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Original article

Potential of diatom consortium developed by nutrient enrichment for biodiesel production and simultaneous nutrient removal from waste water

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ABSTRACT

Because of the decreasing fossil fuel supply and increasing greenhouse gas (GHG) emissions, microalgae have been identified as a viable and sustainable feedstock for biofuel production. The major effect of the release of wastewater rich in organic compounds has led to the eutrophication of freshwater ecosystems. A combined approach of freshwater diatom cultivation with urban sewage water treatment is a promising solution for nutrient removal and biofuel production. In this study, urban wastewater from eutrophic Hussain Sagar Lake was used to cultivate a diatom algae consortium, and the effects of silica and trace metal enrichment on growth, nutrient removal, and lipid production were evaluated. The nano-silica-based micronutrient mixture Nualgi containing Si, Fe, and metal ions was used to optimize diatom growth. Respectively, N and P reductions of 95.1% and 88.9%, COD and BOD reductions of 91% and 51% with a biomass yield of 122.5 mg L^{-1} day^{-1} and lipid productivity of 37 mg L^{-1} day^{-1} were observed for cultures grown in waste water using Nualgi. Fatty acid profiles revealed 13 different fatty acids with slight differences in their percentage of dry cell weight (DCW) depending on enrichment level. These results demonstrate the potential of diatom algae grown in wastewater to produce feedstock for renewable biodiesel production. Enhanced carbon and excess nutrient utilization makes diatoms ideal candidates for co-processes such as CO_2 sequestration, biodiesel production, and wastewater phycoremediation.

1. Introduction

The level of atmospheric CO_2 is rising because of increased anthropogenic emissions of CO_2. Given the elevated energy demand and limited accessibility to fossil fuels, there is an urgent need to explore renewable, ecofriendly, and cost-effective alternative fuel sources. Algae have high oil content and show rapid biomass production. They can grow on non-cultivable land using wastewater; thus, in contrast to land-based plant sources, they do not compete for land and water for biofuel production. Microalgal biomass can also be used to produce high-value biomolecules (Milledge, 2011). Based on these properties, microalgae are a potential alternative for biofuel production.

Microalgae can utilize low-quality water such as agricultural runoff and municipal or domestic wastewater as a growth medium and source of nitrogen, phosphorus, and minor nutrients. Thus, growing algae in wastewater is economical and environmentally friendly alternative; it can also reduce the cost for nutrients and fresh water for mass culturing while providing a method for wastewater treatment (Oswald, 1988). The quantity of wastewater generated by a city is directly proportional to the amount of water consumed. The use of microalgae for CO_2 mitigation, wastewater treatment, and biofuel production has the potential to maximize the impact of microalgal biofuels on climate change; however, many crucial aspects such as the isolation of algal strains with high growth rates and nutrient uptake, integration of algal growth systems with wastewater systems, improved algal harvesting, and life cycle analysis must be further explored to maximize the potential of algal biofuels.

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Table 1
Showing different nutrient enrichment tested in the experimental study.

Experimental variation	Nutrient source	Concentration
Control	Domestic and industrial waste water(DIW)	18 mg L^{-1} N, 3.8 mg L^{-1} P
Silicate	DIW + Si2	35 mg L^{-1} Si
Silicate + trace metal	DIW + Si2 + trace metal	35 mg L^{-1} Si + Fe + Trace metal solution as in F/2 Si medium (Guillard and Ryther, 1962)
Nualgi	DIW + Si + trace metals	1 ml l^{-1} Nualgi

