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Improved lipid productivity from *Botryococcus braunii* using treated domestic waste water

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Abstract

Microalgae have gained enormous consideration from scientific community worldwide emerging as a viable feedstock for a renewable energy source virtually being carbon neutral, high lipid content, and comparatively more advantageous to other sources of biofuels. Although microalgae are seen as a valuable source in majority part of the world for production of biofuels and bioproducts, still they are unable to accomplish sustainable large-scale algal biofuel production. Wastewater has organic and inorganic supplements required for algal growth. The coupling of microalgae with wastewater is an effective way of waste remediation and a cost-effective microalgal biofuel production. In this article, we primarily discussed the possibilities regarding use of waste water as a culture medium for microalgal cultivation for biofuel production. Wastewaters derived from distillery, paper mill, domestic, and dairy wastes can potentially provide cost-effective and sustainable means of algal growth for biofuels. It is found that treated domestic waste water yielded high lipid productivity and cell density compared to other waste waters.

Keywords: waste water, *b. braunii*, lipid productivity, biofuel

1. Introduction

As the world's natural energy sources become scarce, fossil fuel costs rise. Fossil fuels are a source of air pollution, water pollution, and solid waste. As a result there is a global effort to find clean and renewable liquid fuel sources. Colonial green microalga, B. *braunii*, has been actively studied because of the high levels of hydrocarbons that the microalga produces. *B. braunii* accumulates hydrocarbons (terpenoids) to 30- 70% of its dry weight.

Various industry operations produce wastewater and if this wastewater is discharged in aquatic systems without proper treatment, excess nitrogen and phosphorus in discharged wastewater can lead to downstream eutrophication and ecosystem damage [1]. The negative effects of such nutrient overloading of receiver aquatic systems include nuisance algae, low dissolved oxygen concentrations and fish kills, undesirable pH shifts, and cyanotoxin production. While chemical and physical based technologies are available to remove these nutrients, they are yet to be cost effective [2]. Coupling microalgae culture with wastewater treatment is considered one of the most promising routes to produce biofuel and bio-based by-products in an economically viable and environmentally friendly way since large quantities of freshwater and nutrient required for algal growth could be saved [3].

The cultivation of algae on wastewaters evolved from the use of algae in wastewater treatment [4-5]. The nutrients were removed efficiently in such a symbiotic system. It was demonstrated that algae-based wastewater treatment could remove the nutrients (e.g., N and P) from settled domestic sewage more efficiently than traditional activated sewage process [6-7], indicating a great potential of algae-based wastewater treatment system.

Microalgae are known to be very adaptive and can be grown in salt-water, freshwater or even on contaminated industrial effluents / wastewater without any extra requirements of nutrients and minimal land requirement. Wastewaters are rich in N, P and metals, with concentrations depending on the source (agricultural, municipal, or industrial). Microalgae can therefore potentially perform a dual role for remediation of nutrient pollutants and biomass production for biofuel generation when grown in wastewater, which may improve the cost-efficiency of algal biofuel production.

The purpose of this paper was to study the growth of B. *braunii* in domestic waste water and its consumption of nitrogen compounds and phosphate of the domestic waste water for biofuel production. To meet the future demand of biodiesel, it will be necessary to optimize culture conditions that will allow faster growth of high lipid content micro algae. The objective in this study was to select a better growth medium that will support significantly higher growth rate than the medium currently use to grow this species.

2. Materials and Methods

2.1 Microalgae strain and medium

Botryococcus braunii was isolated from mixed microalgae culture. *B. braunii* was grown on CHU media.

2.2 Cultivation

The cells in exponential period were inoculated (10%, v/v) in a sewage culture medium (sewage water was autoclaved at 121^0 C for 20 min and then twice filtered through whatt man filter paper) to start the culture. All the experiments were carried out at a photo period of 16 h light:08 h dark. Light intensity during the experiment was measured using a light meter (Li- Cor measuring device). In the light phase, flasks were placed in a rotary shaker at 120 rpm for mixing. The cultures were maintained at room temperature (25-27°C) at pH: 8.2 on a fluorescent light with a light dark photoperiod of 16h: 8h. Sterile-air containing 2% (v/v) CO_2 was aerated into the flask through an air sparger at the bottom of the flask. All experiments were conducted in duplicates.

2.3 Growth Measurements

The growth of *B. braunii* was measured via spectrophotometry and biomass dry weight. Optical density for biomass factor was determined at wavelength 550 nm. One ml of sample was appropriately diluted with deionized water and the absorbance of the sample was read at 550 nm.

