Bioresource Technology 157 (2014) 37–43

Contents lists available at ScienceDirect

Bioresource Technology

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

Simulated sugar factory wastewater remediation kinetics using algal–bacterial raceway reactor promoted by Polyacrylate polyalcohol

Abdul Rehman Memon [a,*], John Andresen [b], Muddasar Habib [c], Muhammad Jaffar [d]

[a] Department of Chemical Engineering, Mehran University of Engineering & Technology, Jamshoro, Pakistan
[b] School of Engineering & Physical Sciences, Heriot-Watt University, Edinburgh, Scotland EH14 4AS, United Kingdom
[c] Department of Chemical Engineering, University of Engineering & Technology, Peshawar, Pakistan
[d] Department of Environmental Science and Engineering, Beijing University of Chemical Technology, Beijing 100029, China

HIGHLIGHTS

- Copolymer Polyacrylate alcohol was used for the first time in this research work.
- Polyacrylate polyalcohol induced pH neutralisation of the cultivation medium.
- *C. vulgaris* cultivation yielded in photosynthetic oxygenation of 11 mg l^{-1} d^{-1}.
- Immobilised *C. vulgaris* cultivation resulted in 89% removal of COD in 96 h.

ARTICLE INFO

Article history:
Received 30 October 2013
Received in revised form 11 January 2014
Accepted 14 January 2014
Available online 24 January 2014

Keywords:
C. vulgaris
P. putida
Polyacrylate polyalcohol
Sugar factory wastewater
Wastewater treatment

ABSTRACT

The remediation kinetics of simulated sugar factory wastewater (SFW) using an algal–bacterial culture (ABC) of *Chlorella vulgaris* in association with *Pseudomonas putida* in a raceway reactor was found to be enhanced by 89% with the addition of 80 ppm of copolymer Polyacrylate polyalcohol (PAPA). This was achieved by efficient suspension of the ABC throughout the water body maintaining optimum pH and dissolved oxygen that led to rapid COD removal and improved algal biomass production. The suspension of the ABC using the co-polymer PAPA maintained a DO of 8–10 mg l^{-1} compared to 2–3 mg l^{-1} when not suspended. As a result, the non-suspended ABC only achieved a 50% reduction in COD after 96 h compared to a 89% COD removal using 80 ppm PAPA suspension. In addition, the algae biomass increased from 0.4 g l^{-1} d^{-1} for the non-suspended ABC to 1.1 g l^{-1} d^{-1} when suspended using 80 ppm PAPA.

1. Introduction

Reclamation of industrial wastewater using algal and bacterial consortia (ABC) in a raceway setting has a great potential to address cost and energy issues associated with traditional wastewater treatment (Ruiz et al., 2013). Of main concern is sugar refining, where a ton of sugar beets generates between 0.2 and 0.5 m^3 of sugar factory wastewater (SFW) rich in sucrose and its derivatives (Zver and Glavic, 2005). The SFW effluents are often stored untreated in holding ponds within the factory premises, which can lead to environmental concerns due to their high organic and nutritious contents (Ingaramo et al., 2009). Previous research to harvest nutrients, such as nitrogen and phosphorus as well as energy contained in SFW has been carried out by anaerobic acidification (Alkaya and Demirer, 2011), mixed anaerobic culturing (Ozkan et al., 2010), electrochemical (Guven et al., 2009), adsorption (Parande et al., 2009), fixed bed aerobic biological reactor (Borghei et al., 2008), upflow anaerobic fixed bed bioprocess (Farhadian et al., 2007) and end-of-pipe stabilization in ponds or lagoons (Dilek et al., 2003). However, scale-up issues have affected the implementation of algal–bacterium consortium (ABC) for SFW treatment, in particular the maintenance of pH and DO due to settling of the ABC together with its non-energy intensive suspension that need to be optimised for efficient COD removal rate and improved biomass production.

