ARTICLE IN PRESS

Bioresource Technology xxx (2011) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Materials flow modeling of nutrient recycling in biodiesel production from microalgae

Christine Rösch *, Johannes Skarka, Nadja Wegerer

Institute for Technology Assessment and Systems Analysis (ITAS), Karlsruhe Institute of Technology, P.O. Box 3640, 76021 Karlsruhe, Germany

ARTICLE INFO

Article history:
Received 6 October 2011
Received in revised form 2 December 2011
Accepted 3 December 2011
Available online xxxx

Keywords:
Microalgae
Biodiesel
Nutrient balance
Residual biomass use

ABSTRACT

Biodiesel production based on microalgae as feedstock is associated with a high demand of nutrients, respectively nitrogen and phosphorus. The production of 1 l biodiesel requires between 0.23 and 1.55 kg nitrogen and 29–145 g of phosphorus depending of the cultivation conditions for microalgae. The supply of nutrients can be expected to severely limit the extent to which the production of biofuels from microalgae can be sustainably expanded. The nutrient demand can be reduced if the nutrients in the residual algae biomass after oil extraction are reused for algae cultivation. This modeling work illustrates that for the investigated process chains and scenarios the nutrient recycling rates are in the range from 30% to 90% for nitrogen and from 48% to 93% for phosphorus. The highest recycling values can be achieved by hydrothermal gasification of the oil-free residues.

1. Introduction

Rising oil prices, global warming, and emphasis on renewable energy are attracting contemporary, global interest in biodiesel derived from microalgae as it holds the potential to provide a biomass feedstock without adversely affecting the supply of food. Microalgae are a novel aquatic biomass systems with higher fuel yield potential and lower water demand than traditional energy crops (Posten and Schaub, 2009). While terrestrial plants in temperate climates can achieve a photoconversion efficiency of only below 1%, microalgae can convert up to 5% of the solar energy into chemical energy (Schenk et al., 2008). The enormous capability of algae for the generation of liquid transportation fuels, respectively high-grade jet fuels, is due to the potential of microalgae for producing high concentrations of lipids.

Besides high productivity and oil contents, microalgae provide various potential advantages compared with traditional oil crops, such as rapeseed or oil palms. They are cultivated in ponds or photobioreactors (PBRs) and thus don't compete for arable land and have less negative effects on the environment while their productivity can be much greater. Theoretically microalgae could produce between 10- and 100-fold more oil per acre. However, such capacities have not been validated on a large-scale basis.

To realize these high yields large quantities of nutrients (nitrogen and phosphorus) are needed. This nutrient demand can lead to a non-sustainable development as the production of nitrogen from air is a highly energy demanding process. World phosphate reserves are dwindling in amount and quality and it is assumed that phosphate rock production will peak in around 50–100 years and then decrease as reserves are depleted (Cordell et al., 2009). Moreover, it has been shown in life-cycle assessments, that fertilizers consumption besides harvesting and oil extraction from algae represents a high energy debt which might jeopardize the overall interest of algal biofuel (Clarens et al., 2010; Lardon et al., 2009). Thus, the sustainable supply of nutrients for the production of biofuels from microalgae is of great importance in terms of economics, resource depletion and environmental protection.

Wastewater derived from municipal, agricultural and industrial activities is a source of nutrients for microalgae cultivation that could significantly reduce the operational costs of algal production systems (Lardon et al., 2009). The use of wastewater could reduce nutrient addition for nitrogen and phosphorous by approximately 55% (Yang et al., 2011). For high-lipid algal biomass production the use of waste nutrients could be problematic because the selective enrichment of high-oil algae species could be hampered by the contamination with native algae species and bacteria that are abundant in untreated wastewater and more competitive than the target oil producing algae. Another sustainable option to decrease the demand of nitrogen and phosphorus for microalgae cultivation is nutrient recycling by the reuse of nutrients in the residual algal biomass after oil extraction. This study quantitatively investigates the nutrient demand for different generic algae and cultivation conditions as well as the nutrient recycling rate of biodiesel production for three paths to process the algal residues after the oil extraction: anaerobic digestion, hydrothermal gasification and animal feed production.

---

* Corresponding author. Tel.: +49 721 608 22704; fax: +49 721 608 24806.
E-mail address: christine.roesch@kit.edu (C. Rösch).

0960-8524/$ - see front matter © 2011 Published by Elsevier Ltd.
doi:10.1016/j.biortech.2011.12.016

Please cite this article in press as: Rösch, C., et al. Materials flow modeling of nutrient recycling in biodiesel production from microalgae. Bioresour. Technol. (2011), doi:10.1016/j.biortech.2011.12.016

Table 1
Reported lipid and TAG contents and productivities of microalgae (Prod. = Productivity).

Microalgae	Sufficient nitrogen			Deficient nitrogen			Reference
	Lipids % dw	TAG %dw	Prod. g/(m^3*d)	Lipids %dw	TAG %dw	Prod. g/(m^3*d)	
Summary of 17 Chlorophyta (lab)	13–31, Average 23			18–64, Average 41			Griffiths and Harrison (2009)
Summary of 33 other species except Cyanobacteria (lab)	11–51, Average 25			10–51, Average 27			Griffiths and Harrison (2009)
Summary of 8 species (outdoor)	16–40, Average 26						Griffiths and Harrison (2009)
Neochloris oleobundans (lab)	23	3	16.5 (TAG)	37	18	23 (TAG)	Pruvost et al. (2009)
Neochloris oleobundans (lab)	23	<3		25–37	9–12		Pruvost et al. (2011)
Chlorella vulgaris (lab)	20	<3		20–23	11–14		Pruvost et al. (2011)
Cylindrotheca closterium (lab)	17–20	<3		20–30	7–12		Pruvost et al. (2011)
Nannochloropsis sp. UTEX LB 1999	31	8*		40	21.6*		Tagaki et al. (2000)
Nannochloropsis sp. F&M-M24 (outdoor PBR)	32.3		360 (Biomass)	60		300 (Biomass)	Rodolfi et al. (2009)
Chlorella zofingiensis (lab)	25.8	5*					Liu et al. (2010)

* Calculated from the given values.

2. Background

Microalgae can have lipid contents exceeding 80% by weight of dry biomass (Metting, 1996; Rodolfi et al., 2009) although this is usually in the range of 15–35% and is dependent upon algae strain and growth conditions (Chisti, 2007). Under optimal conditions of growth, algae synthesize fatty acids principally for esterification into glycerol-based membrane lipids, which constitute about 5–20% of their dry content weight (Hu et al., 2008). Unlike the glycerolipids found in membranes, the triacylglycerols (TAGs) do not perform a structural role but instead serve primarily as a storage form of carbon and energy. Thus, one of the key criteria for selection of microalgae strains for biodiesel feedstock production is a high intracellular lipid and TAG content. Total lipids are composed of neutral lipid in the form of energy reserve bodies, as well as glyco- and phospholipids in the structural membranes. Neutral lipids are typically the major constituents of algal lipid-oil in aging or stressed cultures, mainly in the form of TAGs (Hu et al., 2008; Chen et al., 2008).

