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Full-scale bioreactor pretreatment of highly toxic wastewater from styrene and propylene oxide production

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ABSTRACT

The wastewater originating from simultaneous production of styrene and propylene oxide (SPO) is classified as highly polluted with chemical oxygen demand level in the range 5965 to 9137 mg L^{-1}—as well as highly toxic. The dilution factor providing for a 10 percent toxic effect of wastewater samples in a test with *Paramecium caudatum* was 8.0–9.5. Biological approach for pretreatment and detoxification of the wastewater under full-scale bioreactor conditions was investigated. The number of suspended microorganisms and the clean up efficiency were increased up to 5.5–6.58 × 10^8 CFU mL^{-1} and 88 percent, respectively during the bioreactor's operation. Isolates in the *Citrobacter*, *Burkholderia*, *Pseudomonas*, and *Paracoccus* genera were dominant in the mature suspended, as well as the immobilized microbial community of the bioreactor. The most dominant representatives were tested for their ability to biodegrade the major components of the SPO wastewater and evidence of their role in the treatment process was demonstrated. The investigated pretreatment process allowed the wastewater to be detoxified for conventional treatment with activated sludge and was closely related to the maturation of the bioreactor's microbial community.

1. Introduction

Currently, the petrochemical industry plays a major role in the economic growth of every nation and, in the specific case of Russia, is the recipient of major government subsidies. Its products are used in almost all industrial sectors. One typical petrochemical intermediate is styrene, which is important in rubber and plastics manufacture. For economic reasons styrene is generally co-produced together with propylene oxide. The significant use of petrochemical products results in the contamination of different ecosystems. During various stages in the production of styrene and propylene oxide (SPO), such as dehydration, dehydrogenation, and oxidation of organic compounds, highly polluted wastewater is formed. This wastewater contains volatile toxic compounds (Shokrollahzadeh et al., 2008) such as acetophenone, 1-phenyl ethanol, benzene, and phenol, as well as nonvolatile compounds such as mono- and dipropylene glycol, propanol, etc. The chemical oxygen demand (COD) of petrochemical wastewaters is typically very high. In multiple studies from different sites it has variously been estimated as 1620–1896 mg L^{-1} (Calheiros et al., 2009), 2500–4100 mg L^{-1} (Wei et al., 2010), and 2200–4700 mg L^{-1} (Chang et al., 2011). Currently, pollution of the environment by hazardous chemical compounds is one of the most important problems (Mantis et al., 2005). Those petrochemical wastewaters need to be treated before discharging into the environment to avoid river, soil, and air pollution (Stromgren et al., 1995; Chen et al., 1998; Jerez et al., 2002; Chen et al., 2003).

Industrial wastewater treatment methods can be divided into two main groups, physicochemical and biological. Most wastewater treatment processes rely on the use of activated sludge (Soddell and Seviour, 1990; Amann et al., 1998; Blackall et al., 1998; Yang et al., 2011), due to its considerably lower cost in comparison with the physicochemical method, and reasonable efficiency (Shokrollahzadeh et al., 2008; Babaee et al., 2010; Chang et al., 2011). However, some organic substances produced during chemical production are toxic or resistant to biological treatment in conventional biological processes (Adams et al., 1996; Pulgarin and Kiwi, 1996; García et al., 2001; Lapertot et al., 2006; Muñoz and Guieysse, 2006). Meanwhile, the use of chemical and physical techniques requires very expensive pretreatment, usually results in the production of secondary effluent (Sangave et al., 2007) and does not always reduce the pollutant concentration to acceptable levels, necessitating further pretreatment before the water is finally treated with conventional activated sludge (CAS) (Babaee et al., 2010).

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The high COD level in wastewater from SPO production makes CAS processes unsuitable for treatment due to its high toxicity to biota, although most components of the wastewater, such as glycols, are known to be biodegradable (Miller, 1979; Van Hamme et al., 2003; Shokrollahzadeh et al., 2008). Therefore, we proposed a pretreatment process of SPO wastewater using a biotechnological approach for further treatment with activated sludge. The use of a bioreactor, containing highly active specialized microorganisms, immobilized on a carrier, has been demonstrated for the removal of toxic substances, such as phenol, heavy metals and pesticides, from wastewater (Erhan et al., 2004; Wasi et al., 2011; Park et al., 2013). This approach is considered the most promising prospective one for pollutant elimination during SPO wastewater pretreatment. The carrier can maintain a high concentration of bacterial biomass, protect microorganisms immobilized on it from being shocked by any sudden change in pH, temperature, or oxygen concentration, which can lead to the reduction of sludge (Ye and Ni, 2002). In contrast to free microorganisms, immobilized microorganisms can biodegrade higher substrate concentrations (Wang et al., 2007). Bioreactor microflora plays an important role in COD load reduction (Wang et al., 2011). However, there was not sufficient data on microbial communities that could sustain extremely high COD levels as in the case of SPO production before, in which the COD level is more than 6000 mg L^{-1}. Furthermore, isolating and identifying microorganisms responsible for hydrocarbon transformation is important both for fundamental knowledge of microbial metabolic pathways and practical applications. Thus, this study aimed to characterize the microbial community cultivated from a full-scale bioreactor for SPO wastewater.

