

Current status of microalgae to produce biogas through pretreatment of lignocellulosic waste in the era of 21-Century

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Abstract:

Modern day civilization is dependent on energy generation by fossil fuels. But the major drawback of using fossil fuels is environmental pollution. Microalgae are potential candidate for production of various products of interest, such as proteins, mini food, pigments and triglycerides that can be converted into biofuels. Lignocellulosic feedstocks are the most abundantly available raw material of plants that can serve as a promising feedstock for cultivating bacteria, fungi, yeast and microalgae to produce biofuels and other value-added products. Owing to the abundant availability of these low/no cost substrates, can be utilized as feedstocks for cultivating microalgae to generate biogas/biodiesel. Likewise, there is much room to exploit defatted algal biomass to be used as animal/fish feed and oil producing/accumulating genes knowledge in future to produce high and good quality biodiesel and biogas.

Keywords: *Lignocellulosic Substrate; Pre-Treatment; Microalgae/Cyanobacteria; Biogas; Problems; Animal Feed*

1. Introduction

Now-a-days energy resources are inadequate in numbers for desirable production of energy with less frugal resources [1]. Energy and environmental pollution are included in the top ten problems of the world. The desirable amount of oil and other energy products are reduced, and energy requirements reach at elevated point in this modern era. According to World Trend Plus's

Association: Energy Sector in Pakistan, oil consumption was reported at 498.197 barrel/day in 2018 and oil demand is about to increase upto 60%. Fossil fuels are non-renewable resources that are eradicated rapidly and can cause drastic effect on economic cost of any developing country [2, 3]. The depletion of fossil fuel is affecting certain environmental conditions such as abiotic factors like temperature, climate,

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greenhouse effect [4] and ultra-violet radiation [5, 6].

It is reported earlier that Chinese people used microalgae (*Nostoc* sp.) to overcome the famine condition about 2000 year ago [7]. Biomass mainly consisted of compost and manure which may be terrestrial and aquatic [8]. Biomass feedstock is a renewable source of energy for the output of biogas [9] and biofuel through anaerobic microalgal digestion [10] as show in Fig. 1.

Terrestrial biomass is a promising agent to produce renewable energy whereas, aquatic biomass is easily available to be produce energy at large scale without causing any environmental hazards and economically feasible too [8]. The most promising way to obtain maximum energy with less economic cost is decomposition of biomass by using microalgae [11, 12]. Algae is unicellular and simple multicellular organisms with photosynthetic pigments such as chlorophyll a, b, c and other accessory pigments. There are several globally dominating species of

microalgae being cultivated at large scale and produce products of commercial importance with low cost such as *Isochrysis*, *Chaetoceros*, *Arthrospira* and *Dunaliella* [13]. Microalgae effectively break the lignocellulosic wastes without production of toxic material. Advantages of microalgae for biomass treatment is shown in Table I.

Microalgae are cultivated autotrophically, heterotrophically and mixotrophically based on its mode of nutrition. Heterotrophic algae rely on glucose or other organic material as carbon source. Mixotrophically grown microalgae have ability to utilize both organic and inorganic carbon sources such as *Chlorella* sp. and accumulate biomolecules such as carbohydrates, lipids and proteins [19]. Microalgae are capable of producing biofuels like methane [7, 9, 20], biodiesel [3, 21] and bio hydrogen in a simple photobioreactor [22] and biogas as shown in Figure 02

