INT J CURR SCI 2011, 1: 24-29

RESEARCH ARTICLE

Potential of green alga *Chaetomorpha litorea* (Harvey) for biogas production

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Abstract

The anaerobic digestion of the green seaweed *Chaetomorpha litorea* Harvey generated 80.5 L of total biogas per kg of dry biomass under 21 Kg pressure. The carbohydrate content of seaweed and after its digestion (sludge) was 46 and 9 mg/g dry weight, respectively. The protein and lipid contents of seaweed and sludge vary from 6.5 to 9.8 mg/g and from 4.0 to 8.0 mg/g dry weight, respectively. The organic carbon content of the sludge was 12% whereas seaweed (before digestion) contains 19% and the humic acid level of the sludge was 8.17%. The *C. litorea* and sludge were analyzed for the levels of Plant Growth Regulators (PGR). The amount of auxin, gibberellins and cytokinins in the seaweed was 25, 12 and 45 µg/g respectively, whereas the levels of PGRs in the sludge were higher than the previous one.

Key words: anaerobic digestion, methane, biochemical composition, PGRs, *Chaetomorpha litorea*

Article received 10th August; Revised 25th August; Accepted 10th September; ©IJCS – NEW LIBERTY GROUP 2011

Introduction

The production of biogas through anaerobic digestion offers significant advantages over other forms of bioenergy production. It has been evaluated as one of the most energy-efficient and environmentally beneficial technologies for bioenergy production (Fehrenbach et al., 2008). The fossil fuels are being continuously used to a large extent because these forms of energy are non-renewable and their availability will continue to decrease and the cost will continue to go up. This has led to a search for new energy sources. Seaweeds are considered to be an excellent source of energy for biogas production. Production of algae as a second generation biofuel feedstock has been the subject of research in the last decade. Moreover, the recent report of the Food and Agriculture Organization (FAO, 2008) underlines the need to focus on 'non-food' energy crops for the production of 2nd generation biofuels and to develop cost-efficient solutions which directs even more attention to the importance of biofuel production. The microbes such as *Methanobacteria* and *Methanosarcina* are capable to produce methane. Increase in temperature had a declining effect on all substrates even from initial period of incubation (Gomathi et al., 2009). Marine algae consist of polysaccharides (agar, alginate, carrageenan, laminaran and manitol), which zero lignin and low cellulose content, which make them an easy material to convert to methane by anaerobic digestion process (Alberto et al., 2008).

Biogas technology is the transformation of solid waste through anaerobic digestion process to obtain biogas such as methane. In today's energy demanding life style need for exploring and exploiting new resources of energy which are renewable as well as bio-friendly. In rural areas of developing countries various cellulosic biomass (cattle dung, agricultural residues, and algal biomass) are available in plenty which are potential to cater to the energy demand especially in the domestic sector. In India, Khadi and village industries commission started the implementation of the biogas scheme in a planned way. Currently, there are about 2.5 million house hold and community biogas plants installed in India (Kashyap et al., 2003). Biogas refers to the gas product of anaerobic digestion of organic matter present in the biodegradable waste or feedstock such as sewage sludge, municipal solid waste, manure, kitchen waste, algal biomass etc., under anaerobic conditions.

In general, the biogas composed of methane (CH_4) 55-75%, carbon dioxide (CO_2) 25-45% and the rest are hydrogen sulfide (H_2S), nitrogen (N_2) and oxygen (O_2). Biogas as a heating value of 21.48 MJ/m^3 which lower than that of a natural gas (36.14MJ/m^3), after enough cleaning up it may have the same characteristics as a natural gas. Thus biogas can be considered as natural gas substitute for heating and power generation, methane is a green house gas has approximately 21 times the heat trapping capacity of CO_2. The anaerobic biological conversion of organic matter occurs in three steps. The first step involves the enzymes mediated transformation of the insoluble organic material and higher molecular mass compounds such as lipids, carbohydrates and proteins into soluble organic material. This step is called as hydrolysis which is carried out by obligate anaerobes such as clostridia, streptococci etc. In the second step acidogenesis another group of microorganisms ferments the break down products of acetic acid, hydrogen, CO_2 and other lower weight simple volatile organic acids like propionic acid and butyric acid which are in turn converted into acetic acid in the third step methanogenesis. These acetic acid, hydrogen and CO_2 are converted into a mixture of methane and carbon-di-oxide by the methanogenic bacteria. Golueke et al. (1957) originally performed the anaerobic fermentation of algae. It has received a continued attention after the study by Golueke and Oswald including the studies of Vziel (1978) and Eisenbery et al. (1981).

