Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2019.24131

157 (2019) 62–68
July

Improving the efficiency of saline wastewater treatment plant through adaptation of halophilic microorganisms

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Received 15 March 2018; Accepted 16 March 2019

ABSTRACT

In the urban wastewater treatment plant of Bandar Torkaman, total input of solvent solids is about 8–9 g L^{-1} and the electrical conductivity is 14,600 µs cm^{-1}. High salt concentrations cause some problems in biological treatment process. Therefore, this study was designed to improve the effluent of saline wastewater treatment plant in Bandar Torkaman by adaptation of halophilic microorganisms. We designed a pilot plant considering the size of the current wastewater treatment plant and installed an RBC system. Effective parameters were evaluated and halophilic microorganisms in both plants were cultured and identified according to the standard methods in both the treatment plant and the pilot plant. The average electrical conductivity of sewage was 12,000 µs cm^{-1}. We found *Salinibacter* and *Adhaeribacter* species were able to grow in high salt concentrations. Considering the results of the present study, we conclude that the attached growth systems have more efficiency for saline wastewater treatment than the suspended purification systems. In addition, pH (R^2 = 0.344) and rotational velocity (R^2 = 0.094) had the least effect on the efficiency. Changing the system of aerated lagoon and suspended growth to attached growth increases the growth of halophilic microorganisms, thus increasing the efficiency of organic matter removal in wastewater treatment plants.

Keywords: Halophilic microorganisms; Wastewater treatment; Saline wastewater

1. Introduction

Saline wastewater contains high concentration of organic compounds and total dissolved solids (at least 3.5 g L^{-1}), which is caused by leakage from seawater or brackish aquifers and various industrial activities such as leather factories, marine products, pharmaceuticals and oil industry [1,2]. Considering the high cost of physicochemical treatment, biological process is usually considered as the most cost-effective method for treatment of wastewater [3]. Biological treatment of saline wastewater has not been easy. Saline wastewater has not been treated by most popular treatment method due to adverse effects of salt on microbial flora [1]. The high-salt content of saline wastewaters disrupts metabolic functions and cell membranes, denatures enzymes, causes plasmolysis and affects osmotic pressure that are lethal to many microorganisms used in the biological treatment processes [4,5]. Biological treatment of saline wastewater has a low efficiency for removal of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) [4]. In addition, high concentration of salt in wastewater is known to reduce the efficiency of aerobic/anaerobic and conventional activated sludge as well as nitrification and denitrification processes [3]. For biological

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treatment of saline wastewater without the costly dilution process, halophilic and heterotrophic organisms that can tolerate hypersaline environments should be used [5]. The intracellular concentration of salt in halotolerant and halophilic bacteria is low. These bacteria can maintain osmotic balance between their cytoplasm and environment containing high concentration of various organic osmotic solutes [6]. Therefore, utilization of halophilic microorganisms in the biological wastewater treatment systems could improve BOD and COD removal from saline wastewater [6].

Numerous studies have been conducted on biological treatment of domestic and industrial wastewaters and liquid pollutant by using rotating biological contactor (RBC) systems and new methods [7–13]. However, biological treatment of saline wastewater in RBC systems has not been yet studied extensively [14]. Most of the studies on the biological treatment of saline wastewater have been based on use of aerobic and anaerobic halophilic organisms [6]. Kapdan and Erten studied the anaerobic treatment of saline wastewater using *Halanaerobium lacusrosei* [6]. An up-flow anaerobic packed bed reactor was used with synthetic saline wastewater at different initial COD concentration (COD = 1,900–6,300 mg L^{-1}) and salt concentration (0%–5%, w/v). The percentage COD removal reached up to 94% at 19 h hydraulic retention time and 3% salt concentration [6]. Aloui et al. [3] studied the performance of an activated sludge process for the treatment of fish processing saline wastewater. Industrial wastewater containing 1%–6% salt was treated in an activated sludge unit operating in a continuous mode. Inhibition process was found to be significant for salt concentrations higher than 4% [3]. Uygur and Kargi [15] studied the inhibitory effects of salt on biological nutrients from saline wastewater in a sequencing batch reactor. Salt content was variable between 0% and 6% (w/v) at eight different levels [15]. Effects of salt inhibition on the rate and extent of COD, NH^4–N and PO^4–P removals were investigated. Percentage COD removal decreased from 96% to 32% when salt content increased from 0% to 6%. COD removals were above 90% for salt contents below 0.5% [15]. Moreover, in the study by Dincer and Kargi [14,16], a halotolerant bacterium (*Halobacterium halobium*) was added to an activated sludge culture in order to improve the system's performance.

