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Microalgal species growing on piggery wastewater as a valuable candidate for nutrient removal and biodiesel production

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ABSTRACT

Six microalgal species were examined in this study to determine their effectiveness in the coupling of piggery wastewater treatment and biodiesel production. The dry biomasses of *Ourococcus multisporus*, *Nitzschia cf. pusilla*, *Chlamydomonas mexicana*, *Scenedesmus obliquus*, *Chlorella vulgaris*, and *Micractinium reisseri* were 0.34 ± 0.08, 0.37 ± 0.13, 0.56 ± 0.35, 0.53 ± 0.30, 0.49 ± 0.26, and 0.35 ± 0.08 g dwt/L, respectively. The highest removal of nitrogen (62%), phosphorus (28%), and inorganic carbon (29%) were achieved by *C. mexicana*. In the absence of microalgae, the spontaneous precipitation of phosphorus, calcium, and inorganic carbon occurred at slightly alkaline pH. The highest lipid productivity and lipid content (0.31 ± 0.03 g/L and 33 ± 3%, respectively) were found in *C. mexicana*. The fatty acid compositions of the studied species were mainly palmitic, linoleic, α-linolenic, and oleic. The results of our study suggest that *C. mexicana* is one of the most promising candidates for simultaneous nutrient removal and high-efficient biodiesel production.

1. Introduction

The use of petroleum-based fuels has become more restricted due to the declining supply of fossil oils and the demand for the reduction of greenhouse gas emissions that cause global warming. Renewable, carbon-neutral, and economically viable alternatives to fossil fuels are urgently needed in order to avert the impending oil crisis and the dramatic consequences of climate change (Chisti, 2007). Rising oil prices, global warming, and an emphasis on renewable energy have attracted contemporary and global interest in biodiesel derived from microalgae as it holds the potential to provide biomass feedstock without adversely affecting the supply of food.

Microalgae represent an exceptionally diverse, but highly specialized, group of microorganisms adapted to various ecological habitats (Richmond, 2004; Abou-Shanab et al., 2011a, 2011b). Microalgae are novel aquatic biomass systems with higher fuel yield potential and lower water demand than traditional energy crops (Posten and Schaub, 2009). Several microalgal species have biomass production rates that surpass those of terrestrial plants (Dismukes et al., 2008), and many eukaryotic microalgae have the ability to store significant amounts of energy-rich compounds including triacylglycerol (TAG) and starch, which can be utilized for the production of several distinct biofuels including biodiesel and ethanol. In addition, microalgae-based systems can significantly reduce both organic matter and nutrients in municipal and piggery wastewater at minimal energy cost (Gonzalez et al., 2008; Mulbry et al., 2008; Zhou et al., 2012).

Theoretically, microalgae could produce between 10 and 100-fold more oil per acre than terrestrial plants. However, these capacities have not been validated on a large-scale basis, a process that would require large quantities of nutrients (i.e., nitrogen and phosphorus). This nutrient demand could lead to the development of an unsustainable process as the production of nitrogen from air demands high energy input. The global phosphate reserves are dwindling in amount and quality and it is assumed that phosphate rock production will peak in approximately 50–100 years and then decrease as the reserves are depleted (Cordell et al., 2009). Therefore, the sustainable supply of nutrients for the production of biofuels from microalgae is of great importance in terms of economics, resource depletion, and environmental protection. Wastewater derived from municipal, agricultural, and industrial activities is a source of nutrients for microalgae cultivation that could significantly reduce the operational costs of algal production systems

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(Lardon et al., 2009). The use of wastewater could reduce the need for additional nitrogen and phosphorous sources by approximately 55% (Yang et al., 2011). Microalgal cultures offer an effective solution to tertiary and quaternary wastewater treatments due to the ability of microalgae to use inorganic nitrogen and phosphorous for their growth (Oswald, 1988; Kumar et al., 2010). Many studies have been conducted on piggery wastewater treatment using microalgae, even in pilot-scale operations (de Godos et al., 2009). One promising way to make algal biofuel production more cost effective is to integrate wastewater treatment with algae biomass production (Clarens et al., 2010). This study focuses on the potential for using microalgal species isolated from different water bodies in order to increase the removal of nutrients from piggery wastewater in the context of algal biomass production and subsequent lipid accumulation. Molecular identification of the isolates was conducted and the fatty acid composition of the algal biomass was quantitatively determined.

2. Materials and methods

2.1. Algal isolates and cultivation conditions

Six different microalgal cultures were examined in this study. *Nitzschia cf. pusilla* GU732414, *Chlamydomonas mexicana* GU732420, *Chlorella vulgaris* GU732417, and *Ourococcus multisporus* GU732424 were previously isolated by Abou-Shanab et al. (2011a,b), and YSR08 and YSL017 were recently isolated from a freshwater river and lake, respectively, located in Wonju, South Korea. Ten milliliters of each water sample was inoculated into 200 mL Bold's basal medium (BBM) (Kanz and Bold, 1969) in a 500 mL conical flask and then incubated on a rotary shaker at 27 °C and 150 rpm under continuous illumination with white fluorescent light. The algae were subjected to purification by serial dilution followed by plating. The individual colonies were isolated, inoculated into BBM, and incubated at 27 °C under continuous illumination with white fluorescent light at 45–50 μmol/m^2/s for three weeks. Microscopic identification was performed and confirmed using molecular markers.

