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Research article

A novel salt-tolerant bacterial consortium for biodegradation of saline and recalcitrant petrochemical wastewater

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ABSTRACT

Treatment of a saline petrochemical wastewater with BOD_5/COD ratio of less than 0.1 was investigated using a consortium consisted of three isolated salt-tolerant bacteria namely, *Kocuria turfanesis*, *Halomonas alkaliphila* and *Pseudomonas balearica*. Selected bacteria were isolated from petrochemical wastewater containing mineral salt mediums of 3% salinity. A lab-scale activated sludge bioreactor was used for startup in batch mode operation and after obtaining the MLSS concentration of about 3000 mg/L, the operation was changed to continuous flow mode to determine the biokinetic coefficients under different organic loading rates of 0.33–1.21 kg $CODm^{-3}$ d^{-1}. The COD removal efficiency of 78.7%–61.5% was observed for treatment of real saline wastewater with a decreasing trend along with increasing the organic loading rate. In addition, results of kinetic investigation demonstrated that the yield(Y), endogenous decay coefficient (k_d), maximum reaction rate (K_{max}), maximum specific growth rate (μ_{max}) and saturation constant (K_s) were 0.54 mg VSS mg COD^{-1}, 0.014 day^{-1}, 1.23 day^{-1}, 0.66 day^{-1}, and 1315 mg L^{-1}, respectively.

1. Introduction

Saline wastewater is often considered as an effluent which contains high amounts of organic compounds and at least 10,000–15,0000 mg L^{-1} total dissolved solids (TDS) (Lefebvre and Moletta, 2006). The wastewaters discharge from industries such as pesticides, herbicides, organic peroxides, pharmaceuticals, tanneries, petroleum, petrochemical and textile have always been characterized by their high salinity (Lefebvre et al., 2005; Darvishi Cheshmi Soltani et al., 2016a, 2016b; Jorfi et al., 2016). High salinity content in wastewater not only inhibits the metabolic functions of heterotrophic bacteria dealing with biological wastewater treatment, but also reduces the efficiency of remediation process. Isolation and enrichment of salt-tolerant bacteria have been conducted to treat saline wastewaters by different environmentalists (Duan et al., 2015; Tayybi et al., 2016; Kalantary et al., 2014; Jorfi et al., 2017). Saline wastewaters containing recalcitrant organics can be treated through different physical, chemical and biological remediation processes (Jemli et al., 2015; Zhang et al., 2014; Zhao et al., 2016). Although the physical and chemical treatment processes have been applied successfully, but some drawbacks such as chemical consumption, difficult operation, high energy consumption and secondary pollution due to the production of chemical residuals are possible. Conversely, biological processes are characterized as simple, cost-effective, least chemical requirement and efficient processes (Kim et al., 2016; Lu et al., 2014; Pendashteh et al., 2012; Rezaee et al., 2010). Biodegradation of wastewater is among the most effective approaches for removal of organic contaminants from a wide range of industrial wastewaters. Microbial consortium is the key component of biological systems for degradation of targeted contaminants (Boopathy et al.,

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2007; Pronk et al., 2014; Jaafarzadeh et al., 2011). However, direct biological treatment of saline petrochemical wastewater is limited by presence of recalcitrant organics like aromatic and aliphatic hydrocarbons and also chlorinated hydrocarbons leading to low BOD_5/COD ratio in these industrial wastewaters (Jemli et al., 2015). In addition, high salinity leads to either plasmolyzation or reduction of bacterial metabolism (Abou-Elela et al., 2010; Amin et al., 2014). The adverse effects of salinity on biological systems such as conventional activated sludge and extended aeration have been reported by researchers (Kargi, 2002; Mannina et al., 2016a, 2016b; Castillo-Carvajal et al., 2014). In order to overcome these obstacles, the isolation and enrichment of salt-tolerant bacteria as pure cultures have been proposed in previous research (Tan et al., 2016). Success in such activity strictly depends on survival and efficient metabolism of isolated salt-tolerant strains in real saline wastewaters like laboratory incubation conditions. The salt requirement for microorganism growth can be divided into several categories: (i) non-halophiles (less than 0.2 M NaCl); (ii) halo-tolerant (non-halophiles that can tolerate large amount of salts); (iii) slight halophiles (0.2–0.5 M NaCl); (iv) moderate halophiles (0.5–2.5 M NaCl); (v) extreme halophiles (2.5–5.5 M NaCl). Extreme halophiles require NaCl concentrations above 1–1.5% for survival and optimum growth is often obtained at above 2% NaCl (Wang et al., 2013; Zhuang et al., 2010). The halophilic bacteria have special metabolic pathways with high capability for degradation of a wide range of pollutants. The salt-tolerant microorganisms are metabolically different and can adopt to extreme salinity (Darvishi Cheshme Soltani et al., 2013). The main objective of this study was to isolate salt-tolerant bacteria for TDS concentrations of up to 30,000 mg L^{-1}, followed by application of isolated strains in biological treatment of a recalcitrant and saline petrochemical wastewater. The effect of different organic loading rates (OLR) on the function of salt-tolerant consortium and biokinetic coefficients were studied in an activated sludge bioreactor.

