



A comprehensive comparison between halophilic granular and flocculent sludge in withstanding short and long-term salinity fluctuations



Santo Fabio Corsino, Marco Capodici*, Michele Torregrossa, Gaspare Viviani

Dipartimento di Ingegneria Civile, Ambientale, Aerospaziale, dei Materiali, Università di Palermo, Viale delle Scienze Ed. 8, 90128, Palermo, Italy

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ABSTRACT

The effects of salinity fluctuations on the activity of autochthonous halophilic bacteria in aerobic granular sludge (AGS) and flocculent activated sludge (FAS) reactors were investigated. The response of nitrifiers and denitrifiers activity to drastic and moderate salinity shocks in the short-term (ST) and long-term (LT) was examined.

The BOD₅ removal efficiency decreased only in the reactors subjected to the drastic LT salinity increase. Nevertheless, stable performances were achieved 18 days after the shock in the AGS-R1 (90%), whereas after 27 days in the FAS-R1 (82%).

The loss in nitrification efficiency was higher in the FAS reactors and was proportional to the shock intensity. Nitrification activity collapsed from approximately 3.8 mgNH₄-N gVSS⁻¹ h⁻¹ to 0.73 mgNH₄-N gVSS⁻¹ h⁻¹ and from 4.5 mgNH₄-N gVSS⁻¹ h⁻¹ to 0.24 mgNH₄-N gVSS⁻¹ h⁻¹ in the AGS-R1 and FAS-R1, respectively, even if the ammonium oxidation capacity did not completely disappeared. Denitrification activity decreased from 11.44 mgNO₂-N gVSS⁻¹ h⁻¹ to 3.93 mgNO₂-N gVSS⁻¹ h⁻¹ in the AGS-R1 at steady state, whereas in the FAS-R1, it decreased from 12.53 mgNO₂-N gVSS⁻¹ h⁻¹ to 2.09 mgNO₂-N gVSS⁻¹ h⁻¹.

Nitrification and denitrification were completely restored 5 days after ST shock. No significant effects were observed after the moderate shock.

The changes in the total EPS content were lower than 10%, therefore, it was considered negligible. Only drastic shocks caused significant changes in the EPS structure, with an increase of the loosely-bound by 45% in the AGS and 55% in the FAS.

1. Introduction

Nowadays, several industries produce high inorganic salt concentration in their wastewater, inter alia petroleum, chemical and fish canning, tanning, pickling, etc. Because of the massive use of salt during the production process, the disposal of such wastewater in conventional wastewater treatment plants (WWTPs) is not a viable solution, because it is claimed that even small salt concentrations have negative impacts on microorganisms in the activated sludge process [1].

Fish-canning wastewaters are characterized by high salt concentration and by massive fluctuations in salinity. Indeed, in fish-canning industries seasonal and weekly fluctuation in salinity frequently occur. Salinity variations are mainly depending on the fish to be processed (tuna, anchovies, mackerel, etc.), on the manufacturing process

(fish evisceration, canning, machineries cleaning, etc.) and on the production period (winter, summer, etc.). For instance, when fresh fish is processed (spring and summer), process operations, including fishes' evisceration, washing and salting process, require large volumes of brines. Usually, these activities are carried out within a few days per week, during which the wastewater produced is characterized by very high salt concentration (over 200–300 gNaCl⁻¹) [2]. However, wastewater produced by fish processing is generally mixed with fresh water used for cleaning operation, hence the salinity is reduced [3].

During the rest of the year, salinity significantly decreases because the primary activity carried out is the fish canning that does not require the use of brine. However, in some cases, occasional occurrences of moderate salinity increase may occur during this period, according to industry's needs, for instance because of occasional fresh fish

Abbreviations: AGS, aerobic granular sludge; AUR, ammonium utilization rate; BOD₅, biochemical oxygen demand; COD, chemical oxygen demand; EPS, extracellular polymeric substances; F/M, food vs microorganism ratio; FAS, flocculent activated sludge; HRT, hydraulic retention time; LT, long term; MLTSS, mixed liquor total suspended solids; MLVSS, mixed liquor volatile suspended solids; NUR, nitrite utilization rate; OLR, organic loading rate; PLC, programmable logic controller; PN, proteins; PS, polysaccharides; PSD, particle size distribution; SRT, sludge retention time; ST, short-term; TN, total nitrogen; TSS, total suspended solids; UC, uniformity coefficient; VSS, volatile suspended solids; WWTPs, wastewater treatment plants

* Corresponding author.

E-mail address: marco.capodici@unipa.it (M. Capodici).

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processing. Consequently, moderate and drastic increases in salinity generally occur in both the long and short term.

Although chemical-physical treatments are high-efficiency technologies, their high operating costs make that activated sludge process remains the most widely used treatment for organic carbon degradation and nutrient removal. However, high salinity and salinity variations, lead to the arising of the osmotic pressure, thereby decreasing the metabolic enzyme activity and inhibiting the microorganisms' growth. This affects the bacterial metabolism and inevitably the performances of the biological system.

The majority of the saline wastewater related studies reported in the literature dealt with simulated media [4,5]. However, real saline wastewater (i.e. fish canning wastewater), apart from the high salinity, are characterized by high suspended solids (up to 5000 mg L⁻¹), organic matter (COD up to 90000 mg L⁻¹) and nitrogen concentration (TN up to 3000 mg L⁻¹), which make their biological treatment a considerable challenge [6].

