Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2021.27738

239 (2021) 68–83
November

Bacterial bioaugmentation as an efficient approach to enhance the quality of activated sludge-treated effluent

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Received 8 November 2020; Accepted 13 August 2021

ABSTRACT

The present study aimed to investigate the ability of four bacterial isolates to polish and enhance the quality of activated sludge (AS)-treated effluent for potential reuse using bioaugmentation technology following the AS treatment. The expected future increase in the wastewater flow and organic matter load due to the increase in the handling rate of huge containers with more equipment at Alexandria Containers and Cargo Handling Company (ACCHC) necessitate searching for new approache(s) to be integrated with the established AS treatment in order to enhance secondary effluent quality for potential recycling. Four indigenous bacterial candidates (AI 11, AI 12, AO 11, and AO 12) were isolated from the raw wastewater and screened in batch mode to select the most efficient for polishing the secondary effluent. Results indicated variant capabilities among the candidates for the removal of the tested parameters with AO 11 and AO 12 considered the most efficient. They achieved the highest removals (63.39%, 88.66%, 84.32%, and 92.77%) of total suspended solids (TSS), biochemical oxygen demand (BOD), chemical oxygen demand (COD) and oil and grease (OG) from initial concentration (IC) of 112, 97, 185 and 7.33 mg L^{-1}, respectively. Also, AO 12 produced highest total dissolved solids (TDS) increase (IC: 570 mg L^{-1}, 15.79%) indicating high biodegradation ability. AO 11 and AO 12 were selected for bioaugmentation of AS-treated wastewater as individual and mixed culture in a free-living batch mode. AO 11 achieved the highest removal of 52.08%, 37.84%, 32.85% and 74.79% for TSS, BOD, COD, and OG as well as the highest TDS increase (50.82%) from ICs of 96.0, 74.0, 137.0, 8.53, and 610 mg L^{-1}, respectively reaching safe limits for discharging according to their maximum permissible limits (MPLs) stated by the laws. GenBank accession number (NR0428611) with the highest sequence similarity (99.91%) as well as the closest neighbor(s) to the 16S rDNA gene partial sequences identified AO 11 as *Paenibacillus dendritiformis* strain T168. Results present effective, economic and fast solution to enhance the quality of the AS-treated effluents. The study also recommend augmentation of *P. dendritiformis* in the activated sludge unit or as a separate biofilm unit following the AS treatment in order to protect the environment and recycle the produced water in a wider sense and locally to cope with the expected enlargement in the wastewater flow of the ACCHC company.

Keywords: Activated sludge; Bioaugmentation; Enhancement; Oil; Biochemical oxygen demand; Chemical oxygen demand; *Paenibacillus dendritiformis*; Secondary effluent; Water quality

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1. Introduction

Clean and pure water are the main resource necessary to sustain human life. Anthropogenic human activity and overpopulation cause severe water pollution. Maritime container terminal in any sea port is a station which provides a facility for cargo handling where containers are transshipped (charge and discharge) between different transport vehicles using huge equipment called liberees; or discharging on land to be handled by vehicles such as trains or trucks. Container terminals are designated for many purposes such as handling, storing, and possibly loading or unloading of cargo into or out of containers. In the last years, the growing environmental and sanitary impact of road transport and the connected growing costs has given new impulse to ship transport. However, ships activities, although reducing the global impact of land transport, creating new environmental problems in port areas [1]. Sources of pollution in the sea port area are varied (air, water and soil). For example, air pollutants include volatile organic compounds (VOC) emissions from loading and unloading of petroleum products, dry docks, and passenger car traffic as well as combustion products emission from the heavy vehicle and railway traffic [2,3]. Water pollution from ships activities may take place in the sea water or the port area. In the sea water, pollution resulted from leakages of bilge oil, motor fuel from ships, gasoline and diesel oil from pleasure crafts, accidental leakage of oil and chemical substances in loading and unloading of products. In addition, pollution may result from chemical product residues contained in the tanks and of the products used in the washing operations, leaching of antifouling paints used to coat the bottoms of ships and harmful aquatic organisms with ballast water [4]. In the port area, sources of water pollution include operations on terminals and fuel deposits and accidental discharge of oil and other chemicals. It also includes organic compounds, fine particulates, heavy metals, oil and grease, etc. contained in the storm water runoff from port parking lots and vehicle and equipment wash water discharges [4]. Oil and grease (O&G), in particular, pose ecological damages for aquatic organisms, plants, animals, in addition of being equally mutagenic and carcinogenic for human beings [5,6]. They form a layer on the water surface, which decreases dissolved oxygen interchange and exposes the aerobic aquatic organisms to suffocation. Moreover, O&G reduce biological activity in the wastewater treatment plants due to their toxicity and formation of oil film around microbes in the water and on the suspended matter [7,8].

Oil and grease are removed via conventional physical techniques using skimming as well as O&G traps which have low efficiency and the remaining oil causes clogging and sometimes replacement of pipes in the treatment units which increases maintenance and inspection cost [9]. They also generate large amounts of fats and oil residues leading to difficult disposal and handling problems [10]. Activated sludge (AS) is the most common and widest spread technology in wastewater treatment plants worldwide. This technology treating sewage or industrial wastewater using aeration and a biological flock (free-living bacteria and protozoa) [11]. However, the elimination of O&G using AS technology also inhibits microbial growth and causes foaming, filamentous bacteria, and flocculation [12]. In the AS treatment system, the efficient contact of the microorganisms present in the aqueous phase and oil is fundamental and can be achieved by delivering energy to the system, either by mechanical agitation or by electric fields [13]. Inhibition of bacterial growth by industrial influents in wastewater treatment plants (WWTP) was recently documented. Low *Nitrospira* population was detected in the sludge of industrial WWTP, indicating that influent composition affected nitrification and denitrification processes in the AS unit. It was also proved that bacterial richness was positively correlated with carbon, nitrogen, and phosphorus contents in the municipal WWTPs, but negatively correlated with total dissolved solids in industrial WWTPs [14].

The capacity and efficiency of the existing wastewater AS treatment plant at the ACCHC urgently requires to be enhanced due to an expected future increase in the wastewater flow and COD-load with a further increase of the handling rate of huge containers and equipment. Many approaches were investigated to improve the capability of the AS treatment for degradation and removal of wastewater organic load especially O&G. For example, integration between microbial lipases produced by *Pseudomonas* spp. (lipase hydrolysis stage) followed by treatment with adsorbent materials such as zeolite (laterite and amorphous materials) considered as an efficient, easy, and cheap approach for almost complete (99%) O&G removal [15]. Recently, commercial lipase enzymes that have low production cost considered as an alternative new approach for degrading organic matter to clean holding tanks, septic tanks, grease traps, and other systems [16,17]. Similarly, using aerobic digester coupled with AS treatment efficiently enhanced biodegradation of fats and oils reaching a range of 69%–92% compared to 64%–75% removal achieved by activated sludge plant only [10]. Moreover, sorption especially using natural organic sorbents is gaining focus as a secondary treatment for O&G removal from industrial wastewater. This technology has many advantages such as lower cost, high biodegradability, potential recovery or recycling of sorbet O&G as well as durability of some sorbents that may be reused up to a limited number of working cycles. Several kinds of sorbents and mechanisms for oil sorption and recovery are reported [9]. Modifying the conventional AS process with powdered activated carbon (PAC) can greatly enhance the quality of treated wastewater, reduce microbial inhibition by the highly toxic recalcitrant organic compounds, stabilize the treatment system and produce reusable water. In the PACT process, the removal of pollutants is maintained and enhanced by a combination of biodegradation and adsorption [18,19].

