combustion would be the same and cancel each other out.

Bioprocessing Demands

Aspen⁺ was used to calculate the internal process energy requirements of the experimental setup. Figure 1 highlights the system, unit operations and input streams involved. Two final broth concentrations of *cis*, *cis*-muconic acid of 1.85% (option 1) and 4.26% (option 2) were studied. Several process considerations were made in light of the experimental bioreactor, hydrogen reactor, and the succeeding separation and product isolation techniques:

21

- 22 • “FERMENT” – Bioreactor: 30°C, 1bar and 51 hours residence time
- 23 • “HYDROGEN” – Hydrogenation reactor: 30°C, 3.5bar, 3h and electrically charged

- 1 • “EVAPORAT” – Evaporator: 100°C, 3.5bar to remove water
- 2 • “CONDENSE” – Condenser: 20°C, 1bar to recover a large portion of the evaporator
- 3 duty
- 4 • “CRYSTAL” – Crystallizer: 50°C, 1bar to isolate product

5

6The heat streams were based on a simple fuel boiler system that has a heating efficiency of
 785% energy and 45% exergy (Szargut et al., 1988). The hydrogenation reactor is pressurized
 8and electrically charged having an overall electric production efficiency mix of 45% energy
 9and 35% exergy.

10*Feed-Level Demands for Bacterial Growth*

11For each mol *cis, cis*-muconic acid produced, 2 mol sodium hydroxide, and 2.28 mol (option
 121) or 2.12 mol (option 2) hydrogen chloride were incorporated to maintain the fermentation
 13broth at pH 7 during the fermentation and to acidify the fermentation broth to pH 2.5. Benzoic
 14acid and *cis, cis*-muconic acid dissociate in water at pH 7 to the more soluble form of
 15benzoate and *cis, cis*-muconate. The various streams flowing into the bioreactor need to be
 16handled individually to assess their CED, CExD and emissions levels:

17

- 18 • Salts, nutrients and buffer: based on best available technology of the fertilizer industry
- 19 (Brehmer et al., 2008; International Fertilizer Association)
- 20 ○ Salts: $\text{MgCl}_2 \cdot \text{H}_2\text{O}$ – dolomite limestone mining (Brehmer(1), 2008; US
- 21 Department of Energy)
- 22 ■ CED: 0.1GJ/ton, CExD: 0.2GJ/ton
- 23 ■ CO_2 : 5.4kg/ton, N_2O : 0.0kg/ton

Figure 2 is a simple process diagram of the steps involved in glucose production with the following results:

3

• Glucose: based on sucrose content of sugar beets for glucose production in Holland (Brehmer(2), 2008; Ministry of Agriculture Nature and Food Quality)

▪ CED: 8.3GJ/ton, CExD: 8.7GJ/ton

▪ CO₂: 508.0kg/ton, N₂O: 23.3kg/ton

8

9Bioreactor-Feedstock Demands

Four different potential feedstocks were used in the LCA: benzoic acid, impure aromatics, and toluene are petrochemical based and phenol from lignin is biomass based. Since these feedstocks have different energetic and exergetic values and various sources of origin, along with the additional process energy needed to acquire the fraction, their CED, CExD and CO₂eq emission values must be assessed separately.

To obtain benzoic acid and toluene, oil production and refinery are taken into account. Furthermore, as described the stoichiometric carbon content was included in the carbon dioxide emission calculations. An exergy ratio of 1.05 was adapted for the naphtha-based chemicals benzoic acid and toluene, which corresponds to the standard ratio of chemical energy/exergy of naphtha products.

Impure aromatics have the potential to be converted to *cis*, *cis*-muconic acid by *P. putida* KT2440-JD1. These compounds (e.g. benzoic acid) are primarily collected during the BTX purification process. Therefore impure aromatics are considered a major waste product, as they are an uneconomical residue from the distillation of crude oil. Being a waste product, no additional processing inputs are allocated to their production. Consequently, impure aromatics

1 have a CED and CExD demand coupled directly to their chemical formation energy/exergy
2 and no extra CO₂eq emissions are considered.

3 Lignin is a special case being a highly complex molecule. Cleaving lignin via biological
4 delignification (white rot fungi) will release countless different types of aromatic molecules.
5 Due to the vast quantity and diversity of the molecules, economic delignification and isolation
6 techniques are currently unavailable. In any case, phenol derived from lignin is present in
7 higher concentrations and will receive its own theoretical energetic process burden based on
8 its chemical formation energy/exergy and associated agricultural system.

