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Influence of salinity on microorganisms in activated sludge processes: A review

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ABSTRACT

The use of seawater and production of some chemicals produce a lot of saline wastewater. There are many treatment technologies for treating saline wastewater, such as physical, chemical and biological treatment. Biological treatment processes, especially the activated sludge processes, show advantages over other processes due to its cost-effectiveness and avoiding of secondary pollution, and many researches have been performed in this field. In this paper, the progresses of researches about the effect of salinity on activated sludge and its microorganisms were reviewed which included the effect of salinity on sludge structure and properties, microbial species and biomass, microbial physiological changes, and microbial molecules and cells. The mechanisms of the effect of salinity on sludge and the microbes were also summarized. Additionally, the feasibility of treatment of saline wastewater by using the acclimated salt tolerant activated sludge was evaluated. Future research needs were also proposed which include the study on the mechanisms of salt stress on activated sludge microorganisms at cellular and molecular levels and enzyme activity, screening and acclimation of salt-tolerant bacteria including halophiles, and optimizing of process parameters for saline wastewater treatment.

1. Introduction

In order to alleviate the shortage of freshwater resource, seawater has been directly used over the years in many countries. The heavy use of seawater causes the discharge of high salinity wastewater, which results in the salt concentration of wastewater up to 15–45 g/L, while the salt concentration of seawater is 25–35 g/L usually (Sun et al., 2010b). In addition, some industries including petroleum, printing and dyeing, paper, chemical, and pesticide industries discharge a large amount of wastewater which contains highly inorganic saline, recalcitrant or toxic organic pollutants, and the these wastewaters can have salinities ranging from 3.5 wt% to 20 wt% (Woolard and Irvine, 1995; Lefebvre et al., 2005). In wastewater treatment, saline wastewaters are defined as salinity at least 1 wt% and hypersaline wastewaters generally contain more than 3.5 wt% salinity (Shi et al., 2012). In recent decades, the inflow of saline and hypersaline wastewater to treatment plants has increased considerably, represents as much as 5% of the worldwide wastewater treatment streams (Lefebvre et al., 2007).

At present, the treatment methods of chemical and physical technology used in the disposal of salinity wastewater are adsorption method, membrane separation, ion exchange or electrodialysis, etc (Fan et al., 2011; Neilly et al., 2009; Dincer and Kargi, 2000). However, these treatment methods always lead to some problems such as high cost, secondary pollution, and as a consequence, these technologies are only applied in certain conditions. By contrast, biological treatments especially activated sludge process are preferred, because of their better economic performance, moreover they can avoid secondary pollution (Boopathy et al., 2007; Shi et al., 2014). Some researches focus on the effect of salt-tolerant sludge on saline wastewater treatment process and removal efficiency, such as two stage biological contact oxidation process, sequencing batch reactor (SBR), biofilm sequencing batch reactor (BSBR), upflow anaerobic sludge blanket (UASB), anaerobic

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filter, anaerobic aerobic process, etc (Gao et al., 2013; Ferrer-Polonio et al., 2016b; Jiang et al., 2016; Wang et al., 2005; Lu et al., 2011). Predecessors researched the nitrogen removal efficiency and improved methods in the condition of salinity from different aspects, such as saline concentration, operational conditions, removal rate of organic pollutant and nitrogen removal efficiency (Belkin et al., 2007). Due to the differences of process and wastewater quality, there are various opinions on the treatment process of saline wastewater by using activated sludge especially the treatment effect, some researchers found that the removal efficiency of wastewater was decreased in saline wastewater but other researchers studied that the removal efficiency of saline wastewater was not affected (Aslan and Simsek, 2012; Zhao et al., 2013). Besides, the microorganisms of activated sludge were acclimated into salt tolerant microorganisms by increasing influent salinity gradually and then the microorganisms had good capacity of nutrition removal at high saline conditions (Rene et al., 2008). Researches showed that activated sludge regains the ability of nitrogen removal by adding the cultivated microbes into traditional activated sludge process (Kulkarni, 2013; Figueroa et al., 2008). Base on so many researches about the treatment process and removal efficiency of saline wastewater, there are many related articles which have summarized activated sludge treatment, organic removal and biological nitrogen removal performance (Chowdhury et al., 2010; Lofrano et al., 2013).

