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Review

미세조류 탈지세포잔류물의 미생물 배양 및 바이오에너지 생산으로의 재활용

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Recycling of Lipid-extracted Algae Cell Residue for Microorganisms Cultivation and Bioenergy Production

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Abstract

Microalgae is one of the promising biodiesel feedstock with high growth rates compared to those of terrestrial oil crops. Despite its numerous advantages, biodiesel production from microalgae needs to reduce energy demand and material costs further to go to commercialization. During solvent extraction of microalgal lipids, lipid-extracted algae (LEA) cell residue is generated as an organic solid waste, about 80-85% of original algal biomass, and requires an appropriate recycling or economic disposal. The resulting LEA still contains significant amount of carbohydrates, proteins, N, P, and other micronutrients. This review will focus on recent advancement in the utilization of LEA as: (i) utilization as nutrients or carbon sources for microalgae and other organisms, (ii) anaerobic digestion to produce biogas or co-fermentation to produce CH_4 and H_2, and (iii) conversion to other forms of biofuel through thermochemical degradation processes. Possible mutual benefits in the integration of microalgae cultivation-biodiesel production-resulting LEA with anaerobic digestion and thermochemical conversion are also discussed.

Keywords: *Lipid-extracted algae (LEA), Bioenergy, Mixo/heterotrophic culture, Anaerobic digestion, Thermochemical conversion*

1. Introduction

Microalgae are considered as a renewable source of liquid and gaseous biofuels and an efficient biological capturer of carbon dioxide and nitrogen oxides. Microalgae represent one of the most sustainable and promising biodiesel feedstock, demonstrating particularly high growth rates compared to terrestrial green plants. They can also thrive in harsh environments such as seawater, alkaline lakes, municipal and industrial wastewater. Thus, they do not require fresh water or arable land for cultivation. Unfortunately, several recent studies on life cycle assessment (LCA) and related reviews on microalgae biodiesel have suggested that a considerable amount of energy input is required to produce biodiesel during cultivation, harvesting, extraction and refinery stages, mostly due to the aqueous nature of the cultivation mixture and intracellular constituents. In fact, a negative energy balance has been observed in some LCA studies[1-5], indicating that the sustainability of microalgae as a biofuel feedstock of the future is still questionable. The cost of carbon source, nutrients such as nitrogen and phosphorous, and water supply in the cultivation stage and multi-step downstream processing sacrifices economic profitability of the produced biodiesel. Therefore, despite its numerous advantages, biodiesel production from microalgae has a further way to go to independent commercialization by reducing energy demand and material costs.

There are several options to improve the economic feasibility and environmental sustainability of microalgal biodiesel production. The use of more economical non-conventional nutrients source has been considered as a potential substitute of traditional nutrition in the cultivation stage. A wide range of studies have been carried out on the utilization of non-conventional nutrient sources including wastewaters, flue gases, organic wastes, organic fertilizers, and so on, for microalgae

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growth and lipid production [6-10]. Another way to improve the economic feasibility and environmental sustainability is to recycle process byproducts or wastes for valuables-producing purposes without disposing them as wastes. These byproducts and wastes such as biogas, biohydrogen, bioethanol, and pyrolysis-based products can be utilized for further energy production and recycled for the cultivation stage again to produce microalgae biomass [11-16].

Lipid in the form of triacylglycerol is usually recovered through organic solvent extraction from lipid-accumulated microalgae cells. These extracted lipids then undergo transesterification to produce biodiesel. Cell disruption may be employed depending on extraction and transesterification methods. If methanol is used in the reaction, fatty acid methyl esters (FAMEs) are produced as biodiesel with glycerol as a byproduct. During solvent extraction, lipid-extracted algae (LEA) cell residue will remain in the extraction unit as an organic solid waste, like excessive sludge in wastewater treatment processes. It requires an appropriate recycling or economic disposal. It is known that the mass of the resulting LEA after lipid extraction is about 80-85% of original algal biomass [17]

Among many possible options of LEA utilization, we are interested in facts that major constituents of LEA come from algal cell wall, membrane-based carbohydrates and proteins and that some microalgae can grow mixotrophically or heterotropically utilizing organic carbons as well as inorganic carbon like CO_2 autotrophically. This review will focus on recent advancement in the utilization of LEA as a nutrient source for cultivating microalgae and other microorganisms. Recycling for microalgae cultivation is intended to have a closed-loop integration of microalgal biodiesel production. LEA will be mostly used in fermentation or anaerobic digestion processes of microorganisms. Possible mutual benefits in the integration of microalgae cultivation-biodiesel production-resulting LEA with anaerobic digestion and thermochemical conversion are also discussed.

