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Biological purification processes for biogas using algae cultures: A review

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Abstract: Bioenergy is a type of renewable energy made from biological sources including algae, trees, or waste from agriculture, wood processing, food materials, and municipalities. Currently, the uses of renewable fuels (bioethanol, biodiesel, biogas and hydrogen) are increased in the transport sector worldwide. From an environmental and resource-efficiency perspective biogas has several advantages in comparison to other biofuels. The main components of biogas are methane (CH₄) and carbon dioxide (CO₂), but usually biogas also contains hydrogen sulphide (H₂S) and other sulphur compounds, water, other trace gas compounds and other impurities. Purification and upgrading of the gas is necessary because purified biogas provides reductions in green house gas emissions as well as several other environmental benefits when used as a vehicle fuel. Reducing CO₂ and H₂S content will significantly improve the quality of biogas. Various technologies have been developed and available for biogas impurity removal; these include absorption by chemical solvents, physical absorption, cryogenic separation, membrane separation and biological processes are environmentally friendly and feasible. Furthermore, algae are abundant and omnipresent. Biogas purification using algae involved the use of algae's photosynthetic ability in the removal of the impurities present in biogas. This review is aimed at presenting the algal characteristics, scientific approach, gather and clearly explain the main methods used to clean and purify biogas, increasing the calorific value of biogas and making this gas with characteristics closest as possible to natural gas through algae biological purification processes.

Keywords: Algae, Biogas, Biological Purification, Renewable Energy

1. Introduction

Bioenergy should play an essential part in reaching targets to replace petroleum-based transportation fuels with a viable alternative, and in reducing long-term CO_2 emissions, if environmental and economic sustainability are considered carefully. The world continues to increase its energy use, brought about by an expanding population and a desire for a greater standard of living. This energy use coupled with the realization of the impact of CO_2 on the climate, has led us to reanalyze the potential of plant-based biofuels [1]. The term biofuel is referred to as liquid or gaseous fuels for the transport sector that are predominantly produced from biomass. A variety of fuels can be produced from biomass resources including liquid fuels, such as ethanol, methanol, biodiesel, Fischer-Tropsch diesel, and gaseous fuels, such as biohydrogen and biogas.

The process of biogas production from algal biomass is an

alternative technology that has larger potential energy output compared to green diesel, biodiesel, bioethanol, and hydrogen production processes. Moreover, anaerobic digestion can be integrated into other conversion processes. The organic fraction of almost any form of biomass (from plants, algae and other microorganisms), including sewage sludge, animal wastes and industrial effluents, can be broken down through anaerobic digestion (AD) into CH₄ and CO₂ mixture called as "biogas". The first methane digester plant was built at Bombay, India in 1859 [2, 3]. AD approaches steadily growing role in the renewable energy mix in many countries. AD is the process by which organic materials are biologically treated in the absence of oxygen by naturally occurring bacteria to produce 'biogas' which is a mixture of CH₄ (40-70%) and CO₂ (30-60%) with traces of other gases such as hydrogen, hydrogen sulphide and ammonia [4]; the biogas process also produces potentially useful by-products in the form of a liquid or solid 'digestate' [5].

Normally, biogas is comprised of CH₄, CO₂, and other trace gas compounds gases such as water vapour, H₂S, halogenated hydrocarbons, siloxanes, ammonia, nitrogen, and oxygen [4]. Biogas is a valuable fuel which is produced in digesters filled with the feedstock like dung or sewage. All types of biomass can be used as substrates for biogas production as long as they contain carbohydrates, proteins, fats, cellulose, and hemicelluloses as main components. The composition of biogas and the methane yield depends on the feedstock type, the digestion system, and the retention time. In general, the use of plant biomass for energy generation today is problematic because of the competition with food or feed production. This is because most of the plants used for energy generation today (crop plants, sugar cane, sugar beets, canola, etc.) have to be grown on arable land. Low demand alternatives like switchgrass are only beginning to emerge. Algae have got a number of potential advantages compared to higher plants because of faster growth rates and the possibility of cultivation on non-arable land areas or in lakes or the ocean, therefore attenuating food and feed competition [6,7]. Of the potential sources of biogas the most efficient producers of biomass are the photosynthetic algae (micro and macroalgae).

Photosynthetic pigments, including chlorophyll, have an important role since it provides the oxygen and the source of energy for all living things. Plant and algae growth is affected by the photosynthesis speed which depends on the availability of CO_2 . Biological CO_2 fixation by algae is another such form; i.e. sunlight being used to reduce CO_2 to carbon. Capturing CO_2 from flue gases is the precautionary principle which needs preventive action, at both national and international levels to minimize this potential action [8]. A promising approach therefore seems to be the use of fast-growing algae species for anaerobic fermentation to produce biogas, which then can substitute natural gas resources.

To utilize biogas as a transport fuel, CO₂ and H₂S must be removed from the concentration to leave biomethane. Biogas purification is the process where any impurities are removed such as sulphides and ammonia. Biogas upgrading on the other hand is the process which removes CO₂ and the end product is bio-methane. The bio-methane which has been upgraded is suitable for injection into the national gas grid or vehicle fuel [4]. Biogas needs cleaning for two main reasons; the first is to improve the calorific value of the product gas and the second is to reduce the chance of damaging downstream equipment which is due to the formation of harmful compounds [9]. Thus, biogas has a wide availability and renewable nature due to the organic materials and microorganisms required for biogas synthesis. Biogas purification methods can be divided into two generic categories:

1 Those involving physicochemical phenomena (reactive or non-reactive absorption; reactive or non-reactive adsorption).

Those involving biological processes (contaminant consumption by living organisms and conversion to less harmful forms). Biological processes are widely employed for CO_2 and H_2S removal, especially in biogas applications.

For CO₂ capture from biogas, physical and chemical absorption methods are generally applied with fewer complications; however, these methods are needed to post treat the waste materials for regeneration of cycling utilization. The biological methods of CO₂ capture from biogas are potentially useful [10]. Biological processes are widely employed for H₂S removal, especially in biogas applications [11]. Furthermore, biogas is an environment friendly, clean, cheap and versatile fuel. Consequently, the purpose of the current paper is to present an integrated review of the biogas production methodologies and purification process, algal characteristics, approaches and clearly explain the main methods used to clean and purify biogas, increasing the calorific value of biogas and making this gas with characteristics closest as possible to natural gas through algae biological purification processes

2. Growth Characteristics of Algae and Importance

Algae are the most important primary producer in aquatic ecosystem [12]. Many species of algae are present such as; green, red and brown algae which belong to the group of Chlorophyta, Rhodophyta and Phaeophyta, respectively. Algal growth is found in a wide range of habitats, like fresh water, marine water, in deep oceans, in rocky shores, the plank-tonic and benthic algae can become important constituents of soil flora and can exist even in such extreme conditions as in snow, sands/desert or in hot springs, open and closed ponds, photo bioreactors, sewage and wastewater, desert as well as CO₂ emitting industries etc [13]. Generally they are found in damp places or water bodies and are common in terrestrial as well as aquatic environments. Algae, a broad category encompassing eukaryotic microalgae, cyanobacteria and macroalgae, can be cultivated to produce biomass for a wide range of applications [14].

