Environmental impact of microbial protein from potato wastewater as feed ingredient : comparative consequential life cycle assessment of three production systems and soybean meal

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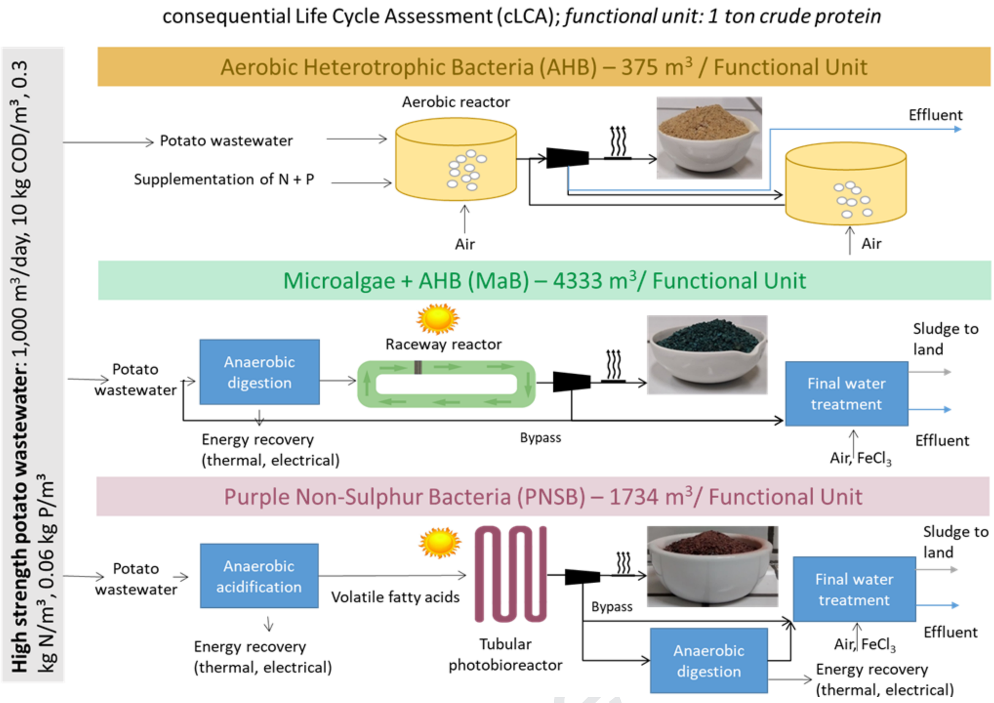
consequential Life Cycle Assessment (cLCA); *functional unit: 1 ton crude protein*
High strength potato wastewater: 1,000 m³/day, 10 kg COD/m³, 0.3 kg N/m³, 0.06 kg P/m³
Aerobic Heterotrophic Bacteria (AHB) – 375 m³ / Functional Unit
Aerobic reactor
Potato wastewater
Supplementation of N + P
Air
Effluent
Air
Microalgae + AHB (MaB) – 4333 m³/ Functional Unit
Raceway reactor
Potato wastewater
Anaerobic digestion
Energy recovery (thermal, electrical)
Bypass
Sludge to land
Final water treatment
Effluent
Air, FeCl₃
Purple Non-Sulphur Bacteria (PNSB) – 1734 m³/ Functional Unit
Potato wastewater
Anaerobic acidification
Energy recovery (thermal, electrical)
Volatile fatty acids
Tubular photobioreactor
Bypass
Anaerobic digestion
Sludge to land
Final water treatment
Effluent
Air, FeCl₃
Energy recovery (thermal, electrical)

Environmental impact of microbial protein from potato wastewater as feed ingredient: Comparative consequential Life Cycle Assessment of three production systems and soybean meal

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Keywords

resource recovery, nutrient recovery, novel protein, protein transition, single-cell protein, purple phototrophic bacteria

Abstract

Livestock production is utilizing large amounts of protein-rich feed ingredients such as soybean meal. The proven negative environmental impacts of soybean meal production incentivize the search for alternative protein sources. One promising alternative is Microbial Protein (MP), i.e. dried microbial biomass. To date, only few life cycle assessments (LCAs) for MP have been carried out, none of which has used a consequential modelling approach nor has been investigating the production of MP on food and beverage wastewater. Therefore, the objective of this study is to evaluate the environmental impact of MP production on a food and beverage effluent as a substitute for soybean meal using a consequential modelling approach. Three different types of MP production were analysed, namely consortia containing Aerobic Heterotrophic Bacteria (AHB), Microalgae and AHB (MaB), and Purple Non-Sulfur Bacteria (PNSB). Production of MP was modelled for high-strength potato wastewater (COD=10 kg/m^3) at a flow rate of 1,000 m^3/day. LCA results were compared against soybean meal production for the endpoint impact categories human health, ecosystems, and resources. Soybean meal showed up to 52% higher impact on human health and up to 87% higher impact on ecosystems than MP. However, energy-related aspects resulted in an 8 to 88% higher resource exploitation for MP. A comparison between the MP production systems showed that MaB performed best when considering ecosystems (between 13-14% better) and resource (between 71-80% better) impact categories, while AHB and PNSB had lower values for the impact category human health (8-12%). The sensitivity analysis suggests that the conclusions drawn are robust as in the majority of 1,000 Monte Carlo runs the initial results are confirmed. In conclusion, it is suggested that MP is an alternative protein source of comparatively low environmental impact that should play a role in the future protein transition, in particular when further process improvements can be implemented and more renewable or waste energy sources will be used.

List of acronyms

Acronym	**Meaning**
AHB	Aerobic Heterotrophic Bacteria
CHP	Combined Heat and Power
COD	Chemical Oxygen Demand
CP	Crude Protein
DM	Dry Matter
kWh	kilo Watt hour
LCA	Life Cycle Assessment
LCI	Life Cycle Inventory
MaB	Microalgae – aerobic heterotrophic Bacteria
MP	Microbial Protein
MFE	Mineral Fertilizer Equivalent
N	Nitrogen
ORP	Open Raceway Pond
P	Phosphorus
PM	Particulate Matter Formation
PNSB	Purple Non-Sulfur Bacteria
SCP	Single-Cell Protein
SM	Supplementary Material
SRT	Sludge Retention Time
tkm	ton kilometer
TSS	Total Suspended Solids
UASB	Upflow Anaerobic Sludge Blanket
UF	Ultra Filtration
VFA	Volatile Fatty Acids

1 Introduction

Intensive livestock production is relying on protein meals as feed ingredients. Global demand for protein meals is predominantly satisfied by soybean meal production, for which an annual increase of 1.6% until 2027 is expected (OECD/FAO 2018). This increase continues to be driven mainly by protein feed demand in China. In the EU, a current issue of concern is the dependency on imported soybean meal that accounts for 64% of the protein-rich feed material, of which 97% is imported (EU 2019). Most of the global soybean and soybean meal production originates from the USA, Brazil, and Argentina (OECD/FAO 2018). In particular the production increase of soybean cultivation in recent decades in South America, has raised concerns about environmental impacts as a result of land use change. These impacts include, amongst others, greenhouse gas emissions and biodiversity loss (Castanheira and Freire 2013, Chaudhary and Kastner 2016).

The increasing global demand for protein and concerns about the environmental impacts of feed and food production have led to a call for a protein transition (Aiking and de Boer 2018). Amongst the many actions necessary for this transition to take place is the search for novel feed protein sources with a lower environmental impact than traditional protein crops. Indeed, the EU recently called for urgent action to replace imported protein with alternative European sources (Denanot 2018). Novel protein sources include for instance insects, duckweed, yeasts, other fungi, macro- and microalgae (incl. cyanobacteria) as well as bacterial protein (excl. cyanobacteria). Of the above yeasts, microalgae, and bacteria can be classified as microbial protein (MP) or single-cell protein. The proponents of MP bring forward a number of compelling arguments, such as (Matassa et al. 2016):

- Protein content comparable to soybean meal: microalgae contain 40-70% and bacteria 50-80% crude protein on a dry matter (DM) basis, compared to soybean meal with 45-57% DM.