Chaetomorpha is also called Bee Hoon seaweed, it is seasonally abundant, grow in tangles in the seagrass lagoon among the seagrass. It grows in long unbranched strands. The global distribution of *C. litorea* includes the coast of Red Sea, Indian Ocean, Pacific Ocean and brackish areas of Mediterranean coasts. The aim of the study is to demonstrate the feasibility of converting *C. litorea* for methane production to provide fuel for cooking purposes for the people living in the coastal areas through family type biogas plant. It also reduces pressure on forest and accentuates social benefits and provides organic manure for the agricultural crops.

Materials and methods

Sample collection

Fifty kg of *Chaetomorpha litorea* Harvey was collected from the shallow backwater areas of Muttukadu, near Chennai. It was brought to laboratory, cleaned, washed with tap water and sun dried for 4 days. The sample alga was kept at 55°C in a hot air oven for 24 h and recorded its dry weight. It was powdered and stored in a clean plastic container for further study.

Biogas plant

It was made as per the description of KVIC (Khadi and Village Industries Commission) model (Lichtman, 1983). It consists of deep well floating drum made of steel. The feed (slurry) was loaded through inlet and collected (sludge) through outlet. The system collects the gas which has kept at a relatively constant pressure, as more gas is produced the drum gas holder consequently raises. As the gas is consumed the drum then it falls. The biomass slurry moves through the system as the inlet is lower than the outlet tank creating hydrostatic pressure. The completely digested material can flow up a partition wall which prevents fresh material from short circuiting the system before flowing into the outlet tank. The fermentor used in the present study had a fixed lower dome and an upper floating dome.

Preparation of seed culture and algal slurry

Fifty kilogram of cow dung was mixed with tap water in the ratio of 1:1 (w/v) and loaded in the fermentor for developing the seed culture of methanogenic bacteria for 30 days. The algal slurry was prepared by mixing one kilogram of dry powdered seaweed with tap water in the ratio of 1:1 (w/v). The initial pH of the slurry was 7.0. The slurry was then loaded in the fermentor contained the seed culture of methanogenic bacteria through the inlet.

Production of Biogas

The biogas produced was collected from the floating drum and measured every day and the time taken to burn the particular volume of gas was recorded by using the biogas stove. The volume of biogas produced was calculated subsequently.

Collection of sludge sample

` The digested slurry was collected from the outlet of the fermentor and recorded the pH. The sludge sample was dried, powdered and stored for further analysis.

Isolation and identification of microorganisms

The bacterial isolates isolated from both *C. litorea* and fresh sludge were identified by following the routine microbiological and biochemical tests using the key provided by Bergy's manual of Determinative bacteriology (Holt et al; 1994; Krieg and Garrity, 2001). The morphological characteristic of the bacterial isolates were recorded and they were identified as per the procedure given in the Bergy's manual for systematic bacteriology (Sneath, 1986).

Biochemical parameters of Chaetomorpha litorea (Seaweed and Sludge)

The biochemical constituents of the dry algal sample and sludge (after anaerobic digestion) such as total protein (Bradford, 1976), total carbohydrate (Dubois et al., 1956), total lipid (Folch et al., 1957), total organic carbon (Walkley and Black, 1934), humic acid (Welte et al., 1952) were estimated and recorded. The average of the triplicate considered for the single reading and expressed in milligrams and percentage of dry weight. The level of PGRs such as auxin (Gordon and Paleg, 1957), Gibberellins (Holbrook et al., 1961) and Cytokinin (Syono and Torry, 1976) were also recorded.

