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Biodegradation of organic pollutants in saline wastewater by halophilic microorganisms: a review

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Abstract Agro-food, petroleum, textile, and leather industries generate saline wastewater with a high content of organic pollutants such as aromatic hydrocarbons, phenols, nitroaromatics, and azo dyes. Halophilic microorganisms are of increasing interest in industrial waste treatment, due to their ability to degrade hazardous substances efficiently under high salt conditions. However, their full potential remains unexplored. The isolation and identification of halophilic and halotolerant microorganisms from geographically unrelated and geologically diverse hypersaline sites supports their application in bioremediation processes. Past investigations in this field have mainly focused on the elimination of polycyclic aromatic hydrocarbons and phenols, whereas few studies have investigated N-aromatic compounds, such as nitro-substituted compounds, amines, and azo dyes, in saline wastewater. Information regarding the growth conditions and degradation mechanisms of halophilic microorganisms is also limited. In this review, we discuss recent research on the removal of organic pollutants such as organic matter, in terms of chemical oxygen demand (COD), dyes, hydrocarbons, N-aliphatic and N-aromatic compounds, and phenols, in conditions of high salinity. In addition, some proposal pathways for the degradation of aromatic compounds are presented.

Keywords Halophilic · Biodegradation · Hydrocarbons · Dyes · Pollutants · PAH · Phenols

Introduction

Halophilic microorganisms are capable of growing and carrying out their metabolic functions under hypersaline conditions. Non-halophilic microorganisms show optimal growth below 2 % NaCl, while halotolerant and halo-dependent (halophilic *sensus strictus*) microorganisms can grow in up to 30 % NaCl. Halophilic microorganisms can be classified according to the salt concentrations that they need to grow in as slight halophiles (2–5 % NaCl), moderate halophiles (5–20 % NaCl), and extreme halophiles (20–30 % NaCl). Halophiles have been found in each of the three domains of life: *Archaea*, *Bacteria*, and *Eucarya* (Oren 2002a). Halophiles also exhibit great metabolic diversity; they include oxygenic and anoxygenic phototrophs, aerobic heterotrophs, fermenters, denitrifiers, sulfate reducers, and methanogens (Oren 2002a). The important distinction between *Bacteria* and *Archaea* in hypersaline settings is how they osmoregulate, in general KCl for *Archaea* and compatible solutes for *Bacteria* (Oren 2002b). This affects viability and limits their metabolic capabilities. Currently, the halophiles have great potential in biotechnological processes, especially in bioremediation processes because of their ability to degrade organic pollutants.

Due to industrial activities, saline and hypersaline environments are frequently contaminated with organic compounds (Oren et al. 1992). Additionally, several agro-food, petroleum, textile, and leather industries generate highly saline wastewater with a high organic matter and pollutant content (Lozach 2001; Lefebvre et al. 2005; Diaz et al. 2002). Spillage of those wastewater without prior treatment into freshwater affects aquatic life, water potability, and agriculture (Lefebvre and Moletta 2006).

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It is well known that the degradative efficiency of microorganisms toward pollutants decreases in conditions of high salinity, but the use of halophilic or halotolerant microorganisms can overcome these limitations (Zhuang et al. 2010). Biodegradation of organic compounds in saline environments was reported early on by Oren et al. (1992), but most of the reports on this issue have concentrated on petroleum aliphatic hydrocarbon components. Recently, there has been a renewed interest in pollution control using extremophilic microorganisms, probably due to the increasing problems of pollution and its impact on human health worldwide.

Saline wastewater originates from many industries, such as the production of fertilizers, chemicals, pharmaceuticals, dyestuffs, pesticides, herbicides, some foods, and the meat packing industry. The effluents from oil and gas production, and the mining and mineral industries are also saline (Lin et al. 1998).

The halophilic microorganisms have shown good removal efficiencies of pollutants such as hydrocarbons (Arulazhagan and Vasudevan 2009; García et al. 2005), dyes (Balamurugan et al. 2011; Chan et al. 2012), and phenols (Moussavi et al. 2010), both in water (Fairley et al. 2002; Kapdan and Erten 2007) and in soil (Amoozegar et al. 2008; Zhao et al. 2009). Moreover, some halophiles (Li et al. 2012) can metabolize the pollutants under aerobic and anaerobic conditions, and almost always without the production of toxic degradation intermediates (Haddadi and Shavandi 2013; Leitão et al. 2007). Thus, halophilic organisms have promising potential in the biological, environmentally friendly, treatment of polluted wastewater and soils.

