INTEGRATING MICROALGAE PRODUCTION WITH ANAEROBIC DIGESTION: A BIOREFINERY APPROACH

Enrica Uggetti[*], Bruno Sialve, Eric Trably, Jean-Philippe Steyer

INRA, UR0050,
Laboratoire de Biotechnologie de l'Environnement,
Avenue des Etangs, Narbonne, F-11100, France.

[*]Corresponding author: Enrica Uggetti

Tel: +33 468 42 51 51

Fax: +33 468 42 51 60

E-mail address: enrica.uggetti@supagro.inra.fr

Abstract

In the energy and chemical sectors, alternative production chains should be considered in order to simultaneously reduce the dependence on oil and mitigate climate change. Biomass is probably the only viable alternative to fossil resources for production of liquid transportation fuels and chemicals since, besides fossils, it is one of the only available sources of carbon rich material on earth. Over recent years, interest towards microalgae biomass has grown in both fundamental and applied research fields. The biorefinery concept includes different technologies able to convert biomass into added value chemicals, products (food and feed) and biofuels (biodiesel, bioethanol, biohydrogen). As in oil refinery, a biorefinery aims at producing multiple products, maximizing the value derived from differences in biomass components, including microalgae. This paper provides an overview of the various microalgae-derived products, focusing on anaerobic digestion for conversion of microalgal biomass into methane. Special attention is paid to the range of possible inputs for anaerobic digestion (microalgal biomass and microalgal residue after lipid extraction) and the outputs resulting from the process (e.g. biogas and digestate). The strong interest for microalgae anaerobic digestion lies in its ability to mineralize microalgae containing organic nitrogen and phosphorus, resulting in a flux of ammonium and phosphate that can then be used as substrate for growing microalgae or that can be further processed to produce fertilizers. At present, anaerobic digestion outputs can provide nutrients, CO_2 and water to cultivate microalgae, which in turn, are used as substrate for methane and fertilizer generation.

Keywords: Microalgae, Biorefinery, Co-products, Anaerobic Digestion, Methane production.

1. Introduction

Nowadays, the important increase of the oil demand is placing an enormous pressure on the finite supply of fossil fuel-derived energy and chemicals. For this reason, the development of alternative production chains in the energy and chemical sectors is necessary in order to simultaneously reduce the dependence on oil and mitigate climate change.

Plant-based raw materials (i.e. biomass) have the potential to replace a large fraction of fossil resources as feedstock for industrial production. Due to its high carbon content, biomass is a suitable alternative to fossil resources for production of liquid transportation fuels and chemicals. In addition, biomass resources are locally available in many countries and their use could largely contribute to reduce national dependence on imported fossil fuels.[1]

Beyond their energetic value, microalgae have been widely investigated as sources of chemicals, cosmetics and health products, animal and human feed. In fact, photosynthetic organisms such as higher plants, algae, and cyanobacteria are capable of using sunlight and carbon dioxide to produce valuable organic molecules, such as carbohydrates, lipids, pigments, fibers, etc. Over the recent years, the interest for microalgae biomass has increased in both fundamental and applied research fields aiming at producing biofuels and biochemicals. This paper provides an overview of the various products obtained from microalgae biomass, with a special focus on anaerobic digestion for methane and fertilizer production.

2. Microalgae biorefinery

The biorefinery concept consists in different technologies able to convert any type of biomass to value-added products, biofuels and chemicals. This concept is derived from the petroleum refinery, which uses petroleum to produce multiple fuels and products with applications in various industries. As in oil refinery, a biorefinery aims at generating multiple end-products, and maximizing the value derived from differences in biomass components. In order to design an efficient and cost effective biorefinery, an important stage is the provision of a renewable, consistent and regular supply of feedstock (raw materials used in biorefinery). In this context, microalgae, including all unicellular and simple multi-cellular microorganisms, such as prokaryotic microalgae (e.g. cyanobacteria *Chloroxybacteria*), eukaryotic microalgae (e.g. green algae *Chlorophyta*), red algae (*Rhodophyta*) and diatoms (*Bacillariophta*) play an important role as biorefinery feedstock.[2] These photosynthetic organisms can be cultivated in freshwater, seawater and wastewater, and they can be farmed on non-arable land. Moreover, certain microalgae can tolerate and adapt to a wide variety of environmental conditions (in terms of pH, temperature, light, etc.) and can be produced all year round. Table 1 compares the biomass productivity of microalgae (up to 70 ton dry weight (DW) per ha per year) and conventional agricultural crops together with their raw energy productivity.

Microalgae are typically composed by proteins, carbohydrates, lipids, and other valuable components (e.g. pigments, anti-oxidants, fatty acids and vitamins) (Table 2). These components are valuable for a wide range of applications. The carbohydrates present in microalgae are considered as an appropriate feedstock for microbial growth and generation of various fermentation products. The high lipid content in algal biomass makes it promising for biodiesel production. However, special attention to the fractions of lipids stored in microalgae should be paid, and unsaturated fatty acids from microalgae may need to be hydrogenated to improve fuel properties. Finally, the related long-chain fatty acids, pigments and proteins have their own nutraceutical and pharmaceutical applications. However, the technology for the commercial production of microalgae bioproducts is still being under development and investigation. More particularly, additional efforts should be made to reduce the operating costs, that are essentially associated with algal biomass growth (e.g. nutrients, light and CO_2 distribution), harvesting (i.e. isolation of the biomass from the culture, dilution or concentration of algae to suitable levels for further processing), and downstream processing obtaining valuable products or subproducts.

