Marine Pollution Bulletin xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Cultivation of activated sludge using sea mud as seed to treat industrial phenolic wastewater with high salinity

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ARTICLE INFO

Article history:
Received 28 July 2016
Received in revised form 9 November 2016
Accepted 13 November 2016
Available online xxxx

Keywords:
Saline wastewater
Marine activated sludge
Phenol-tolerant
Hierarchical acclimation

ABSTRACT

A technique is proposed to treat saline hazardous wastewater by using marine activated sludge, cultivated with sea mud as seed. Since the developed marine activated sludge had phenol-tolerant microorganisms (MAS-1, MAS-2 and MAS-3) which originated from the ocean, it was envisaged that these bacteria could survive and breakdown phenol in saline environments. In this work, typical phenol-tolerant microorganisms were isolated from the marine activated sludge and identified. After a hierarchical acclimation process, the marine activated sludge was used to treat the industrial phenolic wastewater with high salinity. The marine activated sludge was able to break down phenol and other organic components effectively and efficiently in treating the wastewater with salinity of 5.7% w/v. The results showed a high removal of phenol (99%), COD (80%) and NH_3-N (68%).

1. Introduction

Many industries including food, pharmacy, agriculture, petroleum, manufacturing and mining, discharge a large amount of saline hazardous wastewater to our lands, rivers and oceans, causing significant pollution unless adequately treated (Lefebvre and Moletta, 2006). Properly and efficiently treating these saline hazardous wastewaters with low cost has been an emerging topic to researchers (Fernandez-Torres et al., 2012; Kim and Logan, 2013; Taheri et al., 2012; Yuan et al., 2014). As biological treatment is an efficient way and is easy to scale up, methods utilizing biological resources are of much interest to the researchers.

Activated sludgeis a widely-used biological technique in wastewater treatment with the lowest cost among current techniques (Gander et al., 2000). A traditional method to implement the activated sludge for saline hazardous wastewater treatment is to acclimatize the activated sludge under harsh and challenging conditions for a long period (Kargi and Dincer, 1996; Lu et al., 2014; Panswad and Anan, 1999). However, during the long acclimatization period, the biological diversity of activated sludge will sharply decrease, resulting in a failure of the biosystem, since few typical microorganisms are able to survive in the saline hazardous environment (Lee et al., 2005; Yoshie et al., 2006). To overcome this problem, many works are carried out to isolate special microorganisms, such as salt-tolerant phenol-degrading microorganisms, from saline environments and use them in bioreactors for an enhanced treatment of saline hazardous wastewaters (Bastos et al., 2000; Bonfa et al., 2013; Jiang et al., 2016).

Ocean is a typical saline environment, containing abundant marine microorganisms (Oren, 2008). These microorganisms are salt-tolerant and can even utilize hazardous organics as carbon sources (Moxley and Schmidt, 2012; Tan et al., 2016). In this work, we have cultivated a new activated sludge using sea mud as seed, called marine activated sludge, to treat industrial phenolic wastewater with high salinity. A quick isolation and an identification method of the typical marine microorganisms are presented, showing some characteristics of the functional bacteria. Apart from this work, since the ocean contains many other kinds of tolerant microorganisms, our strategy may offer some possibilities to develop methods in treating other kinds of saline hazardous wastewater.

2. Materials and methods

2.1. Chemicals

All chemicals were analytical reagents (AR). The starch (soluble, from potato) and glucose were purchased from Sinopharm Chemical Reagent, China. Other chemicals were from Sigma-Aldrich, China.

2.2. Cultivation of marine activated sludge

The marine activated sludge was cultivated in seawter using sea mud as seed in an aerobic bioreactor (Fig. 1) with an effective volume of 30 L. The salinity of sea mud and seawater is 3.2 ± 0.1% (w/v), taking

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http://dx.doi.org/10.1016/j.marpolbul.2016.11.026
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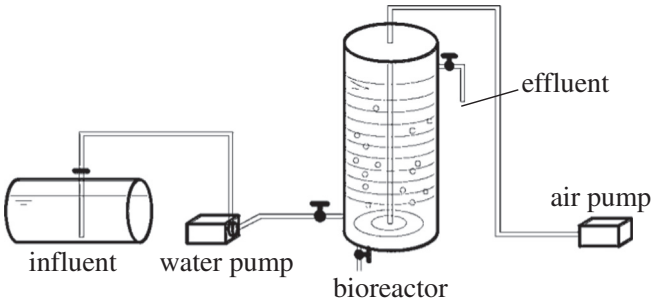

Fig. 1. Schematic diagram of the aerobic bioreactor for cultivation of the marine activated sludge and wastewater treatment.

from the Golden Bay at Weihai, China (37.54 °N and 122.07°E, Yellow Sea). During the cultivation of 60 days, temperature was controlled at 20 ± 1 °C and concentration of dissolved oxygen was maintained at 2.5 ± 0.2 mg/L. The bioreactor was fed once a day by replenishing the supernatant with fresh seawater, containing additional nutrients of 0.1689 g/L starch (soluble, from potato), 0.2064 g/L glucose, 0.0056 g/L NH_4Cl and 0.0028 g/L KH_2PO_4. Sludge volume index (SVI) of the marine activated sludge was measured every 10 days, showing the maturity of the marine activated sludge.