Until now, there are many researches about the influences of salinity on the activated sludge microorganisms. For instance, a certain concentration of inorganic salt (such as Cl^-, SO_4^{2-}, PO_4^{3-}, Mg^{2+}, Ca^{2+}) plays an important role in the growth of microbes, such as maintaining membrane equilibrium, adjusting osmotic pressure, promoting enzyme activity, etc (Gatti et al., 2010). However, when the concentration of inorganic salt is excessively high (>2 wt% salinity), the removal of nitrogen in wastewater will be influenced. Because high salinity can raise osmotic pressure, separate microbial cell plasma, at the same time it can reduce the metabolic enzyme activity, destroy the structure of microbial enzymes and inhibit the growth of microorganisms (Hong et al., 2013). Meanwhile, high salinity can cause activated sludge bulking and loss, and the performances of biological treatment process were badly affected (Wilson et al., 2013). Quick salinity increasing of influent wastewater can lead to the release of intra-cellular constitutes and also the increase of soluble COD (Lefebvre and Moletta, 2006). However, there is only a little literature review in this aspect (Wang et al., 2011).

From the macroscopic and microcosmic angle, this paper summarizes the influence of salinity on the sludge structure and properties, microbial community, physiological effects and internal mechanism of activated sludge, and reviews recent research progresses about microorganisms of activated sludge in the saline wastewater treatment. And this paper can provide some ideas for further studies about impact of salinity on activated sludge treatment.

2. Influences of salt on sludge property and community

2.1. Influences of salt on sludge structure and settlement

Nowdays, the research conclusions about the influence of inorganic salt on the sludge structure and settling property are inconsistent. Some studies suggest that increasing salinity is beneficial to sludge settlement (Moon et al., 2003; Pronk et al., 2014). Because high salt inhibits the rapid growth of the filamentous bacteria, and the size of sludge flocs becomes small and the flocs close together, so that the sludge settling property is increased. For instance, Moussa at al. (2006) studied that the sludge volume index (SVI) gradually reduced and the sludge settling velocity increased when the salinity was increased from 0 to 40 g/L Cl^-. Besides, they found that big activated sludge granules, closed zoogloea structures, loose microbial flocs and a large number of filamentous bacteria existed in the activated sludge when the Cl^- concentration was 0 g/L. However when the Cl^- concentration was 40 g/L, the size of sludge folcs was very small and became extremely close, and filamentous bacteria were hardly seen by microscopy. In their studies, only the nitrifiers lived in the bioreactor as the Cl^- concentration was 40 g/L. In these researches, the use of optical microscopy and scanning electron microscopy (SEM) provides an easy and intuitive way to indicate the sludge morphological changes in salinity wastewater (Ng et al., 2005).

Tokuz and Eckenfelder (1979) studied the effect of sodium chloride and sodium sulfate on the performance of the activated sludge process, when NaCl concentration was less than 35 g/L, effluent suspended solid was less than 10 mg/L, the sludge settling property was not affected, and they found that the effect of sodium sulfate on the system was even less profound. Dahl et al. (1997) studied the biological treatment of high-salinity wastewater as the Cl^- concentration was 20 g/L, they found that the sludge settling property was not affected and the SVI value was 32 mL/g in the activated sludge process. Campos et al. (2002) used the activated sludge to treat high ammonia saline wastewater, they clarified that high salt concentrations (20 g Cl^-/L) did not have long-term effects on the physical properties of sludge, the SVI value was decreased from 42.4 to 11.4 mL/g at the treatment process. Bassin et al. (2012) researched the effects of different salt adaptation on the microbial activity and settling property of sludge in two sequencing batch reactors. And they observed that the SVI decreased with the increase of salinity in both reactors. In the SBR1 as NaCl concentration increased gradually from 5 to 20 g/L, the SVI decreased gradually from 110 to 70 mL/g. In the SBR2 as the NaCl concentrations increased from 10 to 20 g/L, the SVI dropped from 110 to 60 mL/g, a slightly higher as compared to that observed in the SBR1. And also their results showed that a gradual increase in NaCl concentration had a positive effect on the settling properties and the nitrification performance of sludge was not affected. These researches showed that the sludge structure and settling property were not affected in high salinity environment.

