Seaweed Res. Utiln., 38(2) : 83 - 86, 2016

Enhancement of biomethane production from microalgae by biological disintegration

R. YUKESH KANNAH, S. KAVITHA AND J. RAJESH BANU*
Department of Civil Engineering, Regional Campus of Anna University, Tirunelveli
**E-mail : rajeshces@gmail.com*

ABSTRACT

Microalgae-based biofuels have an enormous market potential and it can displace imports of fossil fuels from other countries. Microalgae biomass may be processed generating bioenergy (methane) through anaerobic digestion (AD) process. On the other hand, microalgae biomass has lower biodegradability due to its complex cell wall structure. This in turn limits the production of methane. Therefore, in order to enhance the AD of microalgae, pretreatment (disintegration) processes are essential before AD to improve the generation of methane. Among the pretreatment techniques, biological pretreatment is considered as the best due to mild reaction conditions, less energy consumption and absence of chemical contamination and inhibitory by products. Biological pretreatment includes external addition of purified enzymes, single or mixture of enzymes or adding enzyme secreting bacterial cells. However, commercially available enzymes are very expensive. Therefore, pretreatment of Microalgae biomass with cellulolytic bacteria was considered as best since cellulose is the main constituent of algal cell wall. In the present study, the cellulolytic bacterial pretreatment substantially increased the solubilization percentage, protein and carbohydrate release. Higher methane production of about (0.2 L/g COD) was obtained for biologically pretreated algal biomass when compared to control (0.08 L/g COD), which exposes the efficiency of biological disintegration of algae.

Introduction

Microalgae have been explored in recent years for bioenergy generation owing to their elevated photosynthetic activity, ability to accrue lipids and capability to develop in saline, brackish and waste waters (Park *et al.,* 2011). Now microalgae biomass has become an option for alternative biofuel production. Microalgae biomass may be processed for transformation into bioethanol, biodiesel and/or biogas. Several benefits of microalgae biomass are such as feedstock for biofuel production, food, and biomethanation. After biological pretreatment, the residues obtained from microalgae biomass might be recycled as fertilizer or feed. Similarly, microalgae are indirectly supportive to living organism by trapping CO_2 from atmosphere. However, it has been revealed that biogas production through anaerobic digestion is the most promising technology (Kavitha *et al.,* 2015a). *Chlorella vulgaris* is one of the most attractive algal species for producing biofuels owing to its fast growth and easy cultivation.

There are various pretreatment such as thermal, hydrothermal, ultrasonic, high pressure homogeniser, chemical, microwave, ozonation, mechanical, thermochemical, thermochemo mechanical and biological disintegrations (Rajesh Banu *et al.,* 2001; Roesijadi *et al.,* 2010; Kavitha *et al.,* 2014a; Vimala *et al.,* 2015; Uma Rani *et al.,* 2014) for enhancing microalgae anaerobic biodegradability (Passos *et al.,* 2014; 2015; González Fernandez *et al.,* 2011). Among the various pretreatments, biological pretreatment provides unique advantages compared to chemical or physical processes, as it is environmentally friendly and causes neither pollution nor needs extraordinary equipment (Kavitha *et al.,* 2015c). Therefore, biological pretreatment is the most potential method which operated under mild conditions with enzymatic cell wall degradation. The main objectives of the proposed study are to: i) To collect and analyse initial characteristics of algal biomass. ii) To culture the microalgae biomass in mass. iii) To study the efficiency of bacterial pretreatment for effective algal cell lysis. iv) To assess the anaerobic biodegradability of pretreated microalgae biomass.

Received : Oct. 2016; Revised : Sep. 2016; Accepted : Nov. 2016

Materials and Methods

Collection of sample

The microalgal sample *(Chlorella vulgaris)* was collected from Phycospectrum Environmental Research Centre (PERC), Chennai. Then the sample was stored at 4 ^{0}C and the primary characteristics were analysed.

