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Enhancement of biogas production from microalgal biomass through cellulolytic bacterial pretreatment

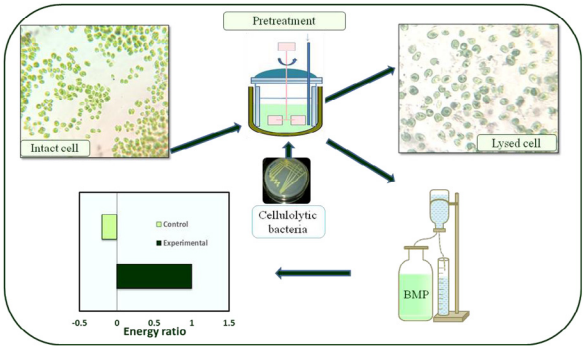
S. Kavitha [a,1], P. Subbulakshmi [a,1], J. Rajesh Banu [a,*], Muthukaruppan Gobi [b], Ick Tae Yeom [c]

[a] Department of Civil Engineering, Regional Campus, Anna University, Tirunelveli, India
[b] Department of Biotechnology, School of Bioengineering, SRM University, Potheri, Chennai, India
[c] Graduate School of Water Resource, Sungkyunkwan University, Suwon, South Korea

HIGHLIGHTS

- Microalgal biomass pretreatment by bacteria enhances liquefaction of about 18%.
- Bacterial pretreatment increases the macromolecular release considerably.
- Experimental microalgae improves the methane to 0.08 gCOD/gCOD comparing to control.
- Methane production rate increased with hydrolysis constant of about 0.24 day^{-1}.
- A positive energy ratio of about 1.04 was achieved in experimental microalgae.

GRAPHICAL ABSTRACT



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ABSTRACT

Generation of bioenergy from microalgal biomass has been a focus of interest in recent years. The recalcitrant nature of microalgal biomass owing to its high cellulose content limits methane generation. Thus, the present study investigates the effect of bacterial-based biological pretreatment on liquefaction of the microalga *Chlorella vulgaris* prior to anaerobic biodegradation to gain insights into energy efficient biomethanation. Liquefaction of microalgae resulted in a higher biomass stress index of about 18% in the experimental (pretreated with cellulose-secreting bacteria) vs. 11.8% in the control (non-pretreated) group. Mathematical modelling of the biomethanation studies implied that bacterial pretreatment had a greater influence on sustainable methane recovery, with a methane yield of about 0.08 (g Chemical Oxygen Demand/g Chemical Oxygen Demand), than did control pretreatment, with a yield of 0.04 (g Chemical Oxygen Demand/g Chemical Oxygen Demand). Energetic analysis of the proposed method of pretreatment showed a positive energy ratio of 1.04.

1. Introduction

In recent years, microalgae have drawn much attention in the energy field as a substitute to fossil fuels. Use of this energetic biomass improves fuel security and minimizes carbon dioxide release (Passos et al., 2013, 2014a). At the same time, microalgae are emerging as the most probable sustainable feedstock owing to their high yield. Among all the known sources of microalgal biomass, *Chlorella vulgaris* was found to contain a high content of organic matter and to possess high photosynthetic activity (Mahdy et al., 2014a). Energy-rich biogas generation from microalgal biomass through the anaerobic digestion (AD) process is a promising technology when considering biomass productivity

* Corresponding author at: Department of Civil Engineering, Regional Centre of Anna University, Tirunelveli 627007, India.
E-mail address: rajeshces@gmail.com (J. Rajesh Banu).
[1] These two authors have contributed equally in this work.

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and the efficient transformation of residues to biofuels (Cho et al., 2013). However, the potential of biogas generation via AD is restricted by the rate-limiting hydrolysis step. This is attributable to the recalcitrant cell walls of microalgal biomass, particularly the multifaceted cell wall arrangement that consists of slowly hydrolyzable cellulose (Alzate et al., 2012). Recalcitrant cellulose has been recognized as the major component of the cell walls of *Chlorella vulgaris* (Aydin, 2016; Ba et al., 2016). Cellulose acts as an obstacle to microalgal biomass, and hence confines the biodegradation of microalgae. (Bruhn et al., 2011; Ba et al., 2016; Munoz et al., 2014). This protects the microalgae from enzymatic attack by methanogenic microbes (Aydin, 2016). Lately many pretreatments (physical, chemical, and mechanical) have been investigated in an attempt to overcome such hydrolysis and to increase methane generation from microalgal biomass. Even though these physical and mechanical pretreatments enhance methane generation efficiently, they provide a negative energy balance in terms of net energy production (the energy spent during pretreatment is not compensated for by the energy gained as methane) as they are highly energy-demanding processes. Thus, biological pretreatment of microalgal biomass can be considered as a probable option to replace the energy-demanding physical and mechanical disintegration process (Mahdy et al., 2014b,c; Wei, 2016; Martin-Ryals et al., 2015). Live microbes including bacteria and fungi possess certain benefits during disintegration. One is the capability to constantly disintegrate organic matter during the different phases of growth and development (Aydin, 2016). Another is that biological pretreatment works under mild working conditions and results in a minimal level of inhibitory by-products (Miao et al., 2013). However commercially available enzymes are relatively high in cost, and they undergo denaturation under harsh environmental conditions. In the present study, in order to overcome these limitations and to attain a positive energy balance with a higher methane output, we planned to disintegrate the microalgal biomass, *Chlorella vulgaris*, with cellulase-secreting bacteria. The bacteria, *Bacillus sps*, used in the present study to disintegrate microalgal biomass is known to be an excellent cellulase-secreting bacteria (Wei, 2016). Cellulase is an essential enzyme that hydrolyzes cellulose. No work regarding bacterial pretreatment of microalgal biomass for enhanced methane generation and energetic balance analysis has been documented in the literature so far. In view of the fact that microalgal biomass is made up of recalcitrant cell walls, the present study focused on the ability of bacterial pretreatment of microalgal biomass with cellulase-secreting bacteria to enhance methane generation in an energy-efficient way. Thus, the objectives of the present study were to: 1) isolate cellulase-secreting bacteria and optimize the growth parameters of the bacteria through response surface methodology (RSM), 2) assess the efficiency of bacterial pretreatment in liquefaction of microalgal biomass, 3) investigate the kinetic modelling of microalgal liquefaction during bacterial pretreatment, 4) evaluate the efficiency of bacterial pretreatment of microalgal biomass in methane generation, and 5) investigate the feasibility of the proposed mode of pretreatment through energy balance analysis.

