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Mass cultivation of microalgae and extraction of total hydrocarbons: A kinetic and thermodynamic study

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HIGHLIGHTS

- Study revealed successful mass cultivation of *B. braunii* AP102.
- Variables affecting the extraction process have been investigated.
- Determination of order of the process, rate constant, equilibrium constant.
- Thermodynamic properties activation energy, Gibbs free energy, enthalpy, entropy.

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ABSTRACT

In this present investigation, the biofuel alga *Botryococcus braunii* (*B. braunii*) was mass cultivated in 10 m^2 open raceway pond at batch mode. This study was mainly focused on the kinetics and thermodynamics approach for total lipid and hydrocarbon extraction. At batch mode cultivation the alga produced highest dry biomass of 23 g m^{-2} d^{-1}. The algal cells were harvested by autoflocculation method and dried. Different pre-treatment methods, solvents systems and optimization of extraction parameters were carried out in the dry biomass containing moisture less than 1 wt%. This reveals ultrasonication combined with chloroform: methanol, extracted maximum of 24.2 wt% total lipid and hydrocarbon. Temperature effects (308–338 K) on kinetics were investigated. The extraction follows first order kinetics and their rate constants and activation energy were determined as 3.0431 $s^{-1}$and 36.7214 kJ mol^{-1} respectively. Thermodynamic activation parameters $\Delta G^{\neq}$, $\Delta H^{\neq}$ and $\Delta S^{\neq}$ were measured, which suggest that the process was endothermic, irreversible and spontaneous.

1. Introduction

In the present scenario, depletion of fossil fuel, climatic changes and environmental pollution are continuously increasing due to escalating global energy consumption. The continuous growth of global population and the ongoing developments makes a rapid increase in the world's demand for energy. To overcome these problems biofuels are the promising alternative energy sources [1]. Substitute for petroleum, renewable energy have been receiving much attention due a number of environmental, economic and societal benefits [2].

Today, several biofuels candidates are proposed to displace fossil fuels inorder to eliminate the vulnerability of energy sector [3,4]. Biofuels produced from crops have become a major controversy due to food versus fuel competition. In this contest, production of biofuel from microalgae is widely accepted. Microalgae are able to produce 15–300 times more oil for biodiesel production than traditional crops based on the areal productivity. Moreover, microalgae have a very short harvesting cycle (3–15 days depending on the algal source and process) allowing multiple or continuous harvests with increased biomass yields when compared with conventional crop plants that are usually harvested once or twice in a year.

Among the different microalgal strains, the green colonial hydrocarbon rich microalga, *Botryococcus braunii* Kützing [5,6] is widespread in freshwater, brackish lakes, reservoirs and ponds [7–9]. *B. braunii* is a promising renewable energy sources since they accumulate very high levels up to 26–86% of lipids on dry weight basis [10]. The important processes involved in biofuel production using microalgae are mass cultivation, harvesting, cell disruption, total lipid and hydrocarbon extraction and finally

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transforming these into liquid fuels. Among these processes, cell disruption and total lipid and hydrocarbon extraction from algal biomass plays an important key role in biofuel production [11].

There are only few methods available in the literature for the extraction of algal lipid, such as mechanical, enzymatic, chemical extraction through different organic solvents and supercritical extraction. However, significant pretreatment methods and extraction kinetic and thermodynamic studies has not been reported for *B. braunii*. Using suitable solvent system is one of the basic processing steps used for recovering total lipids from the biomass for biofuel production. Commercial grade *n*-hexane was used as a solvent for the extraction of oil from biomass for many years [12].

The study presents, the mass cultivation of biofuels alga *B. braunii* in the open raceway pond. The harvested biomass was dried and subjected to different pretreatment methods, which was taken for extraction. The variables affecting the extraction process has been intensively investigated. The studies on the extraction kinetics include: determination of order of the process, rate constant, equilibrium constant and thermodynamic properties like activation energy, Gibbs free energy, enthalpy, entropy and activation thermodynamic parameters.

2. Materials and methods

2.1. Materials

Algal stain *B. braunii* AP102 used in the study was obtained from Centre for Advanced Studies in Botany, University of Madras, Chennai, India. Investigation into the mass cultivation was maintained according to Ashokumar and Rengasamy [13]. The extraction studies were performed using organic solvents dichloromethane, chloroform, methanol, acetonitrile, toluene, isopropanol, diethyl ether, Tetra hydro furan (THF), 1-4-dioxane and *n*-hexane of analytical grade were purchased from Merck, Mumbai, India. All the experiments were done in triplicate and independently validated. The data was summarized with error bars representing the standard error of the mean.