The effect of salt concentration on biodegradation

Salinity affects biodegradation processes in several ways. First, high salt concentrations (>1 %) can cause a loss of microbial activity in conventional activated sludge units, due to cell dehydration or disintegration by osmotic differences across the cell membranes. As a result, low removal performance of chemical and biological oxygen can occur at high salt concentrations (>2 %) (Dincer and Kargi 2000). The strategy to overcome this problem is to adapt the biomass to high salinity or to use halotolerant or halophilic microorganisms (Lefebvre and Moletta 2006; Abou-Elela et al. 2010). Secondly, a high salt content can decrease organic compound solubility in water by a salting out effect, and therefore decrease the bioavailability of the organic compounds (Whitehouse 1984). This problem could be naturally overcome by the biosurfactant production from halophilic hydrocarbon-degrading bacteria (Hao and Lu 2009; Djeridi et al. 2013) or by adding substances to increase the pollutant solubility, such as 2-hydroxypropyl β -cyclodextrin (Sohn et al. 2004). Thirdly, high salinity decreases the dissolved

oxygen content in water, limiting the action of oxygenase enzymes (Von Wedal et al. 1988), and therefore the aerobic biodegradation rate. However, it has been reported that extreme halophilic archaea *Haloferax*, *Halobacterium*, and *Halococcus* have higher biodegradation rates at 2.2 mg L⁻¹ than at 5.3 mg L⁻¹ dissolved oxygen (Al-Mailem et al. 2010). Finally, high salt concentration could inhibit the biodegradation of some intermediates, causing their accumulation in the medium (Alva and Peyton 2003).

The salt content in the saline wastewater produced by various industries varies from 2 to 15 %, although the produced water (PW) obtained from the production of oil and gas can have a salt content of up to 40 % (Bonfá et al. 2011). It has been reported that the negative effect of salt on biodegradation rates can be minimized when microorganisms are immobilized (Diaz et al. 2002).

Thus, bioaugmentation with free or immobilized microorganisms with broad organic pollutant degradation capabilities, at salt concentrations as great as 400 g L⁻¹ can be a useful strategy for the bioremediation of saline environments and the treatment of saline industrial wastewater (Kargi 2002; Oren 2010).

Since the early 1990s, the biodegradation of pollutants at high salt concentrations by halophilic microorganism has been studied (Le Borgne et al. 2008; Oren et al. 1991, 1992). After these initial systematic works, much effort has been directed towards the study of phylogenetic diversity and the physiology of microorganisms living in high salt conditions (Oren 2002b). Recently, research on the degradation pathways, genes, and enzymes involved in pollutant biodegradation by halophilic and halotolerant organisms has been conducted. The performance of these microorganisms under different cultural and environmental conditions has also been investigated.

Biological organic matter removal from saline wastewater by halophilic microorganisms

The wastewater from the pickled vegetable and fish processing industries are characterized by a high organic matter content, expressed as chemical oxygen demand (COD) and high salt content. The COD values seen in this type of wastewater that have a salt content of 7.2 to 10 %, range from 3,400 to 8,160 mg COD L⁻¹ (Abou-Elela et al. 2010; Aloui et al. 2009). These are much higher than the COD values of untreated municipal wastewater (500 mg COD L⁻¹).

COD removal in the biological treatment of saline wastewater can be carried out using activated sludge adapted to saline conditions, but at more than 3 % salt, those inoculants are not effective in the treatment of saline wastewater because they are sensitive to changes in ionic strength, and the biological degradation rates of the organic compounds decrease with

increasing salt concentration (Kargi and Dincer 1998). These problems could be overcome by using pure cultures and consortia isolated from hypersaline environments.

More than 95 % of the COD of pickling processing wastewater can be removed using bioaugmentation with *Halobacter halobium* (Kargi et al. 2000). The COD removal efficiency decreases with increasing feed COD, COD loading rate, and the salt concentration (Dincer and Kargi 2001).

