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Parallel Nutrient Removal and Biogas Production by Chlorella Vulgaris *Cultures*

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Abstract— **In aquatic environments, eutrophication causes algal blooms, oxygen depletion, increase in undesired vegetation, loss of plant beds, fish, coral reef and other species. Eventually, the water bodies become unavailable to utilize for agricultural, recreational, industrial and drinking purposes. Discharge of domestic sewage introducing high levels of nutrients to water bodies is one of the main causes of eutrophication. Secondary treatment may not be adequate for removal of these nutrients. In fact, tertiary treatment methods have been developing for their removal.**

Considering their high nitrogen and phosphorus requirements, growth of algae is an alternate method for biological wastewater treatment with advanced nutrient removal. In addition, photosynthetic activity during treatment process requires carbon dioxide, which may be provided from atmosphere or flue gas. In turn, microalgal wastewater treatment aids sequestration of atmospheric carbon dioxide. *Chlorella vulgaris,* microalga from genus of single-cell green algae, *Chlorella*, has high photosynthetic efficiency, productivity and adaptable to severe environmental conditions. Owing to these properties, *Chlorella vulgaris* is a viable alternative for wastewater treatment systems, in order to provide system flexibility against variations in wastewater compositions.

Microalgal biotechnology has been developed not only for wastewater treatment, but also for a variety of consumer products such as pharmaceuticals and nutrient supplements. Apart from consumer products, microalgae can be used for production of biofuels. Extracted oil from microalgal biomass can be converted into biodiesel. The cell residues can then be converted into biomethane, bioethanol or biohydrogen. Biomethane production from microalgal biomass has received attention, since biogas obtained from anaerobic digestion can be used for electricity generation. In addition, biomass residues after anaerobic digestion can be converted into fertilizers. Utilization of these fertilizers provides sustainable agriculture and reduces production costs of microalgae.

Production cost of methane from microalgae is higher than other feedstocks, due to microalgae cultivation costs involved in these systems. Therefore, integrated wastewater treatment and biomethane production can be the most feasible approach to reduce production cost. When coupled with carbon dioxide sequestration and wastewater treatment, microalgae can provide possible solutions to environmental problems and simultaneously create valuable consumer products and biofuels. Although cellulose and lignin contents of microalgae are almost zero and anaerobic process stability is high, hydrolysis of microalgal cell wall is problematic. The biodegradation of algal biomass can be improved by different pretreatment methods, such as microwave pretreatment; ultrasound pretreatment; thermal pretreatment including drying, heating, thermal hydrolysis or high pressure thermal hydrolysis; chemical pretreatment and biological pretreatment.

This study investigated (1) the nutrient (nitrogen and phosphorus) removal from wastewater, (2) pretreatment of the produced algal biomass by heat, autoclave and thermochemical methods, (3) and anaerobic digestion of the pretreated and non-pretreated algal biomass to produce biogas. To this purpose, a semi-continuous photo-bioreactor was operated for investigation of nutrient (N and P) removal efficiency of unialgal culture, *Chlorella vulgaris*. Then, the produced algal biomass with and without pretreatment were subjected to Biochemical Methane Potential tests.

The results indicated that the autoclave pretreatment is superior than other pretreatment methods, increasing methane yield by 79% in the reactor with initial COD concentration of 19000 ± 500 mg/L, and resulting in 33% increase in methane yield in the reactor with 34000 ± 1500 mg/L initial COD concentration, when compared to reactors fed with same amount of untreated microalgae as substrates.

Keywords—Algae; Chlorella vulgaris; anaerobic digestion; biogas; pretreatment; nutrient.

I. INTRODUCTION

Balance, adequacy and availability of nutrients, including but not limited to nitrogen and phosphorus, have direct effects on life on Earth. Following industrial revolution, human manipulation on these key nutrient cycles has been significant. Increased atmospheric carbon dioxide concentrations due to fossil fuel consumption clearly illustrates negative anthropogenic effects on ecosystems [1]. This increase causes elevation in global temperatures and uncontrolled sequestration of carbon-dioxide on ecosystems. These manipulations adversely affect not only ecosystems, but also human well-being, having influencing environmental health, public health and economy [2].