In this sense, even though economics are strictly correlated with the biochemical composition of the biomass, Williams and Laurens (2010)[5] emphasized that the “biofuel only” option is unlikely to be economically viable and other sources of revenue are needed to make the system profitable. For this reason, the main challenge prior to any biorefinery development is the optimization of efficient and cost effective production of transportation biofuels, biomaterials and biochemicals, by using all biomass components as co-products.

2.1 Pharmaceuticals, food and feed

Many microalgae naturally contain omega-3 fatty acids which can be purified to provide a high-value food supplement.[6] In addition, eicosapentanoic acid (EPA) as well as decosahexaenoic acid (DHA) have pharmaceutical applications in the treatment of heart and inflammatory diseases (e.g. asthma, arthritis, headache and psoriasis) as well as in the prevention and cure of cancer, AIDS, to control and lower cholesterol, or to boost the immune system and body detoxification.[7]

The antioxidants produced from microalgae to protect the photosynthetic cells from oxidative stress can be used in the medical field to limit or prevent health problems, such as atherogenesis, cancer, neurodegenerative diseases, infant retinopathy, muscular degeneration and renal failure.[8] In addition, hydrocarbons contained in microalgae can replace the paraffinic and natural waxes in the production of facial masks for the cosmetic industry.

Microalgae are also used in pharmaceuticals or in cosmetics as a source of chlorophyll pigment and they are currently gaining importance as a food additive due to their strong naturally green color. Traditionally the above mentioned compounds have been obtained by solvent extraction. However many researchers are nowadays focusing on more sustainable extraction techniques. As an illustration, supercritical CO_2 extraction was recently applied for successful lipid extraction on *Botryococcus braunni*, *Chlorella vulgaris*, *Dunaliella salina* and the cyanobacteria *Arthrospira (Spirulina) maxima.*[9] However, CO_2 can only extract the neutral lipid fraction and, in order to achieve higher yields, alternative extraction techniques combined with polar extraction solvents (e.g. microwave-assisted extraction, ultrasound-assisted extraction, extraction with pulsed electric field, bead-beating-assisted extraction, Soxhlet extraction, pressurized fluid extraction, and others) were also reported in the literature, each having their own advantages and disadvantages.[10]

Many algal species have been also examined by various researchers for their biochemical compositions to be suitable as substitute or primary livestock feed. Indeed, it has been reported that microalgae can play a key role in high-grade animal nutrition food, from aquaculture to farm animals. Comprehensive nutritional and toxicological evaluations demonstrated the suitability of algae biomass as a valuable feed supplement or substitute in conventional animal feed sources.[11]

2.2 Fuel products

2.2.1 Biodiesel

The viability of microalgae for biodiesel production has been investigated by a number of studies.[12, 13, 14] Authors pointed out that, in spite of a certain dependence of the oil yield of the algal strain, the oil content of microalgae is generally much higher than for other plant crops. In fact, many species of algae produce amounts of lipids as high as 50–60% of their dry weight. Various methods for lipid extraction from microalgae were reported in literature, the most common methods being expeller/oil press, liquid–liquid extraction (solvent extraction), supercritical fluid extraction and ultrasound techniques.[13]

Concerning the species the most suitable for biodiesel production, *Botryococcus braunii*, *Chlorella vulgaris*, *Nannochloropsis* sp., *Nitzschia laevis*, *Parietochloris incise* and *Schizochytrium* sp. have oil contents higher that 50% dry weight.[15] However, only few strains are nowadays commercially produced and there is a strong need for screening for new strains or modifying the existing strains in order to reach an optimal lipid content for efficient biodiesel production.[16]

2.2.2 Bioethanol

Bioethanol from algae represents a significant potential due to their low percentage of lignin and hemicellulose compared to other lignocellulosic plants and to the important amount of carbohydrates, typically galactose (23%) and glucose (20%) which are energy-rich compounds[17] In fact, certain species of microalgae have the ability of producing high levels of carbohydrates instead of lipids as reserve polymers. The starch accumulated within the chloroplasts or the cytoplasm[18] is a source of carbohydrates that can be extracted to produce fermentable sugars. Bioethanol from biomass could therefore be obtained by means of biochemical processes (i.e. fermentation), thermo-chemical processes or gasification. The microalgae *Chlorella vulgaris*, more particularly, has been considered as a promising feedstock for bioethanol production as it can accumulate up to 37% (dry weight) of starch.[19] *Chlorococum* sp. was also used as a substrate for bioethanol production under different fermentation conditions.[19] Bioethanol can be produced directly from the microalgae biomass or from the exhausted biomass following lipid extraction. For example, Harun et al. (2009)[20] tested the effect of different fermentation conditions and parameters on accumulation of bioethanol and found that the lipid-extracted microalgae gave 60% higher ethanol concentrations than the dried and intact microalgae. In this way, microalgae could be used for the production of both lipid-based biofuels and for ethanol biofuels from the same biomass, thus increasing their overall economic value.

In addition, CO_2 produced as by-product from the fermentation process can be recycled as carbon source for further microalgae cultivation. This aspect is discussed in further details below.

2.2.3 Biohydrogen

In the case of biohydrogen production, microalgae can either produce themselves biohydrogen after derivation of their photosynthetic metabolism, or be used as feedstock for further biohydrogen production by microbial dark fermentation.[21,22] For one side, certain photosynthetic microalgae and cyanobacteria are capable of directly producing biohydrogen through photobiolysis involving the oxidation of ferredoxin by the hydrogenase enzyme, but only when the cellular metabolism is restricted, ie. under medium (S) starvation and low light intensity. In that case, the reduced ferredoxin are reoxidized by transfering their electrons to the hydrogenase. However, hydrogenases directly compete with many other metabolic processes for the partitioning of electrons, and are strongly inhibited by the presence of the oxygen, produced concomitantly by photosynthesis. To avoid such inhibition, a two steps growth, so-called indirect biopholysis, is recommended where the microalgae grows in the first stage with no light or medium limitation followed by hydrogen production under medium (S) starvation and lower light intensity.