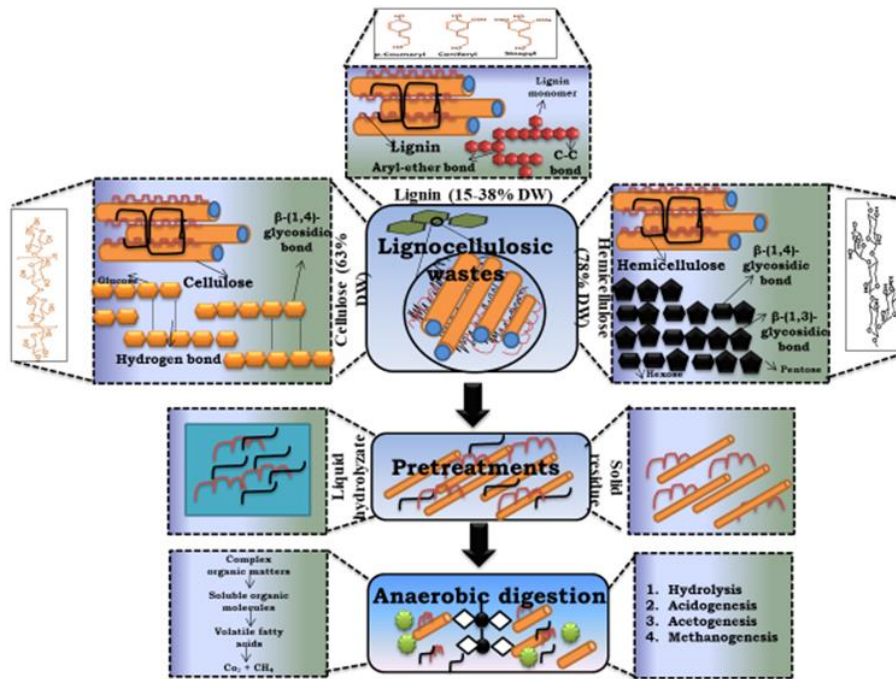


Fig 1: Overview microalgal bio-energy products through pre-treatment of Lignocellulosic wastes

Table I Advantages of using microalgae/cyanobacteria for biogas production

Sr No	Advantages of using microalgae/cyanobacteria for biogas production	References
01	Microalgae/cyanobacteria can grow at high levels of CO ₂ throughout year	[12, 14]
02	Microalgae/cyanobacteria can grow in marine/brackish, freshwater and wastewaters thus less competition for arable land	[15][40][16] [17, 18]]
03	Microalgal biomass is a rich source of other co-products such as gasoline and biohydrogen etc.	[17]
04	Microalgae/cyanobacteria are ubiquitous and serves as a promising candidate to generate high rates of productivity	[5, 16]
05	They play important role in oxygen production. nitrogen cycle and reformed the oxidizing environment from reducing environment.	
06	The requirements for nourishing the microalgae/cyanobacteria is very simple and prosperous agent to convert the solar energy into CO ₂ , also originator of various feedstock for energy producing compound such as lipids, carbohydrates and protein.	

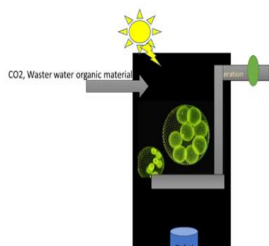


Fig 2: Basic Structure of Photobioreactor

2. Lignocellulosic waste

Food and plant-based waste known as lignocellulosic waste is produced up to 1.3 billion ton/year around the world [23]. Lignocellulose waste is an inexpensive and renewable energy source to produce high yield of biomass approximately 50% [24] and helps in carbon dioxide sequestration [25] globally throughout the year. Lignocellulose

is mainly composed of hemicellulose, lignin, cellulose, phenolics [26] and other inorganic materials [27], their rate of production totally depends on plant species [28]. Major lignocellulosic biomass resources are manure, woodlot arising, crop residues, agro-industrial wastes, sugarcane, bagasse, sawdust and consecrated energy crop Table II.

2.1. Cellulose:

Cellulose is most abundant polysaccharide in biosphere and chief ingredient of higher plants, brown, red and green algae. Cellulose is unbranched polymer, formed by monomer of glucose that are linked by β - 1,4- glycosidic bond. Its degree of polymerization is about to 14000 – 15000 glucose monomer each monomer rotates at 180o angle.