Algae produced more lipids in stressed or unfavorable condition in comparison to optimal growth condition (Hu et al., 2008). The supply of nitrogen can influence the lipid content as well as the lipid composition in microalgae. Nitrogen starvation enhances the biosynthesis and accumulation of lipids, especially of TAG, in numerous species (Hu et al., 2008). The change in metabolism leads to a reduction in cell growth. Thus, TAG productivity under reduced nitrogen supply has to be at least as high as under normal nutrition conditions. The studies of Pruvost et al. (2009) with *Neochloris oleobundans* show that this is possible (Table 1). Very high lipid content of more than 60% can be found in *Nannochloropsis* sp. F&M-M24 grown in photobioreactors under nitrogen deprivation (Rodolfi et al., 2009). The biomass productivity remained high compared to the control culture with normal nutrition (respectively 0.3 g/l/day and 0.36 g/l/day) which indicates a high TAG productivity.

Although both polar and neutral lipids can be converted to biodiesel (Ichihara et al., 2010) neutral lipids is the desirable fraction since TAG are easily transesterified to biodiesel (Doan et al., 2011). Free fatty acids (FFA) can also be converted after esterification. Lipids which are not esterified like phospholipids can cause problems during the conversion process and in engines (Pruvost et al., 2009).

Since usually the total lipid content is indicated in the literature, data on the TAG content of microalgae are sparse. Under normal nutrition conditions their content range from below 3% to 8% of dry weight (Table 1). Besides lipid content, biomass production is also a critical factor to select a favorable microalgae strain for biodiesel production. With respect to lipid accumulation and biomass generation *Nannochloropsis* strains with a rapid and robust growth, lipid contents ranging from 42.5% to 45% as biomass dry weight, and a corresponding fatty acid methyl esters (FAME) yield ranging between 16% and 22% of dry weight are suitable biomass feedstocks for biodiesel production (Doan et al., 2011).

The lipids can be extracted by physical extraction or by solvent extraction following the rupture of the cell wall. One of the problems of this approach is that the wet aquatic biomass requires drying before it can be processed and that large quantities of organic solvents (e.g., hexanes) are needed. The most common way to convert free fatty acids and/or triglycerides into biodiesel is by transesterification with a short chain alcohol (usually methanol).

Homogeneous catalysts, i.e. basic (sodium hydroxide, potassium hydroxide and sodium methylate), and acidic catalysts (sulfuric acid) are commonly used as commercial transesterification catalysts at a concentration of about 1% by weight of oil to boost the rate of the transesterification reaction (Fukuda et al., 2001). However, for some processes using supercritical fluids (methanol or ethanol) it may not be necessary to use a catalyst (Gogate, 2008).

3. Methodology

For the analysis of the nutrient demand three generic microalgae with different characteristics were defined based on data from literature. The functional unit is defined as 1 l of microalgae based biodiesel. The nutrient balance includes the processes of microalgae cultivation (open pond and PBR), harvesting, oil extraction, and esterification to biodiesel as well as the utilization of the oil-free algae residues for the production of energy or animal feed.

3.1. Definition of generic microalgae

For this investigation three generic microalgae (M1, M2 and M3) suitable for cultivation conditions with sufficient and deficient supply of nitrogen are defined. For M1 an average nitrogen supply was assumed. The lipid content for M1 was defined as 25% which corresponds to an average lipid contents (Griffiths and Harrison, 2009) and the TAG content was set to 5%. Cultivation under nitrogen deprivation was assumed for the algae M2. The lipid content was chosen to be 45% which is slightly higher than the average lipid content for Chlorophyta under nitrogen deprivation conditions (Griffiths and Harrison, 2009). The TAG content was assumed to equate to the increase in total lipids compared to M1 (as it is documented in Table 1) and thus to be in the range of 25%. For the al-

Please cite this article in press as: Rösch, C., et al. Materials flow modeling of nutrient recycling in biodiesel production from microalgae. Bioresour. Technol. (2011), doi:10.1016/j.biortech.2011.12.016

gae M3 the lipid content was set to 60% corresponding to the upper range of the values from (Griffiths and Harrison, 2009) for nitrogen deprivation and the lipid content of *Nannochloropsis* sp. F&M-M24 (Rodolfi et al., 2009). The TAG content was enhanced in the range of the lipid increase compared to M1 as well and accounts for 40%.

The nitrogen and phosphorus content of the three generic microalgae were calculated on the basis of the elemental composition of major biochemical components of microalgae (phospholipids, glycolipids, proteins, polysaccharide and nucleic acids) and their fractions on the cell (dry weight) according to Williams and Laurens (2010). For the neutral lipids the elemental composition of a TAG with three 16:0 fatty acids was used, since those are the most frequent fatty acids in Chlorophyceae. The ratio of neutral lipids to glycolipids to phospholipids is assumed to be 35:40:25 (7:8:5) at a lipid content of 25%. The ratio of glycolipids to phospholipids and of proteins to polysaccharides is supposed to remain constant at 40 to 25 and 3 to 2, respectively, and the nucleic acid content was set to 5%. The proportions of lipids, proteins and carbohydrates vary strongly among species and nitrogen supply (Williams and Laurens, 2010). The nitrogen and phosphorus contents for the three generic microalgae are shown in Table 2.

3.2. Definition of process chains and system boundaries

A flow diagram of the investigated microalgae process chains and their system boundaries is shown in Fig. 1. A first nutrient recycling loop is implied at the step where the cultivated algae are harvested (e.g. by centrifugation) and water is separated from the algae. The water soluble nutrients left in the culture medium can be fed back together into the cultivation system. In the next step of the process chain the oil is extracted from the algae. The great majority of the nitrogen and phosphorus remains in the oil-free algal residues which are primarily composed of carbohydrates and proteins. The downstream process chains differ in the technology applied to utilize the algal residues after the oil extraction. Three paths are studied in this work: anaerobic digestion, hydrothermal gasification and animal feed production. Both energy technologies are either in an experimental stage (hydrothermal gasification) or are applied at industrial scale but not yet with microalgae (anaerobic digestion). It is assumed that the nutrients which are leaving the energy production unit can be recycled by supplying them to the cultivation unit. In case of animal feeding, where the algal residues are used for pig fattening, the main part of the nutrients are leaving the animal with the nutrient rich manure which can be fed into the cultivation system. Generally, nitrogen and phosphorus flows that leave the system are referred to as losses.

