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COST EFFECTIVE BIOMASS PRODUCTION OF *CHLORELLA HOMOSPHAERA* AND SCENEDESMUS OBLIQUUS-TWO BIOFUEL POTENT MICROALGAE FROM NORTHEAST INDIA

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Abstract – Microalgal strains are potent for algal lipid generation as well as biofuel production and on the other hand animal manure is a commonly found waste product in Assam which are rich in nitrogen and phosphorus inter alia and is vital for algal growth, therefore in the present study two trials of experiments were conducted to evaluate the biomass in terms of growth and biofuel potential of two indigenous freshwater microalgae *Scenedesmus obliquus* and *Chlorella homosphaera* in three different medium such as Cow dung extract, Goat droppings extract and BG-11 media under controlled laboratory growth conditions in different concentrations (2- 10%) and another set with Urea as an added nitrogen source. It was observed that among the three media at different concentrations *Chlorella homosphaera* grows best in 10% of goat dropping extract achieving a specific growth rate of 0.504(μ/d) and lipid content of 31.5%, similarly, on the other hand, *Scenedesmus obliquus* also exhibited highest growth in 8% of goat dropping extract with added urea achieving an specific growth rate of 0.279(μ/d) and lipid content of 22%. ^{1}HNMR analysis also confirms the presence of diglyceride and monoglyceride along with the triglyceride. Based on our study it can be said that goat dropping extract as organic media can be a cheap, simple and inexpensive alternative method by which more biomass and biofuel can be generated.

INTRODUCTION

Uses of microalgae are numerous in various sectors like biofuel, aquaculture, pharmaceutical, etc and benefits for using microalgae are also abundant as compared to others. Because of which the algae cultivation is increasing day by day. As for algae cultivation nutrient supplementation is critical, chemicals are being abundantly used for the mass propagation of algae which is not cost effective at all. This expenditure on chemical fertilizers/ chemicals in large numbers for growing algae is one of the ultimate reason for the high price of biofuel produced from microalgae (Mata *et al*., 2013). These chemical fertilizers are also one of the members contributing to global warming by releasing a significant amount of CO_2 (Moore *et al*., 1998). A new perspective should be brought forward for handling the waste materials, taking the waste product from one process and making it as the startup material for another process which will be an intelligent approach towards utilization of resources by decreasing pollution and broadening the sustainable approach (Mckendry, 2002 a, b; Cantrell *et al*., 2007). Waste management in such a way involving microalgae biomass production can be very promising (Frac *et al*., 2010). For algal growth in aquaculture, animal manures have a long history as a source of soluble P, N, and C (Bwala and Omoregie, 2009). In India, the applications of manure in a variety of forms are widely available and are an age long process. Cow dung and goat dropping are a very common form of waste material available in this part of the country, in a city like Guwahati (Assam, India) this form of waste is quite common and is abundant in rural areas. Therefore an effort has been made to replace the inorganic media with a low-cost media originated from wastes that are abundantly available. In this study two types of biofuel potent green freshwater algae, *Scenedesmus obliquus* and *Chlorella homosphaera* are used. Their growth was investigated in different media generated from livestock manure. Their biomass was interpreted in terms of optical density, cell density, and dry weight and scrutinized enhanced lipid productivity. The main objective of the work is to scale up microalgae culture system from lab to outdoor conditions using sustainable media for biodiesel production.

MATERIALS AND METHOD

Monoculture establishment and culture conditions

Isolation of microalgae strains was done according to standard procedure (Kawai *et al.,* 2005). The growth conditions inside the culture room were strictly maintained as in which the temperature was maintained at 25 ± 2 ^{o}C. The incident light intensity of 35 - 40μ mol/m^2/s (PAR) was provided by cool white fluorescent tubes (PHILIPS, 45W). The light and dark phase (photoperiod) in each culture rack was maintained at 18/6 h using an automatic electronic timer. The studied microalgae species were grown in BG11 medium following standard procedure (Stanier *et al.,* 1971).