2.2. Mass cultivation of B. braunii in open raceway pond

The alga *B. braunii* AP102 was mass cultured in the open raceway pond. This experiment was conducted in a concrete raceway pond (length 6.1 m, width 1.52 m, height 0.3 m) lined with white porcelain tiles having a total working volume of 2000 L of $\approx$10 m^2 surface area. The seed culture (200 L) of *B. braunii* AP102 grown in the mini open raceway pond was transferred to the 2000 L capacity raceway pond containing 1800 L of growth medium and the culture height in the pond was maintained at 0.15 m level. The algal culture was mixed slowly using a paddle wheel during day time to prevent settling and to enhance dissolution of CO_2 from the ambient air and speed of paddle wheel rotates at 80–100 rpm. This experiment was conducted for a period of 18 days in batch mode [13]. Different parameters were analyzed and the microscopic analysis was carried out daily to check the purity of the culture. At the end of the study period the algal biomass was harvested.

2.3. Harvesting of algal biomass

The agitation provided in the open raceway pond was stopped when the culture attain 18th day. The algal cells were autoflocculate and allowed to settle at the bottom pond for 12 h. The harvested algal biomass was washed with fresh ground water and this process was repeated for 3 times in order to remove the excess salts in the algal biomass. The washed algal cells were spread on white high density poly ester sheets and dried in the sun light followed by oven drying at 60 °C for 4 h. Biomass yield ($g\,L^{-1}$) was determined by filtering 20 mL of algal culture using pre-weighed 4.7 cm Whatman GF/C glass fiber filter. The filter with algal biomass was dried at 65 °C for 2 h, which was then cooled to room temperature in a vacuum desiccator and weighed gravimetrically.

2.4. Estimation of total lipid and hydrocarbons

Total lipid and hydrocarbon content of the biomass was determined by Soxhlet method. The powdered algal biomass was packed in a thimble for Soxhlet extraction with 250 mL of solvent. Different solvent systems like dichloromethane, chloroform–methanol (2:1), acetonitrile, toluene, isopropanol, *n*-hexeane-diethyl ether (1:1), THF, 1-4-dioxane and *n*-hexane were used. The extraction time was maintained constant for a maximum of 16 h at a rate of 8 cycles per hour. After extraction, the extract was filtered using Whatman 40 filter paper to remove particles that were entrained during this process. The solvent present in the algal lipid was recovered by vacuum distillation. The yield obtained was expressed in terms of weight percentage of the samples that was calculated gravimetrically. All the experiments were carried out in triplicate form and standard errors bars were plotted.

2.5. Pre-treatment of algal biomass

The alga *B. braunii* accumulates lipids and hydrocarbon in the intercellular matrix and a smaller quantity in the internal protoplasm [7]. In order to extract intercellular lipids and hydrocarbon, it is necessary to destruct the primary cell membrane. These lipids and hydrocarbon accumulate externally in successive outer walls and can be easily recovered from algal biomass with solvents, but it still remains a big challenge to extract these because algal cell membrane acts as a barrier and severely decreases the recovery. Wet algal biomass was subjected to different cell disruption process or pre-treatment methods in order to achieve high yield efficiency before extraction. The different pre-treatment methods employed were:

(i) Ultra-sonication was done using an ultra sonic probe at 24 kHz under a constant temperature of 50 °C ± 1 for 15 min.
(ii) Auto clave method, biomass was autoclaved at temperature of 121 °C, 15 Kpa pressure and time duration of 15 min.
(iii) Deep freezing pre-treatment was carried out using deep freezer and the algal biomass was stored at −20 °C with a holding time of 2 h.
(iv) Microwave pre-treatment, the algal biomass was placed in the microwave oven for 15 min at 500 W and 2455 MHz.

After pre-treatment, the algal biomass was dried in hot air oven to maintain specific moisture content less than 1 wt%. The dried algal biomass was extracted in a Soxhlet apparatus with selected solvent system. When the extraction gets completed, the solvent was removed and the yield was determined.

2.6. Batch extraction

The batch oil extraction process was carried out under laboratory condition. The algal biomass and solvent ratio were taken and placed in the 300 mL screw-cap conical flask and kept inside a temperature-controlled shaker. The oil distributes between the two phases (biomass and solvent) depending on its partition coefficient. The rate at which the transfer of solute takes place from the feed to the extracting solvent depends on the process parameters. The mixing rate was kept constant at 300 rpm throughout the process. Once the required time (10, 20, 30, 40, 50and 60 min) was