In this context, a significant amount of recent research on microalgae photobiohydrogen production has focused on the optimization of process operation as well as the identification of more robust hydrogenase activities, and especially on oxygen-tolerant hydrogenases.[23, 24]

In addition, certain purple non sulfur (PNS) bacteria, e.g. *Rhodobacter* sp. or Rhodospirillum sp., can also produce biohydrogen by photofermentation.[22] This consists in the fermentation of organic compounds (sugars, volatile fatty acids, alcohols) under illumination but in absence of nitrogen in the growth medium. In these microorganisms, the organic compounds are oxidized by a fermentative pathway, ie. under anoxygenic conditions, and the protons are reduced by a nitrogenase, when the cells are under nitrogen starvation.[25] In fact, nitrogenase has a high affinity to nitrogen and any nitrogen source in the medium can cause severe inhibition of the phtotofermentative production of biohydrogen. Moreover, this cellular mechanism requires high amount of energy in the form of ATP molecules, and therefore with low hydrogen yields (<1.5 moleH2 per mole glucose).[25]

On the other side, microalgae can also be used as substrate for dark fermentation to produce hydrogen. The hydrogen productivities are considerably higher with microbial dark fermentation when considering the use of algae as substrate than through photobiological pathways. For this reason, dark fermentative H_2 production from microalgal biomass has received increasing attention over the past few years. It was shown that the use of microalgae *Chlamydomonas* spp., *Chlorella* sp., *Dunaliella tertiolecta* and *Scenedesmus* spp. as feedstock led to hydrogen yields ranging between 17 and 114 mLH_2/gVS (volatile solids).[26]

These results are consistent or even competitive with the biohydrogen yields obtained fromterrestrial plants and agricultural wastes, as previsouly reported byGuo et al. (2010) [27] As pointed out by Cheng et al. (2011), the algal biomass is very suitable as feedstock for biohydrogen by dark fermentation since several strains of microalgae could accumulate carbohydrates in significant amounts.[28] Yang et al. (2010) suggested also to use the residual microalgal biomass after oil extraction processes to produce hydrogen, which suits perfectly with a concept of environmental biorefinery.[29]

2.2.4 Biogas

Anaerobic digestion is a common process to treat organic waste in most of the developed countries across the world. During the past few years, it has been largely implemented because of the increase in the economic subsidies for generation of electricity from biogas. In certain countries (such as Germany and Sweden), biogas is also used as transportation biofuel, after purification upgrading to biomethane. In the following, we will focus on the anaerobic conversion of microalgae biomass to methane. Special attention will be paid to the vast range of possible inputs on anaerobic digestion and outputs resulting from the process (e.g. biogas and digestate).

3. Anaerobic digestion of microalgae

Anaerobic digestion is a microbial process of degradation and stabilization of organic materials under anaerobic conditions, leading to the formation of biogas and digestate (with liquid and solid phases). The process is carried out by heterogeneous microbial populations involving multiple biological and substrate interactions. Anaerobic digestion (also called methanogenic fermentation, or methanogenesis) is widely applied to the treatment of liquid wastewaters (in particular for the treatment of effluents from food, pulp, paper and chemical industries) and solid waste originating from agriculture (e.g. manure and plant residues) or from urban activities such as sewage sludge in wastewater treatment plants and the organic fraction of municipal solid wastes (OFMSW)).

3.1 Substrate for anaerobic digestion

3.1.1 Microalgae

During the past years, interest has grown in favor of anaerobic digestion of microalgal biomass, leading to studies on various freshwater and marine microalgae, and using different process combinations. Over the past five years, investigations tested a wide range of process temperatures, reactor configurations, pretreatment methods as well as the use of co-substrates. Due to the specific cell wall properties, anaerobic digestion efficiency is often strain specific.[30,31] Indeed, a significant variability of the methane yield (from 140 up to 400 $mLCH_4/gVS_{influent}$) is observed in the literature, likely due to different operating conditions of the digester (i.e. bioreactor type, hydraulic retention time and the digestion temperature[30]) in combination with microalgal strain selection and cultivation conditions that are responsible of variations in protein, carbohydrate and lipid cellular contents, as well as cell wall structure.[32]

Recently, Frigon et al. (2013)[33] tested under similar operating conditions a selection of 15 freshwater and 5 marine microalgae in order to identify a microalgal strain suitable for large scale production of methane. The Biochemical Methane Potential (BMP) tests were performed using a microalgae:sludge inoculum ratio of 2:1 based on volatile solids concentration. Results showed no significant difference in the maximum methane yield between freshwater microalgae (330 $mLCH_4/gVS_{influent}$) and marine microalgae (300 $mLCH_4/gVS_{influent}$) although it varied greatly within the tested strains (230-410 $mLCH_4/gVS_{influent}$).