Bioaugmentation of activated sludge systems with specialized bacterial strains was reported a long time ago [20] as a powerful tool to improve flocculation and degradation of recalcitrant compounds. Sludge bioaugmentation enhances biodegradation of recalcitrant compounds, either through the activity of the inoculated strain or after transfer of degradative plasmids to activated sludge bacteria which plays an important role in the natural genetic exchange between inoculated and indigenous sludge bacteria, and in the construction of new genetically

modified organisms. Selecting the appropriate microbial candidate(s) is considered the most critical factor for a successful bioaugmentation process [21–24]. Michalska et al. (2020) reported that among 10 bacterial strains tested, *Pseudomonas putida* OR45a and *P. putida* KB3 can be considered the best candidates for bioaugmentation of the AS to improve the aerobic treatment of landfill leachate. They degraded aromatic compounds (primarily catechol, phenol, and cresols) at a concentration of 1.0 mg/mL and survive in 12.5% of the Kalina pond leachate (KPL). Genomic analyses of these two strains revealed the presence of the genes encoding enzymes related to the metabolism of aromatic compounds. They also exhibited the ability to produce biosurfactants as well as high resistance to ammonium (above 600 μg/mL) and heavy metals (especially chromium) which enable the exploitation in the bioremediation of the AS contaminated with the KPL [21]. In another type of wastewater rich in nitrogen-containing organic pollutants (quinoline, pyridine, and indole), other bacterial candidates {*Comamonas* sp. Z1 (quinoline degrader) and *Acinetobacter* sp. JW (indole degrader)} were successfully used to remove these recalcitrant pollutants. The bioaugmented AS was highly effective at the starting period and the operation stage fed with raw wastewater compared to the non-augmented AS. High-throughput sequencing analysis indicated that nitrogen-containing organic pollutants could shape the AS microbial community structure and that bioaugmentation could induce the formation of the effective microbial community. Results suggested that bioaugmentation could facilitate the enrichment of functional genes related to xenobiotics biodegradation and metabolism, probably leading to improved performance and could promote the removal of nitrogen-containing organic pollutants in bioaugmented AS, which should be an effective strategy for wastewater treatment [22].

In addition to the ability of sludge bioaugmentation to significantly remove highly toxic contaminants, it could effectively reduce the toxicity of the recalcitrant compounds included in the industrial wastewater on the AS microbial community that leads to the failure of the treatment process. Park and Oh [23] confirmed that augmentation of a *Pseudomonas* population (designated PCO) into the AS bioreactor played a key role in detoxifying up to 100 mg/L of APAP {acetaminophen (N-acetyl-p-aminophenol)} fed as a sole carbon, nitrogen, and energy source. PCO could remove APAP at levels up to 590 mg/L without inhibition and could also metabolize APAP-derived metabolites, 4-aminophenol, hydroquinone, and 1,4-benzoquinone at varying levels. PCO introduced to AS bioreactor at various volumes (5%, 25%, and 50% of the total), showed significantly enhanced APAP transformation rates (1.5, 1.9, and 2.3/d) compared to 1.2/d achieved by the control (non-augmented AS). Such results will help in the design of a biological technology (bioaugmentation) for treating APAP-bearing waste streams [23]. Hexavalent chromium Cr(VI) toxicity was inhibited during the denitrification of nitrogen contaminated wastewater using *P. brassicacearum* LZ-4, immobilized in sodium alginate-kaolin augmented in the AS reactor. In the non-bioaugmented system, the nitrate removal efficiency was decreased by 86.07% at 30 mg/L Cr(VI). Whereas, denitrification was protected with 95% continuous nitrate removal in the immobilized-cell bioaugmentation system, which provides a feasible technique for nitrogen contaminated wastewater treatment in the presence of toxic heavy metals [24].

During the present study, integration between AS treatment and bioaugmentation (seeding of potent exogenous bacteria) with *Paenibacillus dendritiformis* was investigated and recommended as a new approach to improve and upgrade the efficiency and capability of the existing AS treatment plant at ACCHC to cope with the expected increase in the wastewater flow and COD-load due to the further increase of the handling rate of huge containers with more equipment.

2. Materials and methods

2.1. Sampling

Grab wastewater samples were collected from the final drainage effluent of the conventional AS wastewater treatment unit of the ACCHC during the course of the study. Wastewater samples were subjected to physicochemical as well as microbiological characterization before and after the proposed treatment to define the pollution strength of the raw wastewater and calculate the treatment efficiency in order to enhance the efficiency of the existing AS wastewater treatment unit.

2.2. Microorganisms

Four indigenous bacterial species were isolated from the inlet and outlet of the conventional AS aeration basin of the ACCHC. Bacterial species were designated AI 11, AI 12, AO 11, and AO 12 where I refers to the inlet and O refers to the outlet of the AS aeration basin. The selected bacterial species were investigated as individual or mixture for their ability to bio-remediate and enhance the quality of the secondary-treated effluent.

2.3. Media preparation, isolation and purification of the tested bacteria

Dehydrated nutrient broth (NB) and nutrient agar (NA) were used for culturing (HIMEDIA, India). NB medium contained (g/L) Lab-Lemco Powder, 1.0; yeast extract, 2.0; peptone, 5.0 and sodium chloride, 5.0. NA medium contained similar ingredients as NB plus Agar, 15 g/L. They were prepared by dissolving 13.0 and 28.0 g/L from NB and NA dehydrated media, respectively, pH was adjusted to 7.4, sterilized by autoclaving at 121°C for 20 min and freshly used for growth experiments as well as biodegradation assays.

Indigenous heterotrophic bacteria were isolated on NA agar using standard enumeration technique according to the method described by Baird et al. [25] and incubated at 37°C for 24 h. After incubation, bacterial colonies were purified by streaking and incubated under the previously mentioned conditions. Then, they were inoculated onto NA slants, incubated till heavy growth was obtained and kept as a stock in the fridge at 4°C for further investigations.

2.4. Bioremediation bioassays

2.4.1. Screening of bacterial isolates

Indigenous bacterial isolates were screened for bioremediation of secondary effluent to select the most promising candidates for the bioremediation process. Liquid cultures (200 mL, 24 h old) were inoculated individually in 2,000 mL flasks containing 1,300 mL effluent and incubated at room temperature. The test was performed for 7 days where treated samples were drawn at 24 h-interval. Wastewater characterization was performed for the raw and treated samples with three replicates and removal efficiencies of the tested parameters by the tested species were calculated from the average readings.

2.4.2. Batch bioassays using free living bacteria

According to the preliminary screening experiment, two indigenous promising bacterial candidates (AO 11 and AO 12) were selected and employed as free-living individuals or mixture for the remediation of secondary effluent. The selected species were inoculated individually and as mixture in 100 mL NB medium and incubated at 37°C for 24 h till heavy growth was obtained. Then each culture was inoculated into 900 mL effluent reaching a final volume of 1 L after counting the total viable count of bacteria (TVC) for each culture to define the density of the different inocula at the starting point (zero time). The secondary-treated effluent was characterized immediately after sampling collection to define its pollution strength which considered zero time or raw readings. Effluent cultures (individual and mixed), as well as a control sample (one-liter un-inoculated effluent), were incubated for 7 days where samples were aseptically drawn at 24 h-interval. After sampling of the treated effluent, the selected parameters (Temperature, COD, BOD, TSS, TDS, O&G, and TVC), three replicates each, were analyzed to determine their residual levels at each exposure time and their removal efficiency were calculated to determine the effectiveness of the remediation process.

2.5. Molecular identification of the bacterial candidate

Total genomic DNA of the most promising isolate AO 11 was extracted from 5 mL overnight NB culture of the purified isolate [26]. The PCR was performed in a light cycler Eppendorf PCR machine. A 1300 bp fragment was obtained by PCR amplification of the 16S rDNA gene [27] using the primers:

F-start: 5′-AGAGTTTGATCMTGGCTCAG-3′

R-1387: 5′-CGGGCGGTGTGTACAAGG-3′

The PCR mixture was composed of 100 ng of genomic DNA, 30 pmol of each primer, 200 μM of dNTPs, 1U of Taq polymerase, and 10 μL of 10X PCR reaction buffer, the reaction volume was adjusted to 100 μL in a 0.5 mL Eppendorf tube. The PCR amplification conditions were performed by an initial denaturation step at 94°C for 10 min followed by 30 denaturation cycles at 94°C for 1 min, annealing at 60°C for 1 min and an extension at 72°C for 1 min followed by a final extension step at 72°C for 10 min. Amplicons of 16S rDNA were purified using a PCR purification kit (QIAGEN). Each of these purified products was sequenced by the chain terminator method (API model 3730xl, Bioneer, Germany) using the two corresponding PCR primers separately. The resulted DNA sequences were phylogenetically analyzed using the BLAST search program [28]. Multiple sequence alignment and molecular phylogeny were performed using MEGA 5.0 software [29].