Recently, ABC was successfully used without exogenous oxygen for the treatment of: swine slurries (Gonzalez-Fernandez et al., 2011), industrial wastewater (Godos et al., 2011), municipal wastewater (Su et al., 2011) and removal of heavy metals and organic pollutants (Munoz et al., 2006). *Chlorella vulgaris* is the most cultivated green algae due to its tolerance and fast growth rates under varying concentrations of growth nutrients (Dries et al., 2012). *Chlorella vulgaris* was isolated from sugar refinery wastewater

* Corresponding author. Tel.: +92 03333608199.
E-mail address: abdulrehman.memon@faculty.muet.edu.pk (A.R. Memon).

http://dx.doi.org/10.1016/j.biortech.2014.01.047
0960-8524/

suggesting that this alga thrives upon sugar as a feed substrate (Martinez and Orus, 1991). The photoheterotrophic or mixotrophic propagation ability of *C. vulgaris* to feed on organic carbon contained in SFW without exogenous CO_2 coupled with concomitant reduction in the organic loading of SFW is the main strength of its cultivation in SFW. The mixotrophic cultivation of *C. vulgaris* in SFW could hold twin advantage of avoiding exogenous CO_2 and O_2 supply to the ABC consortium, thus making the process more economical. An optimised consortium performance may significantly increase biomass yield that can be further harnessed for potential energy production. *Pseudomonas putida* (*P. putida*) is a harmless NCIMB group 1 obligate aerobe and metabolically versatile heterotroph, which functions best under similar conditions as for *C. vulgaris* (Walker and Weatherley, 1999). *Pseudomonas putida* is also known to utilise sugars and other organics for its growth, which indicates that *P. putida* can be associated with *C. vulgaris* as its growth promoter (El-Masry et al., 2004). The ABC efficiency could be enhanced by using a suspension medium in the reactor such as Polyacrylate polyalcohol, which could also be an improving agent by encapsulating the algae cells in hydrolysed copolymer cages resulting in enhanced photosynthetic efficiency by algae cells (Fang and Liu, 2002). This cationic copolymer is non-biodegradable and its addition in water may result in better bridging of algae cells forming an algae-copolymer-algae matrices in addition to being a pH-regulator agent during the bioremediation process.

The objective of this study was to investigate remediation kinetics of synthetic sugar factory wastewater using an algal–bacterial raceway reactor promoted by copolymer Polyacrylate polyalcohol (PAPA). This study showed that the remediation kinetics of synthetic sugar factory wastewater using an ABC suspended by PAPA significantly enhanced the operational parameters, i.e. pH and DO, which resulted in significant improvement in the nutrients removal rates and algal biomass production. The ABC of *C. vulgaris* and *P. putida* was used in this work for the first time to carry out SFW remediation. The main issue was the reduction in dissolved oxygen (DO) down to 2 mg l^{-1} and decrease in pH down to 5 during benthic free cell cultivation of ABC, which limited the effectiveness of the consortium. However, addition of suitable copolymer dosage for ABC immobilisation restored the pH and DO profile to 7 and 10 mg l^{-1}, respectively, during the critical phase of algae growth resulting in accelerated growth of algae cells as well as removal of major fraction of the organics from simulated SFW.

