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Physical properties and Extracellular Polymeric Substances pattern of aerobic granular sludge treating hypersaline wastewater

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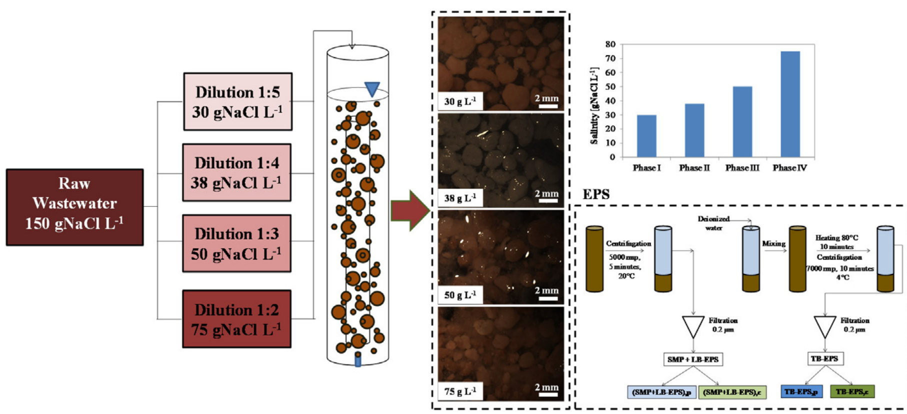
HIGHLIGHTS

- Aerobic granular sludge was successfully used to treat fish-canning wastewater.
- The increase in EPS with salinity was not proportional with the salt concentration.
- The increase in salinity modified the extracellular polymeric matrix.
- The protein EPS content reduced with the increase in salinity.
- Granules stability significantly reduced over 50 g NaCl L^{-1}.

GRAPHICAL ABSTRACT



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ABSTRACT

The modification of the physical properties of aerobic granular sludge treating fish-canning wastewater is discussed in this paper. The structure and composition of the Extracellular Polymeric Substances (EPSs) were analyzed at different salinity levels and related to granules stability. Results outlined that the total EPSs content increased with salinity, despite the EPSs increment was not proportional to the salt concentration. Moreover, the EPSs structure was significantly modified by salinity, leading to a gradual increase of the not-bound EPSs fraction, which was close to the 50% of the total EPSs content at 75 g NaCl L^{-1}. The increasing salt concentration modified also the EPSs composition, causing the gradual reduction of protein content resulting in a decrease of granule hydrophobicity. The results pointed out that the granules stability significantly reduced above 50 g NaCl L^{-1}, suggesting the existence of a salinity threshold above which granules stability is compromised.

1. Introduction

The multitude of studies reported in the literature dealing with the use of aerobic granular sludge (AGS) for the treatment of industrial wastewater is representative of its potentiality in this field (Lotito et al., 2014; Liu et al., 2015; Corsino et al., 2016a). The aerobic granules are characterized by a dense and compact structure that protects microorganisms from toxic compounds potentially contained in the raw wastewater. For this reason, aerobic granular sludge is recognized as capable of coping with a wide variety of high-strength, recalcitrant and complex wastewaters (Maszenan

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et al., 2011; Amorim et al., 2013). Several studies reported the excellent performances of aerobic granular sludge for the treatment of real and synthetic saline wastewater (among others, Pronk et al., 2014; Corsino et al., 2015, 2016a). Although promising, the aerobic granular sludge technology is characterized by specific drawbacks, mainly related to the structural stability of the granules. The structural characteristics of aerobic granular sludge under harsh environmental conditions were largely investigated (Maszenan et al., 2011; Val del Río et al., 2013; Abdullah et al., 2013). However, there is a lack of knowledge about the physical properties of aerobic granules in treating real industrial wastewater under extremely saline environments. Indeed, the majority of the literature studies investigated salinity level generally lower than 30 g NaCl L^{-1} (Val del Río et al., 2013; Pronk et al., 2014). However, many industrial wastewaters are characterized by salinity higher than 30 NaCl L^{-1} like the fish-canning process, because of the use of brine. Moreover, with this regard, the wastewater is generally subjected to salinity increasing, especially during the fish processing phase. Consequently, little is known about the granules characteristics in an extreme salinity environment and their response to further salinity increases. In this context, previous studies demonstrated that the high salt concentration may affect the bacterial EPSs production (Taheri et al., 2012). In a recent study, Wan et al. (2014) noted that not-halophilic microorganisms produced a large amount of EPSs, mainly proteins, to cope with the increasing osmotic pressure, avoiding in such way the plasmolysis.

It is well known that the EPSs play a crucial role in the granules formation and stability (Zhu et al., 2015; Corsino et al., 2016b). Indeed, several studies demonstrated that the EPSs matrix plays a fundamental role in the microbial mechanism of a successful aerobic granular sludge system (Rene et al., 2008; Zhu et al., 2012). Indeed, the EPSs molecules form a buffering layer in order to protect the microbial cells from the bulk environmental conditions (Capodici et al., 2015). In addition, the EPSs may help to protect bacteria living in harsh environmental conditions which generally occur in specific wastewater streams such as fish-canning processing wastewater.

