International Journal of Recent Technology and Engineering (IJRTE)
ISSN: 2277-3878, Volume-8 Issue-3, September 2019

# Phycoremediation and Biogas Production Potential of Chlorella Vulgaris Grown in Secondarily Treated Wastewater

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***Abstract:*** ***Present study aims at exploring parallel nutrient removal from secondarily treated wastewater and biogas production potential of Chlorella vulgaris. The observed growth rate (per day) and doubling time (days) of C. vulgaris was 0.36 and 0.44, respectively. C. vulgaris has removed 31 % COD, 40 % $PO_4$-P , 36 % $NH_4$-N and 38 % TKN from secondarily treated wastewater using open raceway pond. The biomass was analyzed for proteins, carbohydrates, Lipids, fibres, TS and VS. The optimum loading rate for maximum biogas yield was found to be 2 g VS/L. Effect of various pre-treatment methods (thermal, chemical, sonication and thermo-chemical) has also been studied. The biomass and biomass extract (before and after pre-treatments) were also analyzed for solubilization of complex compounds. Thermally pre-treated biomass has increased biogas production by 60 % (480 mL/g VS) relative to untreated biomass (350 mL/g VS). This study has successfully demonstrated that microalgal cultivation in wastewater can be easily adopted in currently available wastewater treatment plants without any major modifications of existing available infrastructure.***

***Keywords : Chlorella vulgaris, phycoremediation, biogas, wastewater treatment, open raceway pond .***

## I. INTRODUCTION

The increase in volume of wastewater beyond the installed treatment capacity of sewage treatment plants is quite common and is leading to the release of untreated water into rivers, wells, and groundwater. On contrary, the availability of freshwater is decreasing, exerting a need to look for alternative wastewater treatment systems to treat and reuse of the water. Drinking of water contaminated with nitrate can cause Blue Baby Syndrome in human infants and release of phosphates to water bodies leads to eutrophication [1].

Currently available technologies for wastewater treatment include adsorption, ion exchange, activated sludge, electrochemical, and membrane filtration [2]. This suffers several disadvantages that include requirement of an external supply of carbon, operation and maintenance cost, need of technical staff, sensitivity to wastewater composition, limited wastewater handling capacity and generation of large quantity of harmful sewage sludge. Treatment of wastewater is depreciated in countryside areas of India due to the improper design of treatment plant, lack of financial resources, lack of technical staff, and poor maintenance. Most of the treatment facilities do not treat wastewater to reach wastewater discharge standards and not maintained to its proper functioning ability.

As reported by Central Pollution Control Board (CPCB-2013) India, 38.25 million cubic meters per day domestic wastewater (DW) is being generated in major cities of India and current treatment capacity for municipal corporations is only 11,787 MLD corresponding to only 31 % of DW generation. CPCB studies also depict that out of 269 DW treatment plants in India, only 231 are operational further reducing the treatment capacity to 21 % of the amount of sewage being generated [3].

*Chlorella vulgaris* is single celled, photosynthetic and nonmotile green microalgae (2-10 µm in size) belonging to Chlorophyta division. *C. vulgaris* reproduce asexually by nonmotile autospores [4]. It was found to contain 25-58 % proteins, 5-40 % lipids 40-61 % carbohydrates as cellular composition [5]. Microalgal cell was is complex in structure and rigid in nature.

Varieties of wastewaters including textile, domestic sewage, municipal, agricultural, anaerobic digester effluent and recalcitrant wastewater have been treated with *C. vulgaris* with 45-97 % N, 28-96 % P and 60-90 % COD removal potential [6]. The microalgae cultivation systems may include closed photobioreactors or Open Raceway Ponds (ORP) [7]. However, open raceway ponds has several advantages over other cultivation methods; low costs for construction, maintenance and operation, easy to scale up and the potential to integrate with wastewater treatment plants [8]. Numerous researchers have reported the biogas production potential of *C. vulgaris* biomass [9]. Biomass pre-treatment methods (thermal, chemical, sonication and thermochemical) can increase biogas production[10].

Present study focusses on phycoremediation potential of *C. vulgaris* for treatment of Sequencing Batch Reactor (SBR) effluent and sustainable biogas generation from microalgal biomass. The effects of different biomass pre-treatments on biogas production have also explored. In conclusion, this study demonstrates the alternative wastewater treatment process that can be incorporated in current wastewater treatment systems without any major modification to available infrastructure.

Manuscript published on 30 September 2019
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## II. MATERIALS AND METHODS

### A. Collection and analysis of Sequential Batch Reactor Treated Water (SBRTW)

Birla Institute of Technology and science (BITS), Pilani Goa campus is having Sequential Batch Reactor (SBR) as a sewage treatment system inside the campus. The current treatment capacity of SBR is 300 $m^3$/day. However, over the period of time, due to increase in population, volume of wastewater increased to 300 $m^3$ daily. Due to the increase in wastewater beyond the installed treatment capacity of SBR, the treatment efficiency of SBR was reduced. Therefore, attempts were made to further phycoremediate Sequential Batch Reactor Treated Water (SBRTW) using Open Raceway Pond (ORP). SBRTW was analyzed different pollutants as per APHA 2005 [11].