To reflect the range of data found in the literature, three scenarios for each process chain have been developed: A worst case scenario which represents the highest reported nutrient losses for each processing step, a best case scenario corresponding to the upper limit of nutrient recovery and a base case scenario usually representing the mean losses for each processing step. Furthermore a sensitivity analysis was conducted to identify the most important losses for the different process chains, of which results can be found in the Supplementary material (Figs. S7–S9). In the following the literature data on the losses in the different processing steps is described. Table 3 gives an overview of the assumed values for the three scenarios.

3.2.1. Microalgae cultivation

The cultivation of microalgae can be associated with losses of nutrient, respectively nitrogen. If nitrogen is supplied as NH_4^+ it can volatilize as NH_3 (Park et al., 2010). In the case that nitrogen is recycled after the algal residues are used the main part of the nitrogen will appear as NH_4^+. The formation and volatilization of NH_3 depend on pH and temperature. The pH of the cultivation medium is assumed to be in the range of 7.5 which is preferred by many microalgae (Park et al., 2010). In this case, only a minor proportion of the nitrogen is available in the form of NH_3.

Tests have shown that microalgae cultivation in bottles containing wastewater with mainly NH_4^+ leads to a loss of less than 7% of the nitrogen content through volatilization (Woertz et al., 2009). This loss was accompanied by an increase of the pH from 7.2 to 10.3. Nitrogen stripping of about 3% from open and closed reactors was measured by Molinuevo-Salces et al. (2010) with diluted digested swine slurry at a pH of 7.5 and temperatures between 30 and 35 °C.

A loss of phosphorus for microalgae uptake can be considered by the forming of poorly soluble compounds of phosphates with metal ions depending on the pH and the concentrations (Grobbelaar, 2004). Due to the assumed pH of 7.5 and the small concentrations in the medium the precipitation of phosphate is supposed to be low and was thus not taken into account in the investigation.

3.2.2. Biodiesel production

For assessing the nitrogen and phosphorus losses during biodiesel production, the values from conventional biodiesel production process were adopted. This process consists of three steps: lipid extraction, refining and transesterification of the TAG to fatty acid methyl esters. The extraction is generally carried out with organic solvents (Robles Medina et al., 1998) which are then separated from the solvent lipid mixture by distillation. With this technology a lipid yield of 98% can be achieved using traditional oil crops (Kaltschmitt et al., 2009). In the refining step the undesired compounds such as phospholipids and glycolipids are removed resulting in a loss of lipids in the range of 4–8% (Kaltschmitt et al., 2009). It was assumed that the glycolipids and phospholipids which are removed by degumming (6%) are added back to the residual algae biomass. In conventional biodiesel production this is often performed to enhance the value of the residual biomass for animal feed and the nitrogen and phosphorus recycling quota since phospholipids contain both elements. A loss of nitrogen and phosphorus occurs by the co-extraction of small amounts of other compounds like proteins. Another loss of these macronutrients occurs because not all phospholipids can be removed by degumming. The nitrogen and phosphorus losses due to protein co-extraction and incomplete phospholipids separation are considered to be in the range between 0.5% and 1.5%. During transesterification the TAG are divided in three low-molecular compounds, called fatty acid methyl esters, and glycerol in a reaction with primary alcohol in the presence of a catalyst (Kaltschmitt et al., 2009). The density of fatty acid methyl esters for diesel engines is between 0.860 and 0.900 kg/l (DIN). In this work, the density of microalgae biodiesel was fixed to 0.864 kg/l in order to calculate the required TAG for the production of 1 l biodiesel (Xu et al., 2006). Assuming the same density for TAG and adding the loss of 2% by solvent distillation and 6% during refining, the required amount of TAG to produce 1 l biodiesel accounts for 0.932 kg/l.

3.2.3. Anaerobic digestion

Anaerobic digestion is appropriate for biomass with high moisture content (80–90%) and thus can be used for microalgae. With the conversion of algal biomass into methane as much energy could be recovered as obtained from the extraction of cell lipids (Sialve et al., 2009), while leaving a nutrient rich waste product that can be fed into the algal growth medium. Microalgae can have a high proportion of proteins that result in low C/N ratios between 6 and 9 (Ras et al., 2011) which can have negative impacts on the performance of the process. This problem may be resolved by co-digestion of microalgal residues with biomass that has a higher C/N ratio. Organic nitrogen and phosphorus compounds are partly

Please cite this article in press as: Rösch, C., et al. Materials flow modeling of nutrient recycling in biodiesel production from microalgae. Bioresour. Technol. (2011), doi:10.1016/j.biortech.2011.12.016

Table 2
Calculated fraction of major cell components and nitrogen and phosphorus of three different generic microalgae.

Cell components (% dw)	M1	M2	M3
Total lipids	25	45	60
TAG	5	25	40
Other neutral lipids	3.75	3.75	3.75
Glycolipids	10	10	10
Phospholipids	6.25	6.25	6.25
Proteins	42	30	21
Polysaccharides	28	20	14
Nucleic acids	5	5	5
Nitrogen	7.76	5.8	4.32
Phosphorus	0.76	0.76	0.76

mineralized in anaerobic digestion and thus will be available for microalgae uptake. The amount of NH_3 losses is determined by the hydraulic retention time (HRT), pH and fermentation temperature influence. The NH_4^+ fraction of total nitrogen in the digester effluent is in between 47% and 69% for a HRT of about 30 days and a temperature of 35 °C (Samson and LeDuy, 1982). A total nitrogen mineralization efficiency of 68% for 28 days HRT and 35 °C was achieved by Ras et al. (2011). Since the microalgae cells are disrupted during the oil extraction, the biodegradability of the algal residues may be higher compared to microalgae with intact cell walls. This is reflected in the base case and best case assumptions (see Table 3).

The mineralized nitrogen in the form of NH_4^+/NH_3 leads to NH_3-emissions during the digestion process as well as storing and processing of the fermentation residues. The pH of the fermentation residues is between 6.6 and 7.6 (Ehimen et al., 2010; Ras et al., 2011; Samson and LeDuy, 1982). A loss of total nitrogen of 16.4% at a pH of 7.55 was observed by Samson and LeDuy (1982) at 35 °C and a HRT of 33 days. This value was adopted for the nutrient balance. The use of the fermentation residues for microalgae cultivation was investigated by Golueke and Oswald (1959). Some studies were also performed on the use of digested manure. These indicate that a solid–liquid separation to reduce turbidity as well as autoclaving or ultra filtration to avoid bacterial contamination may be necessary (Park et al., 2010; Wang et al., 2010).