- High protein yields: up to 20 times more protein per ha per year than soybean and 40 times more than corn.
- Excellent protein quality: essential amino acid profile comparable to that of soybean meal and the majority of feeding trials indicates a similar growth and digestibility performance (for a summary see Pikaar et al. 2018).

Another advantage of MP is that many types can be cultivated on wastewater, and that they present a resource recovery opportunity, for carbon (C), nitrogen (N), and phosphorus (P) (Verstraete et al. 2016). Especially, food and beverage wastewater has been suggested to be suitable for MP production due to its relatively low contamination with pathogens and other harmful pollutants (e.g. heavy metals, exception slaughterhouse wastewater) (Muys 2019). Furthermore, food and beverage wastewater can contain high concentrations of nutrients and carbon that can be utilized for MP production. The present study is utilizing potato industry wastewater, which typically has a COD content of 4-15 g/L, a N content of 0.15 -0.5 g/L, and a phosphorus content between 0.035 - 0.08 g/L. These figures are based on a study of the potato industry in Flanders, more details can be found in supplemental material (SM) section 1.

A literature study indicates that three MP consortia have been cultivated on wastewater, including Aerobic Heterotrophic Bacteria (AHB), Microalgae and AHB (MaB), and purple non-sulfur bacteria (PNSB).

AHB consortia are mainly composed of aerobic chemoorganoheterotrophs, i.e. bacteria that use organic compounds as carbon source and electron donor, and oxygen as electron acceptor. Similar AHB communities are widely used for biological treatment of wastewater in the conventional activated sludge process. Aiming at intensified MP production, a high-rate activated sludge process would be the most suitable technology, with 'activated sludge' to be understood as 'microbial biomass'. High rate technology aims to maximize biomass production through using short Sludge Retention Times (SRT) at which a relatively large proportion of the available organic carbon is directed towards biomass growth. As a consequence of oxidizing a minimum of organics to CO_2, the aeration demand per unit biomass produced is relatively low. High-rate activated sludge is thus far mainly studied and applied in sewage treatment, to redirect organics to anaerobic digestion for energy production or organic commodities (Alloul et al. 2018, Meerburg et al. 2015). The microbial community in high-rate sewage treatment is less rich than in its conventional counterpart (Meerburg et al. 2016), and is containing specific core genera belonging to the Betaproteobacteria and Bacteroidetes (sub)phyla (Gonzalez-Martinez et al. 2016). High-rate communities for food and beverage wastewater treatment are yet to be studied. High rate activated sludge plants with the aim to produce MP are operating at full-scale on a brewery wastewater flow of 1500 m^3/day (Zhejiang-China, Terry et al. 2019) and have been started up at a pilot plant operating on a potato wastewater flow of 80-100 m^3/day (at Clarebout Potatoes Flanders-Belgium, AVECOM 2019)

Microalgae-AHB (MaB) cultivation systems or high-rate algal ponds for wastewater treatment take advantage from a bidirectional product/substrate exchange between photolithoautotrophic microalgae and AHB. In light, microalgae provide O_2 for AHB growth, and the AHB supply CO_2 as a carbon source for the microalgae, therefore minimizing or even avoiding the need for conventional aeration (Van Den Hende et al. 2014). Another rationale to consider heterotrophs in algal reactors for wastewater treatment is that heterotrophs are likely to naturally thrive in non-sterile microalgae cultivation in the presence of influent COD, which consequently improves the COD removal and effluent quality. In addition, MaB biomass was found to bioflocculate and hence settle well, therefore improving harvestability compared to pure microalgal cultures (Valigore et al. 2012). The predominant microalgal genera in MaB systems on wastewater differ by study, but often belong to the genera *Scenedesmus, Acutudesmus, Oocystis, Microspora, Stigeoclonium, Actinastrum*, *Micractinium*, *Pediastrum*, *Monoraphidium, Coelastrum*, *Chlorella*, *Ankistrodesmus* or and *Phormidium* (Van Den Hende 2014). AHB detected in these systems include Bacteroidetes, Verrucomicrobia, Firmicutes, Planctomycetes and Alpha-, Beta-, Gamma-, Delta- and Epsilonproteobacteria (Lee et al. 2013, Su et al. 2011). Microalgae are used as feed products or feed ingredients and especially MaB biomass has been studied as an aquaculture feed (Van Den Hende et al. 2014). The technology for production of microalgae in raceway reactors is commercially available, with a number of full-scale installations mainly in Asia, but also in Europe and other continents.

PNSB are purple phototrophic bacteria belonging to the Alpha- and Betaproteobacteria. Even though they are considered to be the metabolically most versatile microorganisms, anaerobic anoxygenic photoheterotrophy is the growth mode that has received most attention in literature. In this mode, with volatile fatty acids (VFA) as organics, PNSB have a near to 100% carbon yield (biomass-C produced/VFA-C removed) (Alloul et al. 2019). Only PNSB can be grown on infra-red light, which enables the selective enrichment of these organisms in open mixed cultures. Another advantage is that PNSB do not require aeration with O_2 or CO_2 supply for their growth, which is one of the limiting factors in aerobic heterotrophic or photolithoautotrophic growth (Hülsen et al. 2014). PNSB used for, or enriched during, wastewater treatment include *Rhodopseudomonas palustris, Rhodovulum sulfidophilum, Rhodocyclus gelatinosus, Rubrivivax gelatinosus, Rhodobacter sphaeroides*, *Rhodospirillum rubrum*; of which *R. palustris* was found to dominate at longer SRT and *R. sphaeroides* at shorter SRT (Alloul et al. 2019). MP production from PNSB grown on wastewater is an innovative approach, and to the authors' knowledge not yet available at full-scale. Its potential has also been realized by the European Space Agency, which investigates PNSB as a food protein source to grow on fermented organic waste for regenerative life support (Clauwaert et al. 2017).

Of the MP described above, comparative life cycle assessments to evaluate the environmental impacts caused by MP production have so far only been carried out for and micro-algae production systems in photolithoautotrophic or heterotrophic metabolism. Furthermore, no study has investigated the life cycle of MP produced on food and beverage wastewater. From the available LCA studies, conclusions about the environmental sustainability of MP in comparison to soybean meal were mixed. According to Smetana et al. (2017) the autotrophic and heterotrophic growth of *Chlorella vulgaris* and autotrophic growth of *Arthrospira platensis* were considered to have a higher environmental impact than the reference feeds (e.g. soybean meal). However, in their discussion they indicated that the production under heterotrophic conditions with the use of food waste as a carbon source would be most promising. Sfez et al. (2015) evaluated the production of a consortium of MaB flocs on recirculation aquaculture water in an outdoor open raceway pond (OPR). They concluded that valorization of dried MaB flocs as shrimp feed has a lower marine eutrophication and a similar freshwater eutrophication than using MaB flocs for biogas production. In a comparative exergy evaluation of microalgae and soybean meal, Taelman et al. (2015) concluded that soybean meal is the preferred feed ingredient, when benchmarked against phototrophic microalgae production. However, they also suggested that with further optimisation a similar exergy-based resource footprint can be realized. In an earlier study, by Taelman et al. (2013), microalgae (*Nannochloropsis sp.*) were cultivated in an ORP, it was concluded that the production of microalgae can have a smaller exergy based impact than fish feed (8% fishmeal and 25% soybean meal) if the process is further optimized. In addition to these LCA studies Pikaar et al. (2018) also investigated MP production using aerobic heterotrophic fermentation as well as autotrophic production from hydrogen gas and methane. Most of their scenarios show avoided crop land expansion,

CO_2-eq. emissions, and nitrogen pollution by producing MP. They however did not apply an LCA methodology and did compare to an animal feed basket rather than soybean meal production.

All the LCA studies reviewed above employed an attributional modelling approach to assess the environmental impact. This type of LCA aims to answer the question of "how are pollutants, resources, and exchanges among processes flowing within a chosen temporal window" (Weidema et al. 2018). In other words, the results represent the environmental impact of an average product, which is already produced. To investigate the environmental consequences of the implementation of MP production, a more appropriate method is consequential LCA modelling, which seeks to answer the question of "how will flows of pollutants, resources, and exchanges among processes change in response to decisions" (Weidema et al. 2018). Therefore, the results only include products, processes or suppliers that are affected by a change (i.e. the marginal suppliers), for instance through the introduction of a novel and innovative technology to the market. In this regard, a consequential modelling approach is the most appropriate in the context of this study.