In the same manner, the effect of *Staphylococcus xylosum* alone or combined with activated sludge as inoculum in the treatment of wastewater from the pickled vegetable industries has been analyzed at various NaCl concentrations (0.5–3 %w/v) (Abou-Elela et al. 2010). The results showed that the COD removal efficiency depended on the NaCl concentration. At low NaCl concentrations (0.5 and 1.5 %w/v), the COD removal efficiencies for pure and mixed culture are comparable (90 %), and at 3 % NaCl the COD removal efficiencies slightly increase to >94 % for both cultures. In contrast, the COD removal efficiency using activated sludge alone as inoculum decreases from 90 to 64 % at 3 % NaCl. This fact shows that COD from saline wastewater can be reduced by halophilic microorganism without additional treatment.

The performance of halophilic microorganisms in the removal of COD has been also tested in anaerobic conditions. The anaerobic salt-tolerant bacteria *Halanaerobium lacusrosei* has been used in an upflow anaerobic packed-bed reactor using synthetic saline wastewater with various concentrations of salt (0–5 % NaCl) and COD content (1,900–3,400 mg O₂ L⁻¹). The highest COD removal efficiency (94 %) was obtained at low salt and COD content (Kapdan and Erten 2007).

Treatment of saline wastewater polluted with hydrocarbons

Petroleum and gas natural reservoirs contain saline water. During the production of crude oil and natural gas, large amounts of reservoir water are typically also extracted, this reservoir water is known as produced water (PW) (Speight 2007). Although oils and greases are the constituents of PW that have received the most attention, PW also contains organic and inorganic compounds that can vary depending on the extraction site (Veil et al. 2004). PW from gas production contains aromatic hydrocarbons such as benzene, toluene, ethylbenzene, and xylene (BTEX), which are characterized by their low molecular weights and high toxicity.

The PW from oil production also contains polycyclic aromatic hydrocarbons (PAHs) (Jacobs et al. 1992), which persist in soils and sediments for a long time due to their hydrophobic nature. PAHs are toxic, carcinogenic, and mutagenic, and therefore they present problems for human health. The aromatic hydrocarbons of low to medium molecular weight are

relatively soluble in PW compared to the high-molecular-weight non-aromatic fractions of fossil fuels. PAHs contribute to water toxicity because their toxicities are additive, i.e., even though the individual toxicities may be insignificant, the combination of these chemicals results in an increased combined toxicity (Veil et al. 2004).

The biodegradation of hydrocarbons under saline conditions has been extensively studied. The studies on these organic pollutants using halophiles have mainly focused on the isolation and identification of hydrocarbon-degrading halophilic microorganisms from hypersaline environments. In some cases (Kleinstuber et al. 2006; Erdogmus et al. 2013), the proficiency of halophilic isolates in the degradation of several types of hydrocarbons or aromatic metabolites under several culture conditions has been reported. It has been shown that nutrient addition can improve the reduction in COD and hydrocarbon biodegradation (Piubelli et al. 2012; Bonfá et al. 2011). Recently, studies identifying the key enzymes in various metabolic pathways have been conducted (Seo et al. 2007; Zhong et al. 2011).

Aliphatic and aromatic hydrocarbons have been removed by both halobacteria and haloarchaea strains, and differences in the culture conditions and removal efficiencies have been described (Table 1). Furthermore, some members of the *Haloferax* genus have been able to grow on a mixture of some of the intermediates of PAH biodegradation, as the sole carbon source (Bonfá et al. 2011). The intermediates are simultaneously degraded with the mixture of PAHs (naphthalene, anthracene, phenanthrene, pyrene, and benz [a] anthracene; 0.3 mM each) in the presence of 20 % NaCl. This demonstrates the catabolic versatility of haloarchaea to mineralize aromatic compounds. In addition, the growth and extent of PAH degradation by *Haloferax* improved in the presence of 0.05 %w/v of yeast extract (Bonfá et al. 2011).