3.2.4. Hydrothermal gasification

Hydrothermal gasification is an effective thermo-chemical route to convert biomass with high water content in an aqueous phase which is in a supercritical or near-critical state to gases. The resulting gas is rich in hydrogen and methane (i.e. known as syngas). The nutrients are remaining in the effluent and separated unsolved salts. Important losses of nitrogen and phosphorus may occur due to unconverted biomass. In batch experiments with microalgae carbon gasification efficiencies of 68–74% (Haiduc et al., 2009) 93% (Stucki et al., 2009) and 70% (Minowa and Sawayama, 1999) have been achieved with catalysts. The nitrogen in the biomass is converted into ammonia which is predominantly dissolved in the effluent due to its high solubility (Minowa and Sawayama,

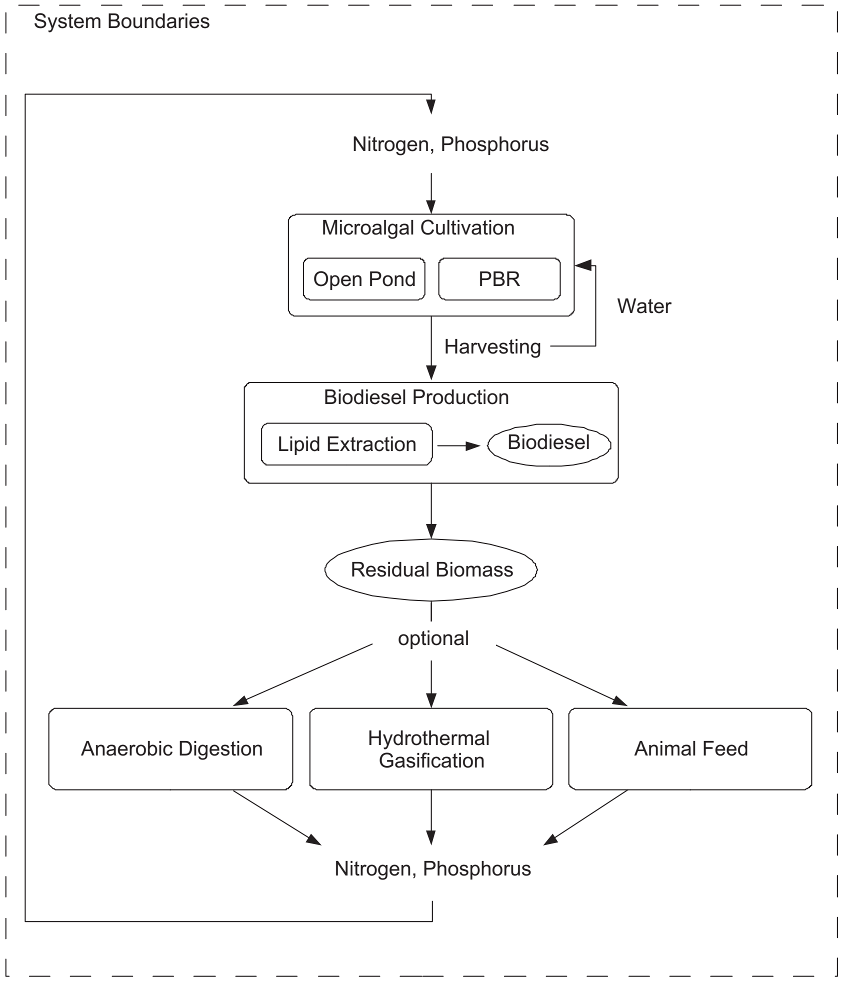

Fig. 1. Process chains and system boundaries of the nutrient balancing for biodiesel production with microalgae.

Please cite this article in press as: Rösch, C., et al. Materials flow modeling of nutrient recycling in biodiesel production from microalgae. Bioresour. Technol. (2011), doi:10.1016/j.biortech.2011.12.016

Table 3
Assumed losses of nitrogen and phosphorus in the different processing steps for three scenarios (WoC = worst case, BaC = base case, BeC = best case).

Processing step/loss	Losses in %		
	WoC	BaC	BeC
Cultivation			
Out-gassing of NH_3	7	5	3
Losses of phosphorus (P)	0	0	0
Biodiesel production			
Losses of nitrogen (N) and P due to protein co-extraction and incomplete phospholipids separation	1.5	1	0.5
Anaerobic digestion			
NH_3 emissions	24	16	8
N and P losses due to Incomplete biomass mineralization	30	20	10
Further losses of N during storage and processing	4.5	3	1.5
Hydrothermal gasification			
N and P losses due to Incomplete biomass mineralization	30	18	7
NH_3 emissions	26	13	0
Losses of P due to salt deposition in the reactor	10	5	0
Animal feed			
Incorporation of N in the animal	29	29	29
Incorporation of P in the animal	31	31	31
NH_3 emissions in the manure	25	15	5
Organically bound N in the manure (not available for algae)	38	25	12
Organically bound P in the manure (not available for algae)	30	20	10

1999; Schubert et al., 2010). According to experiments with salts, between 74% and 100% of the NH_4^+ could be recovered in the separated brine and reactor effluent (Schubert et al., 2010). The loss of nitrogen is probably due to the alkalinity of the effluent emissions which causes emissions of NH_3. Phosphorus exhibits different solubility under supercritical water. Separation tests with phosphates showed a high recovery rate at the salt separator in the range between 75% to 93% and an overall recovery rate in the brine and effluent of 91–100% (Schubert et al., 2010). Thus, the phosphorus will mainly be found in the separated concentrated brine and the nitrogen in the effluent. The effluent of the hydrothermal gasification can contain high concentrations of ammonia and nickel depending on the catalyst that have been applied and thus had to be diluted prior to recycling to the cultivation unit in order to prevent toxic effects on microalgae (Tsukahara et al., 2001). The effluent may also contain trace elements from reactor materials due to corrosion, e.g. by the use of microalgae grown in salt water. However, no problematic concentrations of heavy metals are expected if the trace elements are not allowed to accumulate (Stucki et al., 2009). No results on the use of the separated salts for microalgae cultivation have been published so far. They may contain heavy metals from reactor materials and catalysts as well.

