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Potential applications of halophilic microorganisms for biological treatment of industrial process brines contaminated with aromatics

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Abstract: Saline wastewater contaminated with aromatic compounds can be frequently found in various industrial sectors. Those compounds need to be degraded before reuse of wastewater in other process steps or release to the environment. Halophiles have been reported to efficiently degrade aromatics, but their application to treat industrial wastewater is rare. Halophilic processes for industrial wastewater treatment need to satisfy certain requirements: a continuous process mode, low operational expenditures, suitable reactor systems and a monitoring and control strategy. The aim of this review is to provide an overview of halophilic microorganisms, principles of aromatic biodegradation, and sources of saline wastewater containing aromatics and other contaminants. Finally, process examples for halophilic wastewater treatment and potential process monitoring strategies are discussed. To further illustrate the significant potential of halophiles for saline wastewater treatment and to facilitate development of ready-to-implement processes, future research should focus on scale-up and innovative process monitoring and control strategies.

Keywords: Bioremediation processes, Saline wastewater treatment, Industrial process brines

Introduction

Saline wastewater occur in various industrial sectors around the world, including the petroleum industry, tannery and textile industry, coal gasification and dye or polyurethane production (Castillo-Carvajal et al., 2014; Muddemann et al., 2018). In total, it is estimated that 5% of industrial wastewater contain high amount of salt and quantities are likely to increase in the future (Le Borgne et al., 2008). Due to the fact that saline wastewater mostly originates from heavy industry sectors, they are often contaminated with several toxic and hazardous substances (Woolard & Irvine, 1995; Moussa et al., 2006). Among them are various hydrocarbons, like mono- or polycyclic aromatic hydrocarbons (e.g., phenol, benzene, or anthracene) (Le Borgne et al., 2008). These substances pose a severe environmental and health threat, because they are toxic to most organisms and possess mutagenic and carcinogenic properties (Sun et al., 2019).

Often, saline wastewater is diluted with freshwater before they are released to the environment (Tan et al., 2019). However, on the basis of population- and economic growth as well as other global challenges like climate change, the availability of freshwater will become more challenging in the future (Du et al., 2018; Tong & Elimelech, 2016). Moreover, in some regions freshwater is produced by seawater desalination and brine is generated as a by-product, which has a negative environmental impact when disposed to the sea again, such as salinity gradients or reduction of the amount of flora (Casas et al., 2012). However, treatment of saline wastewater is not only of interest considering the release in the environment, but also its reuse for industrial applications. Brines from seawater or industrial processes can be used by the chlor-alkali industry to produce substances like chlorine, sodium- and potassium hydroxide. The most promising technique in the chlor-alkali industry is the membrane cell process (Brinkmann et al., 2014; Casas et al., 2012). In this process, however, organic and inorganic impurities in the saline wastewater can lead to a cell voltage increase. Therefore, a highly purified brine is needed to avoid a decrease in the membrane efficiency. Consequently, the degradation of pollutants in brines is gaining interest (Brinkmann et al., 2014; Casas et al., 2012; Le Borgne et al., 2008). In the past, it has been reported that physical and electro chemical methods like sorption or oxidation are expensive and energy consuming or inappropriate for large volumes of wastewater. Therefore, biological systems could represent an economically more attractive alternative for saline wastewater treatment (Bonfa et al., 2013; Jin et al., 2012). The biological treatment of saline wastewater with nonhalophilic microorganisms would require a pretreatment to decrease the salt concentration, either by dilution or desalination (Tan et al., 2019). However, these pretreatment strategies would increase operational expenditures significantly. Therefore, halophilic microorganisms are a better choice because direct operation with waste brine is possible (Bonfa et al., 2013; Díaz et al., 2002; Jin et al., 2012). Halophilic bacteria and archaea have already been the target in several studies dealing with the degradation of aromatic compounds, but reports for the application of halophiles in industrial bioremediation are scarce (Krzmarzick et al., 2018). Therefore, the aim of this review is to provide an overview of halophilic microorganisms, degradation of aromatic compounds, sources of saline wastewater polluted with aromatic compounds and other contaminants, and finally shows examples of application of halophilic organisms to treat industrial wastewater streams. Moreover, we address problems in process development which need to be solved for implementation to

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industrial production chains. Finally, future development potential and research goals are outlined.

Degradation of Aromatic Compounds in Halophilic Microorganisms

Halophilic Microorganisms

While nonhalophilic microorganisms show optimal growth at concentrations below 2% NaCl, halotolerant and halo-dependent microorganisms are able to grow in the presence of up to 30% NaCl (Castillo-Carvajal et al., 2014; Margesin & Schinner, 2001). The group of halophiles is highly diverse and present in all three domains of life (archaea, bacteria, eukarya) (Oren, 2002a, 2008; Roberts, 2005). Depending on the level of salt tolerance, halophiles are usually classified into four different groups: halotolerant, slight, moderate and extreme halophilic microorganisms. Halotolerant microorganisms can grow in saline environments, but do not necessarily require high salt concentrations (Zhuang et al., 2010). In contrast, true halophiles are classified as slight (1–3% NaCl), moderate (3–15% NaCl), and extreme halophiles (15–30% NaCl) according to the salt concentration they require for growth (compare sea water salinity: around 3.2%) (Fofonoff, 1985; Kushner, 1978; Oren, 2002a, 2008; Zhuang et al., 2010). In this review, halophilic physiology will not be discussed in detail. However, it is noted that two different strategies for balancing osmotic pressure in high-salt environments have evolved: the salt-in and the compatible solute strategy (Gunde-Cimerman et al., 2018; Oren, 2002a, 2002b, 2008).

The salt-in strategy is mainly used by halophilic archaea and is based on the intracellular accumulation of inorganic ions (K^+ and Cl^-) to provide an osmotic balance (Margesin & Schinner, 2001; Oren, 2002a, 2002b, 2008). The transport of potassium ions can be passive (through a K^+ channel) or active (through ATP-dependent transport systems). Therefore, an energy-dependent mechanism is required, because the intracellular accumulation of negatively charged chloride ions would be repressed by the inside-negative membrane potential (Gunde-Cimerman et al., 2018). Special membrane-bound retinal pigments such as bacteriorhodopsin and halorhodopsin are often found in halophilic archaea like *Halobacterium salinarium* and *Haloferax volcanii*, and enables these organisms to use energy from light directly to power bioenergetical processes (Gunde-Cimerman et al., 2018; Margesin & Schinner, 2001; Oren, 2002b, 2008; Roberts, 2005).

Within the domain of the archaea, the most widespread group are the *Halobacteria* and most of the members of this group require high salt concentrations above 15% (Gunde-Cimerman et al., 2018). To survive at such harsh conditions, the cellular structural components, the intracellular machinery and the intra- and extracellular enzymes are highly adapted (Le Borgne et al., 2008). To maintain enzyme activity and keep proteins soluble at such high intracellular ion concentrations, acidic amino acids are used more frequently on outside regions of proteins than hydrophobic amino acids (Margesin & Schinner, 2001; Gunde-Cimerman et al., 2018). To keep the level of the hydrophobic amino acids low, a high content of the "borderline hydrophobic amino acids" serine and threonine is required (Lanyi, 1974; Oren, 1999). Due to this unique amino acid composition, these microorganisms only have limited ability to adapt to changing conditions, because of protein denaturation at lower salt concentrations (Margesin & Schinner, 2001; Oren, 1999, 2008).

In contrast to accumulating inorganic ions for balancing osmotic potential, some halophilic microorganisms also employ the strategy of biosynthesis and/or accumulation of compatible solutes (Oren, 2008). Compatible solutes are organic, osmotically and highly water-soluble substances. There are a large number of different compatible solutes which can be classified into ionic, zwitterionic, or nonpolar molecules (Oren, 2002b, 2008; Roberts, 2005; Shivanand & Mugeraya, 2011). In addition to their task as salt antagonist, they stabilize DNA, enzymes and whole cells against stress factors such as freezing, drying and heating (Shivanand & Mugeraya, 2011). Thus, they have already been used as cryo-protectant (Roberts, 2005). Further, compatible solutes are often referred to as chemical chaperones, because they stabilize enzymes and assist during proper folding of polypeptide chains (Roberts, 2005; Shivanand & Mugeraya, 2011). This property leads to a high adaptability to changes of the extracellular salinity. The use of compatible solutes is more widespread among halophilic microorganisms, although it requires more energy than the intracellular accumulation of ions, because organic solutes have to be synthesized *de novo* (Gunde-Cimerman et al., 2018; Oren, 2002a).

Degradation Mechanisms of Aromatic Compounds in Halophilic Microorganisms

In the biosphere, aromatic compounds are the second most abundant family of organic constituents beside carbohydrates (Field et al., 1995). They occur frequently in saline waste streams in the petroleum industry (Alva & Peyton, 2003; Bonfa et al., 2011). Beside their frequent occurrence, some aromatic compounds like polycyclic aromatic hydrocarbons (PAH), phenol, toluene, or aromatic amines have toxic, mutagenic and carcinogenic properties (Bonfa et al., 2013; Castillo-Carvajal et al., 2014; González et al., 2012). Several aromatic compounds are considered as a widespread environmental pollutant and hazardous to ecosystems. Furthermore, they are highly persistent in the environment and may accumulate in natural systems (Alva & Peyton, 2003; Bonfa et al., 2011; Gomes et al., 2018; Seo et al., 2009).