Organic stock media preparation from livestock manure

Both cow dung and goat droppings were collected fresh and further sun-dried. Both were then grounded to a fine powder for extraction purposes. 500gm of respective dung powder was added in 1L distilled water in a flask and placed on a magnetic stirrer for 48 hours. Extraction was carried out in room temperature. The mixture was then filtered through Whatman filter paper 1 to get rid of particulate solids if any. The pH of the extract was maintained at 7 and then the stock extract was stored at 4°C for further use as an algae culture media.

Experimental layout

Two sets of experiments comprising organic medium were established at different concentrations from the stock organic media prepared and one set of inorganic medium BG11 was taken for comparison. Medium A (Cow dung: 2%, 4%, 6%, 8% and 10%) Medium B (Goat droppings: 2%, 4%, 6%, 8% and 10%) and Medium C (BG11). The seed microalgal culture with an optical density of 0.708 for *Scenedesmus obliquus*) and 0.631 for *Chlorella homosphaera* recorded at OD680. All the inoculums were introduced in all flasks with different concentration of the manure extract and BG11. In the second trial the same set of the first trial was repeated but with Urea (0.1g/litre) as a nitrogen source such as Medium A (Cow dung: 2%, 4%, 6%, 8% and 10%) + Urea, Medium B (Goat droppings: 2%, 4%, 6%, 8% and 10%) + Urea and Medium C (BG11) + Urea. All of the trial sets were in triplicates. The growth was under observation for seven days. 5ml of aliquots were taken in triplicates daily for study. Lipid estimation and ^{1}HNMR analysis were made for the identification of fatty acids and complex lipids in microalgae. The ^{1}HNMR spectra were recorded on a 400 MHz NMR spectrophotometer (JEOL, JNM ECS, and Japan).

Algal growth and biomass

Growth was determined by cell counting method using Haemocytometer and simultaneously by measuring the optical density of the culture at 680nm using Specord 50 plus spectrophotometer. The cultures were daily monitored under ATC 2000 microscope for tracing contamination. The specific growth rate as follows was calculated according to (Guillard and Ryther 1962).

ì/d = (log ODt – log ODo) / T

where, ODt, ODo were the absorbance at terminal and initial day respectively; and T was the duration (days) between the two measurements.

The time required to achieve a doubling of the number viable cells is termed as doubling time (T2) which is calculated as

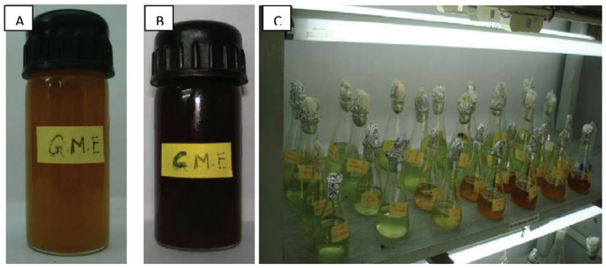
T2=0.6931/ μ

Lipid analysis

Extraction of lipid was carried out by following the (Bligh and Dyer, 1959) method. Total lipid was extracted from algal biomass. The percentage value (%) was determined from the average value of the lipid content of three replicates.

RESULTS AND DISCUSSION

Scenedesmus obliquus and *Chlorella homosphaera* are potent indigenous microalgal strains for algal lipid generation as well as biofuel production. These two potent algae were isolated and cultured using standard protocols. The elemental analysis of the locally available manure powder has also been performed (Table 1) the result of which indicates that nitrogen, calcium and phosphorus are found in high quantities in goat manure than cow dung whereas potash, manganese and iron are high in cow dung.



A: Goat dropping/manure extract; B: Cow manure extract; C: Microalgae culture in different media

It has been observed from the Figure 1 that *Chlorella homosphaera* achieved highest growth in Medium B i.e. Goat Droppings extract at 10% concentration achieving a specific growth rate of 0.504 (μ/d). Other than 10 % concentration of medium B, 6% and 8% concentration have also shown good algal growth then medium C in the first trial and in the second trial as well Medium B achieved higher growth at 6%, 8% and 10% concentration as compared to Medium C. But when medium C (BG11) was compared to medium A (Cow dung extract) the growth of biomass found higher in medium C. The algal growth gradually ceased at cow dung 8% and at cow dung at 10% also the growth phase remained similar to cow dung 8%, no further growth was observed by increasing the concentration. On addition of urea no such changes were observed at 2% to 6% concentration though the growth was little higher than without urea at the similar concentration. The growth seem to increase in cow dung 8% with added urea but on increasing the concentration again the growth seemed to cease (Figure 1).