Algae are a very diverse group of predominantly aquatic organisms of tremendous photosynthetic ecological importance, because they were the beginning of the food chain for other animals. Algae played an important role in self-purification of contaminated natural waters and offered an alternative for advance nutrition removal in water or wastewater [15, 16]. The idea to incorporate microalgae as an agent of bioremediation was firstly proposed by Oswald and Gotaas in 1957 [17]; the biomass recovered was converted to methane, which was a major source of energy [18]. Hence, algae provided the basis of the aquatic food chain and they were fundamental to keep CO₂ of carbon cycle via photosynthesis as a substantial role in biogeochemical cycles [12]. Most algae were photoautotrophic, converting solar energy into chemical forms through photosynthesis.

The mechanisms of algal photosynthesis were very similar to photosynthesis in higher plants and their products are molecularly equivalent to conventional agricultural crops [19]. The main advantages of culturing algae as a source of biomass were as follows: (1) high photosynthetic yields (up to a maximum of 5-6% conversion of light c.f. 1-2% for the majority of terrestrial plants); (2) the ability to grow in fresh, salt and wastewater; (3) high oil content; (4) the ability to produce non-toxic and biodegradable biofuels; (5) many species of algae can be induced to produce particularly high concentrations of chosen compounds–proteins, carbohydrates, lipids and pigments - that are of commercial value; (6) the ability to be used in conjunction with wastewater treatment [13,17–20]. Since algae was a key primary producer global-wide, algae biomass was essential biological natural resources which played an important role in nutrient, food, fertilizer, pharmaceutics and biofuel.

In addition, algae application is widely accepted in practice as one of the best strategies in bioengineering. There are several reasons for this approach: (1) the best growth rate among the plants, (2) low impacts on world's food supply, (3) specificity for CO₂ sequestration without gas separation to save over 70% of total cost, (4) excellent treatment for combustion gas exhausted with NOx and SOx, (5) high value of algae biomass including of feed, food, nutrition, pharmaceutical chemicals, fertilizer, aquaculture, biofuel, etc [13, 20]. Algae an important application for the cultivation of algae is the production of biomass for energy purposes. Due to the energy crisis, renewable energy becomes a popular issue in this world today and there are several alternatives such as bioenergy, solar, wind, tide, geothermal, etc. For bioenergy, algae are the third generation biofuel [20]. For the reasons of the best energy conversion efficiency of sunlight [15] and the highest growth rate [18], algae have the best potential among all the energy crops. Because of the fast growth, many high valuable products are generated, e.g. food, biofuel, etc [Figure 1].

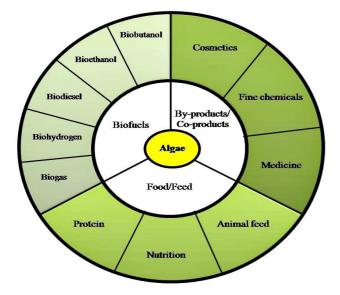


Figure 1. Potential products from algae

Algae produce biomass, which can be converted into energy or an energy carrier through a number of energy conversion processes. They include thermochemical conversion (gasification, direct combustion and pyrolysis), biochemical conversion (anaerobic fermentation, anaerobic digestion and photobiological hydrogen production) and esterification of fatty acids to produce biodiesel [13,18,20]. A lot of studies was indicated the importance of algae in carbon dioxide fixation [12–16,18,20]. Driver et al. [21] stated that algae are an attractive feedstock for the production of liquid and gaseous biofuels that do not need to directly compete with food production. Figure 2 illustrated the detailed information, process including algal stain selection, water type, cultivation methods, growth mode and harvesting methods. Furthermore, the various scenarios for biofuel development from algae are represented. Many options are available with regard to algae type and strain choice, including both eukaryotic algae and prokaryotic cyanobacteria, the source of water for cultivation, cultivation method and mode of growth, the method of algae harvesting and the biofuel conversion process. The understanding of biological phenomena, algal genetics, carbon storage metabolism, photosynthesis and algal physiology, have the potential for significant advances in algal biofuel feasibility [21]. This is being driven by advances in genomic technologies to provide the potential for genetic and metabolic engineering, plus the development of high-throughput techniques for the screening of natural strains for suitable biofuel characteristics.

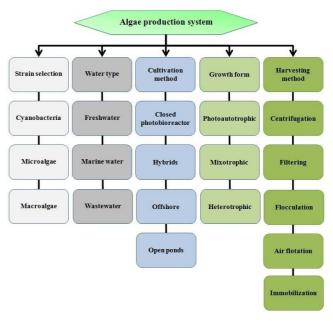


Figure 2. Algae production system

3. Algae Biogas Production Process and Technology

Anaerobic digestion (AD) is a common process for the treatment of a variety of organic materials and biogas production. Macroalgal and microalgal biomass can be AD to produce methane. Recently, microalgae have also become a topic of interest in the production of biogas through anaerobic fermentation [22]. The AD of algae is a prospective environmentally feasible option for creating a renewable source of energy for industrial and domestic needs. Algal AD

23

on is a key unit process that integrates efficiency and beneficially into the production of algal derived biofuels. Both, macro- and microalgae are suitable renewable substrates for the anaerobic digestion process. The process of biogas production from algal biomass is an alternative technology that has larger potential energy output compared to green diesel, biodiesel, bioethanol, and hydrogen production processes [4]. Moreover, anaerobic digestion can be integrated into other conversion processes and, as a result, improve their sustainability and energy balance. Opposite to biohydrogen, bioethanol or biodiesel that only uses determined macromolecules (carbohydrates and lipids), biogas is produced by biological means under anaerobic conditions that converts all algae macromolecules into methane [5, 8].