Bandar Torkaman is a city in the Golestan Province (Iran) with total area of 1,577 km^2, located near to the Gorgan Gulf and Caspian Sea. Wastewater treatment plant of Bandar Torkaman is an aerated lagoon. The characteristics of this wastewater treatment plant were as follows: total input of dissolved solids 8–9 g L^{-1}, BOD_5 63 ± 13 mg L^{-1}, COD 238 ± 35 mg L^{-1} and electrical conductivity 14,600 µS cm^{-1}. Because of the proximity of the city to the Caspian Sea, the amount of dissolved solids and electrical conductivity in the raw wastewater are very high. Currently, the efficiency of the treatment plant is very low due to the quality of salt wastewater produced. Removal of high salt concentrations (greater than 20 g L^{-1}) and BOD is not carried out effectively. On the other hand, sludge sedimentation would be too weak. Studies have shown that increasing the total dissolved solids decreases the rate of biodegradation. Also, in anaerobic processes, there will be a reduction in gas production and a 50% reduction in the activity of methanogenic bacteria at a salt concentration of more than 20 g L^{-1}.

Therefore, this study was designed to enhance the efficiency of saline wastewater treatment in Bandar Torkaman treatment plant by adaptation of halophilic microorganisms, isolate and adapt halophilic microorganisms and study the efficiency of saline wastewater treatment by sequencing batch reactor at pilot scale.

2. Materials and methods

A pilot plant was designed considering the dimensions of the current wastewater treatment plant in Bandar Torkaman. Then, the RBC system was installed. A pilot installation site was designed near the existing wastewater treatment plant to comply with the operating conditions [17].

Effective parameters were adjusted according to the standard method in both the treatment plant and the pilot plant. Culture and identification of halophilic microorganisms were performed according to standard methods [17].

2.1. Pilot design

To simulate operating conditions of the current treatment plant, the pilot (1:100) treatment plant was designed with the following dimensions: length of 136.2 cm, width of 65 cm, depth of 24 cm and volume of 212.5 L. Hydraulic retention time for the existing treatment plant was compared with that of the pilot plant in order to determine its relation to the volume and input flow.

The contents of the pilot reactor were as follows:

- Lagoon tank: made of Plexiglas with a 10 mm thickness (Fig. 1)
- Discus revolver: 20 mm in diameter and 153 cm in length, and containing 15 disks with 3 mm thick
- Rotary disk: 14 disks with 15 cm thick and 9 cm space from each other in vertical axis
- Piston engine: 750 W (1 HP) and 1,500 rpm, connected to the drive for swiveling the axis of the RBC disc with rpm of 10–20
- Peristaltic pump (Heidolph model) with injection rate of 1–150 mL s^{-1}
- Piston blower 4 bolt, model ACO-9610 4 outlet
- A quarry aquarium heater: 300 watts, 68–89 °F, manufactured by Asian Star
- Media: Clay aggregate light expanded (LECA) (additional information is presented in Table 1.

Due to rotation of the RBC and lateral aeration, the simultaneous use of the attached and suspended growth system makes the mixed liquor volatile suspended solids (MLVSS) more accessible to suspended organic matter in less time. Simultaneous application of attach and suspended growth affected the biofilm growth and increased the rate of microbial biosynthesis in the complete mix lagoon. The gradual adaptation of halophilic microorganisms is also provided by providing optimal variables.

2.2. Adaptation of halophilic microorganism and optimization of biological treatment

After starting the pilot plant reactor, the halophilic microorganisms were grown in the media (LECA) at 25°C.

Fig. 1. RBC pilot used in the study.

Table 1
Characteristics of LECA

Commercial name	Clay aggregate light expanded (LECA)
Gradation, mm	10–25
Density, kg m^{-3}	320–400
Pore volume, m^3	0.018
Specific surface area, m^2/m^3	525
Appearance shape	

The bacteria formed 2–3 mm thick biofilm in the complete mix lagoon and the second lagoon (incomplete mixing) of the pilot system after 45 d and continued to grow in the entire complete mixed lagoon. Then the absorption process continues and after reaching the penetration saturation level, which biofilm was sloughed to the bottom of the lagoon in form of non-dense sludge.