The ability of halophilic microorganisms to degrade PAHs decreases with increasing molecular weight (Arulazhagan and Vasudevan 2009), due to their decreased solubility and, consequently, their decreased bioavailability. A consortium collected in Chennai degraded 89 and 74 % of the initial phenanthrene at 50 and 100 mg L⁻¹, respectively, but only 89 and 88 % of the initial pyrene at 5 and 10 mg L⁻¹, respectively, in the presence of 3 % NaCl (Arulazhagan and Vasudevan 2011a). Addition of glucose, sodium citrate, and urea (25 mmol L⁻¹ each) enhanced the PAH utilization due to the increased microbial growth (Arulazhagan and Vasudevan 2011b). The use of chemical dispersing or co-culture with biosurfactant-producing bacteria could overcome the problem of bioavailability. The culture conditions for hydrocarbon biodegradation by halobacteria and haloarchaea under saline conditions are summarized in Table 1.

To improve the process of pollutant biodegradation, various culture conditions and components of the media used for the degradation study can be manipulated. The statistically based experimental designs for screening the nutritional and environmental factors which affect the pollutant biodegradation process

Table 1 Biodegradation of hydrocarbons by halotolerant and halophilic strains

Strains	Pollutants	Removal	Conditions	Reference
<i>Haloferax</i> sp., <i>Halobacterium piscicolsi</i> , <i>H. salinarum</i> , <i>Haloarcula</i> sp., <i>H. hispanica</i> , <i>Halorubrum</i> sp., <i>H. ezzemoulense</i>	<i>p</i> -Hydroxybenzoic acid, Naphthalene, Phenanthrene, Pyrene	Not reported (Optimal concentrations for growth 80–120 mg L ⁻¹)	10–15 d, 37 °C, 20 % NaCl, 150 rpm	Erdogmus <i>et al.</i> 2013
Consortium Qphe-SubIV (<i>Halomonas</i> sp. + <i>Marinobacter</i> sp.) <i>Halomonas</i> sp.	Phenanthrene	90 %, 100 mg L ⁻¹	12 d, 30 °C, 1–17 % NaCl, 120 rpm	Dastgheib <i>et al.</i> 2012
<i>Martellella</i> sp. AD-3 <i>Marinobacter</i> sp., <i>Prolixibacter</i> sp., <i>Balneola</i> sp., <i>Zunongwangia</i> sp., <i>Halobacillus</i> s. <i>Haloferax</i> sp.	Phenol, benzoic acid, and <i>p</i> -hydroxybenzoic acid in produced water Phenanthrene BTEX Naphthalene, Anthracene, Phenanthrene, Pyrene, and Benz [a] anthracene	65–80 %, 2 mM each 100 % 200 mg L ⁻¹ 100 % 120–150 mg L ⁻¹ 30–90 % (depending on the aromatic compound; 0.3 mM each) 65 % of the COD in the PW obtained from an oil refinery, 1345 mg-COD L ⁻¹)	12 d, 38 °C, 10 % NaCl 130 rpm 6 d, 30 °C, 3 % total salt content, 150 rpm 5 d, 30 °C, 5.8–20.3 % NaCl 20 % of NaCl, 168 h, 40 °C, 150 rpm. 10 % of NaCl, 40 °C, 168 h.	Piubelli <i>et al.</i> 2012 Feng <i>et al.</i> 2012 Li <i>et al.</i> 2012 Bonfá <i>et al.</i> 2011
<i>Ochrobactrum</i> sp. VAI	Anthracene Phenanthrene Naphthalene Fluorene Pyrene Benzo(k) fluoranthene Benzo(e) purene Anthracene	88 % (3 mg L ⁻¹) 98 % (3 mg L ⁻¹) 90 % (3 mg L ⁻¹) 97 % (3 mg L ⁻¹) 84 % (3 mg L ⁻¹) 57 % (1 mg L ⁻¹) 50 % (1 mg L ⁻¹) 87 % (3 mg L ⁻¹)	3 % NaCl, 37 °C, 48 h	Arulazhagan and Vasudevan 2011a
<i>Ochrobactrum</i> sp. VAI	Pyrene Anthracene Pyrene Anthracene Pyrene Crude oil <i>n</i> -Octadecane Phenanthrene	83 % (3 mg L ⁻¹) 81 % (3 mg L ⁻¹) 76 % (3 mg L ⁻¹) 88 % (3 mg L ⁻¹) 84 % (3 mg L ⁻¹) 13–47 % 28–67 % 13–30 % 2 g L ⁻¹ each	Medium with glucose Medium with sodium citrate Medium with urea 37 °C, 150 rpm, 5 d, 3 % NaCl	Arulazhagan and Vasudevan 2011b
<i>Haloferax</i> sp., <i>Halobacterium</i> sp., <i>Halococcus</i> sp.	Heptadecane Eicosane	32–95 % 0.5 g L ⁻¹ each	3 weeks, 40–45 °C, 23.4 % NaCl, 180 rpm	Al-Mailem <i>et al.</i> 2010
<i>Haloarcula</i> sp., <i>Haloferax</i> sp.	Heptadecane Eicosane	32–95 % 0.5 g L ⁻¹ each	30 d, 40 °C, 22.5 % NaCl, 120 rpm	Tapilatu <i>et al.</i> 2010