Due to the absence of LCA studies that compare different types of microbial metabolisms and the lack of a consequential LCA on this topic, it is the objective of this study to evaluate the environmental impact of the three different MP production systems introduced using a consequential modelling method.

2 Methodology

2.1 LCA methodology

This study follows the consequential LCA modelling approach as described by Weidema et al. (2009). This approach implies that only the environmental impact of processes that are expected to be affected by a change are accounted for. In the present case for example, MP production alters the state-of-the-art or 'baseline' of potato wastewater treatment process. When implementing MP production the 'baseline' potato wastewater treatment process is replaced. In the model this is accounted for as follows:

$$\textit{Net flow } X_{MP} = \textit{Gross flow } X_{MP} * FU_Q - \textit{Gross flow } X_{base} * FU_Q \quad (1)$$

Where Gross flow X_{MP} is the flow or emission of substance X of the MP production system and Gross flow X_{base} is the flow of substance X of the 'baseline' potato wastewater treatment. FU_Q is a scaling factor (or reference flow) that sets the Gross flows to the volume of wastewater required to produce 1 functional unit (FU). The FU of this cradle-to-plant gate study is the production of 1 t crude protein (CP).

Another feature of consequential LCA is that an increase in demand will be met by suppliers that are more competitive, while a decrease in demand for a product will result in the least competitive suppliers leaving the market. In other words, only the marginal suppliers are affected by a change instead of simply increasing or decreasing the current average production mix. Therefore, the production systems of the three MP types described above were compared to the current marginal supplier for protein feed, namely soybean meal. Multiple suppliers were taken into account and represented in a marginal mix. The process soybean meal at the global market of the consequential system model of ecoinvent 3.4 was taken to represent the marginal market mix for soybean meal. Another frequently discussed protein sources is fishmeal. However, fishmeal production is considered to be declining or stagnating globally (Olsen and Hasan 2012), and products with this characteristic do not qualify as marginal suppliers. Therefore, fishmeal is not investigated in this study. A final feature of the consequential modelling is that by-products are accounted for through substitution of functionally similar products at the market (e.g. those supplied by a marginal supplier), as in this study is the case for the substitution of palm oil by soybean oil (section 3.3).

The geographical location of MP production facilities is Belgium. A zero-burden approach was implemented (i.e. upstream production of the potato and its processing is not accounted for), as potato wastewater is currently considered a waste product that undergoes treatment (Ekvall et al. 2007). Foreground life cycle inventory (LCI) data for MP production and treatment of potato wastewater were derived from a mass balance prepared in excel. All assumptions applied in the mass balance are based on literature data (SM section 1). All exchanges (e.g. emissions, energy demand etc.) were entered into SimaPro 8.4 using data from the ecoinvent 3.4 database, applying the ecoinvent consequential system model for determination of marginal suppliers. To facilitate interpretation of the results they are presented at endpoint level in the categories human health, ecosystems, and resources derived using the ReCiPe v1.13 methodology (Hierarchist version with global normalization). The determination of emission and energy demand/generation is detailed in the SM section 1-4. A sensitivity analysis was carried out using Monte Carlo simulations implemented in SimaPro. The foreground parameters varied were those with the highest impact on the results (background parameters were varied as defined in the relevant ecoinvent processes). Specifically, this concerned the energy related parameters and ammonia emissions. Uniform distributions using the ranges shown in the SM section 5, were implemented to generate 1,000 pairwise comparative runs of MP production systems and soybean meal. Uniform distributions were chosen because more detailed distributions could not be generated from the limited literature information.

2.2 Process descriptions and data collection

2.2.1 Potato wastewater definition

The scale of the MP production as well as the 'baseline' was defined by a model potato processing effluent, with flow rate of 1,000 m^3/day and an influent composition of 10 kg COD/m^3, 0.3 kg N/m^3, 0.06 kg P/m^3. This corresponds to a high-strength potato wastewater as found in potato processing companies in Belgium (SM section 1). The temperature of the incoming process water was assumed to be 40°C, as it is uncommonly found in the potato industry. All effluent is treated before discharge to meet the effluent quality common in the potato industry (Figure 1, section 2.2.6). N (ammonium sulfate) and P (triple superphosphate) were supplied in addition to the wastewater where this was required to either maximize biomass growth or to avoid nutrient limitation during the final effluent treatment (AHB only; biomass composition SM section 1). For photolithotrophic production, values from studies using summer conditions were used (i.e. temperature and light). Any sludge produced that was not used for feed formulation was applied to land, replacing N and P fertilizers. The substitution is estimated for N based on the mineral fertilizer equivalent (MFE) of sewage sludge (Delin et al. 2012).

$$MFE = 87\% - 5\% * \frac{C}{N}\,ratio \tag{2}$$

At a the C/N ratio of 5 in sewage sludge this results in a mineral fertilizer equivalent of 62%, which is the value adopted in this study.

The agronomic effectiveness of P derived from sludge biomass was derived from studies that investigated sewage sludge. In general the results show a wide range of values of between 10-100% when compared to triple superphosphate. The value adopted in the research is 64% derived from the work of Andriamananjara et al. (2016), which is comparable to the work of O'Riordan et al. (1987) who detected average efficiencies between 74-79%. Both studies included sludge that was precipitated with iron or aluminum salts.