2. Characteristics of LEA and recycling strategies

Microalgae biomass contains lipids 7-23%, proteins 6-71%, and carbohydrates 5-64% although those proportions depend on algal species and growth conditions [18]. After extracting lipids for biodiesel production, LEA cell residue is generated. Its amount is close to the total biomass minus lipid content, accounting for about 70-80% of whole algal biomass [17,19]. The reuse or recycle of LEA is important in that it is a tool for waste minimization and a key to enhanced sustainability of biodiesel production. Like excessive sludge of wastewater treatment plants, LEA is an organic waste that is supposed to be disposed by landfill or ocean dumping. Depending on the purpose of its usage, it has several recycling options: (i) anaerobic digestion to produce biogas or co-fermentation to produce CH_4 and H_2, (ii) conversion to other forms of biofuel through pyrolysis or hydrothermal liquefaction, and (iii) utilization as nutrients or carbon sources for microalgae and other organisms.

The resulting LEA after lipid extraction for biodiesel production still contains significant amount of nutrients such as carbohydrates, proteins, N, P, and other micronutrients [20]. It is almost similar to the original whole cells only without the lipid fraction. Therefore, LEA is an excellent resource for the production of other energy sources or for nutritional supply to the cultivation stage of other organisms including microalgae, bacteria, and plants. Because LEA constituents are diverse chemically and biochemically, it is necessary to set a goal and appropriate direction of utilization depending upon the recycling purpose to make microalgal biorefinery more economically feasible and environmentally sustainable [21,22]. Various application of LEA as well as unprocessed whole algae cells have been studied recently for biogas production by fermentation and anaerobic digestion [16,18,23], biohydrogen production [18,24-26], bio-oil production by pyrolysis [13,27-29], bioethanol production, and returning to microalgae cultivation as nutrients [16,20,30,31].

The utilization of LEA can be determined by residual compositions of carbohydrate and protein, or carbon to nitrogen (C/N) ratio. High C/N ratio is beneficial for further production of bioenergy such as biomethane, bioethanol, and biohydrogen. Meanwhile a low C/N ratio with a high protein content may be beneficial as a nutrient source for microalgae, fertilizer, fermentation microorganisms or as a feed supplement for animals, fish and one of the food chain in aquaculture [32,33]. LEA usually has lower C/N value than the original whole algae cells because lipid has been extracted, which is undesirable for direct production of fuels including methane. For such purposes, co-digestion of LEA with additional organic wastes would be necessary. Figure 1 shows several possible prospective recycling options of lipid-extracted algal cell residue (LEA) after biodiesel production, including: (i) recycling for culturing other organisms such as bacterial fermentation and microalgae cultivation after pretreatment such as hydrolysis or saccharification, (ii) recycling for anaerobic digestion, and (iii) recycling for thermochemical conversion of biomass.

The method used to harvest microalgae from a culture also affects the choice of LEA use. Chemical flocculation is not suitable for animal feed due to the presence of coagulant metals such as aluminum and ferric iron known to be toxic to many living organisms. However, anaerobic digestion is not severely inhibited by such components [32]. Non-chemical harvest method such as centrifugation and filtration is recommended for use in animals or for agricultural purpose [18]. The method of lipid extraction is another factor influencing the applications of LEA. Hexane-based extracted LEA is not recommended for bioethanol fermentation or animal feeds unless the extraction solvent is completely removed. However, it can be used for biogas production after a moderate level of evaporation to remove the solvent.

Pretreatment of LEA makes its constituents easily available to organisms to use. It could be classified into thermal, chemical, physical and biochemical methods. Chemical methods such as acidic and alkaline hydrolysis and enzymatic hydrolysis are popular. Thermal decomposition, ultrasonication, and microwave treatments are also gaining attention. The application of a thermo-chemical combined pretreatment is increasing recently. LEAs pretreated by appropriate methods have been used to produce biogas, biohydrogen, lactic acid, succinic acid, and bioethanol [12,34-37]. Alzate *et al.* [38] have performed thermal pretreatment of *Nannochloropsis* sp. LEA for the production of bio-methane. Ansari *et al.* [39] have used ultrasonic pretreatment of *Scenedesmus obliquus* LEA to produce protein and reducing sugars. Alkali-acid pretreated *Lyngbya majuscula* LEA hydrolysate has been used in the culturing *Chlorella vulgaris* to produce lipid [32]. Pancha et al [35] have pretreated *Scenedesmus* sp. LEA with chemicals and enzymes to produce reducing sugars.