2. Materials and methods

2.1. Collection, culturing, and characterization of microalgae

Microalgal biomass was collected from an open raceway algal pond located in Tirunelveli, India. Identification of microalgae was carried out by microscopic examination of collected samples as per the procedure in the Phytoplankton Manual (Sournia, 1978). *Chlorella vulgaris* was found to be the predominant microalgal strain. This microalgal strain was chosen for two reasons: 1) Ease of outdoor cultivation and the presence of a hard cell wall, and 2) biogas generation from microalgal biomass was associated with wastewater bioremediation (Mahdy et al., 2014b). *Chlorella vulgaris* was cultured in 5 L culturing hopkins flasks containing bolds basal medium at ambient temperature (24 ± 1 °C). Continuous illumination was provided using cool fluorescent lights (TL-D 40 W, Philips cool daylight 6500 K). Aeration was provided for uniform mixing of the culture. For harvesting, a microalgal sample was centrifuged at 5000 rpm for 10 min. The initial characteristics of the microalgae, *Chlorella vulgaris*, were as follows: pH, 6.9; total solids (TS), 19.24 ± 0.05 (g/L); volatile solids (VS), 12.28 ± 0.03 (g/L); total COD (TCOD), 20 ± 1.52 (g/L); soluble COD (SCOD), 1.71 ± 0.02 (g/L); and VS/TS (%), 63.8 ± (0.3).

2.2. Isolation, screening and identification of cellulase-secreting bacteria

The bacterium was isolated from the mixed liquid of an activated sludge treatment plant operated by a paper company. A known volume of the mixed liquid sample was spread plated on a carboxymethyl cellulose (CMC) agar plate and incubated at 40 °C for 24 h. Four unique isolates were obtained which differed morphologically. The four isolates were named I1, I2, I3, and I4. All isolates were again screened for cellulase secretion by streaking on CMC agar flooded with 1% Congo red. The most proficient zone-forming isolate was then identified through the 16SrRNA technique.

2.3. Culturing of cellulase-secreting bacteria

The bacterium was cultivated with 1000 mL of carboxymethyl cellulose broth in a conical flask at pH 6.5, 40 °C, and shaking at 160 rpm for 24 h. Then, the cultured cells were collected in the early exponential phase (24 h). These cells were centrifuged at 12,000 rpm and the cell pellet was used as an inoculum for liquefaction of microalgae.

2.4. Optimization of bacterial load for liquefaction of microalgae

100 mL of microalgal sample with a total COD (Chemical Oxygen Demand) of about 20 g/L was harvested from the broth and placed in five 250 mL conical flasks. A different bacterial load, ranging from 0 to 1.1 g dry cell weight/L of bacteria, was inoculated in each flask. The flasks were incubated at 40 °C for 24 h. The level of dissolvable organics was analyzed to optimize the dosage of bacterial load for microalgal liquefaction.

2.5. Optimization of bacterial growth by experimental design through response surface methodology

The factors influencing the growth of cellulase-secreting bacteria were optimized through response surface methodology (RSM). In the present study, a central composite design (CCD) was employed to optimize the parameters (pH, temperature, and time) that influence growth, expressed as dry cell weight (g/L). Using this design, 20 experimental runs were carried out. Because of the initial studies, the ranges of pH, temperature and time were chosen as 4–9, 20–70 °C, and 0–48 h, respectively. The data achieved through growth optimization studies were designed and fitted through the modelling equation as follows:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_11x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_3 + b_{23}x_2x_3 \quad (1)$$

where Y is the predicted response, b_0 is the constant, b_1x_1 and b_2x_2 are the linear coefficients, $b_{11}x_1^2$, $b_{22}x_2^2$, and $b_{33}x_3^2$ are the quadratic