### B. Selection of microalga and inoculum preparation

*Chlorella vulgaris* was selected for phycoremediation of SBRTW. It was received as gift from Dr. Pradeep Dhamole from BITS Pilani, Hyderabad campus. *C. vulgaris* was initially cultivated at 28°C for 15 days in BBM medium at light intensity of 50 µmoles photons/$m^2$-s with 12-hour light/dark cycle. Mixing of culture was performed by manual shaking, thrice a day. This microalgal culture was further scaled up in 20 L aquarium, harvested by silk cloth filtration and used as inoculum for phycoremediation of SBRTW.

### C. Phycoremediation of SBRTW

SBRTW was treated by using 300 L capacity ORP at working depth of 0.2 m. Paddle wheel rotating at 20 RPM was used as mixing apparatus. The average light intensity, pH and temperature was 850 µmoles/$m^2$-s, 6.8 and 30°C, respectively. The 200 L SBRTW and *C. vulgaris* (50 g wet weight) was added in ORP. Initially, for the period of six days, batch mode of operation preferred and later fed batch mode was used for experimentation.

During Fed-batch mode, 33.3 L of culture from ORP was removed and it was replaced with fresh 33.3 L of SBRTW with HRT of six day. The water Samples were analyzed for various pollutants as per APHA 2005 [11].

Three-point sampling was used to study the phycoremediation of SBRTW;

1. Composition of microalgal culture in ORP
2. Composition of fresh SBRTW to be added to ORP
3. Composition of microalgal culture after ORP

After every three days, known volume of microalgal suspension from ORP was centrifuged at 6000 RPM (12 minutes). The moisture from biomass was removed by using oven at 40°C overnight and dry weight was measured. Growth curve was obtained by plotting biomass concentration (mg/L) with respect to time. The supernatant was stored at -4°C and analyzed for COD, $PO_4$-P, $NH_4$-N and TKN. The pollutant removal efficiency was calculated using formulae mentioned below.

% removal= [(A-B)/A] X 100

where, A=initial concentration and B=Final concentration

The overall methodology of experiment is described in Fig. 1

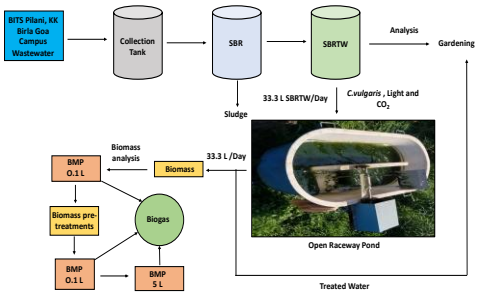


Fig.1 Overall methodology of experiment (SBR-Sequential Batch Reactor, SBRTW- Sequential Batch Reactor Treated Water and BMP-Biomethanation Potential)

### D. Biomass analysis

Moisture, Total Solids (TS), Volatile Solids (VS) and Ash content of biomass was determined. Total carbohydrates and protein concentrations were determined spectrophotometrically by using phenol-sulphuric acid method and Lowry's method, respectively [12,13]. The total lipids were extracted and quantified as per Folch et al [14]. Fibraplus FES-04 (Pelican equipments, Chennai) was used to determine the cellulose, hemicellulose and lignin content of microalgal biomass. The biomass was considered for FTIR spectroscopy as per Vidyadharani et al [15].

### E. Biomass pre-treatments

The biomass was subjected to different pre-treatment methods to understand their effect on Biomethanation Potential (BMP). Known mass of biomass was dissolved in known volume of distilled water and the pretreatment were performed. The pre-treatments were described below,

1. Thermal pre-treatment- autoclaved at 15 psi for 20 minutes.
2. Chemical- treatment with 0.5 M NaOH at 30°C overnight.
3. Sonication- sonication at 20 watts for 5 minutes in ice bath.
4. Thermochemical- treatment with 0.5 M NaOH followed by autoclaving at 15 psi for 20 minutes.

The microalgal suspension was centrifuged at 8000 RPM for 12 minutes to harvest biomass. The supernatant and biomass (after dying at 50°C overnight) was stored at 4°C. The biomass and supernatant were analyzed separately. During anaerobic digestion studies, the biomass along with the liquid was used as substrate.