2.2. DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from the new algal cultures using a Plant Genomic DNA extraction kit (SolGent, Daejeon, South Korea) according to the manufacturer's instructions and protocols. Amplification reactions for the D1–D2 (LSU) coding region of the rDNA were performed on a T-Gradient thermocycler (Biometra GmbH, Gottingen, Germany), using universal eukaryotic primers, ⁻5-AGCGGAGGAAAAGAAACTA-3⁻ (forward) and ⁻5-TACTAGAAxGG TTCG-ATTAGTC-3⁻ (reverse), according to the PCR protocol described by Sonnenberg et al. (2007). The PCR products were purified using the Gel PCR Clean-Up System (Applied Biosystems, Foster, CA, USA). Sequencing reactions were performed using a Dye Deoxy Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) and the sequencing fragments were analyzed on an ABI Prism 377 DNA Sequencer. Ribosomal RNA gene sequences from the isolates were searched in GenBank using BLAST (Altschul et al., 1997). Aligned sequences were checked manually and were edited with Genedoc (Nicholas and Nicholas, 1997).

2.3. Wastewater collection and analysis

Biologically-treated piggery wastewater effluent was collected from commercial water treatment facilities (Phinix Ltd., Seongnam, Wonju Public Livestock Manure Treatment Plant, South Korea), which use an anaerobic/oxic biological process equipped with a rotary disk membrane module for removing organic constituents. The mixed liquor was immediately filtered using 0.2 μm nylon microfilters in order to remove microorganisms and suspended solids. Due to the variation in the wastewater composition with collection time, the wastewater samples were collected in a batch container with a capacity of 1000 L and samples required for the experiments were always stored in 20 L carboys in a cold room maintained at 4 °C. For physicochemical analysis of the wastewater, the solution pH was measured with a pH meter (Thermo Orion 290A, Orion Corporation) and the alkalinity was measured according to the standard methods of the American Public Health Association (APHA, 1998). The total inorganic carbon (TIC) was measured with a Shimadzu TOC-V_{CPH} analyzer. The total nitrogen, ammonium ions (NH_4^+), total phosphorus, and chemical oxygen demand (COD) were measured using a Hach Kit (Hach, USA). The amounts of nitrite (NO_2^-), nitrate (NO_3^-), and phosphate (PO_4^{3-}) were also determined using single-column ion chromatography (761 compact IC, Metrohm Ltd., Switzerland). Metals were analyzed using an ELAN DRC II inductively coupled plasma–mass spectrophotometer (PerkinElmer SCIEX, USA). Morphological analysis was performed by field emission scanning electron microscopy (FE-SEM) using a Hitachi SU-70 (at 10 kV) with energy-dispersive X-ray (EDX) analyses. X-ray diffraction (XRD) patterns were recorded on a Panalytical X'Pert PRO MPD/MRD diffractometer (Netherlands).

2.4. Algal biomass and nutrient removal from wastewater

The microalgal suspension in the BBM was adjusted to an absorbance of 1.5 at an OD of 680 nm. Two milliliters of the microalgal suspension were centrifuged at 3000 rpm for 15 min at 4 °C and the harvested microalgal cells were washed with 15 mg/L of $NaHCO_3$ solution. Two milliliters of each microalgal species were used as the initial algal inocula. The microalgae were grown in Erlenmeyer flasks containing 250 mL of filter-sterilized piggery wastewater as a culture media with agitation at 150 rpm under continuous illumination using white fluorescent light at an intensity of 40 μmol/m^2/s for three weeks. The biomass was determined routinely by measuring the optical density of the samples at 680 nm (OD_{680}). The samples were diluted to appropriate ratios in order to ensure that the measured OD_{680} values were in the range of 0.2–0.6 if applicable. The initial OD_{680} for all of the experimental variations was found at an absorbance of 0.04. The microalgal dry weight (g/L) was measured using the method recommended by the APHA (1998). The initial dry cell weight (DCW) concentrations were kept at 0.25 g/L throughout the experiments. The experiments were performed in triplicate and the average values were reported.

2.5. Lipid extraction and fatty acid profile analyses

Lipids were extracted from the microalga using the methods described by Bligh and Dyer (1959) and Abou-Shanab et al., 2011a. Algae cells were harvested, washed with sterilized 0.85% NaCl solution, and lyophilized. About 0.1 g dried algae powder was weighed into clean screw-top glass tubes and 10 mL of a 1:2 chloroform-methanol (v/v) mixture was added. Tubes were sonicated (Sonic 420, South Korea) at a maximum intensity for 1 h, and then incubated overnight at 27 °C while shaking at 100 rpm. On the following day, an additional aliquot of chloroform (1.25 mL) was added and the extraction mixture was sonicated again for 30 min. The algal solid residues were removed by passing the suspension through a Whatman 934-AH glass fiber filter (Whatman Inc., USA). The filtrate was transferred to another clean screw-top glass tube containing 1.25 mL of water to separate the chloroform and aqueous methanol layers. After centrifugation, a biphasic system