Bioresource Technology 147 (2013) 130–134

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Selection of microalgae for wastewater treatment and potential lipids production

Andriana F. Aravantinou, Marios A. Theodorakopoulos, Ioannis D. Manariotis *

Environmental Engineering Laboratory, Civil Engineering Department, University of Patras, Patras 26504, Greece

HIGHLIGHTS

- Effective synthetic wastewater treatment by saline and freshwater microalgae.
- Marine species exhibited higher growth rates than the freshwater species.
- *Scenedesmus rubescens* and *Neochloris vigensis* gave higher lipids concentration.
- The highest algal lipid content was observed after 20 days of operation.

ARTICLE INFO

Article history:
Received 15 May 2013
Received in revised form 2 August 2013
Accepted 5 August 2013
Available online 11 August 2013

Keywords:
Microalgae
Growth kinetics
Lipids production
Nutrients removal

ABSTRACT

In the present study, ten microalgal strains found in fresh and saline waters were cultured, and used to conduct batch experiments in order to evaluate their potential contribution to nutrient removal and biofuel production. The growth rate of microalgae was inversely analogous to their initial concentration. Three freshwater strains were selected, based on their growth rate, and their behavior with synthetic wastewater was further investigated. The strains studied were the *Scenedesmus rubescens* (SAG 5.95), the *Neochloris vigensis* (SAG 80.80), and the *Chlorococcum spec*. (SAG 22.83), and higher growth rate was observed with *S. rubescens*. Total phosphorus removal at an initial phosphate concentration of 6–7 mg P/L in the synthetic wastewater, was 53%, 25% and 11% for *N. vigensis*, *Chlorococcum spec*., and *S. rubescens*, respectively. Finally, the lipid content was determined at 20th and 30th day of cultivation, and the highest amount was observed at the 20th day.

1. Introduction

The use of microalgae for wastewater treatment has been employed for decades, as a low-cost process, especially in areas with increased ambient temperature and adequate sunshine throughout the year. Treated wastewaters contain high concentrations of nutrients (nitrogen and phosphorus), and a variety of trace elements (K, Ca, Mg, Fe, Cu, and Mn), which are essential nutrients for the metabolism and growth of microalgae (Li et al., 2011a). Secondary effluent can be used as a medium for culturing microalgae (Hammouda et al., 1995; Hoffmann, 1998). Use of microalgae has been proposed for the removal of nutrients in wastewater and has found wide application in recent years for tertiary wastewater treatment (Khan and Yoshida, 2008). The production rate of biofuels from microalgae is of great importance due to their high lipids content. The productivity of the algal lipids is 15–300 times that of conventional crops (Chisti, 2008). Furthemore, the use of algae has advantages over organic farming used for biofuel production as no cropland is required (Chisti, 2008; Hu et al., 2008). Recently, there is an intense research interest in the production of biofuels from microalgae as an alternative energy source due to their high lipid content.

Previous studies have demonstrated successful treatment with microalgae of municipal wastewater, which was rich in nitrogen and phosphorus (Li et al., 2011a,b; Zhou et al., 2011), even at low light intensity (Li et al., 2011b). Only few species of the family of *Chlorella*, such as *Chlorella pyrenoidosa* (Tam and Wong, 1989, 1990; Cheung and Wong, 1981), and *Chlorella vulgaris* (Lau et al., 1995, 1998) have been employed for wastewater treatment. However even though these species have shown excellent efficiency for nutrient removal from wastewater, the concentration of algal biomass and lipids content were not satisfactory for biofuel production (Deng et al., 2009; Mata et al., 2009; Sialve et al., 2009; Li et al., 2008; Chisti, 2007). The organic matter and nutrients content of the secondary effluent, as well as the presence of other heterotrophic microorganisms affect the growth of microalgae. The selection of microalgae for potential biofuel production should take into consideration the high algal cell density, and high lipids content. The use of wastewater in algal cultivation could have a

* Corresponding author. Tel.: +30 2610 996535; fax: +30 2610 996 573.
E-mail address: idman@upatras.gr (I.D. Manariotis).

0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.08.024

double role, the reduction of the wastewater pollution load, and the utilization of microalgae for biomass and energy production.

The aim of this paper was to investigate microalgae species, commonly found in secondary wastewater effluent, for the treatment of synthetic wastewater and potential lipids production. Ten microalgal strains were initially selected based on their presence in secondary effluent and their lipids content and their growth kinetics were determined (phase 1). Batch experiments in larger volume and different initial concentration of microalgae were then conducted with three freshwater strains exhibiting the highest growth rates (phase 2). Nutrient removal and microalgal lipids content were evaluated with different initial biomass concentrations.

2. Methods

2.1. Identification and classification of microalgae

Secondary effluent samples were collected from the University of Patras campus and Amaliada city wastewater treatment plants (WWTP) in Western Greece. The samples were collected in 500-mL polyethylene bottles, and transferred to the Laboratory for qualitative analysis. Identification of phytoplankton organisms was carried out by means of microscopic analysis, using an optical microscope (model DMLB, Leica Microsystems GmbH, Germany). The classification of species was based on taxonomic observations by Canter-Lund and Lund (1996), and the work on taxonomic character by Temponeras et al. (2000).

