Bioresource Technology 144 (2013) 8–13

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

Growth rate, organic carbon and nutrient removal rates of *Chlorella sorokiniana* in autotrophic, heterotrophic and mixotrophic conditions

Sunjin Kim, Jeong-eun Park, Yong-Beom Cho, Sun-Jin Hwang *

Department of Environmental Science and Engineering, Center for Environmental Studies, Kyung Hee University, Seochon-dong, Giheung-gu, Yongin-si, Gyeonggi-do 446-701, Republic of Korea

HIGHLIGHTS

- Growth rate, removal of N, P and organic carbon by *C. sorokiniana* were evaluated.
- The microalgae cultured under autotrophic, heterotrophic and mixotrophic conditions.
- The growth rate and removal rates of N, P was significant in heterotrophic condition.
- Heterotrophic condition was superior compared to other conditions.
- Heterotrophic condition would be useful for application in wastewater treatment.

ARTICLE INFO

Article history:
Received 16 April 2013
Received in revised form 14 June 2013
Accepted 19 June 2013
Available online 27 June 2013

Keywords:
Microalgae
Mixotrophs
Heterotrophs
Autotrophs
Chlorella sorokiniana

ABSTRACT

This study sought to investigate the growth rate and organic carbon and nutrient removal efficiency of *Chlorella sorokiniana* under autotrophic, heterotrophic and mixotrophic conditions. Growth rates of the microalgae were 0.24 d^{-1}, 0.53 d^{-1} and 0.44 d^{-1} in autotrophic, heterotrophic and mixotrophic conditions, respectively. The growth rate of *C. sorokiniana* was significantly higher for that grown under heterotrophic conditions. The nitrogen removal rates were 13.1 mg-N/L/day, 23.9 mg-N/L/day and 19.4 mg-N/L/day, respectively. The phosphorus removal rates reached to 3.4 mg-P/L/day, 5.6 mg-P/L/day and 5.1 mg-P/L/day, respectively. Heterotrophic conditions were superior in terms of the microalgae growth and removal of nitrogen and phosphorus compared to autotrophic and mixotrophic conditions, suggesting that microalgae cultured under this condition would be most useful for application in wastewater treatment systems.

1. Introduction

Generally, microalgae are unicellular organisms which metabolize autotrophically and photosynthesizing using a pigment. However, microalgae growth is possible under heterotrophic or mixotrophic conditions as well as autotrophic conditions depending on specific characteristics of the species (Andrade and Costa, 2007). Some microalgae species which have been observed under all three conditions (autotrophy, heterotrophy, and mixotrophy) are *Chlorella vulgaris* (Mitra et al., 2012), *Haematococcus pluvialis* (Kobayashi et al., 1992), *Spirulina platensis* (Marquez et al., 1993), *C. sorokiniana* (Wang et al., 2012), *Botryococcus braunii* (Zhang et al., 2011), and *C. zofingiensis* (Liu et al., 2011); previous research with these species tended to focus on advanced wastewater treatment and biofuel production.

Heterotrophic microalgae grow using organic carbon as a sole carbon source under lightless conditions. Mixotrophic microalgae metabolize by a combined mechanism with autotrophic and heterotrophic characteristics using both organic and inorganic carbons; they use energy produced from an organic compound for cell synthesis and store chemical energy converted from light energy (Chojnacka and Marquez-Rocha, 2004a). Energy and carbon sources depending on microalgae culture conditions, and pH variation according to the metabolite pathway are shown in Table 1. Autotrophic microalgae use inorganic carbon and produce hydroxyl as a metabolite resulting in increased pH, while heterotrophic microalgae use organic carbon and produce CO_2, resulting in decreased pH. Mixotrophic microalgae use simultaneously both inorganic and organic carbons and produce hydroxyl and CO_2 as metabolites; thereby pH is highly varied.

Results from the literature review regarding microalgae growth rate and removal rates of carbon, nitrogen, and phosphorus are presented in Table 2. The growth rate of autotrophic, heterotrophic, and mixotrophic microalgae ranged from 0.2–0.7 d^{-1},

* Corresponding author. Tel.: +82 31 201 2497; fax: +82 31 203 4589.
E-mail address: sjhwang@khu.ac.kr (S.-J. Hwang).

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http://dx.doi.org/10.1016/j.biortech.2013.06.068

Table 1
Summary of pH change and energy and carbon sources in autotrophic, heterotrophic and mixotrophic metabolic pathways (Chojnacka and Marquez-Rocha, 2004a).

Cultivation type	Energy source	Carbon source	Metabolism
Autotroph	Light	Inorganic	$H_2O + HCO_3^- \rightarrow C(biomass) + 1/2O_2 + 3OH^-$: pH increase
Heterotroph	Organic	Organic	$(1 + a)CH_2O + O_2 \rightarrow C(biomass) + aCO_2 + (1 + a)H_2O$: pH decrease
Mixotroph	Light and organic	Inorganic and organic	$bHCO_3 + cCH_2O \rightarrow (b + (c - a))C(biomass) + 3OH^- + aCO_2$: pH changes are inconsistent

0.4–0.9 d^{-1}, and 0.3–0.6 d^{-1}, respectively. The growth rate of heterotrophic microalgae was significantly higher than the other culture types, while the removal capacities of nitrogen and phosphorus were high, more than 96%, with autotrophic microalgae.

