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Phycoremediation and biogas potential of native algal isolates from soil and wastewater

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HIGHLIGHTS

- Pioneering study to integrate phycoremediation and biogas production.
- First report on phycoremediation and biogas potential of terrestrial and aquatic alga.
- More than 70% reduction in COD by algal isolates.
- Good synergy of algal isolates with native microbes in phycoremediation.
- Biogas yield in the range of 0.401–0.487 m^3 Kg^{-1} VS added.

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ABSTRACT

The present study is a novel attempt to integrate phycoremediation and biogas production from algal biomass. Algal isolates, sp. 1 and sp. 2, obtained from wastewater and soil were evaluated for phycoremediation potential and mass production. The estimated yield was 58.4 sp. 1 and 54.75 sp. 2 tons ha^{-1} y^{-1}. The algal isolates reduced COD by >70% and NH_3-N by 100% in unsterile drain wastewater. Higher productivities of sp. 1 (1.05 g L^{-1}) and sp. 2 (0.95 g L^{-1}) grown in wastewater compared to that grown in nutrient media (0.89 g L^{-1} for sp. 1 and 0.85 g L^{-1} for sp. 2) indicate the potential of algal isolates in biogas production through low cost mass cultivation. Biogas yield of 0.401–0.487 m^3 kg^{-1} VS added with 52–54.9% (v/v) methane content was obtained for algal isolates. These results indicate the possibilities of developing an integrated process for phycoremediation and biogas production using algal isolates.

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1. Introduction

Over the years, the global demand for energy has increased considerably and is being mainly met through conventional energy sources. The current renewable energy resources such as solar, wind, hydro, geothermal and biomass (Converti et al., 2009), represent up to 14% of primary-energy consumption in the world. Out of this, biomass contributes approximately 10% of the renewable energy resources (Antizan-Ladislao and Turrion-Gomez, 2008). Algal biomass has a number of potential advantages over higher plants as a biofuel feedstock. Although algae grow in aqueous media but need less amount of water than terrestrial biomass (Rodolfi et al., 2009). Algae have higher growth rate and are capable of bio-fixing waste/ biogas CO_2 (Chisti, 2007; Mandal and Mallick, 2009). In spite of these advantages, the economics of the processes makes most algal-based technologies unviable in the long run as the cost of algae production (nutrient supplementation) and processing for biofuel production is comparatively high (Chisti, 2007). However, the cost of algal biomass production can be reduced if the nutrient requirement is met through the nutrients present in wastewater. Algae have been utilized for the treatment of variety of industrial and municipal wastewater (Markou and Georgakakis, 2011; Phang et al., 2000; Rawat et al., 2011). The possibility of algal biomass production in wastewaters for biofuel feedstocks, is being increasingly explored (Rawat et al., 2011; Sturm and Lamer, 2011).

Once the algal biomass is generated, it can be used to produce biodiesel, ethanol, hydrogen, biogas or direct burning based on its characteristics (Chisti, 2007; Sturm and Lamer, 2011). However, the high processing cost of algal biomass (e.g., dewatering, oil extraction etc.) makes algal biofuel production economically unviable (Singh and Gu, 2010). Minimal processing of algal biomass is required prior to biogas production which makes it an attractive option. Algal biomass contains considerable amount of biodegradable components such as carbohydrates, lipids and proteins. This makes it a favorable substrate for anaerobic microbial flora and

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can be converted into methane rich biogas (Sialve et al., 2009). In spite of the fact that microalgae have high potential for biogas production, there are only few studies on anaerobic digestion of microalgal biomass utilizing *Chlamydomonas reinhardtii, Scenedesmus obliquus*, (Mussgnug et al., 2010), *Chlorella vulgaris*, *Dunaliella tertiolecta* (Lakaniemi et al., 2011; Ras et al., 2011), *S. obliquus* and *Phaeodactylum triconutum* biomass (Zamalloa et al., 2012).

Although there has been significant work on algal wastewater treatment and some attempts have been made towards anaerobic digestion of algal biomass, very few or no report exist on integration of phycoremediation with the algal biogas production. Hence, the present study is focused on two major objectives, first, to determine the biomass yield and phycoremediation efficiency of two algal isolates obtained from wastewater/soil and secondly, to examine the biogas production potential of algal biomass.

2. Methods

2.1. Algae isolation and identification

The wastewater and soil samples from a nearby drain (IIT Delhi, India) were collected and subjected to blooming process as described by Chinnasamy et al. (2010b). The soil sample (5 g) and wastewater sample (5 mL) were mixed separately in conical flasks containing 50 mL sterile BG11 media and kept under shaking conditions for 30–40 min. The flasks were then incubated in growth chamber for enrichment under 3500–4000 lux with light: dark cycle of 12:12 h for 12–15 days at 25 ± 1 °C. After the growth of algae, 1 mL aliquot from each flask was inoculated on separate petriplates containing sterile BG11 agar media. The inoculated plates were then incubated in the growth chamber under control light and temperature conditions for 15 days. The most dominant algal colony from each plate was then transferred to sterile BG11 slants. Algae from both the samples were identified morphologically using "ICAR Monographs on Algae" (Desikachary, 1959) at National Centre for Cultivation and Utilization of Blue Green Algae, IARI, New Delhi, India.