The cultures were determined gravimetrically and growth was expressed in terms of dry weight (mg/L) [8]. The cultures were harvested by centrifugation at 3000g for 10 min and the cells were washed with distilled water. Then the pellet was freeze dried. The dry weight of algal biomass was determined gravimetrically and growth was expressed in terms of dry weight (g/l).

The biomass yield was calculated. Biomass yield (mg ml-1)

$$= \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Sample taken (ml)}}$$

2.4 Lipid Content

The total lipids were extracted from microalgae biomass using a modified method of Bligh & Dyer, 1959 [9]. The lipids were extracted using a mixture of chloroform/methanol (1:2 v/v). The quantity of lipid residue was measured gravimetrically and expressed as dry weight percentage.

3. Results and Discussion

Algae can grow in various aquatic environment, such as fresh, brackish and marine water, municipal wastewaters, industrial wastewaters, aquaculture wastewaters, animal wastewaters, domestic wastewaters as long as there are adequate amounts of carbon (organic or inorganic), N (urea, ammonium or nitrate), and P as well as other trace elements are present.

Microalgae growth in wastewater

The use of microalgae as a sustainable means of remediating wastewater and producing valuable sub-products has received considerable research attention for decades (Oswald *et al.*, 1959; Oswald, 1988; Ruiz-Marin *et al.*, 2010; Rawat *et al.*, 2011; Park *et al.*, 2011; Pittman *et al.*, 2011; Lohrey and Kochergin, 2012) [10-15]. Microalgae growth in wastewater may offer a source of biomass for biofuel and other potential applications such as biomass production for animal feeds in addition to its remediation potential.

Early research has focussed on the use of microalgae to remove contaminants from secondary effluent as a form of tertiary treatment before discharge into rivers, to prevent eutrophication (Tam and Wong, 1989) [16]. However, a recent report has shown that microalgae can also effectively remove contaminants during secondary treatment stage (Wang *et al.*, 2010) [17]. In this study, it was reported that *B.braunii* grew well in domestic wastewater (DWW).Microalgae can grow in wide varieties of wastewater such as municipal, industrial, artificial and agricultural types (Bajhaiya *et al.*, 2010) [18]. Similarly, *Chlamydomonas* sp. have been reported to remove 100% of NH4+ and NO3- and 33% of PO43− when grown in raw industrial wastewater containing 38.4 mg L-1 NH4+, 3.1 mg L-1 NO3- and 44.7 mg L-1 PO43− (Wu *et al.*, 2012) [19].

Wastewaters contain essential nutrients for microalgae growth such as nitrogen, phosphorus, trace metals, carbon. The concentrations of these nutrients vary in wastewater depending on the source (Metcalf and Eddy, 1991) [20]. Microalgae growth in wastewater has other application in addition to remediation and biofuel. Alginate from microalgae has long been used in pharmaceutical products and as a food additive (Chapman and Chapman, 1980) [21]. The biomass produced by microalgae also has potential as animal feed and for the production of chemical raw materials. In this study we have selected four different types of waste waters i.e ; dairy waste water, paper mill waste water, domestic waste water, and distillery waste water and from Fig:2 it is seen that domestic waste water has shown highest lipid productivity with 0.52 g/l/day and the least with 0.1g/l/day with paper mill waste water.

The choice of microalgae to be grown is very important and finding species that can grow efficiently for high biofuel productivity in wastewater is a key to the success of this strategy. Algae that grow faster even with little lipid content will be ideal because it will reduce the production time and energy demands.

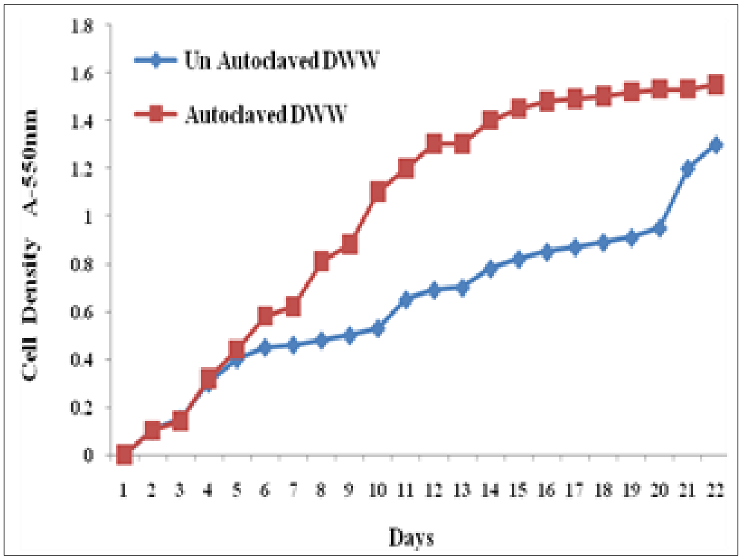

Fig 1: *Growth curves of the* unautoclaved DWW*and* autoclaved DWW is determined by optical density (OD) at 550nm.