Microalgae have several merits and disadvantages for cultivation or wastewater treatment depending on the culture conditions and metabolic pathway employed. Autotrophic microalgae fix CO_2 to organic matter using light energy resulting in reduction of CO_2 (Boelee et al., 2011), and removal capacities of nitrogen and phosphorus are high. However growth rate of autotrophic microalgae is slower than heterotrophic or mixotrophic microalgae and they need sufficient light for cultivation. In addition, operating costs are high when autotrophic microalgae are applied in wastewater treatment (Chen, 1996). In the case of heterotrophic and mixotrophic microalgae, these species are limited and easy to be contaminated with bacteria due to using organic compounds as a carbon source (Perez-Garcia, 2011). Despite these disadvantages, heterotrophic and mixotrophic microalgae can grow at high rates even under lightless conditions and can complement the disadvantages of autotrophic microalgae (Olaizola, 2003; Yang et al., 2000).

Thus, *Chlorella sorokiniana*, which is known to be an adequate strain for wastewater treatment and production of biofuels and may be capable of growth under autotrophic, heterotrophic, and mixotrophic conditions (Su et al., 2011) was selected for the current study. The effects of each culture type on growth rate and removal capacities of organic compounds, nitrogen and phosphorus were evaluated.

2. Methods

2.1. Experimental procedure

A strain of *C. sorokiniana* (UTEX 1670) was obtained from UTEXCC (University of Texas Culture Collection) in USA and was cultured using PM (Proteose Medium) which consisted of $NaNO_3$ 2.94 mM, $CaCl_2{\cdot}2H_2O$ 0.17 mM, NaCl 0.43 mM, $MgSO_4{\cdot}7H_2O$ 0.3 mM, K_2HPO_4 0.43 mM, KH_2PO_4 1.29 mM. The COD of the PM is 1000 mg/L and TN and TP concentrations were 200 and 50 mg/L respectively. For autotrophic culture, 5 g/L of $NaHCO_3$, as an inorganic carbon source, was injected and illuminated at an average intensity 60 μE/m^2/s with fluorescent light for 24 h. For heterotrophic culture, 5 g/L of glucose, as an organic carbon, was injected and cultured under complete darkness using aluminum foil. For mixotrophic culture, both inorganic and organic carbon, 2.5 g/L $NaHCO_3$ and 2.5 g/L of glucose, were added and illuminated fluorescent light for 24 h. Working volume was 600 mL in a 1 L of Erlenmeyer flask, and was inoculated with as much as 10% (v/v) *C. sorokiniana* of 0.3 OD_{540} (optical density at 540 nm). The initial pH was 7 and temperature and shaking speed were maintained at 25 °C and 140 rpm.

2.2. Analytical methods

Samples of 25 mL were taken at an interval of 12 h for 48 h, then sampled every 24 h after that. The pH was adjusted to 7 using 10% HCl and OD was measured using UV/Visible spectrophotometer at 540 nm. Microalgae growth rate was calculated by Eq. (1) (Wang et al., 2010):

$$\text{Growth rate} = (\ln OD_t - \ln OD_0)/t \quad (1)$$

where, OD_0 refers to initial OD value and OD_t signifies OD value after t days.

Liquid samples filtered by a 0.47 μm GF/C membrane filter were analyzed in inorganic carbon (IC), SCOD, total nitrogen (T-N), and total phosphorus (T-P). T-N and T-P were analyzed using TN-TP Autoanalyzer (BLTEC, Korea), and IC and SCOD were measured by TOC analyzer (Shimadzu, Japan) and COD-MR kit (HUMAS, Korea), respectively.

Table 2
Values from the literature regarding microalgae growth rate and removal characteristics of nitrogen and phosphorus depending on culture types.

Culture type	Wastewater	Microalgae	Culture time (d)	Source	Removal efficiency (%)	Growth rate (day^{-1})	Ref.
Photoautotroph	Bristol medium	*Chlorella vulgaris*	21	Ammonia	96	0.219	(Tam and Wong, 1996)
	Secondary-treatment	*Scenedesmus obilquus*	8	Ammonia Phosphorus	99 98	0.768	(Martinez et al., 2000)
	Kuhl medium	*Chlorella zofingiensis*	14	–	–	0.235	(Liu et al., 2011)
Heterotroph	Wastewater from sludge centrifuge	*Chlorella* sp.	9	Ammonia PO_4-P TN COD	78.3 85.6 82.8 83.0	0.948	(Wang et al., 2010)
	Kuhl medium	*Chlorella zofingiensis*	14	Glucose Nitrate	77.8 100	0.769	(Liu et al., 2011)
	Autoclaved centrate	*Chlorella* sp.	7	COD T-N T-P	90.3 89.9 79.0	0.479	(Li et al., 2011)
Mixotroph	Piggery wastewater	Chlorella pyrenoidosa	10	COD Ammonia T-P	55.4 91.2 77.7	0.3	(Wang et al., 2012)
	SOT medium	*Spirulina platensis*	12	Glucose	100	0.66	(Marquez et al., 1993)