However, some researchers suggested that the sludge structure and settling property would become bad in high salinity environment (>1 wt% salinity) (Amin et al., 2014; Zhao et al., 2016). As salinity was above 1 wt%, the settlement of sludge was unbeneficial because of the increase of wastewater density. For instance, Uygur and Kargi (2004) studied the biological nutrient removal from saline wastewater using SBR, and they found that the SVI value increased with the increase of NaCl concentration. When NaCl concentration was increased from 5 to 60 mg/L, the SVI value increased from 50 to 97 mL/g almost linearly. The sludge settling performance was damaged because the microbial biomass of activated sludge reduced under high salt condition. Amin et al. (2014) studied the saline wastewater treatment by sequencing batch reactor with adapted and non-adapted consortiums. Their results illustrated that the SVI values increased with the increase of salinity in both SBRs. And the increased rate of SVI in adapted SBR was below the increased rate in non-adapted SBR. Zhao et al. (2016) researched the SBR treating at different salt concentration (0–3 wt%) wastewater and the treatment performance and microbial community profiles of SBR were discussed. The results showed that as no salt existed, the SVI value was 84 mL/g, however with the addition of salt to 3 wt%, the SVI increased to 116 mL/g steeply. And also they illustrated that the SVI increased due to the high sensitivity of activated sludge microorganisms to salinity variations.

In addition, when activated sludge system is shocked by salinity, sludge flocs are broken and the settling property of activated sludge becomes bad, the removal capacity of pollutant is inhibited (Glass and Silverstein, 1999). Besides in a long-term treatment of salinity wastewater, the activated sludge microorganisms are acclimated into salt-resistant microorganisms, microbial community structure is stabilization and activated sludge has good settleability, and these characteristics are not affected even increasing salinity in smaller increments (van den Brand et al., 2015; Ferrer-Polonio et al., 2015). However, some authors showed that the microbial structure and settling property of salt-tolerant activated sludge became bad if the salinity dropped rapidly, and the removal efficiency of pollutant decreased (Mcadam and Judd, 2008).

These study results of sludge structure and characteristics are largely difference in the high salt environment, and the reason may be the variation of the composition of waste water, sludge types and treatment method (Bao et al., 2009; Luo et al., 2013). In the high salt environment, there are some unfavorable factors on sludge structure and characteristics. For instance, the density of wastewater increases because of the presence of salinity and then increases the buoyancy of suspended solids, which has an adverse effect on settlement of sludge (Wang et al., 2015a). Moreover, the physical properties at high salt concentration could increase the dispersion of sludge system, and this kind of highly dispersed system could cause the loss of activated sludge. However, the amount of sludge microorganism especially filamentous bacterial reduces in the high salt environment, the lighter sludge flocs would outflow with turbid effluent, and the denser sludge flocs would remain, so the sludge structure and characteristics become good (Moussa et al., 2006).

2.2. Changes of sludge biomass and community

Inorganic salt has very important role in the microbial growth. It can promote the enzyme reaction, maintenance of the equilibrium of membrane and the regulation of cell osmotic pressure. Generally speaking, an appropriate amount of inorganic salt can promote growth of microorganisms, which can keep lots of microorganisms living in activated sludge (Bassin et al., 2011b). However, the sludge biomass and community can be changed in high salinity condition. Firstly, high concentration inorganic salt (above 1–2 wt%) may result in plasmolysis and loss of cell activity, cause a part of no salt-tolerant microorganism death, then sludge flocs are disrupted (Ng et al., 2005; Wang et al., 2015a). Secondly, the water density may increase in the high salinity condition, which would result in the outflow of a large number of microorganisms with turbid effluent (Reid et al., 2006). At last, the microorganisms with salt resistance property would survive and grow in the salinity wastewater.

Lots of studies have shown that with the increase of salt concentration, microbial biomass and biodiversity of activated sludge will be greatly reduced, salt-tolerant microorganism which was originally not dominant species increased gradually in activated sludge (Bond et al., 1995; Snaidr et al., 1997; Lefebvre et al., 2006; Wang et al., 2008; Corsino et al., 2015; Cortés-Lorenzo et al., 2016). The relevant microbial or molecular biology tools can carry on effective analysis to the changes of sludge microorganisms and cell structure. Among these tools, polymerase chain reaction (PCR) amplification, denaturing gradient gel electrophoresis (DGGE), fluorescence in situ hybridization (FISH), phylogenetic analysis are the main methods (Ng et al., 2014; Eusébio et al., 2011; Mlaik et al., 2015). Moussa et al. (2006) studied the effect of different NaCl concentration on nitrate bacteria and nitrite bacteria activity and sludge settling performance using SBR, and they found that *Nitrosomonas europaea* and *Nitrobacter* sp. were the only nitrifiers present at high salt levels (at 40 g Cl^-/L) in SBR. Wang et al. (2008) studied the treatment of petrochemical wastewater by two kinds of activated sludge. One of the sludge microorganisms was acclimated to salt-tolerant microorganism, and the other was inoculation activated sludge and not acclimated. They discovered that the main microbes were filamentous bacteria, protozoa species, and total number of bacteria in the mixture was 2.0×10^7 cfu/g in unacclimated activated sludge. However in acclimated activated sludge, the sludge flocs mainly contained cell secretions and a few protozoa, total number of live bacteria in the floc was 1.9×10^7 cfu/g, and the biodiversity greatly reduced.