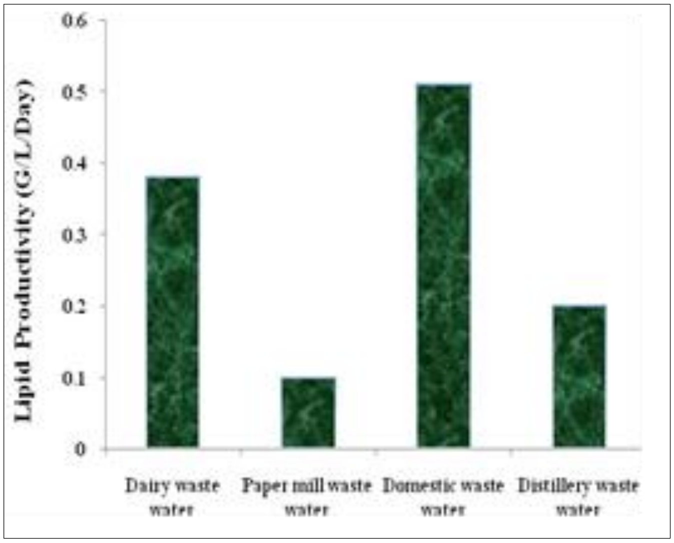

Fig 2: Total lipid productivity determined during exponential phase for four different types of waste waters used.

Challenges associated with growing microalgae in wastewater

Some of the set-backs include the presence of growth inhibiting factors and difficult harvesting processes. Some of these inhibitors include biotic and abiotic factors. Biotic factors can be in the form of viruses, bacteria, zooplankton, grazers, phytoplankton and fungi (Kagami *et al*., 2007; Park *et al*., 2011) [22, 23], which will impede or significantly inhibit the growth of microalgae. These factors will depend on the source of wastewater effluent being used for cultivation. Abiotic contaminants in wastewater such as CO2, NOx, SOx, O2 and NH3+ and heavy metals can also inhibit microalgae growth (Kumar *et al*., 2010) [24]. In contrast, when essential nutrient concentrations in the wastewater are low, in particular trace mineral nutrients, it can result in poor growth, low biomass and low lipid productivity (Christenson and Sims, 2011) [25]. In such cases there is a need to supplement these nutrients in wastewater to achieve high productivity.

Research in the use of wastewater for cultivating microalgae is still very limited as compared to research using synthetic inorganic medium. Lam and Lee (2012) [26] found that only ~30% of published research on microalgae cultivation are based on wastewater as a growth medium with the rest using synthetic media, probably because synthetic media are readily available, less contaminated and yields promising results. However the use of synthetic media might be unsustainable in commercial terms.

Wastewater effluent supporting high microalgae productivity

Wastewater usually contains high concentration of nutrients in terms of N, P, carbon and metals which are an essential requirement for microalgae growth in the presence of sunlight, CO2, O2, optimal temperature and pH. Much of the N is in the form of NH4+ which is an available form of N for microalgae uptake, although this can potentially become toxic and inhibit growth at higher concentrations (Ip *et al*., 1982; Konig *et al*., 1987; Wrigley and Toerien, 1990) [27-29]. Tolerance towards NH4+ is a very important criterion for selection of microalgae to be grown in wastewater. For example, a comparative study of three green microalgae *S. obliquus*, *Scenedesmus platensis* and *Chlorella sorokiniana* grown in piggery wastewater showed that *C. sorokiniana* has a high tolerance to high NH4+ compared to the other species (de Godos *et al*., 2010) [30].

As seen from Fig: 1 autoclaved or treated DWW has shown higher cell density than the un-autoclaved DWW hence, in the further studies autoclaved or treated DWW was used. In agricultural wastewater from piggery manure that contains high concentration of nutrients especially nitrogen, biomass and lipid productivities were recorded to be 700 mg L-1d-1 and 69 mg L-1d-1, respectively (An *et al*., 2003) [31]. Agriculture wastewater from dairy manure has also been shown to promote high biomass and lipid productivities of *Chlorella* sp. attached to supporting polystyrene foam. Biomass productivity based on the algae biomass attached to the foam was 2.6 g m−2 d−1 and total fatty acid content was 230 mg m−2 d−1 (Johnson and Wen, 2010) [32].