Since different environmental factors can affect the growth of the halophilic microorganism, we optimized the environmental variables to achieve maximum biological removal efficiency. The following were used as the optimal conditions: pH 5–9, temperature of 10°C–25°C, the dissolved oxygen (DO) level of 1–2.5 mg L^{-1}, and MLVSS concentration of 1,500–4,500 mg L^{-1}.

Growth of halophilic and halotolerants colloids in different salt concentrations quality are shown in Fig. 2. The optimization response of system variables for simultaneous removal of COD, BOD_5, TSS and TKN are shown in Fig. 3.

2.3. Separation of halophilic microorganism according to the culture medium

Culture-based and non-culture-based methods were used to investigate the diversity of halophilic and halotolerant microorganisms. In the culture-based method, the bacteria were cultured in modified halophilic medium (MHLN), low-nutrient modified Halophilic medium and seawater

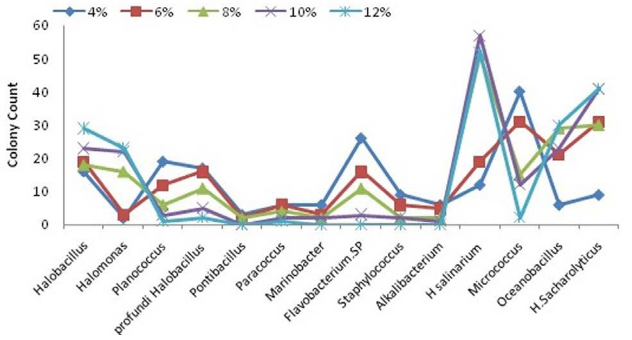

Fig. 2. Growth of halophilic and halotolerants colloids in different salt concentrations.

nutrient agar in aerobic conditions. The isolates were evaluated based on colony differences, gram staining, spore formation and biochemical properties.

In the non-culture method, genomic content of sewage system samples was extracted from separate cultures. By amplifying the 16S rRNA gene and using clone of the gene station, 20% of the recombinant colonies were identified by sequencing.

2.4. Isolation of strains

At this stage, the following steps were taken: pour plate of samples, purification of colonies, microscopic and macroscopic examination of bacteria, optimization of microorganisms, growth curve of microorganisms, identification of bacteria based on biochemical tests, microorganism preservation applied. The same procedures were performed on the sludge samples from the final clarifier tank. All reactions were carried out according to Bergey's manual [18].

3. Results and discussion

3.1. Quality of the wastewater entering the wastewater treatment plant

The main indicators of raw sewage quality are shown in Table 2. The discharge drainage is expected to vary at different times, which can affect the quality of the wastewater. Therefore, combined sampling and average mass load were applied rather than grab sampling method and normal mean.

Estimated changes in the influent parameters of wastewater treatment based on mass load are shown in Table 3.

The maximum mass load of BOD_5 is 1.98 times greater than the average and 2.78 times greater than the minimum mass load. This variation occurs in the Bandar Torkaman treatment plant due to fluctuations in the discharge and infiltration of the wastewater entering the plant, which is due to the fluctuation of the incident. Infiltration in winter is much higher than that in summer and spring (2.8 times). Therefore, the amount of wastewater entering the plant in winter is significantly higher than in summer. However, the fluctuation in the quality of raw wastewater in other treatment plants could be the increase in per capita water