### F. BMP of microalgal biomass

Serum bottles (total viloume-130 mL) was used to carry out the Biomethanation potential assay of algal biomass. BITS Pilani, KK Birla Goa Campus has anaerobic digester running on food wastes collected from institute cafeteria. The liquid digestate from this digester was used as inoculum for the BMP assay. BMP assays were carried out in duplicates. In each bottle, 1 mL micronutrient stock, 1 mL macronutrient stock, 5 mL 5 % (W/V) $NaHCO_3$, 13 mL of DDW and 80 mL of BMP inoculum was inoculated [16]. This combination was referred as negative control. BMP assays were performed at different loading rates (1-5 g VS/L) in serum bottles to optimize the loading rate. The bottles were properly sealed properly and $N_2$ gas was sparged to make system anaerobic. The biogas quantity was determined daily using water displacement process. Methane content was analyzed by using gas chromatography (Thermofischer Trace-1110) using packed sphaerocarb column as per Chavan et al 2018 [16].

The yield of biogas was determined by using following formulae,

Net Biogas production (mL) (A) = (B-C)

Net biogas yield (mL/ g VS) (D) = A / g VS added

Net Methane yield = D X % methane

Where,

B= Total Biogas produced, C= Total biogas produced by negative control.

The loading rate giving maximum yield of methane was selected for further BMP studies. Later, known amount of biomass was subjected to different pre-treatment methods and used as substrate for BMP assay. The pretreatment method and the loading rate giving maximum yield of methane was used to study BMP assay at 5 L reactor volume.

## III. RESULTS AND DISCUSSION

### A. Elemental composition of SBRTW

The composition of SBRTW, ORP outlet and inoculum used for BMP is depicted in Table 1. It was observed that the SBRTW is rich in pollutants that can be further used as nutrients by *C. vulgaris*.

Table1.Elemental composition of SBRTW and inoculum used for BMP

| Parameter (mg/L) | SBRTW | ORP Outlet | BMP Inoculum |
|---|---|---|---|
| COD | 121.34 | 72.94 | 14520.48 |
| $PO_4$-P | 8.69 | 5.40 | 489.77 |
| $NH_4$-N | 42.61 | 22.59 | 1213.56 |
| TKN | 81.23 | 22.41 | 17560.34 |
| pH | 6.72 | 7.5 4 | 8.30 |
| TS % | - | - | 13.55 |
| VS % | - | - | 7.91 |

SBRTW- Sequential Batch Reactor Treated Water, BMP-Biomethanation Potential, ORP-Open Raceway Pond.

It was observed that the SBRTW is rich in pollutants indicating that the SBR is not working efficiently. The SBRTW does not follow the discharge standards specified by Environmental Protection Agency (EPA )-USA and Central Pollution Control Board (CPCB)-India. These pollutants can be utilized as nutrients by *C. vulgaris*. Microalgae have been considered as pollutant scavengers for variety of wastewaters including domestic, agricultural and industrial wastewater. Microalgae can assimilate organic and inorganic forms of C, N and P along with some hydrocarbons and antibiotics.

### B. Phycoremediation of SBRTW

Growth of *C. vulgaris* in SBRTW and Pollutant removal is depicted in Fig. 2. The observed growth rate and doubling time was 0.36/day and 0.44 days, respectively. Numerous studies have reported growth rate and doubling time of *C. vulgaris* ranging from 0.165-1.0 per day and 0.5- 1.5 days, respectively on different growth medium and different types of wastewater [17].

Table 2 represent the pollutant removal from SBRTW using *C. vulgaris*. During phycoremediation using *C. vulgaris*, 31.21 % (31.33 g) COD was reduced with removal rate of 7.49 mg/L-day. Numerous studies has reported COD removal of 40-90 % and COD removal rates of 17-150 mg/L-day in different wastewater with HRT of 10-16 days [18]. The COD removal is less in present study as compared to previous studies. This may be attributed to less HRT of 6 days in present study. The COD removal rate may be increased by increasing the initial microalgal inoculum during phycoremediation studies. However, present study suggests that *C. vulgaris* can utilize organic and inorganic carbon as energy source. Previously it was reported that *Chlorella* sp. can adopt autotrophic, heterotrophic and mixotrophic nutrition mode depending on source of carbon in growth medium [19]. Martinez et al has also reported that *Chlorella* sp. can grow mixotrophically and can utilize light, organic carbon sources (glucose and organic acids) and inorganic carbon dioxide; organic carbon is utilized preferably over inorganic carbon [20].

*C. vulgaris* was observed to remove 39.47 % (3.35 g ) of $PO_4$-P. Various researchers have reported $PO_4$-P removal of 40- 90 % with removal rates of 0.78 to 12.25 mg/L-day using *C. vulgaris* in different kinds of wastewaters with pH range of 6.5 to 9. The $PO_4$-P removal efficiency in present study is similar to $PO_4$-P removal efficiencies reported earlier; however, it can be increased by increasing HRT and amount of initial microalgal inoculum. It was stated that $PO_4$-P from wastewater can be utilized by two main mechanisms; microalgal utilization and $PO_4$-P precipitation at alkaline conditions [21]. In present study, as the pH did not increase beyond 8.0, all the $PO_4$-P removal may be attributed to microalgal utilization.