Moreover, the anaerobic digestion process can be inhibited by ammonia issued from biological degradation of nitrogenous matter and by sulfide causing toxicity effects on various bacterial groups.[32, 34] Toxic effects on AD can also be induced by high sodium levels when marine microalgae are used as a substrate. Optimum sodium concentrations are around 230-350 mg Na^+/L, while inhibitory effects were reported at concentrations higher than 3,500 mg Na^+/L.[34]

The wide and recent interest of the scientific community on microalgae anaerobic digestion is related to its ability to mineralize algal waste containing high amount of organic nitrogen and phosphorus, resulting in a flux of ammonium and phosphate that can then be reused as substrate for microalgae cultivation[35,36] or further processed to obtain fertilizers. Similarly to light, CO_2 and water, the lack of nutrients can be an important obstacle preventing the scaling up of microalgae biorefinery technologies.[5] Here, these nutrients are partially supplied by the outlet of the anaerobic digester. In this context, the microalgae grown in wastewaters, together with other residues, can be used as a digestion substrate and the digestion outputs (nutrients, water and CO_2) can provide substrates for microalgal culture (Figure 1). Then, the methane produced from the anaerobic digestion process can be converted to generate transportation biofuel, heat, or electricity used in microalgae processing.

3.1.2 Co-digestion

The carbon/nitrogen (C/N) ratio is an important factor for guarantying the stability of the anaerobic digestion process. A C/N ratio of 25 to 32 was reported to have a positive effect on the methane yield.[37] At lower C/N ratios, the risk of excess in nitrogen, not needed for biomass synthesis, becomes inhibitory. On the contrary, a very high C/N ratio would lead to nitrogen deficiency for biomass synthesis. Hence, co-digestion can be an alternative to improve process performance by adding a secondary substrate that supplies nutrients lacking in the initial substrate. Combination of two or more substrates could create a synergistic effect by alleviating the nutrient imbalance and, in turn, attenuating the inhibition effects of the individual substrate. As previously mentioned, microalgal biomass generally contains high amounts of nitrogen, therefore a carbon-rich co-substrate could be added to facilitate the methane conversion process. For example, the addition of carbon-rich paper waste to a mixture of *Scenedesmus* spp. and *Chlorella* spp. resulted in an improved methane yield and increased cellulase activity.[38] Similarly, Gonzalez et al. (2011)[39] detected a significant increment of the methane yield when microalgae biomass was digested with swine manure as co-substrate.

3.1.3 Microalgae residue

The microalgae lipid extraction process results in a biomass residue which accounts for approximately 65% of the harvested biomass.[40] This can be considered as a waste with a certain disposal cost that will further increase the already unfavorable economics for biodiesel production from microalgae.[41] However, algal residues contain significant quantities of proteins and carbohydrates, which could undergo anaerobic digestion to produce biogas.[42]

Yang et al. (2011)[43] reported a methane yield of 390 $mLCH_4/gVS_{influent}$ from residual *Scenedesmus* biomass derived from oil extraction processes.

However microalgae biomass residues generated after lipid extraction may cause more severe ammonia inhibition than the whole algae, due to their higher protein contents.[42] As already pointed out, this can be moderated through co-digestion to increase the carbon:nitrogen ratio. An an illustration, co-digestion of algae biomass residue and lipid-rich fat, oil, and grease waste resulted in a specific methane production rate of 540 $mL\ CH_4/gVS_{influent}\cdot d$ with regards to a rate of 150 $mL\ CH_4/gVS_{influent}\cdot d$ when microalgae biomass was digested alone.[444]

The co-digestion of *Chlorella* residues with glycerol, produced from the transesterification process of biodiesel production, was also examined by Ehiment et al. (2009)[45]. These authors showed the effect of the type of solvent used in the oil extraction step on methane yield. In particular, extraction solvents such as chloroform resulted in a repression of methane production. Therefore, where energy generation via anaerobic digestion of microalgae residues is planned, , investigations on possible solvent interferences on the microbial process should be performed before solvent selection. Nonetheless, the solvent inhibitory effects can be reduced by a rinsing step to remove the toxic solvent from biomass. In counterpart, the rinsing process may have important water and energy requirements and could evacuate unbound energy-rich polar molecules, thus reducing the calorific value of the biomass feedstock.[45]

The information available in literature on this subject is still scarce and more investigation is needed to improve knowledge in this interesting option of microalgae biorefinery.

3.2 Products from the anaerobic digestion

3.2.1 Biogas

The biogas produced by anaerobic digestion is characterized by a methane percentage between 60% and 70%, depending of the substrate characteristics.[46]

A number of different pretreatments (thermal, chemical, enzymatic and mechanical pretreatments) have already proved their efficiency to enhance the methane yields.[30] For instance, Passos et al. (2013)[47] detected an increment of the methane yield of 4%, 53% and 62% when a temperature pretreatment of 55, 75 and 95°C was applied, respectively. Similarly, in BMP tests, microwave pretreatment showed an increase of microalgae solubility, leading to a final yield improvement from 12 % up to78% depending on the power applied (from 300 to 900 W).[48]

Some other options, such as an increase in the lipid content, were also proposed to improve the methane yield. However, cultivation strategies (i.e. high light intensity, nutrient starvation) which would raise lipid accumulation in cells, would probably affect the overall microalgae biomass productivity. It is thus not yet clear whether a particular cultivation strategy would be favorable to further increase the methane yields. In spite of recent developments in the field of biomethane production from microalgae, an optimal scenario combining ease of cultivation, high biomass yields and high anaerobic biodegradability has still to be determined.

Furthermore, several operational strategies were recently tested to improve the methane potentials of microalgal biomass. Zamalloa et al. (2012)[49] employed a hybrid flow-through reactor (combining a sludge blanket and a carrier bed) to increase the retention time of the algae biomass and decouple hydraulic and solid retention times. Markou et al. (2013)[50] proposed an increase in biomass carbohydrates through a phosphorus limitation process as an attractive technique to improve the bio-methane yield. Indeed, these authors tested various percentages of carbohydrates in cells and observed a methane yield ranging between 123 and 203 $mLCH_4/gCOD_{influent}$ (chemical oxygen demand) corresponding to 20% and 60% carbohydrates, respectively.

