RESEARCH WRITING

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Mass culture of Botryococcus braunii Kutz. under open raceway pond for biofuel production

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1.Rationale of the work

The use of fossil fuels is been widely accepted as unsustainable due to depleting resources and accumulation of greenhouse gases in environment. Microalgae received considerable attention during recent times as they exhibit higher C02 fixation ability and produces significant quantities of renewable biomass for biofuel, bioenergy and bio productivity. The hydrocarbon rich microalgae are *Botryococcus braunii* and it has a tremendous potential to be used as renewable biomass feedstock and also for production of liquid hydrocarbon. The purpose of the study was to mass culture the algae B braunii, isolated from the fresh water bodies of Andhra Pradesh, India in open raceway pond and assess its growth performance in terms of biomass, pigments, lipids and hydrocarbons.

2. Methods

i. Isolation of *B.braunii*

The single colonies were picked up using micropipette and washed with sterile modified CHU -13 medium for 5 times and allow to grow in modified CHU-13 a far medium and incubated.

ii. Laboratory studies

The experiment was carried out in 500ml flask with 180ml modified CHU-13 medium and inoculated with 20ml of three different isolates of *B.braunii* separately. This was carried out for 30 days during which after 3 days the level of different pigments such as Chlorophyll a, Chlorophyll b, Carotenoids, Carbohydrates, Proteins lipids and biomass were recorded.

iii. Molecular characterization

The three strains were isolated from the Lyophilized algal biomass using GenElute^{TM} Plant DNA mini prep kit. DNA preparation were checked, samples were diluted at concentration of 25ng/μl and subjected for amplification using PCR and product was separated on agarose gel stained with ethidium bromide.

iv. **Acclimatization of B. braunii AP103 for outdoor condition**

The isolate *B.braunii* AP103 produce high biomass yield and lipid content. 20L culture were used as deed material to inoculate in 180 L medium in mini open raceway pond for 2 weeks then culture was mixed after every 30 min during daytime. The alga in pond was subjected for adaptation to open air condition and compensated with chlorine water.

v. **Mass cultivation of B. braunii in open raceway pond**

The seed culture (200L) of *B.braunii* AP103 grown in open raceway pond with a biomass conc. of $0.085gl^{-1}$ was transferred to 2000L pond containing 1800 L of modified CHU-13 Medium. The algae culture was mixed once in every 30 min during day time to prevent enhance CO_2 conc. It was carried out for 15 days and mass, carbohydrate, protein was recorded.

vi. Harvesting of Algal biomass

The algal cell settled at the bottom and was harvested after 12h through auto flocculation on 15^{th} day. The pond was washed with ground water and cells were allowed to settle. The washed algae cells were spread on white plastic sheet and dried in sunlight followed by oven drying at 60^0C for 8h.

vii. Biomass estimation

The biomass was determined by filtering 20 ml of algal culture using pre-weighed 4.7cm wWhattman GF/C glass fiber filter. The filtered algal biomass was dried at 65℃ for 2h, cooled at room temperature in a vacuum desiccator and weighed.

viii. Statistical analysis

Triplicates were maintained for the estimation of different parameters. The result was expressed in $\pm$ SE data and analyzed statistically, using SPSS 17.0 windows.

ix. Hydrocarbon extraction and analysis

10g of the dried algal biomass was homogenized in motor and pestle with n-hexane for 2 min and stored under room temperature for overnight and centrifugal. The extraction process was separated 3 times and extracts were pooled and evaporated under stream of nitrogen to dry at room temperature.

x. Total lipid extraction and fatty Acid analysis

10g of dried algal sample were extracted with chloroform-methanol, and lipid was quantified. The lipid sample was dissolved in benzene and 5% methanolic hydrogen. FAME was analyzed by GC-MS equipped with FID using SPB -1 capillary column with temperature 130-280^0C at 2^0c/Min.

3.Result

The three isolates colonies of *B.braunii* i.e. AP103, AP104, AP105 which were investigated under laboratory conditions showed that AP103 produces more hydrocarbons (11%) and lipid (19%) than the other two isolates. The AP103 performed well than the rest of two isolates in open raceway pond and the daily biomass productivity was observed to be $0.114 g^{-1} d^{-1}$, specific growth rate0.26 div/day 0.38 and generation time 2.64 respectively. This shows that *B.braunii* can produce 68.4g dry tons of biomass ha^{-1} $year^{-1}$.

4. Discussion

The above article states that the *B.braunii* algal sample was collected from the freshwater lake, was isolated and studied in the laboratory. Different pigments were recorded and molecular characterization of the three strains of *B.braunii* was done. The strain AP103 was acclimatized in the outdoor condition for 30 min, where the culture was mixed. Also mass cultivation in the open pond was carried out. Later harvesting of the algal biomass was done after 12 hours and later the biomass estimation was done by filtering the algal culture. The hydrocarbons were extracted from the biomass and analysed, also the analysis of the total lipid extraction and fatty acid was done. The uses of fossil fuels in our day to day lives was also discussed. How biofuels are produced and their uses. The main purpose and also the further scope of this study was discussed**.**

5. Conclusion

In the study conducted it was seen that one of the three of *B.braunii*: AP103 showed amazing potential. It proved to produce renewable biomass for biofuel which can be used indoor and as well as outdoor conditions. This study was conducted to cultivate different strains of B. braunii in open natural parks for biomass and biofuel applications. *B.braunii* was observed for its hydrocarbon, biomass and lipid production in laboratory and outdoor natural conditions. This was carried out using various different methods.

6. Future scope

Further studies and research can lead to betterment of the product. Other than biofuels, algae have applications in human nutrition, animal feed, pollution control, biofertilizers and waste water treatment.

This research paper has helped to understand and give a further insight into the research being done using algae for production of biofuels. It has also increased the scope for further research.