It was observed that, *C. vulgaris* have removed 35.89 % (12 g) $NH_4$-N with removal rate of 2.86 mg/L-day in present study. Various other studies have observed $NH_4$-N removal of 40-94 % with removal rate of 2.78 to 5.72 mg/L-day [17,21]. The $NH_4$-N removal rate is matching with available literature, but the removal efficiency is less as compared to reported values. This suggests that increase in HRT of wastewater might result in increase in treatment efficiency by microalgae. $NO_3$-N is preferred Nitrogen source for microalgae [22]. The treatment mechanisms of $NH_4$-N from wastewater by microalgae include microalgal absorption and $NH_3$ stripping at pH>9.0, presence of abundant urea and elevated temperature [22]. In present study, the temperature did not exceed to 30°C and the pH was observed to be < 8.0; suggesting that $NH_3$ is not significant and $NH_4$-N removal was attributes to microalgal absorption only.

Observed TKN removal and removal rate in present study was 38 % (22.52 g) and 5.36 mg/L-day, respectively. Previously it was reported that *C. vulgaris* grown on varieties of wastewater can achieve TKN removal of 40-80 % with removal rate of 4.45-7.43 mg/L-day [17,21]. The TKN removal efficiency and removal rate were similar to previously reported values but can be increased by using high microalgal concentration initially and HRT. TKN removal is observed to be greater than $NH_4$-N removal in present study suggesting that *C. vulgaris* can utilize $NH_4$-N, $NO_3$-N and $NO_2$-N as Nitrogen source [20].

It was observed that the *C. vulgaris* can significantly remove pollutants from SBRTW, however it does not meet the wastewater discharge limits as specified by Environmental Protection Agency-USA (EPA) and Central Pollution Control Board (CPCB, India). This decrease in efficiency was attributed to short HRT of 6 days. We have observed that, increase in

HRT leading to larval growth and frequent visits of birds to treatment site, raising the issue of human health and hygiene. However, the treated water can be used for agricultural use after disinfection.

Previously it was observed that, the use of treated water for agriculture increases the soil organic content, nitrogen and phosphorous content, thus reducing the use of chemical fertilizers [23].

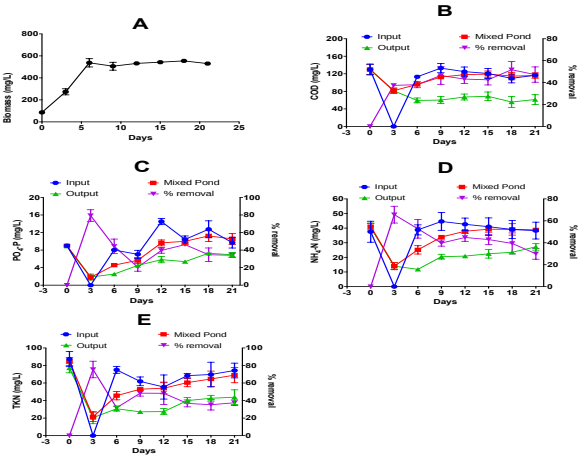


**Fig. 2 Growth of *C. vulgaris* in SBRTW and Pollutant removal. (All the values represent mean and standard error at n=4.)**

Table 2 Pollutant removal from SBRTW using *C. vulgaris*.

| Parameter | COD | $PO_4$-P | $NH_4$-N | TKN |
|---|---|---|---|---|
| Total Inlet (g) | 100.73 | 8.48 | 33.48 | 59.28 |
| Total Outlet (g) | 69.29 | 5.13 | 21.46 | 36.75 |
| Total removed (g) | 31.33 | 3.35 | 12.01 | 22.52 |
| % Removal | 31.21 | 39.47 | 35.89 | 38.0 |
| Removal rate (mg/L-day) | 7.49 | 0.8 | 2.86 | 5.36 |
| Removal rate (mg/$m^2$-day) | 21.89 | 2.33 | 8.36 | 15.68 |

**C. Biomass analysis**

*C. vulgaris* biomass consists of 76 % soluble fibre and 23. 79 % of crude fibre. The crude fibre consists of cellulose, 4.57 %; hemicellulose, 15.58 % and Lignin, 3.64 %; cellulose/hemicellulose =0.29 . The literature regarding fibre composition of *C. vulgaris* is not very well documented. However, it was reported that *Chlorella pyrenoidosa* consists of 0.3 % cellulose and 0.5 % hemicellulose [24]. It was also reported that *Chlorella* sp. contains 8.6 % hemicellulose and 15.4 % cellulose; cellulose/hemicellulose =1.79 [25]. The lower cellulose/hemicellulose ratio (0.29) was obtained in present study as observed (1.79) by Northcote et al. Lower the cellulose/hemicellulose ratio, higher the biodegradability of biomass and can be utilized as a feedstock for biogas production [26]. The presence of lignin in *Chlorella* sp. was not always clear. Zhu et al reported that the lignin is not present in *Chlorella* sp. [27]. However, Zhou et al has reported the presence of lignin in biomass in less amount [28]. Cellulose, hemicellulose, and lignin play an important role as fibrous barrier to protect the cells from external environment [29]. The high lignin content provides the robustness to algal cell walls. Table 3 represent fibre composition of *C. vulgaris* biomass.