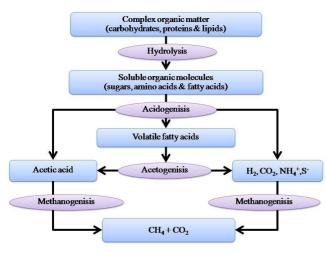


Figure 3. Stages of Anaerobic Digestion (methane fermentation process)

AD is an application of biological methanogenesis which is an anaerobic process responsible for degradation of much of the carbonaceous matter in natural environments where organic accumulation results in depletion of oxygen for aerobic metabolism. Since AD is a process by which almost any organic waste can be biologically converted in the absence of oxygen. This process, which is carried out by a consortium of several different microorganisms, is found in numerous environments, including sediments, flooded soils, animal intestines, and landfills. Accordingly, this is a complex process, which requires specific environmental conditions and different bacterial populations. Mixed bacterial populations degrade organic compounds, thus producing, as end-product; a valuable high energy mixture of gases (mainly CH₄ and CO₂) termed biogas [9]. Methane fermentation is a complex process, which can be divided up into four phases: hydrolysis, acidogenesis, acetogenesis/dehydrogenation, and methanation (Figure 3). These four stages are involved in the breakdown of organic matter on the path to methane production; stages fermentation (or include hydrolysis, acidogenesis), acetogenesis and eventual methanogensis (1). Hydrolysis involves the conversion of complex molecules and compounds-carbohydrates, lipids and proteins - found in organic matter into simple sugars, long chain fatty acids and amino acids, respectively. Acidogenesis in turn converts these

into volatile fatty acids, acetic acid, CO_2 and H_2 . Acetogenesis converts the volatile fatty acids into more acetic acid, carbon dioxide and hydrogen gas. Methanogens have the ability to produce methane by using the carbon dioxide and hydrogen gas or the acetic acid produced from both the acetogenic or acidogenic phases [10,11].

3.1. Anaerobic Digestion of Macroalgae Biomass

Macroalgae is one such source of aquatic biomass and potentially represents a significant source of renewable energy. The average photosynthetic efficiency of aquatic biomass is 6-8%, which is much higher than that of terrestrial biomass (1.8-2.2%). Macroalgae are fast growing marine and freshwater plants that can grow to considerable size (up to 60 m in length). Annual primary production rates (grams $C m^{-2} yr^{-1}$) are higher for the major marine macroalgae than for most terrestrial biomass [23]. Macroalgae can be subdivided into the blue algae (Cyanophyta), green algae (Chlorophyta), brown algae (Phaeophyta) and the red algae (Rhodophyta). Either Freshwater macroalgae or marine macroalgae (kelp or seaweed) could be used for solar energy conversion and biofuel production [23]. Macroalgae received a large amount of attention as a biofuel feedstock due to its prolific growth in natural habitat of freshwater system, eutrophic coastal water fouling beaches and coastal waterways.

Macroalgae can be converted to biogas by process of AD to biogas (~ 60% CH₄) [24]. Research conducted in the 1980's on macroalgae (giant brown kelp (Macrocystis)) [25] still provides a bench mark for biogas yields for a number of macroalgal species, but since this time there have been developments in AD technology and an enormous increase in its use. In comparison to terrestrial biomass crops, macroalgae contain little cellulose and no lignin and therefore undergo a more complete hydrolysis. AD has been used to dispose and process this material for the production of biogas; the AD of macroalgae biomass could meet two currently important needs, the mitigation of the eutrophication effects and the production of renewable energy. Because of the abundance of seaweed/ freshwater macroalgae biomass its conversion can be highly desirable and convenient, mostly for countries with long coastlines or eutrophic environments [26].

Investigations on the use of macroalgae of the brown algae division in processes of methane fermentation were conducted by Vergara-Fernàndez [27]. He was examining the possibility of applying to this end the biomass of Macrocystis pyrifera and Durvillea antarctica macroalgae and a substrate based on the mixture of these species. His study proved that for all substrates tested the yield of biogas production was comparable and reached 180.4±1.5 dm³/kg d.m.d. Singh and Gu [28] and Parmar et al. [29] were also analyzing the yield of biogas production with the use of microphytobenthos plants as an organic substrate. They achieved the highest technological effects during fermentation of Laminaria digitata brown algae belonging to the order Laminariales. In that case, methane production was high and reached 500 dm³ CH₄/kg o.d.m. The Macrocystis use of sp. enabled achieving $390-410 \text{ dm}^3 \text{ CH}_4/\text{kg o.d.m.}$, whereas upon the use of *Gracilaria sp.* and *Laminaria sp.* methane production accounted for $280-400 \text{ dm}^3 \text{ CH}_4/\text{kg o.d.m.}$ and $260-280 \text{ dm}^3 \text{ CH}_4/\text{kg o.d.m.}$, respectively [30].

The feasibility of biogas production from macroalgae collected from the Orbetello lagoon. Maroalgae biomass collected from the same lagoon was used for biogas production in batch reactors. He demonstrated that it is possible to produce CH₄ directly from macroalgae, preserving the spontaneous epiphytic microorganisms, as microbial starter of the digestion process. Moreover, it is possible to foster CH₄ yield by using anoxic sediments collected from the same lagoon as a further microbial inoculum. In fact, the addition of sediment improved the degradation activity, accelerating the removal of volatile fatty acids (VFA) from the medium and their conversion into methane, reducing the digestion time and increasing CH₄ yield [31]. The promising results obtained despite the harsh conditions (high salts, sulphur and heavy metals concentration) have been favoured, in our opinion, thanks to a pre-existing adaptation and mutual interactions within the native microorganisms. The bacterial pool was highly adapted both to biotic and abiotic factors, that is to macroalgal tissue composition and to the salts and toxic components present in water and sediments. Furthermore, this approach solely based on the exploitation of the intrinsic degradation potential of the reference ecosystem, proved to be suitable for a selective and non-intensive anaerobic digestion of macroalgae. In the review by Debowski et al. [30] presented the effectiveness of biogas production with the use of macroalgae as a substrate in methane fermentation processes (Table 1). Huesemann et al. [32] stated that AD of macroalgae was technically feasible at scale and it has been suggested that it could be a cost-competitive with anaerobic digestion of terrestrial biomass and municipal solid waste.

Table 1. Effectiveness of biogas production with the use of macroalgae as a substrate in methane fermentation processes.

