Bioresource Technology 210 (2016) 146–152

Contents lists available at ScienceDirect

Bioresource Technology

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

Performance, 5-aminolevulinic acid (ALA) yield and microbial population dynamics in a photobioreactor system treating soybean wastewater: Effect of hydraulic retention time (HRT) and organic loading rate (OLR)

Shuli Liu [a], Guangming Zhang [b,a,*], Jie Zhang [a], Xiangkun Li [a], Jianzheng Li [a]

[a] School of Municipal and Environmental Engineering, Harbin Institute of Technology, Harbin 150090, China
[b] School of Environment and Resource, Renmin University of China, Beijing 100872, China

HIGHLIGHTS

- Effects of HRT and OLR on a photobioreactor performances were investigated.
- The highest biomass production of 2655 mg/L and ALA yield of 7.40 mg/g-biomass were achieved.
- HRT and OLR have important impacts on microbial dynamics.

ARTICLE INFO

Article history:
Received 29 November 2015
Received in revised form 11 January 2016
Accepted 13 January 2016
Available online 19 January 2016

Keywords:
Photobioreactor
Soybean wastewater
Hydraulic retention time
Organic loading rate
ALA yield

ABSTRACT

Effects of hydraulic retention time (HRT) and influent organic loading rate (OLR) were investigated in a photobioreactor containing PNSB (*Rhodobacter sphaeroides*)-chemoheterotrophic bacteria to treat soybean wastewater. Pollutants removal, biomass production and ALA yield in different phases were investigated in together with functional microbial population dynamics. The results showed that proper HRT and OLR increased the photobioreactor performance including pollutants removal, biomass and ALA productions. 89.5% COD, 90.6% TN and 91.2% TP removals were achieved as well as the highest biomass production of 2655 mg/L and ALA yield of 7.40 mg/g-biomass under the optimal HRT of 60 h and OLR of 2.48 g/L/d. In addition, HRT and OLR have important impacts on PNSB and total bacteria dynamics.

1. Introduction

Purple non-sulfur bacteria (PNSB) have been applied to treat various nutrient wastewaters such as soybean wastewater, molasses wastewater, fermented starch wastewater, citric acid wastewater (Liu et al., 2015; Prachanurak et al., 2014; Yetis et al., 2000; Zhi et al., 2010). PNSB can utilize organic substances in wastewaters to produce valuable biomass resources including bacteriochlorophylls, biopolymers, 5-aminolevulinic acid (ALA), carotenoids, CoQ_{10}, etc (Kang et al., 2012; Kien et al., 2010; Kuo et al., 2012). Among these valuable products, ALA has drawn increasing interests as a photodynamic chemical, herbicide or insecticide, which has been widely applied in medical, agricultural biotechnological fields (Carmichael, 1992; Kang et al., 2012; Sasikala et al., 1994; Sasaki et al., 2002). ALA production by PNSB has great potential because of simple biosynthetic process, low cost and widely substances (Choorit et al., 2011; Sasaki et al., 1998). Since ALA is of increasing demand in many fields, more and more attention are devoted to increasing ALA production by PNSB (Choorit et al., 2011; Chung et al., 2005).

PNSB wastewater treatment is usually carried out in a photobioreactor. Controlling operation parameters is critical to the treatment performance of and products yields in a photobioreactor system. Previous studies focused on enhancing PNSB biomass and ALA yield by regulating conditions including pH (Chung et al., 2005), metal ions addition (Tangprasittipap et al., 2007), synthetic substrates (Ano et al., 1999), ALA dehydratase inhibitor (Ano et al., 2000) and metabolic engineering (Kang et al., 2012). Moreover, soybean wastewater was proved to be a popular culture for

* Corresponding author at: School of Municipal and Environmental Engineering, Harbin Institute of Technology, Huanghe Road 73, Harbin 150090, China. Tel.: +86 82502680; fax: +86 10 62511042.
E-mail address: zgm@ruc.edu.cn (G. Zhang).

http://dx.doi.org/10.1016/j.biortech.2016.01.030
0960-8524/

PNSB growth and biomass accumulation (Lu et al., 2011; Wu et al., 2012). Hence, soybean wastewater was selected for PNSB biomass and ALA production in this study.

Two functional microorganisms exist in the photobioreactor system, one is chemoheterotrophic bacteria, the other is photosynthetic microorganisms including microalgae or PNSB. Photosynthetic microorganisms have longer growth period than chemoheterotrophic bacteria (Park and Craggs, 2011; Chu et al., 2015). The specific growth rate of PNSB is generally slower than that of chemoheterotrophic bacteria, so PNSB requires relatively longer hydraulic retention time (HRT) than chemoheterotrophic bacteria in the photobioreactor system (Eroglu et al., 1999; Koku et al., 2003). Moreover, it was reported that HRT and organic loading rate (OLR) had obvious impacts on biomass and carotenoids productions in the photobioreactor for treating acid wastewater in our previous study (Liu et al., 2016). Hence, regulating HRT was critical to the pollutant removal efficiency, PNSB biomass and valuable materials production of PNSB-chemoheterotrophic bacteria wastewater treatment system. Few studies are reported about the effects of HRT on PNSB biomass, ALA production and functional microorganism population dynamics. Different levels of PNSB biomass, ALA yield and pollutant removal were influenced by varying HRTs. Similarly, as another key parameter, OLR has significant impacts on the efficiency of photobioreactor system. Soybean wastewater was proved to be a good culture medium for PNSB growth and ALA production (Liu et al., 2015), so it was used for biomass and ALA production of PNSB in this study.