2.2. Algal growth and biomass determination

For estimation of biomass production potential of the isolated algal strains, batch scale studies were performed. Erlenmeyer flasks containing 50 mL sterile BG11 media were inoculated with 10% (v/v) algal suspension taken from stock cultures (optical density ≈ 2.0 at 680 nm). The inoculated flasks were incubated in growth chamber as described earlier (Section 2.1) for 18 days. Flasks were removed at regular intervals (48 h) and the samples were analyzed for growth estimation w.r.t. dry cell weight and chlorophyll a (chl-a) content.

2.3. Wastewater collection

Two wastewater samples were collected from Village Mubarakpur, Haryana (India). The grey water generated from approximately 400 households runs through uncovered channels (drainage lines) and gets accumulated in a pond (drainage pond) along with agricultural run-off. The samples were collected from the sources viz., drainage line and drainage pond. Raw wastewater samples were characterized for various parameters such as chemical oxygen demand (COD), nitrate-nitrogen (NO_3-N), total dissolved phosphorous (TDP), ammonia-nitrogen (NH_3-N), pH, total dissolved solids (TDS) and total suspended solids (TSS).

2.4. Biomass production and phycoremediation potential

To examine the biomass production and wastewater nutrient removal potential of the isolates, batch experiments were conducted under control temperature and light conditions for 12 days. Unsterile and sterile neat wastewater samples were used as growth medium. The experiments were carried out in 500 mL capacity Erlenmeyer flasks with 200 mL working volume. Flasks were inoculated with 10% (v/v) stock culture (optical density ≈ 2.0 at 680 nm). The inoculated flasks were incubated in growth chamber as described earlier in Section 2.1. Samples (2 mL) were withdrawn regularly after every 48 h for the analysis of chl-a content. At the end of experiment, homogenized broth from each flask was centrifuged at 8000 rpm for 10 min and the supernatant was collected for the analysis of pH, COD, NO_3–N, TDP, and NH_3-N in order to examine the phycoremediation potential of the algal isolates.

2.5. Biochemical composition and stoichiometric methane potential (SMP) of algal isolates

In order to examine the biochemical composition and the SMP, algal isolates were cultivated in 1 L capacity Erlenmeyer flasks with 500 mL growth medium and incubated in the growth chamber. The biomass was harvested through pH assisted auto-flocculation at pH 10.2–10.6 by adding 1 N NaOH followed by centrifugation. The harvested biomass was then dried at 60 °C for 24 h. Elemental composition (C, H, N) of algal biomass was determined through CHN analyzer and volatile solid (VS) content was determined through standard methods (APHA, 2005), respectively. For determining the biochemical composition of algal biomass, standard protocols were followed. Total carbohydrate content of the biomass was estimated using phenol–sulphuric acid method after hydrolysing the sample with 0.25 N HCl on boiling water bath and neutralizing it with sodium carbonate. The color intensity of the samples was measured at 490 nm (Dubois et al., 1951). For lipid content estimation, an aliquot (0.5 g) of the dry cell biomass was blended with 100 mL of distilled water and the mixture was kept in a microwave oven at a high temperature (about 100 °C) for 5 min in order to disrupt the algal cell wall. Total lipids were then extracted using chloroform–methanol mixture (1:1 v/v) mixed with the sample in the proportion of 1:1 through modified Bligh and Dryer's method (Lee et al., 2010). The mixture was then allowed to separate, the lower solvent phase removed and passed through a Whatman #1 filter paper and the filtrate was saved in a vial. Another 5 mL of chloroform was added to the remaining pellet and aqueous phase, and homogenized another 2 min. The resultant mixture was added to the previous filtrate by passing it through the Whatman #1 filter paper. The filtrate was allowed to separate in graduated cylinder, and the volume of the lower chloroform layer was recorded. The lipids were then gravimetrically determined by placing 0.5 mL aliquots of the chloroform layer into pre-weighed aluminum pans (three pans per sample), allowing the samples to evaporate in a hood overnight, recording the weights, and then converting to percent lipids. Protein content was calculated by multiplying the total nitrogen content (obtained through CHN analyzer) by 6.25 (Zhong et al., 2012).

The expected SMP for algal biomass was calculated by multiplying specific methane yields (m^3 CH_4 kg VS^{-1}) for carbohydrates (0.851), lipids (1.014) and proteins (0.415) with their respective content (% of TS) of biomass. These calculated yields, calculated are the maximum possible yields as this theoretical approach does not take into account the needs for cell maintenance and anabolism (Sialve et al., 2009).

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