Macroalgae taxon	Quantity of biogas/methane		
Durvillea antarctica	179.3±80.2 dm ³ CH ₄ /kg d.m. d		
Gracilaria sp.	280–400 dm ³ /kg o.d.m.		
Laminaria sp.	260–280 dm ³ /kg o.d.m.		
Laminaria digitata	500 dm ³ /kg o.d.m.		
Macrocystis	390–410 dm ³ /kg o.d.m.		
Macrocystis sp.	189.9 dm ³ CH ₄ /kg o.d.m.		
Macrocystis pyrifera	181.4±52.3 dm ³ CH ₄ /kg d.m. d		
M. pyrifera+Durvillea antarctica	164.2±54.9 dm ³ CH ₄ /kg d.m. d		
D : $I_{m} = II_{m} + \Gamma_{m} $	40.0–54.0 dm ³ /kg		
Pilayella+Ectocarpus+Enteromarpha	29.2–39.4 dm ³ CH ₄ /kg		
Ulva sp.	200 dm ³ /kg o.d.m.		
Ulva lactuca	157–271 dm ³ CH ₄ /kg o.d.m.		

3.2. Anaerobic Digestion of Microalgae Biomass

Microalgae are highly productive and are able to produce large quantities of biomass more efficiently [13,14,16]. Generally, the composition of microalgae is $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ [13], and microalgae have been found to have several constituents, mainly including lipids (7–23%), carbohydrates (5–23%), and proteins (6–52%). The chemical compositions of microalgae are mainly dependent on the species and culture conditions. Microalgae AD is a key unit process that integrates efficiency and beneficially into the production of microalgae derived biofuels. The first authors to report on the anaerobic digestion of microalgae biomass were Golueke et al. [33]. They investigated the anaerobic digestion of *Chlorella vulgaris* and *Scenedesmus*, microalgae species grown as part of a wastewater treatment process.

The technical feasibility data on the anaerobic digestion of algal biomass have been reported for many species of algae. Among the microscopic algae, the following cultures have been successfully used for the production of methane: the mixed culture of Scenedesmus sp. and Chlorella sp., the mixed culture of Scenedesmus sp., Chlorella sp., Euglena sp., Oscillatoria sp., and Synechocystir sp., the culture of Scenedesmus sp. alone, and together with either Spirulina sp., Euglena sp., Micractinium sp., Melosira sp., or Oscillatoria SP. The production of biogas through AD offers significant advantages over other forms of bioenergy production. Since AD consists of organic carbon degradation into organic acids and biogas. Biogas mainly consists of methane (around 65%), which is carbon most reduced state, and carbon dioxide (around 35%), which is its most oxidized state. Other gases (normally less than 1%), such as nitrogen, nitrogen oxides, hydrogen, ammonia and hydrogen sulphide are also formed [34, 35].

Table 2. Effectiveness of biogas production with the use of microalgae as a substrate in methane fermentation processes.

Macroalgae taxon	Quantity of biogas/methane
Arthrospira platensis	481±13.8 dm ³ /kg o.d.m.
Chlamydomonas reinhardtii	587±8.8 dm³/kg o.d.m.
Chlorella kessleri	335±7.8 dm ³ /kg o.d.m.
Chlouella en lo ania	150 dm ³ CH ₄ /kg o.d.m.
Chlorella vulgaris	240 dm ³ CH ₄ /kg o.d.m.
Dunaliella salina	505±24.8 dm ³ /kg o.d.m.
Euglena gracilis	485±3.0 dm ³ /kg o.d.m.
Phaeodactylum tricornutum	350±3.0 dm ³ CH ₄ /kg o.d.m.
Scenedesmus obliquus	210±3.0 dm ³ CH ₄ /kg o.d.m.
S. obliquus	287±10.1 dm ³ /kg o.d.m.
Scenedesmus sp.+Chlorella sp.	986 dm ³ /kg o.d.m.
	$180\pm 8 \text{ dm}^3/\text{dm}^3 \text{ d}$
Scenedesmus sp+Chlorella sp.	573±28 cm ³ /dm ³ d
	818±96 cm ³ /dm ³ d
Spirulina maxima	240 dm ³ CH ₄ /kg o.d.m.
Spirulina platensis	280±0.8 dm ³ CH ₄ /kg o.d.m.

Sialve et al. [35] stated that an organic matter composition can be converted stoichiometrically into methane for calculating the theoretical methane yield. Thus, lipids (1.014 L/g VS), followed by proteins (0.851 L/g VS) and carbohydrates (0.415 L/g VS) have the highest theoretical methane yield. Indeed, inducing a particular macromolecule accumulation in microalgae cells has proven to successfully increase the methane yield. Research conducted with carbohydrate-enriched cyanobacteria *Arthrospira platensis* by phosphorus limitation attained a methane yield of 0.203 L/g COD when biomass had 60% of carbohydrates in respect to 0.123 L/g COD when the carbohydrate content was 20% [36]. In the review by Dębowski et al. [30] presented the effectiveness of biogas production with the use of macroalgae as a substrate in methane fermentation processes (Table 2).

The biogas yield of plants is generally limited by the greater or lesser proportion of lignocellulose, which is difficult to recycle. Efficiency of biogas production is related to the species-dependent, efficiency of cell degradation and presence or absence of molecules. However, the use of microalgae with a low lignocellulose content, for example Chlorella vulgaris, Phaeodactylum tricornutum and Spirulina platensis, permits an almost complete utilization of the organic substance. Golueke et al. [33] demonstrated the ability of microalgae to pass through an anaerobic digester intact and remain undigested. The authors noted that microalgal cells are known to be able to effectively resist bacterial attack and found intact microalgae cells in digestate leaving a digester after a 30-day hydraulic retention time. The composition of the biogas and the yield could be varied depending on the cell contents, the cell wall components and the stability of the cell wall. In particular the protein content of the cell plays a decisive role. Depending on the type of algae, the biogas yield was between 280 and 400 L/kg total volatile solids. Generally the variability is related to two main aspects: (i) the macromolecular composition, and (ii) the cell wall characteristics of each microalgae species. The difference in anaerobic biodegradability due to the macromolecular composition lies on the methane potential of different organic compounds in microalgae cells. Consequently, pretreatment techniques have been used to solubilize particulate biomass and improve the anaerobic digestion rate and extent.

4. Pretreatment Methods for Increased Biogas Production from Algae

Algae anaerobic biodegradability is limited by their complex cell wall structure. Thus, pretreatment techniques are being investigated to improve algal methane yield. Various pretreatment technologies have been developed in recent years. These pretreatment technologies aim to make AD faster, potentially increase biogas yield, and make use of new and/or locally available substrates, and prevent processing problems such as high electricity requirements for mixing or the formation of floatinglayers. Pretreatment methods can be divided into four categories: thermal, mechanical, chemical and biological processes (Figure 4).