3. As carbon/nutrient source for microalgae and other microorganisms

Microalgae cultivation in these days is not confined to photoautotrophic cultivation. It has been expanded to mixotrophic or heterotrophic manners. The extent of achievable biomass production is generally limited in photoautotrophic cultivation because increased cell concentration imposes cellular shading effect and hinders light availability for cell growth. This is the reason why most large-scale photobioreactors have shapes of shallow raceway and cylindrical forms with small diameters or thin plate types having a limited thickness. The use of gaseous carbon dioxide as a carbon source also requires a long retention time. Thus, long tubular form of reactors are usually employed. Some microalgae can grow on organic molecules, such as monosaccharides (glucose and fructose, among others), organic acids (acetate, among others), glycerol, and amino acids [30,40,41].

3.1. General consideration

A plausible strategy to reduce the significance of light requirement by cells and to improve cell growth rate which is limited by autotrophic culture would be a heterotrophic or mixotrophic cultivation of microalgae. Although heterotrophic cultivation without light is possible for some microalgal species, mixotrophic cultivation has been gaining advantages over heterotrophic one in that it usually shows higher biomass productivity [42,43]. A mixotrophic culture can assimilate CO_2 and organic carbons simultaneously. In addition, both respiratory and photosynthetic metabolism operates concurrently. Mixotrophic growth requires relatively lower light intensities than a photoautotrophic one. Many studies have demonstrated that mixotrophic cultivations of various microalgae with glucose or other organic carbons can result in higher final biomass and lipid content than autotrophic cultivation [35,43,44].

Besides carbon, nutrients like nitrogen and phosphorus, and some minerals are also essential for the growth of microalgae. It is known that the production of 1 L of biodiesel requires 0.23 - 1.55 kg of nitrogen and 29-145 g of phosphorus depending on cultivation conditions and the microalgal strain used [45]. The production of biomass and biofuels including biodiesel can be limited by sustainable supply of nutrients as well as carbon source. In order to overcome this limitation and reduce the cost for nutrients, many researchers have investigated the usage of alternative nutrient sources such as wastewater, flue gas, organic wastes, fertilizer and recycling of nutrients from used medium or production processes [46]. Various wastewaters such as industrial, municipal, dairy, and food wastewaters and organic fertilizers can be used for microalgae cultivation [47,48]. Recently LEA biomass residue has been attracted attention as an economical way of microalgae production and microalgal biofuel refinery since it contains a substantial amount of organic carbon and nutrients such as nitrogen, phosphorus and micronutrients. The burden of replenishing carbon source and nutrients in continuing microalgae cultivation could be greatly reduced if carbon and nutrients in the LEA after lipid extraction can be recycled and used in the cultivation process.

3.2. Use in mixo/heterotrophic cultivation of microalgae

In the context of mixotrophic or heterotrophic cultivation of microalgae, LEA cell residue is a good candidate to provide organic carbons because carbohydrates and proteins, which are sources of carbon and nitrogen, will remain after solvent extraction of lipids. The use of LEA in microalgae cultivation is based on the utilization of mixotrophic or heterotrophic metabolism of microalgae for growth. Indeed, microalgal LEA has been used as a nutrient source for growth of not only microalgae, but also other organisms such as bacteria, fish, livestock, and plants [32,41,45]. To use LEA in microalgae cultivation, an appropriate pretreatment involving enzymatic or chemical hydrolysis is necessary to degrade carbohydrate polymers to its consisting monosaccharides which can facilitate their availability to microalgae cells as carbon and nutrient sources.