Cow dung could not be considered as an alternative media in as compared to BG11 as the growth in cow dung media could not surpass the growth in BG11 or BG11 with urea as a replaced nitrogen source, but cow dung at 8% can be used as alternative of inorganic media (BG11) for mass cultivation though the growth is little less than inorganic media but can be cheap and sustainable source for mass production. But in case of Medium B in the first trial only *Chlorella homosphaera* outshines and exhibits much better growth than BG11 or BG11+ urea. Starting from 2% concentration *Chlorella homosphaera* exhibited a steady growth till 10% concentration with increasing concentration the growth also seemed to amplify. Again in the second trial with added urea the growth was found better as compared to cow dung and from 6% concentration onwards the growth surpassed the growth observed in both Media A and C. It was observed that at 6% concentration onwards of goat manure the growth was found better than BG11. Goat manure can definitely be an alternative to BG11 for sustainable mass cultivation of *Chlorella homosphaera* isolated from Assam, Northeast India. In case of *Scenedesmus obliqus* in the first trial the growth was found highest in Medium B i.e. goat droppings extract at 8% concentration with added urea (Fig. 2) achieving a specific growth rate of 0.279 (μ/d). The algal growth gradually ceased at goat manure 10% on addition of urea in trial B. The growth was observed to increase gradually in Medium B from 2-8% concentration then the growth ceases at 10% concentration. Similar pattern was observed in case of added urea. In case of Medium a highest growth was observed in 2% concentration both with and without added urea. BG11 was found to be better than the initial concentration but it was observed that both goat dropping extract and cow dung extract can be a better alternative than inorganic media.

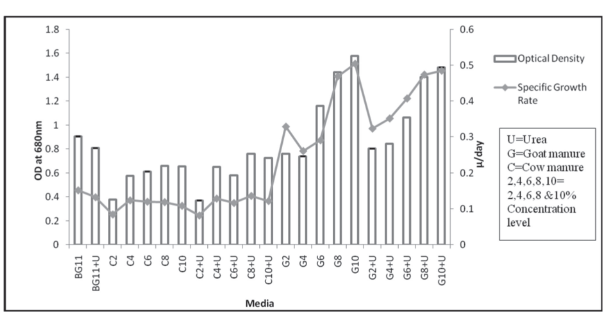


Fig. 1. Growth of *Chlorella homosphaera* in three Mediums (A, B and C) for both trial A and B.

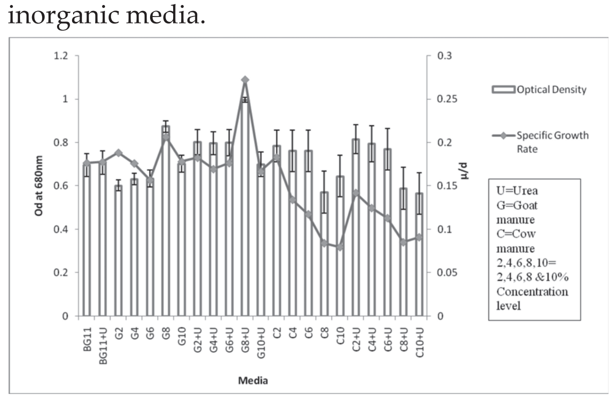


Fig. 2. Growth of *Scenedesmus obliquus* in three Mediums (A, B and C) for both trial A and B.