Table II Major lignocellulosic wastes and their composition

Sr NO:	Lignocellulose wastes	Cellulose (wt %)	Hemicellulose (wt %)	Lignin (wt %)	References
01	Corn stalks	35.0	16.8	7.0	[29]
02	Sugarcane bagasse	40.0	27.0	10.0	
03	Wheat straw	32.9	24.0	8.9	
04	Soft wood (spruce)	44.7	22.9	30.6	[30]
05	Hard wood (beech)	45.6	25.9	23.8	
06	Grass (switch grass)	32.2%	24.4 %	23.3%	

2.2. Hemicellulose

The second most important polymer in this biosphere is hemicellulose which has random and amorphous structure, consisted of heteropolymer such as xyloglucan, glucomannan, arabinoxylan, glucuronoxylan, galactomannan and xylans [31]. Due to this, its chemical composition deviated from species to species, however, xylans and glucomannan are present in hard wood or soft wood respectively [31]. Hemicellulose makes up 15 – 35% plant biomass that are joined with cellulose by hydrogen bond, to lignin through covalent bond

2.3. Lignin

Lignin are large, complex and crosslinked aromatic polymers that are formed by oxidative combinative conjugation of 4-hydroxy-phenyl-propanoids [32]. Lignin monomers are linked together through C-C and aryl ether linkage associating with varieties of functional group such as methoxy, phenolic hydroxy and adjacent aldehyde [33]. Divergence in composition of bond and functional group depend on the plant's species biomass. Lignin provide strength and protect from stress condition by association with cellulose and hemicellulose.

3. Pre-treatment of lignocellulosic waste:

Pre-treatment is a critical procedure for the biochemical renovation of lignocellulosic biomass into bioethanol, biogas, biohydrogen [34]. This process is essential to modify the cellulosic biomass for making large quantity of cellulose which is converted into the carbohydrate polymers (fermentable sugars) by the action of specific enzymes [35]. Pre-treatment of lignocellulosic wastes is completed through the acidic, basic and enzymatic courses [36]. Recent research reveals that enzymatic hydrolysis is best-identified technique to get high yield of biomass Fig, 3.

3.1. Acidic Hydrolysis

Acidic method is one of the efficient methods to treat the lignocellulosic wastes through weak or strong acid hydrolysis specifically dilute acid hydrolysis that is most effective to gain high yield of biogas [28]. There are two types of weak acid hydrolysis usually:

- Extreme temperature for low solids loading ($T > 160\text{ }^{\circ}\text{C}$, 5-10 wt% substrate concentration).

- Low temperature for high solids loading ($T \leq 160$ °C, 10-40% substrate concentration).

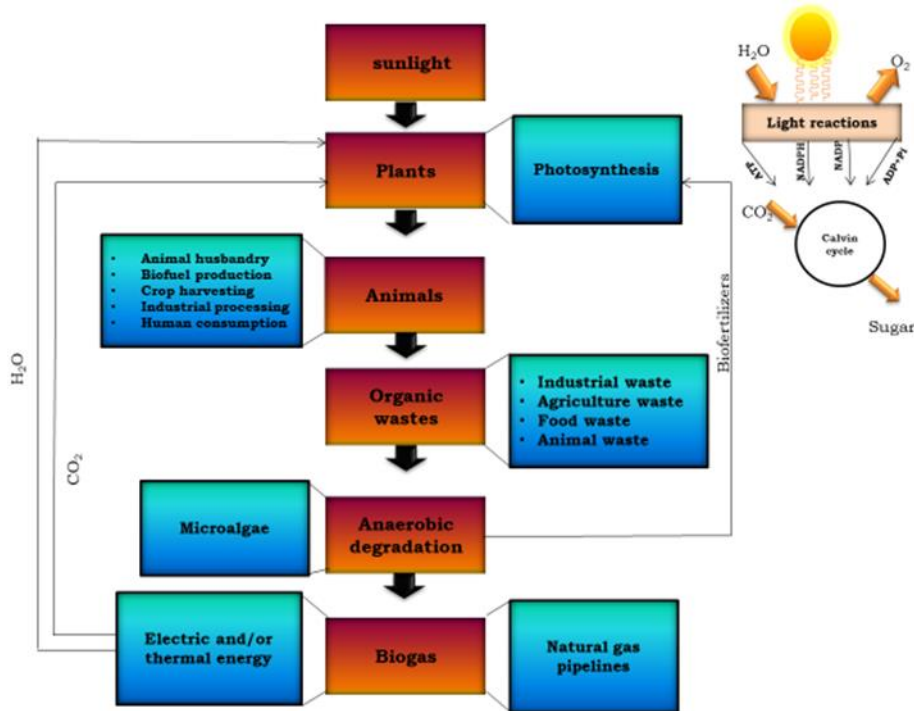


Fig. 3. Schematic representation of pre-treatment of lignocellulosic biomass procedure