Concerning biogas quality, an important factor affecting CH_4 proportion in the biogas is the pH, which controls the speciation of the carbonate system and the release of CO_2. Rates and yields of CH_4 formation also often increase with digestion temperature.[22] However, since microalgae hardly contain sulphurated amino acids (Becker, 2007)[51], their digestion releases a lower amount of hydrogen sulfide than other types of organic substrates.

Biogas could thus be reused for microalgae growth, promoting the interesting possibility to close the flux of products and effluents. In fact, the exploitation of biogas energy within a co-generation process can produce a gas mixture mainly composed of CO_2 with the same quality as turbine gas. A comparison between flue gas from turbines, water heaters and ovens, refinery activities, coal ovens and fuel injection, reveals that the turbine gas composition is characterized by the lowest concentrations in toxic compounds (NO_x, SO_x, C_xH_y, CO, heavy metals and particles). Thus, the product resulting from biogas combustion can be a suitable source of inorganic carbon for microalgal cultures with low concentrations of toxic compounds. Moreover, the oxidized form of nitrogen and sulfur present in high concentrations in flue gas can contribute to fulfill microalgae nutrient requirements.

It is known that microalgae incorporate inorganic carbon as a primary nutrient, and not limiting carbon conditions is one of the key conditions to optimize microalgal production. On average, algae consume 1.83 g CO_2 to produce 1 g of biomass.[12] Thus, biological CO_2 fixation by microalgae is considered to be a promising mean for fixing CO_2, combining environmental and economic advantages, by contributing to prevent global warming on one hand and supplying carbon for microalgae for the other hand.

Moreover, even though CO_2 fixation is often mentioned in literature, an accurate CO_2 mass balance taking into account the final biomass disposal is necessary to determine the environmental impact of the overall process. In the case of fuel generation, the biomass originates from atmospheric CO_2 and will be ultimately converted back into CO_2 when the fuel is burned and, in this case, the process could be considered as carbon neutral rather than a carbon sink. More discussion about the environmental impact of biofuel products generated by microalgae can be found in Lardon et al., (2009).[52]"

CO_2 consumption rates reported in literature in bubbled columns reactors varied between 0.2 and 27 $g/m^2 \cdot d$, depending on the microalgae culture and operational conditions.[53] Traviesco et al. (1993)[54] as well as Doušková et al., (2009)[55], fed microalgae with biogas produced by anaerobic fermentation of a sugar cane distillery stillage. They observed that algae were able to consume CO_2 directly from biogas as well as from other sources in a range of concentrations between 2% (v/v) and 56% (v/v) of CO_2 in the mixture. Moreover, Park and Craggs (2011a; 2011b)[56,57] showed an increase in algal/bacterial production by about 30%, concomitantly to a significant nutrient removal enhancement due to CO_2 addition. A supplement in CO_2 can also maintain the pH at a suitable value (usually 8), thus preventing inhibition of algal growth by ammonia.[58] Furthermore, a pH less than 8 can reduce nitrogen removal by physicochemical processes such as ammonia volatilization, and may increase algal nutrient assimilation.

These facts highlight the large adaptability of microalgae to different substrates, which is an important added value for a microalgae-based biorefinery. Indeed, microalgae culture can be coupled to a number of industrial chains for low cost wastewater treatment and generation of bioproducts.

3.2.2 Digestate (liquid and solid phase)

Besides biogas, anaerobic digestion processes generate liquid and solid phase effluents (digestate) that are rich in phosphorus and organic nitrogen compounds, ideal for use as organic fertilizer. Within the management process of this product (direct spreading, drying, liming) the separation between solid and liquid phases is suitable for an optimal exploitation of the different components. Many options for nutrient extraction from the digestate are nowadays explored in order to produce high quality fertilizers (e.g. ammonia stripping for ammonium sulfate production and phosphorus precipitation through struvite formation). The separation process, that can be improved by addition of organic or mineral flocculants, produces a liquid fraction, rich in mineralized elements that can be directly spread or precipitated (e.g. struvite) (Türker et Celen, 2007)[59] and a solid fraction, usually composted, dried and/or exploited as an organic supplement.[560]

The different forms of digestate are characterized by different bio availabilities. Some components are absorbed on the organic fraction of suspended solids. This absorption is a function of the chemical properties of the components and the physico chemical properties of the solids. Generally, 40 to 86% of the organic matter is present in the solid fraction (Moller, 2012)[61] while the liquid phase is characterized by a low organic matter content. The solid fraction contains about 75% of phosphorus, which is directly absorbed or trapped with calcium, magnesium and nitrogen.[61] Similarly, complex reactions are responsible for the distribution of microelements in liquid or solid phase after the post-treatment. For example, with liquid swine manure, copper, zinc and manganese were absorbed on the smaller particles (between 1 and 60 µm) and were preferably mobilized in the liquid phase after separation.[72]

On the other hand, the recycling of nutrients from wastewater highlights the need for the characterization of the quality of the digestate, with special attention to pathogens and heavy metal concentrations. Although anaerobic digestion is classified as a process that significantly reduces pathogens, their elimination strictly depends on the microbial species, digester temperature and retention time.[63] Likewise, pH, anaerobic conditions, nitrogen and volatile fatty acids can affect some pathogens.[63] However, information about this aspect is still scarce, and evidence from literature points out the necessity to consider the variability of the digestate composition and the concentrations in pathogens and heavy metal as important factors. Therefore, further efforts are required to determine the operating conditions able to enhance fertilizer properties and pathogen reduction, as well as to promote the digestate nutrient recycling.