9 The potential feedstocks are:

10

- 11 • Benzoic acid: derived from oxidized toluene (Patel, 2003; Szargut et al., 1988)
 - 12 ▪ CED: 62.4GJ/ton, CExD: 65.5GJ/ton
 - 13 ▪ CO₂: 3735.0kg/ton, N₂O: trace ≈ 0.0kg/ton
- 14 • Impure aromatics: derived from an oil cracker (Internal calculations)
 - 15 ▪ CED: 25.4GJ/ton, CExD: 27.4GJ/ton
 - 16 ▪ CO₂: 0.0kg/ton, N₂O: 0.0kg/ton
- 17 • Toluene: derived from benzene (Panke et al., 1998; Patel, 2003; Szargut et al., 1988)
 - 18 ▪ CED: 56.4GJ/ton, CExD: 59.2GJ/ton
 - 19 ▪ CO₂: 4190.0kg/ton, N₂O: trace ≈ 0.0kg/ton
- 20 • Phenol derived from lignin: derived from wheat stover grown under Dutch conditions
21 (Brehmer(1), 2008; Brehmer(2), 2008)
 - 22 ▪ CED: 11.2GJ/ton, CExD: 13.8GJ/ton
 - 23 ▪ CO₂: 682.0kg/ton, N₂O: 3.9kg/ton

2Limitations

3For the conversion of benzoic acid to *cis*, *cis*-muconic acid no net consumption of co-factors
4like NADH are required, while for each mol toluene or phenol one mol NADH is produced or
5consumed, respectively. Hence the biomass yield (g dry cell weight (dcw)/g glucose) might
6increase with toluene and decrease with phenol. This hypothesis and its impact on the overall
7system dynamics were not taken into account. Furthermore, other aromatic feedstocks are also
8foreseeable, but were not assessed.

9Apolar compounds such as phenol and toluene are generally not well soluble in water and
10often toxic to whole cells as they accumulate in the cytoplasmic membrane. As a consequence
11the membrane loses its integrity and increases its permeability (Ramos et al, 2002). Technical
12solutions to this problem are two-liquid-phase media, gas-phase biocatalysis and feeding
13under controlled conditions such as the pH-stat fed-batch process (Schmid et al., 2001). For
14the LCA, the pH-stat fed-batch process is the only biological production system considered.
15Residual minerals can be separated from the product by (re-)crystallization from methanol
16(Mizuno et al., 1988). This option and the impact on the overall system dynamics was
17however not taken into account.

18

19Calculations

20Bioreactor System

21In the small-scale pH-stat fed-batch process a molar production yield (mol *cis*, *cis*-muconic
22acid/mol benzoic acid) of 96% was achieved with *P. putida* KT2440-JD1 (van Duuren(2)

1et al., submitted). This is further assumed for all four different feedstocks, as they are all
2similar aromatic compounds that can be converted to *cis*, *cis*-muconic acid.

3*Energy and Emission*

4Aspen⁺ lists the resulting energy duties of the internal bioprocessing in terms of flows, i.e.
5GJ/h. Consequently, the bioprocessing demands in CED, CExD and CO₂eq emission values
6per ton pure adipic acid can be calculated for both final broth concentrations of *cis*, *cis*-
7muconic acid and are presented in Table I. The feed-level demands for growth, maintenance,
8and pH regulation are listed in Table II for the bioreactor at both final broth concentrations of
9*cis*, *cis*-muconic acid per ton pure adipic acid, which provide the basis for calculating the
10associated CED, CExD and CO₂eq emission values. Hereby it is assumed that for option 2 the
11duration and medium consumption is similar, independent of the different specific
12productivity and final concentration of *cis*, *cis*-muconic acid. Furthermore, for each of the
13four feedstocks based on the stoichiometric conversion rate and respective feed level per final
14product, the associated CED, CExD and CO₂eq emission per ton pure adipic acid were
15calculated (Table III). Figure 3 illustrates the resulting potential CED, CExD and CO₂eq
16emissions induced for option 1 and 2 compared to the traditional petrochemical production
17route of adipic acid. As described, the greenhouse gas emissions are combined and expressed
18as the total CO₂eq emission, but special consideration should be paid to the major reduction
19potential N₂O.