2.2. Algae strain collection and culturing conditions

Ten different algae strains were investigated and were selected on the basis of their presence in municipal wastewater and lipids content; eight of these were obtained from the bank SAG Culture Collection of the University of Göttingen (*Scenedesmus rubescens* SAG 5.95, *Botryococcus braunii* SAG 807-1, *Neochloris vigensis* SAG 80.80, *Chlorococcum spec.* SAG 22.83, *Euglena gracilis* SAG 1224-5/15, *Chlorella vulgaris* SAG 211-11b, *Chlorella protothecoides* SAG 211-10a, *Phaeodactylum tricomutum* SAG 1090-1a) and the remaining two from the Laboratory of Microbiology, Department of Biology, University of Patras (*Dunaliella tertiolecta* CCAP 19/6B, *Nannochloropsis gaditana*). Prior to the experiments, the microalgae were preserved in BG-11 medium (Feng et al., 2011) which contained the following ingredients: Na_2CO_3 (20 mg/L), $NaNO_3$ (1500 mg/L), Na_2Mg EDTA (1 mg/L), ferric ammonium citrate (6 mg/L), citric acid·$1H_2O$ (6 mg/L), $CaCl_2{\cdot}2H_2O$ (36 mg/L), $MgSO_4{\cdot}7H_2O$ (75 mg/L), K_2HPO_4 (30.5 mg/L), H_3BO_3 (2.86 mg/L), $MnCl_2{\cdot}4H_2O$ (1.81 mg/L), $ZnSO_4{\cdot}7H_2O$ (0.222 mg/L), $CuSO_4{\cdot}5H_2O$ (0.079 mg/L), $CoCl_2{\cdot}6H_2O$ (0.050 mg/L), $NaMoO_4{\cdot}2H_2O$ (0.391 mg/L); this medium was used to simulate effluent from secondary WWTP. The BG-11 medium was supplemented with 26.0 g/L NaCl for the marine strains and this was used as marine synthetic water. Before the start of the experiments, all algae strains were cultivated in BG-11 to obtain stable characteristics.The experiments were carried out in two phases. In the first phase (phase 1) batch experiments were conducted using the ten microalgae strains. Each strain was grown in 1.3-L sterile conical flasks. The cultures consisted of 900 mL sterile synthetic wastewater and 100 mL of preculture algae. The synthetic wastewater used in algae cultures consisted of BG-11 medium to simulate the effluent from secondary wastewater treatment plants. Algal precultures were derived from cultures, which were in the exponential growth phase. The flasks were kept in a walk-in room and were incubated under controlled environmental conditions; temperature of 21 ± 2 °C, photosynthetic radiation intensity 150 μmol m^{-2} s^{-1} (Lightscout, Quantum Light Meters), and a continuous supply of air 3 L/min (air pump, HP-400, Sunsun, Zhejiang, China) filtered by a 0.22 μm syringe filter. The illumination was provided continuously by two 36 W (cool daylight) fluorescents lamps, which were placed 20 cm above the culture's surface. The light intensity at the surface of the conical flasks was 22 μmol m^{-2} s^{-1}. The experiments were conducted in a walk-in incubator room under controlled environmental conditions. All procedures were performed under aseptic conditions. The nutrients and all components were sterilized. All mediums and cultivation apparatus were sterilized with an autoclave sterilizer at 121 °C for 20 min. Moreover a 0.22 μm filter was used to filter the air supplied to the flasks. The conical flasks were covered with sterilized hydrophobic cotton wool to prevent contamination. The duration of the experiments was 30 days, and samples were taken at regular intervals, to evaluate the phytoplanktonic production. All experiments were performed in duplicate.

In the second phase (phase 2) three strains (*S. rubescens*, *N. vigensis* and *Chlorococcum spec.*) were selected for further study. Batch experiments were conducted using synthetic wastewater in larger 2.8-L sterile conical flasks. The cultures consisted of 1800 mL sterilized synthetic substrate (BG-11) and 200 mL preculture algae. The flasks were incubated under the same environmental conditions as in phase 1. The duration of the experiments was 30 days, and samples were taken one to two times per week to evaluate nutrient removal, microalgae growth rate, and lipids content.

2.3. Analytical methods

Microalgal biomass was determined by the measurement of total suspended solids (TSS) according to standard methods (APHA et al., 1998). Since the algal cultures were axenic and samples were aseptically handled, the biomass change was attributed to algae. Total phosphorus (Total-P) was determined by the ascorbic acid method after digestion of the sample with ammonium persulfate (APHA et al., 1998). Absorbance was measured at 880 nm with a spectrophotometer (U-1100, Hitachi, Tokyo, Japan). Nitrate nitrogen (NO_3–N) was determined spectrophotometrically by the method of 2.6 – dimethylofenolis (ISO, 1986).

The lipids content of microalgae were measured by the modified method of Folch et al. (1957). A measured quantity of dry algal biomass (approximately 100 mg) was homogenized and extracted three times with a chloroform: methanol (2:1) mixture. The biomass was removed by filtration through a filter paper and the extracted lipids transferred quantitatively to a tared Erlenmeyer flask. The procedure was repeated three times in order to extract all the lipids. Weight measurements were made on a precision analytical balance (AE200, Mettler Instrumente AG, Zurich, Switzerland). The flask was placed in an oven at 90 °C until all reagents was removed. The flask was allowed to cool to ambient temperature in a desiccator and then was weighed. The weight difference corresponded to intracellular lipids.

2.4. Data analysis

The specific growth rate (μ) was determined from the growth phase by the following equation:

$$\mu = \frac{\ln C_t - \ln C_0}{t} \rightarrow \ln C_t = \mu t + \ln C_0 \quad (1)$$

where C_t is the concentration of biomass (g/L) at time t (d), and C_0 the initial concentration of biomass (g/L). The value of the specific growth rate (μ) was determined by the slope of the plot of $\ln C_t$ versus t (Li et al., 2011b).