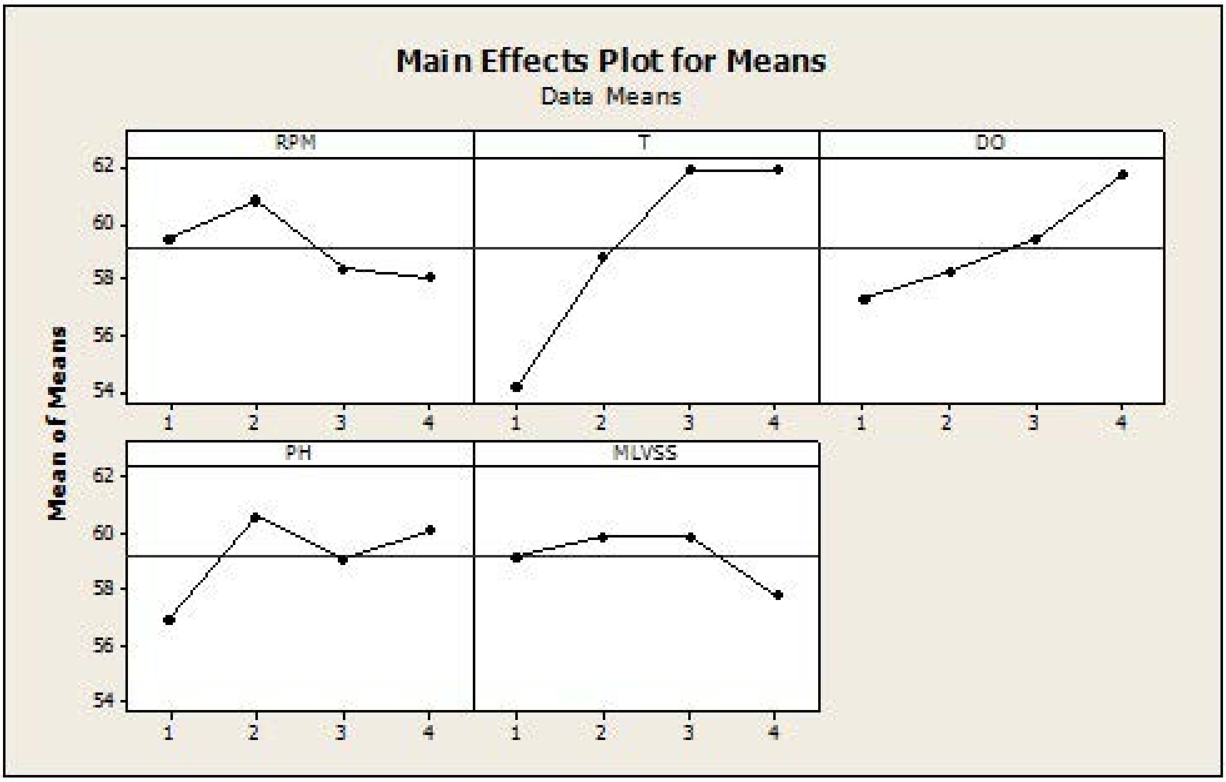

Fig. 3. Optimization response of system variables for simultaneous removal of COD, BOD_5, TSS and TKN.

Table 2
Values of influent parameters of Bandar Torkaman wastewater treatment

Parameter	Maximum	Minimum	Mean	Standard deviation	Coefficient of variation
BOD_5, mg L^{-1}	74.4	21.56	51.7	16.2	31.3
COD, mg L^{-1}	230.2	64	108.4	8.7	8
TSS, mg L^{-1}	99.7	41.1	70.1	21.5	30.6
TKN, mg L^{-1}	19.92	6.67	12.1	5.3	43.8
EC, mg L^{-1}	18,950	7,480	11,918	19.6	13.69

Table 3
Estimated changes in influent parameters of wastewater treatment based on mass load

Parameter (kg h^{-1})	Maximum	Minimum	Mean	Standard deviation
Q × BOD_5	22.76	8.19	12.08	8.5
Q × COD	41.43	15.78	20.12	11.8
Q × TSS	29.62	9.06	15.11	12.1
Q × TKN	3.3	0.89	1.23	1.2

consumption of household purposes. This situation, which is not carefully considered in the design of the wastewater treatment system of the Bandar Torkaman, has caused the system's control criteria, such as calculating the required air, based on the maximum organic and mass loading, is not correct and the result of the biological treatment of the system is not desirable.

3.2. Identification of halophilic microorganisms

5 kg of sludge samples from same area wastewater treatment plant was received, which according to the obtained reports contained a very high concentration of halophilic microorganisms. Two samples from the raw wastewater and two samples from the clarifier were examined in a

microbiology laboratory to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration. In addition, indicators for studying the chemical indices of raw wastewater are as follows:

- (A) Determination of MIC and MBC indices for the study of the effect of chemical indicators of raw wastewater
- (B) Identification of total halophilic microorganism in wastewater and sludge
- (C) Differential diagnosis of dominant halophilic microorganism in sludge

The preliminary results of microbiological studies indicated that the appropriate amounts of halophilic microorganisms, which were from the strain of *Aspergillus* spp. and *Bacillus* sp., were confirmed by their high concentration of NaCl salt (11.5% concentration) agar nitrate medium. The next study focuses on the types and concentrations of other salts in raw sewage.

Until this stage, there is no need for sludge loading due to the number and potential of halophilic microorganisms in sludge.