20

21**Results and Discussion**

22*Reduction of Fossil Fuel and Related Greenhouse Gas Emission*

1The production of *cis*, *cis*-muconic acid from benzoic acid by *P. putida* KT2440-JD1 in a pH-
2stat fed-batch process (van Duuren(2) et al., submitted) was used as a model system for the
3limited LCA of a combined biological and chemical process for the production of adipic acid.
4The data in Table II and III reveal that glucose, sodium hydroxide and the feedstock for the
5carbon backbone of *cis*, *cis*-muconic acid play important roles in the total fossil fuel energy
6demand and greenhouse gas emission of the system. For the low final product concentration
7of option 1, use of the chemical pure laboratory benzoic acid feedstock did not contribute to
8an environmental impact decrease (Figure 3). Compared to benzoic acid, impure aromatics
9led to an increase in the overall fossil fuel reduction potential: for option 1 from -17.8GJ/ton
10and -35.3GJ/ton to 15.4GJ/ton and -1.2GJ/ton CED and CExD, respectively and for option 2
11from 10.7GJ/ton and 2.0GJ/ton to 43.9GJ/ton and 36.2GJ/ton CED and CExD, respectively.
12This reveals the huge potential of employing impure aromatics over benzoic acid as a
13feedstock. The robustness and productivity of *P. putida* KT2440-JD1 with impure aromatics
14however remains to be determined. The other pure petrochemical feedstock toluene has a
15neutral fossil fuel reduction potential for option 1 at 0.0GJ/ton CED and -16.7GJ/ton CExD.
16The most sustainable option would be using the biobased material lignin for phenols: for
17option 1 and 2 the highest calculated reduction potential of CED (30.4 and 58.9 GJ/ton
18respectively) and CExD (13.8 and 51.1 GJ/ton respectively) are achievable. When phenol
19from lignin is used as a carbon source the CED would decrease 29% (30.4 GJ/ton) for option
201 and 57% (58.4 GJ/ton) for option 2 compared to the traditional petroleum-based production
21route. As the biological delignification and isolation techniques improve, a switch to a lignin-
22based feedstock is foreseeable (Chakar and Ragauskas, 2004).
23For both product concentrations the CO₂eq emissions are reduced for all of the different
24carbon backbone feedstocks (petrochemical and biomass-based). For option 2 the reduction is

1higher, which relates to a lower consumption of glucose and ammonium sulphate per ton
 2product. A reduction in CO₂eq emission can especially be achieved when the N₂O emission is
 3also restricted in the combined biological and chemical process for the production of adipic
 4acid (Figure 3). As a first option, it might be advantageous to use the protein rich co-product
 5slurry of the glucose production to offset ammonium sulphate of the feed-level demands. This
 6would reduce the N₂O emissions of the feed-level demands for ammonium sulphate to
 7practically zero, achieving a decrease of an average of 47% or 38% of the CO₂eq emissions
 8for option 1 and 2, respectively. Therefore, in Figure 3 the optimized CO₂eq extinction is
 9added representing these calculations. For option 1 and 2, the optimized CO₂eq emissions
 10concern a reduction in the magnitude ranging from 9.8 to 13.1 and 14.0 to 17.4 ton CO₂eq/ton
 11adipic acid, respectively.

12Increasing the final product concentration of *cis, cis*-muconic acid in the bioreactor will result
 13in a reduction of glucose and NPK per ton product reducing the environmental impact,
 14assuming that the requirements of *P. putida* KT2440-JD1 for growth and production are
 15constant. By increasing the final concentration less water is present, which directly results in
 16less bioprocessing energy per ton adipic acid. The required energy is directly proportional to
 17the concentration of *cis, cis*-muconic acid in the broth. It is imperative that any future
 18biotechnological work should aim for the highest solid loading possible. The bulk of the
 19bioprocessing energy is attributed to the hydrogenation reactor, therefore this effect is greater
 20for this process step than any other unit. As shown in option 2 with 4.26% *cis, cis*-muconic
 21acid, the energy required for the hydrogenation was 15.1GJ/ton whereas in option 1 with only
 221.85% 35.1GJ/ton was required (Table I). In option 2 together with the lower feed-level
 23demands this reduced the bioprocessing energy by more than 50%, which even facilitated an
 24energy mitigation potential for benzoic acid. A further doubling of the final product

1concentration to beyond 8% would result in a highly competitive system, which might be
 2feasible since *P. putida* KT2440-JD1 can grow in mineral medium up to 8.7% *cis, cis*-
 3muconic acid (van Duuren(2) et al., submitted). Alternatively, the high energy intensity of the
 4hydrogenation-evaporation process of the *cis, cis*-muconic acid-containing fermentation broth
 5could be reduced by extracting *cis, cis*-muconic acid from the acidified broth with e.g. cold
 6diethyl ether (Schmidt and Knackmus, 1984). This will result in a much higher concentration
 7of *cis, cis*-muconic acid in cold diethyl ether, and the evaporation will require much less
 8energy since the boiling temperature of cold diethyl ether (34.6°C) is much lower than water.
 9Furthermore, the solvent used for extraction purifies the product from salts and should have a
 10high extraction efficiency, since this has a large influence on the environmental impact.