Table 1 (continued)

Strains	Pollutants	Removal	Conditions	Reference
<i>Gammaproteobacteria</i>	Benzene Toluene	100 % 20–25 µM	30 °C, 2 weeks, 11.6 % NaCl	Sei and Fathepure 2009
Halophilic coccus TM-1	Crude oil and low molecular weight organic substrates	Not reported	37 °C, 18 % NaCl, 150 rpm, 5 d	Hao and Lu 2009
Consortium	Phenanthrene	89 % and 74 % (50 and 100 mg L ⁻¹)	3 % NaCl, 5 d, 37 °C, 150 rpm	Arulazhagan and Vasudevan 2009
	Fluorene	89 % and 78 % (50 and 100 mg L ⁻¹)		
	Pyrene	89 % and 88 % (5 and 10 mg L ⁻¹)		
	Benzo(e)pyrene	70 % (1 mg L ⁻¹)		
<i>Halomonas</i> sp., <i>Ralstonia</i> sp. <i>Dietzia</i> sp.	Diesel fuel	95 %	7.5 % of NaCl, 84 d	Kleinsteuber et al. 2006
<i>Halomonas</i> sp.	Benzoic acid, <i>p</i> -hydroxybenzoic acid, Phenylpropionic acid, Ferulic acid, <i>p</i> -aminosalicylic acid	Not reported	10 % NaCl, 48 h, 37 °C	Garcia et al. 2005

by non-halophilic microorganism have been performed (Farag and Soliman 2011; Xia et al. 2012), but in saline conditions this procedure has been poorly explored. Factorial design techniques have advantages over the one-factor-at-a-time approach to pollutant removal in order to carry out biostimulation and bioaugmentation strategies. With those approaches, it is possible to know the significant factors and to get their optimization in the biodegradation process. Ghevariya et al. (2011) increased the chrysene degradation (from 40.79 to 85.96 %) by halotolerant *Achromobacter xylosoxidans* using Central Composite Design.

Knowledge of the metabolic pathways in pollutant biodegradation is important as it allows predicting the fate of the degradation products in the environment (Le Borgne et al. 2008). In case of halophilic microorganisms, information about the metabolic mechanisms, enzymes, and genes involved in PAH biodegradation is scarce. Similar to the non-halophilic microorganisms, the degradation pathway of PAHs in saline conditions by halophilic microorganisms involves their oxidation to salicylate that can then be further converted to either catechol or gentisic acid (Feng et al. 2012). The catechol ring is cleaved by 1,2-dioxygenase (*ortho* pathway) or 2,3 dioxygenase (*meta* pathway). Biodegradation products produced by these oxidative enzymes have been examined in several recent biodegradation studies (Fig. 1).

The halophilic bacterium *Marteletella* sp. degrades PAH through the gentisic acid rather than the catechol pathway (Feng et al. 2012). The catechol 1,2 dioxygenase enzyme was found to be involved in PAH biodegradation by *Haloferax* sp., *Halorubrum* sp., and *Halobacterium piscisalsi*, which are halophilic archaea isolated from Çamalti Saltern in Turkey (Erdogmus et al. 2013).

Catechol 2,3-dioxygenase rather than catechol 1,2-dioxygenase was involved in the degradation of benzene and toluene in the presence of 29 % NaCl by *Arhodomonas* sp., isolated from Great Salt Lake, Utah, USA (Sei and Fathepure 2009; Le Borgne et al. 2008). These findings demonstrate that halophilic archaea and halophilic bacteria are able to metabolize PAH, and that some of them share the same catabolic pathways. Haloarchaea probably evolved from methanogens, upon which they had to switch from a strictly anaerobic chemolithoautotrophic to an aerobic photo-organoheterotrophic lifestyle. This switch was accompanied by a massive gene gain from the *Bacteria* domain (Khomyakova et al. 2011).