The degradation of aromatic compounds has frequently been reported for halophilic microorganisms (Arora, 2015; Bonete et al., 2015; Castillo-Carvajal et al., 2014; Fuchs et al., 2011; Krzmarzick et al., 2018; Li et al., 2019; Nogales et al., 2017; Vaillancourt et al., 2006). For the aerobic and anaerobic degradation of small aromatic compounds, the pathways and involved enzymes are known and are shortly described in this review.

The first task of degrading aromatic compounds is to break the energetically stable aromatic ring shared by all aromatic compounds, which can be done either in the presence or absence of oxygen (Fuchs et al., 2011; Heider & Fuchs, 1997). Under aerobic conditions, the aromatic ring is cleaved at an intradiol bond, an extradiol bond or independently of a diol (Burroughs et al., 2019), whereas aromatic rings in anaerobic pathways are reduced to cyclohexane derivatives (Heider & Fuchs, 1997).

One of the most studied oxidative aromatic degradation pathways is the β-ketoadipate pathway, which starts with the conversion of aromatic compounds to catechol (e.g., phenol, benzene, or benzoate) or protocatechute (e.g., 4-hydroxybenzoate) (see Fig. 1) (Fuchs et al., 2011; Li et al., 2019; Vaillancourt et al., 2006; Wells & Ragauskas, 2012). Both molecules are then cleaved by oxygenases to *cis-cis*-muconate and 3-carboxy-*cis-cis*-muconate, respectively (ortho-cleavage pathway). The end products of this pathway (succinyl- and acetyl-CoA) finally enter the TCA cycle (Vaillancourt et al., 2006; Wells & Ragauskas, 2012). In contrast, when using the meta-cleavage pathway, catechol and protochatechute are finally transformed into pyruvate and acetaldehyde (Fuchs et al., 2011). Both pathways have been described for halophilic bacteria, like *Halomonas* or *Marinobacter*

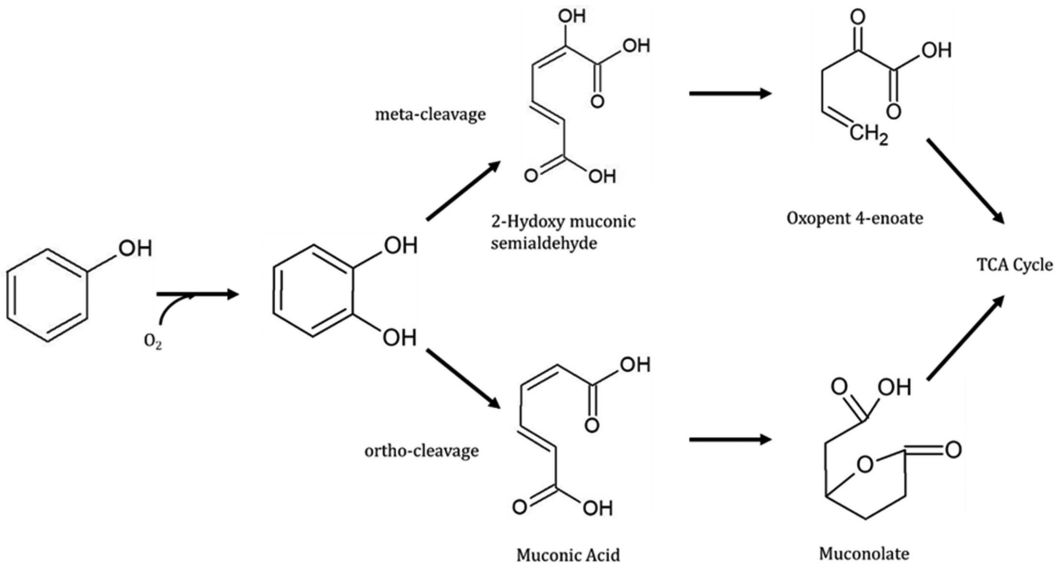

Fig. 1. Aerobic pathway of phenolic degradation (meta- and ortho-cleavage) (Li et al., 2019).

species, for various aromatic compounds like phenol, benzene or aniline (Li et al., 2019; Nicholson & Fathepure, 2004). For several *Pseudomonas* strains, which are halotolerant bacteria, it was reported that toluene is degraded, however, the strains used different pathways (Bordel et al., 2007; Duetz et al., 1994; Otenio et al., 2005; Yu et al., 2001). Reported pathways for toluene degradation were the conversion into 3-methylcatechol, *o*-cresol, *p*-cresol, or catechol (via benzoate), eventually resulting in the conversion to CO_2. Several studies showed also the degradation of toluene by various halophilic bacteria like *Marinobacter*, *Planococcus*, and *Arhodomonas* (Berlendis et al., 2010; Dalvi et al., 2014; Desouky et al., 2015). For the latter one, a degradation pathway of toluene via 3-methylcatechol and 4-hydroxy-2-oxovalerate into the TCA cycle was proposed, based on genomic and proteomic analyses (Dalvi et al., 2014). In 2002, Fairly et al. (Fairley et al., 2002) reported that only one ring cleavage oxygenase (gentisate 1,2-dioxygenase, Fu & Oriel, 1998) exists for archaea. Moreover, Wells et al. (Wells & Ragauskas, 2012) reported in 2012, that the β-ketoadipate pathway is not utilized by halophilic archaea and archaea in general. However, more recent studies reported the use of the β-ketoadipate pathway by halophilic archaea like *Haloferax*, *Haloarcula* of *Halobacterium* species, to degrade, for example, phenol or 4-hydroxy benzoate (Acikgoz & Ozcan, 2016; Erdoğmuş et al., 2013).

The halophilic archaea *Haloferax* sp. D1227 was the first halophilic archaeon reported to grow solely on aromatic compounds, such as benzoate, cinnamate, and phenylpropanoate, using the enzyme gentisate 1,2-dioxygenase, which does not belong to either intradiol or extradiol oxygenases. Rather, gentisate is cleaved between the carboxyl- and the hydroxyl group to form maleylpyruvate (Emerson et al., 1994; Fu & Oriel, 1998). This pathway is also present in halophilic bacteria like *Martelella* strains (Huang et al., 2015). The gentisate pathway is active during the haloarchaeal degradation of aromatic compounds like 4-hydroxybenzoate, 3-hydroxybenzoate, anthranilate and salicylate (Fairley et al., 2002, 2006; Fu & Oriel, 1998; Vaillancourt et al., 2006). Nonhalophilic microorganisms use the same pathways for the degradation of aromatic compounds as halophiles, as the same degradation pathways via catechol, gentisate or protochatechute pathway have been reported (Ladino-Orjuela et al., 2016; Nair et al., 2008).

As reported in literature, anaerobic bacteria mostly metabolize aromatic compounds via the benzyl-CoA pathway, which is degraded to acetyl-CoA and finally CO_2 is released (Evans & Fuchs, 1988; Fuchs et al., 2011; Heider & Fuchs, 1997). Few hydroxylated aromatic compounds are degraded anaerobically via the polyphenolic intermediates phloroglucinol and resorcinol. These compounds are later also dearomatized and transformed to acetyl-CoA (Fuchs et al., 2011).

Fuchs et al. pointed out that the anaerobic degradation of the polycyclic aromatic naphthalene via benzyl-CoA appears to be not yet clear (Fuchs et al., 2011). Nevertheless, Nzila et al. recently summarized the anaerobic degradation of naphthalene, which happens via the nonaromatic molecule *cis*-2-carboxycyclohexylacetyl-CoA and later to CO_2 via acetyl-CoA (Meckenstock & Mouttaki, 2011; Nzila, 2018). Likewise, the degradation of other PAHs occurs via multiple step pathways resulting in the production of CO_2 (Nzila, 2018). The aerobic degradation of PAHs in halophilic bacteria and archaea has been reported for several halophilic strains like *Haloarcula* sp., *Haloferax* sp., or *Halomonas* sp. and has recently been reviewed (Bonete et al., 2015; Erdoğmuş et al., 2013; Ghosal et al., 2016; Govarthanan et al., 2020; Oren, 2019). Two aerobic degradation mechanisms of PAH's are described for nonhalophilic bacteria. The first one starts with the hydroxylation of an aromatic ring to a *cis*-dihydrodiol. This intermediate is metabolized via ortho- or meta-cleavage, resulting in the formation of catechol, which is finally degraded via the TCA cycle (Ghosal et al., 2016; Mallick et al., 2011). Additionally, the bacterial cythocrom P450-mediated pathway was reported for aerobic PAH degradation (Ghosal et al., 2016). This pathway initially forms *trans*-dihydrodiol molecules as shown for the degradation of benzo[*a*]pyrene by *Mycobacterium vanbaalenii* PYR-1 (Moody et al., 2004).

Industrial Saline Wastewater

Sources and Composition of Saline, Aromatic Compounds Containing Wastewater

Aromatic compounds are widespread among industrial saline wastewater (oil refineries, food-processing sites, tannery industry, etc.), which can contain concentrations from the mg l^{-1} to

even the g l^{-1} range, depending on the industry of origin, process conditions or dilution factors (Garcia et al., 2005; Le Borgne et al., 2008; Ramos et al., 2015; Woolard & Irvine, 1995). The efficient degradation of organics in industrial wastewater is therefore beneficial for environmental considerations as well as for potential reutilization of the purified brines in industrial processes. As the composition of industrial wastewater often changes, and companies rarely publish the exact species and amount of contaminants in their wastewater, most studies about bioremediation of saline wastewater use synthetic brines with model contaminants. Nevertheless, this chapter aims to summarize potential sources of saline wastewater and wants to give an overview of their aromatic contaminants and potential co-contaminants.