Pretreatment methods have been studied in order to disintegrate microalgae cells, solubilise the organic content, and increase the anaerobic digestion rate and extent. Thermal pretreatments have been the most widely investigated already in continuous reactors and leading to net energy production [36, 37]. Mechanical pretreatments have mostly been investigated in batch assays using algae cultures [38]. Thermal pretreatments have been the most widely studied already in continuous reactors and leading to net energy production [39]. Mechanical pretreatments were less dependent on algae species, but required a higher energy input if compared with chemical, thermal and biological methods [38]. Chemical pretreatments have been proved successful, particularly when

combined with heat [39]. Enzymatic pretreatment seem to improve microalgae hydrolysis [40], which is promising due to its low energy input.

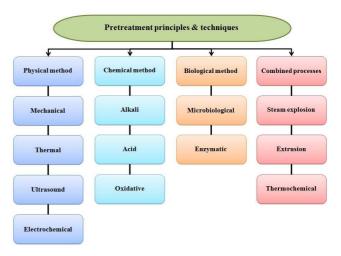


Figure 4. Pretreatments for improving algae biogas production

4.1. Pretreatment Methods for Increased Biogas Production from Macroalgae

Pretreatment of the algae is thus needed to aid both mechanical transport (pumping) as well as microbiological AD. Biogas can be derived via anaerobic fermentation of any organic matter, including the cellulose and hemicellulose within macroalgae, although the biomass must be subjected to pretreatment processes in order to liberate the sugars needed for fermentation. The effect of the pretreatment technologies, thermal treatment, thermochemical treatment, mechanical treatment, wet oxidation, hydrothermal pretreatment, steam explosion, plasma-assisted pretreatment and ball milling. One option is mechanical pretreatment of the algae; however a method which can handle the long fibrous material in macroalgae species is needed. Another method, which is relatively untested but promising, is enzymatic pretreatment which during recent years has been tested on many substrates to investigate effect on biogas potential [41].

The mechanical pretreatment effectively broke up the structure of all macroalgae into homogenous slurry. Mechanical pretreatment could increase the soluble COD-concentration of the tested algae by 1.5 to 3 times compared to raw algae. Enzymatic treatment increased it by 1.3 to 1.7 times. The best results were achieved by combining mechanical and enzymatic treatment where the concentration could was increased 3.5 times compared to raw algae [42]. A mechanical pretreatment phase is usually the first step not only for methane [43]. Nielsen and Heiske [44] was discussed the effect on methane yield of U. lactuca by various pretreatments including mechanical maceration and autoclavation. Sodium hydroxide soaking at room temperature prior to AD led to a 18% increase in methane potential in macroalgae as (Palmaria *palmata*), possess a high methane potential $(308 \pm 9 \text{ mL g}_{VS}^{-1})$ [45]. Nielsen and Heiske [44] studied four macroalgae species-harvested in Denmark-for their suitability of bioconversion to methane. In batch experiments (53 °C)

methane yields varied from 132 ml g volatile solids(-1) (VS) for *Gracillaria vermiculophylla*, 152 ml gVS(-1) for *Ulva lactuca*, 166 ml g VS(-1) for *Chaetomorpha linum* and 340 ml g VS(-1) for *Saccharina latissima* following 34 days of incubation. With an organic content of 21.1% (1.5-2.8 times higher than the other algae) *S. latissima* seems very suitable

for anaerobic digestion. However, the methane yields of U. lactuca, G. vermiculophylla and C. linum could be increased with 68%, 11% and 17%, respectively, by pretreatment with maceration. Nielsen and Heiske [44] data of methane potentials in different macroalgae with pretreatments were presented in Table 3.

Table 3. Methan	e potentials oj	f different mac	roalgae with pret	reatments.
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Macroalgae taxon	Pretreatment	Methane yield (ml g VS ⁻¹)	Methane production (ml g algae ⁻¹ ')				
Batch screening of methane potentials of different macroaigae ^a							
Chaetomorpha linum	Washed, chopped	166 ± 43.5	11.4 ± 2.98				
Chaetomorpha linum	Washed, macerated	195 ± 8.7	13.4 ± 1.46				
Saccharina latissima	Washed, chopped	340 ± 48.0	68.2 ± 9.63				
Saccharina latissima	Washed, macerated	333 ± 64.1	66.8 ± 12.87				
Gracillaria vermiculophylla	Washed, chopped	132 ± 60.0	17.3 ±4.88				
Gracillaria vermiculophylla	Washed, macerated	147± 56.3	19.3 ± 7.39				
Ulva lactuca	Washed, chopped	152 ± 18.7	9.9 ± 1.21				
Ulva lactuca	Washed, macerated	255 ±47.7	16.5 ± 3.08				
Pretreatments of U. lactuca ^b							
Ulva lactuca	Unwashed, chopped	174±23.3	12.8 ± 3.33				
Ulva lactuca	Unwashed, macerated	271 ± 16.2	17.6 ± 1.12				
Ulva lactuca	Washed, chopped	171 ±22.3	12.2 ± 1.06				
Ulva lactuca	Washed, macerated	200 ± 11.0	14.3 ± 1.53				
Ulva lactuca	Washed, 110 C/20 min	157 ± 13.4	11.3 ±0.96				
Ulva lactuca	Washed, 130 C/20 min	187 ± 23.2	13.4 ± 1.72				
Ulva lactuca	Dried, grounded	176 ±17.3	95.6 ± 9.42				

Note: ^a34 days of incubation; ^b42 days of incubation (source: Nielsen and Heiske, 2011)

4.2. Pretreatment Methods for Increased Biogas Production from Microalgae

The digestibility of microalgal biomass varies significantly even between closely related species [46]. CH₄ yields from microalgae vary due to variation in cellular protein, carbohydrate and lipid content, cell wall structure, and process parameters such as the bioreactor type and the digestion temperature. Regarding the cell wall characteristics, it is mostly composed of organic compounds with low biodegradability and/or bioavailability, such as cellulose and hemicellulose. This tough cell wall hinders the methane production, since organic matter retained in the cytoplasm is not easily accessible to anaerobic bacteria [47]. AD is carried out by heterogeneous microbial populations involving multiple biological and substrate interactions. Anaerobic biodegradation can be divided into four main phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis (before mentioned). AD (sometimes also called methanogenic fermentation) is widely applied in digestion of manure, sewage sludge and organic fraction of municipal solid wastes in industrial and agrarian societies. Anaerobic digestion of microalgal biomass has been studied from many freshwater and marine microalgae in various combinations. Rigid eukaryotic cell walls of microalgae can limit the anaerobic digestion of the biomass [33,47]. Pretreatment techniques were pointed out as a necessary step for microalgae cell disruption and biogas production by Chen and Oswald [47]. The effectiveness of pretreatment methods on biogas production depends on the characteristics of microalgae, i.e., the toughness and structure of the cell wall, and the macromolecular composition of cells. For instance,

Scenedesmus sp. has one of the most resistant cell walls, since it is composed by multilayers of cellulose and hemicellulose on the inside, and sporopollenin and politerpene on the outside [48].