Treatment of saline wastewater polluted with phenol

As a consequence of its serious health and ecological concerns, phenol has been included in the priority list of hazardous substances of the Environmental Protection Agency (EPA) and the European Union (EU) (Busca et al. 2008).

Table 2 Biodegradation of phenol by halotolerant and halophilic strains

Strain	Pollutant	Removal	Conditions	Reference
<i>Halomonas campisalis</i>	Phenol	100 %, 130 mg L ⁻¹	30 °C, 2.5–10.0 % NaCl,	Alva and Peyton (2003)
	Catechol	100 %, 16 mg L ⁻¹	140 rpm, pH 9.5, < 6d	
<i>Penicillium chrysogenum</i>	Phenol	100 %, 300 mg L ⁻¹	25 °C, 5.8 % NaCl, 160 rpm,	Leitão et al. (2007)
<i>Arthrobacter</i> sp. W1	Phenol (P)	100 %, 200 mg L ⁻¹	30 °C, 5.0 % NaCl, 150 rpm, 36 h	Wang et al. (2009)
	Catechol (C)	100 %, 100 mg L ⁻¹	30 °C, 10 % NaCl, 150 rpm, 60 h	
	P+C	200+100 mg L ⁻¹		
Consortium composed of <i>Arthrobacter</i> sp., <i>Pseudomonas aeruginosa</i> , <i>Bacillus</i> spp., <i>Halomonas salina</i> , <i>P. putida</i>	Phenol	300 mg L ⁻¹	37 °C, 5.0 % NaCl, 150 rpm, 4 days	Gayathri and Vasudevan (2010)
		88 %		
		77 %		
		63–71 %		
		70 %		
<i>Halomonas</i> sp. strain PH2	Phenol	100 %, 1,100 mg L ⁻¹	0.2 % glucose, 2.0 % NaCl, pH 7, 150 rpm, 80 h	Ravikumar et al. (2011)
<i>Halomonas organivorans</i> ,	Phenol	88 %, 280 mg/L	30 °C, 18.0 % NaCl, pH 7, 150 rpm, 7 days	Haddadi and Shavandi (2013)
<i>Arhodomonas aquaeolei</i>		75 %	37 °C, 17.4 % NaCl, pH 7.2, 150 rpm	Bonfã et al. (2013)
<i>Modicisalibacter tunisiensis</i>		89 %		

insufficient to effectively treat this kind of heterogeneous wastewater. The recent application of microbial aggregates (i.e., periphyton) technologies to improve in situ pollutant removal of surface waters has been reported (Wu et al. 2014).

Periphyton comprising of heterotrophic and phototrophic microorganisms has been proven to possess significant potential in the removal of miscellaneous contaminants in non-saline environments (Wu et al. 2012), but the application of detoxifying periphyton communities in high salinity conditions has been barely characterized. Periphyton communities could be contrived and/or incorporated into bioreactors based on cell immobilization technology to treat multiple pollutants.

Treatment of saline wastewater polluted with N-aromatic or N-aliphatic compounds

Nitro-substituted aromatic compounds, such as nitrobenzene and nitrophenol, are used in the manufacturing of azo dyes, explosives, pharmaceuticals, and pesticides (Oren et al. 1991). The concentration of inorganic pollutants varies with the type of industrial wastewater; however, the more usual range of salt content is between 20 and 150 g L⁻¹ (Afzal et al. 2007; Li et al. 2010; Ogugbue et al. 2011; Lefebvre and Moletta 2006). During the chemical and biological breakdown of these products in soil and water, some nitroaromatic derivatives are released into the environment (Oren et al. 1992). Oren et al. (1991) tested two halophilic strains, *Haloanaerobium*

praevalens and *Sporohalobacter marismortui*, which reduce *p*-nitrophenol and other nitro-substituted aromatic compounds, to produce the respective aromatic amine derivatives.