Dilute H_2SO_4 is most used acid for hydrolysis at 160-220 °C for few minutes. Organic acids such as maleic acid, fumaric acid can also be used for dilute acid pre-treatment. The hydrolysate produced is a mixture of various valuable organic carbon, in the form of sugars and acts as a potential source to support microalgal growth and production of other targeted compounds such as oil and pigments [26].

Treatment of lignocellulosic waste with concentrated strong acids such as H_2SO_4 and HCl have been extensively used since they are powerful agents for cellulose hydrolysis with less enzyme requirement. Some advantages of using concentrated acid hydrolysis are high monomeric sugar yield as well as slight temperature conditions are required. The most common drawback is alteration of natural cycles (Carbon, Nitrogen and water cycle).

3.2. Alkaline Method

Three bases are commonly used in lignocellulosic pre-treatment for generation of high energy amalgams such as biogas, biohydrogen and methane etc.

- Calcium Hydroxide
- Sodium Hydroxide
- Ammonia

Lignocellulosic biomass must be discoloured through microbial enzymes which perform output chemical hydrolysis because enzymes demonstrate the high substrate and reaction specificity, activate beneath minor circumstances and not produce by-products [49]. Due to this enzymatic method is precious for chemical, fuel, food and textile industries as well as in washing powder constructions and in paper making Table III.

Table III Effect of Biogas production through various Pre-treatment methods

Sr. No	Pre-treatment method	Enhancement of Biogas %	Advantage	Disadvantage	References
01	Acidic	0-166	Destruction of lignin and hemicellulose	<ul style="list-style-type: none"> • High charge of acid • Deterioration problem in digester • Establishment of inhibitory substance 	[37]
02	Basic	0-207	Solubilizes the lignin and hemicellulose	<ul style="list-style-type: none"> • High alkali meditation in digester • Elevated cost of chemical 	
03	Enzymatic	0-208	Low energy feasting	<ul style="list-style-type: none"> • Unceasing adding mandatory • High cost of enzymes 	[38]

4. Wastes water Treatment

In any developing country, quantity of wastewater production every day is gigantic because of growing population and swift industrialization. Due to this, water pollution is one of the greatest life-threatening environmental problem [18, 39]. Countless number of methods are adopted for treating the wastewater that cause pollution in environment, all of these are not economically feasible but one of the best methods for treating wastewater by microalgae that is economical and eco-friendly too. This method is also known as microalgae-based wastewater treatment (MBWT)[40]. One of advantages of using microalgae based wastewater treatment is that they have capability to remove heavy metals, inorganic ions and other organic

material [16]. Following types of wastewater are being treated by cultivating microalgae.

1. Sewage wastewater
2. Municipal Wastewater
3. Industrial wastewater

Both quantitative and qualitative characteristics of lipids content in algal biomass fluctuate due to variable growth conditions such as temperature, pH, light intensity and nutrition media (concentration of nitrogen, iron, phosphate and other inorganic molecules) [10, 21]. The microalgae cultivation is also effected by some other biotic factors such as pathogens and presence or absence of other algal species [41]. Biomass treatment is depending on environmental conditions which influence the production of biogas. Biogas and methane productivities is directly linked to conditions which is provided,

suitable condition gives us high level of biogas as shown in Table IV.

