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Growth of biofuel potent *Chlorella sorokiniana* NCIM 5561 with cow urine

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There are several media and supplements are available in the market for growing microalgae which are artificial, and made for growing microbes. Cow urine is a natural nutritional source for growing algae. It observed while visiting cow farms in the village and for getting surety work has carried out. Microalgae for biofuel is a topic of recent interest as it has overcome the problems related to the previous two generations of biofuel, i.e. freshwater and fertile land availability which was driving food prices high. Selected microalgae species has studied for ten days consistently for analysing its growth with cow urine. And it has observed that a 10% cow urine supplemented culture shows better biomass yield 2.134 g/L while for BG11 broth it is 0.734 g/L.

Keywords: Cow urine, microalgae, media, biofuel, UV-Visible spectrophotometer.

Introduction

Biofuel production and research in the same area is a fascinating researcher's mind for a long time. The fuel sector has a larger share in the economy of any nation. For a country like India, details are available by Sighal *et al.*[1] and by Fang CR *et al.*[2]. Countries like Brazil, Southern America, and South Asia started their biofuel awareness a few decades ago. But that wasn't a grand success for the prior two generations of biofuel based on food crops and their leftovers, which result in food price pump up. Land cultivation shifting from food to fuel crops developed an unbalance of the environment. In spite of biofuels are known for reducing emissions. Overall pollution problem becomes more intensive, due to the razing of forests for the cultivation of more fuel crops. With the third generation of biofuel, we can solve freshwater and fertile land availability for food crop cultivation. From the study of Ugbebor *et al.*[3], we can see an emission comparison between biofuels and fossil fuels from Table 1.

Table 1. Emission study comparison between fossil fuel and biofuel

Sr. No.	Emitted gas	The ratio of emission by fossil fuel to biofuel
1.	CO	1.63
2.	CH_4	1.07
3.	NO_x	1.09
4.	SO_x	Nil[a]
5.	CO_2	5.42

[a]Nil for SO_x as biofuels have zero SO_x emission so ratio can't be taken.

Third generation biofuels are answering the economic dependency, global warming and other environmental issues. Third-generation biofuels based on algal feed stokes.

Microalgae are the unicellular microorganisms that have a simple structure and high growth rate with good lipid content. It makes them a potential source for biofuel production. For microalgae cultivation, we need a nutrition source. The media provides all the necessary nutrients for the growth of algae. From the literature, we know about growing algae on wastewater, but this is not reliable for biofuel production as maintaining the composition of wastewater is a big task in itself. While cow urine is rich in nutrients, they are helpful for crops and photosynthetic microbes. Cow excretions are useful to prepare manures by Singh *et al.*[4].

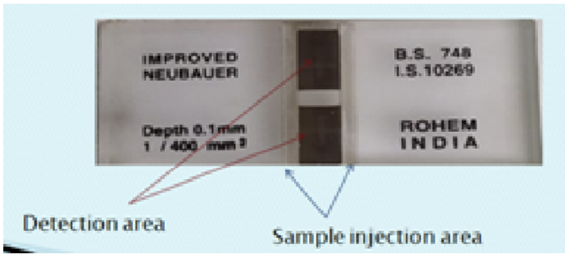
Material and methodology:

Microalgae species *Chlorella sorokiniana* NCIM 5561 is obtained from NCL Pune and initially cultivated in BG11 broth. A fresh cow urine sample has collected from a herdsman.

For cultivation in cow urine, it filtered from pore size of 2.5 µm filter paper then autoclaved with containers and BG11 media at 121°C for 15 min. Time count started after reaching 121°C. After cooling down, microalgae is transfer to the test tubes containing cow urine in 00, 05, 10, 15, 20 volume% of cow urine and rest is BG11 broth. The experiment carried out at 32°C. Light intensity adjusted to 7,000 lux, L/D was 16/8 h and pH adjusted accurately to 7.2 every day using 0.1 *N* HCl and NaOH.

UV-Vis spectrophotometer (Shimadzu, UV-1800) has used for tracking the growth of the samples.

The cell density of *Chlorella sorokiniana* NCIM 5561 has calculated using a hemocytometer under a compound microscope. The hemocytometer is a device with counting chambers designed for blood cell count, refer Fig. 1 for its image and formula for calculating the cell density.



$$\text{Cell density} = \frac{\text{Average of cells per square} \times \text{dilution factor}}{\text{Volume of square}}$$

Fig. 1. Hemocytometer.

The formula for finding dry biomass weight by Shuaijie Chai *et al.*[5] has used for calculations. For lipid extraction method by Bligh and Dyer (1959) has followed.