However, under the high salt environment (>1 wt% salinity), halotolerant bacteria population increased with the increase of saline concentration, and the microbial community could express different degree of tolerance to such an altered stresses of salinity (Lim et al., 2008). For instance, when the NaCl concentration reached 30 g/L, the dominant microorganisms were *Rhodobacter maris* which were reported to be halophilic (Ramana et al., 2008). *Proteobacteria* were dominant bacteria in SBR when treating table olive packaging wastewater, and it could utilize organic contaminant as nutrition (Ferrer-Polonio et al., 2015). In addition, with the increase of salinity, there were some salt-resistant microorganisms dominated at different salinity in activated sludge. For example, *Thiothrix* was slightly suppressed when the NaCl concentration was 20 g/L, and it was capable to remove nitrate in wastewater (Aruga et al., 2002). *Pelagicoccus albus* which was a chemoheterotrophic bacterim, apparently required NaCl for cell growth and it could tolerate the NaCl concentration up to 70 g/L (Yoon et al., 2007). Under the high salt environment, these halotolerant bacteria could endure extreme conditions and grow well, and then play an important role in the degradation of brine wastewater (Sharrer et al., 2007; Yogalakshmi and Joseph, 2010). These studies indicated that the diversity of the halotolerant bacterial community was changed with salt concentration and wastewater characteristics, and they showed that the salinity affected the relative abundance of dominant bacterial populations (Yang et al., 2011; Mohamed and Martiny, 2011; Li et al., 2009).

Additionally, salinity will influence the protozoa species and quantity in activated sludge process. When the amount of salinity exceeds a certain value (as NaCl >5 g/L), the protozoa population are significantly reduced or even disappear at the higher of the salt concentration (Wang et al., 2008; Lu et al., 2011). Some studies illuminated that ciliates could regulate their internal ionic composition and their salt limit was probably 5 g/L (Lefebvre and Moletta, 2006). As the protozoa gradually adapt to the salinity environment, the protozoa in the sludge can grow and breed gradually. And the increase of protozoa population at high concentration of salinity may be due to the acclimatization process of protozoa species. With the acclimation of salt-tolerant sludge microorganism, the protozoa species which couldn't tolerated salt disappeared, and the salt-tolerant protozoa species increased and were dominant species at high salinity wastewater (Salvadó et al., 2001).

Therefore, under the condition of salinity, all kinds of microorganisms in sludge have the trend of reduction. With the adaptation of salinity, these halotolerant microbial species will survive and gradually become predominant microorganisms, and then play an important role in the degradation of brine wastewater. In the later research and application, it should accelerate the filter of salt-tolerant strains especially halophiles, and researches the development of this kinds of microorganisms applicating in real high salt wastewater treatment.

3. Influence on sludge microbial physiology

3.1. Influence on osmotic pressure

Usually, microbial growth survival requires appropriate osmotic pressure in the environment, as generally microorganisms survive only under the condition of appropriate environmental osmotic pressure. Meanwhile, the solution concentration is proportional to osmotic pressure, and the higher the inorganic salt concentration in the solution is, the higher osmotic pressure will be (Bassin et al., 2011a). In isotonic condition (e.g. 0.85 wt% NaCl) microbial metabolism has the best performance and growth status. In pure water or low salt condition (e.g. 0.01 wt% NaCl) microbial cells are likely to swell or even break as water molecules in solution may infiltrate into microorganisms.

In high salinity environment (e.g. 2 wt% NaCl), sludge microbial cells plasmolysis could occur without acclimated. Because the water molecules within microbial cells could infiltrate into external environment, and microbial growth could be inhibited even die (Ozalp et al., 2003). Kincannon and Gaudy (1968) pointed out after adding 30 g/L NaCl into traditional activated sludge treatment system, environmental osmotic pressure increased rapidly which led to microbial cell lysis and then the removal rate of BOD decreased to 30%. Burnett (1974) discovered that when activated sludge system was shocked by 32–38 g/L NaCl, microbial death occurred and the removal rate of effluent BOD fell of 73%, the reason could be that microorganism couldn't tolerate such a high osmotic pressure. At the same time, the concentration of suspended solids in effluent increased. Some researches also showed that inoculation sludge microbial survival rate which was measured by plate count method was below 40% and the removal efficiency of pollutant was seriously influenced after inoculating microorganisms from freshwater to saline environment where the salt content was 2 wt% (Ozalp et al., 2003). Therefore, it is generally acknowledged that the treatment effect of pollutant is unsatisfying with the use of freshwater microorganisms when the salt content in the environment exceeds 20 g/L (Dincer and Kargi, 1999).

