A perspective on novel cascading algal biomethane biorefinery systems

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Abstract

Synergistic opportunities to combine biomethane production via anaerobic digestion whilst cultivating microalgae have been previously suggested in literature. While biomethane is a promising and flexible renewable energy vector, microalgae are increasingly gaining importance as an alternate source of food and/or feed, chemicals and energy for advanced biofuels. However, simultaneously achieving, grid quality biomethane, effective microalgal digestate treatment, high microalgae growth rate, and the most sustainable use of the algal biomass is a major challenge. In this regard, the present paper proposes multiple configurations of an innovative Cascading Algal Biomethane-Biorefinery System (CABBS) using a novel two-step bubble column-photobioreactor photosynthetic biogas upgrading technology. To overcome the limitations in choice of microalgae for optimal system operation, a microalgae composition based biorefinery decision tree has also been conceptualised to maximise profitability. Techno-economic, environmental and practical aspects have been discussed to provide a comprehensive perspective of the proposed systems.

Keywords: Anaerobic digestion; Biomethane; Biogas upgrading; Microalgae; Biorefinery; Circular Bioeconomy.

1 Introduction

1.1 Biofuels in the Future Bioeconomy

Transitioning from a fossil based economy to a renewable biological resource based bioeconomy is required to reduce environmental impacts including greenhouse gas (GHG) emissions in food, energy and material production (Sanz-Hernandez et al., 2019). Within the future bioeconomy, biofuels will play a significant role in energy systems including advanced transport biofuels and agriculture (Sanz-Hernandez et al., 2019). To maximise profitability and sustainability, biofuels can benefit from revenues associated with co-products generated in a biorefinery system, and by the optimal choices of resources, methods of production and processing, and use of co-products.

1.2 Biomethane: A Renewable Biofuel

Gaseous biofuels, particularly biogas produced via anaerobic digestion (AD), present significant benefits including; *1)* the possibility for use in electricity, heat and /or transport; *2)* lower capital and operational costs compared to other advanced bioenergy technologies like gasification and pyrolysis; *3)* ability to incorporate a wide variety of feedstock; and *4)* scope for nutrient recycling (Bohutskyi et al., 2015). Biomethane (97% CH_4 following the removal of CO_2 from biogas) can therefore be a versatile renewable biofuel that can effectively substitute natural gas in complex energy systems, viz., transport and agriculture (Wall et al., 2017). This enables biomethane to play a significant role in decreasing emissions from energy sectors including electricity, heating and cooling, heavy industries and transport (Wall et al., 2017).

1.3 Advances in Biogas Upgrading to Biomethane using Microalgae

By 2026, in the European Union, biomethane must effect a GHG emissions reduction of 65% as compared to the fossil fuel comparator (FFC) to be deemed sustainable as a biofuel in the transport sector (European Commission, 2018). Innovations in both feedstock and biogas upgrading technologies are urgently needed. Besides the choice of feedstock (the primary contributor towards the overall emissions), emissions associated with energy used in biogas upgrading are significant (Valli et al., 2017). Traditional upgrading technologies employing physicochemical techniques, viz., membrane separation, chemical adsorption and pressure swing adsorption have a significant energy penalty (3-6% of the energy content of biogas processed) and a high cost (Angelidaki et al., 2018). Photosynthetic biogas upgrading using microalgae could overcome the economic and environmental drawbacks associated with traditional biogas upgrading (Marín et al., 2018). In this process, CO_2 is removed from biogas by a carbonate rich solution generating bicarbonate. The bicarbonate rich solution is used as an inorganic carbon source for microalgae cultivation at a relatively high pH (Bose et al., 2019). A high areal productivity of microalgae, rapid CO_2 fixation, and ability to grow in a wide range of environments (Jankowska et al., 2017) would increase the sustainability of photosynthetic biogas upgrading. Although a two-step bubble-column and microalgae cultivation using a circulating algal solution has been found to be effective (Meier et al., 2015), being a novel technology, the choice of operational parameters and the microalgae species for optimal performance is still under research.

1.4 Cascading Algal Biomethane Biorefinery System (CABBS) in a Circular Bioeconomy

Effective utilisation of highly efficient natural microalgae "bio-factories"('t Lam et al., 2018) is imperative to ensure economic and environmental sustainability of photosynthetic biogas upgrading. Indeed, without effective resource management through principles of input reduction, eco-design, minimisation and recycling of waste, a bioeconomy system often fails to achieve its objective benefits (D'Amato et al., 2018).

This mandates a shift from the traditional "take-make-dispose" linear economic model towards a circular economy through the integration of different sectors and technologies.

Simultaneous production of biofuels and high value products for food, feed, and chemicals, often referred to as a microalgae biorefinery is currently receiving significant research attention to maximise the use of microalgae ('t Lam et al., 2018; Chew et al., 2017). Integrating a microalgae biorefinery and AD system for biomethane production via CABBS presents an interesting opportunity for a circular bioeconomy system as shown in Figure 1. In addition to simultaneous biogas upgrading and CO_2 supply for microalgae growth, digestate (residue from AD) can be used as a nutrient source to grow microalgae (Chen et al., 2018). However, to maximise the benefit of such combined systems, an assessment of each implementation possibility is required. To the best of the authors' knowledge, present literature focuses on the microalgae strain selection based on the targeted product (such as biodiesel, biogas and protein). However, due to the considerable variation in microalgae composition from the growing conditions, determining the use of microalgae based on its final composition would amplify the positive impact of the integrated systems with microalgae.

In this paper, possibilities for AD based circular biomethane-biorefinery systems have been explored with an emphasis on the following:

1. Establish the state of the art of microalgae use for biomethane generation via AD, including; *biogas generation*, *biogas upgrading* and *digestate treatment*.

2. Develop a decision tree for an effective microalgae biorefinery with a focus on AD from the perspective of microalgae composition.

3. Explore and illustrate possible configurations of the proposed CABBS layout.

4. Provide a perspective on the corresponding economic, sustainability and practical challenges of such integrated systems.

2 Microalgae in Biogas and Biomethane Systems

Producing biomethane via AD entails three fundamental steps: *1)* biogas generation; *2)* biogas upgrading to biomethane; and *3)* digestate treatment and re-use. Herein, the synergies and limitations of applying microalgae in each of these steps have been reviewed focusing on the benefits to both microalgae cultivation and utilisation.

2.1 Biogas Generation from Microalgae

Theoretical specific methane yields (SMY_{PCL}) of any biomass can be estimated from its chemical composition, as described in the equation (1) (Sialve et al., 2009). The SMY of 1.014 L/gVS, 0.415 L/g VS and 0.446 L/gVS from lipids (L), carbohydrates(C) and proteins(P) were found to adequately predict biogas from the AD of microalgae (Heaven et al., 2011). The chemical oxygen demand (COD) of the biomass can also be used to estimate the theoretical SMY (SMY_{COD}) under mesophilic conditions as described in equation (2) (Ras et al., 2011)

$$SMY_{PCL} = 0.446 * P + 0.451 * C + 1.014 * L \quad (1)$$

$$SMY_{COD} = 0.380\, mLCH_4\, g^{-1}COD * (CODg^{-1}VS) \quad (2)$$

The presence of a broad range of carbohydrates (7 to 69% Volatile Solids (VS)), lipids (1 to 63% VS) and proteins (15 to 84% VS) makes microalgae an attractive substrate for AD, either directly or after the extraction of specific fractions (Saratale et al., 2018). In practice, however, ideal biogas generation is seldom achieved. Although non-structural carbohydrates such as starch are readily digestible, high concentrations could lead to build-up of volatile fatty acids (VFAs) in the digester and inhibit AD (Li et al., 2017). Sub-optimal C:N ratios owing to the presence of proteins (levels of around 6-9) (González-Fernández et al., 2012a), lead to increased NH_3^+ accumulation, accelerating digester failure through inhibition of methanogenesis and VFA accumulation (Li et al., 2017; Magdalena et al., 2018). The presence of long chain fatty acids in lipids can cause severe inhibition to AD, requiring long retention times to achieve biodegradation (Rasit et al., 2015). In addition, the presence of complex structural carbohydrates, viz., cellulose and hemicellulose in the microalgal cell walls limit their practical methane yield by encapsulating the more readily digestible fractions (Saratale et al., 2018). For un-treated microalgae, especially freshwater species like *Scenedesmus obliquus*, therefore, the biodegradability is often below 50% (Table 1). In contrast, due to the presence of less complex cell walls, most un-treated cyanobacteria such as *Spirulina platensis* show biodegradability of above 60%. However, a higher yield from AD of untreated cyanobacteria is hindered due to their high protein content.

Pre-treatment of microalgae to rupture cell walls and increase the availability of digestible fractions is one of the most promising techniques to enhance SMY from microalgae, clearly observed from the data in Table 1. Several methods including physical (mechanical and thermal); chemical, (acidic and alkaline); and biological (aerobic digestion and enzymatic) pre-treatments have been proposed (Jankowska et al., 2017; Saratale et al., 2018), each with its own merits and disadvantages. For example, ultra-sonic pre-treatment was reported to increase the SMY by 128 MJ/kgTotal Solid (TS) or 90%, but required 128.9 MJ/kg Total Solid (TS) of input energy for *Scenedesmus* sp. (González-Fernández et al., 2012a). In contrast, a 15% increase in SMY was reported by enzymatic pre-treatment with a much lower energy expenditure (Passos et al., 2016). Co-digestion of microalgae with suitable feedstock having a high C:N ratio can optimise the C:N ratio and improve digestion conditions (Saratale et al., 2018). A 46% increase in the SMY was observed by mixing a microalgae culture dominated by *Microcystis* spp. with corn straw to ensure a C:N ratio of 20 (Zhong et al., 2013). Herrmann et al., (2016) co-digested *Spirulina platensis* with barley straw, beet silage, and *Laminaria digitata* respectively at a C:N ratio of 25. Improvements in biomethane yield (22.5% as compared to mono-digestion of *Spirulina platensis*) at a higher organic loading rate of 2 g/VS/L/day, were obtained with 55% beet silage as co-substrate (VS basis). Alternatively, changing microalgae growth environment for a more favourable biochemical composition for AD has also been proposed to improve biogas yield (Jankowska et al., 2017).