Aromatic amines are reagent precursors for the manufacturing of dyes, pesticides, rubber, fertilizers, surfactants, and foods. As a result of their broad usage, amines are common constituents in industrial effluents (Lawrence 2004). Aromatic amines can also be produced by the reduction of azo dyes, and due to their toxicity, a subsequent treatment is required to eliminate them (Saratale et al. 2011). However, the removal efficiencies of aromatic amines such as aniline decrease with increases in the amine and salt concentrations (Li et al. 2010).

Jin et al. (2012) demonstrated that the addition of 10–70 mM sodium acetate increases the biodegradation of aniline, phenylamine by the halophilic bacterium *Dietzia natronolimnaea* JQ-AN. In the presence of 3 % NaCl, only 60 % of the initial content of aniline was degraded after 5 days of incubation at 150 rpm and 30 °C. The addition of 40 mM sodium acetate significantly improved the microbial growth, and the aniline degradation reached 87 %. The authors proposed that aniline was metabolized via catechol as the first intermediate by *D. natronolimnaea* JQ-AN under aerobic conditions, and then further biodegraded through the tricarboxylic acid cycle to yield small organic compounds. As is observed with the degradation of other organic pollutants, the addition of co-substrates to the medium could significantly increase the removal efficiency of the pollutants. Campo et al. (2011) studied the aerobic biodegradation of several amines (5–6 mg L⁻¹) in two saline industrial effluents containing 3

and 7 % NaCl using consortia from two industrial bioreactors. The salinity did not affect the biodegradation rates of tris (2-hydroxyethyl) amine (triethanolamine and *N,N*-bis (2-hydroxyethyl) methylamine (methyldiethanolamine), but *N,N*-diethylethanolamine and *N*-(2-aminoethyl) ethanolamine were degraded faster in the presence of 3 % NaCl than in 7 % NaCl. Aniline disappeared after 48 h with both salt concentrations. In contrast, 100 % of cyclohexylamine and 4,4'-methylenedianiline were degraded in the presence of 3 % NaCl after 24 h, and the concentrations of these two chemicals remained unaltered in the presence of 7 % NaCl. These results show that the extent of biodegradation and the rate depend on the type of amine, and the salt concentration, with a maximum of concentration of 3 % NaCl.

The presence of high levels of histamine is detrimental to the quality of foods; Tapingkae et al. (2010) isolated three halophilic archaea from fish sauce and identified these as *Halobacterium piscisalsi* HPC1-2 and *Natrinema* sp., HDS3-1 and HDS1-1. The HDS3-1 strain exhibited a biodegradation efficiency of 80 % for 5 mM histamine under hypersaline conditions (25 % NaCl) without releasing any toxic intermediates, which suggests the activity of a salt-tolerant histamine dehydrogenase.

Treatment of saline wastewater polluted with dyes

Azo dyes are the largest class of dyes used in the textile processing and paper printing industries (Saratale et al. 2011). These dyes are usually synthesized to resist oxidative attacks and bind strongly to fibers, thereby generating more intense and longer lasting colors (Chen et al. 2003). The biodegradation of azo dyes in a culture broth with low salinity has been extensively studied, and it has been shown that the process is influenced by the temperature, pH, dye structure and concentration, carbon and nitrogen sources, oxygen concentration, and agitation (Solis et al. 2012; Saratale et al. 2011).

Although the wastewater from the textile industry are of high salinity due to the presence of salts used in dye baths (30–100 g L⁻¹), the studies on the biodegradation of dyes in the presence of a high salt content are limited (Ogugbue et al. 2011). Recently, the isolation and identification of halotolerant or halophilic microorganisms capable of degrading dyes has been reported (Oturkar et al. 2011; Chan et al. 2012); however, further studies on the optimal conditions and catabolic pathways for dye biodegradation by these microorganisms, taking into account the salinity of real effluents, are necessary. Decolorization of the azo dye Acid Red B (50 mg L⁻¹) in the presence of 2–5 % NaCl by *Gracilibacillus* sp. GTY has been reported (Uddin et al. 2007). Low decolorization efficiency due to the low growth rate of the strain has also been observed. In contrast, this strain completely degraded the dye in the presence of 15 % NaCl after 96 h of incubation. In

presence of 25 % of NaCl, the decolorization was not satisfactory due to the inhibition of bacterial growth caused by the high salt concentration. The strain expressed an azo reductase enzyme that, in combination with other enzymes, allows the complete degradation of the dye.