Table 3 Fibre composition of *C.vulgaris*

| Fibre Type | % Content |
|---|---|
| Soluble fibre | 76.21 |
| Crude fibre | 23.79 |
| Cellulose | 4.57 |
| Hemicellulose | 15.58 |
| Lignin | 3.64 |

FTIR spectrum of *C.vulgaris* is depicted in Fig. 3. and tentative assignment of bands is depicted in Table 4.

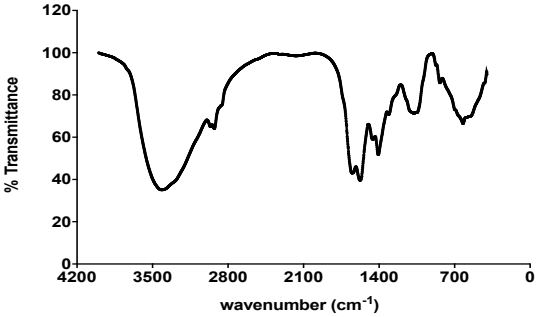


**Fig. 3 FTIR spectrum of *C.vulgaris***

Table 4 Tentative assignment of bands observed in FTIR spectrum of *C. vulgaris*.

| Main peak ($cm^{-1}$) | Band Assignment |
|---|---|
| 3414 | Water v(O-H) stretching, protein v(N-H) stretching |
| 2929 | Lipid-Carbohydrate, mainly $v_{as}(CH_2)$ and $V_s(CH_2)$ stretching |
| 1656 | Protein Amide I bond, mainly v(C=O) stretching |
| 1577 | Protein Amide II bond, mainly ó(N-H) bending and v(C-N) stretching |
| 1465 | Proteins $ó_{as}(CH_2)$ and $(CH_3)$ bending of methyl, and Lipid $ó_{as}(CH_2)$ bending of methyl |
| 1403 | Protein $ó_s(CH_2)$ and $ó_s(CH_3)$ bending of methyl, Carboxylic acid $v_s$(C-O) of COO groups of carboxylates and Lipids $ós(N(CH_3)_3)$ bending of methyl |
| 1078 | Carbohydrate v(C-O-C) of polysaccharides, nucleic acids and other P containing compounds, Vs(>P=O) stretching of phosphodiester bonds |
| 867 | C-H “oop”, aromatics |
| 622 | C-Br stretch, alkyl halides |

FTIR spectrum of *C.vulgaris* confirmed the presence of alkanes, alkenes, aromatics, lipids, proteins, carbohydrates, alcohols and carboxylic acids (Table 4). Similar results were

observed by Vidyadharani et al [15].

The characteristics of *C. vulgaris* biomass are depicted in Table 5. It was found to contain 34 % proteins, 16 % lipids and 38 % carbohydrates. Previously, it was observed that *C. vulgaris* can accumulate 5-40 % lipids inside the cells, this found to be true in our studies [5]. However, it was found that the *C. vulgaris* may contain 37-58 % proteins and 51-61 % carbohydrate [30]. This decrease in proteins and carbohydrate content in our study might be caused by high nitrogen and orthophosphates concentration in SBRTW as compared to absence of orthophosphates in the cultivation medium their studies. Previously, it was observed that N and P limitation can increase the proteins, lipids and carbohydrate content of biomass [31]. The observed VS content is well in agreement with Calicioglu et al [9].

**D. Biomass pre-treatments**

Table 5 represent composition of fresh and post pretreatment *C.vulgaris* biomass and Table 6 represent composition of fresh and post pretreatment *C. vulgaris* extract. It was observed that during pre-treatments VS, proteins, lipids and carbohydrate content of biomass is decreased and proteins, carbohydrates, COD, TKN and NH4-N were increased in microalgal extract as compared to control. This was observed due to solubilization of biomass during different pre-treatments [32]. The observed order of biomass solubilization with respect to different pre-treatment methods as compared to control was thermal > thermochemical > sonication > chemical. Biogas production can be increased by increasing substrate solubilization [9].