However, if salt concentration in the environment is increased gradually, microbes will get used to and then reduce the impact of salt. The reason may be that when salinity in the environment is increased, microbial cells can adjust to the osmotic pressure by pump mechanism (such as contractile vacuole) or synthesize compatible solute. The higher the salinity is, the more energy which microorganisms use for maintaining the microbial functions in high osmotic pressure will be. At the same time the energy which is applied to cell synthesis and cell yield will be low (Russel and Cook, 1995). When microorganisms are shocked by high salinity, they will balance the cells internal osmotic pressure or protect the cells internal protoplasm by themselves regulation mechanism. The regulation mechanism includes that the cells form new protective layer by gathering low molecular weight substances (i.e. amino acid, sugar), regulate metabolism and even change genetic gene (Ganthier et al., 1991). Therefore, it is practicable to obtain salt tolerant microorganisms by selective culture theoretically, and industrial application of salt tolerant microorganisms needs researchers' efforts.

In general, the sludge microorganism would die and the performance of activated sludge is influenced when activated sludge system is in osmotic pressure environment (e.g. saline wastewater), but the performance of activated sludge would be recovered as the sludge microbe adapts to the environment (Uygur, 2006). In practical hypersaline wastewater treatment and also the future research directions, it is feasible to achieve the efficiency of the actual wastewater treatment by choosing the microorganism with good salt-resistance property and treatment effect from acclimated sludge or adding salt tolerant strains into the activated sludge.

3.2. Effect on microbial respiration

The bioactivity of microbes can be reflected by microbial respiration, thereinto, specific oxygen uptake rate (SOUR) of activated sludge is an important indicator of microbial metabolic activity. In natural environment, the appropriate salt content can boost the microbial metabolism and promote the respiration activity of microorganism. Generally, when the SOUR of activated sludge is 20–40 $mgO_2/gVSS \cdot h$, the sludge possesses high bioactivity, if the SOUR is lower than 5–10 $mgO_2/gVSS \cdot h$, sludge microorganisms are poisoned and lack capacity to degrade organic matter (Henze et al., 2002).

Generally speaking, the activated sludge SOUR decreases when the saline concentration increases, which indicates that the sludge microbial bioactivity declines. For example, Wang et al. (2005) studied the effect of salinity shock on the SOUR of SBR, they illuminated that when the NaCl concentration was up to 2 wt%, the SOUR reduced by 35% and removal efficiency of organic matter was dropped by 30%, compared with the control experiment without NaCl shock loading. Li (2006) studied the influence of different sodium chloride concentration on the bioactivity of conventional activated sludge. The study illustrated that the impact on the conventional activated sludge was small when the concentration of sodium chloride was 2 wt%, however the inhibition of sodium chloride on the bioactivity of sludge was enhanced when the sodium chloride concentration was greater than 3 wt%. As the sodium chloride concentration was 8 wt%, the sludge SOUR value was only 23% of the SOUR value when the sodium chloride concentration was 0 g/L. Bella et al. (2013) discovered that the SOUR decreased from 131 mg O_2 L^{-1} h^{-1} to 80.17 mg O_2 L^{-1} h^{-1}, when the salinity was increased rapidly from 0 to 1 wt%. However some other literature showed that the increase of salt concentration caused the increase of respiration rate. For example, Ludzack and Noran (1965) surveyed the effect of salinity on the activated sludge treatment system. The result showed that under the condition of high salt (about 2 wt% Cl^-), the flocculability of sludge decreased, the concentration of suspended solids of effluent rose, and the microbial SOUR increased with the increase of salinity. Panswad and Anan (1999) researched the impact of high sodium chloride (3 wt%) wastewater on an anaerobic/anoxic/aerobic process with and without inoculation of sodium chloride acclimated seeds, the result showed that the SOUR of these two kinds of sludge increased with the increase of sodium chloride concentration in stable state.