2.6. Characterization of the secondary and tertiary industrial effluent

Wastewater was characterized before and after the proposed treatment. The temperature was determined using a digital thermometer while pH (APHA 150-1), TDS (APHA 2540 E), and DO (ASTM D888 – 92 B) were determined using a laboratory Bench Meters. Other parameters including TSS, BOD_5, COD, O&G, and total viable count of bacteria (TVBC) were determined according to methods APHA 2540 D, APHA 5210 B, ASTM D1252, 5520 B (Liquid–Liquid, Partition-Gravimetric Method) and the pour plate technique of the standard plate count method respectively, all of which are described in the Standard Methods for the Examination of Water and Wastewater [25]. After the treatment, residual levels of the selected parameters were determined at each exposure time and their removal efficiency were calculated to determine the effectiveness of the remediation process according to the following equation:

$$\text{Removal efficiency}\left(\text{RE}\%\right)=\left\{\frac{\left(C_0-\text{RC}\right)}{C_0}\right\}\times 100 \quad (1)$$

where C_0 is the initial concentration before treatment (zero time) and RC is the residual concentration after treatment at each exposure time.

3. Results

3.1. Screening of bacterial isolates

Screening the ability of the four indigenous isolates and their mixed culture for polishing secondary treated wastewater of the ACCHC for 7 d (Table 1 and Fig. 1) led to the selection of the most promising isolate. Results indicated the following points:

- A general trend of gradual decreasing of DO levels (3.7 mg/L, zero time) with time was exhibited by most of the tested bacteria attributed to their activity and the consumption of DO during biodegradation. The maximum DO reductions ranged between 94.59% by AO 12, AO 11 and AI 11 and 86.49% by the mixed culture all after 7 d. In contrast, unseeded wastewater (control) recorded the lowest DO consumption (64.86%) indicating the lowest activity. DO levels before and after treatment were lower than the maximum permissible limit (MPL) of 4 mg/L.

- In contrast, TDS levels (raw: 570 mg/L) increased with increasing exposure time (till the 7th day) as a general trend by all the tested isolates due to the remediation process and biodegradation of complex contaminants into simple dissolved salts. They exhibited high variations among them depending on their ability to degrade and/or transform the available contaminants with the bulk increase during the first 24 h. AO 12 showed the highest TDS increase (24.5%) followed by AO 11 (22.8%), AI 12 and AI 11 (21.0%) and finally the mixed culture with the lowest (19.3%) increase. However, the control showed no TDS increases at all; instead, the reduction was recorded reaching the highest (14.0%) after 6 exposure days. TDS levels before and after treatment were lower than its MPL of 2,000 mg/L.
- TSS (raw: 112 mg/L) decreased efficiently after treatment with a general trend of increasing RE of TSS with time reaching their highest removals after the last exposure (7 days). AO 11 achieved the maximum (63.39%, 41 mg/L) while the mixed culture achieved the minimum (38.39%, 69 mg/L) RE both of which are either much lower or slightly above the maximum permissible limit (MPL) of TSS (60 mg/L). On the other hand, the control showed intermediate TSS removal (41.9%, 65 mg/L) after the same exposure.
- BOD (raw: 97 mg/L) showed a remarkable reduction after treatment. Surprisingly the control showed the highest BOD removal (97.9%, 2.0 mg/L) after 2 days. All other cultures showed their highest BOD removal after the 7th treatment day ranging from a maximum (88.66%, 11 mg/L) recorded by AO 12 and a minimum (68.04%, 31 mg/L) by the mixed culture. All the highest removals are equivalent to RCs lower than the MPL of BOD (60 mg/L). Similarly, the control showed the highest COD removal (90.27%, 18.0 mg/L) after 2 days from the initial COD of 185 mg/L. Again, all other cultures showed their highest COD after the 7th treatment day ranging from a maximum (84.32%, 29 mg/L) by AO 12 and a minimum (63.78%, 67 mg/L) by the mixed culture. All the highest removals are equivalent to RCs lower than the MPL of COD (100 mg/L) indicating high ability for organic matter biodegradation.
- O&G (raw: 7.33 mg/L) removal followed a very clear trend by all cultures including the control with regular increase with time reaching the bulk removals after the 7th treatment day. The highest O&G removal ranged between a maximum of 92.77% (0.53 mg/L) by AO 11 and a minimum of 75.4% (18 mg/L) by the mixed culture. All the obtained RCs were lower than the MPL of OG (15 mg/L).

Screening bioassay conclusions summarized in Table 1 revealed that except for the DO in the secondary and tertiary treated wastewater, all the tested parameters reached RCs below their MPLs for safe discharge into the sea. Also, results indicated that AO 11 and AO 12 are the most active towards all the tested contaminants while the mixed culture was the least active although it achieved quite reasonable removals and reached safe discharge limits. Therefore, AO 11 and AO 12 were selected for the next (batch mode) experiment and compared with their mixed culture and the control.

3.2. Bioremediation assays using free living bacteria (batch mode)

Batch mode bioremediation of secondary treated wastewater using AO 11 and AO 12 as an individual and mixed culture for one week (Table 2 and Fig. 2) revealed varied removal efficiencies at the different exposure times that are summarized as follows:

- DO levels (Initial: 5.0 mg/L) exhibited a clear general trend of gradual decreasing reaching the lowest residues after 7 days by all cultures. The highest DO reductions ranged between 98.0% recorded by AO 11 and 90.0% recorded by the mixed culture all after 7 days. In contrast, unseeded wastewater (control) showed the lowest DO (18.0%) consumption indicating the lowest activity. DO levels after treatment were lower than the maximum permissible limit (MPL) of 4 mg/L.
- In contrast, there was a general trend of increasing TDS levels (Initial: 610 mg/L) with increasing time (till the 7th day) by all the tested cultures. AO 11 and AO 12 showed the highest TDS increases (50.82% and 49.18% respectively) while 45.9% and 3.28 % TDS increases were recorded by the mixed and control cultures. However, the control showed TDS increase within the first 24 h only followed by reductions till the end of the experiment reaching the highest RE of TDS (8.2%) after 7 days. However, TDS levels were lower than its MPL (2,000 mg/L) before and after bioaugmentation.

Table 1
Highest and lowest REs% of the different parameters in the treated effluents during the screening bioassay

Parameter	Raw wastewater (mg/L)	Highest RE/SI	Lowest RE	MPL (mg/L)
DO	3.7	94.59%, 0.2 mg/L, AI 11, AO 11 & AO 12	86.49% 0.5 mg/L Mixed culture	4.0
TDS	570	24.5% SI, 710 mg/L AO 12	19.3% SI*, 680 mg/L Mixed culture	2000
TSS	112	63.39%, 41 mg/L AO 11	38.39%, 69 mg/L Mixed culture	60
BOD	97	88.66%, 11 mg/L AO 12	68.04%, 31 mg/L Mixed culture	60
COD	185	84.32%, 29 mg/L AO 12	63.78%, 67 mg/L Mixed culture	100
OG	7.33	92.77%, 0.53 mg/L AO 11	75.44%, 1.8 mg/L Mixed culture	15