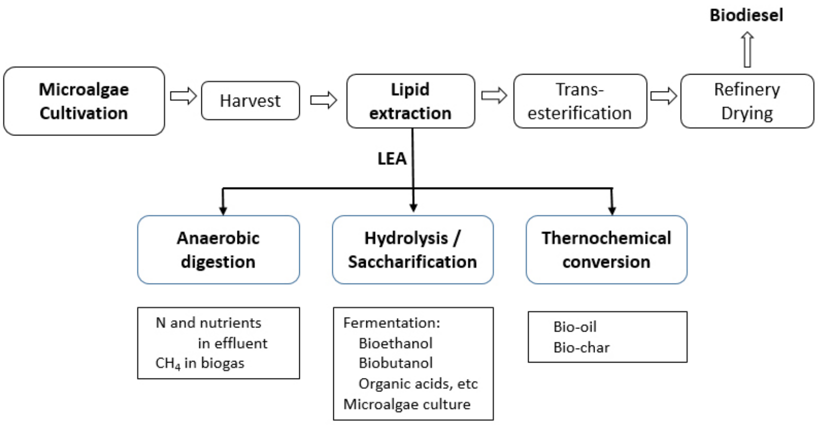

Figure 1. Possible prospective recycling options of lipid-extracted algal cell residue (LEA) after biodiesel production.

Zheng *et al.* [49,50] have prepared LEA hydrolysate by treating with a mixture of cellulase, neutrase and alcalase and returning to a cultivation medium of\ *Chlorella vulgaris* for lipid production. The biomass concentration and lipid productivity were increased under the condition of simultaneous supply of CO_2 in addition to LEA hydrolysate. Enzymatic hydrolysate of *C. vulgaris* LEA has also been evaluated as a nutritional source by Ma *et al.* [51] for mixotrophic growth of the same microalgae and its lipid production. They used cellulase derived from *Trichoderma*, neutrase derived from *Bacillus subtilis*, and alcalase derived from *Bacillus lincheniformis* for the hydrolysis of LEA.

Combining LEA and other carbon source would be a good strategy to balance between N and C levels in a high C demanding usage, if inexpensive carbon source is available. Zheng *et al.* [52] have utilized a mixture of *Chlorella* LEA hydrolysate and molasses to increase lipid productivity of *Chlorella* sp. Maximum biomass concentration and lipid productivity were achieved when the ratio of LEA hydrolysate to molasses was 1:4. It was observed that this LEA mixture contained high amounts of amino acids, oligopeptides, oligosaccharides and minerals which could boost the growth of microalgae. Moon *et al.* [53] have assessed the use of hydrolysate of LEA combined with sugar factory wastewater (SFW) as a low cost nutrient and a carbon source, respectively for microalgal cultivation. They suggested that a combination of LEA hydrolysate and SFW was an economical and efficient growth medium for algal cultivation. Microalgal strain *Ettlia* sp. was able to grow both mixotrophically and heterotrophically using combinations of LEA hydrolysate and SFW. The addition of LEA hydrolysate enhanced the growth rate, while the SFW increased lipid productivity. In a recent study, the use of a mixture of LEA hydrolysate and glycerol as a byproduct from biodiesel refinery was helpful for increasing the growth rate and lipid accumulation. Abomohra *et al.* [20] have studied the utilization of LEA hydrolysate and waste glycerol (WG) for biodiesel production from *Scenedesmus obliquus*. Hydrolysis was performed by acid-alkaline treatment. Increased biomass and lipid productivities were obtained using an appropriate combination of LEA and WG grown in a synthetic wastewater.

Although the expected gain of using LEA is maintaining biomass productivity by reducing the amount of organic waste, the use of LEA often boosts lipid productivity and cell growth. The presence of glucose, arabinose, and xylose in LEA hydrolysate resulted in 54.12% of lipid content and increased *Chlorella minutissima* biomass productivity [54]. Lowrey *et al.* [55] have pretreated *Thraustochytrium* sp. LEA through enzymatic hydrolysis using an alcalase and supplemented the resulting LEA hydrolysate to the usual culture media and found that the biomass and lipid production are both unaffected compared to the use of fresh media. Biomass and lipids produced during LEA-added cultivation were comparable in quantity and quality to the control, suggesting no negative impact of using recycled waste materials.

3.3. Use of water-blooming microalgae

There have been some efforts to derive nutrient source from water-blooming algae for the cultivation of industrial microalgae. Such effort are environmentally important in that the final disposal or reuse is often necessary after collecting water-bloomed algae from bloomed lakes. Maurya *et al.* [32] have prepared algal hydrolysate from LEA of *Lyngbya majuscula* which is a harmful water-blooming cyanobacteria and used it as a growth supplement for the cultivation of *Chlorella vulgaris*. The volumetric productivity of neutral lipids and total protein content of cells were significantly increased by the addition of hydrolysates, demonstrating that LEA hydrolysate from water-boom cyanobacteria could be used as a potential supplement for stimulating the growth of oleaginous microalgae. Jain *et al.* [56] have also shown that algal bloom can be used as a potential nutrient source for microalgae. They prepared hydrolysate of toxic algal bloom using combinations of acid/alkali and autoclave/microwave and used it to cultivate *Chlorella pyrenoidosa*. Acid autoclave treatment resulted in the best result showing increased contents of carbon, nitrogen and phosphorous, which substantially boosted the growth of microalgal cells.