3.3. Variety of halophilic and halotolerant strains

Of 39 isolates, the sequence of 16S rRNA cultures was indexed for 14 strains of sequencing. Phylogenetically, the species *Halobacillus*, *Halomonas*, Planococcus, *Halococcus saccharolyticus*, *Halobacillus profundi*, *Pontibacillus*, *Paracoccus*, *Marinobacter*, *Flavobacterium* sp., *Staphylococcus*, *Alkalibacterium*, *Halobacterium salinarum*, *Micrococcus*, *Halococcus oceanobacillus* were included.

3.4. Dominant halophilic and halotolerant strains

The strains in the sewage were differentiated based on their growth in media containing the predominant salt content of the wastewater. Accordingly, the results of growth and survival of the target microorganisms were obtained by addition of 3%–12% NaCl (98% caustic soda flakes) and 1 mL of the wastewater sample to the four mentioned media. Based on the culture method between sequencing isolates, eight isolates of similarity was less than 98.7% with a standard strain. In the non-culture method, the colonies examined included *Salinibacter* and *Adhaeribacter* species, which are considered as moderate halophiles.

Biological treatment of saline wastewater has a low BOD removal efficiency because of the adverse effects of salt on microorganisms. High salt concentrations cause plasmolysis or deactivate microorganisms [3]. Bacteria use two strategies to prevent plasmolysis. First, halophilic bacteria can regulate their intracellular osmotic pressure by compacting the biochemical molecules that they synthesize themselves or from the environment in the cytoplasm. Second strategy is the selective intake of potassium (K^+) ions into the cytoplasm [19].

Of the 14 strains extracted, six strains were halophilic and eight strains were halotolerant bacteria. According to the least significant difference test, *H. salinarum* was the halophilic bacterial strain present in various salt concentrations. Dan et al. [19] reported that *Halobacterium* species could continue COD removal in presence of 32–45 g L^{-1} salt. He also reported that these bacterial species in activated sludge system could continue COD removal in the presence of 50 g L^{-1} salt [19].

- ANOVA test shows that salt concentration has a significant negative effect on the growth of halotolerant microorganisms and increased wastewater salt has a reversible effect on the number of halotolerants (r = 71%). However, the growth of halophilic bacteria has increased with salt concentration (r = 84%).
- The effect of growth of halotolerant bacteria up to 8% salt concentration (as MIC index) was significant, but for halophilic bacteria, the concentration of 10% had a biasing trend. Usually, with increasing salt concentration due to the reduced bacterial growth, COD removal efficiency decreases. Dincer and Karji [14] reported that COD removal for 1% salt concentration was nearly 93%; however, the COD efficiency removal dropped to 83% for 5% salt and to 60% for 10% salt.

3.5. System optimization to improve efficiency

Optimization of the biological degradation process of organic compounds in urban and industrial wastewaters is very important in order to increase the percentage removal of pollutants by changes in environmental conditions. Various methods have been investigated by researchers to optimize the growth conditions of microorganisms. Today, the design of experiments has been widely used for obtaining optimal conditions in various studies.

After collecting data related to the multivariate variables related to the BOD, COD, MLVSS, RPM, pH and temperature, the software of the laboratory studies MINITAB 16 was used in a factorial environment to evaluate the optimal conditions for better results in wastewater treatment, relatively accurate results from adaptive conditions of the halophilic microorganisms exploitation pilot was extracted. Relative percentages and interaction of significant factors in removal of BOD_5 are shown in Table 4.

In examining the effect of environmental parameters on the removal efficiency of BOD_5 in a pilot, their regression variations were different, with the least effect of pH (R^2 = 0.344) and rotational velocity (R^2 = 0.094). This means that increasing the pH can enhance BOD_5 removal efficiency since halophilic microorganisms can maintain their activity in alkalinity values up to 9.3. Of course, the halotolerant microorganisms this range of salt (3%–12%), but there is no possibility of microbial growth (Fig. 2). The rotational speed variations of the RBC system almost had no effect on

Table 4
Relative percentages and interaction of significant factors in removal of BOD_5

Parameter	Percentage of parameter
Temperature, °C	44
Dissolved oxygen, mg L^{-1}	31
MLVSS, mg L^{-1}	10
pH	9.0
RPM	6.0

Table 5
Comparison of wastewater quality factors (in mg L^{-1}) in existing treatment plant and RBC pilot plant