SI*: Salts increase

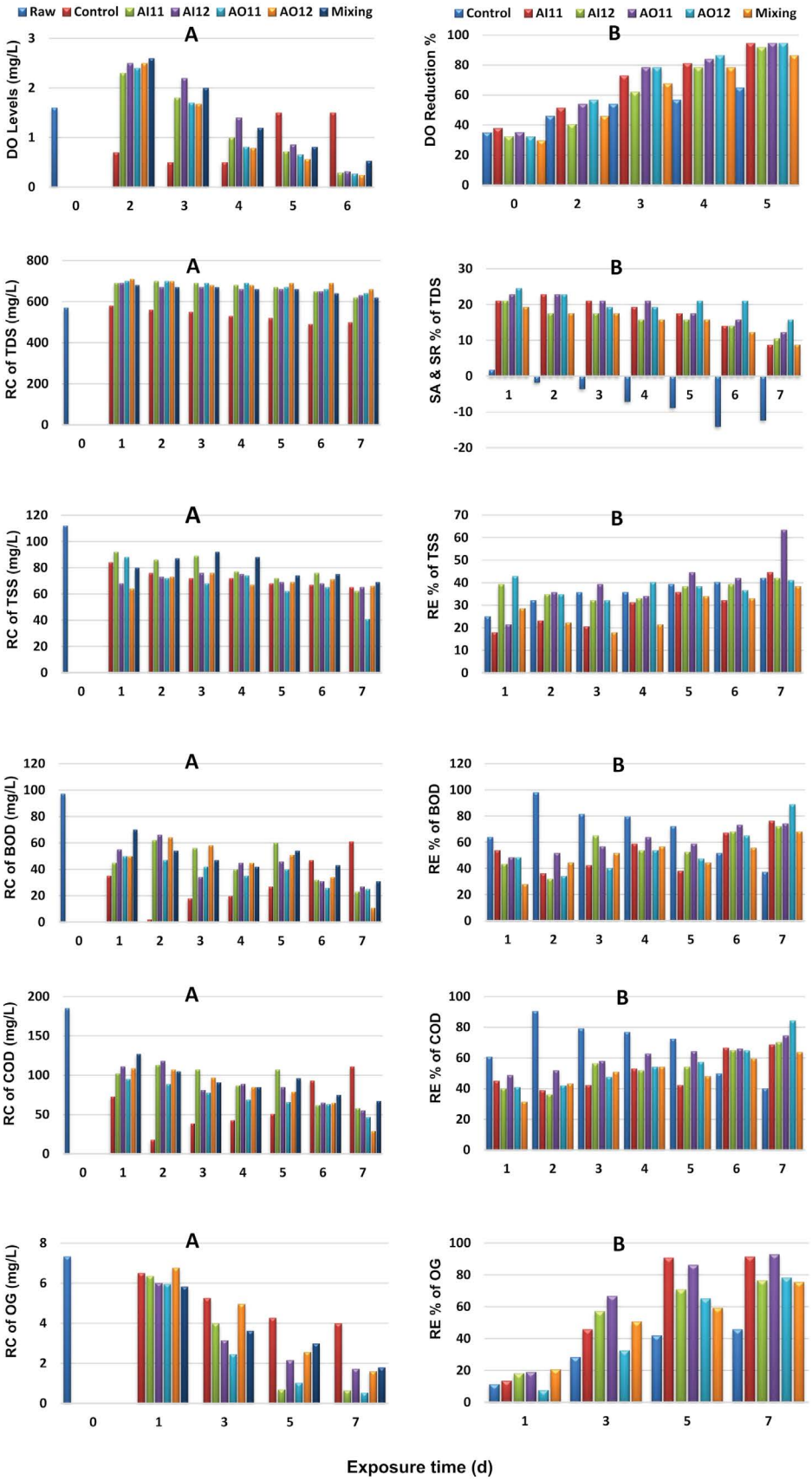

Fig. 1. Residual concentration (RC) (A) and removal efficiency (RE) (B) of the tested parameters in the raw and treated effluent at different exposure times during screening bioassay.

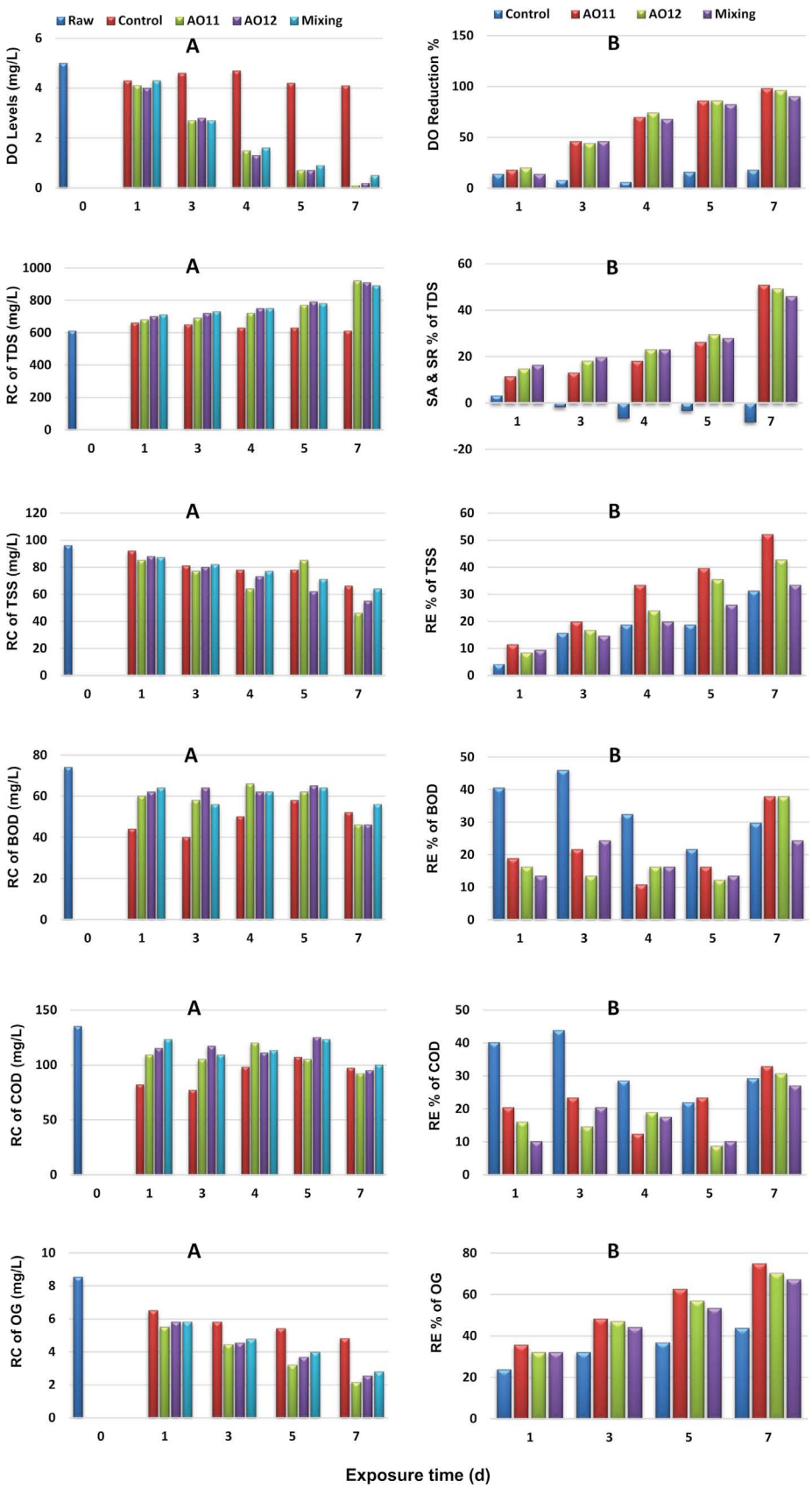

Fig. 2. Residual concentration (RC) (A) and removal efficiency (RE) (B) of the tested parameters in the raw and treated effluent at different exposure times during batch bioassay.

