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Diversity of bacteria in a full-scale petrochemical wastewater treatment plant experiencing stable hydrocarbon removal

Ali Behnami[a], Davoud Farajzadeh[b], Siavash Isazadeh[c], Khaled Zoroufchi Benis[d], Mohammad Shakerkhatibi[e,*], Zohreh Shiri[f], Sajjad Ghorghanlu[g], Adeleh Yadeghari[h]

[a] Department of Environmental Health Engineering, Tabriz University of Medical Sciences, Tabriz, Iran
[b] Department of Cellular and Molecular Biology, Faculty of Biological Sciences, Azarbaijan Shahid Madani University, Tabriz, Iran
[c] Life Science Group, Air Liquid Delaware Research and Technology Center, Newark, DE, USA
[d] Faculty of Chemical Engineering, Sahand University of Technology, Tabriz, Iran
[e] Health and Environment Research Center, Tabriz University of Medical Sciences, Tabriz, 5166614711, Iran
[f] Department of Biology, Faculty of Sciences, University of Tabriz, Tabriz, Iran
[g] Biotechnology Research Center, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
[h] Department of Analytical Chemistry, Faculty of Chemistry, University of Tabriz, Tabriz, Iran

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ABSTRACT

Bacteria play a main role in biological wastewater treatment plants (WWTP). Herein, bacterial diversity was characterized in a full-scale WWTP treating petrochemical wastewater containing a wide range of hydrocarbons. Diversity analysis of the activated sludge was carried out using culture-dependent and culture independent methods. From a total of 20 bacterial species identified using 16S rRNA sequence analysis, *Brevibacillus parabrevis, Cloacibacterium normanense* and *Sphingopyxis soli* were described for the first time from a petrochemical WWTP. The Shannon diversity index as a measure of bacterial diversity showed decreases from 3.181 to 2.614 during warm and cold seasons. The dominant phyla in all samples was *Proteobacteria* comprised 63% of the total isolates. Overall, the results showed that the diversity of bacterial communities was higher in warm months rather than cold months. Although, volatilization has usually a significant role on the removal of volatile organics such as phenol, benzene, toluene, and styrene from the liquid phase, the presence of isolated bacterial species indicates their contribution in removal process. Thus, further studies on the capabilities of isolated bacteria in degradation of specific pollutants will be helpful to deepen scientific understanding of biological treatment of difficult wastewaters.

1. Introduction

Petrochemical processes generate large quantity of wastewater [1] comprise of elevated amounts of chemical oxygen demand (COD), biological oxygen demand (BOD_5), suspended solids, oil and grease, metal salts, volatile compounds etc. [2,3]. Activated sludge process is commonly used as a suitable treatment method to reduce organic compounds from such wastewaters [4,5]. In this process, microorganisms are maintained in liquid suspension by appropriate mixing methods within aeration tanks. The desired microbial concentrations are provided by means of continues return of the settled sludge from secondary clarifiers to aeration tanks [6].

The activated sludge contains of highly complex microbial biomass made of bacteria, viruses, fungi and yeasts [5], in which bacteria are dominant [7]. There are some factors affecting bacterial composition including wastewater characteristics (nutrient contents, pH, temperature, dissolved oxygen, etc.) and operational parameters (solids retention times (SRT), returned activated sludge (RAS), food to microorganisms (F/M) ratios, mixing system, plant configuration, etc.) [4,8,9]. Understanding the composition of bacterial communities be present in the activated sludge can give a clear image on the effect of continuous process manipulation on contaminants removal [10] and provide important guidance in design and stable operation of wastewater treatment system [11]. As a result, it is necessary to understand the dynamics of the bacterial communities to monitor the operational problems, develop strategies for improving process performance and high-efficiency operation of biological processes occurring in wastewater treatment plants (WWTPs) [5,12].

Up to now, a few studies have been characterized the bacterial diversity in full-scale petrochemical wastewater treatment plants

* Corresponding author.
E-mail address: shakerkhatibim@tbzmed.ac.ir (M. Shakerkhatibi).

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(PWWTP) [4,13,14], most of them have been focused on lab-scale systems [15–17]. Because of the complexity of bacterial structure in full-scale WWTPs, implementing the lab-scale results will make possible lots of inaccuracies [7]. Moreover, to our knowledge, no comprehensive investigations have been conducted so far to long-term analysis the dynamic behavior of the bacterial community in activated sludge of a PWWTP.

On the other hand, the majority of previous studies on full-scale PWWTPs have been conducted using culture based isolation and characterization methods [4,13]. In the last two decades, culture independent techniques such as the PCR-based amplification of 16S ribosomal RNA genes have been developed for bacterial community analysis in various environments [8,18]. By applying 16S rRNA, extremely complex bacterial communities being in biological WWTPs [19] and the association of bacterial species with process parameters have been described [8]. However, this technique has not been intensively applied in full-scale PWWTPs. Therefore, more studies on full-scale systems are needed to overcome this shortage and obtain useful knowledge about significant bacterial populations of activated sludge capable degrading specific pollutants, as well as, their diversity and seasonal variations.

Thus, the objectives of this work were (a) study on the performance of activated sludge in hydrocarbon removal; (b) isolation and characterization of bacterial community, and (c) estimating bacterial diversity using Shannon diversity index.