Table IV Methane and biogas yield of some microalgae species by using wastewater

Species	Optimum Temperature	Reactor type	Type of Waste(s)/ Water	Methane (L CH ₄ g/VS)	Biogas Yield (ml/gVS)	References
Spirulina platensis	30	CSRT/Semi continuous/ Batch	Sewage Sludge	0.26-0.32	525-640 ml/gVS	[42]
Chlorella vulgaris	28-31	Batch	Domestic and Municipal wastewater	0.31-0.35	238-99 ml/gVS	[43]
Chlorella sp.	35	Batch	Seafood Processing	0.17-0.32	44 ml CH ₄ /gVS (from chlorella sp.) 193 ml CH ₄ /gVS (from processing of Seafood)	[44]
Scenedesmus obliquus	35-37	Batch	Secondary waste effluents and Municipal wastewater, urban wastewater	0.18	346 L CH ₄ /Kg VS 287 ml/gVS	[45-47]
Chlamydomonas reinhardtii	38	Batch	Wastewater at different level of treatment	0.39	587 ml/gVS	[39, 46]
Botryococcus braunii	35	Batch	Secondary treated sewage from domestic	404 mL/CH ₄ g VS	614.11 L/kg	[48, 49] [50]

			wastewater, Red Nile Tilapia Effluent			
Spirulina maxima	35	Batch		330	361-382 L/Kg VS	[51]
Tetraselmis sp	35	Batch	Urban wastewater treatment	420	419-431 L/Kg VS	[52]

4.1. Effect of temperature

The effect of temperature on microalgae growth display significant role [53]. Researchers found that optimal temperature for microalgal growth is 20-30oC although they can also grow in constant temperature. Microalgae showed less growth when temperature is 16oC but showed increased growth rate as temperature increase up to 28±30oC. The microalgal growth rate is affected at 30oC, decreased at 32oC and almost 17% decline growth rate at 35oC [54]. When temperature is above the optimal temperature about 38oC that led to death of algal cell biomass due to denaturation of protein [55, 56].

4.2. Effect of pH

Microalgae grow in variable ranges of pH but the optimal pH for microalgae is 7-8 to show have maximum growth rate [48]. It is also observed that growth must be influenced by different ranges of pH such as at 6.5 pH growth rate is low but at 7-8 pH showed fastest growth rate [57]. When pH is above optimal range for example 9 than there is continuous decline in growth rate. It is also found that pH should be maintained at optimum range to sustain the growth of microalgae. That's why pH is estimated to be third most important factor (after temperature and light intensity) for microalgae growth [54, 58, 59].

4.3. Effect of light

Diverse form of lights coming from different sources can be categorized as direct sunlight, indirect sunlight, fluorescent light and under dark, effect the growth rate of microalgae [27, 60]. Under dark conditions, microalgae growth is not documented since light is crucial constituent for their growth and microalgal culture is set near to the window to have direct sunlight [61]. In case of direct sunlight, microalgae give maximum rate of growth specifically if fluorescent light is provided. Microalgae growth is also dependant on light intensity, exposure and penetration thus, high light intensity damages the composition of pigments (phycobilin and phycoerythrin their nature different from other plant pigments based on molecular structure and absorption of light) and affects microalgal growth rate [27, 53, 60].

4.4. Effect of Aeration

Continuous aeration is an essential factor for cultivating unicellular form of algae (Chlorella sp.). Thus, without any mean of aeration, no growth is recorded due to death of microalgae biomass cell. Various modes for aeration are in use in a photobioreactor for better growth and enhanced amount algal fuel [62].

5. Inhibitors as by – Product of Lignocellulosic waste treatment

Inhibitors are those compounds which can stop or slow a biochemical process that may be competitive or non-competitive inhibitors. The by-product of pretreatment depends on the nature of chemical reaction and type of method which we use. Inhibitors can stunt the growth of microorganism such as phenolic compound, aromatics, aliphatic acid, furan aldehydes, inorganic ions and other bio-alcohols [63-65].

5.1. Phenolic Compound

We draw out phenolic compound during the pretreatment of lignin and wood extractives. Phenolic compounds are long-familiar with inhibiting activity against microorganism due to various strains of microalgae show the various responses against these types of compounds. Some microalgae/cyanobacteria show complete resistant against phenolic compounds such as *Chlorella saccharophila*, *Scenedesmus quadricauda*, *Chlorella zofingiensis*, *Coelastrum microporum* and *Mesotaenium caldararium* [26].

