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Comparison of two strategies for the start-up of a biological reactor for the treatment of hypersaline effluents from a table olive packaging industry

E. Ferrer-Polonio [a,*], J.A. Mendoza-Roca [a], A. Iborra-Clar [a], J.L Alonso-Molina [b], L. Pastor-Alcañiz [c]

[a] Instituto de Seguridad Industrial, Radiofísica y Medioambiental, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain
[b] Instituto Universitario de Ingeniería del Agua y Medio Ambiente, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain
[c] Depuración de Aguas del Mediterráneo, Avda. Benjamin Franklin, 21, Parque Tecnológico, 46980 Paterna, Spain

HIGHLIGHTS

- Management of brines from table olive processing (FTOP) is a non solved problem.
- Biological treatment of hypersaline effluents (90 mS/cm) from FTOP has been studied.
- Two start-up strategies of the biological reactors for biomass acclimation were compared.
- COD removal efficiencies reached values of 88% phenols were totally degraded.
- An increase of γ-*Proteobacteria* was observed in SBRs when salinity increased.

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ABSTRACT

Biological treatment of hypersaline effluents with high organic matter concentrations is difficult to carry out and it can require a long start-up phase. This is the case of the treatment of fermentation brines from the table olive packaging (FTOP) industries. These effluents are characterized by conductivity values around 90 mS/cm, COD around 15,000 mg/L and total phenols concentration around 1000 mg/L. In this work, FTOP has been treated in two sequencing batch reactors (SBRs) operated in parallel. In each SBR a different start-up strategy has been carried out. In the SBR-2, biomass was previously acclimated to high salinity using simulated wastewater without phenolic compounds, meanwhile in the SBR-1, FTOP was added from the beginning of the start-up. Results indicated more operational problems in the SBR-2 consisting in a higher deflocculation that drove to high turbidity values in the effluent. Besides, at the end of the start-up, the SBR-1 reached higher COD removal efficiencies than SBR-2 (88% and 73%, respectively). In both reactors, an increase in γ-*Proteobacteria* in the microbial population was observed for increasing conductivities. In addition, phenols were completely removed in both reactors at the end of the start-up, what implied very low toxicity values in the effluent.

1. Introduction

Wastewaters from some industries are characterized by high organic matter and salts concentration. Their treatment by means of biological processes is always complicated, especially when the high wastewater conductivity is combined with some organic compounds, as phenolic compounds, that can inhibit biomass. The main types of industries that generate high salinity effluents are food processing industry (mainly pickled vegetables and fish processing industries), tanneries and petroleum industries [1].

It is well-known that salinity affects the correct performance of an activated sludge process. The effects on the sludge have been summarized in some review papers [1–3]. Salt concentrations above 1–2% may result in plasmolysis and loss of activity of cells. In addition, the physical properties of the activated sludge are affected, decreasing their hydrophobicity, filterability, settlement and bioflocculation [4,5]. However, an acclimation of the microorganisms is possible by means of a gradual salinity increase. Acclimation will not be successful if salinity is increased too rapidly [6], what would imply the release of cellular material and

* Corresponding author. Tel.: +34 96 3877630; fax: +34 96 3877639.
E-mail addresses: evferpo@posgrado.upv.es, evaferrerpol@gmail.com (E. Ferrer-Polonio).

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consequently an increase in soluble COD. On the other hand, a sudden decrease of salinity is also damaging for biomass. This also implies that settling is affected, especially when NaCl concentration is higher than 20 g/L [7].

Some authors have reported the existence of a limiting salt concentration for the achievement of an appropriate organic matter removal with an adapted activated sludge [8]. According to them, the use of halophile microorganisms would be the key to enhance the process performance. Halophilic microorganisms are those that require salt for their survival and can be classified into moderate (3–15% NaCl) and extreme (15–30% NaCl) halophiles [9]. Other authors report slightly different NaCl ranges (5–20% for moderate and 20–30% for extreme halophiles) [10].