Residual biomass, after extraction of valuable fractions is also an effective substrate for biogas generation. AD of lipid extracted *Chlorella vulgaris*, *Nannochloropsis salina* and *Nannochloropsis gaditana* yielded above 0.3 L/g-VS of biogas (Zhao et al., 2014). Amino acid extracted *Scenedesmus sp.* produced 272.8 ± 7.3 mLCH_4/g-VS, a two fold increase in CH_4 yield compared to untreated biomass (Ramos-suárez and Carreras, 2014). Protein extracted *Spirluna platensis* was shown to improve the CH_4 yield by 30.4% to 236.1 mLCH_4/g-VS as compared to untreated biomass (Parimi et al., 2015a). However, the yield was 18 mL/g-VS lower than that of pre-treated *Spirulina platensis* under high pressure without any extraction. The type of pre-treatment for fraction extraction is also crucial for effective digestion of residues. For example, thermal pre-treatment of microalgae during lipid extraction resulted in a 40% increase in SMY compared to that from microalgae residue after lipid extraction without pre-treatment. However, the use of use of chloroform for lipid extraction suppressed biogas production from the residue biomass by inhibiting methanogenesis (Passos et al., 2014).

2.2 **Biogas Upgrading by Microalgae**

Bubbling biogas in an absorption (bubble) column connected to a photobioreactor with circulating algal solution has been established as an effective configuration to optimise biogas upgrading using microalgae (Bose et al., 2019; Meier et al., 2015). In this process, CO_2 is first absorbed by an alkaline solution with carbonate medium (94% CO_2 removal at pH 9.5), which also ensures a total H_2S removal (Bahr et al., 2014; Marín et al., 2018). As shown in Figure 2, microalgae could be cultivated using bicarbonate as the inorganic carbon for photosynthesis, re-generating carbonate in return. Thus, both, a carbonate/bicarbonate cycle and the pH of the circulating medium can be maintained naturally (Bose et al., 2019). Oxygen generated during photosynthesis, leading to elevated levels of O_2 (>0.5%) in the upgraded biomethane is a major challenge to meet gas grid specifications using this process. Additional issues including: *1)* low CO_2 mass transfer; *2)* effective control of process parameters e.g. gas and liquid flow rates; *3)* diurnal variability in operation due to photo-autotrophy; and *4)* fluctuating seasonal operation affecting microalgae growth must be overcome to commercialise the technology (Bose et al., 2019).

Process optimisation and expertise are required to improve the technology readiness. Bose et al., (2019) suggest that more knowledge is required in: gas and liquid flow rates; solution pH; microalgae type and concentration; and gas residence times in the column. For example, although a higher pH improves CO_2 removal, it enhances O_2 and N_2 stripping into the biogas and phosphorus loss from microalgae nutrient solution through deposition by salt formation. This decreases biomethane quality and diminishes microalgae growth rates. Alongside the process parameters, opportune choice of microalgae species could lead to further optimisation of system operations by *1)* improving the hydrodynamics in the bubble column; *2)* catalysing the CO_2 removal in the alkaline carbonate solution using carbonic anhydrase (subject to experimental validation); *3)* decreasing oxygen stripping into the biomethane; and *4)* improving the energy and carbon balance of the overall system. Highly alkaliphilic cyanobacteria such as *Spirulina platensis* is one of the most promising species satisfying all criteria (Bose et al., 2019). The ability to grow mixotrophically would further allow *Spirlulina* sp. to be integrated with digestate cleaning whilst upgrading biogas. *Anabaena* sp. and

Synechococcus sp. have similar properties, although the later would require expensive harvesting techniques due to its unicellular structure. Among the unicellular green algae species, *Scenedesmus obliquus*, *Chlorella sorokiniana* SLA-04; a mutant strain of *Chlorella sp.* AT1 and *Dunaliella salina* NIES-2257 are suitable (Bose et al., 2019). When simultaneous digestate treatment is not desired, alkaliphillic cyanobacteria including *Rhabdoderma* sp. and *Geitlerinema* sp. could also be effectively utilised.

2.3 Digestate Treatment by Microalgae

Digestate from anaerobic digestion, comprises solid (10 to 20%) and liquid (80 to 90% by mass) fractions (Xia and Murphy, 2016). Harmful emissions to air, water and soil through contamination of the un-treated liquid fraction is a major challenge for the biogas industry (Xia and Murphy, 2016). However, most of the hazardous chemicals in the liquid digestate, including heavy metals are essential for microalgae growth (Xia and Murphy, 2016). Therefore, the use of liquid digestate as a nutrient source for microalgae cultivation has been advocated to be advantageous for digestate treatment.

Growing microalgae in liquid digestate, however, has its own challenges. High turbidity; high total ammonium nitrogen (TAN) content between 1000-3000 mgNH_4^+/L; and high COD of 7000 mg/L in the digestate are some inhibitory factors towards algal growth. These would thus limit direct use of digestate for microalgae cultivation (Xia and Murphy, 2016). Therefore, dilution of the digestate is necessary, often performed using wastewater or seawater to increase sustainability (Xia and Murphy, 2016). Furthermore, microalgae cultivated in digestate are prone to biological contamination from bacteria, viruses, and other foreign species (Yan et al., 2016). This directs the current trend in treating digestate with microalgae to use robust chlorophyte species such as *Chlorella* sp. and *Scenedesmus* sp. (Koutra et al., 2018; Xia and Murphy, 2016).

2.4 Combined Biogas Upgrading and Digestate Treatment by Microalgae

Simultaneous biogas upgrading and digestate treatment has been studied quite frequently in the literature. However, maximising the effectiveness of both the strategies has seldom been reached at the same time. While the favourable pH range for digestate treatment varies between pH 7 and 8 (Xia and Murphy, 2016), that for biogas upgrading should be preferably above pH9. As Bahr et al., (2014) reported during biogas upgrading, a decrease in pH from 9.4 to 7 led to a drop in both the CO_2 removal efficiency and biomass productivity when diluted digestate was added replacing mineral salt for *Siprulina platensis* growth. This inherently limits the selection of microalgae species that can simultaneously be favourable for both conditions. *Scenedesmus obliquus*, as well as moderately alkaliphilic cyanobacteria including *Anabaena sp.* and *Synechococcus* sp., able to grow favourably between pH of 7 and 9.5 could be favourable choices. Other mutant species or species cultivated via adaptation could also be effective. Alternately, developing parallel processes for digestate treatment and biogas upgrading with separate microalgae species could be an effective solution. This might complicate the supply of CO_2 and nutrients from the biogas to ensure ideal growth conditions for the species raising both installation and operational costs. No such configurations have yet been reported in literature.

3 Further Opportunities from Microalgae Bioresource

3.1 Bio-products from Microalgae

Opportune use of microalgae, especially after biogas upgrading and/ or digestate treatment is essential to maximise the economic and environmental benefit of the overall process. The use of whole microalgal biomass offers the simplest practical application. Liquid and gaseous biofuels, solids such as biochar, as well as renewable heat and electricity are obtained via thermochemical conversion of whole biomass (Chew et al., 2017). Alternatively, based on composition, specific microalgae species can be used either as food (e.g. *Nostoc*) (Spolaore et al., 2006) or, as soil additives and fertilizers for soil restoration against desertification (Barsanti and Gualtieri, 2018; Lababpour, 2016). Although direct photolysis of microalgae could generate biohydrogen, a process efficiency below 1% limits the commercialisation of this technology (Nagarajan et al., 2017) and hence it is not described in Figure 1. Alternatively, hydrogen can also be generated alongside VFAs via acidogenic fermentation (AF) (Lin et al., 2019). High protein containing microalgae, which naturally inhibits AD due to VFA accumulation, could be advantageous in this regard. Unlike AD, the effluents from AF are rich in VFAs, considered as essential platform chemicals that could widely replace traditional petrochemicals in future biorefineries (Sun et al., 2018). In addition, as VFAs have also been found to enhance microalgae growth, residues from AF could potentially be more advantageous for microalgae cultivation than digestate from AD (Chen et al., 2018). Enhancement of hydrogen and VFA yield through pre-treatment and co-digestion (Sun et al., 2018) could allow attractive integration of AF into the overall biomethane biorefinery with microalgae.

Considering the utilisation of individual fractions, as can be seen in Figure 1, bioethanol, biobutano,l, biohydrogen and organic acids can be obtained from anaerobic fermentation of the carbohydrates in microalgae including polysaccharides (de Farias Silva and Bertucco, 2016). Polysaccharides can also be used in a variety of industries including personal care products (Barsanti and Gualtieri, 2018). Algal oils from lipids can substitute traditional fish or vegetable oils or can be processed to synthesize biodiesel (Posada et al., 2016). Glycerol is a valuable chemical from biodiesel production (10% weight/weight of biodiesel) by transesterification (Fan et al., 2010). Proteins including essential amino acids (e.g. lysine) can be widely applied in nutritional and health supplements post extraction and purification. Carotenoids, chlorophylls and xanthophyll can be extracted for use as additives for both human and animal feed, cosmetics, pharmaceuticals, colouring agents and biomaterials (Chew et al., 2017; Koutra et al., 2018). Phycobiliproteins and astaxanthin from microalgae are already commercialized high value products for use in food and feed and medicines (Chew et al., 2017; Spolaore et al., 2006). Microalgae can also produce limited quantities of other valuable products including polyunsaturated fatty acids (PUFAs), phytosterols, bioactive peptides, toxins, as well as polyketides and antibiotic substances, as summarised in Figure 1.