2. Materials and methods

2.1. Wastewater

Wastewater was collected from the SPO wastewater pretreatment plant (WWPTP) at Nizhnekamskneftekhim (NKNH) Enterprise. NKNH Inc. is a large petrochemical company headquartered in Nizhnekamsk city, Russia. The influent wastewater is characterized as highly alkaline (pH 8.0–12.5) with a high organic load (COD 5965–9137 mg L^{-1}). The main components of SPO wastewater determined by GC–MS were propylene glycol: 784.29–2517.78 mg L^{-1}, 1-phenyl ethanol: 113.62–237.46 mg L^{-1}, phenol: 105.93–182.41 mg L^{-1}, toluene: 80.63–85.87 mg L^{-1}, ethanol: 52.30–398.69 mg L^{-1}, propanol: 22.51–641.20 mg L^{-1}, dipropylene glycol: 20.63–86.75 mg L^{-1}, ethoxy propanol: 18.22–46.77 mg L^{-1}, methanol: 17.17–41.10 mg L^{-1}, acetophenone: 11.07–40.13 mg L^{-1}, ethylene glycol: 10.01–19.52 mg L^{-1}, diethylene glycol: 8.81–11.27 mg L^{-1}, allyl alcohol: 6.22–66.39 mg L^{-1}, propanal: 4.71–19.74 mg L^{-1}, benzyl alcohol: 4.04–8.33 mg L^{-1}, hydroxypropanone: 3.48–8.13 mg L^{-1}, benzaldehyde: 2.41–14.13 mg L^{-1}, acetone: 2.01–179.98 mg L^{-1}, *n*-butanol: 1.41–25.04 mg L^{-1}, ethyl butanol: 0.81–10.53 mg L^{-1}, acetaldehyde: 0.47–14.80 mg L^{-1}, isopropanol: 0.44–198.40 mg L^{-1}, styrene: 0.27–1.95 mg L^{-1}, methyl propyl ketone: 0.24–1.43 mg L^{-1}, hexanone: 0.08–0.31 mg L^{-1}, benzene: 0.01–0.02 mg L^{-1}, and ethylbenzene: 0.001–0.002 mg L^{-1}. Preliminary purification of SPO wastewater is performed under full-scale bioreactor conditions with the participation of freely suspended and immobilized microorganisms (Fig. 1).

2.2. Full-scale bioreactor system

The bioreactor system consisted of two blocks: a preparation block (A) and a biological treatment block (B) (Fig. 1). The system operated at a maximum wastewater feed rate of 25 m^3 h^{-1}. The volume of the bioreactor (6) was 1200 m^3. Block (A) contained a heat exchanger (1), acid (2) and biogenic substances (3) feed tank. Passing through a heat exchanger, the influent wastewater (14) was cooled to 25–35 °C. Before entering the bioreactor the alkaline wastewater was neutralized to pH 6.8–7.5 by sulfuric acid and supplied with superphosphate and ammonium sulfate to create favorable living conditions for microorganisms. Block (B) contained a fermenter (4) used once per year to recreate the microflora during annual bioreactor (6) preventive maintenance. The bioreactor was a round bottom tank which was divided into a zone of flotation (10) and a zone of aeration (9), containing carriers for immobilization (11). The influent wastewater, air for aeration (5) and air for flotation (16) came into the bioreactor at its bottom. The zone of flotation consisted of an annular partition (7) that had windows (8) for the fluid inlet, and provided a separation of the effluent wastewater and microbial biomass. In the upper part of the flotation zone the microbial biomass returned to the aeration zone in the foam form. The effluent wastewater (17) after the bioreactor was then sent to the all-factory wastewater CAS treatment plant. Waste air (13) was treated in a biological filter (12).

The microbial community of SPO bioreactor was obtained from enrichment community of the industrial activated sludge and wastewater slim. The studied microbial community was adapted to gradual increase of the COD by diluting the SPO wastewater. Fiberglass “brushes” and polyurethane foam carriers were used in the SPO bioreactor to immobilize the microflora.