GC-MS

Biogas samples were taken in 0.5 I cylindrical glass vessels with valves at both ends sealed. Separation and quantitative determination of CH_4, CO_2, N_2 and O_2 were performed using a gas chromatograph with gas injection valves, two different columns, and a hot-wire detector (200°C, 150 mA). Methane, N_2 and O_2 were analysed through coloumn (170 cm x 1/8") packed with the molecular sieve 13x as the stationary phase. Carbondioxide was subsequently determined using a column (190 cm x 1/8" o.d) with the porous polymer HayeSep Q (60-80 mesh) as the stationary phase. The separations were performed isothermally at 80°C with helium (25 ml min-I) as the carrier gas. The injected biogas volumes were 290 J.LI (13X column) and 190 J.LI (HayeSep Q column). Mass spectrometric identifications were made with a GC-MS instrument using the analytical methyl silicone column and the temperature program were similar to those described above, resulting in similar chromatograms. Biogas was injected using a gas syringe (500 J.LI) and the carrier gas (30 cm S-I) was helium. Mass spectra (mlz 35-200) were scanned automatically every second. The identifications were based on spectra interpretations.

Results and discussion

In the present study, the potential of green seaweed *Chaetomorpha litorea* in the biogas production was investigated.

Biogas production from Chaetomorpha litorea

The study revealed that one kilogram of dry *C. litorea* produced ca. 80.5 L of biogas. It was burnt for thirty five minutes in the biogas stove. The biogas was analyzed through GC-MS. it contained 65% methane, 19.8% CO_2, 2% nitrogen, less than 0.5% and 1% of hydrogen sulphide and carbon monoxide.

Biochemical parameters of C. litorea

The total carbohydrate content of the seaweed was 46 mg/g whereas sludge had only 9 mg/g. It indicated that the anaerobic microbes utilized polysaccharides for methane production. The protein and lipid content of 6.5 mg/g and 9.8 mg/g present in the seaweed were decreased to 4 mg/g and 8 mg/g respectively in the sludge. The above observations revealed the possible enzymatic activities like amylase and protease of the microbes during the fermentation process on the substrate.

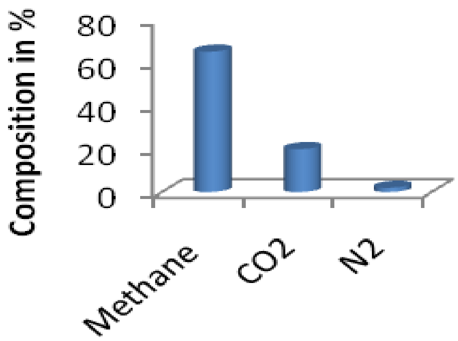


Fig 1. Biogas composition of *C. litorea*

The level of the carbon in the seaweed was estimated as 19% which was decreased to 12% in the sludge, confirmed that for the organisms utilized carbon for their growth. The humic acid in the sludge was estimated as 8.17 %.

Fig 2. Biochemical composition of *C. litorea* and sludge

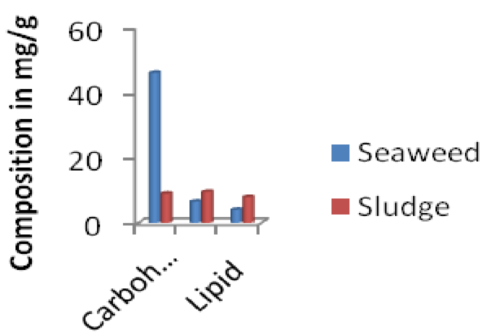
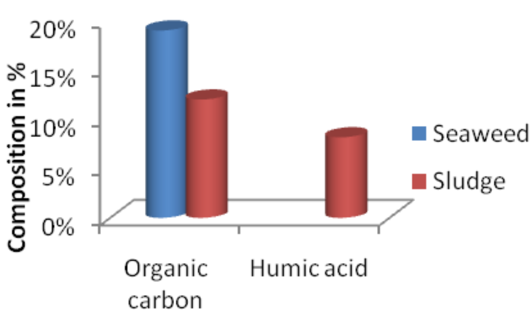