3.2 Economic considerations of Microalgae Bio-products

In general, nutraceutical products attract the highest market prices followed by items for human consumption and personal care, bulk chemicals, animal feed, and biofuels (Barsanti and Gualtieri, 2018; Kothari et al., 2017). Prices for biofuels are primarily driven by fossil fuel prices (van der Voort et al., 2015). Average market prices are typically: 0.4 €/litre to 0.6 €/litre for bioethanol; 0.5 €/litre to 0.7 €/litre for biodiesel (Debnath et al., 2017; van der Voort et al., 2015); and 0.12 €/m^3 to 0.2 €/m^3 for biogas (Barsanti and Gualtieri, 2018; Rajendran et al., 2019a). The production of electricity or heat from biogas, or the injection of biomethane into the gas grid are increasingly incentivised in many countries as advanced forms of renewable energy. In the EU, electricity rates from biogas typically vary between 4 ct€/kWh$_e$ in Hungary to 28 ct€/kWh$_e$ in Germany according to feed-in-tariffs (FiT) (Pablo-Romero et al., 2017). On the contrary, renewable heat is mostly characterised either by low feed-in tariff (e.g. 2.95 ct€/kWh in Ireland) (Department of Communications Climate Action and Environment, 2017) or variable local demands and negotiations at largely fluctuating prices between 1.2 ct€/kWh and 12 ct€/kWh (Herbes et al., 2018). Grid injection of biomethane is increasingly being incentivised within the EU. Tariffs vary between 5.5 ct€/kWh for the

UK (Ofgem, 2019) to 9.5 ct€/kWh in France (Blaisonneau et al., 2017). Considering the assumptions summarised in Box1, the effective price per m^3 of biogas within the EU would then increase to 0.64 €/m^3 for electricity; 0.7 €/m^3 for heat; and 0.48 €/m^3 for biomethane. Despite the relative low prices, encouraging national (in countries such as those within the EU and the USA) and international policies (such as by the Organisation for Economic Cooperation and Development) could set the biofuel sector to rapidly increase at an average compound annual growth rate of over 5% (Figure 3a). Indeed, as can be visualised from the data in Figure 3a, electricity production presents the biggest market opportunity for bioenergy. This is followed by biomethane, with potential to be valued over 5 billion-€ globally by 2025 (Transparency Market Research (TMR), 2018).

The combined health and wellness, food, and animal feed market forms the largest global bulk market for microalgae bio-products, to be worth over a trillion euro by 2025. As evident from Figure 3a, a significant variation in these prices results from the type of application and purity. As an example, while the price of whole microalgae for animal feed is less than 0.3 €/kg in the EU (Spruijt et al., 2016), dried whole *Spirulina*, cultivated in China is being sold for human consumption at 44 €/kg within the EU (BuyWholefoodOnline.eu, 2019). On the other hand, C-phycocyanin, a protein pigment, sells between 180€/kg and 2 million €/kg based on purity (Stanic-Vucinic et al., 2018). In addition, as can be seen within Figure 3a, market growth opportunities for individual product varies significantly. For example, high value chemicals such as isotopically labelled compounds and astaxanthin are already commercially produced from microalgae; and thus have limited market growth potential. In contrast, ω-3 fatty acids could see a growth of over 14% in the near future.

3.3 The Dilemma of Microalgal Use

Most of the low market value products from microalgae including biofuels account for either high market volume or represent significant growth opportunity in future. Products such as whole microalgae for human consumption or essential fatty acids are unique, representing both, a high selling price of around 50 €/kg and a fast growing global market. However, compared to biofuels, they would continue to represent a considerably lower overall market volume in the future. In addition, most often, these high value products are often present in limited quantities in microalgae. To account for the varying usable fraction of microalgae and to analyse the corresponding financial benefits/drawbacks, the equivalent selling price (ESP) per dry weight of whole microalgae for each algal bio-product was evaluated (see *Box 1* with respective assumptions).

BOX 1: Example calculation for equivalent selling price per whole microalgae of algal bio-products

<u>For all products except biodiesel, bioethanol and biogas</u>

➔ Maximum fraction of C-Phycocyanin in microalgae = 20% db* (Stanic-Vucinic et al., 2018)

➔ Current sale price of C-Phycocyanin with minimum purity = 200 €/kg (Stanic-Vucinic et al., 2018)

➔ Equivalent sell price (ESP) of C-Phycocyanin = $200 \times 20\% = 40$ €/kg-db

<u>For biodiesel</u>

➔ Maximum lipid content in microalgae = 60% (Kothari et al., 2017)

➔ Current sale price of biodiesel = 0.7 €/litre (Debnath et al., 2017)

Assuming the density of biodiesel as 0.88 kg/L (Sustainable Energy Authority of Ireland (SEAI), n.d.)

➔ ESP of biodiesel, considering entire lipid fraction to be converted to biodiesel, =
$(0.7 \times 60\%) / 0.88 = 0.48$ €/kg-db

➔ ESP of biodiesel, for a partial conversion of 80% from lipid to biodiesel (Posada et al., 2016) =
$(0.7 \times 60\%) / 0.88 \times 80\% = 0.38$ €/kg-db

<u>For bioethanol</u>

➔ Maximum carbohydrate content in microalgae = 60% (Brányiková et al., 2011)

➔ Current sale price of bioethanol = 0.6 €/litre (Debnath et al., 2017)

Assuming the density of bioethanol as 0.8 kg/L (Sustainable Energy Authority of Ireland (SEAI), n.d.)

➔ ESP of bioethanol, considering entire carbohydrate fraction to be converted to bioethanol =
$(0.6 \times 60\%) / 0.8 = 0.45$ €/kg-db

➔ ESP of bioethanol, for a partial conversion of 64% of carbohydrate fraction (de Farias Silva and Bertucco, 2016) = $(0.6 \times 60\%) / 0.8 \times 64\% = 0.29$ €/kg-db

<u>For biogas as direct commodity</u>

➔ Maximum utilisable fraction of microalgae = 100%

➔ Current sale price of biogas = 0.2 €/m^3 (Barsanti and Gualtieri, 2018)

From Table 1, assuming; 0.5 L-CH_4/g-VS, 60% CH_4, VS content of 92% in the microalgae (Herrmann et al., 2016; Parimi et al., 2015a), density of biogas as 1.1 kg/m^3 (Swedish Gas Technology Centre Ltd (SGC), 2012):

➔ Biogas price per kg = $(0.2 / 1.1)$ = 0.18 €/kg

➔ Biogas generated per dry biomass = $(0.5 \times 0.92) / 0.6$ = 0.77 L-biogas/ g-db

➔ ESP of biogas = 0.77×0.2 = 0.154 €/kg-db

For biogas as electricity

➔ Maximum tariff for electricity = 28 ct€/kWh (Pablo-Romero et al., 2017)

➔ Minimum tariff for electricity = 4 ct€/kWh (Pablo-Romero et al., 2017)

Assuming biogas with lower calorific value of 6.5 kWh/Nm^3 (Swedish Gas Technology Centre Ltd (SGC), 2012); electricity conversion efficiency of 35%; and that biogas being sold for electricity production is priced according to an average electricity tariff of 10 ct€/kWh

➔ Average price of biogas when sold for electricity = $(0.10 \times 6.5 \times 0.35) / 1.1$ = 0.21 €/kg-biogas

➔ Highest ESP of electricity = $0.28 \times 6.5 \times 0.77 \times 0.35$ = 0.49 €/kg-db

➔ Lowest equivalent sale price of electricity = $0.04 \times 6.5 \times 0.77 \times 0.35$ = 0.07 €/kg-db

For biogas as heat

➔ Maximum tariff for heat = 12 ct€/kWh (Herbes et al., 2018)

➔ Minimum tariff for heat = 1.2 ct€/kWh (Herbes et al., 2018)

Assuming biogas lower calorific value of 6.5 kWh/Nm^3; heat conversion efficiency of 90%; and that biogas being sold for heat production is priced as per an average tariff of 3 ct/kWh for heat,

➔ Average price of biogas when sold for heat = $(0.03 \times 6.5 \times 0.9) / 1.1$ = 0.16 €/kg-biogas

➔ Highest ESP of heat from biogas = $0.12 \times 6.5 \times 0.77 \times 0.9$ = 0.54 €/kg-db

➔ Lowest ESP of heat from biogas = $0.012 \times 6.5 \times 0.77 \times 0.9$ = 0.05 €/kg-db

For biogas as biomethane

➔ Maximum tariff for biomethane = 9.5 ct€/kWh (Blaisonneau et al., 2017)

➔ Minimum tariff for biomethane = 5.3 ct€/kWh (Ofgem, 2019)

Assuming biogas lower calorific value of 6.5 kWh/Nm^3 (Swedish Gas Technology Centre Ltd (SGC), 2012), 0.77 L-biogas is generated per g-db

➔ Highest ESP of biomethane = $0.095 \times 6.5 \times 0.77$ = 0.48 €/kg-db 0.77 L-biogas is generated per g-db

➔ Lowest ESP of biomethane from biogas = $0.053 \times 6.5 \times 0.77$ = 0.26 €/kg-db

** db represents dry biomass*

Figure 3b reflects the financial benefits of common microalgae bio-products based on ESP with maximum possible product content. For products making up only a minor fraction in microalgae, such as vitamins C&E or phytosterols, their ESP would drop below that of biofuels. Thus, their extraction would not be rational without co-production of other chemicals or biofuels for added financial benefit. Additionally, for species grown at conditions suboptimal for product accumulation, or those unable to accumulate significant amount of the desired product, the ESP can drop significantly. For instance, β-carotene content of 0.01% in *Nannochloropsis oculata* (Faé Neto et al., 2018) would drop its ESP to 0.04 €/$kg_{dry\ algae}$ compared to 39 €/$kg_{dry\ algae}$ for *Dunaliella salina* with 13% β-carotene content (Sathasivam, 2018).

The current ESP of microalgal biodiesel and bioethanol would be limited to 0.48 and 0.45 €/$kg_{dry\ algae}$, respectively considering complete conversion (Box1). However, these could significantly drop to 0.38 and 0.29 €/$kg_{dry\ algae}$ respectively, considering practical conversion rates (Box1). As for the use of biogas in electricity, heat, or biomethane, individual country policies would alter the optimal use of microalgae for energy production. For instance, in Germany typical electricity, heat, and biomethane FiTs are 13 ct€/kWh (Pablo-Romero et al., 2017), 2.1 ct€/kWh (Herbes et al., 2018) and 7 ct€/kWh (Deutsche Energie-Agentur GmbH, 2019) respectively. Therefore, with an ESP of electricity, heat and biomethane of 0.09, 0.23 and 0.35 €/$kg_{dry\ algae}$, biomethane injection appears to be the most effective use of biogas from microalgae in Germany.