Parameter	Unit location	Complete mixed lagoon		Incomplete mixed lagoon1		Incomplete mixed lagoon2		Clarifier		Total	
		In	Eff	In	Eff	In	Eff	In	Eff	In	Eff
BOD_5	Full scale	56.7	39.2	39.2	30.4	30.4	23.2	23.2	22.8	56.7	22.8
	Efficiency	19.1		16.6		14.6		9.5		59.8	
	Pilot	56.7	36.1	36.1	27.4	27.4	20.7	20.7	16.1	56.7	16.1
	Efficiency	24.7		17.8		17.2		22.2		71.6	
COD	Full scale	103	89.8	89.8	72	72	52.3	52.3	39.8	103	39.8
	Efficiency	18.5		17.1		16.2		10.1		61.36	
	Pilot	103	80.2	80.2	62.4	62.4	43.5	43.5	24.2	103	26.2
	Efficiency	21.3		18.4		20.3		14.3		76.5	
TSS	Full scale	69.2	60.1	60.1	48.2	48.2	41	41	31.3	69.2	31.3
	Efficiency	16.2		20.1		14.7		17		54.7	
	Pilot	69.2	52	52	41.1	41.1	30.6	30.6	21.2	69.2	21.2
	Efficiency	23.6		19.4		17.4		16.2		69.4	
TKN	Full scale	9.23	8.8	8.8	6	6	5.1	5.1	3.9	9.23	3.9
	Efficiency	17.1		20.2		14.3		17.2		57.7	
	Pilot	9.23	7.6	7.6	4.9	4.9	3.2	3.2	2	9.23	2
	Efficiency	21.2		19.3		15.2		19.4		78.33	

the BOD_5 removal efficiency. Of course, this kind of effect is related to the inter-group effects of each of the factors, otherwise the effect of other factors is more than them. In fact, it can be stated that by increasing each of the values of the independent variables, the order of increasing the effect of biological treatment for achieving the maximum efficiency as follows (Fig. 3):

T > DO > MLVSS > RPM > pH

We established a pilot plant at the site of the Bandar Torkaman wastewater treatment plant. The efficiency of removing organic and suspended material increased with the operation of the attached growth system and the growth of halophilic microorganisms (Table 5). These results are consistent with results of similar studies [20–27].

3.6. Engineering and redesign

3.6.1. Conversion of complete mixing lagoon to the RBC system based on combined active sludge

The first lagoon was changed into an integrated activated sludge. Since the microbial concentration of the biologically RBC higher than that of the suspended growth in the lagoon, the higher the biofilm contact area is located on the RBC axis cause the loading process is increased. In addition, there is less need for sludge return in the aerobic reactor in order to achieve higher efficiency. The profile of the modified system was as follows:

- Two 65.8 m long RBC units driven by the axial tool with rotational speed of 14 rpm
- Diameter of the axis was 1.8 m, 1.2 m of which were immersed in the sewage.
- Relative volume of the filling material containing the RBC axes was fourteen-quarters, and a fourth of the empty space was considered to control sloping loss.
- For the design of the integrated RBC system, three superficial aero-turbine units were located within the RBC axes, with 14.7 m distance from one another. The power of each unit was 11.4 kW supplied with 1.2 kg of O^2/HP/h.
- There were three regions for oxygen dispersion 57 m between the RBC axis and the surface aerated device. The first region with average radius of 3 m from the axes, rotated with it, producing 4.1 ± 2.9 mg L^{-1} DO. The second region (7 m wide) was located on the sides of aeration devices and produced 8.5 ± 4.8 mg L^{-1} DO. The third region (47 m long) moved toward the plug flow stream and produced 3.6 ± 2.1 g L^{-1} DO. The total amount of DO required for a favorable biological activity of 4 mg was provided in this lagoon.

4. Conclusions

This study was designed to improve the effluent of saline wastewater treatment plant in Bandar Torkaman by adaptation of halophilic microorganisms. *Salinibacter* and *Adhaeribacter* species are able to grow in high salt concentrations. Considering the results of the present study, we conclude that the attached growth systems have more efficiency for saline wastewater treatment than the suspended purification systems. Changing the system of aerated lagoon and suspended growth to attached growth increases the growth of halophilic microorganisms, thus increasing the efficiency of organic matter removal in wastewater treatment plants.

Competing interests

The authors declare that there is no competing interest.

Authors' contributions

The article is derived from a research project supported by the Golestan University of Medical Sciences. Sampling and analysis were performed by Mahdi Sadeghi, Yousef Kor, Saied Keramat. Data analysis and manuscript preparation were done by Zahra Mehrbakhsh. All authors read and approved the final manuscript.

Acknowledgments

This study has been supported by the Golestan University of Medical Sciences, Iran (Grant no: 930528093).

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