Microalgae complex cell wall structure confers a resistance to biological attack. In fact, species without cell wall (e.g. Dunaliella sp. and Pavlova cf sp.) or containing a glycoprotein cell wall (e.g. Chlamydomonas sp., Euglena sp. and Tetraselmis sp.) showed higher methane yields than those with a more complex cell wall, containing recalcitrant compounds (e.g. Scenedesmus sp. and Chlorella sp.) [49]. Rates and yields of CH₄ formation from microalgal biomass often increase with digestion temperature. For example, [33] reported 5-10% increase in digestibility of microalgal biomass, when the digestion temperature was increased from 35 to 50 °C. Chen and Oswald [47] increased the CH₄ yield by 33% by heat pretreating microalgal biomass at 100 °C for 8 h. In both examples, however, the amount of energy consumed in the heating and pretreatment was higher than the corresponding energy gain from increased CH₄ production [50].

Retention times required to obtain high CH_4 yields from untreated microalgal biomass are relatively long, 20–30 days [51,52]. AD of microalgal biomass has been investigated in batch and fed-batch systems as well as in continuously stirred tank reactors [50]. Zamalloa et al. [52] suggested that anaerobic sludge blanket reactors, anaerobic filter reactors and anaerobic membrane bioreactors should be tested due to their high volumetric conversion rates. In the review by Passos et al. [30] presented the effectiveness of biogas production with the main pros and cons of microalgae pretreatment methods (Table 4). As can be seen, thermal pretreatment seems effective at increasing biogas production, while energy demand is low compared to mechanical ones. Nevertheless, biomass thickening or dewatering is crucial. Scalability may be a handicap for microwave pretreatment. Regarding thermo-chemical pretreatment, studies have shown positive results on microalgae biodegradability increase; however further studies should evaluate the risk of contamination in continuous bench and pilot-scale reactors.

Table 4. Comparison of pretreatment methods for increasing microalgae anaerobic biodegradability.

Pretreatment	Control parameters	Biomass solubilization	Methane yield increase	Pros	Cons
Thermal (<100 °C)	Temperature; exposure time	$\sqrt{\sqrt{2}}$	$\sqrt{\sqrt{1}}$	Low energy demand; Scalability	High exposure time
Hydrothermal (>100 °C)	Temperature; exposure time	$\sqrt{\sqrt{2}}$	$\sqrt{\sqrt{1}}$	Scalability	High heat demand; thickened or dewatered biomass; risk of formation of refractory compounds
Thermal with steam explosion (>100 °C)	Temperature; exposure time; pressure	$\sqrt{\sqrt{2}}$	$\sqrt{\sqrt{2}}$	Scalability	High electricity demand; scalability; biomass dewatering
Microwave	Power; exposure time	$\sqrt{}$	$\sqrt{}$	_	
Ultrasound	Power; exposure time	$\sqrt{}$	$\sqrt{}$	Scalability	High electricity demand; biomass dewatering
Chemical	Chemical dose; exposure time	\checkmark	\checkmark	Low energy demand	Chemical contamination; risk of formation of inhibitors; Cost
Thermo-chemical	Chemical dose; exposure time; temperature	$\sqrt{\sqrt{2}}$	$\sqrt{\sqrt{1}}$	Low energy demand	Chemical contamination; risk of formation of inhibitors; Cost
Enzymatic	Enzyme dose; exposure time; pH, temperature	\checkmark	\checkmark	Low energy demand	Cost, sterile conditions

5. Algae Biogas Impurity Removal and Upgrade Technology

Biogas produced in AD plants or landfill sites is primarily composed of CH_4 and CO_2 with smaller amounts of H_2S , NH_3 and N_2 . Trace amounts of H_2 , VOCs and O_2 may be also present in biogas and landfill gas. Usually, the gas is saturated with water vapor and may contain dust particles. Additionally, organic silicon compounds are usually present in particular with reference to landfill gas, however their presence was highlighted also in AD biogas. The heating value of biogas is determined mainly by the methane content of the gas [53].

The main impurities are CO₂ which lowers the calorific value of the gas and sulfuric acid (H₂S) which could cause several problem on the plants and for human health, in fact on the plants it causes corrosion (compressors, gas storage tank and engines), while it's toxic after its inhalation. Although CO_2 is a major problem in the biogas as its removal is useful to adjust the calorific value and the relative density, and the removal of H₂S can be of crucial point to the technological and economic feasibility of upgrading process of the gas [54]. Biogas production is growing and there is an increasing demand for upgraded biogas, to be used as vehicle fuel or injected to the natural gas grid. To enable the efficient use of biogas in these applications the gas must be upgraded. Since separation of CO₂ and N₂ from CH₄ is significantly important in natural gas upgrading, and capture/removal of CO₂, CH₄ from air (N_2) is essential to greenhouse gas emission control. Removal of CO₂ is done in order to reach the required Wobbe index of gas. As methane has a 23-fold stronger greenhouse gas effect than CO₂, it is important to keep methane losses low, for both economic and environmental reasons.

In general, in the standards requirements on Wobbe index

values and limits on the concentration of certain components such as sulfur, oxygen, dust and the water dew point, as well as a minimum methane volumetric concentration of 96% are defined. There are several different commercial methods for reducing the CO_2 content of biogas. Two common methods of removing carbon dioxide from biogas are absorption (water scrubbing, organic solvent scrubbing) and adsorption (pressure swing adsorption, PSA). Less frequently used are membrane separation, cryogenic separation and process internal upgrading, which are a relatively new method, currently under development. The upgraded biogas is often named biomethane. Various technologies can be applied for removal of contaminants.