3.4. Use in fermentation with other microorganisms

LEA can also be used to support the cultivation of non-algae microorganisms like yeast and bacteria, to produce some valuable products other than biofuels or to substitute expensive substrates needed for fermenting microorganisms. Especially, the use of LEA as a nutrient and carbon source has advantages in ethanol fermentation.

Types of microalgal species to provide LEA and its hydrolysate are versatile. The LEA of *Dunaliella tertiolecta* has been used for bioethanol fermentation by *Saccharomyces cerevisia* after saccharification with a multi-enzyme complex containing arabanase, cellulase, β-glucanase, hemicellulose, and xylanase [57]. Mirsiaghi *et al.* [58] have used LEA of *Nannochloropsis salina* to produce bioethanol [41], in which cells are hydrolyzed using several enzyme mixtures as well as using hydrochloric and sulfuric acid at different process conditions, with a two-step hydrolysis process employing sulfuric acid and enzymatic hydrolysis producing the highest sugar yield. Kavitha *et al.* [59] have applied chemical pretreatment to the LEA of *Botryococcus braunii* for ethanol fermentation by *S. cerevisiae* and found that, among all acid and alkali pretreatment methods, ammonia fiber explosion (AFEX) treatment shows the maximum sugar recovery and ethanol yield.

Talukder *et al.* [34] have utilized the LEA of *Nannochloropsis salina* for the production of lactic acid using *Lactobacillus pentosus*. Gao *et al.* [60] have also investigated the utilization of LEA of *Pseudochoricystis ellipsoidea* for the production of lactic acid or ethanol. They used acid-hydrolyzed LEA as a nutrient source for *Lactobacillus lactis* and *Saccharomyces cerevisiae* in lactic acid and ethanol fermentation, respectively. Similarly, Seo *et al.* [61] have pretreated the *Nannochloropsis salina* LEA by acid catalyzed hot water treatment and utilized it as a substrate for the growth of oleaginous yeast *Cryptococcus* sp. to produce lipid.

The application of microalgae LEA as fermentation substrates is not limited to ethanol and lactic acid production. It is also used for butanol fermentation. Cheng *et al.* [62] have evaluated the potential of butanol fermentation by *Clostridium acetobutylicum* from LEA of *Chlorella sorokiniana*. Using LEA as a substrate, *C. acetobutylicum* produced

3.86 g/L of butanol and achieved butanol yield of 0.13 g/g carbohydrate via acetone-butanol-ethanol (ABE) fermentation. They pointed out that approximately one-third of carbohydrate was not utilized by *C. acetobutylicum.* Thus, further research on fermentation strategies is needed to improve the production yield in butanol fermentation, although it is possible to have biological butanol production from LEA or intact whole algae.

In fermentation, the use of LEA as a protein source seems to be more beneficial than as a carbon source. It has been reported that about one-third of LEA biomass of *Pseudochoricystis ellipsoidea* is composed of proteins that can be an excellent N source for fermentation microorganisms. The N-rich LEA hydrolysate may substitute yeast extract in the fermentation process. Gao *et al.* [60] have used *Chlorella* powder as a source of nutrients for yeast. It not only reduced the doubling time, but also increased lipid accumulation in the yeast, implying the potential that the use of pretreated LEA could substitute yeast extract in the fermentation process. Gu *et al.* [63] have investigated the reuse of LEA of *Scenedesmus acutus* as an N source and found that this alternative nitrogen resource could replace nitrate supply in microalgae cultivation. It is known that protein-rich LEA is not only an effective nitrogen resource, but also a heterotrophic carbon resource in subsequent culturing of microalgae and in versatile fermentation processes for improving overall biomass and product yield compared to a control medium with inorganic carbon and nitrogen only.