Microalgae composition, local incentives, and policies, as well as possibility for considerable CO_2 mitigation could allow biofuel production to be more attractive than food/feed and high value compounds. Such advantages of microalgae biofuels, together with limited market growth opportunities for several high value algal products have led some researchers to raise concerns about shrinkage in the innovation and development of traditional high value microalgal industries (Zhu, 2015). Two fundamental dilemmas can be raised regarding the use of microalgae: *i)* Low priced biofuels (high market size or market growth) vs. high priced products (low market size and market growth); and *ii)* Advanced (biofuels) vs traditional (food and pharmaceutical) bio-products.

3.4 Decision Tree for Selecting Microalgae Biorefinery Pathway

A strategy to address the dilemma of microalgae bio-products, together with improving the economics of low value products is the co-production of multiple products in a biorefinery (Zhu, 2015). Multiple studies have proposed and developed mathematical algorithms for choosing the ideal pathway for processing and utilisation of microalgae, however, focussing on biodiesel production (Gong and You, 2014; Gupta et al., 2017). In contrast, for photosynthetic biogas upgrading with or without digestate treatment, the choice of species is often governed by upstream process(es). Therefore, the strain and the composition of microalgae should be fundamental in defining the target product(s) and corresponding pre-treatment techniques such as cell wall rupturing, if necessary. Figure 4 summarizes the decision-making algorithm proposed for selecting the most suitable biorefinery pathway from a microalgae strain perspective.

As an effective first step for the selection of most preferable bio-product, the ranking of each possible product based on the ESP per kilogram is proposed. Following this, each option could then be ranked in terms of profitability, based on production costs. Practical limitations (conforming to national and international food and drug safety regulations) must also be considered, a discussion of which is presented in Section 5.3. Except for the direct application of whole microalgae (such as in AD, and thermochemical conversion processes) all other products including biodiesel and bioethanol must be obtained from the processing and purification of the extracted fractions of microalgae in a primary biorefinery (Zhu, 2015). This would necessitate a costly primary biorefinery step, estimated to be 3-12.7 €/kg of whole dry biomass ('t Lam et al., 2018; Lupatini et al., 2017). Cost can significantly increase depending on the level of purity. In such cases, profitability from by-products should also be accounted for on a case-by-case basis. In comparison, a cost of only 1-2 €/kg is needed for processing whole biomass for bulk products (such as biogas and animal feed) (Barsanti and Gualtieri, 2018).

Residues from a primary biorefinery can be valorised either as a waste or in a secondary biorefinery step. A similar decision-making step, considering residue composition and net profitability from its processing must be performed to assess the economic viability of a secondary biorefinery step. On the other hand, AD provides an attractive low cost pathway for residue valorisation due to multiple advantages described earlier. Additionally, the potential for coupling of AD and microalgae cultivation, while assisting biomethane production, allows AD to potentially be an integral part of a microalgae biorefinery (Nguyen et al., 2019).

4 Cascading Algal Biomethane Biorefinery System (CABBS)

Process optimisation, together with the selection of an effective microalgae strain and corresponding biorefinery pathway would be essential for the continuous generation of grid quality biomethane and chemicals, food and/or feed, and other biofuels. Considering the microalgae suitable for photosynthetic biogas upgrading, as identified in Section 2.2, in conjunction with the decision tree developed for the associated biorefinery pathway, three possible circular biomethane-biorefinery energy systems have been proposed.

4.1 Composition-based Biorefinery

4.1.1 CABBS with Protein-rich Microalgae

Spirulina, *Anabaena*, *Synechococcus* and *Scenedesmus* comprise between 40% and 63% protein (Becker, 2007; Sialve et al., 2009). Unlike *Scenedesmus* sp. leaner cell walls in cyanobacteria makes their direct application in AD technically feasible without the need for energy intensive and costly cell-disruption techniques. However, considering the opportunities for extraction of valuable compounds from protein, as well as the disadvantages of a high protein content in AD, extraction of protein in a primary biorefinery step is an attractive alternative. As shown in Figure 5a, further purification and concentration then leads to chemically and pharmaceutically valuable pigments such as phycobiliproteins and lectins (Stanic-Vucinic et al., 2018). Based on the lipid content of the microalgae species, the residual biomass can either be extracted in a secondary biorefinery step or used as an effective substrate to generate biogas. However, a detailed economic justification would then be necessary. High protein microalgae can also be utilised directly for food and feed applications without extraction of target fractions, subject to food safety regulations.

4.1.2 CABBS with Lipid-rich Microalgae

Chlorella sorokiniana SLA-04, can accumulate a lipid content of up to 34% (Vadlamani et al., 2017), therefore lipid extraction for biodiesel production could be an effective primary biorefinery. Based on the lipid upgrading technique used, glycerol, PUFAs and other organic compounds can also be extracted as by-products, as shown in Figure 5b. Glycerol can be subsequently utilised as a raw material for production of chemicals/fuels including hydrogen, ethanol and biogas (Fan et al., 2010). Interestingly, other strains of the same species can also accumulate around 30% protein or carbohydrates (Lizzul et al., 2018). These can thus be extracted via protein extraction techniques in a secondary biorefinery step or co-digested with other feedstock in an AD system. Although the former is a costlier alternative, the possibility to produce high value products would require a detailed cost benefit assessment to assess the preferred microalgae utilisation.

4.2 CABBS utilising Pyrolysis

Production of biofuels or bio-chemicals by pyrolysis (decomposition of biomass between 400°C and 600°C in the absence of oxygen), in contrast to fraction extraction, is a feasible alternative (Azizi et al., 2018). The favourable carbon and hydrogen content in microalgae, required for pyrolysis, lack of a need for costly pre-processing, the ability to modify process parameters for effective generation of liquid biofuels, and the similarity of microalgal bio-oil compared to fossil fuels are all advantageous (Azizi et al., 2018; Fermoso et al., 2017). In addition, the potential to co-pyrolyze microalgae and digestate to enhance both liquid and solid yield could provide a major synergistic benefit to the proposed pyrolysis-AD system, shown in Figure 5c. A major by-product of pyrolysis, biochar could be widely applied as an effective soil additive and bio-adsorbent material due to its considerable carbon offset potential (Mumme et al., 2014); as well as in AD to enhance methane yield (Shanmugam et al., 2018). Biochar for biogas upgrading has also been recently investigated by Linville et al., (2017). Additionally, the gaseous fraction of pyrolysis can be utilised to meet the system thermal demands, used for electricity production, or processed into chemicals through thermochemical pathways including Fischer-Tropsch processes (Speight, 2015). Therefore, a sustainable, flexible, and multi-product system can be envisaged. However, being a developing technology, pyrolysis faces several challenges. The presence of excess water in microalgae, requiring energy intensive and costly drying to make the harvested biomass suitable for pyrolysis is a major drawback (Azizi et al., 2018). Furthermore, bio-oil from pyrolysis of microalgae, is acidic, unstable, and contains significant solid and water fractions (Azizi et al., 2018). Therefore, considerable technological advancements and process optimisation is necessary to achieve practical integration of the AD and pyrolysis through CABBS.

5 Environmental, Economic and Practical Challenges and Prospects

Techno-Economic Assessment (TEA) and Life Cycle Assessment (LCA) are two widely used tools for evaluating and optimising the financial feasibility and the environmental impacts of products/ processes respectively (Barsanti and Gualtieri, 2018; Chew et al., 2017). Using a systematic assessment, these tools could provide significant insights into the energetic, environmental and economic benefits of CABBS and identify effective strategies towards its potential commercialisation.

5.1 Environmental Perspectives

LCA studies reveal that biomethane from mono-digestion of *Chlorella vulgaris* may only reduce emissions by 25% in transport, compared to the fossil fuel comparator

(Collet et al., 2011). However, as can be seen from Table 1, *Nannochloropsis salina* or *Spirulina platensis* with a higher biogas yield could provide greater benefits. Alternatively, co-digestion, boosting biomethane yield could also allow higher environmental benefits. Renewable energy generated per fossil energy spent (net energy ratio (NER)) was found to increase from 2.7 to 4.5 when Chlorella sp. was co-digested with sewage sludge due to an increased biogas production of 65% than that of mono-digestion of microalgae (Solé-bundó et al., 2019). Toledo-Cervantes et al., (2017a) calculated CO_{2equiv} emissions 45 times lower than traditional water-scrubber upgrading owing to reduced energy demand, and CO_2 utilisation by the microalgae. Emission benefits from microalgal digestate, enabling nutrient recycling and preventing the release of toxic compounds, have seldom been quantified in literature. Nonetheless, significant indirect emission savings from using recycled nutrient media for microalgae growth can be achieved by substitution of inorganic fertilisers, mostly manufactured by expending fossil energy and finite natural resources (Collet et al., 2014). Therefore, coupling microalgae cultivation with AD in a circular bioeconomy system to provide CO_2 and nutrients, together with the use of renewable energy during microalgae cultivation, harvesting and processing could be environmentally beneficial (Togarcheti et al., 2017).

Co-production of multiple products from a single system in a circular economy framework adds to CO_{2equiv} savings due to the substitution of traditional energy intensive individual production chains (D'Amato et al., 2019). This could considerably enhance the overall emission reduction potential of biomethane generated by the CABBS layout. Further assessments of bio-product choice, suitable extraction pathways, limitations of scale and geographical location are critical as well. For example, energy requirements for drying in pyrolysis without the use of renewable energy can raise the specific emission from bio-oil to above 200 $gCO_{2\ equiv}$ /MJ (Bennion et al., 2015). Un-optimised transesterification of microalgae has been shown to potentially raise the specific emissions from biodiesel to as high as 1000 g$CO_{2\ equiv}$ /MJ (Rocca et al., 2015). Often, additional CO_{2equiv} savings from valorisation of lipid extracted residue may not be sufficient to significantly improve environmental performance (Rocca et al., 2015). In a possible alternative, extracted lipid from *Nannochloropsis* sp. and *Scenedesmus* sp. was used as a substitute for vegetable oil (Posada et al., 2016). When the remaining oil-free cake was used as fish meal, a greater CO_{2equiv} reduction potential was attained compared to combined biodiesel and biogas production (Posada et al., 2016). This was due to the higher emission savings from substitution of vegetable oil and fish meal than replacement of fossil diesel by microalgae biodiesel.