- TSS (Initial: 96 mg/L) decreased efficiently after treatment (Fig. 2) with increasing RE of TSS with time by all cultures reaching their highest removals at the 7th days ranged between a maximum of 52.08% (46 mg/L) by AO 11 to a minimum of 33.33% (64 mg/L) by the mixed culture. These removals are equivalent to residual concentrations much lower or slightly above the maximum permissible limit (MPL) of TSS (60 mg/L). On the other hand, the control showed intermediate TSS removal (31.25%, 66 mg/L) after the same exposure.
- As in the screening experiment, the control showed the highest BOD removal (45.95%, 40 mg/L) after 3 days (Initial: 74 mg/L) (Fig. 2). All other cultures showed their highest BOD removal after the 7th treatment day ranging from a maximum (37.84%, 46 mg/L) recorded by AO 11 and AO 12 and a minimum (24.32%, 56 mg/L) by the mixed culture. All the highest removals are equivalent to RCs lower than the MPL of BOD (60 mg/L). Similarly, the control showed the highest COD removal (43.8%, 77.0 mg/L) after 3 days from an initial concentration of 137 mg/L in the secondary treated wastewater at the starting point (Fig. 2). Again, all other cultures showed their highest COD removals (32.85%–27.01%) with 92 and 100 mg/L RCs by AO 11 and the mixed culture respectively after 7 days. Such residuals are lower than or equal to the MPL of COD (100 mg/L) indicating high ability for organic matter biodegradation.
- O&G removal (initial concentration = 8.53 mg/L) followed a very clear trend by all cultures including the control where removal regularly increased with time reaching

Table 2
Highest and lowest REs% of the different parameters in the treated effluents during the batch treatment

Parameter	Raw wastewater (mg/L)	Highest RE/SA	Lowest RE	MPL (mg/L)
DO	5.0	98% DO, 0.1 mg/L AO 11	90% DO, 0.5 mg/L Mixed culture	4.0
TDS	610	50.82% SA, 920 mg/L AO 11	45.90% SI*, 890 mg/L Mixed culture	2000
TSS	96	52.08%, 46 mg/L AO 11	33.33%, 64 mg/L Mixed culture	60
BOD	74	37.84%, 46 mg/L, Both AO 11 & AO 12	24.32%, 56 mg/L Mixed culture	60
COD	137	32.85%, 92 mg/L, AO 11	27.01%, 100 mg/L Mixed culture	100
OG	8.53	74.79%, 2.15 mg/L, AO 11	67.17%, 2.8 mg/L Mixed culture	15

SI*: Salts increase

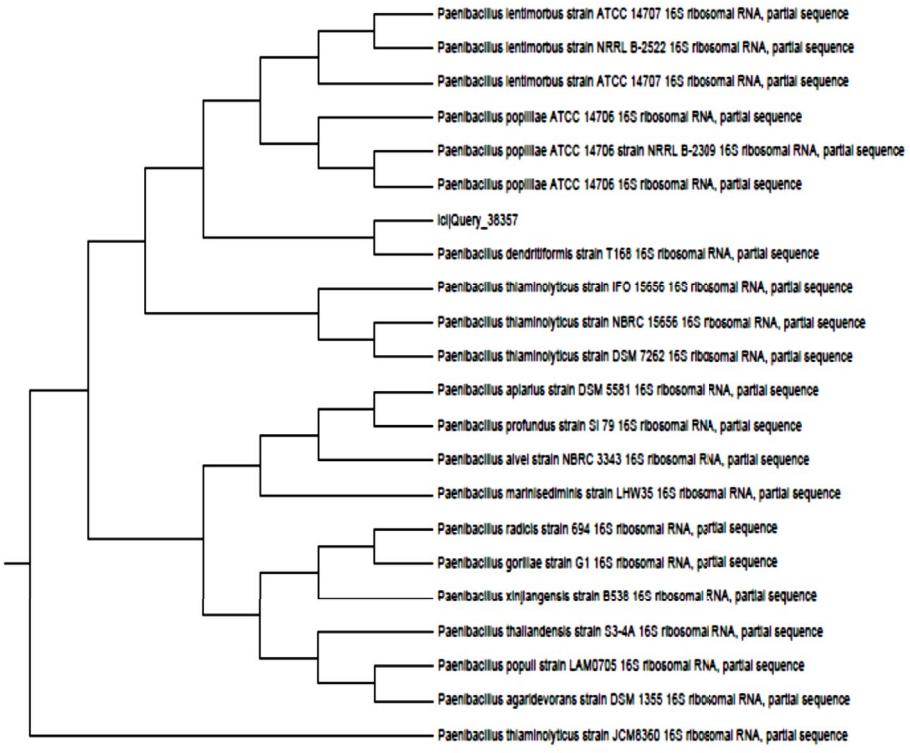

Fig. 3. Phylogenetic relationships of the tested strain *Paenibacillus dendritiformis* strain T168 and the most closely related bacterial species.

Table 3
Stimulation and/or inhibition (%) effect of industrial wastewater on the growth of the tested cultures after 7 d treatment

Cultures	Zero	Exp. Time (d)	Density (CFU/ml)	S^f	I%	Exp. Time (d)	Zero	Density (CFU/ml)	S^f
Control	1×10^6	1	0.019×10^6	–	98.1	1	2×10^6	3.0×10^6	1.5
		2	5.0×10^6	5.0	–	2		–	–
		3	5.0×10^6	5.0	–	3		4.0×10^6	2.0
		4	–	–	–	4		–	–
		5	1.02×10^6	1.02		5		55.0×10^6	27.5
		6	–	–	–	6		–	–
		7	2.0×10^6	2.0		7		123.0×10^6	61.5
AI 11	5×10^6	1	2.0×10^6	–	60	1			
		2	31.0×10^6	6.2		2			
		3	1.0×10^6	–	80	3			
		4	–	–	–	4			
		5	17.0×10^6	3.4		5			
		6	–	–	–	6			
		7	8.0×10^6	1.6		7			
AI 12	2×10^6	1	0.16×10^6		92	1			
		2	5.0×10^6	2.5		2			
		3	0.124×10^6	–	94	3			
		4	–	–	–	4			
		5	62.0×10^6	31.0	–	5			
		6	–	–	–	6			
		7	0.003×10^6	–	99.8	7			
AO 11	$1x\ 10^6$	1	1.0×10^6	1.0	–	1	1×10^6	4.0×10^6	4.0
		2	4.0×10^6	4.0	–	2		--	--
		3	4.0×10^6	4.0	–	3		98.0×10^6	98.0
		4	–	–	–	4		–	–
		5	26.0×10^6	26.0		5		99.0×10^6	99.0
		6	–	–	–	6		–	–
		7	3.0×10^6	3.0	–	7		180.0×10^6	180.0
AO 12	1×10^6	1	2.0×10^6	2.0	–	1	1×10^6	2.0×10^6	2.0
		2	1.0×10^6	1.0	–	2		–	–
		3	4.0×10^6	4.0	–	3		84.0×10^6	84.0
		4	–	–	–	4		–	–
		5	0.2×10^6	–	80	5		135.0×10^6	135.0
		6	–	–	–	6		–	–
		7	0.012×10^6	–	99	7		410.0×10^6	410.0
Mixed Culture	5×10^6	1	1.0×10^6	–	80	1	3×10^6	7.0×10^6	2.3
		2	0.001×10^6	–	100	2		–	–
		3	82.0×10^6	16.4		3		123.0×10^6	41.0
		4	–	–	–	4		–	–
		5	102.0×10^6	20.4		5		137.0×10^6	45.67
		6	–	–	–	6		–	–
		7	10.0×10^6	2.0		7		390.0×10^6	130.0

I % Inhibition %, S^f Growth stimulation fold

the bulk removals after the 7th treatment day (Fig. 2). The highest O&G removal ranged between a maximum (74.79%, 2.15 mg/L) achieved by AO 11 and a minimum of (67.17, 2.8 mg/L) by the mixed culture. All the obtained RCs are lower than the MPL of OG (15 mg/L).

Batch treatment conclusions summarized in Table 2 revealed that all the tested parameters except DO reached RCs below their MPLs for safe discharge into the sea according to the Egyptian Environmental Regulation Laws (No. 48/1982 and 4/1994). Also, results indicated that AO 11 was

the most active towards all the tested contaminants while the mixed culture was the least active although it achieved quite reasonable removals and reached safe discharge limits.