4. Integration with anaerobic digestion

One of options of utilizing LEA is to integrate or incorporate it in anaerobic digestion (AD). Anaerobic digestion a process that breaks down biodegradable organics by microorganisms in the absence of oxygen. It is widely employed to manage organic wastes or to produce fuels (such as methane and hydrogen) for municipal and industrial purposes. The digestion begins with bacterial hydrolysis, by which insoluble organic polymers such as carbohydrates, are broken down to simple sugars, amino acids, and fatty acids that become available for other communities of bacteria. These molecules are further degraded to acetate, hydrogen, ammonia, and carbon dioxide after a series action of acidogenesis and acetogenesis. Finally, methanogens can convert these products to methane and carbon dioxide.

LEA with a high C/N ratio is beneficial for the production of bioenergy such as biomethane and bioethanol, while a low C/N LEA may be advantageous as a fertilizer or a feed supplement for fermentation microorganisms or animals and fish [18]. For large-scale AD facilities, a continuous and steady supply of organic substrates and nutrients would be an economic burden. As the substrate for microalgae-employed AD process, microalgae alone or together with other organic wastes can be used. In either case, the microalgae biomass from LEA provides substrate for AD, and vice versa, the generated AD effluent can be recycled to produce microalgae cultivation stage because AD effluents usually contain high levels of nitrogen and alkalinity which are beneficial for algal growth [64]. This mutual benefit enables us to design a closed-loop AD process by combining microalgae cultivation for biodiesel production and utilizing process byproducts.

Yang *et al.* [65] have proposed a closed-loop process utilizing LEA obtained from biofuel production in anaerobic digestion and microalgal cultivation. LEA was disposed through AD to produce methane. It was found that 60-69% of nitrogen and phosphorus resided in the LEA were released into AD effluent which was then recycled for cultivating microalgae by supplementing it to the growth medium. They achieved higher biomass concentration than that obtained from the process with an artificial culture medium alone.

Sforza *et al.* [23] have performed AD with LEA from *Chlorella vulgaris* to evaluate biogas yield and nutrients recovery. The liquid fraction of AD digestate was used as source of nutrients for the cultivation of microalgae, to assess the possibility of designing a closed-loop nutrient recycling process. The specific growth rate and final biomass concentration increased to levels comparable to those obtained in a defined control medium, after adding soluble sulfate and phosphorous which were insufficient in the liquid digestate. The digested slurry of AD after biogas production was rich not only in nitrogen and phosphorus, but also in micronutrients. Thus, it could be utilized as an organic fertilizer for crop cultivation or horticulture. For example, about 30-60% of N, P, Mg, Ca, S and 15-25% of Mn and Fe present in the LEA were available for recycling after biogas production through AD with LEA of *Auxenochlorella protothecoides* [66].

Although AD is possible with LEA as an organic substrate for fermenting microorganisms in an AD process, using LEA alone as the substrate is not recommended for the purpose of waste volume reduction or biomethane production because the C/N ratio of LEA is relatively small compared to that of usual organic substrates which are fed to an AD process. The efficiency of digestion and biogas production is supposed to decrease when low C/N substrate like LEA is used for AD. Therefore, co-digestion is recommended by adding LEA to on-going AD reactors which are under operation with usual organic substrates such as food wastes, livestock manure or excessive sludge from municipal wastewater treatment plants.

Figure 2 shows the concept of LEA recycling through incorporation into anaerobic digestion with a partial closed-loop strategy. LEA is fed as a part of an organic substrate to an AD reactor where LEA and other organics are degraded to produce biogas containing CH_4 and CO_2. The produced biogas can be upgraded further by purifying CH_4 and the resulting CO_2-rich effluent gas stream can return to microalgae cultivation as a carbon source [14].

5. Microalgae as a fertilizer

The use of microalgae as a potential source of nutrients for plant growth in agriculture and horticulture has a long history of application. Especially as a nitrogen source for plants, microalgae cells, both unprocessed whole cells harvested from the culture and physically disintegrated cells can be utilized. They are desirable when cellular nitrogen content is sufficiently high, or low C/N ratio [67,68]. However, the number of reports on the use of LEA as a source of plant nutrients is still limited.