Eutrophication is one of the direct consequences of human manipulation on nutrient cycles. Discharge of domestic

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sewage, which introduces high levels of N originating from human wastes and P originating from detergents, to water bodies are main causes of eutrophication [3]. In aquatic environments, eutrophication causes algal blooms, oxygen depletion, increase in undesired vegetation, loss of plant beds, fish, coral reef and other species. Eventually, the water bodies become unavailable to utilize for agricultural, recreational, industrial and drinking purposes [4]. In order to avoid deteriorations in public health and environment, it is necessary to separate aforementioned constituents before they reach natural environments.

Growth of microalgae is an alternative method of biological wastewater treatment, considering their high nitrogen and phosphorus requirement for nucleic acids, phospholipids and protein synthesis [5]. It has been reported that microalgal species such as *Botryocoocus*, *Chlamydomas*, *Scenedesmus* and *Chlorella* have high potential of nutrient removal. *Chlorella vulgaris* has been reported to utilize nitrogen and phosphorus from industrial, agricultural and municipal wastewaters, when they are used as source of nutrients. Several studies have been conducted to investigate nutrient removal potential of *C. vulgaris* (Table 1).

TABLE I. WASTEWATER TREATMENT STUDIES USING *CHLORELLA VULGARIS*

Wastewater Type	Cultivation Mode	Nitrogen removal efficiency	Phosphorus removal efficiency	Ref.
Steel making plant industry effluents	Batch	100 % NH_4^+-N 50 % NO_3^--N	N/A	[6]
Digested Dairy Manure	Semi-continuous	99.7 % NH_4^+-N 89.5 % TN	92.0 % TP	[7]
Undigested Dairy Manure	Semi-continuous	100 % NH_4^+-N 93.6 % TN	89.2 % TP	
Wastewater before primary settling	Batch	82.4 % NH_4^+-N 68.4 % TN	83.2 % TP	[8]
Wastewater after primary settling	Batch	74.4 % NH_4^+-N 68.5 % TN	90.6 % TP	
Effluent from aeration tank	Batch	62.5 % NH_4^+-N 50.8 % TN	4.69 % TP	
Centrate from sludge centrifuge	Batch	78.3 % NH_4^+-N 82.8 % TN	85.6 % TP	
Municipal Effluent	Batch	98.1 % NH_4^+-N 90.9 % TN	90.0 % TP	[9]
Municipal Effluent	Semi-continuous	98.4 % NH_4^+-N 93.6 % TN	91.8 % TP	
Municipal Effluent	Continuous	98.3 % NH_4^+-N 95.5 % TN	90.6 % TP	

Biomethane production from microalgal biomass has received attention, since biogas obtained from anaerobic digestion can be used for electricity generation [10]. Advantage of microalgal species over other energy crops such as sugar cane and canola is that, their growth does not require arid lands, which can be allocated for food production [11]. In addition, biomass residues after anaerobic digestion can be used for fertilizer production, which is a sustainable approach for agricultural activities. Microalgal technology also enables reduction of carbon dioxide levels in the atmosphere, while producing valuable end products [12].

Several microalgal species have been studied as feedstocks for biomethane production. These feedstocks include mixed culture of S*cenedesmus spp*. and *Chlorella spp*., the mixed culture of *Scenedesmus spp.*, *Chlorella spp*., *Euglena spp.*, *Oscillatoria spp.*, and *Synechocystir sp.*, the culture of *Scenedesmus sp*. alone, and together with either *Spirulina sp*., *Euglena sp*., *Micractinium sp*., *Melosira sp.*, or *Oscillatoria sp*. Table 2 provides a summary of biomethane production studies using microalgae.

TABLE II. BIOMETHANE PRODUCTION STUDIES USING MICROALGAE

	HRT (day)	Biogas Yield (mL/g VS)	Methane Content(%)	Ref.
Mesophilic Digestion				
Algae Only	28	418.3	73	[13]
Algae Only	30	412.0	61.1	[14]
50/50 Sludge/Algae	28	290.9	64	[13]
Algae (*Scenedesmus* spp. and *Chlorella* spp.) + Waste paper	10	100-140	N/A	[15]
Arthrospira platensis *Chlamydomonas reinhardtii* *Chlorella kessleri* *Dunaliella salina* *Euglena gracilis* *Scenedesmus obliquus* *Zea mays*		481 ± 13.8 587 ± 8.8 335 ± 7.8 505 ± 24.8 485 ± 3 287 ± 10.1 653 ± 37.7	61 66 65 64 67 62 54	[11]
Thermophilic Digestion				
Algae Only	30	493.2	62.1	[14]
50/50 Sludge/Algae (40˚C)	28	449.5	66	[13]
50/50 Sludge/Algae (55˚C)	28	299.7	62	[13]