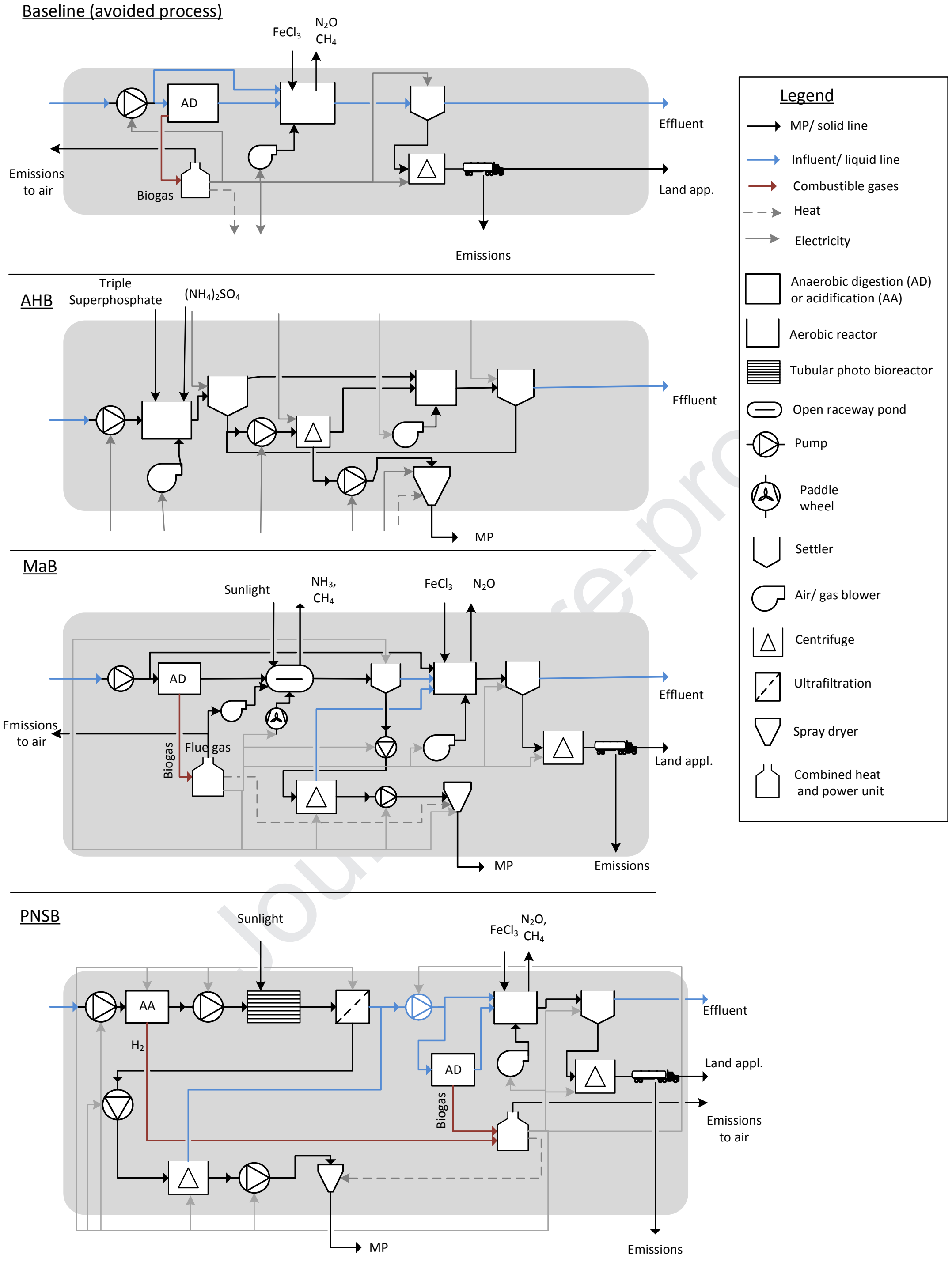

Figure 1: Process flow diagrams of the investigated baseline potato wastewater treatment and the three microbial protein (MP) production scenarios. Grey boxes denote the LCA foreground system boundaries. Abbreviations: AA = Anaerobic acidification; AD = Anaerobic Digestion; AHB = Aerobic Heterotrophic Bacteria; MaB = Microalgae and AHB; PNSB = Purple Non-Sulfur Bacteria; TSP = Triple Superphosphate; UASB = Upflow Anaerobic Sludge Blanket; Land app. = Land application.

2.2.2 Baseline water treatment

The baseline process describes a wastewater treatment process as it is encountered in the potato processing industry. It is comprised of an Up-flow Anaerobic Sludge Blanket (UASB) reactor followed by an activated sludge process. The UASB is used to remove a part of the influent COD, and for biogas generation. The activated sludge process with nitrification-denitrification is applied to meet COD and N discharge limits, while the defined P limit is met via chemical precipitation of $FePO_4$ by dosing $FeCl_3$ (Figure 1, section 2.2.6).

2.2.3 Production of aerobic heterotrophic bacteria (AHB)

AHB cultivation is carried out on potato processing wastewater in two stages. The first stage is a fully aerated tank at an SRT of 1 day. The AHB biomass produced at this stage is separated with a lamella settler, doubling the biomass concentration to 6.86 kg TSS/m^3 (0.7% DM). The liquid from this settler still contains about 3 kg COD/m^3, which is utilized to produce more AHB biomass in a second stage reactor with an SRT of 6 days (modelled as described in section 2.2.6). This biomass is again settled and centrifuged together with the biomass from reactor 1 (25% DM), after which the biomass is dried to obtain a market ready product with a DM concentration of 90%. Supernatants and permeates produced here are again channeled to the second reactor for treatment. As biomass growth is limited by N and P concentrations in the wastewater, these nutrients are added to maximize biomass production. Since N (ammonium sulfate) and P (triple superphosphate) are dosed to match the biomass growth demand, residual concentrations of these elements in the effluent are below the defined standard and therefore no $FeCl_3$ dosing or nitrification-denitrification is required.

2.2.4 Production of a microalgae-AHB (MaB)

MaB biomass is cultivated on the effluent of the UASB reactor in naturally illuminated ORPs, using a paddle wheel for agitation as applied by Van Den Hende et al. (2014). The UASB converts COD to CH_4

enabling the recovery of electricity and thermal energy in a combined heat and power (CHP) unit. Furthermore, the dissolved inorganic carbon present in the UASB effluent is used as a carbon source and pH buffer. The consortium composition of the microalgae and bacteria in the MaB flocs was estimated stoichiometrically. The O_2 demand by heterotrophs is met at a heterotrophs-to-microalgae ratio of about 0.7 (SM section 1.3). However, because not all O_2 is accessible for the heterotrophs, a ratio of 0.6 was assumed. In practice the pH of the ORP may rise to levels above 9 during periods of intense illumination, due to photosynthetic CO_2 removal. To avoid the negative consequences of such a pH rise (i.e. lower biomass production and NH_3 emissions), flue gas from the CHP unit process is sparged to maintain a pH below 8 (Van Den Hende et al. 2014). The biomass concentration realized in the ORP is 0.5 kg TSS/m^3 (Van Den Hende et al. 2014). The biomass produced in the ORP is settled (resulting in 0.1% DM), centrifuged (25% DM), and spray-dried to 90% DM. All supernatants and permeates are treated in the effluent treatment step, that comprises biological COD removal, nitrification-denitrification and $FeCl_3$ addition for P removal. No bypass of the effluent is required as the COD demand for denitrification is satisfied by the effluent from the solid-liquid separation steps.

2.2.5 Production of purple non-sulfur bacteria (PNSB)

The cultivation of PNSB begins with a hydrolytic, acidogenic and acetogenic fermentation step, with the purpose of converting incoming COD into VFA that act as electron donor and carbon source for PNSB biomass growth. The produced H_2 is burned in a CHP generating electrical and thermal energy. Following the anaerobic fermentation, PNSB are produced in an outdoor tubular photobioreactor illuminated with natural light (Figure 1). As in Carlozzi and Sacchi (2001), an SRT of 1 day was used resulting in a biomass concentration of 1.2 kg TSS/m^3. The biomass is harvested using ultrafiltration (UF) to a concentration of 10% DM, followed by a centrifugation step resulting in a DM concentration of 25%, before finally being spray-dried to 90% DM. As the COD_{VFA} removal efficiency of PNSB at SRT 1 day is only 57% and since the acidification step only removes 11% of the COD (SM section 1.1.2), 76% of the incoming COD is still available. To further remove this remaining COD, the tubular photobioreactor is followed by a UASB reactor to remove COD and produce biogas. A bypass of 0.7% (7 m^3/d) of the influent to the UASB is implemented to provide COD for denitrification in the activated sludge process.

2.2.6 Final water treatment to dischargeable quality

All MP production systems are followed by an effluent treatment step. The target composition of the effluent has been defined as the average of the Flemish potato industries (SM section 1). Specifically, the target values are 37 mg COD/L, 7 mg N/L, and 1.5 mg P/L. All treatment systems are based on the activated sludge processes for COD and N removal (including nitrification-denitrification – except AHB) and on chemical P removal by $FeCl_3$ dosage (except for AHB). The biomass yield and associated removal rates were calculated as detailed in SM section 1.4. Following the biological treatment, sludge is settled in a lamella separator and centrifuged to realize a DM of 25%, before disposal of the sludge to land. The $FeCl_3$ dosing is determined based on the stoichiometric ratio of 5.25 g $FeCl_3$/g P removed and multiplied by the efficiency factor of 1.5 (Tchobanoglous et al. 2003). The sludge produced from the precipitation (i.e. $FePO_4$) is added to the biologically produced sludge. Sludge is applied to land replacing N and P fertilizers, except for AHB where all sludge is used as a feed (section 2.2.3).