As we see, two opposite situations come forth about the effect of salinity on microbial respiration. The reason may be that: (1) High salt inhibits the bioactivity of sludge microorganism, so the microbial respiration decreases. (2) However, there are many different species of microbes in activated sludge system, these microbes have different tolerance ability in saline environment. Those microbes which can adapt to high salt environment gradually increase and become dominant microorganisms, so the microbial respiration increases (Wang et al., 2015b; Ferrer-Polonio et al., 2016a). These differences still need the researchers to continue their studies, especially salinity affects the respiration of different microbial species in activated sludge.

4. Influence on microbial internal molecules

4.1. Influence on microbial products

Under the condition of a certain concentration of salt, the microorganisms in activated sludge can accumulate some small molecules in the body as a protectant (for example, osmoprotectant) to resist the adverse environment, and these small molecules include K^+, sugars, alcohols, amino acids and their derivatives, etc (Ontiveros-Valencia et al., 2014). They not only make microorganisms effectively regulate osmotic balance and maintain normal life activities, but also stabilize the cell membrane and cell ultrastructure. Besides they can stabilize and protect the reactive molecules in cells such as peptides, grease, enzyme in the inherent state (Huang et al., 2013; Rene et al., 2008). From a macro view, the sludge has a better settling performance, granule stability and microbial structural integrity, etc (Bassin et al., 2012).

Some research results showed that the sludge volume index, settling performance, sludge morphology, flocs structure were affected when activated sludge system was impacted by salinity. Thus the performance of the activated sludge process was affected (Dincer and Kargi, 2001). Under the condition of no salt or low concentration salt existed, the size of activated sludge flocs is large. From the molecular view, the quantity of extracellular and intracellular storage material which secreted by sludge microbial cell was increased and also the activity of microbial characteristic enzyme was good (Ciggin et al., 2007). And from the microscopic view, sludge zoogloea shape is irregular rotundity or oval, sludge flocs are loose and have close structures, and there are a lot of filamentous microorganisms, protozoan and metazoan existed (Mesquita et al., 2009). The mechanical integrity and structure of activated sludge flocs are mainly composed of filamentous microorganisms (Krasnits et al., 2013; Zhang et al., 2013). When the salt concentration increases to a certain value (e.g. 2 wt% salinity), the secretion of microbial cell (such as extracellular, intracellular storage material) and cellular structure could change, it can be seen that the quantity of extracellular and intracellular storage material increases, and the activity of characteristic enzyme decreases. Then the utilization capacity of nutrients (such as C, N, P, trace elements) by microorganisms is reduced, and microbial growth is restrained (Ye et al., 2009). Also in the high salinity environment, the sludge particles become small, tight, and sludge lacks bioactivity and adsorption capacity (Bassin et al., 2012; Cui et al., 2015).

4.2. Influence on extracellular polymeric substance

Extracellular polymeric substance (EPS) is a kind of polymer material which distribute on the surface of microbial cells, and these materials are advantageous to the microbial cells. EPS are existed extensively in the inside and surface of activated sludge flocs. They can resist the harm of toxic substances to microorganisms (Laspidou and Rittmann, 2002; Wingender et al., 1999).

When impacted by salinity, sludge microorganisms have to resist external adverse environment, so they consume energy, and adjust their metabolic pathway, or secret EPS. Then it results in the decrease of the energy used to grow relatively, and microbial growth rate decreases (Yogalakshmi and Joseph, 2010; Sun et al., 2010a). Hong et al. (2013) studied the effect of salinity on the membrane bioreactor (MBR), and the results indicated that when salinity of MBR was 0 and 35 g/L, the EPS was 13.79 and 39.98 mg/(g MLSS), correspondingly, increasing salt concentration resulted in the increase of EPS concentration. The results also indicated that increased the salinity in the biological wastewater treatment systems, the EPS also increased and this could play an important role in facilitating adherence of bacteria during formation of flocs (Sheng et al., 2010). And some researches also demonstrated that the acclimated activated sludge microorganisms could survive and grow in the saline environment after the domestication of high salt gradually (Sun et al., 2010a). The reason could be that high concentration of EPS existed in vitro of the acclimated salt tolerant sludge microorganisms. EPS has certain protective effect on microorganisms. When saline concentration is changed in the external environment, EPS can reduce the destruction of microorganisms due to maintaining cellular osmotic pressure balance (Johir et al., 2013).