Although cellulose and lignin contents of microalgae are almost zero and anaerobic process stability and digestion efficiency are high, production cost of methane is still higher than other feedstocks. Therefore, integrated wastewater treatment and biomethane production, which is a process first discussed by [13], can be the most feasible approach to reduce production cost [16]. Degree of anaerobic degradation of microalgae can further be improved by several pretreatment methods. Some researchers investigated different pretreatment methods for enhanced methane production such as microwave pretreatment [17]; ultrasound pretreatment [18]; thermal pretreatment including drying [11], heating [19], thermal hydrolysis [18] or high pressure thermal hydrolysis [20]; chemical pretreatment [19] and biological pretreatment [18]. Each pretreatment method brings various benefits and shortcomings. However, thermal pretreatment and its combination with alkali pretreatment has been proven to be effective for many feedstocks such as corn stover and municipal organic wastes and complex materials [21].

In this study, parallel nutrient (nitrogen and phosphorus) removal from wastewater, and anaerobic digestion of pretreated and non-pretreated algal biomass was investigated. A semi- continuous photo-bioreactor was operated for investigation of nutrient (N and P) removal efficiency of unialgal culture, *Chlorella vulgaris*. Then, produced biomass was subjected to heat, autoclave or thermochemical pretreatments. Following pretreatment, biomass was used in Biochemical Methane Production (BMP) Assays.

II. MATERIALS AND METHODS

A. Microalgae

Unialgal culture of *Chlorella vulgaris* (CCAP 211/11B) was obtained from Culture Collection of Algae and Protozoa, UK.

B. Anaerobic Seed

Mixed anaerobic cultures were obtained from the anaerobic sludge digesters of Greater Municipality of Ankara Tatlar Domestic Wastewater Treatment Plant. Characterization of the seed culture is given on Table 3.

TABLE III. ANAEROBIC SEED AND MICROALGAL SLURRY CHARACTERIZATIONS

Parameter	TS (mg/L)	VS (mg/L)	VS (%TS)	tCOD (mg/L)
Anaerobic Seed	38900 ± 566	13300 ± 0.0	32.6	19762 ± 12

C. Batch Cultivation Medium

Enhanced Bold's Basal medium (3N-BBM+V) was used for batch cultivation of *C. vulgaris*. The content of the medium is as follows (g/L); $NaNO_3$: 0.75, $CaCl_2 \cdot 2H_2O$: 0.025, $MgSO_4 \cdot 7H_2O$: 0.075, $K_2HPO_4 \cdot 3H_2O$: 0.075, KH_2PO_4: 0.175, NaCl: 0.025, Na_2EDTA: 0.0045, $FeCl_3 \cdot 6H_2O$: 5.84×10^{-4}, $MnCl_2 \cdot 4H_2O$: 2.46×10^{-4}, $ZnCl_2$: 3×10^{-5}, $CoCl_2 \cdot 6H_2O$: 1.2×10^{-5}, $Na_2MoO_4 \cdot 2H_2O$: 2.4×10^{-5}, Vitamin B1: 0.0012, Vitamin B12: 0.00001.

D. Wastewater

Wastewater used during semi-continuous cultivation studies was obtained from primary clarifier effluents of Greater Municipality of Ankara Tatlar Domestic Wastewater Treatment Plant located in Ankara, Turkey. Wastewater fed to the photobioreactor was collected as five different batches at different dates, namely, . Wastewaters were stored at 0^oC right after the only pretreatment applied to wastewaters, which was screening through a sieve with a pore size of 3 mm, in order to remove coarse particles. Wastewater characterizations and feeding cycles for which they are used are given on Table 4.