Culture conditions

In this current study, Bold Basal culture medium (BBM) was used to cultivate the microalga. The culture medium comprised macronutrients such as $NaNO_3$, $MgSO_4.7H_2O$, NaCl, K_2HPO_4, KH_2PO_4, $CaCl_2.2H_2O$ and micronutrients such as $ZnSO_4.7H_2O$, $MnCl_2.4H_2O$, MoO_3, $CuSO_4.5H_2O$, $Co(NO_3)_2.6H_2O$, H_3BO_3, EDTA, KOH, $FeSO_4.7H_2O$ (Ross *et al.,* 2008). 30 lit. of Cylindrical photobioreactor was used to culture the sample. The culture was mixed using filtered air (0.2 μm membrane filter) flow of 0.6 l/min without any additional CO_2 supply.

Growth dynamics of inoculated microalgae sample

Experimental growth dynamics of microalgal sample were conducted using 1000-ml conical flask with 800 mL of Bold Basal culture medium (BBM). Growth of the inoculated microalga was monitored at regular time intervals (Kavitha *et al.,* 2014c). The optical density method ($OD_é$ = 600 nm) to estimate the biomass concentration (mg/l) by comparing it with a previously generated calibration curve of measured absorbance value.

Isolation of Cellulolytic bacteria

A Cellulolytic bacteria was isolated from paper mill sludge using Carboxyl methyl Cellulose (CMC) agar (Kavitha et al., 2013). The optimum pH, temp and time for bacteria was 6.5 at 40 ^{0}C for 36 hours.

Algal biomass pretreatment with cellulolytic bacteria

100 ml of algal biomass was pretreated with 2 g dry cell weight/l of cellulolytic bacteria.

Biochemical methane Potential (BMP) assay

BMP tests were carried out in serum bottles with a total volume of 150 ml, a useful volume of 120 ml and a gas headspace volume of 30 ml. Two identical serum bottles B1 and B2 were as control (untreated) and experimental value (pretreated) respectively. The substrate (untreated and pretreated microalgae biomass) and inoculum (anaerobically digested sludge) ratio was maintained as 1:2. The following kinetic model was employed to study the cumulative methane production through nonlinear regression modelling according to techniques described by Kavitha *et al.* (2015a; 2014b) using equation 1

$$Y(t) = Y(f_d) \cdot (1-\exp(-k_{hyd} * t))$$

The model was implemented in Mat lab 2012a Version. The parameter estimation and parameter uncertainty evaluation used was calculated with a 95% confidence limit for significance examining and parameter uncertainty investigation.

Analytical methods

The parameters of microalgae such as TCOD, SCOD were analysed as per the Standard methods (APHA, 2005).

Results and Discussion

Initial Characteristics of Sample

Microalgae biomass initial characteristics were characterised as per standard methods and the outcome of results such as Total chemical oxygen demand (TCOD) 3161 ± 100 (mg/L) and Soluble chemical oxygen demand (sCOD) 30 ± 5 (mg/L).

Growth dynamics of inoculated microalgae sample

Microalga growth rate was examined using Bold Basal Medium and the results are given in the figure 1. Daily 1 ml of liquid sample was withdrawn in order to measure the growth rate of microalgae biomass. The experiment was carried out for 40 days to figure out the growth dynamics of microalgal sample. The microalgal growth rate was categorised into different phases (Fig. 1). In the first phase, inoculated microalgal sample custom suitable environment to growth in the culture medium is otherwise known as Lag or accumulation phase (0th to 5th day). During the exponential growth phase, the concentration of microalgal biomass showed steady increase from 6th to 27th day. After 27th till 40th day it showed slight increase in the concentration of biomass and it is named as stationary or stabilisation phase. The maximum turbidity of liquid sample or colour changes were for each sample was observed using a UV–visible spectrophotometer at 600nm. The measured absorbance values was acceptable to estimate the biomass concentration (mg/L) by comparing it with a previously generated calibration curve as stated above. The specific