2. Methods

2.1. Preculturing of C. vulgaris and P. putida

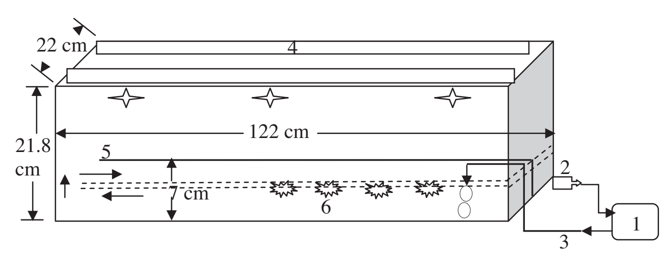
Chlorophyta *C.vulgaris* strain CCAP No. 211/79 was purchased from Culture Collection for Algae and Protozoa Scotland, UK. The preculturing of *C. vulgaris* was carried out in a 250 ml sterilized flask containing BG-11 broth that was exposed to 30-W cool white fluorescent incident light of 2800 ± 100 Lux and temperature of 28 °C ± 2. The BG-11 broth was prepared by dissolving 1.64 g of BG-11 powder in one litre distilled water (DW) in a sterilized bottle along with the addition of 1 ml of separately prepared solution of trace metal mix. The BG-11 powder was composed of the chemicals that included (in mg): $NaNO_3$ (1500), K_2HPO_4 (40), $MgSO_4{\cdot}7H_2O$ (75), $CaCl_2{\cdot}2H_2O$ (36), $C_6H_8O_7$ (6), $_6H_{5+4y}Fe_xN_yO_7$ (6), $C_{10}H_{16}N_2O_8$ (1), Na_2CO_3 (20). The 1 ml trace metal mix solution was prepared using the chemicals such as (in mg): H_3BO_3 (2860), $MnCl_2 \cdot 4H_2O$ (1810), $ZnSO_4 \cdot 7H_2O$ (220), $Na_2MoO_4 \cdot 2H_2O$ (400), $CuSO_4$. $5H_2O$ (800) and Co $(NO_3)_2 \cdot 6H_2O$ (50). The pH of the prepared BG-11 solution was adjusted from 3.8 to 7.1 by adding 1 M NaOH progressively, and the prepared BG-11 solution was sterilised for 2 h at 160 °C. The culture was aerated using 0.5 l min^{-1} of air filtered through a 0.2 μm in-line air filter. The strain was kept under these conditions for 5–7 days before being introduced into the raceway reactor. Pseudomonad *P. putida* strain KT2440, ATCC No. 47054 was purchased from LGC standards Teddington Middlesex, UK. The preculturing of *P. putida* was carried out using the growth medium Luria Bertani (LB). The LB medium was prepared using (in g): Tryptone (10), Sodium chloride (1.81) and Yeast extract (5). The cell-line of 10 ml starter culture was propagated in a test tube at 37 °C for 24 h, which was then transferred into two 100 ml sterilized flasks containing 50 ml of LB medium and 5 ml of sugar water for subculturing at the same temperature along with aeration rate of 0.25 l min^{-1}.

2.2. Simulated sugar factory wastewater

Simulated sugar factory wastewater (SFW) was synthesised based on the composition of wastewater from a UK beet sugar factory since the regular sampling of real wastewater was not possible as the factory runs its campaign for only around four months in a year. Sucrose with 0.5 M was used as the model substrate for carbohydrates contained in the SFW (Skrbic and Gyura, 2007), which reportedly also contains trace quantities of micronutrients required for algae growth such as iron, copper and zinc (Brennan and Owende, 2010). C:N:P ratio of 100:5:1 was used, which contained 30% less N than the standard Redfield ratio. The ingredients to prepare synthetic SFW included (in g l^{-1}): sugar (13), NH_4HCO_3 (0.65), KH_2PO_4 (0.13) and $Ca(OH)_2$ (0.52).

2.3. Lagoon photo tank for mass cultivation of the biomass

Fig. 1 shows the 1.2 m long and 22 cm wide lagoon photo tank (LPT) made of Perspex material mimicking a raceway reactor. The tank contained a working volume of 13 l of artificial SFW and was partitioned on the inside using a 90 cm divider to create wall turbulence for homogeneity of the culture contents and to make the LPT geometry in line with standard raceway lagoons or photobioreactors. An overhead lighting unit (Boss lighting, UK) was used that consisted of two fluorescent tubes of 38 W emitting cool white fluorescent luminance (400–700 nm) to yield an estimated photosynthetic active radiation of 43% (Zemmels et al., 2009). The water in the tank was recirculated at 40 ml min^{-1} to simulate flow pattern as in a raceway reactor as well as to stave off its possible thermal and chemical stratification along with inducing culture renewal cycles and gentle mixing likely without cell-shearing (Chisti, 2007). The surface to volume ratio of the LPT was 89 m^{-1} coupled with a short light path of 22 cm to obtain maximum light absorbance and minimum light attenuation for the optimum growth of the culture.



Legends: 1- Peristaltic pump, 2- Outlet point, 3- Inlet point, 4- Light source, 5- Partition bar, 6- immobilized algae cells.

Fig. 1. Schematic of lagoon photo tank.