TABLE IV. WASTEWATER CHARACTERIZATION

Supply Date	Constituents (mg/L)						Feed Cycle
	TAN	*NO_3^--N*	*PO_4^{3-}-P*	*TN*	*tCOD*	*sCOD*	
1/1/13	25.1 ± 1.0	< 0.1	4.91 ± 0.03	29.4 ± 0.8	351.1 ± 2.81	82.2 ± 0.0	1
1/2/13	31.9 ± 1.3	< 0.1	5.392 ± 0.0	36.9 ± 0.6	368.4 ± 7.96	84.95 ± 1.06	2-4
1/4/13	28.8 ± 0.2	2.3 ± 0.0	1.8678 ± 0.0	35.1 ± 1.79	337.2 ± 1.39	73.0 ± 1.34	5-11
16/4/13	20.6 ± 0.1	4.4 ± 0.1	1.5 ± 0.0	23.9 ± 1.24	253.2 ± 8.44	65.5 ± 1.99	12-16
18/4/13	20.8 ± 0.4	1.5 ± 0.0	1.79 ± 0.0	25.3 ± 1.24	266.6 ± 4.29	67.1 ± 0.28	16-18

E. Analytical Methods

TSS, VSS, tCOD, sCOD and TKN values were determined according to Standard Methods (APHA, 2005). TN, TAN (NH_4^+-N + NH_3-N), NO_3^--N, PO_4^{3-} P analyses were using Lovibond test kit vials Vario 535560, Vario 535600, Vario NitraX 535580, Vario 515810 respectively (Aqualytic, Germany).

Optical density of any sample was measured using macro-cuvettes and spectrophotometer (HACH, DR 2800) at 685 nm wavelength with 1cm light path. Light intensity in culture medium was measured using PAR device (Li-Cor, 250 A) and DO values in photobioreactors were measured using Dissolved Oxygen Meter (Extech, 407510A).

During BMP assays, gas production of each reactor was measured by water displacement method [22]. Gas composition analysis was performed by a gas chromatograph (GC) (Agilent 6890N) equipped with a thermal conductivity detector and capillary column CP-Sil 8 (CP8752, Varian) to detect CH_4 content. The temperatures of the oven, injector and detector were 45, 100 and 250°C, respectively. Helium was employed as a carrier gas at pressure of 28.3 kPa.

F. Experimental Sets and Procedures

Batch Cultivation Photobioreactor (BCP)

Microalgal culture was cultivated in a 3300 L cylindrical bubble column reactor with 3000 ml of working volume. Reactor is constructed with 0.3 cm thick glass. The diameter of the reactor is 8 cm. The BCR is capped with a glass stopper with a tube for gas exit. An aeration tube with inner diameter of 0.5 cm is submerged into the reactor through the stopper and is connected to an air pump (RESUN, AC-9602). Gas inlet and outlet of the reactor are equipped with 0.2 µm syringe filters. The BCP was operated in sterile conditions with 16 h : 8 h day – night cycle. Day cycle was obtained with eight 18 W cool-white fluorescent lamps (OSRAM, L 18W/685) in a cabin isolated from ambient light using black curtains. The closest pair of lamps, which provided light intensity of 120 $\mu mol/m^2 \cdot s$ was, 10 centimeters away from one

side of the reactor. At the opposite side of the reactor, light intensity was 100 μmol/m^2·s. These light intensity values are within the optimum range for *Chlorella vulgaris* cultivation [23]. BCP was continuously aerated with 0.5 vvm (volume air per volume broth per minute) air, in order to supply necessary carbon dioxide for growth and adequate mixing for light-dark cycle achievement. Temperature was 28 ± 2 ^{0}C in culture broth. Initially, pH was set to neutral.

Semi-Continuous Cultivation Photobioreactor (SCP)

In order to obtain microalgal biomass for BMP studies and determine nutrient removal potential of *Chlorella vulgaris*, a SCP for wastewater treatment was designed. The reactor has 40 L inner volume with length, width and height values of 32 cm, 25 cm and 50 cm respectively. The maximum working volume of the photobioreactor is 35L. SCP was equipped with two identical air pumps (RESUN 9602, PRC), each connected to the smallest surface of a pair of spargers with dimensions of 25 cm x 2 cm x 2 cm. Light was provided to SCP with eight cool-white 18 W fluorescent lamps (OSRAM, L 18W/685), placed four by four on largest surfaces of the photobioreactors. The distance between each lamp and the photobioreactor surface is 5 centimeters. Lamps are 8 cm away from and parallel to each other and symmetrically aligned at each surface of the photobioreactor, providing 150 μmol/m^2.s PAR at the surface and 80 μmol/m^2.s in the center of the photobioreactor, when filled with water.

The SCP was continuously illuminated for biomass production; enhancing continuous photosynthesis for 24 h. Aeration was also continuously supplied with 0.5 vvm air. pH was tending to elevate up to alkaline levels, therefore pH was lowered down to 6.0 ± 0.5 using 37% HCl and its increase above 9.3 was avoided, in order to prevent decrease in carbon dioxide uptake capacity and escape of ammonia by stripping.