4.3. Influence on microbial enzyme

Sludge microbial growth need to consume organic matter, and the process is done with the participation of enzymes. These enzymes are very sensitive to the toxic substances, because they are a kind of protein. Hydrolytic enzymes occupy an important position in these enzymes, because the oxidation of organic matter in the organism often through the activity of hydrolytic enzymes (Nabarlatz et al., 2010). Acid phosphatase, alkaline phosphatase, protease, dehydrogenase and esterase are some of the most important enzymatic activities taking part in the biological wastewater treatment (Cadoret et al., 2002; Molina-Muñoz et al., 2010). When wastewater contains some toxic substances, enzyme would be inactivation, which causes the deterioration of activated sludge, and then the removal efficiency of wastewater treatment decreases. A right amount of inorganic salt can promote the metabolism of microorganisms. However when inorganic salt content exceeds a certain amount, the enzyme activity reduces because enzyme may be destroyed in the microbial body (Cortés-Lorenzo et al., 2012). After inoculated to high salt environment, the microorganisms which grow in the freshwater environment need to adjust the process of metabolism in order to adapt to high salt environment, and the microorganisms may produce new enzyme system. In high salt environment, the enzyme synthesis rate is reduced, so the microorganisms need a long time to adapt to the environment (Burgess and Pletschke, 2008).

At present research indicates that in the presence of high saline concentrations (e.g. 2 wt% salinity), the microbial species and quantities possibly reduce, the concentration of suspended solids in effluent increases and the removal efficiency of ammonia and organisms reduces in the biological treatment system (Ye et al., 2009). The reason may be that high salinity in wastewater can interfere with the normal metabolism of microorganism, destroys microbial cell membrane and enzymes, inhibits the growth of microorganisms, reduces the activity of activated sludge microorganisms, and then lead to the decrease of the removal efficiency of wastewater (Uygur, 2006). Cortés-Lorenzo et al. (2012) researched the effect of salinity on enzymatic activities in a submerged fixed bed biofilm reactor for municipal sewage treatment, and the results showed that hydrolytic enzymatic activities were reduced when the salinity was increased from 0 to 44.1 g/L in the influent and consequently the biotransformation of organic matter in the submerged fixed bed bioreactor significantly decreased. Shekoohiyan et al. (2016) studied the peroxidase-mediated biodegradation of petroleum hydrocarbons (TPH) in SBR. In the 37 g/L salinity wastewater, the activity of peroxidase which was mainly produced by *Pseudomonas* spp. and *Bacillus* spp. in the SBR was not affected, and the removal efficiency of TPH in the salinity wastewater was similar to that in fresh media (no salt).

At present, there is only a small amount of literature about the effect of salinity on the microbial enzymes of activated sludge. However, the change of microbial enzymes in different salinity is also a very important factor on the influence of the sludge microbial properties and the removal effect of pollutant. Therefore the influence on enzymes types and activity in activated sludge by the salinity is a very important research in the future.

5. Mechanisms of salt effect on activated sludge floc

It is widely considered that the high salinity (>1 wt% salinity) deteriorates the settleability and wastewater treatment performance of activated sludge (Reid et al., 2006). When salt intrudes in activated sludge system, the mechanisms of salinity effect on activity sludge may be that: (1) The activity of sludge microorganism is inhibited, enzymatic activity of microbe is destroyed and the metabolism of microbe becomes deterioration (Cortés-Lorenzo et al., 2012). (2) Osmotic pressure of external environment increases with the increase of salinity, which sucks the microbial vital water right through its cell wall, causing microbe to dehydrate. And also high osmotic pressure destroys the structure of microbial cells and results in plasmolysis (Ferrer-Polonio et al., 2015). (3) The physical properties of the activated sludge are affected, decreasing their hydrophobicity, filterability and bioflocculation, the microorganisms which couldn't adapt to the salinity environment may die and the structure of sludge flocs is destroyed (Amin et al., 2014). (4) With the acclimation of the salt-tolerant microorganisms in salinity condition, the salt-tolerant microorganisms grow and become the main components of sludge flocs, and then the activity and treatment performance of activated sludge are recovered (Cui et al., 2015) (see Fig. 1).

6. Conclusions

There are a lot of reports on the biological treatment technology of saline wastewater, especially the activated sludge process, and the biological technology can achieve ideal removal. In the process for biological treatment of saline wastewater, salt content affect heavily the activated sludge structure and sludge microorganism. In this paper, the effects of salinity on activated sludge and its microorganisms were reviewed. From a macroscopic view, the changes of sludge structure and sedimentation performance were