3 Results

3.1 Energy balance

Energy generation, energy demand as well as the gross energy flows per FU (i.e. the generated energy during MP production minus the energy consumed during MP production, equation 1) are shown in Figure 2. The gross energy flow for the different MP systems are: AHB of about 1,800 kWh (el)/FU and 9,100 kWh (th)/FU, MaB of about 40,000 kWh (el)/FU and about 52,000 kWh (th)/FU and PNSB of about 8,000 kWh (el)/FU and 8,800 kWh (th)/FU. The large difference in the overall values between the MP systems are partially a function of the MP system characteristics such as their ability to generate energy while producing MP, as is the case for MaB. However, importantly the differences are also a result of the production efficiencies measured as FU_Q (see equation 1), while AHB produces one ton of crude protein (i.e. the FU) on FU_Q=375m^3 of wastewater, MaB requires FU_Q=4,333m^3, and PNSB FU_Q=1,734 m^3. These different efficiencies are also reflected in the base values which are calculated using the same FU_Q. In accordance with equation 1, these base values, or the energy production/ demand of conventional baseline potato wastewater treatment, are subtracted from the values obtained for the MP production systems (i.e. because the process is avoided). The resulting value is denoted as $NetAHB_{MP}$, $NetMaB_{MP}$ and $NetPNSB_{MP}$ respectively in Figure 2. It can be observed that all processes have a net electrical and thermal energy demand; with the exception of MaB that has a net positive electrical energy balance of about 2,500 kWh. The reason that MaB has a net electricity production is that biomass growth in this system is partially autotrophic and N is incorporated in microalgal biomass without COD demand. Therefore, compared to the baseline, more COD can be anaerobically digested in the MaB scenario as no COD needs to be bypassed for N removal in the denitrification effluent treatment step. As a result, the overall electricity balance is positive as more biogas and energy is produced. The main processes contributing to the net electricity demand are in particular harvesting, production (for PNSB) and avoided energy generation (AHB, PNSB; Figure 2). However, heat demand generally exceeds electrical energy demand, and is dominated by the drying process (9100 kWh/FU AHB, 8500 kWh/FU MaB, 9600 kWh/FU PNSB).

In addition to the energy data described above, detailed and comprehensive life cycle inventory data are provided in SM Table 6. In this section only the energy related information were presented in detail as they have a pivotal impact (see section 3.3).

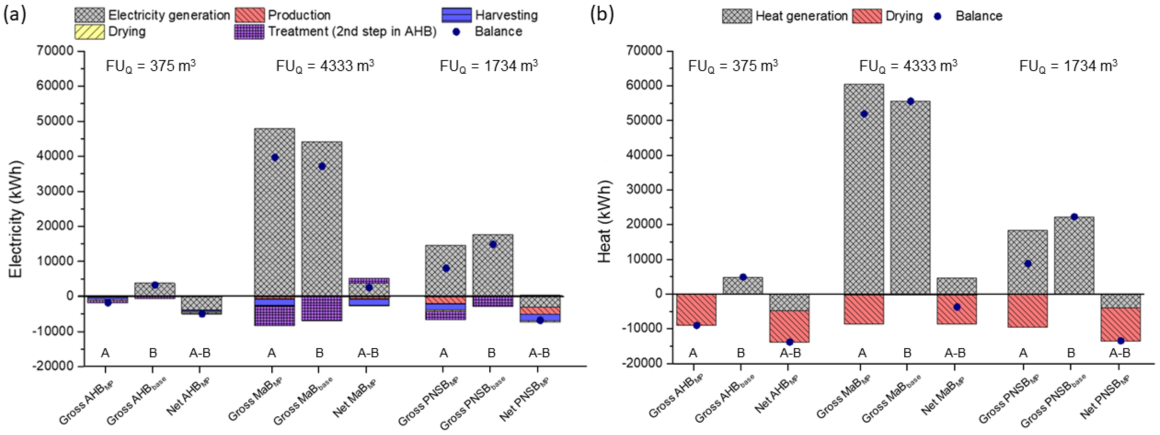

Figure 2: Energy balance per functional unit for the three MPs, showing MP production and baseline values (i.e. Gross) and the combination of both expressed as Net$_{MP}$ (equation 1 and supportive A, B letter code). Figure A shows the electrical energy balance and figure B shows the heat energy balance. Abbreviations: AHB = Aerobic Heterotrophic Bacteria; MaB = Microalgae and Bacteria consortium; PNSB = purple non-sulfur bacteria. The term 'base' indicates the energy production or demand of the baseline systems. FU$_Q$ indicates the wastewater flow required to produce one FU.

3.2 Impact assessment

Results show that for the human health and the ecosystem categories MP cause lower impacts than soybean meal, while they cause a higher impact in the category resources (Figure 3, life cycle inventory SM Table 6). In detail, for human health (Disability Adjusted Life Years - DALYS) AHB and PNSB obtain a similar score of 48-52% relative to soybean meal, while the impact of MaB is somewhat higher at 60%. For ecosystems impacts (species/year) again AHB and PNSB have similar values of 28 and 26% relative to soybean meal, with MaB showing a clearly lower value of 13%. For resource depletion (US Dollar - USD) AHB has the highest impact (100%), followed by PNSB with 91% of the impact relative to AHB. MaB show an impact of only 20% of that of AHB and soybean meal production causes an impact of 12% relative to AHB.

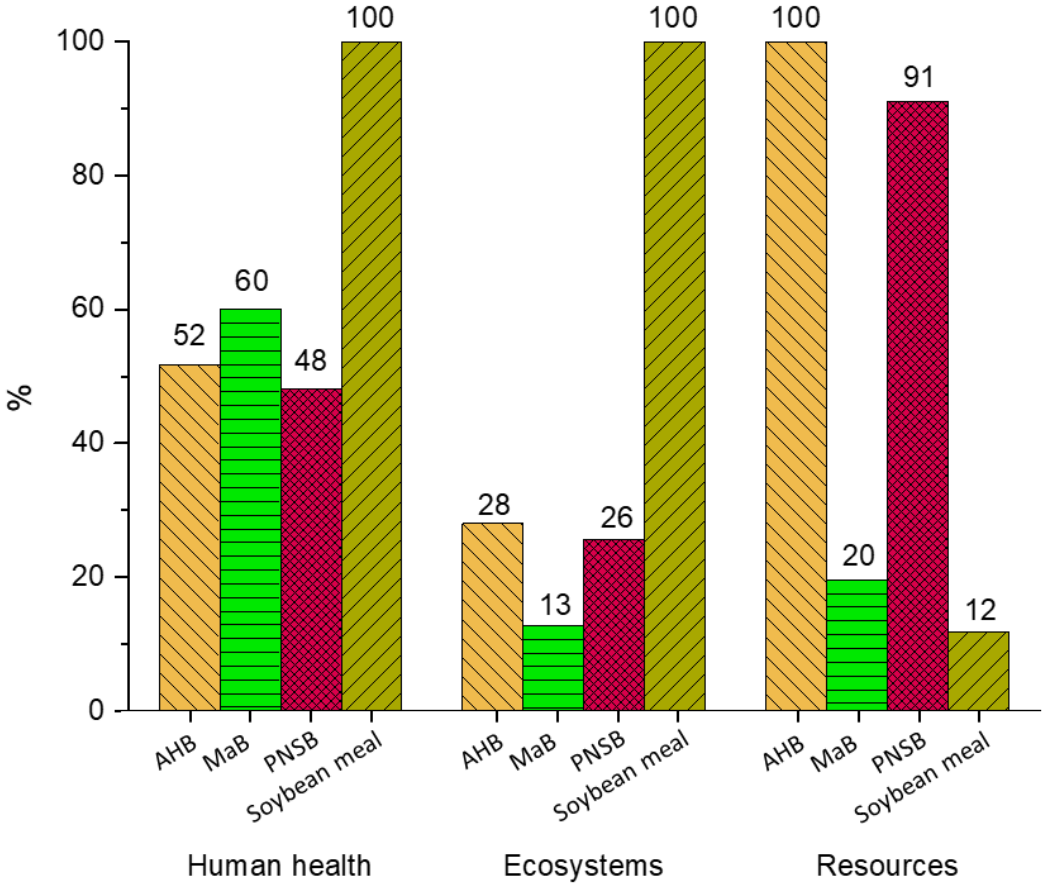

Figure 3: Comparative endpoint impact assessment of microbial protein production compared to soybean meal production. The figure shows the relative impact in percent in comparison to the process with the highest impact per category.

Figure 4 shows the contribution of individual mindpoint categories to the endpoint impacts. It clearly displays that impacts on human health are mainly a function of climate change indicators with the exception of MaB where most of the endpoint impact is originating from particulate matter formation. The main contributors to ecosystem impacts are climate change and agricultural land occupation for AHB and PNSB. MaB show comparatively low impacts in this category. For soybean meal the agricultural land occupation, natural land transformation and climate change impacts have high contributions. In particular, soybean meal shows three times higher contribution in agricultural land occupation and nearly double the natural land transformation than MP. It is hence these two impact categories that are responsible for the big difference on the ecosystems endpoint impacts above. In this study, resource use is measured as fossil depletion only, and hence simply reflects the results as shown in the impact assessment of Figure 3.