SCP was started with 0.5 L microalgal culture and 3.5 L wastewater. Characteristics of the inoculated culture broth are given on Table 5. For the first four cycles, photobioreactor was operated in fed-batch mode. Afterwards, operation mode was switched to semi continuous. Rather than setting hydraulic residence time and performing feeding / wasting operation on diurnal basis, feedback from the reactor was gathered periodically to determine optimum time for wasting and feeding. That is, soluble nitrogen (TAN and NO_3^--N) concentrations in the reactor were measured frequently as a feedback from the system, since wastewater obtained were N-limiting. When a minimum of 75% soluble nitrogen removal was achieved on mass basis, photobioreactor was fed with fresh wastewater. Wasting and feeding volumes were determined not only considering the feedback, but also providing a relatively constant ratio between microalgae and soluble nitrogen on a mass basis, at the beginning of each cycle.

TABLE V. CULTURE BROTH CHARACTERISTICS

NO_3^--N (mg/L)	PO_4^{3-}-P (mg/L)	sCOD (mg/L)	OD (Abs.)
19.6 ± 0.94	44.2 ± 1.1	149.5 ± 0.7	3.92 ± 0.006

In addition to optical density and soluble inorganic nitrogen species, PO_4^{3-}- P concentrations in the reactor at the beginning and end of each cycle were determined.

G. Microalgae Harvesting

Effluents of SCP were collected at the end of each cycle, centrifuged at 4000 rpm for 30 minutes. The recovery efficiency was calculated measuring optical densities of the effluents and final supernatants and found out to be over 90%. Characterization of microalgal slurry is given on Table 6.

TABLE VI. MICROALGAL SLURRY CHARACTERIZATION

Parameter	Untreated Microalgae
TS (mg/L)	33250 ± 70
VS (mg/L)	27950 ± 70
VS as %TS	84.1
TSS (mg/L)	32186 ± 702
VSS (mg/L)	26753 ± 685
VSS as %TSS	81.2
tCOD (mg/L)	42943 ± 285

H. Microalgal Slurry Pretreatment

For pretreatment studies, three 500 mL autoclave bottles were filled with 200 mL microalgal slurry. Table 7 summarizes pretreatment procedures followed. After pretreatment, all bottles were stored at 0^{o}C prior to characterization and BMP assays. Characterizations of pretreated microalgal slurry are given on Table 8.

TABLE VII. PRETREATMENT METHODS SUMMARY

Pretreatment Type	Temperature	Pressure	pH	Duration
Heat	121 ^{0}C	Atmospheric	Neutral	120 min.
Autoclave	121 ^{0}C	5 psi	Neutral	5 min.
Thermochemical	121 ^{0}C	Atmospheric	Alkaline (12.0)	120 min.

TABLE VIII. CHARACTERIZATION OF PRETREATED MICROALGAL SLURRY

Parameter	Untreated Microalgae	Pretreated Microalgae		
		Heat	*Autoclave*	*Thermochemical*
TS (mg/L)	33250 ± 70	34200 ± 283	30340 ± 300	33700 ± 990
VS (mg/L)	27950 ± 70	28350 ± 495	27091 ± 236	27300 ± 707
VS as %TS	84.1	82.8	81.0	81.0
TSS (mg/L)	32186 ± 702	32875 ± 318	27966 ± 416	32933 ± 76
VSS mg/L)	26753 ± 685	27275 ± 177	23200 ± 200	26350 ± 150
VSS as%TSS	81.2	83.0	83.0	80.0
tCOD (mg/L)	42943 ± 285	41768 ± 289	40659 ± 425	42721 ± 236

I. BMP Assay

Reactors of 100 mL total volume and 71 mL effective volume were used in the experiments. The test reactors were seeded with various concentrations of untreated or pretreated microalgal slurry, as shown on Table 9 and 6 mL of concentrated anaerobic seed sludge. Two COD values, approximately 19000 mg/L and 34000 mg/L were initially maintained in each test reactor for untreated microalgae and each pretreatment type All reactors were completed up to effective volume with distilled water. Control reactors were run without any algal biomass but seed sludge. After addition of all the constituents, effective volumes of the reactors were purged with nitrogen gas for 3 minutes and headspaces were purged for an additional minute. Reactors were capped with rubber septa and placed in a constant-temperature room at 35±1^{o}C for batch mode incubation. Daily gas productions were measured and gas compositions were analyzed.