3.2.5. Animal feed

Microalgae are the natural food source of many aquaculture species (Spolaore et al., 2006) and thus the main applications for algal biomass as animal feed are fish feed including e.g. larval nutrition for molluscs or penaeid shrimp and coloring for farmed salmonids (Muller-Feuga, 2000). Microalgae were found also to be a suitable feed supplement for poultry and pigs, particularly as substitute for conventional protein sources, whereas feeding studies on ruminants are limited (Becker, 2004). In this study the use of the protein-rich algal residues after oil extraction are regarded as substitute for pig fattening because the market for pig feed is considered to be large enough to absorb significant quantities of residual algal biomass as feedstock. The perceptual losses and recoveries of nitrogen and phosphorus only refer to the fraction of algal biomass in the feed. They are assumed to equate to the total losses and recoveries of nitrogen and phosphorus in the feed. First losses occur by incorporation of the nutrients into the animal. A standard feeding mixture and a growth of 210 kg per animal place and year was assumed (DLG e.V., 2005). Based on this, 71% of the nitrogen and 69% of the phosphorus contained in the feed are excreted by the pig as manure. Approximately 50% of the nitrogen in the manure is in the form of NH_4^+ (Galler, 1989). The other part consists of poorly soluble nitrogen compounds. However, Kebede-Westhead et al. (2006) found that about 80% of total nitrogen was in the form of NH_4^+ in piggery manure. The high amount of NH_4^+ in the manure can lead to NH_3 emission between 10% and 30% of the nitrogen content during storage (DLG e.V., 2005). To reduce these losses a short storage time is assumed. Besides, a small loss of NH_3 can occur by autoclaving the manure before supplying it to the culture medium. With regard to phosphorus, a high recycling rate of 80% can be achieved due to the fact that manure is consisting mainly of water soluble inorganic phosphates (Galler, 1989).

4. Results of nutrient balance

The results of the mass flow balance show that to produce 1 l algae biodiesel around 19 kg algal biomass in the case of sufficient nitrogen supply and in the case of deficient nitrogen supply 3.7 kg algal biomass with the model algae M2 and 2.3 kg biomass with the model algae M3 are required (see Table 4). The differences are due to the varying TAG content. The nitrogen demand for the production of 1 l biodiesel with microalgae amounts to 1.52 kg (sufficient supply), 0.23 kg nitrogen (deficient supply, M2) and 0.11 kg nitrogen (deficient supply, M3). For phosphorus the corresponding figures are 142 g, 28 g, and 18 g respectively. These numbers illustrate that microalgae cultivation with deficient nitrogen supply is by far more favorable with regard both to nitrogen and phosphorus demand. With the downstream processes anaerobic digestion and hydrothermal gasification a substantial part of nitrogen and phosphorus can be recovered and reused for microalgae cultivation. Fig. 2 illustrates that by anaerobic digestion more than 60% of the nitrogen can be recycled within the process chain for the base case. For the worst case this amounts to 47% and reaches 79% for the best case (see Figs. S1 and S2). The greatest lost of nitrogen is due to the incomplete conversion of algal biomass in the digester, which is also the most important loss when looking at the different scenarios. Another significant loss is due to emissions of NH_3. For phosphorus the recycling rate is significantly higher than for nitrogen. Almost 80% of the phosphorus can be recycled for

Please cite this article in press as: Rösch, C., et al. Materials flow modeling of nutrient recycling in biodiesel production from microalgae. Bioresour. Technol. (2011), doi:10.1016/j.biortech.2011.12.016

Table 4
Demand for nitrogen and phosphorus for the production of 1 l microalgae biodiesel.

Cultivation method	Sufficient nitrogen (M1)	Deficient nitrogen (M2)	Deficient nitrogen (M3)
Required TAG	932 g	932 g	932 g
TAG content in microalgae	5%	25%	40%
Required mass of algae	18,642 g	3728 g	2330 g
Required mass of nitrogen	1520 g	227 g	106 g
Required mass of phosphorus	142 g	28 g	18 g

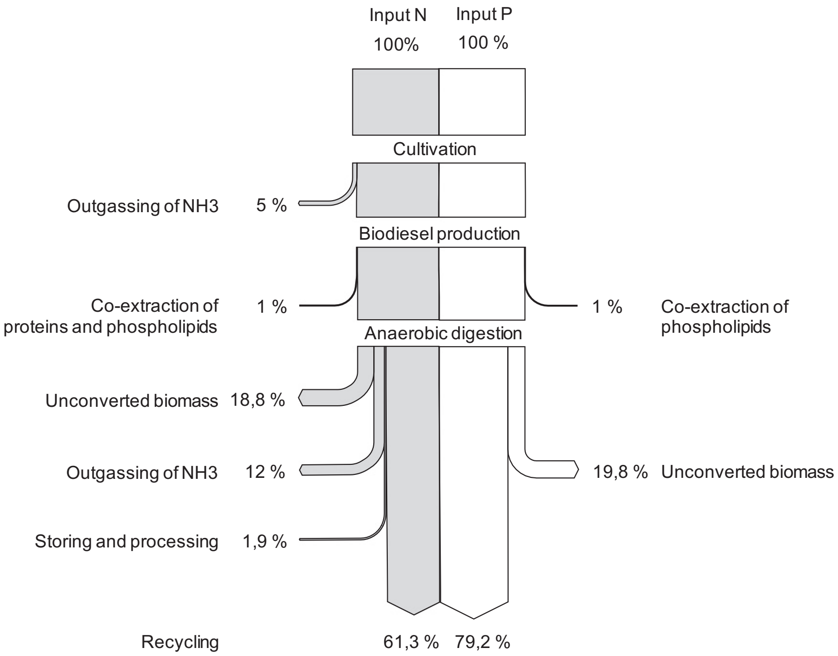

Fig. 2. Nitrogen and phosphorus losses and recycling rate for biodiesel production with microalgae and anaerobic digestion of the residual algal biomass.

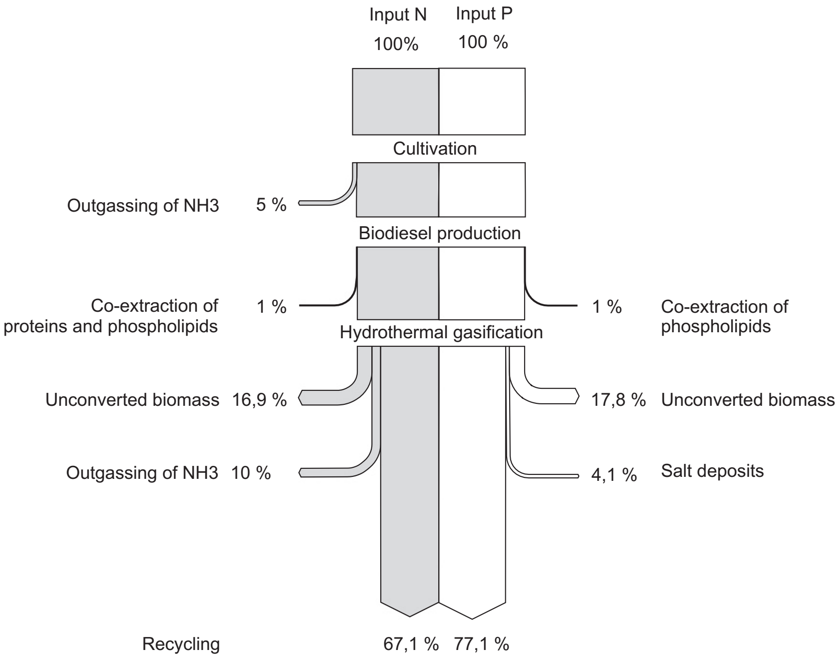

Fig. 3. Nitrogen and phosphorus losses and recycling rate for biodiesel production with microalgae and hydrothermal gasification of the residual algal biomass.