Presently, negative emission technologies are also being investigated to achieve effective removal of atmospheric CO_2 with biofuel production. As different alternatives, burial of whole or fraction extracted microalgae (Sayre, 2010), production of long-lived potentially carbon negative chemicals like construction materials (Greene et al., 2017) or biochar for fertilisers (Sayre, 2010) are effective routes for long-term carbon sequestration by microalage. Indeed, if used as feedstock for chemical production, rather than direct application for energy generation to release the captured CO_2, the proposed CABBS can lead to significant capture of atmospheric CO_2 with simultaneous biomethane generation. Such meticulous selection of microalgae usage could therefore allow the proposed CABBS configuration to be potentially referred to as a Bioenergy with Carbon Capture and Storage (BECCS) system, speculatively sequestering the CO_2 from biogas over long geological time intervals, as shown in Figure 6.

The increased requirement of water and land is however, a major disadvantage of photosynthetic biogas upgrading as compared to conventional upgrading technologies. Nevertheless, by careful choice of algal products significant overall environmental benefits can be obtained. For example, locally sourced proteins from microalgae grown on marginal lands would have a much lower emissions and resource footprint in Europe than protein imported from commercial facilities in South America (Rösch et al., 2019).

5.2 Techno-economic Perspectives

Production of biomethane from microalgae is still not commercially viable without incentives (Rajendran et al., 2019b). For the proposed CABBS configuration, the capital cost would increase significantly as compared to traditional biogas upgrading facilities (Toledo-Cervantes et al., 2017). For yearlong operations, a closed photobioreactor would be essential; this is currently limited by high cost and economic scalability (Bose et al., 2019). Nevertheless, a significantly lower operational energy demand from photosynthetic biogas upgrading and microalgae harvesting from closed PBRs would result (Bose et al., 2019). Furthermore, the generation of additional revenue from microalgal bio-products would considerably increase the economic benefit of photosynthetic biogas upgrading. Toledo-Cervantes et al., (2017a) predicted a payback of 5 years for a 300 Nm^3 biogas/hr photosynthetic biogas upgrading system with 20 g/m^2/day microalgae productivity in an open pond, while selling biomethane at natural gas prices of 0.3 €/Nm^3. No incentives were required as the added revenue generated from microalgae sale as fertiliser at 0.08 €/kg was highly profitable.

Direct production of biomethane and electricity from mesophilic AD of *Cyanothece sp.* BG0011 was estimated at 6.7 ct€/kWh and 11.7 ct€/kWh respectively, when grown at a scale of almost 300 tonnes per year of microalgae (Wu et al., 2019). Based on the individual country FiT, therefore, either electricity or biomethane production could also yield considerable economic returns. Low purity C-phycocyanin was produced at 249 €/kg product or 21.5 €/kg dry biomass; the opportunity to sell it at 500 €/kg (43.3 €/kg dry biomass ESP) was found economically advantageous (Chaiklahan et al., 2018).

Conversely, the cost of protein production at 12.7 €/kg dry biomass would be too high for economic benefit. Extraction of high value protein isolates (>2000 €/kg) by further purification steps could be beneficial (Lupatini et al., 2017). Indeed, co-production of high value and bulk compounds including biofuels, with waste valorisation for added biomethane production could increase the economic benefits further. Nonetheless, such opportunities are yet to be explored in detail.

5.3 Practical Challenges and Perspectives

Integration of a microalgae biorefinery, together with photosynthetic biogas upgrading via CABBS could accelerate the commercialisation of both the technologies. However, besides economic and environmental benefits, several practical challenges must be overcome to increase the choice of product from microalgae. To use microalgae for food/feed and nutraceutical applications, it must adhere to both local and global rules regarding food safety and human health (Chew et al., 2017). This is because the microalgae composition can vary significantly based on the growth environment, leading to accumulation of toxins, heavy metals, allergens, pathogens and pesticides (García et al., 2017; van der Spiegel et al., 2013). To cite instances, the growth of microalgae without adequate quality control, under non-standard conditions has shown to result in toxin concentrations above tolerable daily intake values (Roy-Lachapelle et al., 2017). Lead accumulation, up to 100 times above the safety limit for specific human consumption of 0.05 mg/kg wet weight has been reported for *Chlorella* PY-ZU1 when grown using undiluted digestate from swine manure (Cheng et al., 2015).

Impact of trace compounds in biogas such as volatile organic compounds on microalgae composition is yet to be determined by the scientific community. Therefore, microalgae growth conditions within CABBS, especially when using digestate for microalgae cultivation is essentially non-standardised. Choice of specific microalgae species, together with assessment of its chemical properties within the proposed system is therefore urgently required to assess the complete potential for the simultaneous production of food/feed, chemicals and biomethane in a future circular bioeconomy.

6 Conclusions

In this paper, three alternate configurations have been proposed for cascading algal biomethane biorefinery system for simultaneous production of biomethane and/or biofuels, food/feed and chemicals. In a novel approach as compared to recent review literature (Table 2), all significant factors necessary for developing a comprehensive perspective have been holistically identified and reviewed together with practical limitations and legislative challenges. In addition, a decision-making strategy was developed to facilitate maximisation of financial sustainability of the proposed CABBS layout. It is postulated that the system could be considered a carbon capture system and be an element of a negative emission technology.

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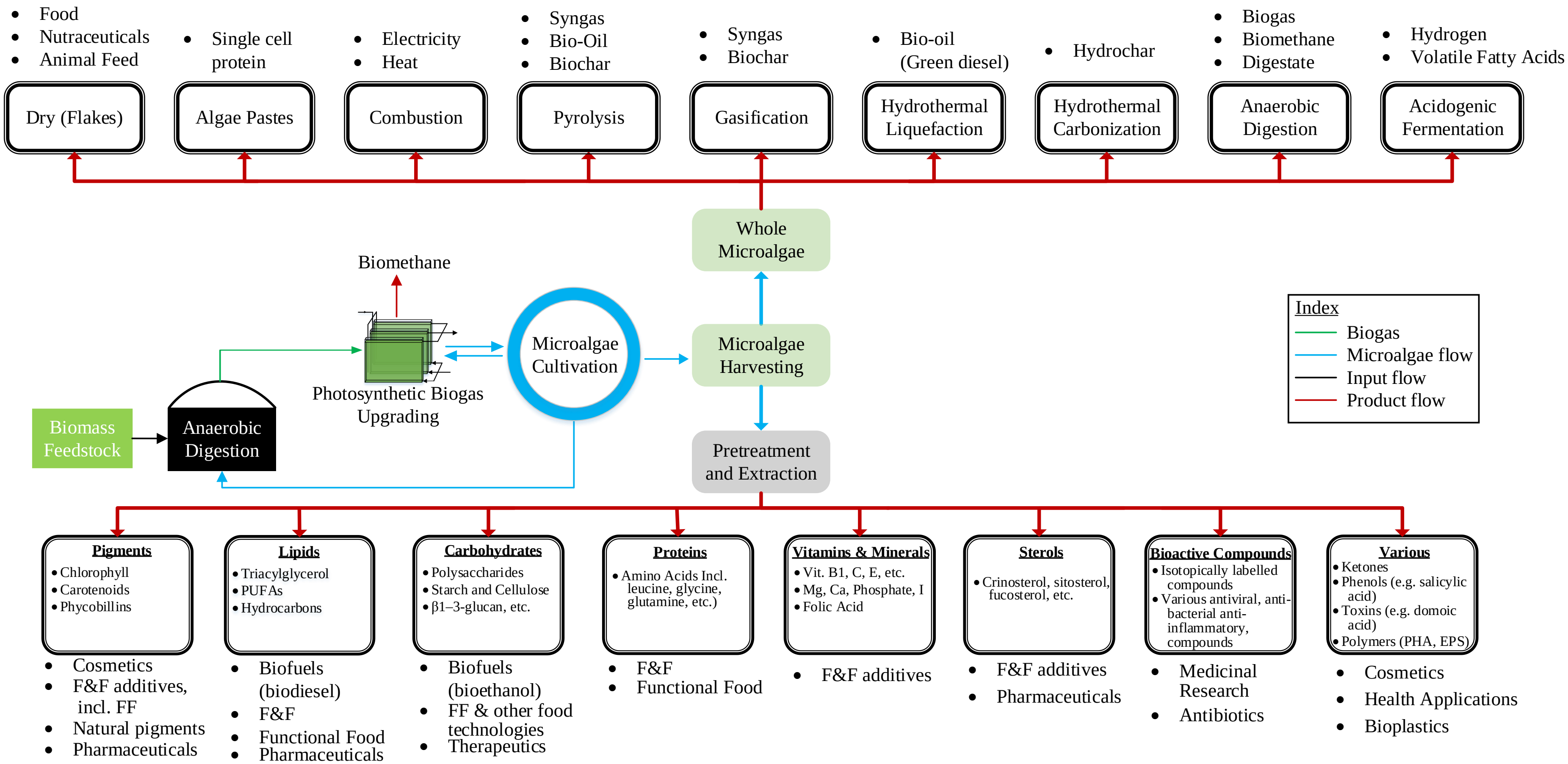


Figure 1 Schematic representation of a cascading algae biomethane biorefinery system with summary of different microalgae bio-products and possible applications in food, feed and energy; F&F: Food and Feed; FF: Functional Food