5.2. Furans

Furans are union of furfural and Hydroxymethyl furfurals (HMF) produced during the pretreatment of sugar and lignocellulosic waste with acids or base [66]. Knowledge about the effect of furans on microalgae growth is scarce but a certain amount of furfural and HMF stimulate the inhibition of growth and ethanol production in diverse species of microalgae such as *Saccharomyces cerevisiae*, *Zymomonas mobilis*, *Pichia stipites* and *Candida shehatae*.

5.3. Levulinic Acid

Levulinic acid is produced by the decomposition of HMF and 1.16g/L of levulinic acid can stunt the growth of microalgae i.e *Skeletonema costatum*, *Chlorella vulgaris* and *Agmenellum quadruplicatum* [66].

5.4. Fatty acid

Fatty acids are powerful part of lignocellulose waste material that are constituent of wood of various trees such as *Eucalyptus*. Fatty acids also act as inhibitory agent in various species but their peculiar types such as oleic acid, palmitic acid and linoleic acids have pronounced inhibitory effect on many microalgae species such as *S. capricornutum*, *Monoraphidium contortum* and *Chlorella vulgaris* [26].

6. Biogas productivities

Microalgal species required specific medium for their growth or normal functioning on the substrate to produce large quantity of biogas yield/productivity [17, 67]. These are also helpful for treating the agro-industrial wastes help reducing the environmental pollution Table V.

7. Problems associated with anaerobic digestion of microalgae

Anaerobic digestion is not best known or beneficial method because a lot of complications faced while dealing with microalgae by cultivating on agro-industrial wastes [46]. Some of the challenges are:

Low concentration of digestible substrate

Microalgae biomass is unique substrate for gaining high energy foodstuffs with lower commercial rate or low economically rate. Micro-algae with low volatile solid piling quantity can be utilized as digestible substrate for extraction of less concentrated biomass [57].

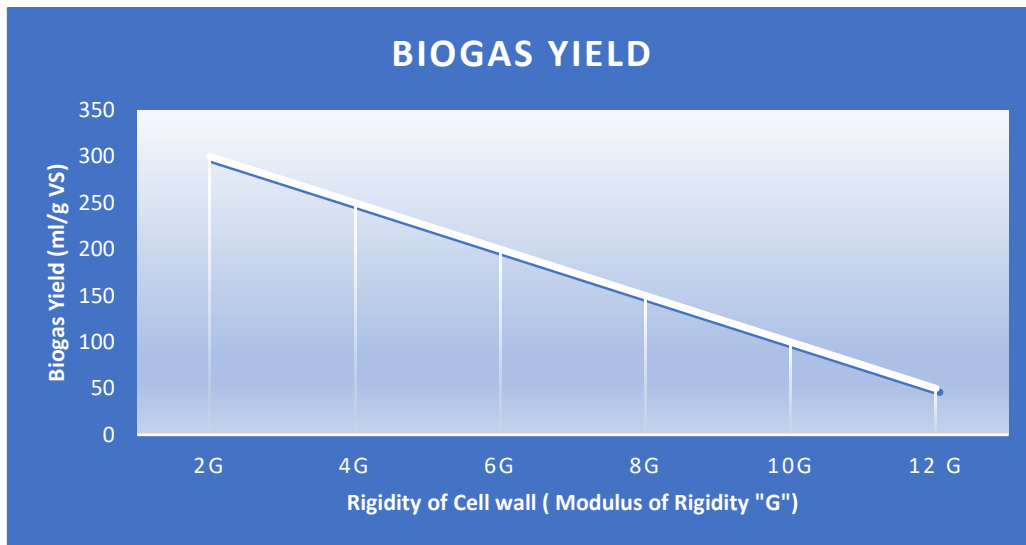
7.1. Cell wall degradability

Cell wall degradability is major problem for anaerobic digestion because its rigidity effect the biogas productivity due to incomplete breakdown of cell wall [15, 57]. Impregnation of carbohydrates and supplementary biomolecules make the cell wall more rigid. Thus, biogas productivity from microalgae depends on the rigidity of cell wall [52]. Cell wall provide protection to inner organic matter of microalgae from bacterial infection. Bacteria can easily grow

and proceed anaerobic digestion after breaking down of microalgal cell wall. [46]
Fig, 4.