Table 5 Characterization of *C.vulgaris* biomass after pre-treatments

| Method | A | B | C | D | E |
|---|---|---|---|---|---|
| Moisture % | 6.8 | ).26 | 0.86 | 12.17 | 13.23 |
| TS % | 93.2 | 0.74 | 9.14 | 87.83 | 86.77 |
| VS % | 85.71 | 7.42 | 3.29 | 72.39 | 69.54 |
| VS/TS | 0.92 | ).74 | ).82 | 0.82 | 80.14 |
| Ash % | 14.9 | 2.58 | 6.71 | 27.61 | 30.46 |
| Protein % | 30.26 | 9.38 | 0.87 | 18.26 | 20.91 |
| Lipids % | 16.45 | - | - | - | - |
| Carbohydrate % | 38.31 | 1.49 | 9.86 | 18.56 | 17.89 |

* A-control, B-Thermal, C-Sonication, D-Chemical and E-Thermochemical

Table 6. Characterization of *C.vulgaris* extract after pre-treatments

| Method | A | B | C | D | E |
|---|---|---|---|---|---|
| Protein mg/g | 38.56 | :8.6 | 125.86 | 89.64 | 135.89 |
| Carbohydrate mg/g | 29.38 | :.39 | 79.63 | 74.59 | 78.41 |
| Soluble COD/g | 4128.89 | 45. | 7895.19 | 7658.43 | 9586.17 |
| TKN % | 0.38 | .69 | 0.61 | 0.53 | 0.61 |
| $NH_4$-N mg/L-g | 6.43 | ).35 | 26.58 | 24.53 | 28.32 |

* A-control, B-Thermal, C-Sonication, D-Chemical and E-Thermochemical

**E. Biogas production**

Biogas production from *C. vulgaris* biomass at different loading rate, scale of operation and the result of various biomass pre-treatments is depicted in Fig. 4 and Table 6, respectively. Maximum biogas production was observed at loading rate of 2 g VS/L followed by 3 g VS/L with biogas yield of 452 mL/ g VS and 386 mL/ g VS, respectively with increase of 31. 5 % and 12.61 %, respectively. The range of biogas yield and methane yield at different loading rates were found to be 340-452 mL/ g VS and 185- 224 mL/ g VS, respectively.

Previous studies have reported that the optimum VS loading for anaerobic digestion was 1.4-2.9 g VS/L with biogas yield of 250- 360 ml /g VS while using vegetable waste as substrate [33]. Numerous studies have also reported the biogas yields ranging from 238- 630 mL/ g VS while using different substrates (microalgae, food wastes, dairy manure and vegetable wastes) for anaerobic digestion [9]. At high VS loading > 3 gVS/L, there was increase in cumulative biogas production but yield of biogas was not increased. This might be due to the effect of accumulation of various toxic metabolites including aromatics, ammonia, volatile fatty acids [34]. Further research needs to be carried out to confirm this assumption.

Effect of different pre-treatments on biogas production from microalgal biomass was studied at optimum loading rate of 2 g VS/L. Biomass pre-treatments increase the solubilization of biomass components leading to their bioavailability to be used as nutrients by anaerobic microorganisms [9]. The biogas yield (mL/ g VS) obtained by untreated, thermal, sonication, chemical and thermo-chemical treated biomass was 350, 482, 360, 374 and 430, respectively. The maximum biogas increase was observed in biomass pretreated with thermal; 60 % followed by thermochemical; 28 %, sonication; 24% and chemical method; 18 %. It was reported previously that thermal pretreated biomass can produce 29-70 % higher biogas as compared to untreated biomass [10], this found to be true in present study as well. Sonication pre-treated biomass was reported to produce 6-130 % more biogas (384 mL/ g VS) as compared to untreated biomass [35]. However, maximum biogas yield obtained by sonication treated biomass in present study is less as compared to previous observation in Lee et al study ; this might be due to the use of high sonication dose ( 2500 J/mL) as compared to 300 J/mL in present study [35]. Thermochemical pretreatment can increase biogas production up to 44 % [10]. In present study, thermochemically pre-treated biomass produced 28 % more biogas as compared to untreated biomass. Anaerobic digestion studies at 5 L scale using untreated and thermally pre-treated biomass replicated the results of 0.1 L scale studies. This indicate that the anaerobic digestion can be further scaled up to understand more about the feasibility and cost effectivity of both processes; thermal pre-treatment and anaerobic digestion.

**Table 7 Biogas production from *C.vulgaris* at different loading rates, different scales of experiment and effects of biomass pre-treatment methods**

| Sr. NO. | Method | Scale | Duration (Days) | Loading rate (g VS/L) | Biogas Yield (mL/g VS) | Methane yield (mL/g VS) |
|---|---|---|---|---|---|---|
| 1 | No treatments | 0.1 L | 29 | 1 | 343.5 | 185.49 |
| | | | | 2 | 452 | 289.28 |
| | | | | 3 | 386.83 | 224.36 |
| | | | | 4 | 346.38 | 207.82 |
| | | | | 5 | 341.5 | 194.65 |
| 2 | Control | 0.1 L | 30 | 2 | 350 | 174 |
| | Thermal | | | | 482.25 | 279.71 |
| | Sonication | | | | 359.75 | 215.85 |
| | Chemical | | | | 374.75 | 206.11 |
| | Thermochemical | | | | 430.87 | 224.06 |
| 3 | Control | 5 L | 33 | 2 | 370.65 | 492.45 |
| | Thermal | | | | 207.56 | 290.55 |