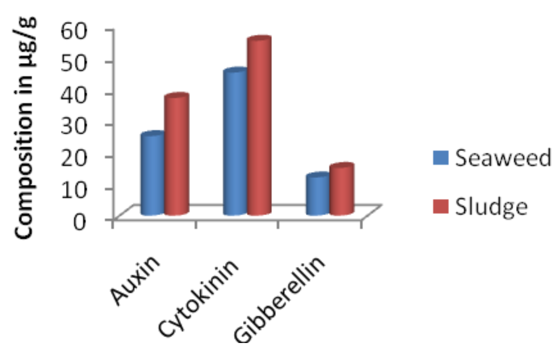
Fig 3. Biochemical composition of *C. litorea* and sludge



Plant Growth Regulators in C. litorea (Seaweed and Sludge)

The *C. litorea* dry and sludge samples were analyzed for the levels of PGRs. The amount of auxin present in the seaweed was 25 µg/g whereas the amounts of gibberellins and cytokinin were 12 µg/g and 45 µg/g, respectively. The amount of auxin in the sludge sample was 37 µg/g and the gibberellins and cytokinins were 15 µg/g and 55 µg/g, respectively. The increment of PGRs in the sludge could be due to the synthesis and contribution of PGRs by microbial population involved in the biogas production. The *C. litorea* sludge could be a good source of manure since it contained PGRs, which promote the growth and yield of plants. Therefore, *C. litorea* could be an ideal source for biogas production as well as manure as byproduct (sludge), useful in organic farming.

Fig 4. Plant growth regulators of *C. litorea* and sludge



Microbial studies on C. litorea and sludge

A total of eight different bacteria were isolated from both *C. litorea* and sludge and identified based on their morphological and biochemical characteristics. The pH of the seaweed slurry was 7.0 while the sludge was 9.0. *Chaetomorpha litorea* sample contained four different bacteria namely *Alteromonas* sp., *Acetovorax* sp., *Pseudomonas* sp., and *Azomonas* sp. whereas the sludge sample contained four different bacteria such as *Reseobacter* sp., *Rugomonas* sp., *Rhizomonas* sp. and *Kurthia* sp. It was noted that the bacteria occur in the seaweed did not present in the sludge could be due to the intolerance to anaerobic condition, high temperature, pH etc., prevailed during the fermentation process.

Conclusions

Investigation was made on the green alga, *Chaetomorpha litorea* collected from the backwaters of Muttukadu near Chennai for its potential in production of biogas. The biogas obtained through anaerobic digestion was 80.5 L /kg seaweed under the pressure of 21 kg, which had 65% of methane and it burnt for nearly 30 minutes in the biogas stove reaching the high boiling temperature in the short duration of time. *Chaetomorpha litorea* can be considered as the suitable substrate for biogas production. The sludge could be used as the manure since it was rich in humic acid which considered as store house of nutrients and helps in the plant growth. In addition it contained PGRs like auxin, gibberellins and cytokinin in high levels when compared to the slurry. Thus the sludge could be suitable manure for the various economically important agricultural crops. If energy and fertilizer shortage become more acute and pollution regulations concerning odor become stricter methane generation may become a feasible process in waste management systems.

Acknowledgement

Authors are grateful to Mr. Abhiram Seth, Managing Director, Aquagri Processing Private Ltd., Manamadurai, Sivagangai District, Tamil Nadu, India who has financially supported the project.

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