The purposes of this study were to assess the efficiency of soybean wastewater treatment under different HRTs and OLRs in a photobioreactor; to investigate the impacts of different HRTs and OLRs on PNSB biomass and ALA yield in the continuous culture photobioreactor; to analyze the functional microorganism population dynamics depending on different HRTs and OLRs in the photobioreactor.

2. Methods

2.1. Inoculum

A purple non-sulfur bacteria (PNSB) strain (*Rhodobacter sphaeroides* ATCC17023) was obtained from China General Microbiological Culture Collection Center (CGMCC) in this study. It was cultured in a thermostat shaker (static, 30 °C) under light-microaerobic conditions with PYG medium for 36 h. PYG medium comprised 10 g/L polypepton, 5 g/L yeast extract and 1 g/L glucose, pH of PYG medium was adjusted to 6.8–7.0. The density of *R. sphaeroides* at logarithmic growth phase in the initial inoculum was 6.8×10^8 CFU/mL. Bacteria except PNSB in the wastewater were mostly chemoheterotrophic bacteria and the density was 3.2×10^5 CFU/mL using the plate count method.

2.2. Wastewater

A soybean wastewater was collected from a local soybean milk producer. Soybean wastewater of different nutrients loadings contained chemical oxygen demand (COD) of 6200 and 9300 mg/L (COD: TN: TP = 200: 15: (1). The characteristics of wastewater (containing COD of 6200 mg/L as an example) were as follows: COD, total nitrogen (TN) and total phosphorous (TP) were 6200, 408 and 30 mg/L, respectively; protein concentration was 4077 mg/L; and pH was about 7.0 ± 0.2. Fe^{2+} solution contained 40 mmol $FeCl_2 \cdot 4H_2O$ and 1 L H_2O. Fe^{2+} solution of 10 mL was added into 1 L wastewater each time.

2.3. Photobioreactor design and operating conditions

Two parallel photobioreactors were kept at 30 °C in an electrothermal incubator. The photobioreactor was performed according to the description of the previous study (Liu et al., 2016). The details were described as follows. Each photobioreactor unit consisted of two parts: (1) 1 L gas-tight glass was used as the photobioreactor. Each photobioreactor had a reaction volume of 800 mL. A magnetic stirrer ensured homogeneous mixing at the bottom of photobioreactor. (2) The illumination was provided by two 60 W incandescent lamps.

The light intensity was adjusted to 3000 lux by changing the distance between bioreactor and incandescent lamp. Light-microaerobic conditions were provided for *R. sphaeroides* growth. Micro-aerobic condition was maintained by flushing air inflow.

After initial *R. sphaeroides* preincubation for 48 h with PYG medium, the reactor was fed with continuous influent and effluent of a soybean wastewater. Uniform initial HRT, Uniform initial HRT, inoculation of *R. sphaeroides* and pH were 96 h, 550 mg/L and 7.0. The detailed information of parameters about operations was formulated in Table 1.

2.4. Analytical methods

COD, TN, TP concentrations and biomass were detected according to APHA standard methods (Clesscerl et al., 1998). Protein concentration was determined according to a Lowry method of protein determination kit (SHANGHAI LABAIDE).

5-Aminolevulinic acid hydrochloride was provided by Tokyo Chemical Industry (Tokyo, Japan). The intracellular ALA concentration was measured according to the description (Liu et al., 2010). The ALA yield (mg/g-biomass) was calculated by Eq. (1):

$$Y = 1000 \times C/W \quad (1)$$

where *Y* denotes the ALA yield, *C* (mg/L) denotes the ALA concentration at 96 h, *W* (mg/L) denotes the dry biomass at 96 h. Sample collected from photobioreactors was centrifuged at 9000 rpm for

Table 1
Parameters and performances of the photobioreactor system during continuous operation.

Parameters and performances	Time periods				
	1–20 d (I)	21–40 d (II)	41–52 d (III)	53–70 d (IV)	71–90 d (V)
HRT (h)	96	60	48	60	60
Influent COD (mg/L)	6200	6200	6200	9300	6200
OLR (g/L/d)	1.55	2.48	3.10	3.72	2.48
Average COD removal efficiency (%)	88.6	89.6	75.1	54.4	89.5
COD removal loading rate (g/L/d)	1.37	2.22	2.33	2.02	2.22
Protein removal (%)	90.6	91.7	72.7	62.6	91.7
Biomass production (mg/L)	2642.0	2653.0	2192.7	2474.7	2655.0
ALA yield (mg/g-biomass)	6.85	7.31	4.44	5.23	7.40
ALA production (mg/L/d)	4.52	7.76	4.87	5.18	7.86