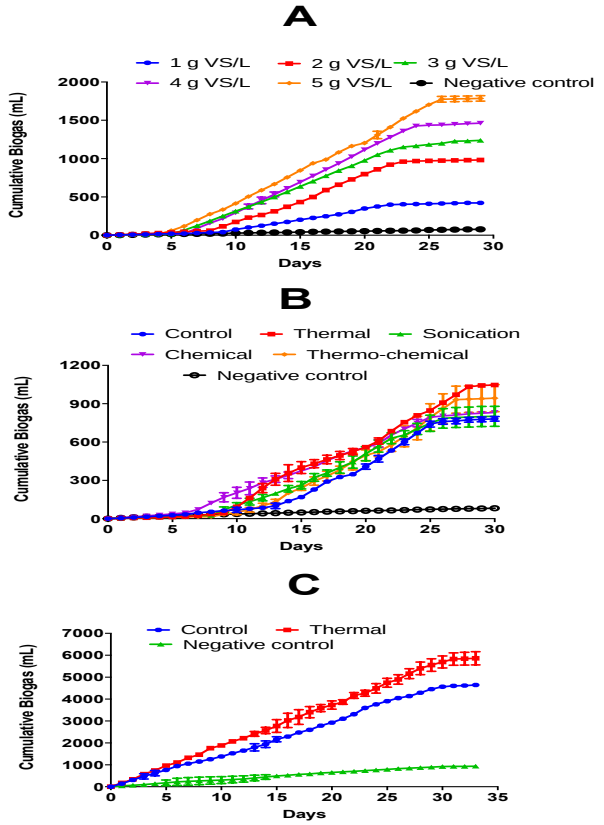


**Fig. 4 Biogas production from C.vulgaris A-Biogas** production at different loading rate, B- biomass pre-treatments and biogas production and C-biogas yield from fresh biomass and thermally pre-treated biomass at 5 L scale. (VS-Volatile solids; all the values represent mean and standard error at n= 2).

## IV. CONCLUSION

Most of the conventional wastewater treatment facilities focuses mainly on COD and BOD removal, however *C. vulgaris* is efficient in removal of COD, nitrogen and phosphorous. It can remove 30 – 40 % of these pollutants from wastewater. TKN removal is higher compared to $NH_4$-N removal suggesting that $NH_4$-N is not the preferred N source. Moreover, microalgal treatment systems can be installed at sewage treatment plants without any major modification to available infrastructure. Thermal pretreatment of biomass can increase the biogas production up to 60 %. Integration of microalgal cultivation with wastewater treatment may serve two major purposes; handling of wastewater and sustainable generation of clean energy fuel, biogas.

## ACKNOWLEDGMENT

Authors would like to thank BITS Pilani, Centre of Research Excellence for providing the funds to carry out this research under the theme Wastewater and Energy. Authors would also like to thank Ms. Megha Shinge (Karnataka University, Dharwad) for FTIR analysis.

## REFERENCES

1. Knobeloch L, Salna B, Hogan A, et al. Blue babies and nitrate-contaminated well water. Environ. Health Perspect. 2000;108:675–678.
2. Kightlinger W, Chen K, Pourmir A, et al. Production and characterization of algae extract from Chlamydomonas reinhardtii. Electron. J. Biotechnol. 2014;17:14–18.
3. J.S. Kamyotra and R.M. Bhardwaj. Municipal Wastewater Management in India. India Infrastruct. Rep. 2011. 2011.
4. Manisha SCA. Growth, survival and reproduction inChlorella vulgaris andC. variegata with respect to culture age and under different chemical factors. Folia Microbiol. (Praha). 2007;52:399–406.
5. Becker EW (E. W. Microalgae : biotechnology and microbiology. Cambridge University Press; 1994.
6. Lim S-L, Chu W-L, Phang S-M. Use of Chlorella vulgaris for bioremediation of textile wastewater. Bioresour. Technol. 2010;101:7314–7322.
7. Pulz O, Gross W. Valuable products from biotechnology of microalgae. Appl. Microbiol. Biotechnol. 2004;65:635–648.
8. Z. J. Pei, Q. Wu, E. Mao, et al. Microalgae Mass Production Methods. Trans. ASABE. 2013;52:1275–1287.
9. Calicioglu O, Demirer GN. Integrated nutrient removal and biogas production by Chlorella vulgaris cultures. J. Renew. Sustain. Energy. 2015;7:033123,1-14.
10. Campo G., Rıggıo V.A., Cerutti A., Ruffino B., Panepınto D. ZM. Thermal, Alkali And Thermo-Alkali Pretreatments Applied On Monospecific Microalgal Biomass To Improve Anaerobic Biogas Production. Desalin. Water Treat. 2018;127:165–170.
11. Eaton A, Franson M. Standard methods for examination of water and wastewater. 21th End. Washingt. APHA, AWWA. 2005.
12. Dubois M, Gilles KA, Hamilton JK, et al. Colorimetric Method for Determination of Sugars and Related Substances. Anal. Biochem.2009;394:192–201.
13. González López CV, Del M, Cerón García C, et al. Protein measurements of microalgal and cyanobacterial biomass. Bioresour. Technol. 2010;101:7587–7591.
14. Folch J, Lees M, Stanley GHS. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957;226:497–509.
15. Vidyadharani G, Dhandapani R. Fourier transform infrared ( FTIR ) spectroscopy for the analysis of lipid from chlorella vulgaris. Appl. Biol. 2016;61:16753–16756.
16. Chavan R, Mutnuri S. Tertiary treatment of domestic wastewater by *Spirulina platensis* integrated with microalgal biorefinery. Biofuels. 2018;1–12.
17. Wang L, Min M, Li Y, et al. Cultivation of Green Algae Chlorella sp. in Different Wastewaters from Municipal Wastewater Treatment Plant. Appl. Biochem. Biotechnol. 2010;162:1174–1186.
18. Wang H, Lin K, Hou Z, et al. Sorption of the herbicide terbuthylazine in two New Zealand forest soils amended with biosolids and biochars. J. Soils Sediments. 2010;10:283–289.
19. Lalucat J, Imperial J, Parés R. Utilization of light for the assimilation of organic matter in Chlorella sp. VJ79. Biotechnol. Bioeng. 1984;26:677–681.