3.3. Population dynamics of the tested cultures

During the screening bioassay, AI 11 and AO 12 showed the highest start-up density both recording 2×10^6 CFU/mL. AO 11 and the mixed culture showed the intermediate start up density (1×10^6 CFU/mL) while AO 12 showed the lowest start-up density recording 0.16×10^6 CFU/mL. AI 12, AO 11 and the mixed culture showed the highest growth stimulation recording 31, 26, and 20.4 – fold their initial densities respectively all after 5 treatment days (Table 3) indicating their ability to use the included contaminants as carbon and energy sources. This was followed by AI 11 and the control cultures showing 6.2- and 5-fold stimulation in their growth respectively both after 2 days and finally AO 12 with the lowest stimulation (4.0-fold) and mostly inhibition with time.

During the batch treatment, the mixed culture showed the highest start-up density recording 7×10^6 CFU/mL, AO 11 showed the intermediate start-up density recording 4×10^6 CFU/mL while AO 12 showed the lowest start-up density 2×10^6 CFU/mL. AO 12 showed the highest growth stimulation (410-fold) followed by AO 11 (180-fold), the mixed culture (130-fold) and finally the control with lowest growth stimulation (61.5-fold) their initial densities all after 7 treatment days (Table 3) indicating that AO 12 and AO 11 are the most adapted strains for inhabiting and deal with this sort of industrial wastewater where they assimilate the included pollutants like carbon and energy sources and duplicate their biomass but efficiency wise AO 11 was the most active for pollutants removal.

3.4. Molecular characterization of AO 11

The most promising isolate AO 11 was molecularly identified. The 16S rDNA sequences of AO 11 were submitted to Gene Bank sequencing data and aligned against the 16S rDNA sequences of the Ribosomal Database project. Gene Bank accession number (NR0428611) with the highest sequence similarity (99.91%) as well as the closest neighbor(s) to the 16S rDNA gene partial sequences (S1: supplementary material) identified AO 11 as *Paenibacillus dendritiformis* strain T168 (Fig. 3).

3.5. Statistical analysis

Correlation coefficients (Pearson's r) at confidence level 95% among the different contaminants in the contaminated effluents during the screening bioassay are illustrated in Table 4A and Fig. 4A. Few significant correlations were detected among the tested parameters. DO showed insignificant correlations (at $P < 0.05$) with all the tested parameters although it is a very critical factor that strongly affects the bioremediation process through its effect on biological activities. TSS showed significant positive correlations (at $P < 0.05$) with TDS and COD. BOD showed only significant positive correlation (at $P < 0.05$) with COD. However, OG showed insignificant correlations (at $P < 0.05$) with all the tested parameters. A matrix plot (Fig. 4A) is used to assess the relationship among several pairs of variables at once. It is an array of individual scatterplots. It displays a plot for every possible combination of variables and it is useful when there are many variables and with the need to see relationships among all pairs of variables. During the batch experiment and except of the significant positive correlation (at $P < 0.05$) between TSS and OG, none of the tested parameters had any correlations with each other (Table 4B and Fig. 4B).

4. Discussion

Biological treatment approaches, especially microbial processes considered highly efficient, clean, environmentally friendly and economic for treating all kinds of wastewater. Microorganisms or their derivatives (enzymes, surfactants, etc.) proved to be value – added resources that guarantee sustainable approaches for decontaminating recalcitrant contaminants in affordable manner. Wastewater used in the present study was secondary treated using activated sludge technology, therefore, the effluent was moderately polluted and contained slightly high levels of the tested contaminants that required good polishing treatment to reach quality appropriate for reuse/recycling or safe discharge. Environmental laws in Egypt stated the maximum permissible limits (MPLs) of the different tested pollutants for the safe discharge into open environments. These limits are set to minimize the ecological disturbances and protect aquatic as well as soil environments from hazardous discharges.

Screening bioassay revealed high efficiency of two isolates (AO 12 and AO 11) that showed the highest acclimatization for inhabiting and deal with this sort of industrial wastewater with low-moderate pollution load. The biodegradation capacity of pollutant loads in the secondary treated effluent was found to be time and species dependent and accordingly resulted in varying levels of contaminants removal efficiencies. Among the four tested indigenous bacterial isolates, two (AO 11 and AO 12) showed the highest ability to clarify wastewater indicating that they have the required degrading enzymes and secondary metabolites which seem to enhance their growth and their degradation ability [30]. However, the combination of the indigenous microbial isolates (consortium) showed the lowest degradation ability which may be attributed to antagonistic effects among them. In this regard and according to the achieved results, the individual culture of AO 11 showed higher activity towards the tested contaminants compared to AO 12 individual and AO 11-AO 12 mixed cultures. It could achieve 52.08%, 37.84%, 32.85%, and 74.79% removal of TSS, BOD, COD, and O&G with final RCs of 46, 46, 92, and 2.15 mg/L respectively in the treated effluent, all of which are lower than their MPLs (60, 60, 100, and 15 mg/L) indicating very good effluent quality to be safely discharged or reused.

AO 11 that showed the highest capabilities towards O&G degradation and removal of the other included contaminants was molecularly identified as *P. dendritiformis* strain T168. *P. dendritiformis* belong to Phylum: Firmicutes, Class: Bacilli, Order: Bacillales and Family: Paenibacillaceae. It is a motile, endospore-forming, Gram-positive facultative

Table 4
Correlation coefficients (Pearson's *r*) among the different parameters (contaminants) during screening and batch bioassays

Screening bioassay						
	DO	TDS	TSS	BOD	COD	OG
DO		0.472	0.430	0.491	0.612	0.172
TDS	0.472		0.944*	0.842	0.806	0.267
TSS	0.430	0.944*		0.825	0.894*	0.137
BOD	0.491	0.842	0.825		0.917*	0.664
COD	0.612	0.806	0.894*	0.917*		0.379
OG	0.172	0.267	0.137	0.664	0.379	
Batch treatment						
	DO	TDS	TSS	BOD	COD	OG
DO		0.325	0.783	0.666	0.529	0.734
TDS	0.325		0.843	0.922	0.974	0.881
TSS	0.783	0.843		0.986	0.942	0.997*
BOD	0.666	0.922	0.986		0.985	0.996
COD	0.529	0.974	0.942	0.985		0.965
OG	0.734	0.881	0.997*	0.996	0.965	

*Statistically significant correlation at *P*-value = 0.05 (2-tailed)

anaerobic rod that is found in many different environments including the plant rhizosphere, insect larva, and a variety of soils [31,32]. It was discovered in forest soil samples collected from Brazil [33] and from marine soil, specifically that located near the Red Sea in Saudi Arabia [34]. This bacterium is biologically significant due to its potential as an active bioremediator to clean up environments contaminated with polycyclic aromatic hydrocarbons (PAH) and motor sludge [35–37]. It is an efficient biocontrol agent against crop fungal diseases which increases crop yield and enhances its quality [38]. The above mentioned characteristics of *P. dendritiformis* clearly explained its high efficiency in dealing with oil-contaminated wastewater in relatively short time (7 days) especially at the low levels that are difficult to be treated.

Although AS is the most common and widest spread technology in wastewater treatment worldwide [11], O&G in the wastewater is known to inhibit the AS microbial growth and cause foaming, filamentous bacteria, and flocculation [12–14]. Enhancing AS efficiency to treat toxic industrial wastewater was investigated and achieved through the application of many approaches and modifications. Examples included integration between AS treatment with aerobic digester [10], *Pseudomonas* spp. lipases (lipase hydrolysis stage) followed by treatment with adsorbent materials such as zeolite [15–17], natural organic sorbents [9] and powdered activated carbon (PAC) [18,19], all of which can greatly enhance the quality of treated wastewater, reduce microbial inhibition by the highly toxic recalcitrant organic compounds, stabilize the treatment system and produce reusable water. Moreover, biotechnological processes involving microbial lipases are currently considered the most attractive catalysis for O&G hydrolysis due to their flexibility, ease of mass production at low cost and ability to cope with different types of industrial wastewater [39–42], especially coupled with composite materials (aluminium oxide, nano particles, amorphous zeolite, and laterite) as effective adsorbents to treat complex wastewater [15,43].