2.3. Isolation and identification of phenol-tolerant microorganisms

To study the phenol-tolerant bacteria in the marine activated sludge, we isolated and purified three main strains (named as MAS-1, MAS-2 and MAS-3) from it. The liquid culture medium contained 0.5 g peptone, 0.5 g phenol, 0.1 g K_2HPO_4, 0.05 g $MgSO_4$, 0.01 g $CaCl_2$, 0.01 g $FeSO_4$ and 1000 mL seawater with regulated pH at 7.5. The solid isolation medium contained 20 g agar, 1.1 g phenol (same concentration as the industrial phenolic wastewater used in this work), 1 g NH_4SO_4, 0.5 g K_2HPO_4, 0.05 g $MgSO_4$, 0.01 g $CaCl_2$, 0.01 g $FeSO_4$ and 1000 mL seawater with regulated pH at 7.5.

The strain was isolated and purified by a quick method described as follows: a 5 g sample of the marine activated sludge was put into a 500 mL conical flask filled with 200 mL of the liquid culture medium. The flask was shaken in a mixing table at 25 °C and 150 rpm for one week to enrich microorganisms. An inoculating loop containing the enriched culture solution was used to draw curvatures on the slants of solid culture medium in prepared test tubes (30 mm × 200 mm). Then the tubes were cultured in an incubator at 25 °C for 4 days to obtain single colonies on slants. Subsequently, colonies with highest growth rate (large mass) were selected. Isolation and purification were conducted multiple times to obtain purely cultured phenol-tolerant strains. The isolated microorganisms were used respectively in the liquid cultivate medium at 25 °C to biodegrade the phenol. The removal of phenol was measured after 48 h using the spectrophotometry (Folsom et al., 1990). Besides, pH of the liquid cultivate medium was adjusted from 1 to 14 with temperature at 25 °C to measure their biodegradability of phenol. The pH with the highest removal amount was regarded as the optimum pH, while the pH with a removal higher than 50% was concluded in bioactive range of pH. Also, temperature was changed from 5 to 45 °C with pH at 7.5 to measure the biodegradability of phenol. The temperature with the highest removal amount was regarded as the optimum temperature, while the temperature with a removal higher than 50% was concluded in bioactive range of temperature. To identify these phenol-tolerant microorganisms, Gram staining was implemented (Li et al., 2012) and 16S rDNA was performed by Takara Biotechnology (Dalian) Co., Ltd. using the amplification-sequencing method (Kim et al., 2004; Yi and Chun, 2004). The determined 16S rDNA sequence was analysed by BLAST (Basic Local Alignment Search Tool) in GenBank.

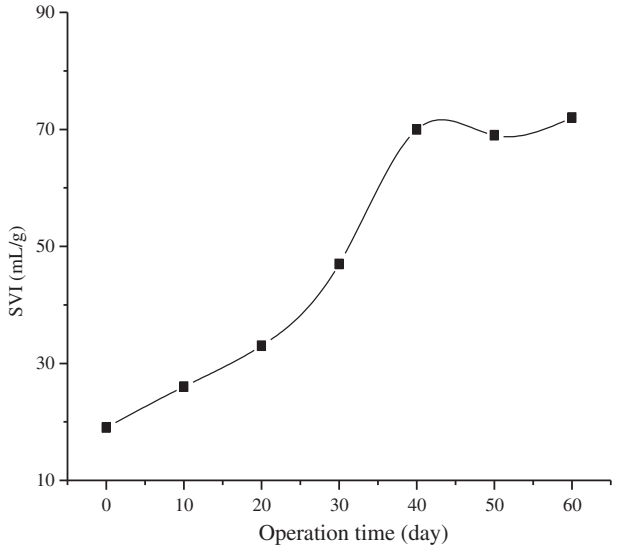

Fig. 2. Change of SVI over the operation time.

2.4. Wastewater treatment

Besides the lab-scale biodegradation of phenol, an industrial phenolic wastewater taken from the Weihai Chemical Plant (Shandong, China) was used in this work to evaluate the practical biodegradability of the marine activated sludge. pH, salinity, concentration of phenol, COD and NH_3-N of the industrial phenolic wastewater were measured to be 11, 5.7%, 1100 mg/L, 4700 mg/L and 67 mg/L, respectively. The pH of industrial phenolic wastewater was adjusted to 7 by simply adding HCl solution. The industrial phenolic wastewater was mixed with seawater in ratios of 1:4, 2:3, 3:2 and 4:1, respectively, and used as influent every several days for sludge acclimation (Table 2). Start from the 28th day, no seawater was added to the influent. Industrial standard methods were used to measure the concentration of phenol (4-aminoantipyrine spectrophotometry), COD (permanganate index) and NH_3-N (naphthol blue spectrophotometry) (Chinese-SEPA, 1997).

3. Results and discussion

3.1. Cultivation process

SVI (sludge volume index) is a typical value to evaluate the physiochemical properties and bioactivity of activated sludge (Dick and

Table 1
Characteristics of the phenol-tolerant microorganisms.

Strain	MAS-1	MAS-2	MAS-3
Removal of phenol (48 h)	89%	81%	87%
Optimum pH	7 (active among 5–9)	7 (active among 5–9)	7 (active among 5–9)
Optimum temperature	35 °C (active among 20–40 °C)	35 °C (active among 25–40 °C)	35 °C (active among 20–40 °C)
Optimum salinity	3% (active among 1–6%)	3% (active among 1–6%)	3% (active among 1–6%)
Gram straining	Negative	Positive	Negative
Species	*Oceanimonas* sp.	*Arthrobacter* sp.	*Vibrio* sp.

Please cite this article as: Tan, S., et al., Cultivation of activated sludge using sea mud as seed to treat industrial phenolic wastewater with high salinity, Marine Pollution Bulletin (2016), http://dx.doi.org/10.1016/j.marpolbul.2016.11.026