20. Martínez ME, Camacho F, Jiménez JM, et al. Influence of light intensity on the kinetic and yield parameters of Chlorella pyrenoidosa mixotrophic growth. Process Biochem. 1997;32:93–98.
21. Li Y, Chen Y-F, Chen P, et al. Characterization of a microalga Chlorella sp. well adapted to highly concentrated municipal wastewater for nutrient removal and biodiesel production. Bioresour. Technol. 2011;102:5138–5144.
22. Matusiak K. Studies on the purification of wastewater from the nitrogen fertilizer industry by intensive algal cultures. I. Growth of Chlorella vulgaris in wastes. Acta Microbiol. Pol. 1976;25:233–242.
23. Mohammad MJ, Mazahreh N. Changes in Soil Fertility Parameters in Response to Irrigation of Forage Crops with Secondary Treated Wastewater. Commun. Soil Sci. Plant Anal. [2003;34:1281–1294.
24. Gai C, Zhang Y, Chen W-T, et al. An investigation of reaction pathways of hydrothermal liquefaction using Chlorella pyrenoidosa and Spirulina platensis. Energy Convers. Manag. 2015;96:330–339.
25. Northcote DH, Goulding KJ, Horne RW. The Chemical Composition and Structure of the Cell Wall of Chlorella pyrenoidosa. Biochem. J. 1958;70:391–397.
26. Ghosh S, Henry MP, Christopher RW. Hemicellulose conversion by anaerobic digestion. Biomass.1985;6:257–269.
27. Zhu S, Wang Y, Huang W, et al. Enhanced Accumulation of Carbohydrate and Starch in Chlorella zofingiensis Induced by Nitrogen Starvation. Appl. Biochem. Biotechnol. 2014;174:2435–2445.
28. Zhou N, Zhang Y, Gong X, et al. Ionic liquids-based hydrolysis of Chlorella biomass for fermentable sugars. Bioresour. Technol. 2012;118:512–517.
29. Safi C, Zebib B, Merah O, et al. Morphology, composition, production, processing and applications of Chlorella vulgaris: A review. Renew. Sustain. Energy Rev. 2014;35:265–278.
30. Kumar K, Dasgupta CN, Das D. Cell growth kinetics of Chlorella sorokiniana and nutritional values of its biomass. Bioresour. Technol. 2014;167:358–366.
31. Ana Cláudia Freitas Margarites JAVC. Increment of carbohydrate concentration of Chlorella minutissima microalgae for bioethanol production. Int. J. Eng. Res. Appl. 2014;4:80–86.
32. Cho S, Park S, Seon J, et al. Evaluation of thermal, ultrasonic and alkali pretreatments on mixed-microalgal biomass to enhance anaerobic methane production. Bioresour. Technol. 2013;143:330–336.
33. Babaee A, Shayegan J. Effect of organic loading rates (OLR) on production of methane from anaerobic digestion of vegetables waste. Bioenergy Technol. Linköping- Sweden; 2011. p. 411–417.
34. Hecht C, Griehl C. Investigation of the accumulation of aromatic compounds during biogas production from kitchen waste. Bioresour. Technol. 2009;100:654–658.
35. Lee K, Chantrasakdakul P, Kim D, et al. Ultrasound pretreatment of filamentous algal biomass for enhanced biogas production. Waste Manag. 2014;34:1035–1040.

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