Please cite this article in press as: Rösch, C., et al. Materials flow modeling of nutrient recycling in biodiesel production from microalgae. Bioresour. Technol. (2011), doi:10.1016/j.biortech.2011.12.016

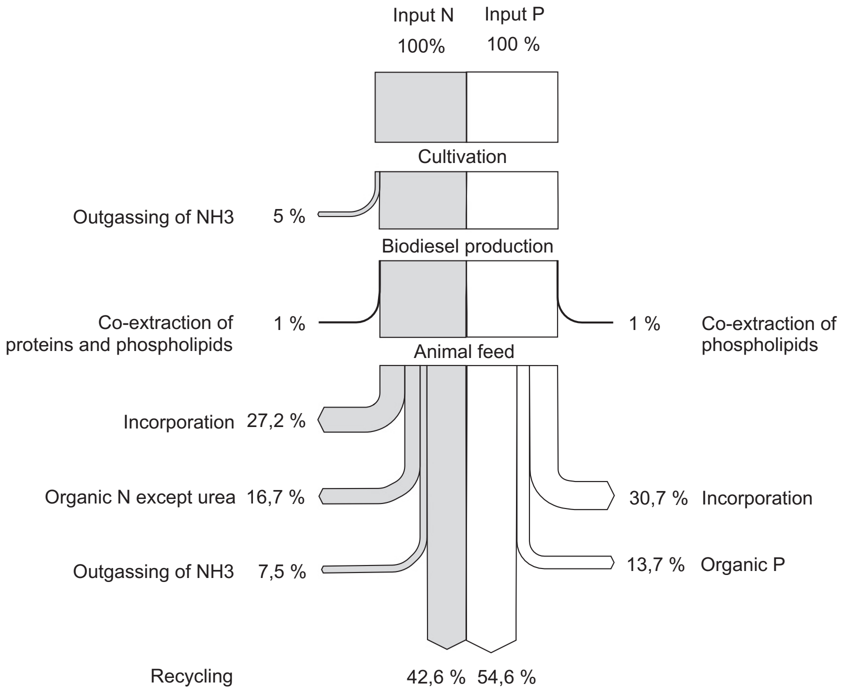

Fig. 4. Nitrogen and phosphorus losses and recycling rate for biodiesel production with microalgae and use of the residual algal biomass as animal feed.

microalgae cultivation (see Fig. 2), within a range of 69% to 90% for the case scenarios (Figs. S1 and S2). Incomplete biomass conversion turned out to be the main loss factor.

A higher recycling rate can be achieved by hydrothermal gasification of the residual algal biomass. Around 67% of the nitrogen can be reused that way (Fig. 3) and for the best case even 90% (Fig. S4) (worst case 47%, see Fig. S3). This is mainly due to the higher conversion efficiency of the biomass assumed for the hydrothermal gasification process. Therefore, a higher conversion of organically bound nitrogen to NH_4^+ was supposed. Besides incomplete conversion, losses occur due to the volatilization of NH_3. The loss of phosphorus is slightly higher for the process of hydrothermal gasification compared to anaerobic digestion. Fig. 3 illustrates that losses amount for about 23% and for the best case for only 7.5% (Fig. S4). The main losses of phosphorus are related to unconverted biomass and salt deposits.

Applying the residual algae biomass for animal feed instead of energy conversion will result in a much lower nutrient recycling rate (Fig. 4 and Figs. S5 and S6) of 43% (30–57%) for nitrogen and of 55% (48–62%) for phosphorus. This is mainly due to the incorporation of nitrogen and phosphates in animal products and organic nutrients.

The sensitivity analysis (Figs. S7–S9) shows that for anaerobic digestion and hydrothermal gasification incomplete biomass conversion during the fermentation/gasification process is the most critical factor for the resulting recycling rates of nitrogen and phosphorus. When the residual biomass is used as animal feed the most critical factor is the incorporation in the animal of nitrogen and phosphorus.

It can be concluded that with respect to the recycling of nitrogen and phosphorus the use of algal residues for energy production is much more favorable than its use as animal feed supplement. Comparing the two energy technologies hydrothermal gasification show slightly better recycling results than anaerobic digestion. The main losses identified for nitrogen and phosphorus along the process chain are unconverted biomass and ammonia emission from the culture medium and effluents of the downstream processes. Optimization of the conversion processes, respectively with regard to emissions of NH_3, could significantly increase the recycling rate for both nitrogen and phosphorus.

5. Discussion

The results from the nutrient balance indicate a high recycling potential in the biofuel production with microalgae if the oil-free algal residues are used for energy production, respectively for hydrothermal gasification and anaerobic digestion. These results however have to be confirmed by large-scale and long-time experiments because the studied technologies are either in an experimental stage or only be applied for microalgae on a pilot scale for a restricted period of time. Moreover, only few investigations on methane production with transesterified algal residues have been conducted with fresh-water microalgae, e.g. residues of *Chlorella* (Ehimen et al., 2010) and almost none with salt-water microalgae. The results from anaerobic digestion of *Chlorella* indicate that there is a demand to improve the conversion efficiencies. Besides, it was observed that methane production could be increased by more than 50% if the C/N ratio of the substrate was enlarged by co-digesting of microalgae residues together with a carbon source, such as glycerol from biodiesel production.

The nitrogen and phosphorus demand for the cultivation of microalgae for biodiesel production depends on the TAG content of the microalgae since these lipids are best suited for biodiesel production. There is an inverse relationship between the TAG content and the required algal biomass to produce 1 l biodiesel: The higher the TAG content of the microalgae, the lower the demand for algal biomass and thus the demand for nitrogen and phosphorus to cultivate microalgae. The nitrogen demand for biodiesel production can be further decreased by the selection of microalgae which accumulate TAG if cultivated under deficient supply of nitrogen which is the case for many microalgae species (Hu

Please cite this article in press as: Rösch, C., et al. Materials flow modeling of nutrient recycling in biodiesel production from microalgae. Bioresour. Technol. (2011), doi:10.1016/j.biortech.2011.12.016

et al., 2008). Besides, microalgae with a higher lipid and TAG concentration compared to microalgae with lower contents of lipids and TAG facilitate the lipid extraction (Rodolfi et al., 2009). A positive side effect of microalgae cultivated with deficient nitrogen supply is the higher C/N ratio of the algal biomass which leads to a higher methane yield in anaerobic digestion, respectively if microalgae are used as a single substrate (Ras et al., 2011). Thus, the selection of naturally occurring or engineered microalgae which can be cultivated with deficient nitrogen supply and have a high lipid and TAG content can significantly reduce the nutrient demand for biodiesel production.