11*Biological Process*

12Compared to existing small-scale biological production systems (Bang and Choi, 1995;
 13Mizuno et al., 1988; Schmidt and Knackmuss, 1984), the small-scale pH-stat fed-batch
 14process with *P. putida* KT2440-JD1 has allowed for a further optimization of the biological
 15production of *cis, cis*-muconic acid (van Duuren(2) et al., submitted). The volumetric
 16productivity (g/L) of the process could be further improved by applying a cell-recycle system,
 17since this will increase the cell density. Choi et al. (1997) were the first who addressed the
 18cell-recycling issue for *cis, cis*-muconic acid production, and reached a volumetric
 19productivity of 5.5 g/(L·h). Unfortunately, they were unable to maintain a continuous recycle
 20system for more than 2 days due to membrane fouling. By combining the pH-stat fed-batch
 21bioprocess with a cell retention system, the volumetric productivity of a cell-recycle
 22bioreactor is expected to increase significantly, assuming that the pH-control still is accurate
 23at high cell densities. While this only has a minor impact on the energy consumption (Table I

1(bioreactor)), it does have a major impact on the economic parameters of an industrial scale
 2bio-reactor. Another positive effect is that recycling of biomass would reduce the
 3consumption of glucose and NPK, which lowers the environmental impact of the bioprocess.
 4To retain the cells, new process technology in the form of membrane separation must first be
 5resolved with cell fouling stipulating the main hurdle.

6

7**Conclusions of the Limited LCA**

8The environmental impact concerning the CED, CExD and CO₂eq emission of the
 9manufacturing of the nylon-6,6 precursor adipic acid can be reduced by replacing the current
 10petroleum-based chemical process with a combined biological and chemical process that is
 11based on a small-scale pH-stat fed-batch bioprocess to produce *cis*, *cis*-muconic acid using *P.*
 12*putida* KT2440-JD1 (van Duuren(2) et al., submitted). The usage of different carbon
 13backbone feedstocks (both petrochemical and biomass-based), the final product concentration
 14in the fermentation broth, and the level of N₂O emission have a large influence on the
 15environmental impact. At a final concentration of 1.85% *cis*, *cis*-muconic acid in the
 16fermentation broth, the CED is only improved when using impure aromatics or phenol
 17derived from lignin. Should the bioprocess be further optimized to increase the final
 18concentration of *cis*, *cis*-muconic acid to 4.26%, the CED and CExD are reduced for all the
 19assessed feedstocks (benzoic acid, impure aromatics, toluene, and phenol). Additionally, the
 20CO₂eq emissions for option 2 are reduced more as for option 1. In order to further reduce the
 21CO₂eq emission compared to the petrochemical process, it is assumed that the N₂O emission
 22can be actively decreased in the combined biological and chemical process by using the

1protein rich co-product slurry of the glucose production to offset ammonium sulphate of the
2feed-level demands. The highest reductions were achieved with impure aromatics and phenol.
3Several possibilities exist to improve the environmental and economical impact of the
4bioprocess, like i) improvements of *P. putida* KT2440-JD1 by enhancing the catechol 1,2-
5dioxygenase activity, reducing the sensitivity of the enzyme to putative product inhibition by
6*cis*, *cis*-muconic acid in the cell, increasing the possibly limiting transport of *cis*, *cis*-muconic
7acid through the cell membrane, and extension of the range of aromatic feedstocks that can be
8co-metabolized to *cis*, *cis*-muconic acid; ii) enhancement of the volumetric productivity of the
9bioprocess and reduction of the demand for nutrients by recycling the biomass; iii) reduction
10of the energy demand of the hydrogenation-evaporation process by extraction of *cis*, *cis*-
11muconic acid from the fermentation broth with e.g. diethyl ether.

12A huge potential for employing impure aromatics has been revealed as well as for phenol
13derived from the biological delignification and isolation techniques. Yet, the robustness and
14productivity of *P. putida* KT2440-JD1 with impure aromatics remains to be determined, while
15the biological delignification and isolation techniques have to be improved considerably
16before a switch to a lignin-based feedstock is foreseeable.

17

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