As oilfields are a widespread source of contamination for saline environments, plenty of studies deal with the treatment of wastewater contaminated with petroleum hydrocarbons (Al-Mailem et al., 2010; Dastgheib et al., 2012; Pugazhendi et al., 2017). During oil and gas extraction, so-called "produced water" is generated as a by-product in large volumes, because water is pumped from subsurface reservoirs, along with gas and oil (Fathepure, 2014; Veil et al., 2004a). The volume of produced water is equal to or even higher than the extracted volume of crude oil and in 2007, over 20 billion barrels of produced water have been generated alone in the United States (Clark & Veil, 2009; Piubeli et al., 2012b; Pugazhendi et al., 2017; Veil et al., 2004a). Produced waters are not only characterized by their high salt content, but it has also been reported that produced water contains toxic chemicals, heavy metals and organics (Al-Mailem et al., 2017; Díaz et al., 2002; Fathepure, 2014; Neff et al., 2011). Wastewater from petroleum industry is a complex mixture of organic substances like cycloalkanes, aliphatic, mono-, and polyaromatic compounds. Those wastewater mostly contains toluene, benzene, ethylbenzene, xylene, phenol, 2,4-dimethyl-phenol, or PAHs like naphthalene as aromatic contaminants (González et al., 2012; Piubeli et al., 2012a; Veil et al., 2004b). Information about the composition of produced water can also be found elsewhere (Neff et al., 2011). Due to the hazardous properties of wastewater originating from oil processing industries, the contamination of ecosystems like beaches, salt marshes, or salt lakes should be avoided. The disposal of contaminated wastewater into the environment could be realized by biological pretreatment of those saline wastewater with halophilic biological systems.

Another source of industrial wastewater originates from the coal chemical industry, where chemical products are generated from coal-based processes. Wastewater from this industrial sector might be reused and purified by membrane-based technologies, ultrafiltration and reversed osmosis, generating high saline liquid leftovers, contaminated with a complex mixture of organic compounds (Ge et al., 2019; Bian et al., 2020). The inorganic resources present in these wastewater (NaCl, $NaNO_3$, and Na_2SO_4) can be recovered by fractional crystallization. However, it is known that organic contaminants in the wastewater can negatively affect the crystallization reactions. For example, it was shown that phenol and quinolone affects the average crystal size remarkably, while phenol decreases the solubility of sodium sulfate during the crystallization. As the crystal size is important for the crystallization process itself and the further downstream processing, removal of the contaminants is beneficial for process efficiency (Bian et al., 2020; Cisternas & Rudd, 1993; Ge et al., 2019; Su et al., 2018).

During polyurethane production the generation of a high-saline process water (10–15% NaCl) is reported, which contains aromatic amines and phenol as well as formate in significant amounts. High pH values make this wastewater a difficult target for bioremediation, and require a pretreatment in order to lower the pH (Mainka et al., 2019; Muddemann et al., 2018). However, once the organic content is removed, this process water represents an ideal substrate, for example, for the chlor-alkali electrolysis in order to generate chlorine gas and sodium hydroxide.

The tannery industry is generating large saline waste streams, because salt is used to preserve the fresh skins (Lefebvre et al., 2005). The resulting soak liquor waste wastewater contains phenolic compounds (phenol, *p*-cresol, etc.) and aromatic carboxylic acids (benzoate, phthalate, phenylacetate, etc.) as organic contaminants (Lefebvre et al., 2005; Reemtsma & Jekel, 1997). Currently, biological treatment of tannery wastewater commonly relies on the use of activated sludge (Durai et al., 2011). However, it is noted that tannery wastewater composition can be highly complex and may negatively affect biological processes due to the presence of salts, tannins and sulfide (Munz et al., 2008).

In the textile and dye industry, saline wastewater containing aromatic compounds are generated. Dying wastewater contains high salt concentrations (4–10% NaCl), because NaCl is used to maximize the dye fixation on textiles (Liu et al., 2013). The release of dye-containing wastewater negatively affects the aquatic ecosystem because photosynthetic activities decrease which results in the depletion of dissolved oxygen in the water (Guadie et al., 2018). It has been demonstrated that azo dyes, a widely used group of dyes in textile industry, are transformed to aromatic amines by skin microbiota. As aromatic amines have mutagenic and potentially carcinogenic properties, the treatment of azo dyes is highly important to avoid release into the environment (Brüschweiler et al., 2014; Brüschweiler & Merlot, 2017; Dafale et al., 2008; Yurtsever et al., 2016). In literature, several azo dyes were used as model contaminants for biodegradation experiments as they are frequently used in the textile industry. Among them are, for example, Reactive Red 184 (Guadie et al., 2018), Reactive Black 5 (Işık & Sponza, 2008), or Acid Orange 7 (Liu et al., 2013).

The treatment of saline wastewater using microorganisms from conventional wastewater treatment plants or freshwater organisms will operate poorly in terms of degradation efficiency (He et al., 2017; Kargi & Dincer, 1996). Therefore, an adaptation of the microbial community to higher salinities is necessary. If higher salinities over 15% NaCl occur, even the use of halophilic or halotolerant is essential (Amin et al., 2014; Dinçer & Kargi, 2001; Kargi & Dinçer, 1998).

As most of the industrial saline wastewater do not only contain aromatic contaminants, but also aliphatic (e.g., alkenes in petroleum wastewater) or inorganic components (e.g., ammonium or heavy metals), the influence of the wastewater composition on the degradation efficiency is crucial and has to be investigated (Deng et al., 2014; Jiang et al., 2016a, 2016c; Lofthus et al., 2018; Pereira et al., 2019). For instance, it is reported, that phenol-containing wastewater is often contaminated with heavy metals, and found that phenol-degradation by the halotolerant fungi *Debaryomyces* sp. JS4 was lower, when Co(II) and Ni(II) were present in the medium (Jiang et al., 2016a).

In conclusion, various saline wastewater in different industrial sectors are generated, which contain aromatic and other contaminants. It is of high interest to treat these wastewater and degrade potential pollutants, because the release of toxic compounds into the environment could be prevented or organic free wastewater could be reutilized in other industrial approaches. Often the biological treatment of saline wastewater is inhibited by high salt concentrations, thus, efficient microbial systems for the

degradation of the present contaminations should be found and investigated.

Sources for Halophilic Microorganisms Able to Degrade Aromatics

For industrial wastewater treatment processes, it is highly important to find halophilic or halotolerant strains able to degrade the specific aromatic compounds present in the wastewater. Multiple studies reported the discovery of halophilic communities or halophilic strains around the world, suitable for bioremediation purposes (Bertrand et al., 1990; Oren et al., 1991, 1992; Rosenberg, 1983). Halophilic and halotolerant strains and communities were found at salt lakes, salterns, soda lakes, or coastal areas, as well as in seawater samples (Bertrand et al., 1990; Deng et al., 2014; Lofthus et al., 2018; Oren et al., 1991, 1992; Pereira et al., 2019; Todorova et al., 2014). Adapted microorganisms could be isolated from saline environments, which were contaminated with aromatic compounds, like from oil spills. An *Achromobacter* strain was found in oil-contaminated seawater and was able to degrade PAHs like phenanthrene with an efficiency of 50.6% (Deng et al., 2014). Similar results were shown for two other bacteria (*Bacillus methylotophicus* and *Pseudomonas sihuiensis*), isolated from sample spots at the Lagao do Peixe National Park in Brazil. These strains showed a degradation efficiency for phenanthrene (originated from petroleum contamination) of 33% under marine environment conditions. Additionally, microorganisms at natural, uncontaminated spots (e.g., from uncontaminated seawater samples) have also been the target of investigations to study their ability to degrade contaminants present in various industrial wastewater (Brakstad et al., 2015; Cuadros-Orellana et al., 2006, 2012; García et al., 2004; Lofthus et al., 2018). It was for example shown, that aromatic compounds (PAH's and phenols) from a produced water was degraded by marine microorganisms sampled from uncontaminated seawater (Lofthus et al., 2018). Another study investigated the composition of microbes in seawater contaminated with oil (Brakstad et al., 2015). The authors could show, that the abundance of specific degrading bacteria increased, when seawater was contaminated with oil. It was discovered, that Gammaproteobacteria are responsible for the oil compound degradation. Within this group, *Cycloclasticus* and *Marinobacter* were correlated with the biodegradation of aromatic hydrocarbons.

Thus, in Table 1 we want to give information about halophilic strains, found to degrade aromatic compounds, their place of origin, as well as the salinity they were grown in.

Existing and Potential Applications of Halophiles in Industrial Bioremediation Processes

This chapter focuses on halophilic bioremediation processes in bioreactor-scales, their potential to be transferred into industrial scale and future research subjects. Therefore, it will be discussed of which parts a biological process for saline wastewater treatment have to consist, and which requirements such processes have to meet.