2.3. Sampling

The sampling points were the influent wastewater (14), mix liquor from the bioreactor (6) and the effluent wastewater (17). The influent and effluent wastewaters were used for chemical and toxicological analyses. The mix liquor samples from the bioreactor were analyzed for their suspended cultivated microbial community and so were collected aseptically. Mix liquor samples were collected in the first, 16th and 32nd week after initiation of the bioreactor system. For assessment of the immobilized microbial community, we extracted the immobilized material after bioreactor shutdown at the 40th week. Mix liquor and carrier samples were transferred into sterile glass tubes and analyzed by microbiological methods within 6 h after sampling. In accordance with the mode of SPO production the system pauses after the 40th week for preventive maintenance. At the same time the sample of immobilized bacteria was collected aseptically and stored by laboratory BIOSTAT cultivation with diluted SPO wastewater. Before annual launching of the bioreactor, microbial community accumulation was performed in the full-scale fermenter (4) with the presence of diluted SPO wastewater and growth stimulating additives such as peptone and yeast extract. Initiation of the bioreactor system starts with microbial-free carriers. At the same time, SPO wastewater was mixed with microbial biomass from the fermenter (4). The fermenter works until the bioreactor biomass reaches 3×10^7 CFU mL^{-1}, thereafter the reactor performs without replenishment from the fermenter (4).

2.4. Microbiological analysis

Mixed liquor samples were inoculated on various rich and selective media such as meat-extract agar medium, yeast growth medium, N-free medium, Czapek-Dox medium, and King's B medium at different dilutions (10^{-2}–10^{-7} dilutions). For microbial sludge sample we used dilutions series up to 10^{-10}. The composition of these media is described below: meat extract medium—per liter fish meal pancreatic hydrolyzate (18 g), NaCl (2 g), agar (20 g), yeast growth medium—per liter glucose (40 g), peptone (10 g), yeast extract (4 g), agar (20 g), N-free medium—per liter sucrose (20 g), K_2HPO_4 (0.2 g), $MgSO_4 \cdot 7H_2O$ (0.2 g), NaCl (0.2 g), K_2SO_4 (0.1 g), $CaCO_3$ (5 g), agar (20 g), Czapek-dox medium—per liter sucrose (30 g), $NaNO_3$ (3 g), K_2HPO_4 (1 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), KCl (0.5 g), $FeSO_4 \cdot 7H_2O$ (0.01 g), agar (15 g), and King's B medium—per liter peptone (20 g), K_2HPO_4 (1.5 g), $MgSO_4 \cdot 7H_2O$ (2.8 g), glycerol (8 mL), agar (15 g). The pH was adjusted to 6.8–7.2.

After 3–5 days of incubation at 28 °C, different colonies were selected from the cultivated plates and repeatedly inoculated into agar plates until pure cultures were obtained. Total numbers of counted microorganisms were reported as CFU mL^{-1} for suspended microflora and CFU g^{-1} for immobilized microflora. The ratios of different bacterial types in the cultivated microbial community were estimated by identification of each colony on Petri dishes using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS).

The ability of dominant isolates of the bioreactor's microbial community, which were obtained during the study, to degrade wastewater compounds was assessed by their growth in a liquid medium using one of the main components of SPO wastewater as a single carbon source for 5 days. For this purpose we used a mineral medium (g L^{-1}): $(NH_4)_2SO_4$—1, $MgSO_4$—0.25, KH_2PO_4—3, $Na_2HPO_4 \cdot 12H_2O$—4.5 (pH 6.8–7.2). The main components of SPO wastewater were added to the mineral medium in the following scheme: propylene glycol—10 g L^{-1}, dipropylene glycol—10 g L^{-1}, the remaining components (ethylene glycol, diethylene glycol, styrene, 1-phenyl ethanol, acetophenone, benzene, toluene)—2 g L^{-1}. We also assessed the ability of these strains to biodegrade the components of SPO wastewater by assessing their growth in the influent wastewater. Optical density values at 600 nm were used to monitor growth. We used −, + and ++ for OD_{600} values of less than 0.1, 0.1–1, and more than 1, respectively. All the experiments were performed in triplicate.

2.5. Identification of microorganisms

The dominant isolates chosen from the microbial community and occupying a major fraction of the total in the bioreactor were identified. These isolates were identified using molecular-genetic analysis of 16S rRNA gene sequences. Genomic DNA from pure cultures of microorganisms was isolated using phenol-chloroform extraction (Maloy, 1989). The DNA concentration of the resulting solution was