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Performance assessment of biofuel production in an algae-based remediation system

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ABSTRACT

The production of biofuel from microalgae has been an area of great interest as microalgae have higher productivities than land plants, and certain species have high lipid constituents which are the major feedstock for biodiesel production. One way to enhance the economic feasibility of algal-based biofuel is to couple it with waste remediation. This study investigated the technical feasibility of cultivating *Chlorella* sp. and *Nannochloropsis* sp. with fish water for biofuel production. The remediation potential of *Chlorella* sp. was found to be higher but the lipid yield is lower, when compared to *Nannochloropsis* sp. Lipid productivities were found to be similar for both types of algae at 1.1–1.3 mg L^{-1} h^{-1}. The fatty acid profiles of the obtained lipids were found suitable for biofuel production, and the calorific values were high at 30–32 MJ/kg. The results provide insights into lipid production in *Chlorella* sp. and *Nannochloropsis* sp., when coupled with waste remediation.

1. Introduction

The worldwide need for energy is continuously increasing, due to increasing population and industrialization. The basic sources of energy are petroleum, natural gas, coal, hydro and nuclear (Kulkarni and Dalai, 2006). The major disadvantage of using petroleum based fuels is atmospheric pollution. Petroleum diesel combustion is a major source of greenhouse gas and other air contaminants including oxides of nitrogen and sulphur as well as volatile organic compounds (Hossain et al., 2008). With the need to reduce carbon emissions, and the dwindling reserves of crude oil, bioenergy (from biofuels) is one important alternative fuel which can help to mitigate the greenhouse gas emissions. Biodiesel, an important biofuel, is obtained by the trans-esterification of triglyceride oil with monohydric alcohols. It has been well-reported that biodiesel can be obtained from canola and soybean, palm, sunflower oil as a diesel fuel substitute (Lang et al., 2002; Spolaore et al., 2006). Biodiesel can also be prepared from waste cooking oil, fish oil, chicken fat and algae (Sharif et al., 2007). The use of biodiesel will decrease the dependency on petroleum-based fuels.

The first-generation biofuels is based on sugar and starch crops (for ethanol) and oilseed crops (for biodiesel). However, due to the negative impact on global food markets like competitive consumption of crops and arable land, these are very restricted. The more recently developed technologies use lignocellulosic biomass for biofuel. The cost of cellulosic feedstock is lower than the first-generation feedstocks. However, the cellulosic resources are naturally resistant to being broken down into their constituent parts, and thermal chemical pathways like gasification and pyrolysis are required. Alternatively, a combination of chemical methods and biochemical conversion pathways via specialized bacteria and yeasts can be used. Frequently, these technologies for converting cellulosic biomass into liquid fuels are difficult for commercial exploitation (Boyer 2006).

Besides energy, food security in aquaculture is also a worldwide concern. Increasing global population, coupled with increased per capita seafood consumption, results in a constantly growing demand for seafood. Global seafood consumption has reached 130 million metric tons in 2011, which is an increase of more than 30 million tons in 10 years (Food and Agricultural Organization (FAO) statistics). The new World Bank report projects that in 2030, aquaculture will produce half of the world's supply of fish, including fish for food and other products such as fishmeal (World Bank Report, 2013). Meanwhile, 62% of the consumed seafood will come

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from fish farms, which will grow production to meet rising demand especially from Asia, where roughly 70% of fish will be consumed.

As intensive aquaculture utilizes large quantities of clean freshwater, the prerequisite to healthy aquaculture is good water quality. When decomposing, the surplus feed and fish excreta can reduce the oxygen content and increase nutrient concentration in the receiving water, thus rendering treatment or replenishment of the water necessary (Gal et al., 2003). Sustainable water treatment technologies are needed to reduce nutrient and chemical discharge into receiving waters.

Algae are important bioremediation agents and are being used in many wastewater facilities. Algae cultivation on aquaculture wastewater aims at removing the nitrogen loads mainly, and producing biomass at the same time. On the other hand, oil productivity of many microalgae greatly exceeds the oil productivity of the best producing oil crops (Hossain et al., 2008). With the advantages of large oil content, low land occupation and efficient photosynthesis, algae have been considered an alternative energy resource that can technically and economically overcome the problems associated with production of first and second generation of biofuels. By coupling biofuel production to waste water remediation, there will be substantial savings from the 'free' nutrient feeds which can minimize the cost for algae cultivation, and therefore biofuel production.

This work evaluates the technical feasibility of coupling the production of biofuels from *Chlorella* sp. with the remediation of waste water from the aquaculture industry. While there have been many reports mentioning the benefits of coupling wastewater remediation with biofuel production (Chinnasamy et al., 2010; Rawat et al., 2011; Fathi et al., 2013), few have demonstrated its remediation efficacy together with biofuel production potential. Therefore, the objective of this work is to investigate the lipid yields, productivities and fatty acids profiles under simulated light conditions where phytoremediation of aquaculture waste is in effect. Two algae species, namely *Chlorella* sp. and *Nannochloropsis* sp., were used in this work due to their high oil content.