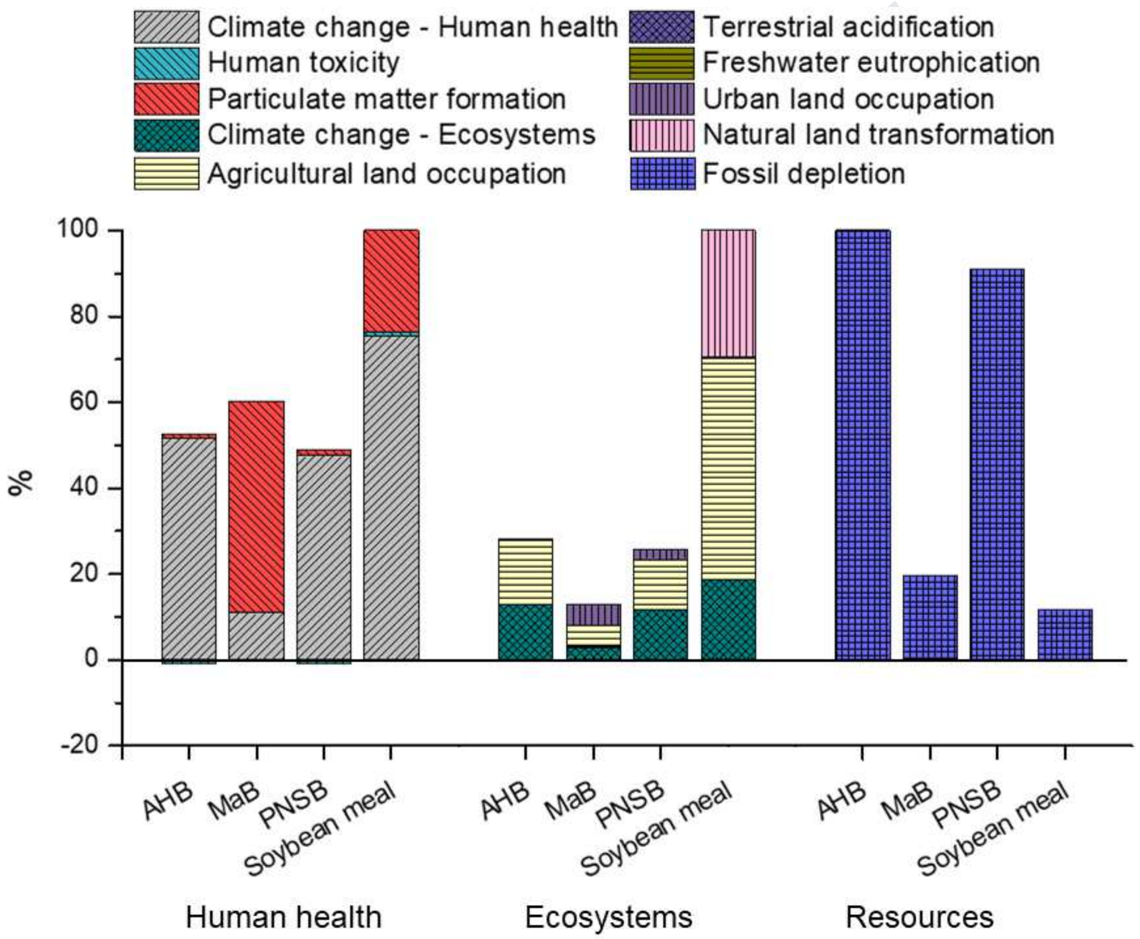

Figure 4: Contribution of individual mindpoint categories to the endpoint impacts in terms of human health, ecosystems and resource depletion (details can be accessed in Table 7 of the SM).

3.3 Contribution analysis

In order to further understand the reasons for the observed impacts, results are presented as a function of the contributing processes. Figure 5 shows that the contributions across all endpoint impact categories are a result of heat demand (SM Table 8). The exception to this is MaB, where the main contribution to human health impact is derived from direct emissions (0.006 DALYS). This is mainly due to gaseous emissions of NH_3 from the ORP (section 2.2.4). Another reason for this is that MaB has a relatively low heat demand and is even a net electricity producing system. Therefore, the relative impact originating from thermal energy related processes is reduced and impacts originating from electrical energy is even avoided (-0.0005 DALYS). In addition to thermal energy, electrical energy demand has a considerable impact in particular on human health and resources use. It can also be noted that all systems avoid environmental and resource related impacts through the reduced utilization of iron (III) chloride (from -0.0004 DALYS for MaB to -0.0006 DALYS for AHB; -7.1 USD for MaB to -10.9 USD for AHB). This can be realized because MP incorporate P in their biomass and hence less $FeCl_3$ needs to be dosed for P precipitation. However, for the same reason MP systems have an impact through a reduced production of N- and P-rich sludge that is used as fertilizer on land. This reduced nutrient application results in an increase in demand for N and P fertilizers from other sources, which is reflected in the graph by impacts generated through N and P fertilizers (up to 0.00029 DALYS for P in AHB and up to 0.001 DALYS for N in MaB; up to 0.000001 species/ year for P in AHB and up to 0.000008 for N in for MaB). For AHB it must also be noted that the addition of ammonium sulfate to avoid N limitation also contributes to all impact categories. This effect is less pronounced for triple superphosphate that is dosed to avoid P limitation.

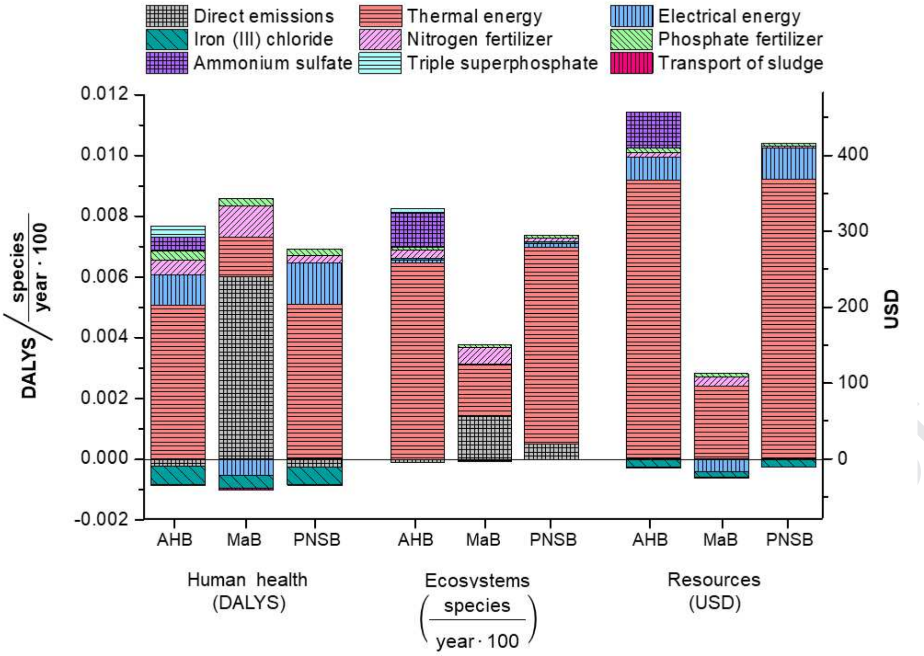

Figure 5: Contribution of processes to impacts at endpoint level. Disability Adjusted Life Years – DALYS, United States Dollars – USD

Investigation of the results for soybean meal shows that for human health, key contributions are a consequence of clear cutting of secondary forests in Argentina and primary forests in Brazil; contributing more than 71% of the caused impacts (Figure 6). Other important impacts are a result of the soybean production process itself (17%) and arable land use change to annual cropping in Brazil (6%). A fraction of the impact (6%) is avoided due to reduced production of palm oil, that is substituted by the soybean oil which is a co-product of the soybean meal production. For ecosystem impacts, species loss is mainly caused by clear cutting primary forests in Brazil (33%). A similar contribution is made by the production process of soybean in the US (28%) followed by production in Brazil (10%), Argentina (9%), and the supplies from the rest of the world (8%). Finally, again clear cutting of secondary forests in Argentina plays a key role (6%), with the remaining processes contributing less than 7%. The impact of palm fruit production is not explicitly shown in the figure as individual processes have an avoided impact of less than 5% and are thus aggregated in the sum of the remaining processes (see SM Table 8 for a detailed breakdown of impacts). Resource use is a function of the ethylene, petroleum, and gas production. Ethylene must be produced because palm oil production is avoided and petrochemical based substitute for a fraction of the palm kernel oil is now produced. Overall it can be concluded that land occupation and land use changes are key contributing categories.