Nitrogen recycling within the studied process chains is strongly dependent on the NH_3 emission occurring during cultivation as well as the downstream process. Shifting the type of nitrogen supply from NH_4^+ to NO_3^- in microalgae cultivation could prevent these losses. However, in the effluents of hydrothermal gasification and in the fermentation residues, NH_4^+/NH_3 are the most important nitrogen compounds. The volatilization of NH_3 from the cultivation medium highly depends on the pH and further on temperature, cultivation mode and air circulation. A slightly alkaline pH was assumed for the nutrient balance, but there are also microalgae that prefer a high pH, such as *Spirulina* (Borowitzka, 1999). The production of these species results in a higher loss of nitrogen during cultivation, if NH_4^+ is present in the medium. On the other hand, some microalgae species grow even better if nitrogen is supplied in the form of NO_3^- (Li et al., 2008). If the effluent streams in the process chains shall be used for their cultivation the maximum productivity may not be achieved. An alternative could be the interposing of a nitrifying step. This would also prevent NH_3 emission from the culture medium.

Another loss of nitrogen and the main loss of phosphorus are due to incomplete conversion in the downstream process. The unconverted material could be used as fertilizer in agriculture, since slowly degradable organic compounds are important for soil quality. The nutrients would thus be used for plant growth but cannot be recycled within the microalgae cultivation system. A loss of phosphorus can further occur by the precipitation of poorly soluble compounds (Grobbelaar, 2004). In this study building of these compounds was assumed to be low and not taken into account. However, depending on the pH and the metal load precipitation could occur resulting in a small loss of phosphorus. In this case, a higher demand of phosphorus for microalgae cultivation would be required. The sludge of precipitated phosphorus compounds might also be applied as agricultural fertilizer depending on the solubility of the compounds and the contained metals.

It is assumed that the phospholipids are added back to the residual algal biomass after refining the extracted lipids because the phospholipids located in the cell walls make up a large share of the phosphorus content in microalgae (Williams and Laurens, 2010). Thus, for a high recycling rate their presence in the downstream process chain is necessary.

The use of the effluents from anaerobic digestion and hydrothermal gasification for the cultivation of microalgae has been investigated in only few studies so far, e.g. Li et al. (2008) or Tsukahara et al. (2001). These experiments indicate that nutrient recycling could be feasible. A critical point for using effluents of hydrothermal gasification could be that heavy metals can accumulate in the effluent of the gasification plant due to adsorbing and absorbing of the heavy metals by the microalgae. Thus, concentrations may be reached which exceed the tolerance of the microalgae. Further studies are needed to investigate the long-term effect of applying the effluent for microalgae cultivation. The composition of the separated effluents that contain the major part of phosphorus, as well as the suitability of the salts for the use in microalgae cultivation has not been analyzed yet. Also, the disposition of unconverted material was not addressed in the studies so far.

6. Conclusions

Nitrogen and phosphorus balancing shows that a high amount of supplied nitrogen and phosphorus for the cultivation of microalgae can be recycled. Inevitable nutrients losses could be reduced by optimizing the process conditions for nutrient recycling and made up with wastewater nutrients. Thus, the development of suitable treatment technologies to recycle nutrients from algae biofuel processing residuals for use in algae cultivation is of great importance. The ability to recycle nutrients within microalgae process chains could become one of the great advantages of microalgae farming over traditional farming once the technical challenges for nutrient recycling have been overcome.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2011.12.016.

References

Becker, E.W., 2004. Microalgae in human and animal nutrition. In: Richmond, A. (Ed.), Handbook of Microalgal Culture. Blackwell Science, Oxford, pp. 312–351.

Borowitzka, M.A., 1999. Commercial production of microalgae: ponds, tanks, tubes and fermenters. J. Biotechnol. 70, 313–321.

Chen, G.Q., Jiang, Y., Chen, F., 2008. Variation of lipid class composition in *Nitzschia laevis* as a response to growth temperature change. Food Chem. 109, 88–94.

Chisti, Y., 2007. Biodiesel from microalgae. Biotechnol. Adv. 25, 294–306.

Clarens, A.F., Resurrection, E.P., White, M.A., Closi, L.M., 2010. Environmental life cycle comparison of algae to other bioenergy feedstock. Environ. Sci. Technol. 44, 1813–1819.

Cordell, D., Drangert, J.-O., White, S., 2009. The story of phosphorus. Global food security and food for thought.. Global Environ. Change 19, 292–305.

DLG e.V., 2005. Bilanzierung der Nährstoffausscheidungen landwirtschaftlicher Nutztiere. Arbeiten der DLG 199. Frankfurt am Main.

Doan, T.T.Y., Sivaloganathan, B., Obbard, J.P., 2011. Screening of marine microalgae for biodiesel feedstock. Biomass Bioenergy 35, 2534–2544.

Ehimen, E.A., Sun, Z.F., Carrington, C.G., Birch, E.J., Eaton-Rye, J.J., 2010. Anaerobic digestion of microalgae residues resulting from the biodiesel production process. Appl. Energy 88, 3454–3463.

Fukuda, H., Kondo, A., Noda, H., 2001. Biodiesel fuel production by transesterification of oils. J. Biosci. Bioeng. 92, 405–416.

Galler, J., 1989. Gülle: Anfall, Lagerung, Verwertung, Umwelt. Stocker.

Gogate, P.R., 2008. Cavitational reactors for process intensification of chemical processing applications: a critical review. Chem. Eng. Process. 47, 515–527.

Golueke, C.G., Oswald, W.J., 1959. Biological conversion of light energy to the chemical energy. Appl. Environ. Microbiol. 7, 219–227.

Griffiths, M.J., Harrison, S.T., 2009. Lipid productivity as a key characteristic for choosing algal species for biodiesel production. J. Appl. Phycol. 21, 493–507.

Grobbelaar, J.U., 2004. Algal nutrition: mineral nutrition. In: Richmond, A. (Ed.), Handbook of Microalgal Culture. Blackwell Science, Oxford, pp. 97–115.

Haiduc, A.G., Brandenberger, M., Suquet, S., Vogel, F., Bernier-Latmani, R., Ludwig, C., 2009. SunCHem: an integrated process for the hydrothermal production of methane from microalgae and CO_2 mitigation. J. Appl. Phycol. 21, 529–541.

Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M., Darzins, A., 2008. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. Plant J. 54, 621–639.

Ichihara, K., Yamaguchi, C., Araya, Y., Sakamoto, A., Yoneda, K., 2010. Preparation of fatty acid methyl esters by selective methanolysis of polar glycerolipids. Lipids 45, 367–374.