A state-of-the-art saline wastewater treatment process should consist of a continuous process operation mode, a suitable halophilic microbial systems, and potential process monitoring approaches. Moreover, requirements of biological processes for saline wastewater treatment, such as a robust process behavior against disturbances, a continuous process mode, corrosion-resistant reactor systems and a simple monitoring and control strategy (see Fig. 2) are discussed. Moreover, certain main process characteristics are important to evaluate a process. Important criteria for an efficient process are the degradation efficiency, the hydraulic retention time (HRT), requirements for additional substrates, and the reactor volume (Fig. 2). The efficiency of a biological remediation process is usually evaluated by the removal efficiency (biodegradation) of organic pollutants (Tan et al., 2019). The hydraulic retention or residence time (HTR) is the ratio between the bioreactor volume and the feed flow rate (David et al., 2019; Deowan et al., 2015). It is an important process criteria, as it describes the duration for how long cells and/or substrates remain inside the bioreactor. When lower HRTs are used in processes, usually lower reactor volumes are required, as the organic loading rate (OLR) is increased. In contrast, higher HRTs are reported to achieve better removal efficiencies (Deowan et al., 2015). A summary of bioprocesses for the degradation of aromatic compounds in saline wastewater can be found in Table 2.

For the integration of bioremediation processes into industrial process chains, in general a continuous process mode is necessary, as high volumes of wastewater streams are continuously generated. Usually, liquid dilution rates of wastewater streams are larger than the specific growth rates of halophiles. To keep the liquid dilution rate in the range of growth rates, or even below, but to simultaneously apply high wastewater flows, large bioreactors have to be used. Alternatively, the use of cell retention systems offers the possibility to decouple growth rate and wastewater dilution rate. Therefore, lower bioreactor volumes are needed, which would reduce operational (OpEx) as well as investment (CaPex) costs (Mainka et al., 2019). The size of bioreactors is especially relevant for saline bioprocesses, because more expensive, highly corrosion-resistant materials have to be used. In comparison to conventional continuous stirred tank reactors (CSTRs), cell retention systems can prevent cell wash out, and thus, might accelerate the adaptation of microbial systems to changes in wastewater compositions (Jang et al., 2013). Several cell retention processes for the treatment of saline wastewater have already been reported. In general, two strategies have evolved regarding cell retention in the reactor: retaining cells in suspension (e.g., with membranes) or immobilizing cells to a carrier material. Alternatively, the cell suspension can be settled and cell-free medium withdrawn from the fermenter. The settling and withdrawal strategy is mostly used in conventional wastewater treatment process, but only allows a discontinuous process mode.

Additionally, to develop a successful bioremediation process, a suitable microbial systems has to be chosen. The microbial system has to be able to deal with the conditions present in the specific wastewater. In case of saline wastewater, the microbial system has to be selected according to (1) the potential contaminants to enable efficient degradation and (2) the salt concentration of the wastewater. The microbial system should also be robust against variations in composition and salinities of different wastewater batches. Salinities up to 15% NaCl can be handled by moderate halophilic or halotolerant microorganisms, whereas salinities above 15% NaCl usually require extremely halophilic microorganisms. However, the process development for extremely halophilic strains might be more complex as with moderately halophiles with respect to the cultivation, but also due to technical aspects (e.g., use of highly corrosion-resistant bioreactor setups). Also, at sudden drops in wastewater salinities, extreme halophiles might be contaminated with moderate halophiles, because growth rates at low saline environments are usually lower for extreme halophiles compared to moderate halophiles. In

Table 1. Overview of Halophilic or Halotolerant Microorganisms, Able to Degrade Aromatic Compounds and Their Place of Origin

Strain(s)/culture	Origin	Type of halophile	Salinity (%)	Aromatic compounds	Reference
Arthrobacter strains	Soils/bottom sediments with waste from chemical and salt mining industry (Verkhnekamskoe potash deposit, Permkrai, Russia)	Halotolerant bacteria	6%	Naphthalene, salicylate, gentisate, diesel fuel, tetradecane, octane, phenanthrene	Plotnikova et al. (2011)
Halophilic bacterial population (mostly related to *Halomonas* species)	Water and sediment of salterns and hypersaline soils in South Spain close to oil refineries and food-processing industries	Moderately halophilic bacteria	10%	Benzoate, *p*-hydroxybenzoate, cinnamate, salicylate, phenylacetate, phenol, *p*-coumarate, ferulate, *p*-aminosalicylate	Garcia et al. (2005)
Halomonas organivorans	Saline soils from Isla Cristina (Spain)	Moderately halophilic bacterium	10%	Benzoate, *p*-hydroxybenzoate, cinnamate, salicylate, aminosalicylate, phenylpropionate, phenol, *p*-coumarate	García et al. (2004)
Geobacillus sp.	Production water (oil/water mixture) of the oil field TPS in Tunisia	Halotolerant (and thermophilic) bacterium	3%	Benzoate, *p*-hydroxybenzoate, protochatechute, vanilliate, phenol, *m*-cresol	Chamkha et al. (2008)
Halomonas sp. IMPC	Table-olive fermentation	Moderately halophilic bacterium	8%	*p*-Coumarate, benzoate, protochatechute, *p*-hydroxybenzoate, *p*-methoxy-benzoate, cinnamate, caffeate	Abdelkafi et al. (2006)
Halophilic archaeal strain (family of Halobacteriaceae)	Brine and sediment from Dead Sea (Jordan, December 2002)	Extremely halophilic archaea	34% (dead sea)	*p*-Hydroxybenzoate, benzoate	Cuadros-Orellana et al. (2012)
Halophilic consortium (*Halomonas* and *Marinobacter* strains	Saline soil sample contaminate with oil from industrial activity or accidents (Iran)	Moderately halophilic bacteria	1–17%	Phenanthrene	Dastgheib et al. (2012)
Halomonas sp. C2SS100	Production water from saline oilfield (Tunisia)	Moderately halophilic bacterium	5–8%	Crude oil [aliphatic hydrocarbons (C_{11}–C_{22})], carbazole (degradation activity was weak)	Mnif et al. (2009)
Haloferax sp., *Halobacterium* sp., *Halococcus* sp.	Soil and water samples from hypersaline coastal areas (supertidal "sabkha" from Kuwait and Abu Dhabi)	Extremely halophilic archaea	6–24%	*n*-alkanes (C_8–C_{40}), benzene, toluene, phentanthrene, biphenyl, naphthalene, *p*-hydroxybenzoic acid	Al-Mailem et al. (2010)
Debaromyces sp.	Activated sludge from a pharmaceutical plant (Wuhan, China)	Moderately halophilic fungus	5%	Phenol	Jiang et al. (2016a)
Haloferax sp. D1227	Top 5 cm of coarse, sandy soil surrounding an oil well (Grand Rapids, Michigan, USA)	Extremely halophilic archaeon	10–15%	Benzoate, cinnamate, phenylpropanoate	Emerson et al. (1994)
Haloferax sp., *Halobacterium piscisalsi*, *Halobacterium salinarium*, *Halorubrum ezzemoulense*, *Halorubrum* sp.	Brines samples from Çalmatı salterns (Turkey, 2007)	Extremely halophilic archaea	20%	*p*-Hydroxybenzoic acid, naphthalene, phenanthrene, pyrene	Erdoğmuş et al. (2013)

Table 1. Continued

Strain(s)/culture	Origin	Type of halophile	Salinity (%)	Aromatic compounds	Reference
Haloarcula sp. A235	Brine, salt, and saline soil samples from different (salt) lakes in Turkey in September 2000 and 2001 (see Ozcan et al., 2006)	Extremely halophilic archaeon	20%	Phenol	Acikgoz and Ozcan (2016)
Halomonas campisalis	Isolated near Soap Lake (WA, USA)	Moderately halophilic bacteria	0–15%	Phenol, catechol	Alva and Peyton (2003)
Haloarcula sp. EH4	Interface water sediment in a salt-marsh (Aigues-Mortes, France)	Extremely halophilic archaeon	15–30%	Acenaphthene, phenanthrene, anthracene, 9-methyl anthracene	Bertrand et al. (1990)
Haloferax alexandrinus st. KCTC 12962 BO3, BO6, AA31, AA35 *Haloferax* sp. CS1-9 BO7, MM17, PR13 *Haloferax* sp. HSC4 MM27 *Haloferax sulfurifontis* JCM 12327 CL47	Uyuni Salt Marsh, Bolivia, Cabo Rojo marine salterns, Puerto Rico, sabkhas (salt flats), Saudi Arabia, Dead Sea, Jordan, and Cahuil marine salterns, Chile	Extremely halophilic archaea	20%	Benzoate, *p*-hydroxybenzoate, salicylate, naphthalene, anthracene, phenanthrene, pyrene, benzo[*a*]anthracene	Bonfa et al. (2011)
Haloferax volcanii DSMZ 3757	Mud samples (1 m depth) in water close to the shore at the northern end of the Dead Sea	Extremely halophilic archaeon	20%	Anthracene, only with yeast extract: naphthalene, phenantrene, pyrene, benzo[*a*]anthracene	Bonfa et al. (2011), Mullakhanbhai and Larsen (1975)
Dietzia natronolimnaea JQ-AN	Industrial wastewater from Zhejiang Dragon Chemical Group Company (Hangzhou, China)	Moderately halophilic bacterium	0–6%	Aniline	Jin et al. (2012)
Unknown halophilc mixed culture	Soil samples from salterns at the Great Salt Lake Minerals Corporation (Utah, USA)	Moderately halophilic microorganisms	14%	Phenol	Woolard and Irvine (1995)
Halophilic enrichment culture	Two soil samples from the Great Salt Plains National Wildlife Refuge, OK, USA	Halophilic microorganisms	14.6%	Benzene and toluene	Nicholson and Fathepure (2005)
Bacillus methylotrophicus and *Pseudomonas sihuiensis*	Samples from National Park of Lagao do Peixe (sediment, sediment with seawater, seawater sample), Brazil	Halotolerant bacteria	3.5%	Aliphatic hydrocarbons (C8 to C33) and PAHs (anthracene, phenanthrene and pyrene)	Pereira et al. (2019)
Marine microorganisms from seawater samples	Seawater from Trondheimsfjord, Norway (depth: 80 m)	Halotolerant and/or halophilic microorganisms	3.4%	Produced water from Ula platform (North Sea): napthalenes, PAHs (2–3 rings and 4–6 rings), phenols	Lofthus et al. 2018)
Achromobacter sp. HZ01	Crude oil-contaminated seawater near the Mabianzhou Island, Daya Bay, Huizhou, China	Halotolerant bacterium	3%	Diesel oil, *n*-alkanes, anthracene, phenanthrene, and pyrene	Deng et al. (2014)
Arhodomonas sp. strain Seminole	Enriched microbial consortium (Nicholson & Fathepure, 2004)	Halophilic bacterium	14.6%	Benzene, toluene, phenol, 4-hydroxybenzoate, protochatechute, and phenylacetate	Dalvi et al. (2014)