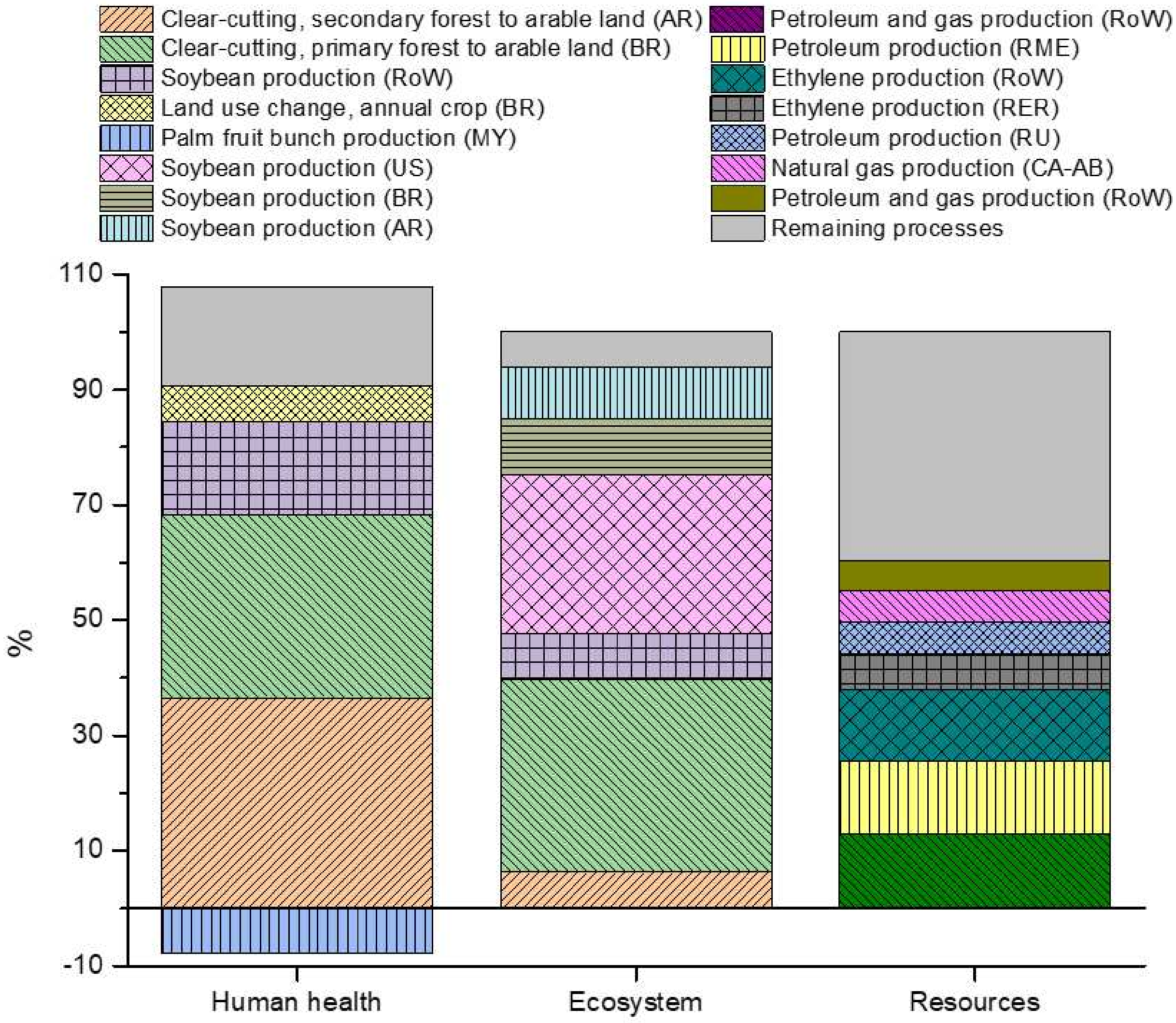

Figure 6: Contribution of processes to impacts at endpoint level for soybean meal at the global market. The figure shows only the process that contribute more than 5% to the total impact. BR – Brazil, US – United States, RoW - Rest of World, RER – Rest of Europe, RME - Middle east, RU – Russia, AR – Argentina, CA-AB – Canada Alberta.

3.4 Sensitivity analysis

The results show that the overall conclusions of the LCA are robust (Figure 7). Namely, there is no case in which more than 50% of the 1,000 Monte Carlo runs suggest that soybean meal exceeds impacts of MP on ecosystems or human health. In particular, the impact on ecosystems is robustly lower for MP, with a maximum of 5% of the Monte Carlo runs indicating the opposite. For human health results show that 30% of the scenarios for AHB and 7% of the scenarios for PNSB could have a lower impact than soybean meal. Similarly, in 15% of the runs for MaB the impact of soybean meal could be higher. With regards to the impact on resources it can be seen that no scenario, for neither AHB nor PNSB, results in a better performance of these MP. For MaB however, 29% of the runs obtained a result with a lower impact than soybean meal.

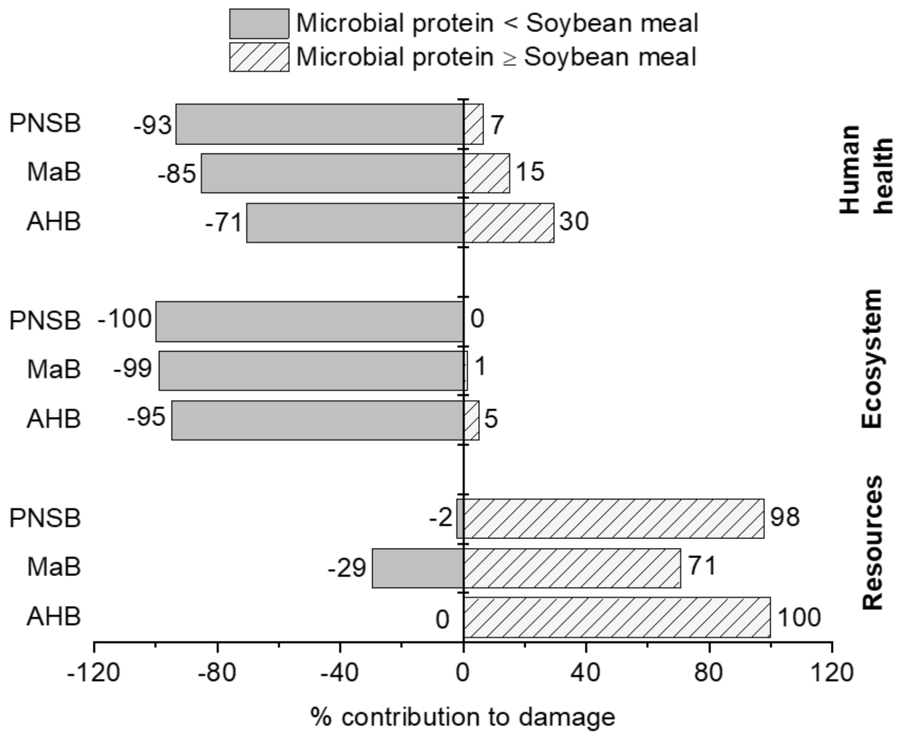

Figure 7: Pairwise sensitivity analysis (1,000 runs in a Monte Carlo simulation) of microbial protein and soybean meal per endpoint impact category. Microbial protein < soybean meal indicates the percentage of runs where the impact of soybean meal

exceeds that of microbial protein. Microbial protein ≥ soybean meal indicates the percentage runs where the impact of soybean meal is lower or equal to that of microbial protein.