The use of digestate as substrate for microalgae growth is particularly interesting for the reduction of the process inputs in a biorefinery concept coupling wastewater treatment, microalgae culture and anaerobic digestion. Indeed the outlet of the anaerobic digesters fed with microalgae or other biomass contains about 50% of the initial nitrogen that can be reused as a source of nutrients and water for microalgae growth.

In a context of nutrient recycling, the liquid phase of the digestate was tested as a possible source of nitrogen for algae cultivation. In fact, the digestate liquid is characterized by low organic matter and phosphorus concentrations, counterbalanced by high potassium and nitrogen concentrations (up to 80% in the form of ammonium) (Table 3). Moreover, the micro-element composition of digestates (Table 4) can cover the nutrient requirements of a microalgae population.[66]

Many studies report the use of digestate from urban wastewater treatment, manure, abattoir residue or swine slurry for microalgal growth.[63,64,65,66,67] Bchir et al, (2011)[70] obtained a high biomass production of $5.29 \cdot 10^6$ cell/mL associated with an important content of chlorophyll (65.32 mg/L) after 42 days of culture of *Spongiochloris* sp fed with abattoir digestate. Chen et al. (2012)[72] tested a long-term cultivation of freshwater algae in anaerobic digested manure effluents and indicated that *Chlorella* and *Scenedesmus* were able to grow in high nutrient loads (40, 100 and 200 g/L TN). However, Bjornsson et al. (2013)[73] show a magnesium limitation in *Scenedesmus* sp. growth with liquid swine manure digestate.

A few studies also tested the digestate of microalgal biomass as substrate for microalgal growth. Doušková et al., (2009)[55] tested a pilot scale reactor for biogas production and subsequent microalgae cultivation. The process consisted of a 50 L mesophilic reactor fed in semi-continuous mode with pure stillage. The reactor was followed by a photobioreactor constituted by a set of glass bubbled columns in a thermostatic bath continuously illuminated. These researchers determined experimentally that the growth rates of microalgae grown on digestate were similar to those obtained with urea as substrate (16gDW/L).

Several experiments also pointed out the existence of inhibitory effects on microalgal growth, especially with manure wastewater or digestate as substrate (Table 5). Among the observed effects, high ammonia concentrations were often responsible of microalgal growth inhibition.[74,80] Indeed, although ammonia can be an excellent source of nitrogen for microalgal growth, free ammonia is toxic for most strains of microalgae due to its uncoupling effect on photosynthetic processes in isolated chloroplasts.[81]

Another cause of microalgae growth inhibition is light limitation mainly due to mutual shading caused by a high biomass density.[67,82,83] No particular effect of digestate turbidity on microalgal growth has yet been reported in literature. However, it should be noticed that the digestate is diluted in almost all the experiments reported in literature.[51,66,63]

Nevertheless, once the inhibitory factors have been identified, their effect can be easily overcomed by substrate dilution or carbon dioxide addition (for pH and ammonia concentration control) or, in the case of self-shading, a periodical harvesting could prevent high microalgal concentrations.[67] In this sense, Cho et al. (2013)[84] used urban wastewater for microalgae growth, by testing 1) the effluent from a primary settling tank, 2) the effluent from an anaerobic digestion tank and 3) a digestate dilution. According to their results, *Chlorella* sp. showed the highest biomass production (3.01 g dry cell weight/L) when digestate was diluted with wastewater rejected from a sludge concentrate tank (10:90, v/v).

It should also be taken into account that, depending on the digester performance, digestate may contain volatile fatty acids and microorganisms already present in the substrate or produced by the anaerobic flora. Similarly, in the liquid phase, it is possible to observe residue from the flocculation processes used for solid/liquid separation.

Thus, the variability of digestate composition has an important potential impact that has not yet been carefully studied.

3.3 Anaerobic digestion in microalgae-based biorefinery

During the past recent years, different applications of microalgae anaerobic digestion have been integrated in a biorefinery concept moving the role of anaerobic digestion from a waste treatment to an organic matter conversion unit. Razon (2012)[85] proposed a process in which ammonia sulfate from the digestate is stripped, converting the ammonia to a solid form. Thus, it can be easily separated by gravity settling and processed into crystals further used as fertilizer, while the liquid part (~70%) can be used in agriculture or returned to the algal culture.

With similar objectives, De Schamphelaire and Verstate (2009)[86] proposed a closed loop system integrating an algal growth unit for biomass production, an anaerobic digestion unit to convert the biomass to biogas and a microbial fuel cell to treat further the effluent of the digester and produce electricity. To close the loop, nutrients from the digester are returned to the algal growth unit.

A recent study[87] investigated the selection of methanotrophic bacteria to produce polyhydroxybutyrate (PHB), which is a biodegradable polyester. In this case, biogas was used to feed microalgae and to stimulate methanotroph bacteria. Moreover, these researchers found that the symbiotic cooperation between microalgae and methanotroph bacteria led to the formation of harvestable bioflocs.

These studies show that it is possible to develop new interesting solution to integrate anaerobic digestion into a biorefinery concept. In this perspective, it is advisable to integrate different processes in order to generate new valuable products maximizing overall efficiency, while reducing operating costs and environmental impacts. To do this, multidisciplinary research on systems biology, strain development, systems design, modeling and biorefining is required.