When CO_2 and other impurities are removed during the upgrading process, the methane concentration increases and thus the resulting biomethane can be utilized as an alternative to natural gas. Starr et al. [55] articulated on the carbon capture technologies that upgrade biogas by removing its CO_2 content. There are quite a few different technologies on the market today. The main unit operations used are absorption, adsorption, membrane separation and cryogenic separation; further information about these unit operations and their associated technologies shown in Table 5. A common factor of all of these techniques is that the removed CO_2 is normally released back into the atmosphere. In some cases, if its quality is high enough, it can be used for industrial purposes such as increasing the CO_2 concentration for photosynthesis in greenhouses or for carbonation in food production.

Strevett et al. [56] investigated the mechanism and kinetics of chemo-autotrophic biogas upgrading. In this experiment, different methanogens using only CO_2 as a carbon source and H_2 as an energy source were examined. The selection between mesophilic and thermophilic operation temperatures is typically based on whether the completion of reaction or the rate of reaction is of primary concern. Thermophilic methanogens exhibit rapid methanogenesis, while mesophilic bacteria give more complete conversion of the available CO₂ [56]. They selected *Methanobacterium thermoautotrophicum*. The organism works optimally at temperatures of 65–70 °C and has a specific requirement for H₂S, so both unwanted components are removed. A synthetic biogas of 50–60% CH₄, 30–40% CO₂ and 1–2% H₂S was mixed with H₂ to a final mole fraction of H₂: CO₂ equaling 0.79:0.21. The gas mixture was fed to the hollow fibers packed with organisms. This biological system can effectively remove CO_2 and H_2S , while approximately doubling the original CH_4 mass. Alternative physicochemical treatment methods only remove the contaminating gas components, without changing CH_4 mass. Furthermore, physicochemical treatment generates additional waste and unwanted end products. The purified biogas contains about 96% CH_4 and 4% CO_2 , while H_2 and H_2S were not detected [56].

Unit operation	Technology	Acronym	Description of process
Absorption	High pressure water scrubbing	HPWS	Water absorbs CO ₂ under high pressure conditions. Regenerated by depressurizing
	Chemical scrubbing	AS	Amine solution absorbs CO ₂ . The amine solution is regenerated by heating
	Organic physical scrubbing	OPS	Polyethylene glycol absorbs CO2. It is regenerated by heating or depressurizing
Adsorption	Pressure swing adsorption	PSA	Highly pressurized gas is passed through a medium such as activated carbon. Once the pressure is reduced the CO_2 is released from the carbon, regenerating it
Membrane	Membrane separation	MS	Pressurized biogas is passed through a membrane which is selective for CO ₂
Cryogenic	Cryogenic separation	Cry	Biogas is cooled until the CO_2 changes to a liquid or solid phase while the methane remains a gas. This allows for easy separation

Table 5. Current biogas upgrading technologies (adopted from Starr et al. [55]).

Table 6. Con	parison table o	fCO_2	fixation i	n the up	take rate and	consumption efficiency.
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Culture	Species	CO ₂ source	biomass	Uptake rate (mg/L/day)	consumption efficiency (w/w)	Reference
Pure	<i>Chlorophyta</i> sp. <i>Chlorella</i> sp.	10% CO ₂	8.2 g/m ² /d 13.2 g/m ² /d	4 (10% CO ₂) 6 (10% CO ₂)	9%	Hase et al. [57]
	Chlorella sp. Spirulina platensis	1, 5, 10% CO ₂	2.25 g/L 2.91g/L	83 (10% CO ₂) 70 (10% CO ₂)	4% 2% a	Ramanan et al. [58]
Mixed	-	Diffusion from air	22 t as C	19 (50cm)	44%	Green et al. [59]
	-	Injected CO ₂ 11.5 kg/day as C	10.2 kg/day as C	187	148%	Weissman and Tillett [60]
	Dominant species ^a	Diffusion from air	0.126 g/L (TSS ^c)	162	123%	Tsai [20]
	Dominant species b	Diffusion from air	0.136 g/L (TSS ^c)	175	131%	Ramaraj [13]

Note: a the genera Chlorella, Oscillatoria, Oedogonium, Anabaena, Microspora and Lyngbya

^b the genera Anabaena, Chlorella, Oedogonium and Oscillatoria; ^ctotal suspended solids (as a biomass)

6. Biogas Purification Using Algae Biological Biogas Purification Methods and Techniques

Microalgae are a group of unicellular or simple multicellular photosynthetic microorganisms that can fix CO₂ efficiently from different sources [12–16], including the atmosphere, industrial exhaust gases, and soluble carbonate salts. Furthermore, combination of CO₂ fixation, biofuel production, and wastewater treatment may provide a very promising alternative to current CO₂ mitigation strategies. Presence of chlorophyll and other pigments help in carrying out photosynthesis. The true roots, stems or leaves are absent. Mostly they are photoautotrophic and carry on photosynthesis, some of these are chemo heterotrophic and obtain energy from chemical reactions as well as nutrients from preformed organic matter. Beside the plants, since algae had high potential CO₂ fixation in the current knowledge.

Microalgae can fix CO_2 using solar energy with efficiency ten times greater than terrestrial plants [13, 16]. The issue of greenhouse gas attracts an enormous attention worldwide recently. When atmospheric CO_2 concentration increased, it would gradually disturb the balance of global climate to cause unusual and astounding phenomena on earth. Therefore, we require the rapid development of bio-carbon-fixation technology to eliminate the adverse effects of CO_2 , to transfer atmospheric CO_2 through the carbon cycle and to promote carbon balancing ecologically. Currently, many innovative alternatives of physical, chemical and biological technologies of CO_2 mitigation are rapidly developed.

At present, algae application of CO_2 sequestration has developed as a popular topic and the current interests are including: species, power plant flue gas utilization, reactor design, growth condition, growth kinetics and modeling. The most studies in the literature concerned the maximum CO_2 uptake rate by the artificial photo-bioreactors [12, 13, 20]. Among those techs, bio-eco-technology is the most natural and ecological way to accomplish the designed targets by the utilization of "self-designed" bio-functions of nature [12, 13, 15, 16]. The different sources and approaches of algal CO_2 uptake rate and consumption efficiency was presented in Table 6. Accordingly, algae production has a great potential for CO_2 bio-fixation process and deserves a close look.