4 Discussion

4.1 Environmental impact of microbial protein

To the knowledge of the authors, the present study is the first to investigate the cultivation of MP on food and beverage wastewater, using the case of potato wastewater. The results show that MP exert a lower impact than soybean meal within the categories human health and ecosystems, but a higher impact when resource exploitation is considered. A similar conclusion is reached by Taelman et al. (2015), based on an exergy analysis of a small scale plant producing microalgal biomass and comparing it to soybean meal. They suggest that this system leads to higher resource exploitation than soybean meal feed production, in particular because of the high fossil energy use. However, their sensitivity analysis suggests that the microalgal MP production can become more resource efficient when energy related optimizations are implemented at larger scale. Indeed, for resources also the sensitivity analysis of the present study suggests that MaB could become more resource efficient under certain circumstances.

Smetana et al. (2017) carried out a sensitivity analysis for different microalgae production systems i.e. autotrophic and heterotrophic in different reactors ORP, photobioreactor and aerobic fermenter for *Chlorella vulgaris* and *Arthrospira platensis*. They concluded that: “The most promising scenario […] would be the combination of microalgae production in heterotrophic conditions with the use of food waste as a source of carbon and photovoltaic energy generation for cultivation.”. However, their results still indicate that even under these conditions soybean meal production would have a lower environmental impact when measured as CO_2-eq. Yet, their assessment did not account for land use changes caused by the demand for soybean meal. If land use changes would be included, their findings may be more in line with the present study, as the results indicate that land use changes contribute substantially to the impact. Furthermore, Smetana et al. (2017) suggested that MP cultivation under heterotrophic conditions is preferable. The present study can only confirm this conclusion for the human health category where the heterotrophic production systems for AHB and PNSB exert the lowest impact. The partially photolithoautotrophic MaB system shows higher impacts in the human health category, due to NH_3 emissions from the ORP which occur at times of high photosynthetic activity and an associated rise in pH. Further lowering the pH may eliminate this impact, but it needs to be further investigated whether more intensive flue gas sparging or other approaches can achieve this.

In conclusion, the common denominator of the present and past studies is that for the MP investigated, production can be preferable to soybean production at large scale (energy) optimized conditions.

4.2 Energy use and other process improvements

Results suggest that MP production generally has a high energy demand, reflected in all endpoint impact categories. Indeed, there is a consensus in the LCA studies that the most resource demanding processes in the MP production systems are heat demand for drying, heating of reactors as well as electricity demand for harvesting (Smetana et al. 2017, Taelman et al. 2015).

In the present study, one exception to the above claim is the MaB process. While also for MaB energy related issues play are role; Figure 5 shows that in the human health and ecosystem categories thermal energy demand plays a minor role. MaB is therefore relatively energy efficient, which is realized through the (partially) autotrophic metabolism. The autotrophic metabolism enables biomass production without the utilization of COD. Together with the biomass, N is removed from the wastewater. As a consequence, less COD is bypassing the UASB reactor to supply carbon to the denitrification process in the effluent treatment step. In fact, no by-pass is implemented in this study as the required COD is provided by the reject water/ biomass from solid-liquid separation (section 2.2.4). As a result, the MaB system produces more biogas than the baseline scenario, resulting in a net positive electricity production and a comparatively low thermal energy demand (section 3.1). The MaB production system therefore has the advantage of maintaining and even increasing energy recuperation, while producing MP.

Another pathway to tackle energy related issues in MP production is to satisfy the energy demand with currently unused waste heat or renewable electricity sources. With regards to thermal energy, low-grade waste heat that is currently not utilized could be used. For example, waste heat of refrigerator systems that are common in the potato industry could be explored as a source of heat (Patel and Kar 2012). Electricity demand for production and harvest is relatively high in the PNSB system due to the operation of the tubular photobioreactor and a poor settleability of the biomass. This may further be reduced, as there are indications that PNSB can be cultivated in ORP (García et al. 2019). If this can be followed by a conventional settler as the first solid-liquid separation step and not by using UF as in this research, energy demand can be further reduced. Another key impact for MaB and PNSB is the demand for $FeCl_3$ for P removal. Currently, a number of potato processing industries have installed P removal through struvite precipitation (Desmidt et al. 2013). Struvite is considered a slow-release P fertilizer and a recent LCA study of struvite produced from municipal wastewater treatment plants suggests that environmental benefits can arise in the categories cumulative energy demand, global warming potential and acidification, through reduction of flocculant demand and replacement of N and P fertilizers (Amann et al. 2018).

In conclusion, energetically MaB production is most promising, but also for the other MP production systems energy demand can further be reduced and other process improvements may be implemented in the future. As a consequence, a further reduction of environmental impacts in particular in the resource category can be expected.

4.3 Future research needs

Results of the study show that, in particular for the human health and ecosystems categories, the impacts of soybean meal exceed that of any MP by 40% and 70% respectively. The analysis shows that these are strongly related to land use changes in the tropical regions and to arable land occupation by the production of soybean. Indeed, literature does confirm the importance of land use changes, but it also demonstrates that it can be rather variable. Castanheira and Freire (2013) showed that CO_2-eq. emissions vary between 0.1 and 17.8 kg CO_2-eq/kg of soybean produced depending on whether land use change is avoided or whether rainforests or other types of land use are converted to soybean production. Given this variability, the importance of determining the location of the marginal soybean production and associated land use change is crucial. While the present study makes use of the widely accepted ecoinvent 3.4 data base to determine marginal suppliers, it could be of research interest to evaluate the present results against the outcomes obtained when applying the methodology to determine marginal suppliers, as proposed by Weidema et al. (2009). In that manner the impact of the uncertainty in the currently used data could be explored.

However, more important than considering the direct land use changes, may be indirect land use change. Schmidt et al. (2015) proposes that indirect land use changes can play a more significant role than direct land use change caused by the clear cutting of land for crop cultivation. Adopted to the present study, their proposal is that the additional area of soybean production on, for example, agricultural land in the US, will result in a situation where the crop that is replaced by soybean must be produced elsewhere. They argue that this production, or the indirect land use change, will almost always involve deforestation and hence have substantive environmental impact. These considerations would imply that, if indirect land use changes are accounted for adequately, impacts of soybean meal production could be even higher than found in this study. Currently however, the ecoinvent database does not follow the modelling methodology as proposed by Schmidt et al. (2015), but future studies should aim to also include these aspects.

Finally, the present LCA does not account for additional functionalities of MP. In particular the MaB (i.e. the microalgae specifically) and PNSB do not only contain protein, but also valuable essential fatty acids, carotenoids, and vitamins (Chumpol et al. 2017). In a strict implementation of the consequential LCA methodology environmental impacts of by-products are accounted for through substitution. For soybean meal this is the case of the soybean oil that is substituting other vegetable oils at the market. Future LCA studies may therefore seek to include these other functionalities of MP by substituting comparable products at the market.

5 Conclusions

- From the results of this study it can be concluded that Microbial Protein (MP) is an novel protein source that should play a role in the future protein transition. The comparison with soybean meal shows that MP produced on potato wastewater exerts lower impacts in the human health and ecosystem categories. However, thermal and electrical energy demand as well as avoided energy production from the baseline result in a higher impact of MP than soybean meal for the endpoint impact category resources.
- Between the three MP production systems studied, MaB has the lowest impact in categories ecosystems and resource use, while AHB and PNSB have lower values for human health. Therefore, another tentative conclusion is that the autotrophic growth mode has a low environmental impact, in particular because the energy recuperation is maximized.
- Future research should explore further reduction in energy demand and the use of alternative energy sources, especially with focus on heat demand.

- Future LCA studies should also account for the fact that MP do not only contain protein, but also valuable components such as polyunsaturated fatty acids, carotenoids, and vitamins.
- Accounting for the impact of indirect land use changes for soybean production may further increase the environmental impact gap between MP and soybean meal.

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Highlights

- Microbial protein has lower impact than soybean meal for human health & ecosystems
- Microalgae-bacteria consortia have least impact on resource use & ecosystems
- Environmental impacts of microbial protein are mainly energy-related
- Land use and land use change are key contributors to impact of soybean meal

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: