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Solar driven mass cultivation and the extraction of lipids from *Chlorella variabilis*: A case study

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ABSTRACT

Microalgae are the potential third generation energy feedstock with environmental benefits. In order to reduce the overall cost of production, a saline tolerant isolate CSIR-CSMCRI's *Chlorella variabilis* (ATCC PTA 12198) was grown in open solar salt pans using sea water during the summer season at a temperature of 45 ± 3 °C. In the present case with an ability to proliferate in hypersaline environment (3–8 °Be′), *C. variabilis* was cultivated in a vast area of 772 m^2 with a total cultivation volume of 360 m^3 with an average biomass productivity of 34.59 $g/m^2/d$. The cultivated *C. variabilis* was harvested by auto-settling and had an average calorific value of 3885 kCal/kg. The lipid extraction using Chloroform:Methanol (1:2) yielded a total lipid content of 22.59% (w/w) with a calorific value of 9288 kCal/kg. The total lipid extract in hexane yielded 10.58% (w/w) of lipid. The fatty acid methyl ester (FAME) profile of hexane extracted lipid of *C. variabilis* showed its suitability for biodiesel production. Application of solar energy in energy intensive extraction process improved the energy output to input ratio minimizing the energy input of 33.12 MJ/kg biomass without affecting its lipid profile. Furthermore, an unmodified diesel motor vehicle was run successfully using the neat biodiesel. The entire process is cost effective and energy efficient leading to the sustainable development of microalgae-based biofuel for future commercialization.

1. Introduction

Microalgae as a source of biofuel have many advantages over traditional crops including the potential to be grown on marginal land and coastal land using water sources/effluent not suitable for agriculture. Microalgae can also act as an energy feedstock for several fuels [17], e.g. the incineration/gasification of spent microalgal biomass; the utilization of crude algal oil for direct combustion for usage in other transportation fuels such as diesel, gasoline and jet fuel [32]; the preparation of biogas through the anaerobic digestion of the biomass [26,43]; biohydrogen; and bioethanol via the fermentation of carbohydrates derived from algae or directly through algal photosynthesis [13,24,28,40]. Microalgae grow faster than other plants, besides having better oil content [7].

Switching over from low-volume high-value products (like phycobiliproteins, carotenoids, etc.) to high-volume low-value products (like biofuel) from microalgae on a large scale is one of the biggest challenges to be faced in the uninterrupted supply of biomass in an economically feasible manner [2]. Lipids are synthesized as a reserve material during the nutritional and environmental stress conditions. Phosphorus along with carbon and nitrogen is the major nutrient for the growth of microalgae besides trace metals such as Mg, Ca, Mn, Zn, and Cu, and vitamins [18,23,27]. Temperature plays an important role in the microalgal metabolism [29]. Closed photobioreactors and open ponds may be used for the cultivation of microalgae on a large scale depending on the desired product. However, the closed system has many limitations, e.g. adequate light availability for individual cells and harvesting, thus making the process more costly and tedious than the open system [5,6,34]. Production of 10 MG per year in open raceway ponds costs around 12.74 $ per gallon whereas oil produced from biomass grown in photobioreactor costs 32.57 $ per gallon [15].

Open systems can be either permanent structures (rolled concrete/bricks) or temporary (using plastic liners) [31,36]. For uninterrupted microalgal biomass supply, the raceway pond is the most preferred open system [7,9,19,21]. Basically, these cultivation systems require

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relatively low construction and operating costs for which pilot systems can be made on unutilized land [5,6,36]. Open systems are economically advantageous, although they have the problem of airborne contamination. In the last few years, various strains of *Chlorella* sp. have been studied with a strong focus on open cultivation for CO_2 sequestration in order to reduce the effects of global warming [16,41].

Chlorella sp. is one of the microalgae with the greatest potential for biofuel production [10,12,14,20,22,25,38]. *Chlorella* sp. is considered as one of the best feedstock for biofuel production due to its higher growth rate as well as high lipid and carbohydrate content. *Chlorella* sp. also has the ability to obtain its nutrients through autotrophic or mixotrophic mode or from various types of waste water, besides, its tolerance towards flue gases make them a promising candidate for further scale up at large scale [44].

It is very difficult to select a particular culture technology in terms of consistent biomass productivity as mass culture systems vary with different geographical locations, culture strategies (batch, continuous, or semi-continuous systems), salinity, etc. [4]. According to Weissman et al. [39], biomass productivity ranges from 10 to 50 g/m^2/d with an average of around 20 g/m^2/d.

At present, there is no literature available on the mass cultivation of microalgae in solar salt pans using sea water as the medium according to our knowledge. Therefore, we decided to prepare a model for the mass cultivation of halotolerant microalgae (*Chlorella variabilis*) in open solar salt pans using sea water as the growth medium. Further, in order to minimize the energy consumption for extraction of non-polar lipids, solar energy was used.