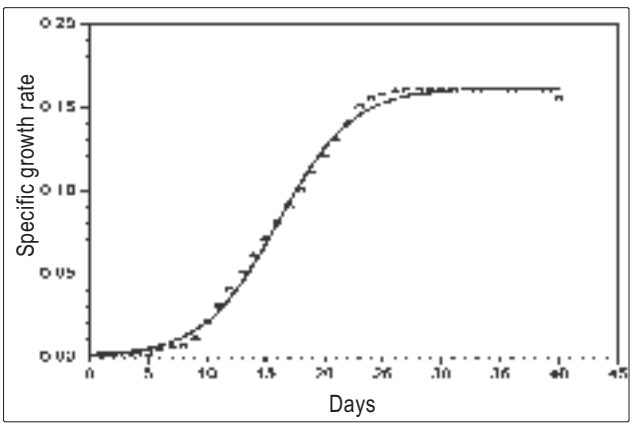

Fig. 1. *Specific growth rate of inoculated microalgae growth*

growth rate (¼) of inoculated microalgae sample was calculated based on calculation described by Kavitha *et al.* (2014c) using equation 2.

$$\mu = \frac{(\text{Ln } N_t - \text{Ln } N_0)}{(t_t - t0_)}$$

Where N_t is the microalgae biomass density (g/L) at time t (expressed in hours) and N_0 is the microalgae density at time zero (0^{th} hour). Such maximum growth rate was estimated during the exponential growth phase based on the best slope fit to the experimental data and making sure reproducible results were obtained. Whenever needed, an additional experiment was performed to confirm reproducibility.

Bacterial pretreatment

During bacterial pretreatment, the cell wall of microalga was broken and the intracellular constituents were transfer to the supertant (liquid portion). The transformation of constituents into the supertant leads to increase the soluble chemical oxygen demand (sCOD) value. The cell walls of many species of microalgae are multi-layered and contain a relatively large proportion of cellulose. Cellulose is a linear polymer of 1, 4-D-anhydroglucopyranose units, that formulates it as extremely firm and opposed to degradation. Biomass is first subjected to cellulolytic bacterial pretreatment of the cell wall to cell lysis (Rajesh Banu *et al.,* 2016; Kavitha *et al.,* 2015b). After the pretreatment, the cell wall of microalgae was viewed in the microscopic to analysis the pretreatment efficiency. The figure 2 and 3 (microscopic image) show the cell structure of raw microalgal sample and bacterial pretreated sample respectively. The outcome of bacterial pretreatment shows higher sCOD concentration as 540 mg/L when compared to control (untreated sample).