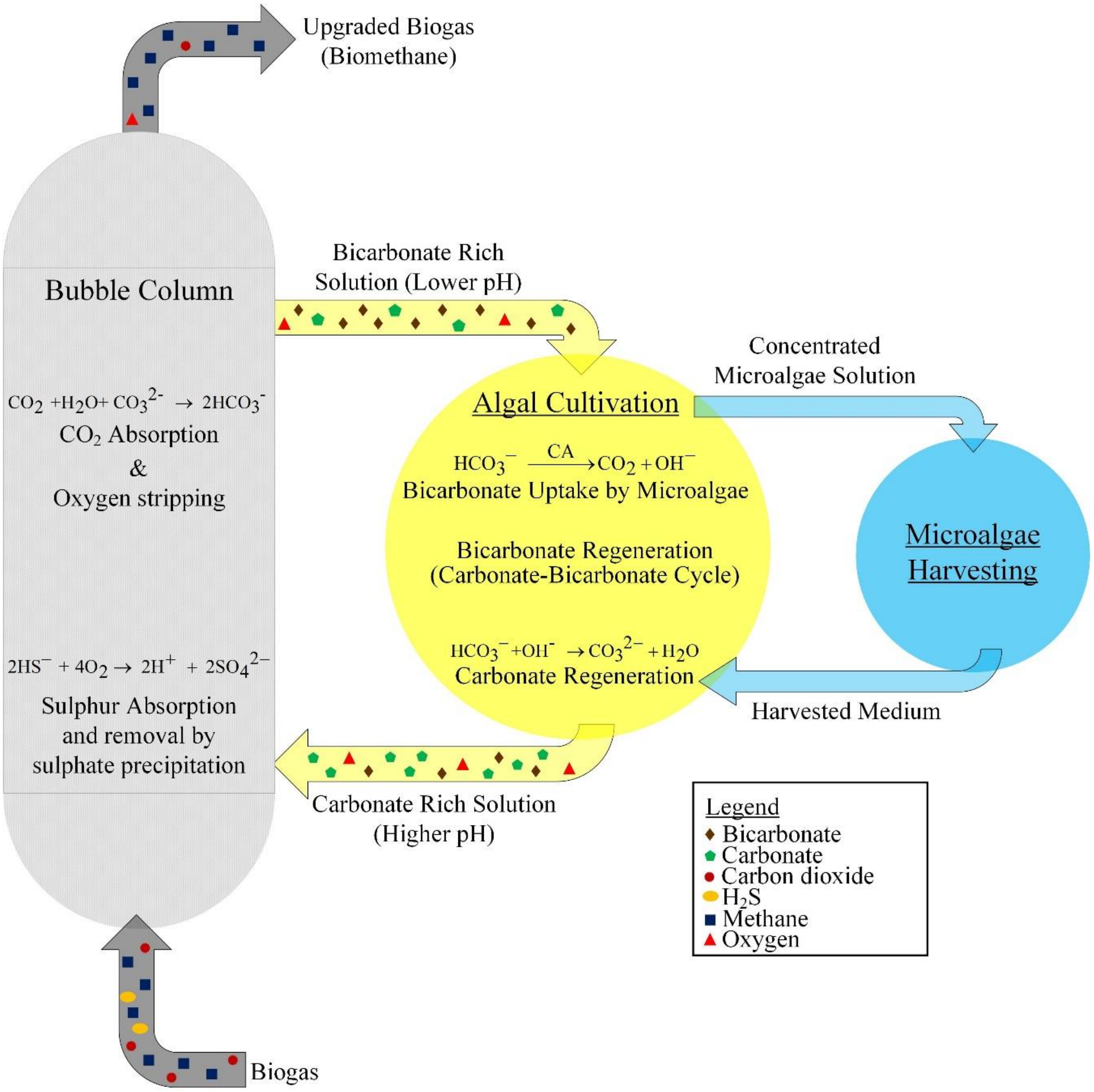
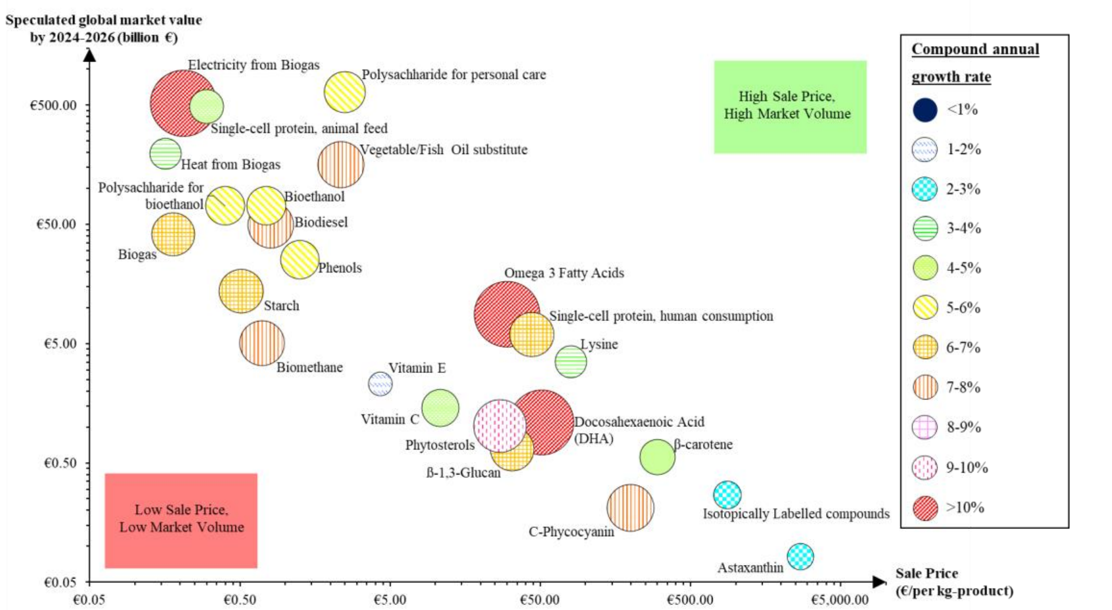
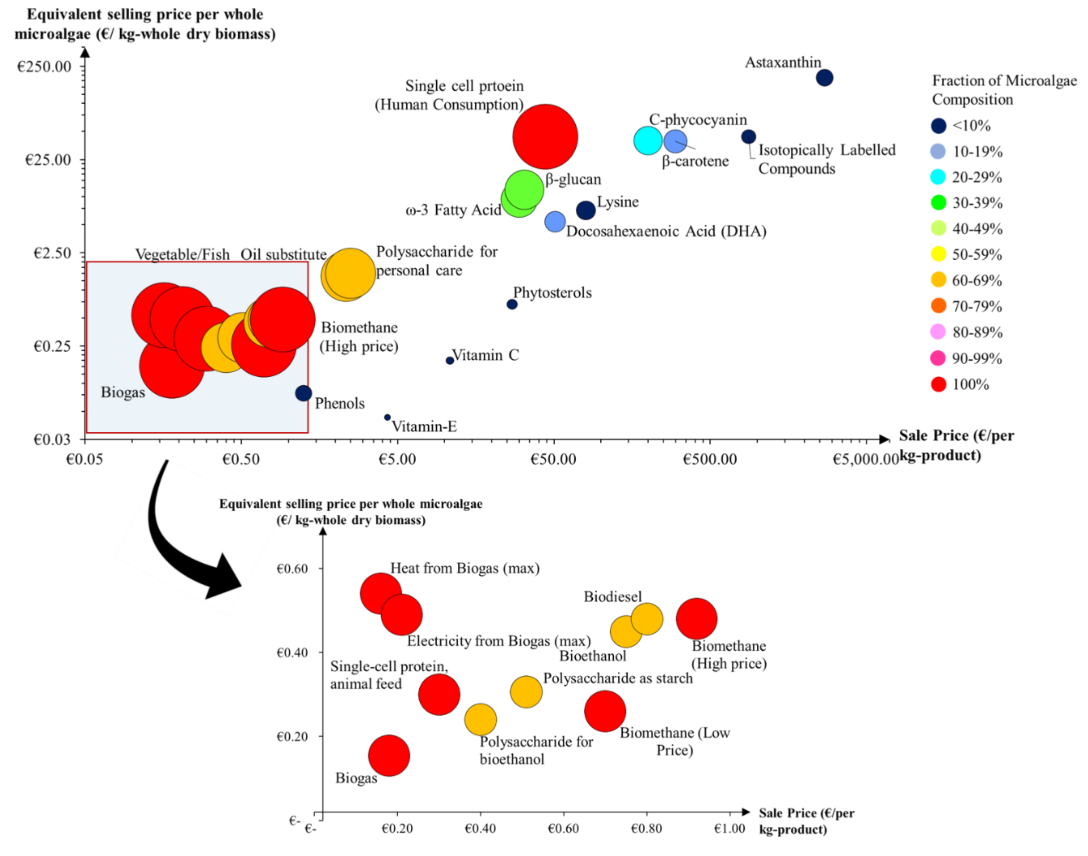


Figure 2 Schematic of photosynthetic biogas upgrading in an alkaline (carbonate) algal solution via carbonate/bicarbonate cycle followed by microalgae harvesting (The number of markings of each chemical species are indicative only to their relative quantity and not in absolute terms) (Bose et al., 2019)



(a)



(b)

Figure 3 Evaluation of market opportunity and economic possibilities with respect to the current market sale price of some common bio-products from microalgae, considering (a) the speculated global market value and market growth up to 2024-26, in which, the bubble size and hatching style represents the compound annual growth rate (CAGR) of each product and (b) the equivalent selling price of corresponding bio-products respectively. The bubble sizes in Figure 3(b) indicate the maximum fraction of microalgae utilisable for the corresponding bio-product considering maximum conversion. All references are explained and included in detail in Table A1 in the appendix.