Biogas purification/scrubbing using algae involved the use of algae's photosynthetic ability in the removal of the impurities (mainly CO_2 and H_2S) present in biogas, leaving a purified biogas containing almost pure methane, which could be used for energy generation. Biological purification technology is worth examining because has double impact. The method about removing CO₂ from biogas by microalgal culturing using the biogas effluent as nutrient medium and effectively upgrade biogas also simultaneously reduce the biogas effluent nutrient [61]. Using biogas as a source of carbon dioxide has two main advantages: the biomass production costs are reduced and the produced biomass does not contain harmful compounds, which can occur in flue gases. Hendroko et al. [62] verified xhibit that microalgae (Scenedesmus sp.) in laboratory experiments using biogas slurry as growing medium and biogas are given periodically generate 21% of CO_2 compared with 24% of controls. They summarized: digestion slurry with seed cake JatroMas cultivar as raw material is able to increase growth of microalgae Scenedesmus sp. higher than standard media; microalgae Scenedesmus sp. is able to capture CO_2 gas in bio-methane; with integration of slurry and bio-methane intake, there is tendency Scenedesmus sp. growth is more increasing; Mutualism symbiosis among slurry, bio-methane and microalgae Scenedesmus sp. will give impact to increasing of CH₄ content in bio-methane. In other word, microalgae can be work as purification biologic from bio-methane [62].

There are several authors [10, 62, 63] reported that *Arthrospira* sp, *Chololera vulgaris* SAG 211-11b, *Chlorella* sp. MM-2, *Chlorella* sp. MB-9, *Chlorella vulgaris* ARC1, *Chlamydomonas* sp. dan *Scenedesmus* sp. was a positive synergy with biogas. The productivity of the system with Zarrouk media and biogas almost 5 times higher than that for the same media without biogas when piggery waste was used, the utilization of biogas brings a productivity gain of about 2–5 times higher [63].

Kao et al [64] demonstrates that the microalga *Chlorella* sp. MB-9 was a potential strain which was able to utilize CO₂ for growth when aerated with desulfurized biogas ($H_2S < 50$ ppm) produced from the anaerobic digestion of swine wastewater. The demonstrated system can be continuously used to upgrade biogas by utilizing a double set of photobioreactor systems and a gas cycle-switching operation. Furthermore, they demonstrated that the efficiency of CO₂ capture from biogas could be maintained at 50% on average, and the CH₄ concentration in the effluent load could be maintained at 80% on average, i.e., upgrading was accomplished by increasing the CH₄ concentration in the biogas produced from the anaerobic digestion of swine wastewater by 10%.

Some literatures mentioned about the cultivation microalgae using biogas as CO₂ provider. Kao et al. [64] used biogas that contained $20\pm2\%$ CO₂ for *Chlorella* sp. culture with variation of light intensity which was at cloudy and at sunny day. Kao et al. [10] used biogas that contained $20\pm1\%$ CO₂ for *Chlorella* sp. culture with variation flow rate of biogas which was 0.05; 0.1; 0.2; 0.3 vvm. Douškova et al. [65] investigated the potential of biogas as CO₂ provider for *Chlorella vulgaris*; and optimization of biogas production from distillery stillage is described. The growth kinetics of microalgae *Chlorella* sp. consuming biogas or mixture of air and CO₂ in the concentration range of 2–20% (v/v)

(simulating a flue gas from biogas incineration) in laboratory-scale photo-bioreactors. It was proven that the raw biogas (even without the removal of H_2S) could be used as a source of CO₂ for growth of microalgae. The growth rate of microalgae consuming biogas was the same as the growth rate of the culture grown on a mixture of air and food-grade CO₂. Several species of algae can metabolize H_2S [66]. Using a biological system to remove H_2S has similar benefits to using one to remove CO₂: lower upkeep costs, more environmentally sustainable and non-hazardous waste.

Furthermore, Tongprawhan et al. [67] used oleaginous microalgae to capture CO₂ from biogas for improving methane content and simultaneously producing lipid. They screened several microalgae for identify their ability to grow and produce lipid using CO2 in biogas. Finally, they reported a marine Chlorella sp. was the most suitable strain for capturing CO_2 and producing lipid using biogas (50% v/v CO_2 in methane) as well as using 50% v/v CO2 in air. Sumardiono et al. [68] established to evaluate the design of the photobioreactor system for purifying biogas through the culturing of microalgae. This system represented a simple promising way for the current forthcoming technologies of biogas purification. It helps to decrease the concentration of CO_2 in biogas concomitantly producing microalgae biomass. The microalgae Nannochloropsis is able to use CO₂ from biogas produced from the anaerobic digestion of tannery sludge. The results show that cultivation of microalgae under the biogas to scrub out CO_2 and promote enrichment of methane in the biogas in this work and obtained scrubbing of 27% from 30%.

The biocapture of CO_2 by microalgae can be applied to improve the quality of biogas by reducing the CO_2 content as this would lead to an increase in the methane content [69]. The microalgae Chlorella sp.was analysed in terms of conditioning biogas. As a result the biogas components CO_2 and H_2S could be reduced up to 97.07% and100%, respectively. Also an increase of microalgae cell count could be documented, which provides interesting alternatives for the production of algae ingredients. Consequently, the algae biological purification is an alternative to other biogas purification methods.

7. Conclusion

Biogas is a promising and valuable renewable energy source. Biogas can be utilized in several ways; either raw or upgraded. As a minimum, biogas has to be cooled, drained and dried immediately after production, and almost always it has to be cleaned for the content of CO₂, H₂S and other impurities. Using the photosynthesis of algae to remove the CO₂ from biogas is an alternative method that solves the problems of the common non-biological methods. Algae are self-sustaining with the addition of minimal nutrients and light. Algae were used as a biological method to remove CO₂ through Algae has several advantages photosynthesis. over conventional chemical CO2 removal methods because algae is inexpensive to obtain, requires only light and minimal nutrients in addition to the CO₂ for growth, and the waste can be harvested for biofuels. Several species of algae can metabolize H₂S. The H₂S content in biogas, at levels higher than 300–500 ppm, damages the energy conversion technique. Today biological cleaning reduces the content of hydrogen sulphide to a level below 100 ppm. Using a biological system to remove H₂S has similar benefits to using one to remove CO₂: lower upkeep costs, more environmentally sustainable and non-hazardous waste. Maintaining a pure culture would increase the efficiency of the algae in processing CO₂. Using biological metabolism to purify biogas is a promising means of biofuel production. The incorporation of algae in photobioreactors to purify biogas has several advantages over conventional chemical methods of CO₂ removal. Obtaining algae is relatively inexpensive because culturing algae requires minimal nutrients for their growth. Growth of the algae requires a light source as well, which does not necessarily have to be expensive if illumination is provided by natural sunlight, which is not limited in supply.

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