Bioaugmentation of activated sludge systems with specialized bacterial strains is considered a powerful tool to enhances biodegradation of recalcitrant compounds and overcome their toxicity against AS microbial community. This is performed natural genetic exchange between inoculated and indigenous sludge bacteria, and in the construction of new genetically modified organisms. However, selecting the appropriate microbial candidate(s) is considered the most critical factor for a successful bioaugmentation process [21–24]. For example, *Pseudomonas putida* OR45a and *P. putida* KB3 can be considered the best candidates for bioaugmentation of the AS to improve the aerobic treatment of landfill leachate containing aromatic compounds (catechol, phenol, and cresols) at a concentration of 1.0 mg/mL and survive in 12.5% of the Kalina pond leachate (KPL) [21]. *Comamonas* sp. Z1 (quinoline degrader) and *Acinetobacter* sp. JW (indole degrader) were successfully used to treat wastewater rich in nitrogen-containing organic pollutants (quinoline, pyridine, and indole) [22], while a *Pseudomonas* population augmented into AS bioreactor played a key role in detoxifying of up to 100 mg/L of APAP {acetaminophen (N-acetyl-p-aminophenol)} [23] and *Pseudomonas brassicacearum* LZ-4 immobilized in sodium alginate-kaolin augmented in AS reactor could protect denitrification process with 95% continuous nitrate removal in the presence of highly toxic hexavalent chromium Cr(VI) [24]. In the present study, bioaugmentation with *P. dendritiformis* strain T168 efficiently enhanced TSS, BOD, COD and O&G removal in only 7 days when applied as a polishing post-treatment stage after AS remediation.

The low affinities towards degradation of organic matter (OM) which is shown by BOD and COD removal

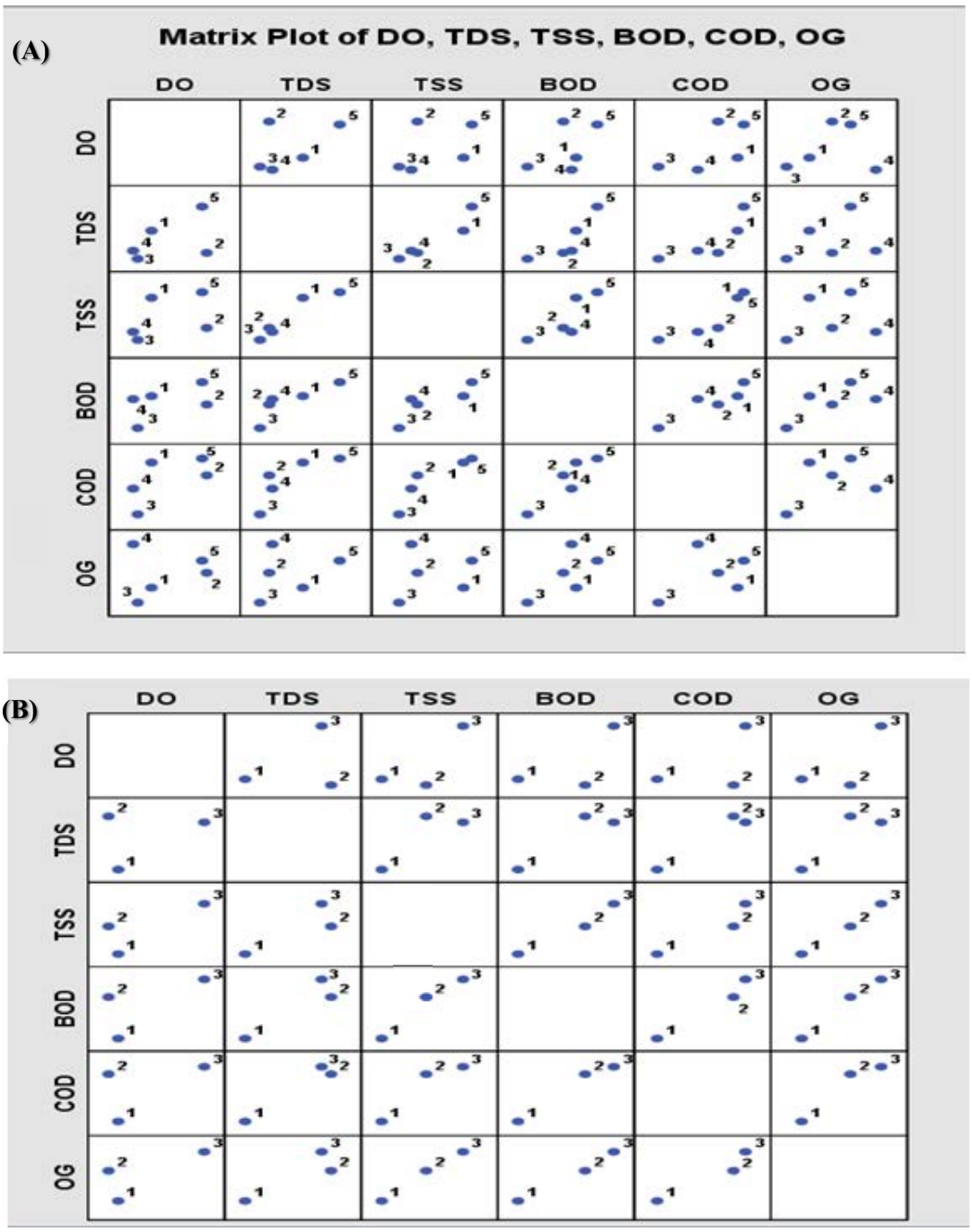

Fig. 4. Matrix plot of the tested parameters in the treated effluent during (A) the screening, and (B) the batch bioassay.

exhibited by *P. dendritiformis* strain T168 may be attributed to the OM portions that are either slowly or non-biodegradable [44] where 66%–70% of the tested influent COD was readily biodegradable, 10%–14% was slowly degradable and 17%–21% was non-biodegradable. It can also be related to the operation mode (batch) with the short exposure which is also confirmed by other workers using the continuous operation mode for treating effluents rich in organic load. It is always expected that continuous treatment enhances the RE of the included contaminants compared to batch operation. These facts are shown with 75% increase in the COD removal with increasing sludge retention time (SRT) to 15 days [45] as well as 90% and 100% removal of COD and ammonium by using membrane reactor [46].

Oil and grease removal from wastewaters is often challenging and involves the combination of different treatment technologies, according to the specifications for the treated water and the O&G substances involved [9]. Enhancing the quality of activated sludge-treated effluent

for potential reuse, environment protection or to cope with expected future enlargement plans could be achieved by integrating some treatment approaches (physical, chemical, or biological) either in sequence or combination; pre- or post-AS process. For example, COD removal was enhanced when seawater and ferric salt flocculation were used as chemical integration step to enhance biological treatment during 110 days operation with seawater being more effective [45]. Modification of AS treatment with chemical or physical methods such as gravity separators, dissolved air flotation, coagulation/flocculation, electro-coagulation/flotation [47], membrane separation as using powdered activated carbon (PAC), although highly effective, many of these treatments have limitations such as high operational costs, production of hazardous sludge or high energy requirements [9,18,19,43].

Supporting AS community with resistant specialist exogenous bacteria greatly helps in upgrading the system to cope with the threats posed by toxic contaminants such as O&G on the indigenous AS microorganisms such as inhibition of bacterial growth, reducing microbial community structure and diversity which in turn adversely affect treatment performance and stability [14]. According to the results achieved in the present study, it is clear that AS bioaugmentation with *P. dendritiformis* either in the main AS process (bioaugmentation into the aeration tank) or as a polishing step in a pre- or post-treatment stage can lead to very high-quality effluent in a very short time (hours) especially if *P. dendritiformis* is fixed as a biofilm and used in continuous treatment mode. It could achieve 52.08%, 37.84%, 32.85%, and 74.79% removal of TSS, BOD, COD, and O&G with final RCs of 46, 46, 92, and 2.15 mg/L, respectively in the treated effluent, all of which are lower than their MPLs (60, 60, 100, and 15 mg/L) indicating very good effluent quality to be safely discharged or reused.