Nevertheless, despite extremely saline environments promote the EPS production, it seems that the stability of the granules would not increase accordingly. In this regard, Ismail et al. (2010) reported several issues related to the granule structure and strength due to high concentration of sodium. Particularly, Ismail and co-workers found that at 20 g Na^{+} L^{-1} the deterioration of the granule strength was likely due to the replacement of Ca^{2+} in the matrix by the abundantly available Na^{+}.

In accordance with the literature, the EPSs structure is constituted by two fractions: the former is bound to the bacterial cells, usually named "tightly-bound EPSs" (TB), whereas the other is constituted by the so-called "loosely-bound EPSs" (LB) (Basuvaraj et al., 2015). Actually, there is a third fraction which is the soluble one, namely soluble microbial products (SMP), which is not bound to the cells. It was observed that the contribution to the sludge aggregation given by the soluble and the loosely-bound EPSs fractions is marginal if compared with the tightly-bound EPSs fraction (Liu et al., 2010).

Considering the importance of the EPSs on the physical properties of aerobic granular sludge as well as in maintaining well-functioning AGS reactors, a thorough analysis concerning the production and the characterization of the EPSs structure in the treatment of a real industrial wastewater under extreme saline conditions is of extreme importance. For this purpose, the objective of this study was to analyse the variation of the aerobic granules properties treating fish-canning wastewater, focusing on the production and characterization of the EPS structure and its relationship with the granules stability with the increased salinity levels.

2. Material and methods

2.1. Experimental set-up

AGS was cultivated for 6 months from conventional activated sludge (CAS) fed with a synthetic acetate-based wastewater in a granular sequencing batch airlift reactor (GSBAR). During cultivation, the influent salinity was gradually increased up to 25 g NaCl L^{-1}. Once the granules reached their full maturation, they were fed with the real saline wastewater. The real wastewater was collected from a fish-canning industry producing salted anchovies. Since the salinity of the raw wastewater was approximately equal to 150 g NaCl L^{-1}, it was diluted with tap water in order to obtain different salt concentrations during each period. The GSBAR was operating for 61 days, divided into 4 periods each characterized by a specific salinity level. In details, the salinity levels tested during this study were 30 g NaCl L^{-1}, 38 g NaCl L^{-1}, 50 g NaCl L^{-1} and 75 g NaCl L^{-1}. The NaCl concentrations were achieved by progressively reducing the dilution factor (from 5 to 2) of the raw wastewater (Table 1). The reactor used for the experiment was a column-type (100 cm height) with a working volume of 3.5 L (internal diameter: 8.6 cm) and was characterized by an internal riser (50 cm height) with an internal diameter of 5.4 cm. Air was introduced via a fine bubble diffuser at the base of the reactor at a flow rate of 3 L min^{-1} so that the hydraulic shear forces, expressed in terms of up-flow air velocity, were approximately 2.4 cm sec^{-1}. The effluent was discharged by a solenoid valve placed at 35 cm from the base of the reactor. Thus, the volumetric exchange ratio (VER) was 50% for each cycle.

Each experimental phase lasted as far as steady state conditions, in terms of nutrient removal efficiency and granules physical properties (EPS, hydrophobicity, size), were reached. For further details concerning the structure and operation of the GSBAR as well as the wastewater characterization the reader is addressed to Corsino et al. (2016a).

2.2. Analytical methods

EPSs extraction was carried out by combining the Heating Method described by Le-Clech et al. (2006) to extract the soluble and the tightly bound fractions, and the method described by Yan et al. (2015) to extract the loosely bound fraction. The specific method is as follows: a 50 mL of mixed liquor sample was centrifuged at 5000 rpm at room temperature (Thermoscientific centrifuge) and the supernatant filtered through a 0.20 μm membrane (Millipore). In this way, both the soluble (SMP) and the loosely bound fractions were extracted. The precipitate was resuspended with deionized water to its original volume, then put in a thermal bath (Thermoscientific cryostat) at 80 °C for 10 min. The sample was again centrifuged at 7000 rpm for 10 min at 4 °C, then the supernatant was filtered through a 0.20 μm membrane. During this step, the tightly-bound EPSs were extracted. In this study the soluble and the loosely-bound EPS were considered together as not-bound EPS (NB-EPSs), because their contribution to the granules stability is significantly lower compared with that of the tightly-bound EPSs (Corsino et al., 2017). Subsequently, each sample was analyzed in terms of carbohydrate and protein content according to the phenol–sulphuric acid method with glucose as the standard (DuBois et al., 1956) and by the Folin method with bovine serum albumin as the standard (Lowry et al., 1951), respectively. Each EPSs fraction was then referred to the volatile suspended solid (VSS) concentration within the reactor.

The measurements of the total suspended solids (TSS) and VSS in the mixed liquor and in the effluent were carried out according