Table V Microalgal Biogas Yield and Biogas productivity

Sr. No	Microalgae	Biogas yield ml/g DM	Biogas Productivity (P) ml/1V	References
01	Desmodesmus.sp	456	1436.40	[12]
02	D. armatus	440	1318.53	
03	H. pluvialis	413	1261.63	
04	S. obliquus	433	548.47	
05	C. vulgaris	510	1944.80	
06	C. sacharophilum	303	887.79	
07	Geminella.sp.	448	981.12	
08	G. terricola	316	449.77	
09	P. tricornutum	0.337L	–	[15]
10	Isochrysis galbana	0.22L	–	[68]
11	Scenedesmus sp.	646	–	
12	Chlorella sp.	652	–	[9]
13	Cladophora glomerate	0.20m ³ /m ³ d	–	
14	Chara fragilis	0.12m ³ /m ³ d	–	
15	Spirogyra neglecta	0.23m ³ /m ³ d	–	



Fig, 4. Rigidity of cell wall in terms of Modulus of Rigidity) Between Biogas yield (ml/g VS along Y- axis) and Microalgal cell wall rigidity (Modulus of Rigidity (G) along X- axis.

Solubilization and surface area effect the production of biogas by bacteria on inner organic matter [34, 69]. The solution for this problem is shown in Fig, 5.