2. Experimental

2.1. Isolation and identification of a salt-tolerant microorganism

The isolation and enrichment of salt-tolerant bacteria capable of degrading hydrocarbons was carried out, based on method described by Jorfi et al. (2013). Briefly, a low TDS recalcitrant wastewater containing various hydrocarbons originated from a petrochemical industry in the southwest of Iran was poured into a 250 mL flask containing 100 mL enrichment culture solution. The enrichment cultures contained (g/L) K_2HPO_4, 6.3; KH_2PO_4, 1.8; NH_4Cl, 1; $MgSO_4{\cdot}7H_2O$, 0.1; $CaCl_2{\cdot}H_2O$, 0.1; $FeSO_4{\cdot}7H_2O$, 0.1; $MnSO_4{\cdot}H_2O$, 0.1 and 1 ml/L of trace elements solution, pH 7.0. The trace elements solution contained (g/L) H_3BO_3, 0.03; $ZnSO_4{\cdot}7H_2O$, 0.01; $CoCl_2{\cdot}6H_2O$, 0.02; Na_2MoO_4, 0.006; $CuSO_4{\cdot}2H_2O$, 0.001(Jorfi et al., 2013). All of the culture mediums were sterilized by autoclaving. The enriched medium was supplemented with 5 mL saline wastewater (characteristics are presented in Table 2) as the sole source of carbon and energy. Experimental flasks were also incubated at 31 °C on a shaker (IKM 4000 ci, Germany) at 180 rpm during one week. The bacterial growth was observed through the measurement of the absorbance at 600 nm (OD_{600}). Afterwards, 5 mL enriched culture was added into another 250-mL flask with 95 mL fresh saline wastewater + enrichment medium (Budsabun, 2015). This procedure was repeated six times. To isolate pure salt-tolerant strains, 1 mL culture supernatant was diluted to 10^{-5} times and spread onto the saline wastewater coated mineral agar plates and incubated at 31 °C for 3 days. There appeared several colonies and pure cultures of each morphologically distinct colony were obtained by repetitive streaking onto the saline wastewater + agar mineral medium. Thereafter, colonies demonstrated sufficient growth were determined as salt-tolerant strains capable of treating saline wastewater and maintained on nutrient agar slant contained 0.5 M NaCl at 4 °C.

2.2. Identification of salt-tolerant strain

The boiling approach was applied to extract the genomic bacterial DNA for identifying the salt-tolerant bacteria (Freschi et al., 2005; Freschi and Oliveira, 2005). Furthermore, the16S rRNA gene amplification and sequencing were conducted via following universal primers: fD1 (5′-AGA GTT TGA TCC TGG CTC AG-3′) and rD1 (5′-AAG GAG GTG ATC CAG CC-3′) (Weisburg et al., 1991). Each reaction was run with 50 μL mix using I-Taq Maxime PCR Premix (iNtRON Biotechnology, Korea). PCR was performed via the conditions below: denaturing step of 95 °C for 5 min, then 35 cycles of denaturing for 30 s at 95 °C, annealing for 30 s at 52 °C, and extension for 1.5 min at 72 °C, followed by a final extension at 72 °C for 15 min. Moreover, a 3730XL DNA analyzer instrument (Applied Biosystems) was applied to purify and also perform the sequencing of PCR substances under contract by bioneer Inc (South Korea). All original sequence fragments were edited and assembled using DNA Sequence Assembler v4 (2013). The sequence data were analyzed by comparison via nucleotide-nucleotide BLAST from NCBI (http://www.ncbi.nlm.nih.gov) and classified by Ribosomal Database Project Classifier tools (http://rdp.cme.msu.edu). Evolutionary analysis was carried out in MEGA6 by maximum likelihood algorithm using Kimura-2-parameter distances (Tamura et al., 2011) and 1000-bootstrap replication.

2.3. Lab scale bioreactor

A 20 L cubic glass reactor comprised of 12 L for aeration tank and 8 L for settling tank was used to perform biodegradation experiments. A vertical wall with a distance of 0.5 cm at the lower end was provided to separate settling tank from the aeration tank (Fig. 1). Vacuum force derived from the action of aerators led to the continuous recycling of settled sludge from the settling tank to the aeration tank. The reactor was aerated by means of an aerator pump with an injection rate of 6 L_{air} min^{-1}. The aeration tank was mixed with diffused air. A peristaltic pump with adjustable flow rate of 2–6 L $h^{-1}$was used to inject the influent wastewater. Petrochemical saline wastewater was obtained from a local petrochemical corporation, Mahshahr city, Iran.

2.4. Bioreactor startup

Isolated pure strains were transferred to 500 mL flasks containing nutrient broth and incubated in a shaker incubator (Model: IKM 4000, Germany) at 180 rpm and 31 °C to obtain sufficient growth ($OD_{600\ nm} = 2$) as initial seed for aeration tank. Thereafter, 75% of aeration tank volume was filled with liquid culture medium and the remaining volume of25% with raw saline wastewater. Furthermore, nitrogen and phosphorus were provided by addition of desired amounts of NH_4Cl and K_2HPO_4/KH_2PO_4 respectively to obtain the C:N:P ratio of 100:5:1. The reactor was first operated in the batch mode for approximately 9 weeks and DO was adjusted to 3–6 mg/L. The principal objective of this step was to reach the MLSS concentration of 2000–3000 mg/L. After each 24 h aeration period, the aerators were switched off and sludge settling occurred immediately. Afterwards, around 2 L of the supernatant was decanted and fresh raw wastewater with COD value of 1100–1300 mg $L^{-1}$was replaced. The proportion of influent wastewater was increased gradually and reached to 10 L during 9 weeks. Improvement of COD removal was considered as a criterion