Benthic diatoms contribute greatly to reduce nutrient level and increases O_2 levels in wastewater bodies and enhances the benthic food web. Diatoms are estimated to contribute ~40% of total primary production in Oceans, which is equal to the biomass of all tropical rain forests. Diatoms due to their efficient carbon concentrating mechanisms (CCM) play a significant role in carbon sequestration to the deep ocean and are major contributors to the "biological carbon pump" (Bowler et al., 2009). Although diatoms exhibit numerous characteristics required for biofuel production such as an elevated growth rate, rich lipid content, ability to grow under diverse environmental conditions, and species diversity, they are the least-represented species in mass-scale experiments for biofuel and biomolecule production (Hildebrand et al., 2012). The productivity of diatoms in natural environments is largely influenced by the availability of silica but a limited iron supply is also known to negatively influence diatom growth in oceans (Takeda, 1998). Iron and silica can readily form complexes when dissolved in water, which may not be readily available for diatoms thus, enrichment experiments should evaluate metal ion bioavailability. At a high Si:P ratio, diatoms are known to dominate other microalgal species such as blue-green algae (Holm and Armstrong, 1981) This means that Si and Fe enrichment can help shift the nutrient balance towards diatom dominance. Mesocosm experiments conducted by many researchers have stressed on the importance of Si in producing an algal community dominated by diatoms (Litchman, 2007). Therefore, in the present study, we assessed the effects of silica, Fe, and trace metals, which are the three main medium components in triggering diatom growth in wastewater. We analyzed the potential of a diatom consortium developed using nutrient enrichment with Si, Fe, and trace metals grown in urban wastewater for nutrient removal and biomass and lipid production. We also explored the potential of the nano-nutrient mixture Nualgi to trigger diatom growth and lipid production.

2. Methods

2.1. Sample collection and study area

Hussain Sagar lake is situated in the heart of the cities Hyderabad and Secunderabad and is fed by four major inlets. The lake covers an area of 5.7 km^2 with an average depth of 5.00 m. It was a major drinking water source for the city till 1920. Due to urbanization of the city, sewage and industrial wastewater were discharged into lake, greatly contributing to cultural eutrophication of water. Water samples were collected from the lake to test physiochemical parameters. Samples for preparing the diatom consortium were collected using standardized protocols (Kelly et al., 1998).

2.2. Growth studies

Two sets of experiments were conducted in same time and conditions to evaluate growth and lipid productivity of algae in wastewater and the effect of algal growth on nutrient removal. Our goal was to grow an algae consortium dominated by diatom algae. We want to develop a diatom consortium rather than using a single strain because using different strains of diatoms isolated from the same water can eliminate issues related to adaptation and the time required to establish pure cultures. We used the patented commercial micronutrient mixture Nualgi®, which has an alumina-modified nano-silica base coated with inorganic salts of the major nutrient Fe and trace metals including Mn, Co, S, Ca, Mg, Zn, and B (US patent application No.: 70275856). The diatom consortium was prepared by dislodging diatoms growing on rock samples collected from lake water followed by culture for 30 days in filtered lake water with silica enrichment using Nualgi® 1 mL L^{-1} with 20% exchange of wastewater every 5 days. The resulting culture contained an algal consortium dominated by diatoms; this was used as an inoculum for further experiments. Different experimental variations were studied by conducting enrichment experiments (Table 1). The cultures were grown in a culture room at 26 ± 2 °C with a 12-h:12-h light:dark cycle at a light intensity of 100 μmol photons m^{-2} s^{-1}. The cultures were hand-shaken twice per day. All experiments were conducted in triplicate. Growth kinetics was studied by cell counting and determining the specific growth rate (Furnas, 2002) and measuring biomass at stationary phase. Diatom samples were counted at 0.1 mm depth using a hemocytometer and compound microscope.

2.3. Diatom identification

Identification of diatoms in the consortium before and after Si enrichment was carried out following standard protocols (Taylor et al., 2007) and using taxonomic guides (Prescott, 1962).

2.4. Analysis of physiochemical parameters

Physico-chemical parameters of water such as dissolved oxygen, pH, total hardness, biological oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids, total nitrogen, total phosphate, and electrical conductivity were analyzed before and after the growth period using standard methods (APHA, 1985).

2.5. Fatty acid profiling

Total lipids were extracted according to the modified Bligh and Dyer protocol of Folch method (Bligh and Dyer, 1959) for algal lipids. The lipids extracted from each sample were analyzed by gas chromatography-mass spectrometry (Suman et al., 2012).

2.6. Statistical analysis

Growth data, represented the mean ± standard deviation, was statistically analyzed. SPSS version 21.0 software (SPSS, Inc., Chicago, IL, USA) was used for all the statistical analysis. One-way ANOVA was used to compare the means between groups to identify the significance level of variations.

3. Results and discussion

3.1. Growth studies and nutrient enrichment

Nutrient enrichment with Si favors diatom growth, as diatoms require silica for cell wall biogenesis. Enrichment with Nualgi, a silica-based nutrient mixture containing Fe and metal ions,