Several studies demonstrated the feasibility in treating saline wastewater by means of halotolerant biomass [4,7]. However, the main bottleneck is that the performance of such salt-adapted systems is usually limited to low salinity levels [8,9]. A viable approach to overcome the effect of high salinity on bacterial activity is the use of granular sludge biomass [7,10]. Although granular sludge technology offers several benefits compared with flocculent activated sludge, the ability of granules in withstanding highly salinity environment, while preserving satisfactorily nutrients removal performance, is limited [8,10,11]. In the majority of these studies, dealing with simulated saline wastewater, no significant variations in salinity occurred, meaning that either the salinity was constant or it was stepwise increased [4,11]. In the literature is reported that rapid salinity fluctuations could significantly affect the performance even of salt-adapted bacteria [10]. Nevertheless, no studies aimed at examining the stress of salinity shock on biological systems, neither granular nor flocculent activated sludge, are available in the literature.

To overcome the bottleneck of the biological salt-adapted systems, many researchers speculated the possibility of using halophilic biomass for the treatment of high salinity wastewater [10,12]. Indeed, compared with halotolerant microorganisms, halophilic bacteria better withstand high saline environments similar to their natural habitats [13,14]. In this context, autochthonous halophilic activated sludge was successfully used to treat fish-canning wastewater [14]. The biological system was successfully tested at 30 gNaCl L⁻¹ of salinity. High biomass activity and nutrients removal efficiencies higher than 90% were observed, thereby confirming the capability of halophilic biomass in withstanding highly saline environments. Based on the literature review arise that, if on the one hand it was already demonstrated that salinity fluctuations seriously affect the metabolism of microorganisms that have been successfully acclimated to a steady salinity [15,16], on the other hand there is a lack of knowledge about their effects on halophilic biomass.

With this purpose, this work is aimed at investigating the effects of moderate and drastic fluctuations in salinity on the biological activity of autochthonous halophilic bacteria cultivated from a real fish canning wastewater. Salinity shocks were applied in both the short and the long-

term, in order to simulate seasonal and weekly fluctuations. Particularly, the study was aimed at assessing the effects of salinity fluctuations on nitrification and denitrification kinetics of the autochthonous halophilic biomass in forms of flocculent and granular sludge. A comprehensive comparison between the granular sludge and the flocculent biomass was performed with the aim of demonstrating the potential benefit deriving from the halophilic biomass granulation.

2. Material and methods

2.1. Experimental set-up

Four sequencing batch lab-scale reactors, two with aerobic granular sludge (AGS) and two with flocculent activated sludge (FAS) were monitored in this study. The AGS reactors, named AGS-R1 and AGS-R2 (4 L of volume) and the FAS reactors, named FAS-R1 and FAS-R2 (8 L of volume) were subjected to drastic (R1 ones) and moderate (R2 ones) salinity increases, both in the long term (Run I) and in the short term (Run II). Both AGS and FAS were operated with a cycle length equal to 12 h. The AGS reactor cycle was featured as follows: 60 min of feeding (from the bottom of the reactors), 700 min of aeration, 5 min of settling and 5 min of effluent withdrawal. The FAS cycle, instead, included 30 min of influent feeding (aerated), 10 h of aeration, 60 of anoxic mixing followed by 5 min of aeration to favour nitrogen stripping, and lastly 20 min of settling and 5 min of effluent discharge. A Programmable Logic Controller (PLC) automatically handled the cycling operations. In all the reactors, the volumetric exchange ratio was equal to 50% for each cycle, so the feeding daily flow was equal to 4 L and 8 L for the AGS and the FAS reactor respectively. The hydraulic retention time (HRT) was therefore equal to 24 h for each reactor. Sludge retention time (SRT) was maintained close to 23 days by daily purging a known amount of sludge from the reactors. The SRT was calculated by the following Eq. (1):

$$SRT = \frac{V \cdot MLTSS}{q_w \cdot MLTSS + q_d \cdot TSS_{effluent}} \quad (1)$$

where, V is the volume of the reactor, MLTSS is the total suspended solids concentration in the mixed liquor, q_w and q_d are the volumes of sludge wasted and of wastewater treated on a daily basis, respectively, and TSS effluent is the TSS concentration in the effluent. Based on the TSS concentration in the effluent and the MLTSS concentration, the q_w was controlled to maintain the SRT approximately 23 days. The main process parameters, including the organic loading rate (OLR), the total (MLTSS) and volatile (MLVSS) suspended solids concentration in the mixed liquor and the food to microorganism ratio are summarized in Table 1.

2.2. Wastewater

The fish-canning wastewater was collected from a local industry producing canned anchovies. The wastewater was characterized by high organic matter content (BOD₅ 20,000 mg L⁻¹) and nitrogen (TN > 3000 mg L⁻¹), high total suspended solids concentrations (4500 mg L⁻¹), and high sodium chloride concentration

Table 1
Summary of the main process parameters.

Parameter	Units	Run I		Run II	
		AGS	SBR	AGS	SBR
OLR	[kgBOD m ⁻³ d ⁻¹]	1.96 ± 0.21	1.96 ± 0.21	1.73 ± 0.29	1.73 ± 0.29
MLTSS	[gTSS L ⁻¹]	10.31 ± 0.56	5.56 ± 0.95	11.02 ± 0.23	5.81 ± 0.34
MLVSS	[gTSS L ⁻¹]	7.91 ± 0.39	4.41 ± 0.44	8.14 ± 0.41	4.96 ± 0.32
F/M	[kgBOD kgVSS ⁻¹ d ⁻¹]	0.24 ± 0.03	0.42 ± 0.07	0.21 ± 0.05	0.34 ± 0.06
SRT	[days]	23	23	23	23

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