2. Materials & methods

2.1. Experimental setup

2.1.1. Organism

Oleaginous CSIR-CSMCRI's *C. variabilis* (ATCC PTA 12198) isolated from Diu, India (N20°41.341′; E70°53.734′) was grown in a CSMCRI-modified Zarrouk's medium consisting of g/l $NaHCO_3$ 25; $NaNO_3$ 2.5; K_2HPO_4 0.5; K_2SO_4 1.0; NaCl 1.0; $CaCl_2$ 0.04; Na_2EDTA 0.08; $MgSO_4 \cdot 7H_2O$ 0.2; $FeSO_4 \cdot 7H_2O$ 0.01; 1 ml of A5 trace metal solution consisting (%) H_3BO_3 0.286, $(NH4)_6Mo_7O_{24}$ 0.002, $MnCl_2 \cdot 4H_2O$ 0.18, $CuSO_4 \cdot 5H_2O$ 0.008, and $ZnSO_4 \cdot 7H_2O$ 0.022, and 1 ml of B6 trace metal solution consisting (%) NH_4NO_3 0.0002296, $K_2Cr_2(SO_4)_4 \cdot 24H_2O$ — 0.000096, $NiSO_4 \cdot 7H_2O$ — 0.004785, $Na_2SO_4 \cdot 2H_2O$ — 0.001794, $Ti(SO_4)_3$ 0.0040 and $Co(NO_3)_2 \cdot 6H_2O$ 0.004948. All the chemicals used were of commercial grade.

2.1.2. Inoculum development

The inoculum was initially prepared in 20 l carboys (Zarrouk's medium, 25 ± 5 °C) from a 1 l Erlenmeyer flask (Zarrouk's medium, 700–800 lux, 25 ± 5 °C). Thereafter, the inoculum was further scaled up in the open plastic tanks (sea water with critical nutrients) with dimensions of 1.1 × 1.1 m, each having a depth of 10 cm. The average solar irradiation during inoculum preparation of *C. variabilis* ATCC PTA 12198 in the open tanks was 854.5 ± 20 W/m^2 during April–June 2011. The air temperature during daytime was 45 ± 3 °C and during night was 30 ± 3 °C during April–June 2011.

2.1.3. Cultivation site

The Institute's own Experimental Salt Farm (21°47.488′ N; 72°07.316′ E; elevation 28 ft.) was chosen as the mass cultivation site. The proximity to the seacoast also helped us in agitating the ponds at night. During times with less wind movement, the cultures were agitated manually three times a day using a perforated pipe for proper agitation and mixing (Fig. 1). Sea water being utilized for mass cultivation of *C. variabilis* comprises of dissolved inorganic phosphate 1.06 ppm, organic phosphate 15.94 ppm, total phosphate 17.002 ppm and nitrate 17 ppm. The availability of abundant sunlight and prevailing high temperature conditions during the day were the other factors which were favorable for the selected strain.

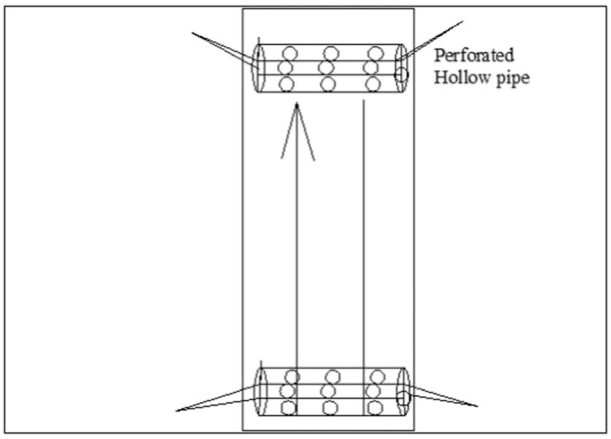

Fig. 1. Design of the perforated hollow pipe for manual agitation of *Chlorella variabilis* culture in open solar salt pan.

2.1.4. Microalgal cultivation setup

Nine solar salt pans 18 m^2 in size, six pans 90 m^2 in size, and nine pans 140 m^2 in size were prepared. Plastic (LDPE) liners were spread out in individual ponds to avoid percolation of the culture. The whole cultivable area was 772 m^2 with a culture volume of 360 m^3 and a culture depth of 30 cm.

2.1.5. Culture media

The *C. variabilis* was grown in each of the ponds using pristine seawater. The previously described two inoculum raising tanks were supplemented with 0.5% $NaHCO_3$ (w/v), 0.12% $NaNO_3$ (w/v) and 0.00125% $FeSO_4$ (w/v) to adapt the microalgae for further use as an inoculum in 10% v/v basis.

2.2. Mass cultivation

The cultivation was carried out during the peak summer season in Gujarat, India with a 45 ± 3 °C ambient air temperature. The water temperature range was 40 ± 3 °C during the entire cultivation. The desired culture for the mass cultivation needed was initially grown in two inoculum-raising ponds with an area of 18 m^2 each (Fig. 1). The ponds were monitored regularly by measuring the pH, biomass yield and environmental parameters including total salinity, dissolved oxygen, pH, electrical conductivity, nitrate content, and total dissolved solids, were monitored regularly using a multi-parameter probe (Thermo Fisher, USA) and the OD was measured at 540 nm using a UV–visible spectrophotometer (Cary Bio 50, Varian Inc., USA) involved in mass cultivation of *C. variabilis*.

The average solar irradiation during the mass cultivation of *C. variabilis* ATCC PTA 12198 was 8545 ± 20 Wh/m^2 for a time period of April–June 2011. The air temperature during daytime was 45 ± 3 °C and during night was 30 ± 3 °C during April–June 2011.

A cell concentration of 5 g/l (wet basis) was used to inoculate 7 more tanks with an area of 18 m^2 each and 3 tanks with an area of 90 m^2 each. The pH, the OD at 540 nm, the biomass yield, and the environmental parameters were monitored on a regular basis. The agitation of the ponds was done manually three times a day using a hollow pipe tied with strings at its ends for up to 18 days. After 20 days of cultivation, it was observed that the biomass had settled automatically at the bottom of the ponds. The supernatant from each pond was transferred into an empty tank for charging the next batch as the inoculum and the settled biomass was collected and sun-dried. The environmental parameters were monitored regularly using a multi-parameter probe (PCD 650,