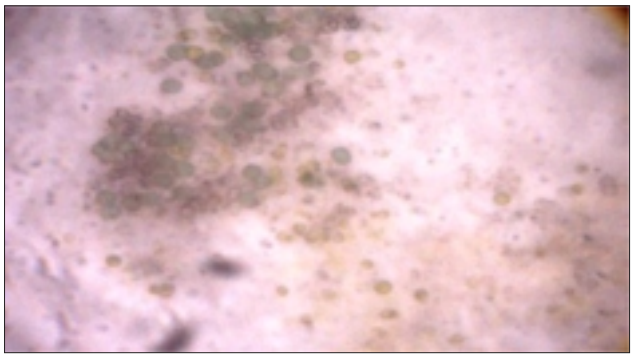

Fig. 2. *Microscopic image of untreated microalgae biomass*

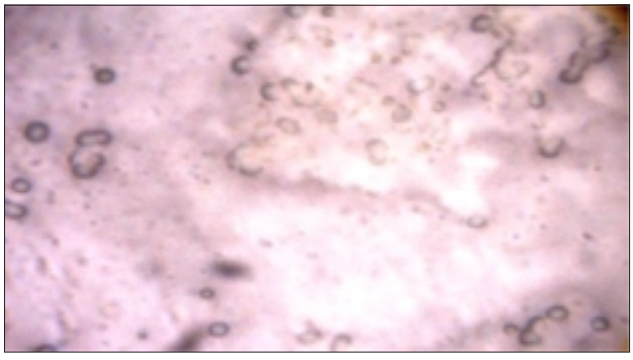

Fig. 3. *Microscopic image of pretreated microalgae biomass*

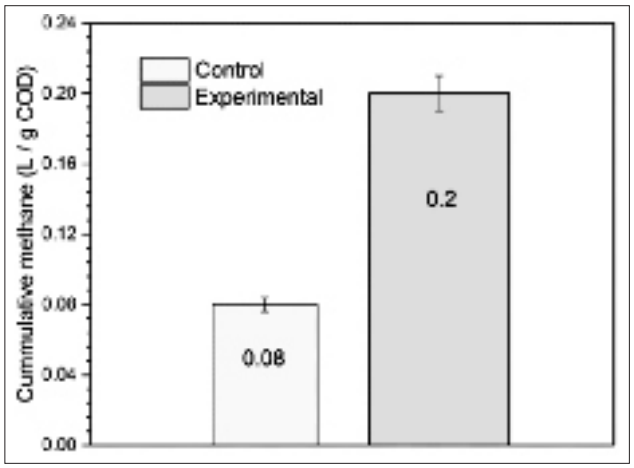

Fig. 4. *Cumulative methane production*

Anaerobic biodegradability assay

Anaerobic biodegradability assays have been employed to evaluate and compare the biogas production efficiency of pretreated and raw microalgae sample. The initial TCOD was found 3161 ± 100 mg/L for both control and pretreated samples. During the start of the experiment the initial sCOD of control and pretreated sample were found to be 30 and 540 mg/L. To let methanogenic bacteria to get access to the cellular materials (Rajesh Banu *et al.,* 2009; Merline *et al.,* 2013; Kavitha *et al.,* 2015c) present inside the cells of microalgae, overall biodegradability and methane production. The outcomes of BMP assay as given in the figure 4 reveals that the higher biogas production was achieved for biologically pretreated microalgae biomass than the untreated microalgae biomass.

Based on the results it can be concluded that using bacteria with cellulolytic activity is the promising and potential pretreatment method for pretreating the microalga biomass efficiently. Eventhough the biogas production improvement was not as high as like that of mechanical pretreatments, it is still capable due to its low energy needs, ease operation and mild operating conditions. The maximum methane yield was 0.08 L/g COD and 0.2 L/g COD in control (raw microalgae) and experimental (pretreated microalgae) condition.

Acknowledgements

The authors are thankful to Dr. V. Sivasubramanian, Director, Phycospectrum Environmental Research Centre, Chennai for research support.

References

APHA 2005. Standard Methods for the Examination of Water and Wastewater, 21st edition. American Public Health Association, Washington, D.C.

González-Fernández, C., B. Sialve, N. Bernet and J.P. Steyer 2011. Impact of microalgae characteristics on their conversion to biofuel. Part II: focus on biomethane production. *Biofuels Bioprod. Biorefin.* 6: 205–218.

Park, J.B.K., R.J. Craggs and A.N. Shilton 2011. Wastewater treatment high rate algal ponds for biofuel production. *Bioresour. Technol.,* 102: 35–42.

Passos, F., E. Uggetti, H. Carrère and I. Ferrer 2014. Pretreatment of microalgae to improve biogas production; a review. *Bioresour. Technol.,* 172: 403–412.

Passos, F., R. Gutiérrez, D. Brockmann, J.P. Steyer, J. García, I.Ferrer 2015. Microalgae production in wastewater treatment systems, anaerobic digestion and modeling using ADM1. *Algal Res.,* 10: 55–63.

Roesijadi, G., S.B. Jones, L.J. Snowden Swan and Y. Zhu 2010. Macroalgae as a Biomass Feedstock: A Preliminary Analysis. US DOE, PNNL-19944.