2. Materials and methods

2.1. WWTP description and sample collection

This study was carried out on an extended aeration activated sludge system treating Tabriz Petrochemical Company (TPC) wastewater with an average design flow rate of 4800 m^3/d. The PWWTP consist of coarse screens, API separator, equalization basin, coagulation-flocculation and dissolved air flotation (DAF) systems, followed by aeration tanks, primary clarifiers, coagulation and secondary clarifiers as well as a sand filtration unit (Fig. 1). Currently, a major part of treated effluent from PWWTP is currently used for firefighting and irrigation purposes within the company.

Wastewater samples were collected in February, May, August and November 2015. Samples were taken from the raw influent and final treated effluent, as well as the inlet and outlet of aeration tanks. At each sampling run, one liter of suspended activated biomass was taken under aseptic conditions in sterile 1500 mL glass bottle from the points selected in aeration tanks (6 points) as shown in Fig. 2. Samples were kept on ice during transportation to the laboratory intended for microbiological analysis.

2.2. Analytical procedure

Dissolved oxygen (DO), temperature and pH were recorded on-site using DO-meter (Aqualytic, AL20OXi) and pH-meter (EDT RE 357), respectively. BOD_5 and COD were measured using BSB-OxiDirect-0604527 and thermo-reactor (Al-125-Aqualytic)/spectrophotometer (Al-100-Aqualytic), respectively. Other parameters including phenol, total dissolved solids (TDS), alkalinity, mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS) and sludge volume index (SVI) were measured in accordance with the standard methods for the examination of water and wastewater [20].

To assess the performance of activated sludge reactor, phenol, benzene, toluene and styrene were considered as mostly occurring aromatic organic compounds in petrochemical wastewater. Samples collected from the inlets were diluted at the ratio of 1:4 and centrifuged at 4000 rpm (8 min) to measure benzene, toluene and styrene. Dichloromethane was used to air-assisted liquid-liquid micro-extraction according to the method described by Ghorbanpour et al. [21]. A gas chromatograph (Agilent, 7890A) equipped with a flame ionization detector was used to analysis of benzene, toluene and styrene.

2.3. Bacterial community analysis

2.3.1. Isolation of cultivable aerobic heterotrophic bacteria

10 mL of each sample was suspended in 90 mL of Tween 80 (0.1% w/v) plus 0.85% (w/v) saline solution and serially diluted (10-fold) in the same normal saline solution. Both plate count agar (PCA) and R2A media (Merck, UK) were used to grow the heterotrophic bacteria. Inoculated plates were incubated at 30 °C for 48 h. For further purification, single and different bacterial colonies grown on the cultivated plates were isolated by streak-plating method. The active bacterial cells were grown on nutrient agar slant and kept at 4 °C. For long-term storage, pure bacterial cultures were prepared as glycerol stocks (30%) and frozen at −80 °C [22].

2.3.2. Biochemical identification

Initial identification of isolates was carried out by a light microscope (×1000) (Zeiss, Argentina S.A) in order to determine the morphology and Gram stain. Also, regular biochemical tests for taxonomic classification at the genus or species level were performed in accordance with Bergey's manual of determinative bacteriology, including colony morphology, form and size of cells, cyst formation, motility, Gram staining, reduction of nitrate to nitrite, Voges–Proskauer test, production of H_2S and indole, fermentation of D-glucose, D-sucrose, D-lactose; and enzyme activities such as oxidase, urease, and catalase [23].

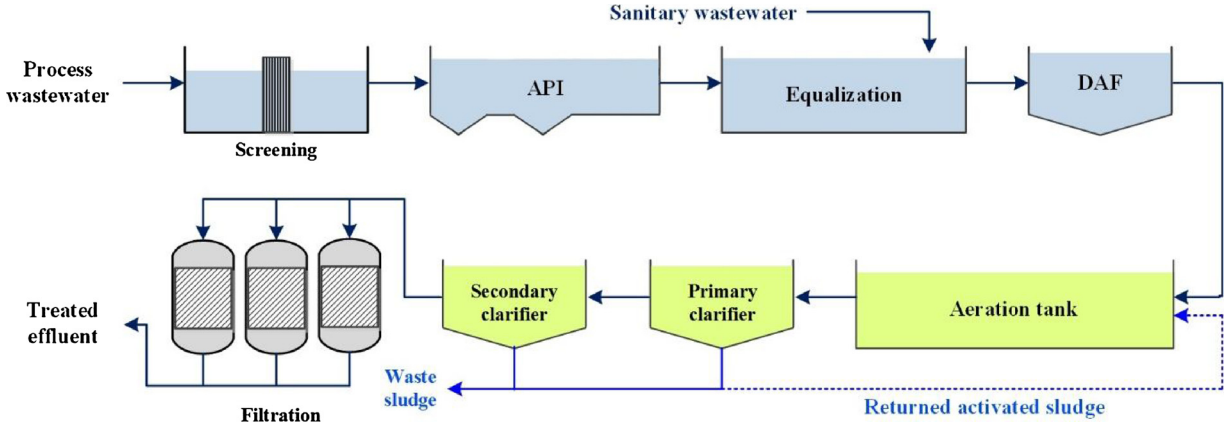

Fig. 1. Schematic flow diagram of PWWTP.