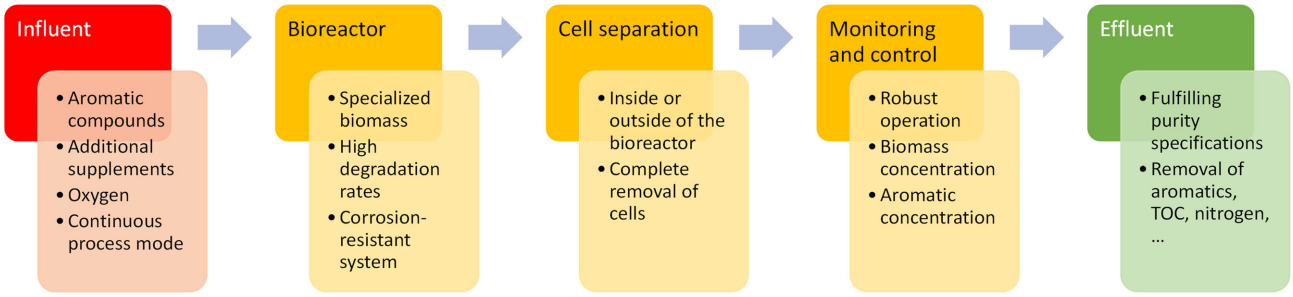

Fig. 2. Overview of process requirements for the implementation of halophilic bioremediation in industry. Red box: influent wastewater stream containing contaminants and additional supplements. Yellow boxes: bioreactor system containing biomass to degrade contaminants in the wastewater. Cell suspension is separated into cell-free effluent and broth remaining in the bioreactor. Cell separation can take place inside or outside the reactor. Control loop for a robust process control. Green box: treated wastewater stream within purity specifications.

general, the microbial system to be used in bioremediation processes can be chosen either from a microbial consortia originating from saline habitats or natural halophilic isolates from a strain collection [e.g., DSMZ (Deutsche Sammlung für Mikroorganismen und Zellkulturen)].

In addition to the operation mode of a bioremediation process and the used microbial system, also a process monitoring strategy contributes to a high process performance. Developing a monitoring strategy for wastewater treatment processes raises several questions. Those questions highly depend on the process and the process requirements. Therefore, a general statement for the need and the exact approach of process monitoring tools is impossible. Nevertheless, process monitoring is of utmost importance to guarantee the quality of the treated wastewater. Thus, it is important to first decide what parameters have to be measured. More precisely, it has to be decided if specific analyte concentrations (e.g., aniline concentration in the effluent, biomass concentration, etc.) are needed or if more unspecific parameters like the total organic carbon (TOC), the biological oxygen demand (BOD), or the chemical oxygen demand (COD) are sufficient (Bourgeois et al., 2001; Zhang & Li, 2009). The next questions would be how frequently data of the parameters are needed and what the most suitable measurement method is. Is it sufficient to take effluent samples once per day or is it necessary to obtain real-time data of the parameter? If only low numbers of data points per day are required, methods like HPLC, spectrometric methods or enzymatic essays might be used, as they offer high accuracy and well established protocols. However, if a permanent monitoring of the wastewater effluent is necessary, online hard or soft sensors offer data acquisition in real-time. Finally, the functionality of the monitoring of saline wastewater treatment processes is the applicability of the methods to high salt concentrations. Does the salt matrix disturb the method? Does dilution increase the measurement insecurity? Does the sensor corrode in the saline environments?

Wastewater Treatment with Halophilic Mixed Cultures

Using a microbial consortia from saline origins for treating saline wastewater increases the chances of accumulating species adapted to the environment and able to degrade the occurring contaminants. Such a strategy would finally allow to enrich special microorganisms able for the degradation of contaminants in the specific wastewater. In the following section, we summarized existing works using halophilic mixed cultures to treat aromatics-containing wastewater (e.g., phenol, etc.).

For instance, activated sludge from sea mud was used to degrade phenol containing wastewater mixed with seawater (Tan et al., 2017b). The cultivation was performed in an aerated 30 l bioreactor in a discontinuous way, as supernatant in the bioreactor was replaced by fresh medium once per day. The medium contained fresh nutrients like glucose (0.21 g l^{-1}), starch (0.1689 g l^{-1}) and in smaller amounts NH_4Cl and K_2PO_4. The salinity of the phenol-containing seawater–wastewater mixture was increased in the influent from 3.7% to 5.7%. The activated sludge was characterized, and three halophilic species (*Oceanomonas* sp., *Arthrobacter* sp., *Vibrio* sp.) could be identified in the activated sludge. All three species showed sufficient phenol removal capacities as over 80% of initial phenol concentration could be removed in batch experiments within 48 hr. The performance in the bioreactor experiments was similar as COD removal was always between 70% and 85%. Also, the phenol concentrations in the effluent never exceeded 40 mg l^{-1} (influent concentrations: 220–1 100 mg l^{-1}). Nevertheless, at the beginning of every new seawater–wastewater mixture, the performance of phenol and COD degradation decreased, probably due to an acclimatization phase. From this study we can learn, that activated sludge enriched with halophilic microorganisms is able to degrade aromatic compounds in slightly saline wastewater with an efficiency of over 70%. As a result, the process could be developed further by investigating the influence of the three halophilic strains found in the sea mud on the aromatic degradation. Furthermore, the operation mode should be changed into a continuous mode and an active cell retention system, like membrane-based technologies, should be used to reduce bioreactor volumes for following scale-up studies.

In addition to aerobic treatment processes also anaerobic treatment of saline wastewater containing aromatics has been described (Wang et al., 2017). Three upflow anaerobic sludge blanket (UASB) reactors (two saline reactors, one nonsaline reactor) with 3.5 l working volumes were inoculated with activated sludge from a municipal wastewater treatment plant in Hefei (China) and compared according to phenol degradation efficiency (Wang et al., 2017). It was shown, that phenol removal decreased at higher phenol (2 g l^{-1}) and Na^+ concentrations (20 gNa^+ l^{-1}). Synthetic wastewater with phenol, catechol, resorcinol and hydroquinone as aromatic contaminants was used at Na^+ concentrations of 10 and 20 g l^{-1}. The HRT was set to 48 hr, while the upflow velocity was 1.3 m h^{-1}. COD and phenol removal efficiencies always reached values above 95% and 98%, respectively, at influent concentrations of 100–500 mg l^{-1} phenol and 10 gNa^+ l^{-1}. However, the used microbial system suffered from a phenol shock when the influent concentration of phenol was increased to 1000 mg

Table 2. Bioprocesses for the Treatment of Saline Wastewater, Containing Aromatic Contaminants. Processes are Compared According to Their Process Parameters Like Reactor Volume, Operation Mode, HTR, and Removal Efficiency. Additionally, Information is Given About the Salt Content of the Wastewater, Additional Nutrients, and the Used Microorganisms

Wastewater source	Reactor volume	Operation mode	Salt concentration	Contaminants	Removal efficiency (%)	Nutrient supplementation	Microorganism	Reference
Synthetic wastewater	10 l	Continuous (HTR = 4.7–5.7 hr), immobilized cells	0–6.5%	Phenol	99	Salts, oxygen	*Oceanomonas* sp.	Tan et al. (2017a)
Synthetic wastewater	20 l	Continuous (HTR = 5 days), membrane-based cell retention	0–1.5%	Phenylphenol, acetanilide, bisphenol A, etc.	20–60	–	Activaled slduge (anaerobic)	Song et al. (2016)
Synthetic wastewater	625 l	Sequencing batch, immobilized cells	5%	Phenol	100	Salts, oxygen	*Camomonas* sp. JB	Jiang et al. (2016b)
Industrial wastewater	1 l	Continuous (HTR = 10 hr), membrane-based cell retention	15%	Formate, aniline, phenol, and MDA	100	Salts, glycerol, oxygen	*Haloferax mediterranei*	Mainka et al. (2019)
Industrial wastewater	30 l	Sequencing batch	3.7–5.7%	Phenol	80	Ammonium, phosphate, starch, glucose, oxygen	Activated sludge from sea mud	Tan et al. (2017b)
Synthetic wastewater	250 ml	Continuous (HTR = 3–14 hr), membrane based cell retention	Controlled to 50 mS (with NaCl)	Phenol	100	Salts and trace elements	*Pseudomonas putida* ATC 11172	Praveen and Loh (2016), Praveen et al. (2015)
Synthetic wastewater	1 l	Sequencing batch	14%	Phenol	99.5	Ammonia, phosphorus, iron, inorganic salts	Halophilic mixed culture	Woolard and Irvine (1995)
Synthetic wastewater	3.5 l	Continuous (HTR = 48 hr)	10–20 $gNa^+ l^{-1}$ = 2.4–4.8% NaCl	Phenol, catechol	95–98	Macro- and micronutrients	Activated sludge (anaerobic)	Wang et al. (2017)

l^{-1}. Thus, the phenol removal efficiencies were reduced to only 70–80%. After 10–20 days the removal efficiency was increased again to 95–97%. This study showed, that an anaerobic process for the removal of aromatics using activated sludge is possible. Nevertheless, this process could only be operated at lower salt concentrations. Poor process performance for anaerobic systems was also reported in other studies, which showed the inhibition of the anaerobic microbial community due to high salinities (Ng et al., 2014; Svojitka et al., 2017).