Ross, A.B., J.M. Jones, M.L. Kubacki, T. Brideman 2008. Classification of microalgae as fuel and its thermochemical behavior. *Bioresour. Technol.,* 99: 6494 – 6504.

Rajesh Banu, J., Do Hac-Uan, S. Adish Kumar and Ick-Tae Yeom 2001. Effect of low temperature thermochemical pretreatment on sludge reduction capacity of MBR treating secondary treated dairy wastewater. *Water Quality Research Journal of Canada.* 46(4): 312-320.

Rajesh Banu, J., S. Kaliappan and Ick-Tae Yeom 2009. Combinative treatment of domestic wastewater using anaerobic and solarphoto catalytic treatment" has been accepted for publication in the *Water Quality Research Journal of Canada.* 44 : 4.

Rajesh Banu, J., U. Ushani, S. Merline and S. Kaliappan 2016. Evaluation of operational parameters for biodegration of bacterialy disintegrated sludge. Desalination and water treatment.

Merline, Adish Kumar, S. Kaliappan, Ick-Tae Yeom and J. Rajesh Banu 2013 Biological pretreatment of non-flocculated sludge augments the biogas production in the anaerobic digestion of municipal solid waste. *Environ. Techn.,* 34(13-16): 2113-2123.

Kavitha, S., S. Adish Kumar, K.N. Yoga Lakshmi, S. Kaliappan and J.Rajesh Banu 2013. Effect of enzyme secreting bacterial pretreatment on enhancement of aerobic digestion potential of waste activated sludge interceded through EDTA. *Bioresour. Technol.,* 150: 210–219.

Kavitha, S., C. Jayashree, S. Adish Kumar, S. Kaliappan and J. Rajesh Banu 2014a. Enhancing the functional and economical efficiency of a novel combined thermo chemical disperser disintegration of waste activated sludge for biogas production. *Bioresour. Technol.,* 173: 32–41.

Kavitha, S., C. Jayashree, S. Adish Kumar, I.T. Yeom and J. Rajesh 2014b. The enhancement of anaerobic biodegradability of waste activated sludge by surfactant mediated biological pretreatment. *Bioresour. Technol.,* 168: 159–166.

Kavitha, S., C. Jayashree, S. Adish Kumar, I.T. Yeom and J. Rajesh 2014c. Improving the amenability of municipal waste activated sludge for biological pretreatment by phase-separated sludge disintegration. *Bioresour. Technol.,* 169: 700–706.

Kavitha, S., R. Yukesh Kannah, I.T. Yeom, K.U. Do and J. Rajesh Banu 2015a. Combined thermo-chemo-sonic disintegration of waste activated sludge for biogas production. *Bioresour. Technol.,*197: 383–392.

Kavitha, S., S. Kaliappan, S. Adish Kumar, I.T. Yeom and J. Rajesh 2015b. Effect of NaCl induced floc disruption on biological disintegration of sludge for enhanced biogas production. *Bioresour. Technol.,* 192: 807–811.

Kavitha, S., S. Saji Pray, K.N. Yogalakshmi, S. Adish Kumar, I.T. Yeom and J. Rajesh Banu 2015c. Effect of chemo-mechanical disintegration on sludge anaerobic digestion for enhanced biogas production. *Environ. Sci. Pollut. Res.,* 23: 2402– 2414.

Uma Rani, S. Kalippan, S. Adish Kumar, Ick-Tae Yeom and J. Rajesh 2014. Enhancing the anaerobic digestion potential of dairy waste activated sludge by two step sono-alkalization pretreatment. *Ultrasonics Sonochemistry,* 21(3): 1065-1074.

Vimala Ebenazar, Arul, S. Adish kumar, I.T. Yeom and J. Rajesh Banu 2015 Effect of deflocculation on the effciency of low-energy microwave pretreatment and anaerobic biodegradation of waste activated sludge" *Applied Energy.* 145: 104-110.