2. Materials and methods

2.1. Materials

Freshwater *Chlorella* sp. and marine *Nannochloropsis* sp. were gifts from a local fish farm and their identities verified using 18S rRNA sequencing. A Guillard F/2 media, Micro Algae Grow, was purchased from Florida Aqua Farms Inc. The Nessler reagent set, nitrite and nitrate testing reagents (Nitriver 3, Nitraver 5), and chemical oxygen demand (COD) vials were purchased from Fluke South East Asia Pte Ltd. Methanol, hexane, chloroform and dichloromethane were purchased from Merck Co. and used without purification. All other chemicals and solvents were obtained from Sigma–Aldrich Co.

2.2. Verification of algae strains

The algae DNA were extracted using DNAzol® reagent (Thermo Fisher Scientific Inc.). One ml of the reagent was added to a pellet of approximately 3×10^7 cells. After gentle pipetting to lyse the cells, the mixture was centrifuged at 10, 000 × *g* for 10 min at room temperature. The DNA in the supernatant was precipitated with absolute ethanol and washed twice with 75% ethanol. The air-dried DNA was then solubilized in 8 mM NaOH and the final pH adjusted to 7.5 using 0.1 M HEPES. PCR was performed in 50 μl reactions containing 25 μl of GoTaq® qPCR Master Mix (Promega), 1 mM concentrations of each primer, and 10 ng of DNA.

The 18 S rRNA gene specific to *Chorella* sp. was amplified using primers (Zhu et al., 2005), EUK528f (5′-CCGCGGTAATTCCAGCTC-3′) and CHLO02r (5′-CTTCGACCCCCAACTTTC-3′), resulting in a 410 bp product. For *Nannochloropsis* sp., the primers used are EUK345f (5′-AAGGAAGGCAGCAGGCG-3′) and EUK499r (5′-CACCAGACTTGCCCTCYAAT-3′), resulting in a 149 bp product. The conditions used for PCR amplification were as follows: initial denaturation at 94 °C for 5 min followed by 30 cycles of denaturation (94 °C for 1 min), primer annealing (1 min, 55 °C for *Nannochloropsis* sp. and 53 °C for *Chlorella* sp.) and chain extension (2 min at 72 °C), before a final extension at 72 °C for 10 min. A clean up of the PCR product was performed using the Wizard® SV Gel and PCR Clean-up System (Promega) with the manufacturer's instructions before the purified product is sent to AITBiotech Pte Ltd (Singapore) for sequencing.

2.3. Cultivation of microalgae

Chlorella sp. was routinely cultured in the Guillard F/2 commercial media, Micro Algae Grow, according to the manufacturer's recommended dilution (1:1000) with tap water. *Nannochloropsis* sp. was cultured in the media with the addition of 30 g/L NaCl. Fish water was obtained during water changes for catfish (*Pangasius hypophthalmus*) reared in Temasek Aquaculture Facility, Temasek Polytechnic (TP), Singapore. The rearing of catfish was approved by TP Institutional Animal Care and Use Committee (IACUC control number: 2012-50A).

2.4. Experimental setup

Batch cultivation (1 L) of the microalgae was performed using the obtained fish water in glass bottles and air-aerated with a flow rate of 10–15 ml/min. The growth and nutrient profiles of the water were monitored for 48 h. Illumination was provided by fluorescent lamps with a luminance of 3000 lux (42 μmol m^{-2} s^{-1}), equivalent to the natural light intensity on a cloudy day (Stevens, 1969) and indoor lighting. Temperature has a diurnal range of 28–30 °C. The flasks were placed on a tray with the fluorescent light placed 8 cm away from the side of the flasks. The various trays were covered with opaque boxes, with artificial light as the only light source. pH was not controlled but closely monitored during the experiments. Throughout the study, the only form of agitation was provided by air aeration (10–15 ml/min) via single tubing in each cultivation bottle.

For each flask, 3 mL of algae suspension was removed at time points of 0, 3, 24, 27, 48 h and direct counts were performed using Neubauer hemocytometer and an Olympus light microscope. The cell optical density was also measured at 682 nm using a spectrophotometer (HACH DR2800). Relative growth was calculated by the following formula:

$$\text{Relative growth} = \frac{\text{Cell concentration at time } t}{\text{Cell concentration at } t} \quad (1)$$

2.5. Harvesting and analytical methods

Stipulated amounts of algae suspension (50 ml) were removed from the cultivation flasks and subjected to centrifugation at 2650 × *g* for ten minutes. The supernatants were removed and analyzed for ammonia, nitrite and nitrate. The biomass was oven-dried at 60 °C for lipid extraction.

Ammonia, nitrite and nitrate concentrations were determined standard methods (Standard Methods, 2000). Absorbance readings were obtained using the HACH DR2800 spectrophotometer. The% reduction in ammonia and nitrate was calculated by the following