^{1}H-NMR spectra allow the quantification of the moieties present in samples which give rise to well assign peaks. Only a few classes of lipids, like the carotenoids, some steroids and the chlorophylls, contain protons whose peaks do not appear due to overlapping with one another. The ^{1}H-NMR spectras of algal lipids obtained in our study (*Chlorella homosphera & Scenedesmus obliqus*) are presented in Figure 3 and Figure 4 which shows all the characteristic signal of glycerides, fatty acids and other components. It is very interesting to note the symmetrical pattern of the glyceryl proton signal at 4.10–4.70 ppm indicating the presence of a triglyceride moiety. However, the unsymmetrical pattern indicates the presence of a diglyceride and monoglyceride along with the triglyceride. Monoglyceride is also clearly distinguishable from other glycerides by the multiplets at 3.60–3.80 ppm (Kumar *et al.*, 2011). The presence of multiplets at 3.60–3.80 ppm along with the unsymmetrical pattern at 4.10–4.70 ppm clearly reveals the presence of monoglyceride along with triglycerides in the algae lipid in NMR tube. Other signals are indicated at 2.10–2.30 ppm due to –CH2– attached to carbonyl

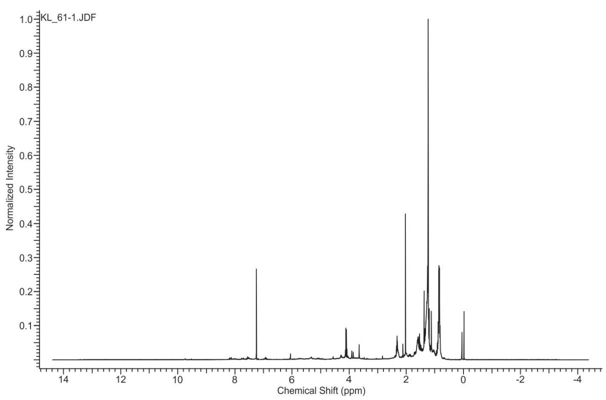


Fig. 3. ^{1}HNMR of *Chlorella homosphera* lipid

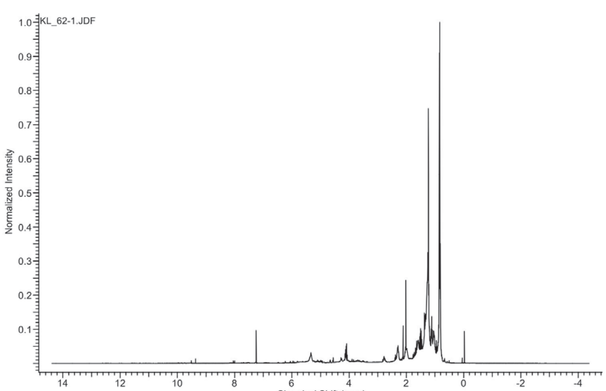


Fig. 4. ^{1}HNMR of *Scenedesmus obliquus* lipid

Table 1. Elemental Composition of Organic sources

Parameters	Cow dung powder (%)	Goat droppings powder (%)
Nitrogen	0.05	0.63
Potash	1.0585	0.56
Phosphorus	0.0197	0.23
Calcium	0.3485	1.88
Manganese	0.0395	0.0005
Iron	0.9493	0.0027

group and a signal at 2.81–2.84 ppm due to – CH2– between the two unsaturated conjugated groups of fatty acids/esters having more than one double bond. The appearance of two resolved triplets at 0.95 and 0.85 ppm are due to the terminal methyl of PUFA, C3 double bond and all other fatty esters/ acids, respectively. PUFA (Poly unsaturated fatty acid) of the algae lipids can also be estimate by developed methodology(Kumar *et al.,* 2011a). As the peak at 0.67 ppm in the proton NMR spectra of lipid is assigned specifically to the methyl group.Some peaks in the spectral region from 9 to 10 ppm are easily assignable to the protons on carbons á, â and ã in the chlorin macrocycle of chlorophylls or pheophytins present in the lipid.

CONCLUSION

Both goat droppings and cow dung which is waste product can be utilized for mass cultivation of microalgae which will be a sustainable method as well as will produce huge amount of algal biomass which can be further utilized for biofuel. Therefore the present study indicates that these organic extracts are potential for cultivation of both *Scenedesmus obliqus* and *Chlorella homosphaera.* Specific growth rate of both the algal species in selected organic extracts exceeded the growth in that of BG11. Therefore, based on the present study we recommend cow dung extract and goat manure extract as media for these biofuel potent microalgal growths.

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