Future perspective of bioenergy from microalgae

Based on current knowledge, it is unlikely that microalgae will produce biofuel which is cost competitive with fossil fuels without major advances in technology. Most significant improvements are expected to be in the area of strain selection, cultivation, harvesting and oil extraction (Pittman *et al*., 2011) [33]. Integrating microalgae cultivation in wastewater for the dual purpose of remediation and biofuel production can potentially accelerate the commercialisation of algae biofuel.

Microalgae strains from the genus of each of these five species have previously been shown to grow on wastewater conditions (Li *et al*., 2011, Zhou *et al*., 2011; Shimura *et al*., 2012) [34-36]. Chlorophyte algae such as *Chlorella* and *Chlamydomonas* are often dominant in wastewater stabilization ponds, with organic loading, and ammonia and sulphide tolerance indicated as potential factors in determining species dominance (Konig *et al*., 1987; Pearson *et al*., 1987) [37, 38]; however, the exact mechanisms of wastewater tolerance are unclear and none of these specific species have previously been evaluated for biofuel or wastewater treatment characteristics. Non-adapted microalgae are very sensitive to wastewater.

Microalgae growth in wastewater can be enhanced by autoclaving the water (Cho *et al*., 2011) [39]. We observed significant increase in growth rate of culture collection *B.braunii* when grown in autoclaved DWW compared to untreated DWW. Although algae and heterotrophic bacteria have a mutualistic relationship in facultative ponds with algae providing O2 for the bacteria, which in turn provide CO2 and inorganic N and P for the algae, wastewater microorganisms including some anaerobic bacteria and viruses can be toxic or outcompete microalgae species (Cho *et al*., 2011) [39]. Absence of microorganisms could be one explanation for improved growth in autoclaved media, although breakdown of toxic organic compounds following autoclaving could be another reason. However, the isolated strains grew well in non-autoclaved, untreated wastewater and there was no significant increase in growth in autoclaved (DWW). Even under autoclaved conditions, growth of the indigenous strains was better than culture collection strains suggesting that other factors also influence strain toxicity to wastewater to which the indigenous strains have adapted.

To identify the mechanisms underlying the ability of

B.braunii to grow well in wastewater conditions, carbon utilisation was first examined. *B.braunii* was assessed to examine whether they were more efficient than the other indigenous strains at growing mixotrophically, and therefore able to utilize external carbon more efficiently. Microalgae in general have the ability to change their metabolic processes in response to environmental conditions (Devi *et al*., 2012) [40]. Some algae can grow photoautotrophically by the use of light and CO2 through photosynthetic processes and some heterotrophically by the use of organic carbon sources (Brennan and Owende 2010; Devi *et al*., 2012) [41]. In heterotrophic cultivation, cell growth and biomass and lipid productivity can be significantly influenced by the nutrients present in the cultivation medium (Devi *et al*., 2012).

This oxidative condition poses a challenge to growth of algae in DWW. The ability of algae to survive in high oxidative conditions will be important in determining strain suitability for DWW cultivation. Therefore, it can be deduced that their high growth rates and increased biomass productivity in DWW is at least in part due to enhanced oxidative stress tolerance.

This study has therefore demonstrated that selected *B.braunii* microalgae strain is able to provide high biomass productivity, however, if this strain is to be utilised as feedstock for biodiesel production, then it was important that lipid productivity characteristics be determined. The lipid productivity values determined for the indigenous strain were substantially higher than those observed in many previous wastewater cultivation experiments (Pittman *et al*., 2011) [33]. Neutral storage lipid accumulation was slightly higher under untreated wastewater conditions than autoclaved conditions, probably due to the stress induction of lipids. Studies have shown that intracellular lipid content of algae increase when they are grown in either nutrient stress or environmental stress condition (Sharma *et al*., 2012; Adams *et al*., 2012) [42, 43]. For example *C. reinhardtii* and *S. subspicatus* grown in nutrient stress condition were observed to show significant increase in neutral lipid in comparison to when they were grown in non stress condition (Dean *et al*., 2010) [44]. In a similar study, TAG were found to significantly increase in *Nannochloropsis* sp following cultivation in a nutrient stressed medium (Pal *et al*., 2011) [45].

4. Conclusions

This study has shown that *B.braunii* strain isolated from wastewater tanks can grow very efficiently in domestic waste water (DWW) and display high biomass productivity. This strain is likely to have adapted to the secondary effluent conditions by increased oxidative stress tolerance. In addition, *B.braunii* showed high lipid productivity values that indicate suitable biodiesel quality, demonstrating that they have potential as a future feedstock for biofuel applications which could be coupled to wastewater pollutant remediation.

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