Halomonas spp. strains have decolorized Remazol Black B dye under both anaerobic and microaerophilic conditions in a wide range of NaCl concentrations (up to 20 % (w/v)) (Asad et al. 2007). The optimal conditions for dye removal were pH 9–11 and 35–40 °C. High performance liquid chromatography (HPLC) chromatograms showed that the dye was reduced to aromatic amines, which are toxic intermediates.

Another strain of *Halomonas*, namely *Halomonas* sp. GTW, was able to anaerobically degrade 100 % of Reactive Brilliant Red K-2BP in the presence of 10–15 % NaCl and yeast extract at 30 °C, and pH 6.5–8.5 (Guo et al. 2008). The identification of the metabolites was not performed in this case.

Due to textile effluents containing a complex mixture of dyes, research on halophilic strains with the ability to degrade several pigment classes is desirable. A *Pseudomonas aeruginosa* and *Bacillus circulans* consortium was able to anaerobically decolorize 100 mg L⁻¹ Reactive Black 5 with a removal efficiency of 93 % in the presence of 5 % NaCl. In addition, the consortium had the ability to decolorize other azo dyes, such as Reactive Violet 13, Reactive Orange 16, Reactive Red 11, Reactive Red 141, and Direct Yellow 12, with discoloration efficiencies of 80–90 %, whereas Acid Orange 7, Direct Green 6, and Acid Yellow 36 were decolorized with efficiencies of 40–65 % (Dafale et al. 2008).

The use of anthraquinone as a redox mediator for the enzymatic reduction of Reactive Brilliant Red X-3B was assessed by Tan et al. (2009). The dye removal was performed enzymatically and, to a low extent, by biosorption onto microbial cells of the salt-tolerant bacterium *Exiguobacterium* sp. The optimal NaCl concentration for color removal and bacterial growth was 15 % (w/v); at this concentration, the organism reduced 1,000 mg L⁻¹ of the dye to less than 200 mg L⁻¹ in 25 h. To our knowledge, the potential use of archaea in biodegradation of azo dyes has been not reported.

Although the studies on the degradation of azo dyes under saline conditions have been generally conducted under anaerobic conditions, some researchers have studied the removal of azo dyes under aerobic conditions by halotolerant bacteria (Chan et al. 2012). It has been reported that the halotolerant *Bacillus lentus* BI377 was able to degrade Reactive Red 120 in microaerophilic and aerobic conditions (Oturkar et al. 2011). However, its maximum decolorization rate was at 1 % NaCl, a very low salt concentration compared to actual textile wastewater (3–10 % NaCl). Therefore, it is necessary to characterize halophilic or halotolerant strains able to degrade dyes in aerobic conditions at actual salt concentrations seen in the wastewater.

In addition, the use of sequential anaerobic–aerobic processes to mineralize azo dyes in non-saline conditions using pure cultures or consortia have been extensively reported (Solis et al. 2012). In fact, the biodegradation of azo dyes by non-halophilic strains requires a sequential anaerobic–aerobic process, in which the aromatic amines produced in the first step are oxidized in the second step under aerobic conditions (van der Zee and Villaverde 2005; Mohanty et al. 2006; Pandey et al. 2007). Unfortunately, similar research in saline conditions is scarce.

Also, dye degradation in saline conditions using microbial aggregates, immobilization of facultative dye degraders, or consortia on irregular solid material, which facilitates the creation of aerobic and anaerobic microenvironment, can be investigated to mineralize those recalcitrant compounds as has been reported for non-halophilic microorganism (Wu et al. 2014; Barragán-Huerta et al. 2007; Barragán et al. 2007). Application of periphyton technology could be useful in the treatment of complex effluents from textile industries.

Conclusion

Halophilic microorganisms have the ability to degrade diverse organic pollutants in the presence of high concentrations of salt. For this reason, these organisms may play an important role in environmental biotechnology for the removal of organic pollutants from multiple types of industrial saline wastewater. Although it has been shown that halophilic and halotolerant microorganisms are capable of degrading several organic pollutants and, in some cases, exhibit higher versatility than non-halophilic microorganisms, their potential has been insufficiently explored. Therefore, it will be necessary to perform more in-depth investigations into the growth conditions and degradation mechanisms of these types of microorganism either in model systems or using actual industrial effluents.

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