Results and discussion

Cow urine supplementation increased biomass productivity appreciably. For pure media, OD_{600} ranges from 0.015 to 0.68. And for the cow urine supplementation media, it ranges from 0.045 to 1.37 (for 10% cow urine), on 7th day and then growth was slower.

For a better understanding of CU supplementation on selected species, the OD_{600} results are plotted against the no. of days as shown in Fig. 2. Results of the hemocytometer hold good approximation with OD_{600} readings. Cell count

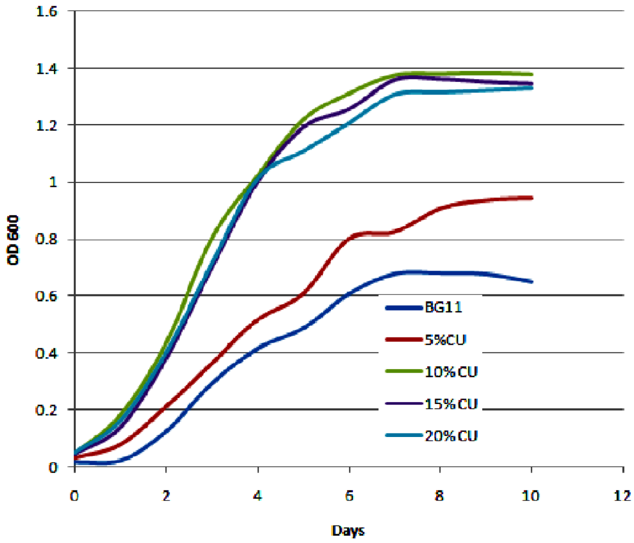


Fig. 2. OD_{600} Observation for all samples for 10 days.

was highest, for 10% CU culture, it noted to 54.8 million cells per ml.

For BG11 media, only 0.734 g/L of dry biomass has obtained, while for 10% and 15% cow urine supplementation it is 2.134 g/L and 2.034 g/L respectively. From this data, we can observe that biomass production is increased 2.91 times the real productivity of *Chlorella sorokiniana* NCIM 5561, in BG11 media.

Cow urine supplementation increased the biomass productivity and also the lipid content of *Chlorella sorokiniana* NCIM 5561 microalgae cells. For 15% CU lipid content per unit dry biomass noted to highest that is 33.43% for 10-day old cultures.

For details of the lipid content and the cell biomass productivity from the various samples on the tenth day of culture, check the Fig. 3. On comparing the results of lipid productivity of BG11 media with 15% cow urine supplemented sample, we see that cow urine supplementation increased lipid productivity from 18.93% to 33.43% of the dry biomass produced. It is about 1.76 folds the real productivity, while the change in a cost input is not significant.

We are interested more in increasing the lipid productivity for each L of the sample, than in each gram of dry biomass. So we should consider the total lipid produced in each L of the sample.

The conversion formula between per cent lipid content in

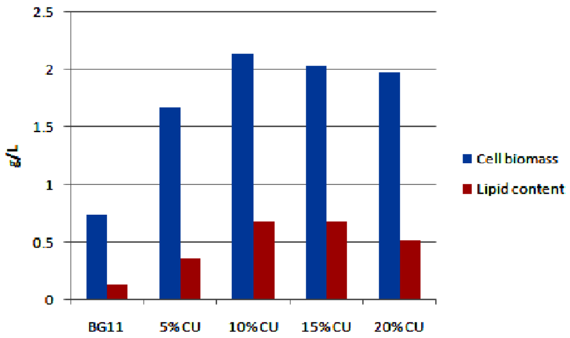


Fig. 3. Biomass and lipid productivity from the 10-day old culture.

dry biomass to total lipid produced in each L of the sample as follows:

$$\frac{\text{Per cent lipid content of dry biomass} \times \text{dry biomass produced in L of the sample}}{100} = \text{Total lipid produced per L of the sample}$$

It found that the total lipid productivity for 10% CU supplemented culture is 0.683 g/L. And for 15% CU supplemented sample it is 0.68 g/L. There is a small difference in the result of the above discussed two cultures. Still, 10% CU supplementation is advised, for large-scale, it may show a significant difference.

Conclusions

Cow urine is a cost-effective supplement for better growth and lipid productivity *of Chlorella sorokiniana* NCIM 5561. This strain is a potential source for biofuel production as its lipid content is good enough for this use. With cow urine supplementation, it reaches 32.07% of dry cell weight. We can couple microalgae cultivation and cow farming together for the economic feasibility of biomass production.

10–15% of CU dosage shows a positive impact on overall productivity. These results could further optimized for the betterment of biofuel generation. *Chlorella sorokiniana* NCIM 5561 species is important regarding biofuel production.

Algal growth can readily track by using UV-Visible Spectrophotometer or Hemocytometer with microscopy.

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