5. Conclusions

Screening of the four bacterial candidates indicated variant capabilities among them for the removal of the tested parameters with AO 11 and AO 12 considered the most efficient. They achieved the highest removals (63.39%, 88.66%, 84.32%, and 92.77%) of TSS, BOD, COD, and OG from initial concentration (IC) of 112, 97, 185, and 7.33 mg L^{-1}, respectively. Also, AO 12 produced the highest TDS increase (IC: 570 mg/L^{-1}, 15.79%) indicating high biodegradation ability. The most efficient two cultures AO 11 and AO 12 were selected for the remediation of wastewater as individual and mixed culture in a free-living batch mode for 7 days. AO 11 achieved the highest removal of 52.08%, 37.84%, 32.85%, and 74.79% of TSS, BOD, COD, and OG as well as the highest TDS increase (50.82%) from ICs of 96.0, 74.0, 137.0, 8.53, and 610 mg/L^{-1}, respectively reaching safe limits for discharging according to their maximum permissible limits (MPLs) stated by the laws. Gene Bank accession number (NR0428611) with the highest sequence similarity (99.91%) as well as the closest neighbor(s) to the 16S rDNA gene partial sequences identified AO 11 as *P. dendritiformis* strain T168. Results of the present study recommended augmentation of *P. dendritiformis* in the activated sludge unit or as a separate biofilm unit following the AS treatment to cope with the expected enlargement in the wastewater flow of the company and enhancing the effluent quality.

Conflict of interest

All authors declare that they have no conflict of interest.

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Supplementry information

***Paenibacillus dendritiformis* strain T168 16S ribosomal RNA, partial sequence**

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Paenibacillus dendritiformis strain T168 16S ribosomal RNA, partial sequence	1953	1953	100%	0.0	99.91%	NR_042861.1
Paenibacillus thiaminolyticus strain NBRC 15656 16S ribosomal RNA, partial sequence	1881	1881	96%	0.0	99.80%	NR_113795.1
Paenibacillus thiaminolyticus strain IFO 15656 16S ribosomal RNA, partial sequence	1881	1881	96%	0.0	99.80%	NR_040887.1
Paenibacillus thiaminolyticus strain DSM 7262 16S ribosomal RNA, partial sequence	1866	1866	96%	0.0	99.41%	NR_114808.1
Paenibacillus popilliae ATCC 14706 16S ribosomal RNA, partial sequence	1860	1860	96%	0.0	99.51%	NR_040888.1
Paenibacillus popilliae ATCC 14706 strain NRRL B-2309 16S ribosomal RNA, partial sequence	1855	1855	96%	0.0	99.32%	NR_115980.1
Paenibacillus popilliae ATCC 14706 16S ribosomal RNA, partial sequence	1855	1855	96%	0.0	99.32%	NR_114456.1
Paenibacillus lentimorbus strain ATCC 14707 16S ribosomal RNA, partial sequence	1796	1796	96%	0.0	98.16%	NR_114457.1
Paenibacillus lentimorbus strain ATCC 14707 16S ribosomal RNA, partial sequence	1792	1792	96%	0.0	98.07%	NR_040889.1
Paenibacillus lentimorbus strain NRRL B-2522 16S ribosomal RNA, partial sequence	1773	1773	96%	0.0	97.58%	NR_115981.1
Paenibacillus apiarius strain DSM 5581 16S ribosomal RNA, partial sequence	1716	1716	100%	0.0	95.85%	NR_040890.1
Paenibacillus radicis strain 694 16S ribosomal RNA, partial sequence	1714	1714	100%	0.0	95.85%	NR_148658.1
Paenibacillus thiaminolyticus strain JCM8360 16S ribosomal RNA, partial sequence	1701	1701	96%	0.0	96.09%	NR_115600.1
Paenibacillus xinjiangensis strain B538 16S ribosomal RNA, partial sequence	1700	1700	100%	0.0	95.57%	NR_043221.1
Paenibacillus populi strain LAM0705 16S ribosomal RNA, partial sequence	1698	1698	100%	0.0	95.57%	NR_145559.1
Paenibacillus gorillae strain G1 16S ribosomal RNA, partial sequence	1692	1692	100%	0.0	95.48%	NR_136879.1
Paenibacillus profundus strain SI 79 16S ribosomal RNA, partial sequence	1692	1692	100%	0.0	95.47%	NR_132304.1
Paenibacillus marinisediminis strain LHW35 16S ribosomal RNA, partial sequence	1692	1692	100%	0.0	95.48%	NR_132694.1
Paenibacillus thailandensis strain S3-4A 16S ribosomal RNA, partial sequence	1683	1683	100%	0.0	95.30%	NR_041490.1
Paenibacillus agaridevorans strain DSM 1355 16S ribosomal RNA, partial sequence	1681	1681	100%	0.0	95.29%	NR_025490.1
Paenibacillus alvei strain NBRC 3343 16S ribosomal RNA, partial sequence	1668	1668	100%	0.0	95.01%	NR_113577.1

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Query  1    GTCGAGCGGAGCTGATGGAGTGCTTGCACTCCTGATGCTTAGCGGCGGACGGGTGAGTAA  60
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Sbjct  30   GTCGAGCGGAGCGGATGGAGTGCTTGCACTCCTGATGCTTAGCGGCGGACGGGTGAGTAA  89

Query  61   TACGTAGGTAACCTGCCCTTAAGACCGGGATAACTCACGGAAACGTGGGCTAATACCGGA  120
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Sbjct  90   TACGTAGGTAACCTGCCCTTAAGACCGGGATAACTCACGGAAACGTGGGCTAATACCGGA  149

Query  121  TAGGCGATTTCCTCGCATGAGGGAATCGGGAAAGGCGGAGCAATCTGCCACTTATGGATG  180
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Sbjct  150  TAGGCGATTTCCTCGCATGAGGGAATCGGGAAAGGCGGAGCAATCTGCCACTTATGGATG  209

Query  181  GACCTACGGCGCATTAGCTAGTTGGTGGGGTAACGGCTCACCAAGGCGACGATGCGTAGC  240
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Sbjct  210  GACCTACGGCGCATTAGCTAGTTGGTGGGGTAACGGCTCACCAAGGCGACGATGCGTAGC  269

Query  241  CGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAG  300
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Sbjct  270  CGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAG  329

Query  301  GCAGCAGTAGGGAATCTTCCGCAATGGACGCAAGTCTGACGGAGCAACGCCGCGTGAGTG  360
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Sbjct  330  GCAGCAGTAGGGAATCTTCCGCAATGGACGCAAGTCTGACGGAGCAACGCCGCGTGAGTG  389

Query  361  ATGAAGGTTTTCGGATCGTAAAGCTCTGTTGCCAGGGAAGAACGCTATGGAGAGTAACTG  420
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Sbjct  390  ATGAAGGTTTTCGGATCGTAAAGCTCTGTTGCCAGGGAAGAACGCTATGGAGAGTAACTG  449

Query  421  TTCCATAGGTGACGGTACCTGAGAAGAAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGG  480
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Query  481  TAATACGTAGGGGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTC  540
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Sbjct  510  TAATACGTAGGGGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTC  569

Query  541  ATGTAAGTCTGGTGTTTAAACCCGGGGCTCAACTCCGGGTCGCATCGGAAACTGTGTGAC  600
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Query  601  TTGAGTGCAGAAGAGGAAAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGG  660
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Query  661  AGGAACACCAGTGGCGAAGGCGACTTTCTGGGCTGTAACTGACGCTGAGGCGCGAAAGCG  720
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Query  721  TGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGCTAGGT  780
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Sbjct  750  TGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGCTAGGT  809

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Sbjct  810  GTTAGGGGTTTCGATACCCTTGGTGCCGAAGTTAACACATTAAGCATTCCGCCTGGGGAG  869

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Query  901  GTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCCTCTGACCGTC  960
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Sbjct  930  GTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCCTCTGACCGTC  989

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Sbjct  990  CTAGAGATAGGGCTTCCCTTCGGGGCAGAGGTGACAGGTGGTGCATGGTTGTCGTCAGCT  1049

Query  1021 CGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCA  1060
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Sbjct  1050 CGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCA  1089
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