TABLE IX. COMPONENTS OF BMP ASSAY REACTORS

Reactor Ccode	Pretreatment Type	Microalgal Slurry Volume (mL)	Total VS (mg/L)	Total COD (mg/L)
C	-	0	2997	4454
A1	Untreated	49	21596	34091
A2	Untreated	24.5	12297	19272
H1	Heat	49	22563	33279
H2	Heat	24.5	12780	18866
At1	Autoclave	49	21694	32514
At2	Autoclave	24.5	12345	18484
Tc1	Thermochemical	49	21838	33937
Tc2	Thermochemical	24.5	12418	19195

III. RESULTS AND DISCUSSIONS

A. Batch Cultivation of Chlorella Vulgaris

Unialgal culture of *Chlorella vulgaris* had cultivated in 3NBBM + V medium for 56 days in batch mode. Correlation of TSS with optical density values was determined. TSS values were estimated further in semi- continuous cultivation studies using Equation (1), which represents the relationship between TSS and OD measured at 685 nm wavelength.

$$[TSS(mg/L)] = 203.77\ [OD_{685}\ (Abs.)]\ ;\ R^2 = 0.998 \quad (1)$$

B. Semi Continuous Cultivation of Chlorella Vulgaris

In order to produce microalgal biomass for BMP studies and to determine nutrient removal potential of the culture, semi- continuous cultivation reactor had been operated for 21 days. During the first four cycles with fed- batch operation, N/TSS_{algae} ratio was 0.18, 0.22, 0.18 and 0.18 respectively. When semi- continuous cultivation was started, N/TSS_{algae} value was fixed at 0.13.

Initial optical density in the reactor was 0.7 during the first cycle and decreased to 0.5 by the second cycle. Then, initial optical densities per cycle gradually increased up to 0.7 at the beginning of cycle 10. Starting from cycle 11, nutrient concentrations of the fed wastewater decreased. As a result, lower optical density value corresponded to the constant N/TSS_{algae} ratio. Until cycle 10, final optical density of each cycle had a tendency to increase compared to the previous cycle. Maximum optical density value was observed at day 10 as 1.472, at the end of cycle 8 (Fig. 1a).

As N/TSS_{algae} values were taken into account for determination of the wastewater amount to be fed in SCP at the beginning of each cycle, daily volume of added wastewater varied in relation to the optical density reached at the end of the previous cycle. Without altering the ratio, daily wastewater volume and effective reactor volume were changed. Although wastewater volume changed between 8.5 L and 14.5 L, effective reactor volume was kept constant at 25L (Fig. 1b).

Since TAN, which is converted into NO_3^--N in the SCP, cannot be considered as treated, nitrogen removal efficiencies were calculated by taking into account the difference between sum of TAN and NO_3^--N concentrations in influent wastewater and effluent from each cycle. Ortho-P removal efficiencies were also calculated, considering influent concentration in wastewater and effluent of each cycle. As shown in Fig. 1c, nitrogen removal efficiencies were above 80%, except for cycles 4, 5, 7 and 10. However, the removal efficiencies in these four cycles were acceptable in terms of feeding protocol. In order to completely remove nitrogen fed to the SCP in each cycle, N/TSS_{algae} ratio was reduced from 0.18 to 0.13. Then, cycle duration was increased. Maximum N removal efficiency of 99.6% was achieved at the end of cycle 9. When wastewater strength decreased, removal efficiencies were over 90%, with the maximum value of 92.5% at cycle 18.

Phosphorus removal efficiencies were unclear for the first four cycles, due to the interference of culture broth (3N BBM + V) with wastewater phosphorus concentrations (Table 5). When this effect disappeared, the wastewater phosphorus concentrations in primary clarifier effluents of treatment plant started to decrease. Therefore, remaining phosphorus from previous cycles interfered with phosphorus removal efficiency calculations, until the phosphorus content in the reactor became lower than the concentration in feeding wastewater, which corresponds to cycle 13. As shown in Fig. 1d, phosphorus removal efficiency in SCP was over 80% afterwards. Maximum phosphorus removal efficiency of 91.2% was observed at the end of cycle 17.