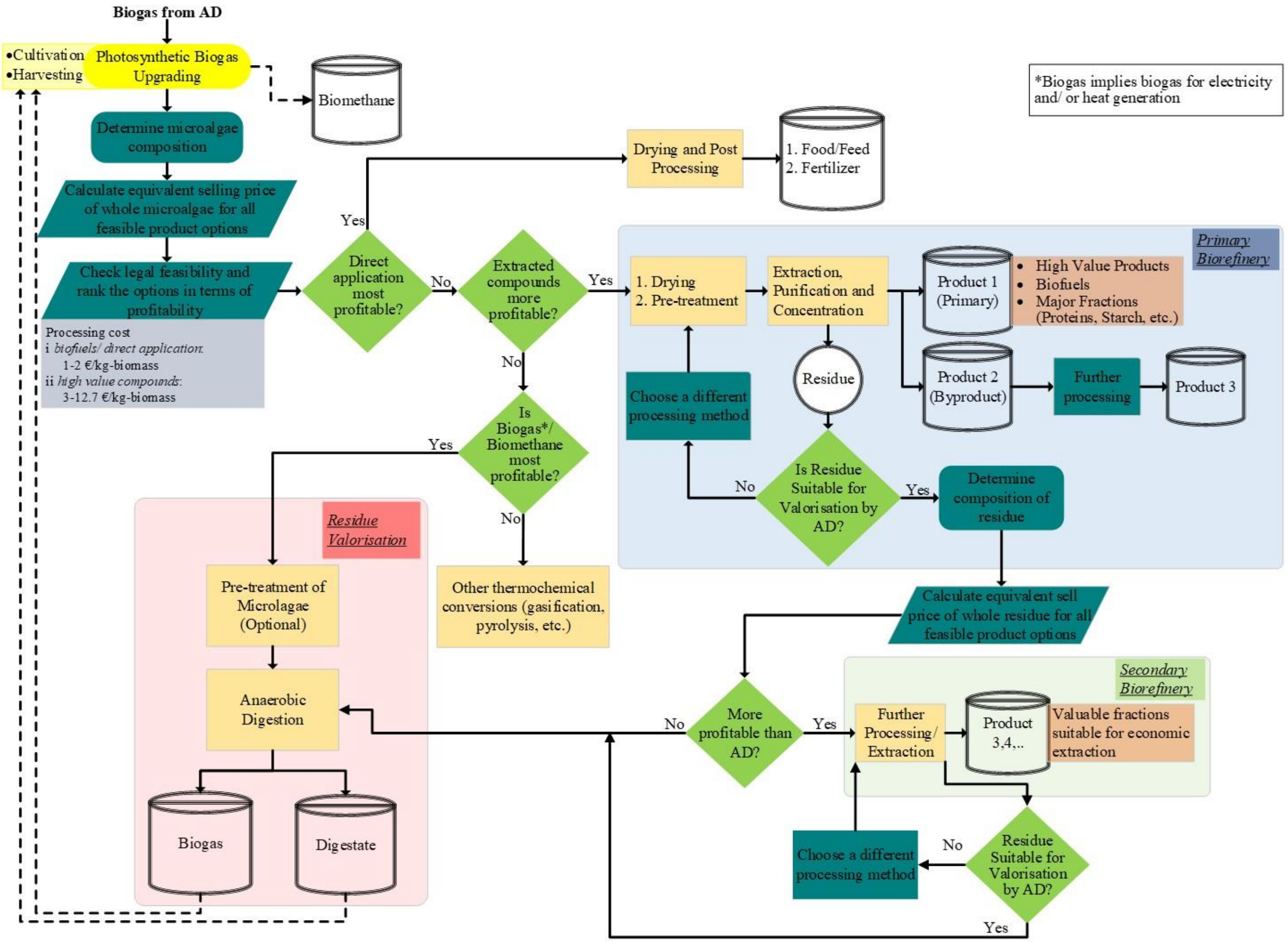


Figure 4 Decision making algorithm for microalgae application in a biorefinery approach from the perspective of microalgae composition

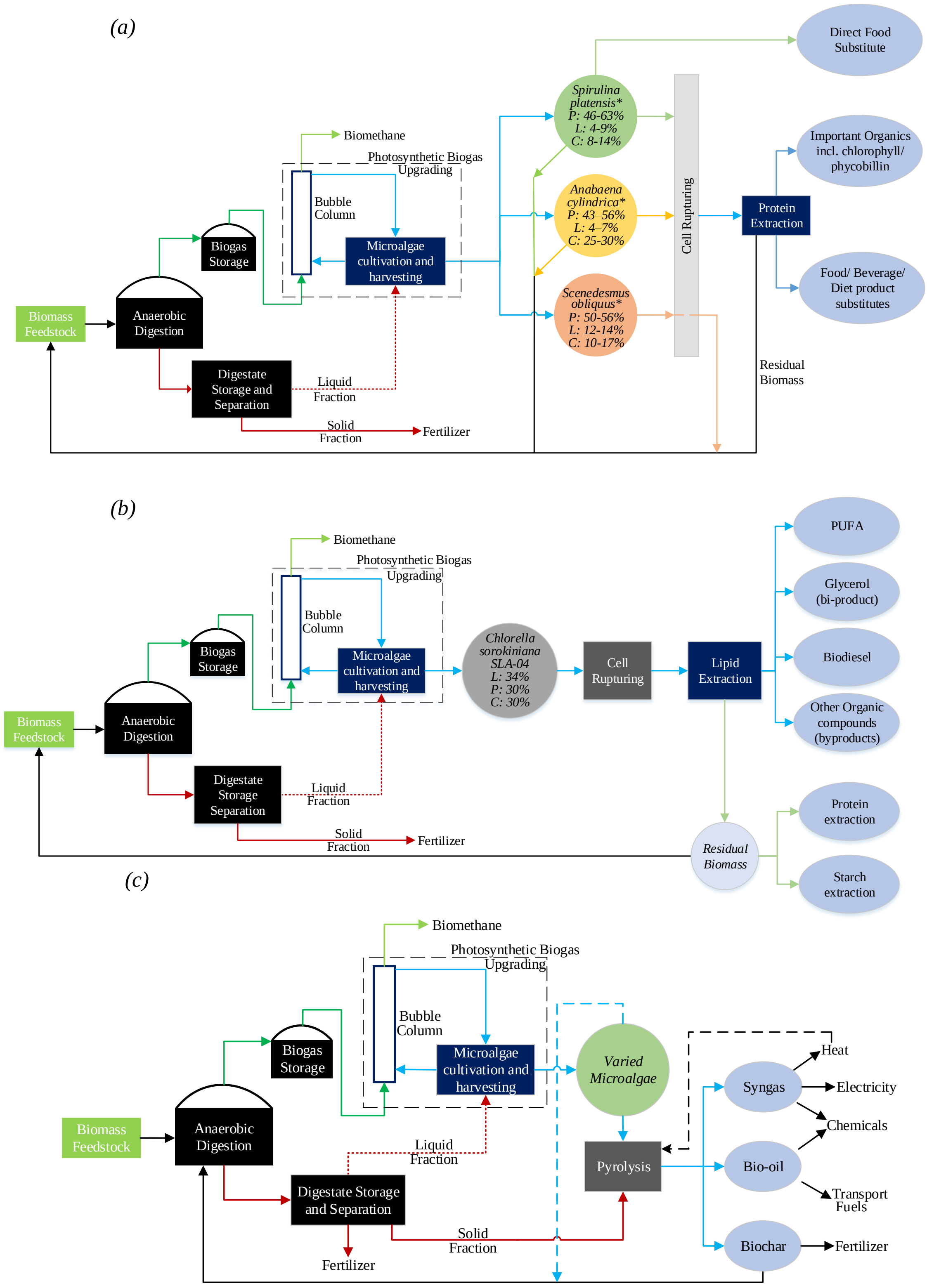


Figure 5 Cascading Algal Biomethane Biorefinery System (CABBS) (a) with protein rich microalgae for photosynthetic biogas upgrading; (b) with lipid rich microalgae for photosynthetic biogas upgrading; (c) integrating pyrolysis with AD systems and photosynthetic biogas upgrading

**Microalgae composition obtained from Becker (2007) and Lizzul et al., (2018), where, P is protein, L is lipid and C is carbohydrate*

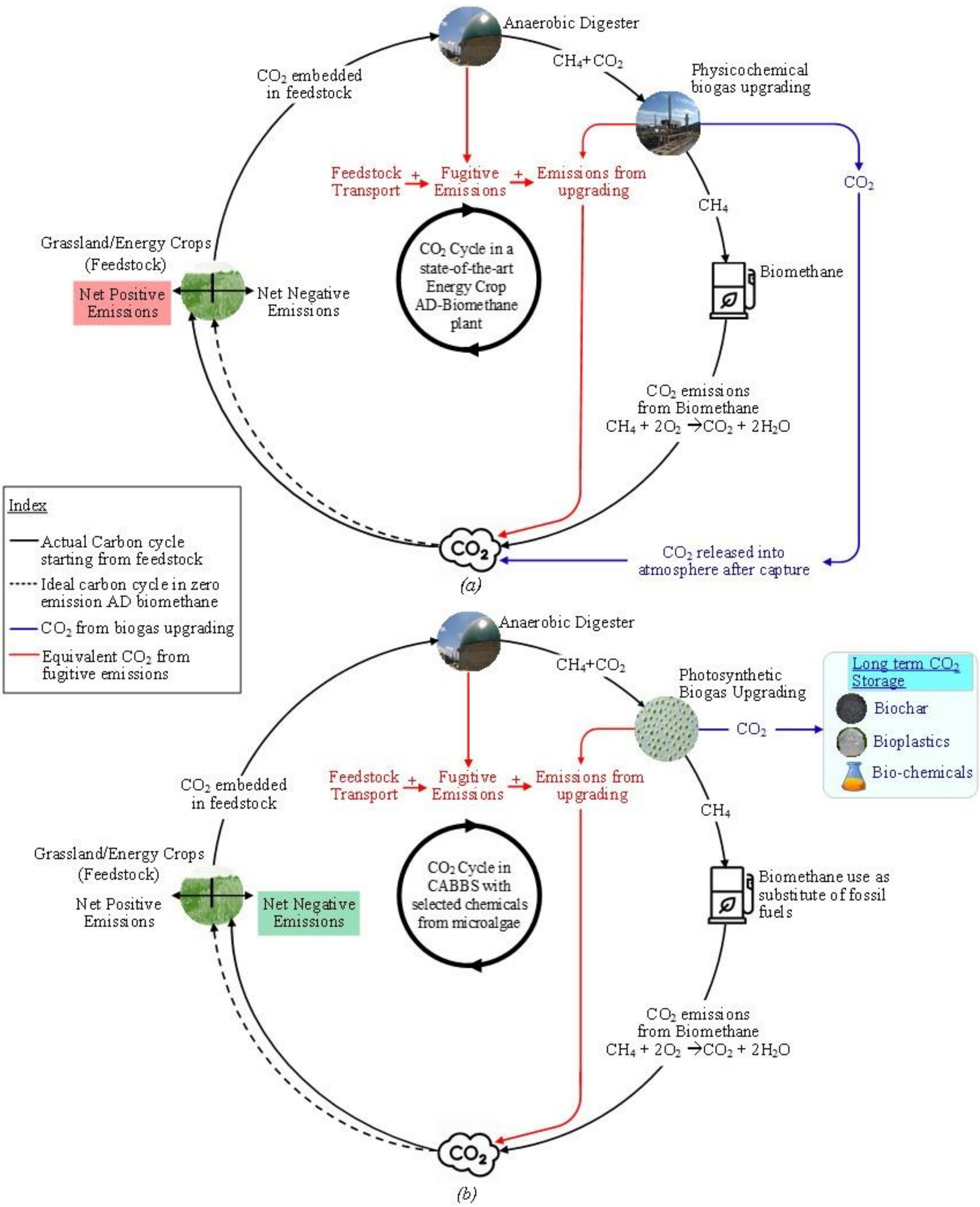


Figure 6 Comparison of net CO_2 flow in (a) state-of-the-art grass/energy crop based AD plant with physicochemical biogas upgrading and (b) proposed CABBS layout with production of selected chemicals from microalgae to be regarded as a potential Bioenergy with Carbon Capture and storage (BECCS) system.