3.4 Economic and environmental aspects

In spite of the increasing interest in anaerobic digestion of microalgae, little information on the economic aspects of this process is available in literature. Delrue et al (2012)[88] carried out an economic study of biodiesel production from microalgae considering anaerobic digestion as a treatment of microalgae residue. According to this study, the price of 1 liter of biodiesel varies between 1.94 and 3.35 €. Among the major bottlenecks identified in this study, the cultivation steps and the downstream processes play an important role. This indicates that more efforts are needed in order to reduce cultivation costs, optimize microalgae productivity and improve technologies for biomass valorization. Overall, anaerobic digestion methane yield positively impacts the net energy ratio, contributing to 33% of the total energy production. A recent study on the potential of microalgae as feedstock for methane production[85] found a cost of energy in the order of magnitude of 0.087-0.170 €/kWh^{-1}. This study considered the microalgae biomass cultivated in a 400 ha (4 km^2) raceway pond with inputs of fresh water, nutrients and sunlight. The harvesting step consists on a settling stage with flocculants followed by a dissolved air flotation. Then an anaerobic process is carried out at 30°C and the water and nutrients from the pre-concentration and anaerobic digetsion stage are recirculated and the CO_2 from the flue gas is used for algae cultivation.

However, the wide range of data available in literature makes difficult an economical comparison between processes and even between units of the same process. Moreover, the economic studies available are based on theoretical models; the availability of data from real and large scale plants would certainly help to get more reliable information about the economic viability of microalgae biorefinery. An accurate economic and environmental study is especially needed for the most recent biorefinery solutions presented above.

From an environmental point of view, only few studies on microalgae biorefinery and anaerobic digestion have been recently published.[52,89,40,42] Concerning the environmental impact, the study carried out by Lardon et al. (2009)[52] confirmed the potential of microalgae as an energy source but emphasized on the imperative necessity of decreasing the energy and fertilizer consumption. Collet et al. (2011)[90] pointed out the electricity consumption as the main source of impacts and suggested that improvement of the efficiency of the anaerobic process under controlled conditions could be a possible solution for decreasing process consumption. Benemann et al. (2012)[91] found that oil production from microalgae coupled with the anaerobic digestion of microalgae residue does not require fossil energy inputs and does not produce greenhouse gas emissions.

4. Perspectives and further research

This paper has emphasized several crucial points of microalgae-based bioprocesses that need to be developed in order to upgrade the potential of microalgal anaerobic digestion and to find new renewable and carbon-neutral products and energy sources.

Firstly, challenges regarding microalgal culture need to be solved. In fact, in spite of the increasing interest and the number of studies conducted in this field, there are still problems related to the high building and operating costs, the difficulty in controlling and optimizing the culture conditions, contamination by bacteria or microalgae, predators, unstable light supply and weather changes.

The selection of the most valuable microalgae strains for anaerobic digestion still requires research efforts. In this context, the genetic improvement can be a tool to create microalgae strains with high productivity and high methane potential that could improve anaerobic digestion efficiency.

Anaerobic digestion effectiveness could also be enhanced by the study and implementation of innovative pretreatments or co-digestion processes as well as reactor configurations and operation strategies.

Another bottleneck is the harvesting process, which is a crucial step for biomass production with low costs and low energy requirements.

Moreover, the benefit in closing the loop of microalgae biorefinery would require the extension of the actual limited knowledge on digestion of algal biomass residue. Another interesting aspect that deserves further attention is the quality of digestate and its properties as a substrate for microalgae growth and/or as fertilizer.

We report here some example of process coupling; however more biorefinery configurations incorporating a whole range of different installations should be further explored. In this context, a number of industries could combine their material flows in order to reach a complete utilization of all biomass components. In this way the residue from one industry (e.g. lignin from a lignocellulosic ethanol production plant) could become an input for other types of industry.

In line with the promising results produced from laboratory studies, a scaling-up of the technology from the laboratory to the pilot plant has now become essential in order to verify the sustainability of the process.

Finally, the increasing interest in developing industrial-scale microalgae-to-biofuel technology requires a detailed assessment of the costs and the potential environmental impacts of the entire process chain, from biomass production to the biofuel combustion. Almost all environmental and economic assessments found in literature have been indeed based on assumptions and extrapolations from laboratory experiments and small-scale outdoor systems. Last but not least, the emissions of major greenhouse gasses (e.g. nitrous dioxide and methane) during the microalgae cultivation stage have been ignored and real data remain necessary to improve life cycle assessment.

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6. Tables and Figures

Table 1. Biomass and raw energy productivities of land-based plants and microalgae culture (adapted from Dismukes et al. 2008).[3]

	Biomass productivity (dry tons/ha·y)	Raw energy productivity (GJ/ha·y)*
Corn grain	7	120
Sugarcane	73-87	1230-1460
Woody biomass	10-22	
Mixed grasses	3.6-15	61-255
Rapa seeds	2.7	73
Microalgae*Tetraselmis suecica*	10-22	700-1550
Microalgae *Arthrospira (Spirulina)*	27, 60-70	550, 1230-1435

* Assuming heat of combustion, theoretical maximum energy content

Table 2. Distribution of the biochemical fractioning of a microalgae cell.[4]

Biochemical compartment	**Function**	**Mass concentration (%)**
Proteins	Structure and metabolism	40-60
Lipids	Structure and energetic reservoir	5-60
Carbohydrates	Structure et energetic reservoir	8-30
Nucleic acids	Support, vector and regulator of the genetic information	5-10

Table 3. Comparison between total nitrogen and phosphorus concentrations (mg/L) for different effluents (adapted from Cai et al., 2013)[64].