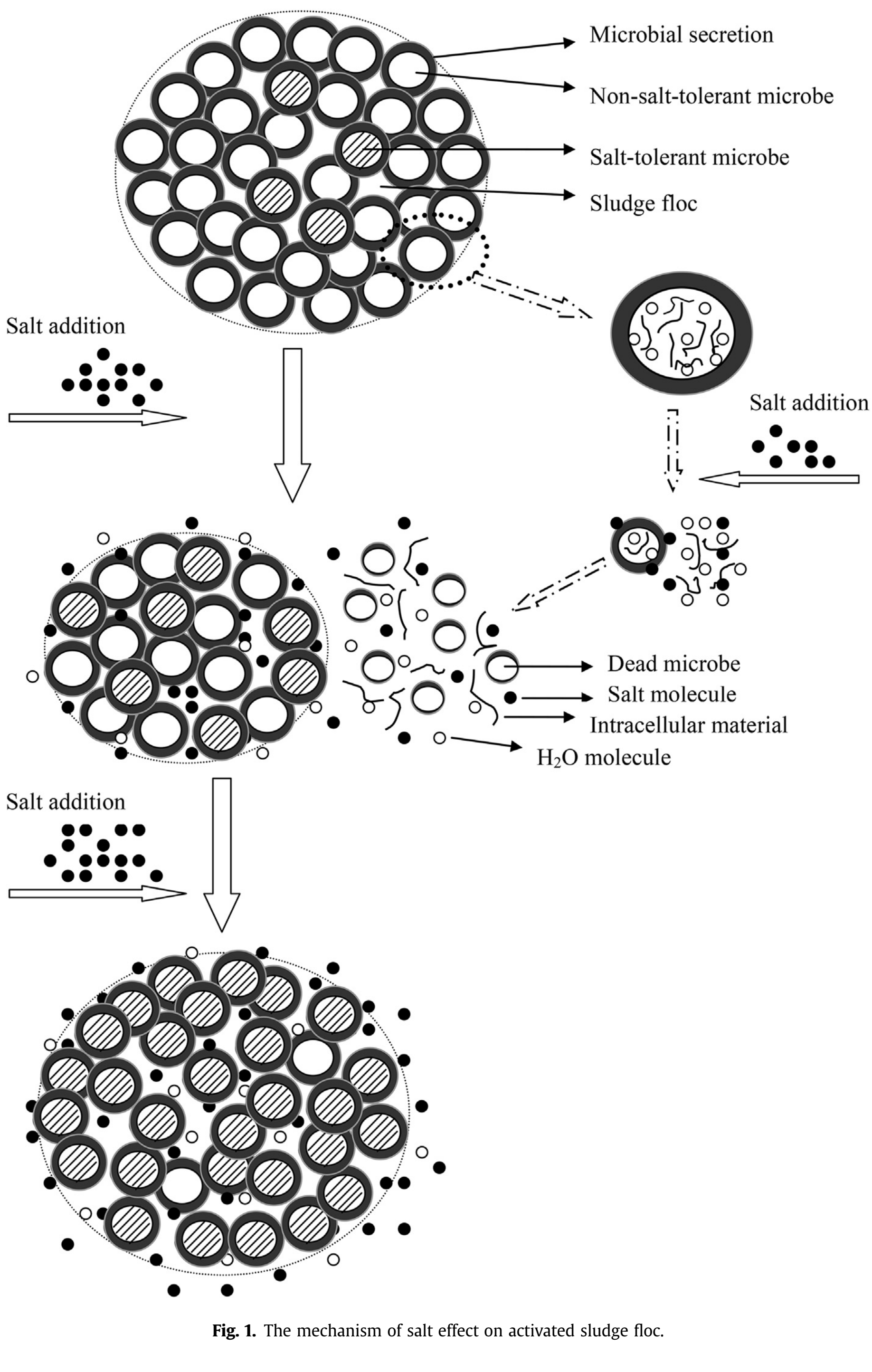

Fig. 1. The mechanism of salt effect on activated sludge floc.

expounded. And from a microcosmic view, the effects of salinity on microbial species and biomass, microbial physiological changes, and microbial molecules and cells were also summarized. Besides, the mechanism of salinity effect on activity sludge and its microorganisms were discussed. In addition, the future researches were also proposed. For example, the variations of the microbial species and morphologies in different bioreactors, salt tolerant mechanisms of microorganisms and removal efficiency of pollutants in saline wastewater need to be further studied. Meanwhile investigations are proposed on the salt stress mechanisms of activated sludge microorganisms at cellular and molecular levels and enzyme activity, screening and acclimation of salt-tolerant bacteria including halophiles, and optimization of process parameters. Taking into account that many researches on biological treatment of saline wastewater were carried out at laboratory scale, demonstrations and applications of biological treatment technology for high salt wastewater treatment at field scale are needed.

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