Nutrient removal efficiencies observed in this study are in consistency with similar studies. For example, ammonium and phosphorus removal efficiencies of high rate algal ponds were reported as 89% and 49% respectively [24]. [9] conducted batch, modified semi- continuous and continuous cultivation experiments of *Chlorella vulgaris* in municipal effluent. It was

found that ammonia-N, total nitrogen and total phosphorus can effectively be removed by 98.0%, 90.3 – 93.6% and 89.9 – 91.8% respectively.

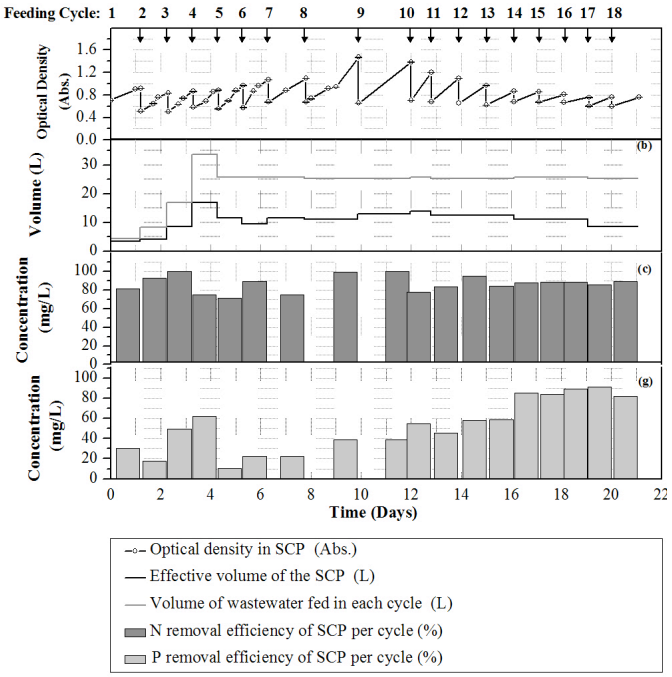

Fig. 1. SCP Operation Kinetics and Nutrient Removal Efficiencies: (a) OD variations in SCP; (b) Fed wastewater volume and effective volume variations in SCP; (c) N removal efficiency of SCP per cycle; (d) P removal efficiency of SCP per cycle.

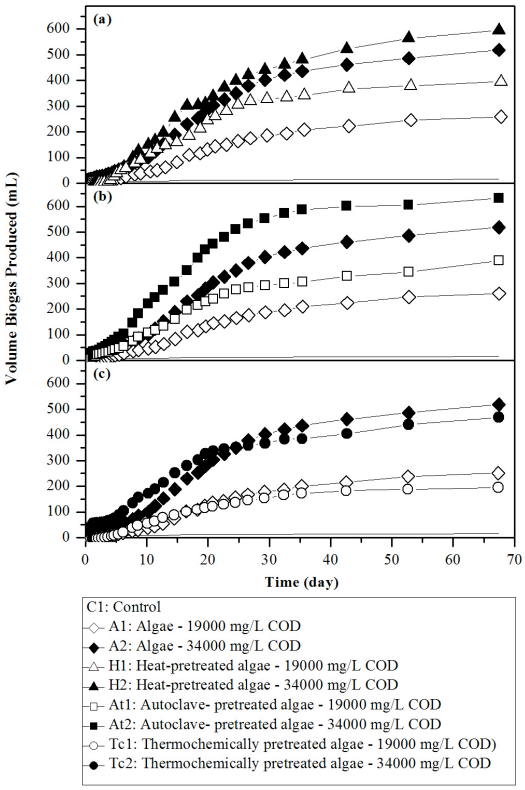

Fig. 2. Cumulative biogas production data of pretreated microalgae reactors with 19000 ± 500 and 34000 ± 1500 mg/L COD: (a) Untreated Algae and Heat- pretreated Algae; (b) Untreated Algae and Autoclave- pretreated Algae; (c) Untreated Algae and Thermochemically- pretreated Algae

C. BMP Assay

In order to investigate effect of pretreatment on AD of microalgae, eight anaerobic batch reactors with initial COD values of either 19000 ± 500 mg/L or 34000 ± 1500 fed with untreated or pretreated microalgae had been operated for 66 days. Reactors were evaluated in terms of biogas production potential, biogas and methane yields. Gas production values were calculated after subtraction of cumulative biogas volume produced in control reactor. In all reactors, gas production was observed; however, varied among reactors with different COD values. Fig. 2 depicts cumulative biogas production data of batch reactors with initial COD values of 19000 ± 500 mg/L and 34000 ± 1500 mg/L, containing untreated or pretreated microalgae as substrates.