Effluent	**Origin**	**Total Nitrogen**	**Total Phosphorus**
Urban wastewater	-	15-90	5-20
Digestate	Dairy manure	125-3456	18-250
	Poultry manure	1380-1580	370-382
	Sewage sludge	427-467	134-321
	Food waste and dairy manure	1640-1885	296-302

Table 4. Comparison of macro and micro element concentrations (mg/L) from different digestates.[65]

Element	**Bovine manure**	**Activated sludge**	**Pig manure**	**Poultry manure**
K	116	12	366	592
Na	38	31	111	214
Mg	60	32	225	54
Ca	171	267	174	42
Fe	9.1	3	38	2.5
Cu	0.04	0.02	0.02	0.04
Zn	0.44	0.16	0.08	0.1
Co	0.02	0.12	0.09	0.12
Mn	0.12	0.26	1.15	0.1
Cr	0.002	0.012	0.05	0.047

Table 5. Potential effects of the liquid digestate phase to microalgal growth

Component	Potential effect	Reference
Turbidity	Partial absorption of light energy	
Nitrogen concentration	Toxicity of the ammoniac form is pH is not regulated	74 75
Volatile fatty acids concentration	Impact on the population equilibrium due to the stimulation of heterotrophic bacteria growth. Long chain fatty acids (>C14) can be toxic for some species.	76 77
Flocculants	Coagulation effect leading to biomass sedimentation and performance limitation but also the bioavailability of essential nutrients such as phosphorus	
Microorganisms	Potential ecological impact (competition) and sanitary (depending on the microalgal exploitation industry)	
Heavy metals	Cellular toxicity, accumulation and potential sanitary impact (depending on the microalgal exploitation industry)	78
Organic trace elements	Potential cellular toxicity	79

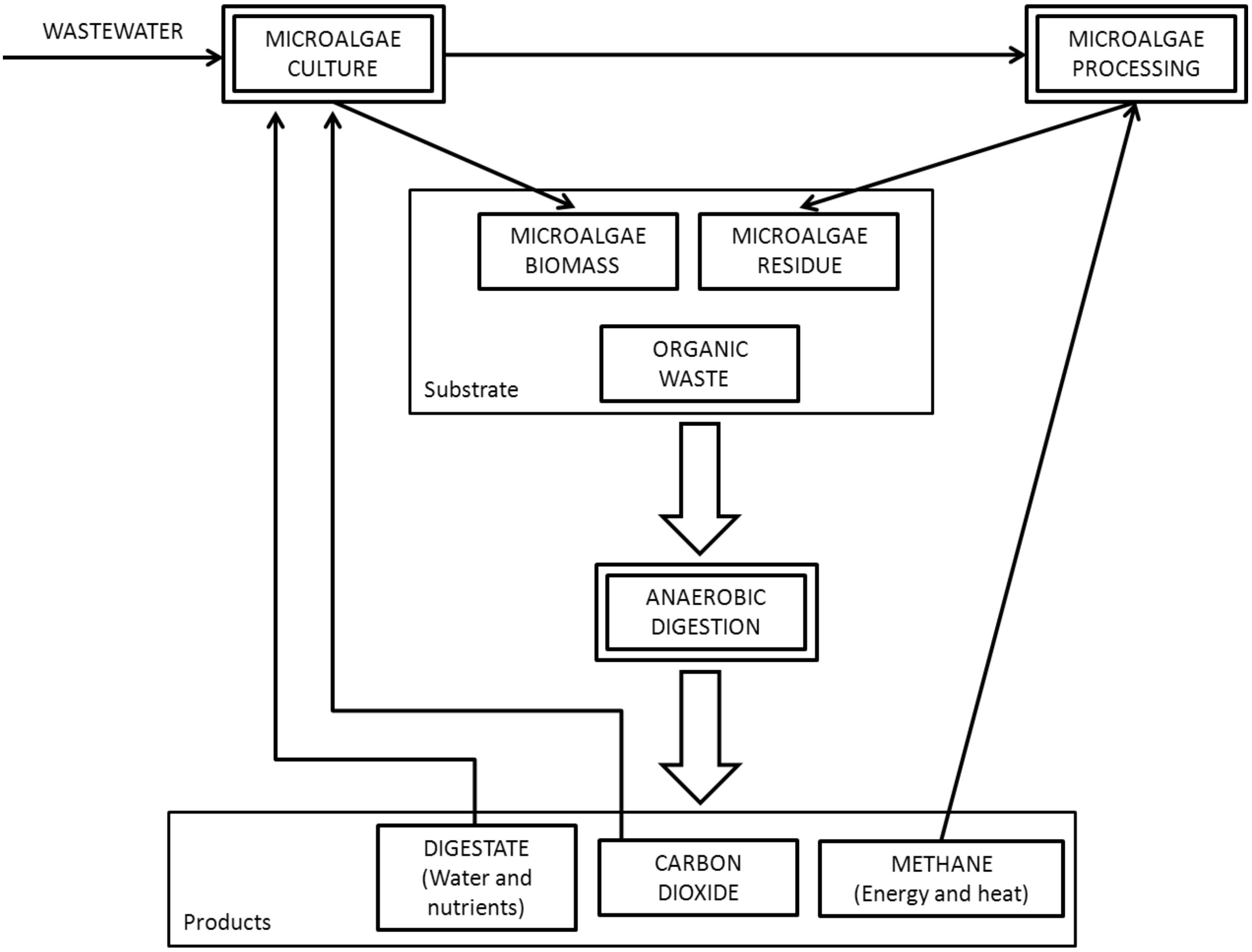

Figure 1. Flux of materials in anaerobic digestion of microalgae biomass