Similarly, another report showed comparable result by using activated sludge to treat saline wastewater in a 20 l anaerobic MBR (anMBR) system (Song et al., 2016). In this study, activated sludge from the Wollogong Wastewater Treatment Plant (Australia) was used to degrade 33 trace organic compounds (trOC) belonging to the four key groups of contaminants (i.e., pharmaceuticals, personal care products, industrial chemicals, and pesticides), among them are also aromatic compounds like phenylphenol and bisphenol A. The salinity in the reactor was increased from 0% to 1.5% with a rate of 0.1% per day. Only at NaCl concentrations below 1%, COD removal efficiency was 98%. When salt concentrations exceeded levels higher than 1%, COD removal efficiency decreased to 80%.

Wastewater Treatment with Halophilic Mono Cultures

Besides using halophilic microbial communities for the remediation of industrial waste streams, it is also possible to select halophilic strains according to their ability to degrade the present aromatic contaminants. Thus, a bioprocess for saline wastewater treatment can be tailored according to the existing conditions.

For example, an *Oceanomonas* sp. strain was used as inoculum for a biological contact oxidation reactor (BCOR), in order to degrade phenol in slightly saline wastewater [0–6.5% (wt/vol) NaCl] (Tan et al., 2017a). For cell retention, the cells were immobilized to polypropylene fibers in a 10 l reactor filled with seawater. The medium was additionally supplemented with ammonium, phosphate, calcium, magnesium and iron salts. Moreover, no additional carbon source was necessary. Because *Oceanomonas* is strictly aerobic, the reactor was aerated and the dissolved oxygen concentration was controlled to a minimum of 2.5 mg l^{-1} O_2 at a temperature of 25°C. The immobilization allowed liquid feeding rates of 1.33–2.13 l h^{-1}, which results in HRTs of 4.7–7.5 hr. As a result, saline wastewater containing phenol concentrations up to 1 500 mg l^{-1} and 6.5% (wt/vol) NaCl was successfully treated with removal efficiencies of 99%. Nevertheless, only synthetic wastewater under laboratory conditions was used in this study. Therefore, the investigation of long-term performance using real industrial wastewater would be interesting. Moreover, as salt concentrations were low in this study (below 6.5% NaCl), co-contaminations with other slightly halophilic microorganisms are possible. To test the robustness of the present approach against contaminations, unsterile industrial wastewater could be used as cultivation medium.

At a smaller scale of 625 ml, a system was developed which used magnetically immobilized *Comamonas* sp. JB for the degradation of phenol, *o*-cresol, *m*-cresol, and *p*-cresol in a synthetic wastewater containing up to 5% NaCl (Jiang et al., 2016b). In parallel, two other reactors were inoculated with *Comamonas* sp. JB, one without immobilized cells and the other with nonmagnetically immobilized cells. The wastewater was replaced in the reactors every 12 hr, which corresponds to a sequencing batch mode. Compared to nonimmobilized cells and nonmagnetically immobilized cells, the magnetically immobilized cells showed higher removal rates. In contrast to nonimmobilized cells, the magnetically immobilized cells even achieved high phenol removal rates of over 70% at salinities above 3% NaCl. The process performance could also be improved, by including electrodes into the bioreactor system for electrical stimulation of the cells. With this integrated system, phenol removal rates of 100% could be achieved, due to an increase of phenol hydroxylase activity from 0.135 to 0.31 U mg^{-1}. Besides high removal efficiencies, this systems lacks several factors, which are important for the implementation into industrial process chains. First, the volume of less than one liter is low, which makes scale-up studies necessary. Second, the process was not operated in a continuous mode. Therefore a strategy handling high volume waste streams has to be developed.

In contrast, using membranes is a common approach for retaining cells in the bioreactor. We have reported such a membrane-based retention system for the degradation of phenol, aniline, 4,4′-methylenedianiline (MDA) and formate in high-saline MDA process water using the extreme halophilic archaeon *Haloferax mediterranei* (process scheme, Fig. 3) (Mainka et al., 2019). The reported system allows not only higher hydraulic dilution rates D for the process water ($D = 0.1$ h^{-1}, equals a HRT of 10 hr), but also monitoring and control of the biomass concentration in the reactor through a feedforward control strategy. The feedforward strategy controls the biomass concentration by adjusting the two parameters (1) glycerol concentration in the feed and (2) the ratio R of cell-free permeate flow F_H and the feed flow F_F (Fig. 3). The removal efficiencies for the aromatic compounds were up to 100% for all of the four organic contaminants. The additional carbon source glycerol was used as growth substrate in order to maintain high biomass concentrations and enable high degradation rates of the contaminants. Glycerol is a cheap and widely available carbon source. However, the utilization of other substrates such as acetate or lactate from waste sources is also possible (Erian et al., 2018; Pflügl et al., 2014; Russmayer et al., 2019). In terms of scale-up, we reported the construction and utilization of a 21 l corrosion-resistant bubble column bioreactor (BCR) equipped with a membrane-based cell retention setup (Mahler et al., 2018). While studying the BCR, we showed the successful cultivation of halophilic cultures in pilot-scale. Compared to the lab-scale bioreactor, the process parameters and yields were similar in the BCR, even though oxygen supply and mixing was provided only through bubbles and not by stirring. As a result, such a system reduces the operational costs at larger scales and makes the setup easier to maintain. As the experiments were performed under laboratory conditions, it would be interesting to investigate the effect of changing wastewater conditions. Therefore, a long-term cultivation would be interesting, where the composition of the wastewater is altered, in a way that simulates conditions found in a real industrial environment.

Another study used a forward osmotic hollow fiber filtration unit for cell retention in a continuous bioprocess for the degradation of phenol (influent concentration 0.6–2.5 g l^{-1}) with halotolerant *Pseudomonas putida* ATC 11172 (Praveen et al., 2015; Praveen & Loh, 2016). Additionally, an extractant impregnated membrane (EIM) was used as a partitioning phase to prevent inhibitory phenol concentrations in the aqueous phase by temporarily removal of phenol (Praveen & Loh, 2016). With this system, removal efficiencies of 100% were reached at HRT of 3–14 hr. However, the used volume of 250 ml was low and a scale-up to reactor-size should be carried out, in order to investigate potential scale-up effects. Moreover, problems with membrane fouling

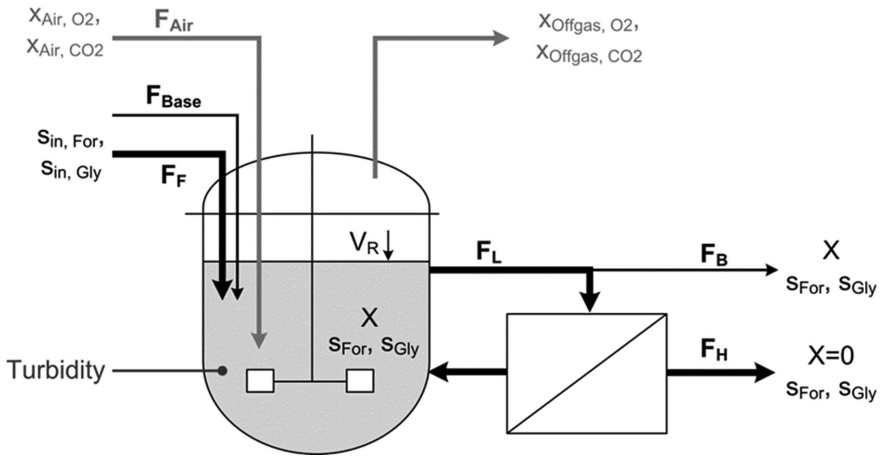

Fig. 3. Scheme of the cell retention setup. A constant feed (F_F) supplies the cells with fresh substrate and media components. Base (F_{Base}) is added to hold the pH on a constant level of 7.0. A pump continuously circulates the cell suspension as loop flow (F_L) through the membrane module to separate cell-free harvest (F_H). Bleed flow (F_B) is continuously removed to eliminate cells and sustain steady state conditions. To guarantee a constant reactor volume (V_R) flows for Feed, Base, Harvest, and Bleed have to meet the following equation: $F_F + F_{Base} = F_H + F_B$. Biomass is monitored using a turbidity probe and a soft sensor that is driven by measurements of off-gas composition (Mainka et al., 2019).

occurred, which were caused by proteins attached to the membrane (Praveen & Loh, 2016).

In summary, state-of-the-art bioprocesses for the treatment of saline wastewater, containing aromatic contaminants, deliver good performances according to aromatic degradation efficiencies. Various biological systems were used and different cell retention system could be applied successfully. A comparison of the presented processes can be found in Table 2.