In order to determine methane production yields of reactors, methane contents were determined on volume percentage basis. Measurements were started after the headspaces of reactors were washed with produced biogas; that is, when produced biogas volume exceeded three-folds volume of headspace. Weighted average percent methane values content in reactors were used for determination of methane yields and are given on Table 10.

TABLE X. BIOGAS AND METHANE YIELDS OF TEST REACTORS

Parameters	Reactor Code							
	A1	*A2*	*H1*	*H2*	*At1*	*At2*	*Tc1*	*Tc2*
Biogas Yield (mL/g VS_{added})	390	379	580	428	595	476	315	350
Methane Yield (ml CH_4 / g VS_{added})	223	249	393	291	398	332	195	258

As shown on Table 10, among reactors fed with untreated microalgae, maximum biogas production yield of 390 mL/ VS_{added} was achieved in A1, with initial COD concentration of 34000 ± 1500 mg/L whereas maximum methane yield was achieved as 249 ml CH_4 / g VS_{added} in reactor A2, which initially contained 19000 ± 500 mg/L COD. Maximum positive effect of pretreatment on biogas yield was reported as 595 mL/ VS_{added} for reactor At1, which contained autoclave-pretreated microalgae with the initial COD concentration of 34000 ± 1500 mg/L. The maximum methane yield was also observed in this reactor. Among reactors with lower initial COD concentrations of 19000 ± 500 mg/L, maximum biogas and methane yields were observed as 476 mL biogas/ VS_{added} and 332 ml CH_4 / g VS_{added} respectively in At2, which contained autoclave-pretreated microalgae. It can be clearly seen from Table 2 that these findings are in consistency with literature. Compared to reactors with untreated microalgae with came initial COD concentrations; namely, A1 and A2,

the increase in methane yields were 79% and 33% respectively for low-COD and high-COD reactors (3). In reactors fed with heat – pretreated microalgae, higher performance in terms of biogas and methane yields were observed, when compared to that of reactors with untreated microalgae. However, thermochemical pretreatment caused even lower or slightly higher performance than untreated microalgae utilization as substrate, for low-COD and high-COD reactors.

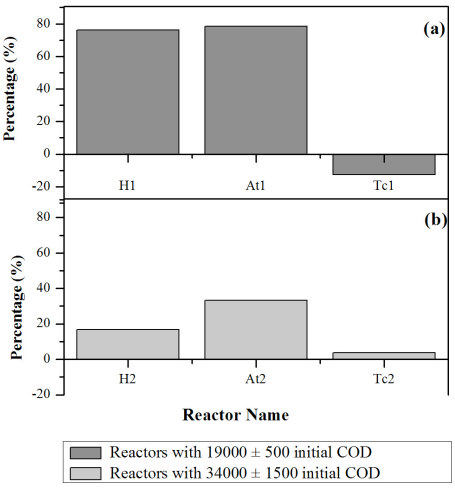

Fig. 3. Cumulative biogas production data of pretreated microalgae reactors with 19000 ± 500 and 34000 ± 1500 mg/L COD: (a) Untreated Algae and Heat- pretreated Algae; (b) Untreated Algae and Autoclave- pretreated Algae; (c) Untreated Algae and Thermochemically- pretreated Algae

IV. CONCLUSIONS

In this study, semi- continuous nutrient removal potential from primary clarifier effluents of domestic wastewater treatment plant, using microalgae was investigated. In the test runs, it was found that 99.6% of inorganic nitrogen and 91.2% of inorganic phosphorus present in domestic wastewater can be removed by *Chlorella vulgaris.*

Besides nutrient removal potential of *Chlorella vulgaris*, it was also revealed that harvested microalgae from wastewater treatment systems can be converted into bioenergy via biomethane production. Results of this study showed that methane yield of 249 ml/ g VS_{added} can be obtained by anaerobic digestion of *Chlorella vulgaris* without pretreatment. After autoclave pretreatment, methane yield can be increased to 398 ml/ g VS_{added}, which corresponds to 79% of the value without pretreatment.

Considering outcomes of the present study, it can be stated that microalgal nutrient removal is a feasible method for decreasing stress on ecological integrity due to nutrient discharge. Moreover, biomass produced as an end product of microalgal nutrient removal systems can be used as a renewable energy source.

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