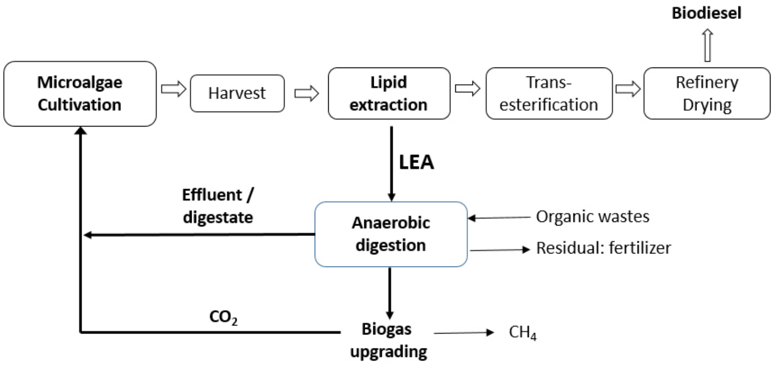

Figure 2. Incorporating LEA recycling into anaerobic digestion with a partial closed-loop strategy.

LEA is rich in nitrogen because proteins and nucleic materials are not removed during lipid extraction to produce biofuel. It is also rich in phosphorus and other mineral contents that are required for good performance as a fertilizer. Therefore, LEA that is rich in N, P, and other minerals can be a good nutritional source for terrestrial crops and aquaculture. It is also good for culturing microalgae. It is known that magnesium as part of photosynthetic apparatus, i.e., chlorophyll, of microalgae is an important source of plants with the same purpose.

There are some reports on the final use of AD effluents or digestate slurry as a possible substitute of chemical fertilizer. As mentioned in the previous section, effluent slurry from AD employing *Auxenochlorella protothecoides* LEA after biogas production is rich in nitrogen, phosphorus, and micronutrients. Thus, it could be utilized as an organic fertilizer for crop cultivation or horticulture [66]. Similarly, Juarez *et al.* [69] have discussed the potential use of residual effluent from AD with microalgae (co-digestion of microalgae mixture and pig manure).

Although LEA with a low C/N ratio is not recommended for use directly in an AD process for bio-methane production, LEA with a low C/N ratio can be utilized as plant fertilizer, animal feed, or nutrient source for various microorganisms [46,60,70]. LEA has been used to reclaim barren soil along with agricultural residues[71]. *Nannochloropsis salina* LEA can increase organic carbon content when it is applied to the soil [72]. LEA of *Chlorella. vulgaris* and *Lyngbya majuscula*, a filamentous cyanobacteria, has been utilized as a fertilizer substitute for *Zea may*s L [67].

In conclusion, LEA and its hydrolysate can be supplemented to the culture of microalgae and other various organisms as nutrients to promote growth, which can augment biomass and lipid yield as well as reduce the burden of waste disposal and cost of replenishing culture media. Also when long chain carbohydrates present in the LEA contain fermentable sugars that can be sufficiently hydrolyzed, they can be utilized to produce various value-added products and biofuels through bacterial fermentation and anaerobic digestion.

6. Integration with thermochemical processes

A thermochemical conversion is a decomposition process using hydrous pyrolysis to reduce complex organic materials such as ligno-cellulosic biomass, agricultural and animal wastes, plastics, tires, and municipal organic solid wastes or sewage sludge. Under pressure and heat, long chain polymers can decompose into low molecular weight hydrocarbons. Thermochemical conversion is classified into carbonization, torrefaction, hydrothermal liquefaction, pyrolysis, gasification, and so on depending on the involved conversion conditions such as temperature and process time, and the major final products (solid, liquid, or gas). The most popular method to obtain a liquid form of fuel is pyrolysis in which biomass decomposes thermally under oxygen-limited condition at around 450-550 ℃ and a liquid form of final product, so called bio-oil, is obtained [13,15].

Since LEA and intact microalgae cells are organic biomasses, it is possible to treat or dispose them with a new or on-going thermochemical conversion process. The nutrient recycle for microalgal cultivation is also possible by pyrolysis or hydrothermal liquefaction (HTL) applications of microalgae or LEA. Barbera *et al.* [73,74] have applied a flash hydrolysis (FH) process to intact *Scenedesmus obliquus* biomass. Flash hydrolysis is a sort of fast hydrothermal liquefaction under 280 ℃ and 70 bar for 9 sec. They have shown that FH is a viable way to fractionate a wet biomass into a solid fraction in which most lipids are retained and a liquid phase that is rich in N, P, and other micronutrients is obtained. Up to 70% and 60% of phosphorus and nitrogen can be recovered in bioavailable forms. They are suitable for recycling to microalgae cultivation. However, the achievable biodegradability is not high in HT than in AD. In addition, energy profitability is sacrificed due to employed thermochemical conditions.