Monitoring Approaches for Saline Wastewater Processes

The monitoring of critical process parameters depends on requirements of the wastewater treatment process. Parameters which can be monitored, are physical (temperature, pressure, etc.), chemical (e.g., analyte concentration, TOC or COD), or biological (e.g., biomass concentration, etc.) characteristics (Biechele et al., 2015; Bourgeois et al., 2001; Vanrolleghem & Lee, 2003). In general, a process monitoring strategy should help to obtain stable and reproducible bioprocesses which meet the desired quality criteria for the treated wastewater (Biechele et al., 2015). Although process monitoring is applied to saline and nonsaline processes, saline conditions and an industrial environment cause challenges to the design and usability of sensors and measurement methods. First of all, the high salt concentrations require materials to withstand the corrosive conditions. Moreover, measurement methods have to overcome interference problems with the salt matrix.

In the case of bioremediation of aromatics containing saline wastewater, the monitoring of biomass concentration, specific aromatic concentrations, and/or total organic concentrations (TOC, COD, or BOD) are potential approaches and are discussed in the following section.

Biomass monitoring

The determination of biomass related parameters, like absolute biomass concentration or specific growth rate, is an important topic for bioprocess development, as cells are the biocatalyst (Tamburini et al., 2003). For instance, in production bioprocesses often a correlation between specific productivity and specific growth rate can be found, thus monitoring the growth rate can help to maintain optimal production parameters (Looser et al., 2015, 2017; Zhang et al., 2005). Furthermore, the biomass concentration in wastewater treatment processes is a crucial parameter, not only important for degradation efficiency, but also as the disposal of waste biomass contributes to the operational costs of a wastewater treatment plant (Canales et al., 1994; Kayranli & Ugurlu, 2011; Wei et al., 2003). Methods published for the determination and estimation of biomass range from cell count methods on agar plats, to indirect gravimetric determination of cell dry weight to online methods like turbidity measurements and soft sensor estimations (Finn et al., 2006; Biechele et al., 2015; Kager et al., 2017; Mainka et al., 2019).

If a dependency between biomass concentration and process performance of bioremediation processes exists, the biomass concentration can be used as control parameter for process optimization. Control parameters need to be available in real-time, thus, data of the biomass concentration have to generated permanently. Thus, microbiological methods like cell count on agar plates or offline methods, such as gravimetric determinations or optical density (OD) measurements, are not suitable. Potential alternatives include online methods such as the measurement of turbidity with a turbidity probe, near infrared spectroscopy (NIRS), dielectric spectroscopy or mass balancing based on offgas data (Finn et al., 2006; Kiviharju et al., 2008; Mainka et al., 2019; Münzberg et al., 2017).

Turbidity probes are based on different optical measurement principles, such as transmission, reflection and transflection measurement (Münzberg et al., 2017). Probes based on reflection or backscattering are often preferred for in-line measurements, because these principles do not depend on the optical path length and are more resistant to probe fouling (Münzberg et al., 2017). Wavelengths for turbidity measurements in bioprocesses were reported at 600 nm or (860 ± 30) nm (Vojinović et al., 2006; Münzberg et al., 2017). However at 860 nm might be beneficial when used in colored systems, as the light absorbance is lower (Münzberg et al., 2017). For using turbidity probes to determine biomass concentration in bioreactors, an *a priori* calibration with offline biomass data, like the cell dry weight, is needed. Also other process parameters, like agitation or aeration, can influence the turbidity signal alter it in terms of turbidity-biomass-correlation (Gregory & Thornhill, 1997). Limitations using turbidity probes are occurring due to nonlinear correlations of turbidity and biomass

concentration (Gregory & Thornhill, 1997; Kiviharju et al., 2008; Münzberg et al., 2017). Those effects, however, can be avoided by special probe models where samples are passed into a degassed measurement chamber (Kiviharju et al., 2008; Olsson & Nielsen, 1997). Also, other suspended solids in the medium (e.g., dirt particles) can disturb the turbidity measurement and interfere with the biomass (Vojinović et al., 2006). Therefore, industrial wastewater should be particle free, if accurate turbidity data are needed.

A more sophisticated method for the determination of biomass concentration in cell broth would be the use of NIRS-probes. For biomass determination with NIRS-probes, a spectrometer scans wavelength ranges from 400 to 2 500 nm (Finn et al., 2006; Tamburini et al., 2003). Information about biomass concentration can, for example, be found in regions from 910 to 930 or 2260 to 2270 nm (Cervera et al., 2009; Finn et al., 2006). In order to extract biomass concentration data, it is common to pretreat the absorbance spectra, for example, calculating the second derivative, and build a chemometric model to estimate biomass concentrations (Cervera et al., 2009; Ge et al., 1994). Nevertheless, the NIRS measurements were found to be affected by the aeration rate, the agitation speed, and also the temperature, which has to be considered when building a biomass model (Ge et al., 1994; Scarff et al., 2006). One advantage of NIRS measurement for biomass estimation is the possibility of online *ex situ* measurements, realized by either flow-through cells or fiber optic process behind a glass wall (Cervera et al., 2009; Ge et al., 1994). Thus, no probes have to be inserted into saline medium containing bioreactors. Moreover, NIRS measurements were also reported for the determination of substrates and products in fermentation broths. Among those molecules, carbon sources such as glucose, ethanol, glycerol, or lactose were determined, but also ammonia or phosphate were reported to be measured (Cervera et al., 2009; Finn et al., 2006). The monitoring of such metabolites or by-products is important, when the total organic content in the wastewater should be removed. Also the accumulation of ammonia should be avoided in some cases, for example, for chlor-alkali electrolysis, thus online monitoring of ammonia could be beneficial for process efficiency and security (Brinkmann et al., 2014).

Alternatively, soft sensors offer the possibility to generate information about biomass concentration by using different process variables. Those variables are measured by several probes and are processed by a software-based algorithm to estimate biomass concentration (Biechele et al., 2015; Kiviharju et al., 2008). The underlying principles for soft sensors are either model-driven or data-driven. Whereas model-driven soft sensors are based on mass and energy balances, data-driven soft sensors use historical process data for online estimation (Biechele et al., 2015). Process variables which are commonly used for biomass soft sensors are offgas values (CO_2, O_2), base consumption or dissolved oxygen (pO_2) (Biechele et al., 2015; Kager et al., 2018; Kiviharju et al., 2008; Sundström & Enfors, 2008). In comparison to biomass estimation with hard sensors, also soft sensors need offline reference values of biomass concentration to develop and calibrate the underlying models. Nevertheless, soft sensors are able to extend the usability of probes and sensors commonly used for bioprocess and which are often already implemented in existing processes. Therefore, besides the software tools, no investments for additional devices and probes are necessary. Especially for the harsh conditions at treatment plants for industrial saline wastewater, avoiding sensitive probes is beneficial. Thus, soft sensors for the determination of biomass concentration in saline bioprocesses offer great potential for future process development. This potential could already be shown, as we have recently established a soft sensor for real-time estimation of biomass concentration based on offgas measurements and substrate concentrations in wastewater feeds (Mainka et al., 2019). The soft sensor was developed for the extremely halophilic archaeon *H. mediterranei*, used for continuously treating an industrial saline wastewater, containing various aromatic compounds.

Monitoring aromatic concentrations

The monitoring of concentrations of specific aromatic compounds (like aniline or phenol) can be useful, if, for example, thresholds of these compounds in the effluents have to be met. When the wastewater contains more than one aromatic compound, HPLC methods offer the possibility to measure several compounds at once and, depending on the method, with high accuracy. However, samples have to be taken, either manually or automatically, prepared and the HPLC measurement time considered (e.g., 30 min). HPLC measurements are time consuming and data are only generated discontinuously. Nevertheless, for purposes, which are uncritical in terms of time like for the comparison of concentrations of wastewater batches with environmental specifications, HPLC methods are suitable and sufficient. In contrast, if monitoring data are needed for control purposes, data have to be generated in real-time, which can, for example, be realized by sensors. In literature, several (bio)sensors and methods for the detection and quantification of aromatic compounds are described (Buerck et al., 2001; Gutés et al., 2005; Korkut et al., 2016; Mu, 2006; Rahemi et al., 2020; Zhang & Li, 2009).

Online measurement methods for aromatic compounds in wastewater treatment processes need to be highly sensitive and be able to detect also low concentrations. This is true, as the purpose of wastewater treatment plants is the removal of contaminations, and thus, contaminant concentrations should be close to zero. Therefore, the limit of detection of a potential sensor need to be even lower as the threshold, if the concentration of contaminants in a wastewater have to be decreased below a certain threshold. Otherwise, additional offline measurements are necessary to check the removal efficiency of the process.