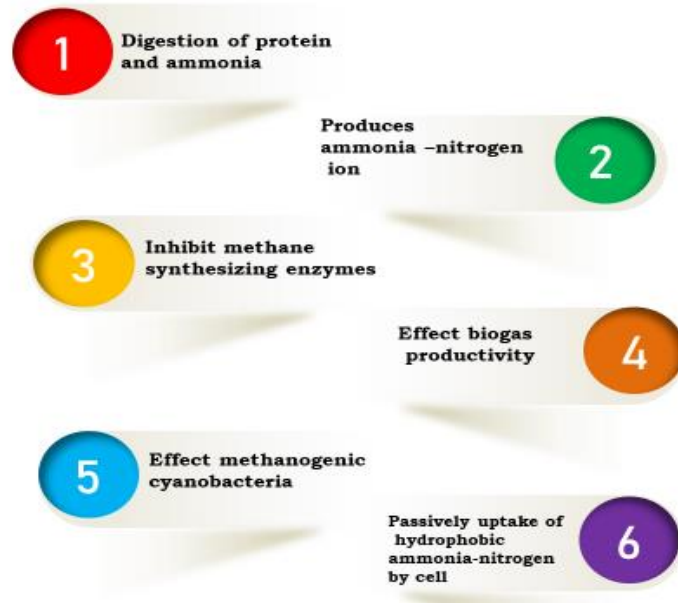


Fig. 5. Solution of Cell wall rigidity for enhancing Biogas Production

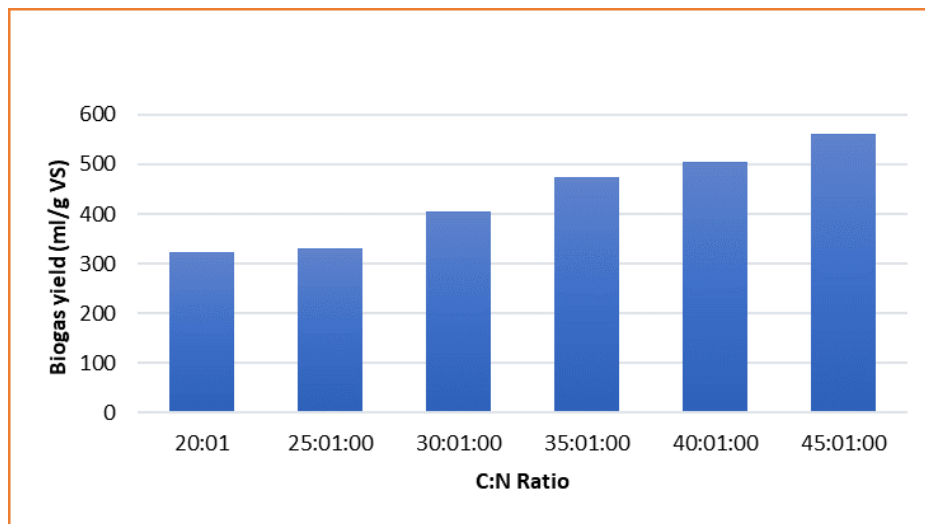


Fig. 6. Effect of Carbon-Nitrogen Ratio on Biogas Yield

7.2. Carbon to Nitrogen Ratio (C: N)

Carbon to nitrogen ratio increase the complications for anaerobic digestion of microalgal biomass which indirectly affect the production of biogas [70]. High amount of nitrogen in the form of ammonia have inhibitory effect on anaerobic digestion, thus, nitrogen cause negative effect on biogas productivity indirectly [71] [50]. This problem can be solved by adding nutrients in the form of high carbon substrate for digestion that will reduce inhibitory effect of ammonia [71] as shown in Fig. 6.

7.3. Ammonia-Nitrogen toxicity

By hydrolysis of nitrogen containing compounds (Protein, urea, and Keratin), which are impregnated in algal cell cause ammonia- nitrogen toxicity. Thus, ammonia-nitrogen concentration is passively entered in the cell by inhibiting methane synthesizing enzymes. Due to movement of ammonia-nitrogen ions imbalance the potassium concentration and decreased the biogas productivity [17]. This problem can be solved by removing acetogenic, hydrolytic and methanogenic bacteria from microalgal substrate. By removing bacteria, biogas production and anaerobic digestion increased. Optimum concentration of ammonia-nitrogen is 3000 mg/L in a reactor above this figure may affect the productivity of biogas [71] as shown in Fig. 7.

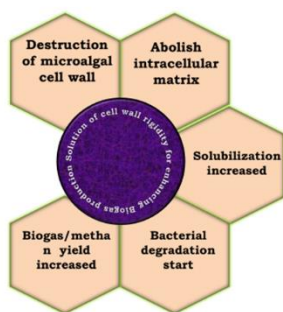


Fig. 7. Effect of Ammonia-Nitrogen toxicity on Biogas Yield

7.4. Effect of Salinity

Microalgae (*Macrocyctis pyrifera* and *Tetraselmis sp.*) are cultivated in saline-habitat to protect the agricultural land. Lower alkaline earth metal is required for better anaerobic digestion in saline environment. Higher concentration of alkaline in soil will change the pH and cause toxicity for methanogenic bacteria. Higher salinity cause dehydration in bacterial cell due to higher osmotic pressure that cause inhibitory effect on anaerobic digestion. Sodium, aluminium and magnesium change the chemistry of saline environment that is toxic for anaerobic processing of algal biomass and indirectly effect the biogas production [40].

7.5. Role of Sulfide:

Microalgae in freshwater have less amount of sulfurated amino acid so during digestion H₂S is produce in less amount. Marine water has measurable amount of oxidized sulphide compounds that act as electron acceptor for sulphide reducing bacteria to convert organic compounds into H₂S that cause corrosion in apparatus (photobioreactor). Thus, this extra amount of H₂S must be removed from apparatus which may affect less biogas productivity [40].

8. Conclusion:

Energy requisition now-a-days touched at higher level resulting in depletion of non-biodegradable resources. That's why truncated energy production effects economic value of any industrial country to overcome this crisis by using algal lignocellulosic biomass undergoes anaerobic digestion. To contract high yield of bioenergy product over exploitation of agro-industrial wastes, wastewater and other enduring biomass that is unsurpassed way to improve economy or to marmalade the natural environment.

List of Abbreviation

HMF_ Hydro methyl furfurals

MBWT_ Microalgal based wastewater treatment

ACKNOWLEDGEMENTS

Not applicable

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