Kaltschmitt, M., Münch, E.W., Müller-Langer, F., 2009. Produktion und Nutzung von Pflanzenölkraftstoffen – Pflanzenölgewinnung in Großanlagen. In: Kaltschmitt, M., Hartmann, H., Hofbauer, H. (Eds.), Energie aus Biomasse. Springer, Berlin, pp. 712–725.

Kebede-Westhead, E., Pizarro, C., Mulbry, W.W., 2006. Treatment of swine manure effluent using freshwater algae: production, nutrient recovery, and elemental composition of algal biomass at four effluent loading rates. J. Appl. Phycol. 18, 41–46.

Lardon, L., Helias, A., Sialve, B., Steyer, J.P., Bernard, O., 2009. Life cycle assessment biodiesel production from microalgae. Environ. Sci. Technol. 43, 6475–6481.

Li, Y., Horsman, M., Wang, B., Wu, N., Lan, Q.C., 2008. Effects of nitrogen sources on cell growth and lipid accumulation of green alga *Neochloris oleoabundans*. Appl. Microbiol. Biotechnol. 81, 629–636.

Liu, J., Huang, J., Sun, Z., Zhong, Y., Jiang, Y., Chen, F., 2010. Differential lipid and fatty acid profiles of photoautotrophic and heterotrophic *Chlorella zofingiensis*: assessment of algal oils for biodiesel production. Bioresour. Technol. 102, 106–110.

Metting, F.B., 1996. Biodiversity and application of microalgae. J. Ind. Microbiol. 17, 477–489.

Minowa, T., Sawayama, S., 1999. A novel microalgal system for energy production with nitrogen cycling. Fuel 78, 1213–1215.

Molinuevo-Salces, B., García-González, M.C., González-Fernández, C., 2010. Performance comparison of two photobioreactors configurations (open and closed to the atmosphere) treating anaerobically degraded swine slurry. Bioresour. Technol. 101, 5144–5149.

Muller-Feuga, A., 2000. The role of microalgae in aquaculture: situation and trends. J. Appl. Phycol. 12, 527–534.

Park, J., Jin, H.-F., Lim, B.R., Park, K.-Y., Lee, K., 2010. Ammonia removal from anaerobic digestion effluent of livestock waste using green alga *Scenedesmus* sp.. Bioresour. Technol. 101, 8649–8657.

Posten, C., Schaub, G., 2009. Microalgae and terrestrial biomass as source for fuels – a process view. J. Biotechnol. 142, 64–69.

Pruvost, J., Van Vooren, G., Cogne, G., Legrand, J., 2009. Investigation of biomass and lipids production with *Neochloris oleoabundans* in photobioreactor. Bioresour. Technol. 100, 5988–5995.

Pruvost, J., Van Vooren, G., Le Gouic, B., Couzinet-Mossion, A., Legrand, J., 2011. Systematic investigation of biomass and lipid productivity by microalgae in photobioreactors for biodiesel application. Bioresour. Technol. 102, 150–158.

Ras, M., Lardon, L., Sialve, B., Nicolas, B., Steyer, J.P., 2011. Experimental study on a coupled process of production and anaerobic digestion of *Chlorella vulgaris*. Bioresour. Technol. 102, 200–206.

Robles Medina, A., Molina Grima, E., Giménez Giménez, A., Ibannez González, M.J., 1998. Downstream processing of algal polyunsaturated fatty acids. Biotechnol. Adv. 16, 517–580.

Rodolfi, L., Zittelli, G.C., Bassi, N., Padovani, G., Biondi, N., Bonini, G., Tredici, M.R., 2009. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnol. Bioeng. 102, 100–112.

Samson, R., LeDuy, A., 1982. Biogas production from anaerobic digestion of *Spirulina maxima* algal biomass. Biotechnol. Bioeng. 24, 1919–1924.

Schenk, P.M., Thomas-Hall, S.R., Stephens, E., Marx, U.C., Mussgnug, J.H., Posten, C., Kruse, O., Hankamer, B., 2008. Second generation biofuels: high-efficiency microalgae for biodiesel production. Bioenergy Res. 1, 20–43.

Schubert, M., Regler, J.H., Vogel, F., 2010. Continuous salt precipitation and separation from supercritical water. Part 1: Type 1 salts. J. Supercrit. Fluids 52, 99–112.

Sialve, B., Bernet, N., Bernard, O., 2009. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. Biotechnol. Adv. 27, 409–416.

Spolaore, P., Joannis-Cassan, C., Duran, E., Isambert, A., 2006. Commercial applications of microalgae. J. Biosci. Bioeng. 101, 87–96.

Stucki, S., Vogel, F., Ludwig, C., Haiduc, A.G., Brandenberger, M., 2009. Catalytic gasification of algae in supercritical water for biofuel production and carbon capture. Energy Environ. Sci. 2, 535–541.

Tagaki, M., Watanabe, K., Yamaberi, T., Yoshida, T., 2000. Limited feeding of potassium nitrate for intracellular lipid and triglyceride accumulation of *Nannochloris* sp. UTEX LB1999. Appl. Microbiol. Biotechnol. 54, 112–117.

Tsukahara, K., Kimura, T., Minowa, T., Sawayama, S., Yagishita, T., Inoue, S., Hanaoka, T., Usui, Y., Ogi, T., 2001. Microalgal cultivation in a solution recovered from the low-temperature catalytic gasification of the microalga. J. Biosci. Bioeng. 91, 311–313.

Wang, L., Li, Y., Chen, P., Min, M., Chen, Y., Zhu, J., Ruan, R.R., 2010. Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae *Chlorella* sp.. Bioresour. Technol. 101, 2623–2628.

Williams, P.J.B., Laurens, L.M.L., 2010. Microalgae as biodiesel & biomass feedstock: review & analysis of the biochemistry, energetics & economics. Energy Environ. Sci. 3, 554–590.

Woertz, I., Feffer, A., Lundquist, T., Nelson, Y., 2009. Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock. J. Environ. Eng. 135, 1115–1122.

Xu, H., Miao, X., Wu, Q., 2006. High quality biodiesel production from a microalga *Chlorella protothecoides* by heterotrophic growth in fermenters. J. Biotechnol. 126, 499–507.

Yang, J., Xu, M., Hu, Q., Sommerfeld, M., Chen, Y., 2011. Life-cycle analysis on biodiesel production from microalgae: water footprint and nutrients balance. Bioresour. Technol. 102, 159–165.

Please cite this article in press as: Rösch, C., et al. Materials flow modeling of nutrient recycling in biodiesel production from microalgae. Bioresour. Technol. (2011), doi:10.1016/j.biortech.2011.12.016