As mentioned above (section "Monitoring Approaches for Saline Wastewater Processes"), NIRS measurements are able to determine carbon sources like glucose or glycerol, studies were also published using NIRS to measure aromatic compounds (Buerck et al., 2001; Mattioda et al., 2005). However, problems occur when aromatics are dissolved in aqueous solutions, as the OH-group of water absorbs strongly at 1450 and 1940 nm, and thus, overlaps with the CH peaks of aromatics (1600–1900 nm) (Buerck et al., 2001; Bürck et al., 1992). Therefore, an NIR–EFA sensor (EFA = evanescent field absorption) was developed, which enables the extraction and enrichment of apolar hydrocarbons into the cladding of quartz glass fiber sensor, according to the Nernst distribution law (Buerck et al., 2001). Prior to determining aromatic concentrations, spectra of pure aromatics were measured and calibration models were generated using partial least squares (PLS) method. As this sensor could be promising for monitoring aromatic compounds at wastewater treatment processes, still several things have to be taken into account. The first one is, that concentrations in a mixture of several different aromatics cannot be determined, but a cumulative concentration parameter could be calculated by including data from a filter photometer. Moreover, the response time of the sensor to concentration changes can range from 2 to more than 20 min, depending on the aromatic species. Also, the sensor has to be implemented inside the bioreactor or a

Table 3. Sensors for the Determination of Aromatic Compounds

Aromatic compound	Measurement principle	Probe	Linear conc. range	Reference
Catechol	Enzyme-based	Glassy carbon electrode	0.036–2.5 µM	Maleki et al. (2017)
Catechol	Electrochemical oxidation	Three-electrode system (working electrode: platinum or copolymer, counter electrode: platinum, reference electrode: calomel (SCE)	5–80 µM	Mu (2006)
Catechol (cat), hydroquinone (hyd)	Electrochemical detection	Glassy carbon electrode	cat: 0.5–40 µM hyd: 0.13–56.6 µM	Nazari et al. (2018)
Hydroquinone, 4-aminophenol	Enzyme-based	Electrode modified with TiO_2	4-Aminophenol: 0.05–2 µM	Rahemi et al. (2020)
3-Methoxyaniline	Electrochemical detection	Glassy carbon electrode	0.1 nM–0.1 mM	Rahman et al. (2019)
Hydroquinone, catechol, resorcinol (res)	Electrochemical oxidation	Glassy carbon electrode	hyd: 1–200 µM cat: 4–200 µM res: 3–400 µM	Wei et al. (2014)

bypass construction, as it has to be in contact with the medium. Therefore, stability and performance tests for saline wastewater are still necessary. Moreover, potential interferences with cells have to be investigated.

In contrast to measure cumulative concentration parameters of aromatics in general, as happened with NIRS measurements, sensors detecting only specific aromatics and being able to differentiate between these compounds have also been reported (Maleki et al., 2017; Mu, 2006; Nazari et al., 2018; Rahemi et al., 2020; Rahman et al., 2019; Wei et al., 2014; Zhang & Li, 2009). The principles used for the measurement range from optical based systems over enzymatic biosensors to electrochemical detectors. Aromatic compounds which were reported to be measured with sensors are, for example, aniline, catechol, hydroquinone, resorcinol, or 3-methoxyaniline (Maleki et al., 2017; Mu, 2006; Nazari et al., 2018; Rahemi et al., 2020; Rahman et al., 2019; Wei et al., 2014; Zhang & Li, 2009). Such systems offer the possibility of measuring specific compounds and are suitable for processes where only a small amount of different aromatics are present. However, at microbial degradation processes of aromatics or any other organic contamination, potential intermediates of the degradation pathways could accumulate. Thus, this accumulation could remain undetected if the sensors can measure the original contaminant but not possible intermediates. Nevertheless, sensors able to measure specific aromatic compounds could be used for quantifying contaminant concentrations in wastewater feeds. Those concentration data could consequently be used for feedforward control strategies, where the bioremediation process is controlled based on composition information of the influent. An overview of the aromatic (bio)sensors and their measurement principles is given in Table 3.

Monitoring TOC

Monitoring organics concentration in wastewater treatment plants is commonly carried out by measuring cumulative concentration parameters like TOC, COD, or BOD (Assmann et al., 2017; Bourgeois et al., 2001; Vanrolleghem & Lee, 2003). Those parameters are all measured in different ways. Therefore, the obtained results offer different possibilities to be interpreted. The standard BOD_5 method measures the consumption of dissolved oxygen of a microbial system during 5 days in a test sample (Vanrolleghem & Lee, 2003). The long timespan makes this measurement method obviously not suitable for real-time monitoring. However, BOD methods were developed which deliver results with 5–10 min (Bourgeois et al., 2001; Vanrolleghem & Lee, 2003). The COD analysis gives information about the oxidation ability of organic compounds in wastewater samples, by using strong oxidizing agents (e.g., dichromate, $Cr_2O_7^{2-}$) under acidic conditions (Bourgeois et al., 2001; Kayaalp et al., 2010; Vanrolleghem & Lee, 2003). Drawbacks of COD analysis are that biologically inert organic compounds cannot be differentiated from biodegradable content and the generation of hazardous waste (e.g., chromium) (Bourgeois et al., 2001; Guan et al., 2019). Simultaneously to organic substances, chloride ions in saline samples react with chromate ions and therefore increase measurement errors (Kayaalp et al., 2010). TOC analysis only measures the concentration of organic compounds, and is considered to be the most 'true' index of the total organic contamination in wastewater (Bourgeois et al., 2001). In general, the offline TOC analysis is based on the principle to convert organic matter into CO_2, either by using a catalyst at high temperatures (650–900°C) or by oxidizing organics with UV light and persulfate reagent, subsequently measuring the amount of formed CO_2 gas (Assmann et al., 2017; Bourgeois et al., 2001; Vanrolleghem & Lee, 2003). In both cases, inorganic carbon needs to be eliminated prior to measure TOC. Also, both methods have some drawbacks. The high temperature method is sensitive to salts, as salts could produce a melt on the catalytic surface. When using the persulfate method, incomplete oxidation could occur, if the pH is too low or the wastewater sample is too turbid (Vanrolleghem & Lee, 2003). Moreover, TOC measurements cannot give information about the biodegradability of wastewater samples (Vanrolleghem & Lee, 2003).

For the real-time measurement of TOC or COD-values, online spectrometers can be used. Those probes use the principle of many organic substances to absorb light at specific wavelengths (e.g., 254, 350, or 465 nm) (Guan et al., 2019; Mills & Fones, 2012). Online spectrometers can detect organic matter in concentration ranges down to ppb, and are widely used in wastewater treatment plants for monitoring and control purposes (Van Den Broeke et al., 2006). As the use of online spectrometers only delivers indirect measurement values, a calibration procedure with offline values is required (Langergraber et al., 2003). However, suspended solids influence spectroscopic measurements, due to light scattering and shading. Therefore,

compensations are necessary to obtain accurate measurements (Van Den Broeke et al., 2006).

Conclusion and Outlook

In this review, we showed the potential to use halophilic microorganisms for biological treatment of industrial saline wastewater contaminated with aromatic compounds. In that course, we highlighted the need for treating aromatic compounds-containing wastewater, suggested requirements for industrial wastewater processes, gave examples of halophilic bioremediation processes reported in the literature and discussed potential aspects of further research and development topics.

Studies dealing with the degradation of aromatics in saline wastewater showed the proof of principle of using halophiles, as high removal efficiencies for the tested contaminants were reached. Also, reports showed that continuous bioprocesses in lab-scale bioreactor systems including cell retention units are working successfully.

Nevertheless, for the implementation of halophilic bioremediation processes into industrial production environments, several problems still have to be solved or investigated. Most studies used lab scale systems with a volume well below 20 l. One reason might be the technical effort of large-scale experiments, but also investment costs play an important role. Therefore, experiments in larger scales are necessary to investigate potential scale-up effects. For that reason, collaborations with industrial project partners could intensify the research of large-scale bioremediation processes for saline wastewater. In particular, the usage of cell retention system in large scales should be examined considering long-term process performance. In addition, wastewater used in scientific studies were mostly synthetic wastewater with only little number of tested contaminants. For addressing the complexity of most industrial wastewater, more studies should be performed using real industrial waste streams. To do so, industrial collaborations would be again a suitable way to take the research a step further.

The industrial implementation of halophilic bioremediation processes also has to solve issues on the level of process technologies. For instance, corrosion-resistant bioreactor systems are needed when saline wastewater are used, in order to reduce technical problems like leakages in pipes, pumps, or valves. Furthermore, a complete halophilic bioremediation process necessitates a suitable process monitoring system for critical process parameters like biomass concentration, TOC levels or contaminant concentrations. Such a system could consist of both, hard and soft sensors. A complete monitoring and control strategy for a bioremediation process of aromatics containing wastewater should consist of different sensor systems. Online sensors for the measurement of aromatic concentrations in the feed, biomass estimation in the bioreactor based on soft sensors and the measurement of an organic sum parameter (e.g., TOC) in the effluent would enable monitoring the main process parameters. Those parameters could then be used as input parameters for a control strategy, which would help to improve and maintain process performance over longer operation periods. Although hard (e.g., TOC measurement) and soft sensors (e.g., for biomass estimation) are already established, more effort is required for further development of these systems. Information gathered by specialized sensors can help to improve process understanding, which could result in higher process efficiency.

Future research in the field of bioremediation of saline wastewater should also pay attention to environmental regulations concerning maximum levels of contaminants allowed to be released into the environment. As regulations differ between countries and also depend on the industrial sectors, information about maximum TOC or COD levels allowed for release should already be included during the process design phase.

In conclusion, halophilic organisms are promising catalysts for purification of saline, industrial wastewater. Combined with systematic bioprocess development which allows to establish efficient microbial degradation systems which meet industrial specifications, the time is ready for the application of halophilic cultures in large scale industrial systems.

Author Contributions

Conceptualization: Thomas Mainka, Stefan Pflügl; writing—original draft preparation: Thomas Mainka, David Weirathmüller; writing—review and editing: Stefan Pflügl, Christoph Herwig; visualization: Thomas Mainka; funding acquisition: Christoph Herwig; and supervision: Stefan Pflügl, Christoph Herwig.

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Conflict of Interest

The authors declare no conflict of interest.

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