On the other hand, although the primary target of pyrolysis of microalgal biomass or LEA is to produce bio-oil, some insolubilized fractions of biomass constituents are collected as bio-char, a solid state end product [10]. It has been reported that a bio-char is derived from pyrolysis of microalgal biomass or its LEA and can be used as a chemical fertilizer substitute. Johnson *et al.* [75] have developed a one-step conversion of algal biomass to biodiesel using tetramethyl ammonium hydroxide (25% in methanol) at 250-550 ℃ with the formation of algal char as a potential fertilizer.

Wang *et al.* [76] have pyrolyzed LEA of *Chlorella vulgaris* in a fluidized-bed reactor and obtained 31% of bio-char as well as 53% of bio-oil, showing that 94% of the energy content of algal biomass is recovered. They suggested that the high inorganic content (P, N and K) of the bio-char could provide nutrients for crop production as a fertilizer. Chang *et al.* [77,78] have prepared a bio-char from LEA of *Chlorella* sp. through a slow pyrolysis (10 ℃/min) that could be used as a porous fertilizer with high-N (>10%) and minerals. Francavilla *et al.* [79] have performed a fast pyrolysis for a solid residue of *Dunaliella tertiolecta* after extraction of valuables. At pyrolysis temperature of 600°C, the bio-oil yield was maximized (45.13 wt.%), while the bio-char yield reached 29.34 wt.%. Along with the potential use of bio-oil as fuel, the utilization of the bio-char as fertilizer or sorbent for soil remediation was also discussed.

Figure 3 shows the scheme of LEA recycling by incorporating it into thermochemical conversion processes. As an effort to design a closed-loop strategy and mitigate CO_2 emission, the CO_2-containg exhaust gas from pyrolysis or hydrothermal liquefaction can be supplied to microalga cultivation as a carbon source [80]. This scheme can be applied for not only recycling of LEA, but also for the disposal of excessive algae sludge which might be generated from an algal pond or recovering algal biomass from water-bloomed natural water resources.

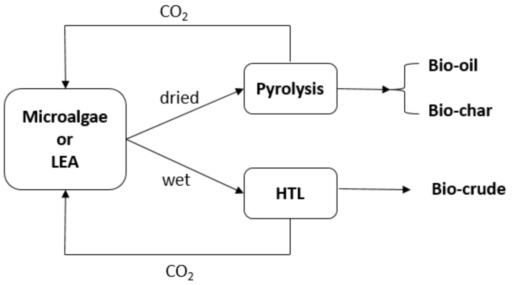

Figure 3. Incorporating LEA recycling into thermochemical conversion with a partial closed-loop strategy. HTL, hydrothermal liquefaction.

7. Perspectives and conclusions

Since the production cost of biodiesel from microalgae is currently not comparable to that of petro-diesel, to improve the economic sustainability of microalgal biodiesel, it is necessary to utilize LEA, a by-product generated from the lipid-extraction step, for valuable purposes. Recycling LEA is also an inevitable option to reduce the amount of final disposal of organic wastes and to mitigate CO_2 emission to the atmosphere.

Because LEA contains carbohydrates, proteins, nucleic acids, and various minerals, it is useful to recycle as carbon and nutrients for growth of microalgae, various microorganisms, and other higher organisms. It is also useful as a substrate of fermentation, anaerobic digestion or thermochemical conversion to produce other kinds of bio-energy or value-added products. According to literature review here, LEA has been successfully utilized as nutrients source for fermentation microorganisms, higher organisms including plants, livestock, and fish, as well as for cultivating microalgae.

Economic and environmental sustainability can also be improved through CO_2 recycling in which CO_2 produced during fermentation, anaerobic digestion and thermochemical conversion is returned to the cultivation of microalgae. Especially the recycling of LEA to anaerobic digestion and thermochemical conversion seems to have good prospects because LEA can be fed to reactors under operation employing other organic substrates already unless the fraction of LEA addition is too high compared to original organic substrates of biomass to disturb on-going AD or pyrolysis processes. However, recycling to a fermentation process to produce bioethanol and other bioproducts still requires more research and developments to be successful since LEA needs to undergo chemical or enzymatic hydrolysis steps to use it. In addition, usual fermentation bacteria need a carefully selected carbon source because they might be sensitive to LEA-originated intermediates and possible inhibitors.

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