Table 1 Theoretical and actual specific methane yield (SMY) in batch tests without pre-treatment, after pre-treatment and following fraction (lipid and protein) extraction of multiple microalgae species reported in literature

Microalgae	Theoretical SMY (g/L-VS)	Experimental SMY, Batch g/L-VS				Reference
		Without pre-treatment	After Pre-treatment	After Lipid Extraction	After Protein Extraction	
Chlamydomonas reinhardtii 6145	0.399	0.351				(Fernández-Rodríguez et al., 2019)
C. reinhardtii cw15	0.433	0.381				
		0.046**				(Mahdy et al., 2016)
Chlorella vulgaris	0.465	*	0.242			
Chlorella vulgaris	0.479	0.24				(Mendez et al., 2015)
Aphanizomenon ovalisporum	0.532	0.2877				
Synechocystis sp.	0.476	0.38				
Borzia trilocularis	0.482	0.2842				
Chlorella vulgaris 211/11B	0.543*	0.24				(Ras et al., 2011)
Chlorella vulgaris UTEX 395	0.604	0.337		0.314		(Zhao et al., 2014)
Nannochloropsis sp.	0.682	0.357		0.399		
Nannochloropsis salina	0.749	0.557		0.383		
Nanofrustulum sp.	0.882	0.507		0.304		
Phaeodactylum tricornatum CCMP 632	0.629	0.337		0.339		
Nannochloropsis gaditana	1.568	0.17		0.203		(Hernández et al., 2014)
Isochrysis T-ISO	0.866	0.188		0.215		
Scenedesmus almeriensis	0.646	0.165		0.203		
Tetraselmis maculata	0.738	0.205		0.236		
Scenedesmus sp.	0.35	0.084	0.133			(González-Fernández et al., 2012b)
Scenedesmus sp.	0.417	0.14		0.212	0.273	(Ramos-suárez and Carreras, 2014)
Spirulina platensis	0.569	0.357				(Herrmann et al., 2016)
Spirulina platensis	0.422**	0.37				(Aramrueang et al., 2016)
Spirulina platensis	0.558	0.254	0.254		0.163	(Parimi et al., 2015b)
Spirulina platensis	0.488	0.298	0.298			(Du et al., 2018)
Hydrodictyon reticulatum	0.574	0.17	0.384			(Lee et al., 2014)

SMY: Specific methane yield. The full names of microalgae species listed are as follows: C. reinhardtii - Chlamydomonas reinhardtii; P. tricornatum - Phaeodactylum tricornatum; H. reticulatum- Hydrodictyon reticulatum; A. ovalisporum - Aphanizomenon ovalisporum;

** Calculated based on the reported COD content in biomass using equation (2)*

*** Calculated based on the reported protein, carbohydrate and lipid fractions using equation (1)*

**** Results from continuous 1L Continuous Stirred Reactor (CSTR)*

Table 2 Comparison of the present review article and recent review papers (since 2015) focussing on the use of microalgae in AD systems and development of microalgae biorefinery

Reference	Microalgae in AD systems			Microalgae Biorefinery					Proposals of Comprehensive Biorefinery systems	
	Biogas Production*	**Photosynthetic Biogas Upgrading**	**Digestate treatment**	**Microalgae Processing****	**Bio-products*****	**Life Cycle Perspectives**	**Dilemma of Biorefinery**	**Decision Making strategies**	**Layout Development**	**Practical & Legislative Considerations**
This Study	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓
(Bose et al., 2019)	✗	✓	✗	✓	✗	✗	✗	✗	✗	✗
(Koutra et al., 2018)	✓	✗	✓	✓	✓	✗	✗	✗	✗	✓
(Gifuni et al., 2018)	✗	✗	✗	✓	✓	✗	✗	✗	✗	✗
(’t Lam et al., 2018)	✗	✗	✗	✓	✓	✗	✗	✗	✗	✗
(Saratale et al., 2018)	✓	✗	✗	✗	✗	✗	✗	✗	✗	✗
(Chen et al., 2018)	✓	✓	✓	✓		✗	✗	✗	✓	✗
(Chew et al., 2017)	✗	✗	✗	✓	✓	✓	✗	✗	✗	✓
(Jankowska et al., 2017)	✓	✗	✗	✓	✓	✗	✗	✗	✓	✗
(Kothari et al., 2017)	✗	✗	✗	✓	✓	✓	✗	✗	✗	✗
(Xia and Murphy, 2016)	✗	✗	✓	✗	✓	✗	✗	✗	✓	✓
(Neves et al., 2016)	✓	✗	✗	✓	✓	✗	✗	✗	✗	✗
(Posada et al., 2016)	✗	✗	✗	✓	✓	✓	✓	✗	✓	✗
(Zhu, 2015)	✗	✗	✗	✓	✓	✗	✓	✓	✗	✓
(Muñoz et al., 2015)	✗	✓	✗	✗	✗	✗	✗	✗	✗	✗

Includes review on pre-treatment of microalgae
**Includes cultivation, harvesting and extraction of useable fractions from microalage*
***Including both technological and economic aspects of microalgal bio-product*

Appendix

Table A1 References for product content in microalgae and economics (sale price, compound annual growth rate and speculated market value) of bio-products

Product	Sale Price	Product (% dry weight)	Compound Annual Growth Rate (CAGR) and Speculated Market value (billion Euros)			
	Reference	**Reference**	**Keyword**[1]	**News Source**[2]	**Date**[3]	**Reference**[4]
Biogas	Calculated in Box 1	Assumed 100%	Global Biogas Market	PR Newswire	16/01/2017	
Biodiesel	Calculated in Box 1	(Kothari et al., 2017)	Global biodiesel market	Business Wire - BZX	25/08/2017	(MarketWatch Inc, n.d.)
Bioethanol	Calculated in Box 1	(Brányiková et al., 2011)	Bioethanol	Zion Market Research	13/12/2018	
Biomethane	Calculated in Box 1	Assumed 100%	-		Dec 2018	(Transparency Market Research (TMR), 2018)
Biogas for Electricity	Calculated in Box 1	Assumed 100%	Distributed Energy market	PR Newswire	21/05/2019	(MarketWatch Inc, n.d.)
Biogas for Heat	Calculated in Box 1	Assumed 100%	Global District Heating	Zion Market Research	19/03/2018	(Globenewswire, n.d.)
SCP* (Feed)	(Spruijt et al., 2016)	Assumed 100%	Compound Feed	Market Research Future	07/05/2019	
SCP (Human food)	(BuyWholefoodOnline.eu, 2019)	Assumed 100%	Neutral Alternative Protein Market	ACPS**	08/03/2019	(MarketWatch Inc, n.d.)
Oil (human consumption)	(Xue et al., 2018)	(Kothari et al., 2017)			2018	(Value Market Research, 2018)
Starch			Modified Starch	Business Wire	02/08/2019	
Personal care products	(Barsanti and Gualtieri, 2018)	(Brányiková et al., 2011)	Beauty & Personal Care Products	PR Newswire	22/11/2018	(MarketWatch Inc, n.d.)
Lysine	(Bulk Powders, 2019)	(Koyande et al., 2019)	Lysine	All Company Press Releases	29/03/2019	
Vitamin E	(FeedInfo News service, 2017)	(Kothari et al., 2017)	Global Vitamin E Market	Zion Market Research	23/01/2019	(Globenewswire, n.d.)
Astaxanthin	(Li et al., 2011)	(Sathasivam, 2018)	Astaxanthin	Orbis Research	19/06/2019	
Vitamin C	(Tay, 2017)	(Miller and Brown, 1992)	Vitamin C	ACPS	22/03/2019	(MarketWatch Inc, n.d.)
Beta-Carotene	(DEINOVE, 2015)	(Sathasivam, 2018)	Beta Carotene	PR Newswire	18/10/2018	
Isotopically Labelled Compounds	(Kothari et al., 2017)	(Kothari et al., 2017)	Stable Isotope Labelled Compounds	ACPS	13/08/2018	
C-Phycocyanin	(Stanic-Vucinic et al., 2018)	(Stanic-Vucinic et al., 2018)	Phycocyanin	Future Market Insights	29/08/2019	(Globenewswire, n.d.)
Ω-3 Fatty acids	(Barsanti and Gualtieri, 2018)	(Sahin et al., 2018)			Nov 2018	(Verified market Research, 2018)
DHA			Global Algal DHA	Coherent Market Insights	10/12/2018	(Globenewswire, n.d.)
ß-1,3-Glucan	(Pharmacompass, n.d.)	(Schulze et al., 2016)	Beta-glucan Market Size	PR Newswire	09/01/201	(MarketWatch Inc, n.d.)
Phytosterols		(Stonik and Stonik, 2018)	Phytosterols	ACUMEN Research and Consulting	09/04/2019	(Globenewswire, n.d.)
Phenols	(Argus Media Group, 2018)	(Li et al., 2007)	-	-	Feb 2019	(imarc, 2018)

[1]Keyword indicates the keyword to be entered to search for the news release cited in the present work; [2]News source indicates the name of the organisation that must be selected to obtain the news release cited in the present work;[3]Date indicates the date of news release; [4]All data accessed before 31.08.2019;
[*]SCP: Single Cell protein; [**]ACPS: All Company Press releases