One of the hypersaline effluents coming from industry is the fermentation brine from the table olive processing (FTOP). The finality of table olive processing is to make edible the olive fruit. This is performed by the following steps: (1) Debittering; treatment with sodium hydroxide solution (1–2% w/v) to remove the olive natural bitterness (in this stage, oleuropein is hydrolyzed) [11]. (2) Rinsing cycles for eliminating the alkali excess. (3) Fermentation; olives are submerged in brine (4–8% w/v) of sodium chloride for several months. The wastewater volume generated in all stages is about 3.9–7.5 m^3 per ton of green olives [12]. FTOP contributes to the 80–85% of the global pollution of wastewater generated during the production in these types of agro-food industries [13]. However, it represents only 20% of the total volume. This is the reason why it is important to segregate the FTOP to treat it separately.

FTOP is characterized by high conductivities (around 90 mS/cm) combined with high organic matter content (between 7 and 20 g/L of CDO), and phenols compounds (between 700 and 1500 mg/L). These features will entail very high environmental impacts if these effluents are not correctly managed [14]. The traditional management of these effluents consisted in either their disposal in lagoons for water evaporation or their transport to large municipal wastewater treatment plants for their blending with the municipal wastewater. However, the increasing legislation strictness and environmental awareness have led to study different alternatives for the management of these effluents.

Biological treatment of olive oil mill wastewater has been reported in many research works [15,16]. These effluents are characterized by COD ranges between 35 and 200 g/L (around 10% of this organic matter corresponds to phenolic compounds), and high total solid content. If olive mill wastewaters (OMW) and FTOP are compared, COD of OMW is higher than COD of the FTOP. However, conductivity of the FTOP is considerably higher than that of the OMW. In fact, conductivity values of the FTOP are around 10 times higher than those reported for OMW. A direct biological treatment of the fermentation brines has not yet been reported in the bibliography. There are only a few works in which the removal of phenolic compounds from saline wastewater has been studied [17,18], but they are performed with simulated water. However, there are several studies that consider the treatment of other table olive packaging effluents; as the global wastewater [19,20], the global wastewater excluding fermentation brines [18,21], the alkaline debittering wastewaters [22,23]and olive washing water [24]. In other papers, FTOP is treated by other techniques, such as electro-coagulation [25] or the biological treatment is combined with chemical or electrochemical processes [26,27].

The aim of this work is to perform a direct biological treatment of FTOP wastewater from a table olive packaging industry without previous dilution or physico-chemical treatment, by a gradual adaptation of activated sludge to high salinity and polyphenols. Difficulties for the treatment of these wastewaters are not only focused on high salinities but also on the eventual inhibitory effect of the polyphenols concentration. The experiments were performed in two sequential biological reactors (SBRs), and two different start-up strategies have been compared.

2. Material and methods

2.1. Analysis

For the tests, two different samples from the table olive packaging industry (FTOP 1 and FTOP 2) were used. The characterization of fermentation brines included the analysis of pH, conductivity, soluble COD (filtered to 0.45 μm), total phenols (Folin–Ciocalteu method), phenolic profile (analysis of simple phenolic compounds with UPLC-PDA analysis), sodium, chloride, turbidity, suspended solids (SS), volatile suspended solids (VSS) and total antioxidant activity (TAA). For the characterization of the SBRs effluents, pH, conductivity, soluble COD (filtered to 0.45 μm), turbidity and total phenols were monitored. In the last days of the start-up, in order to check phenols degradation, total phenols, phenolic profile, TAA and toxicity were measured. SS, VSS and microbial community analysis by fluorescence in situ hybridization (FISH) were measured to characterize the biomass in SBRs.

pH and conductivity measurements were carried out with pH-Meter GLP 21+ and EC-Meter GLP 31+ (CRISON), respectively. Turbidity was determined with a Turbidimeter D-112 from DINKO INSTRUMENTS. Suspended solids (SS) and volatile suspended solids (VSS) were measured according to APHA, 2005 [28]. Sodium and chloride ions and soluble COD were analyzed using kits and a Spectrophotometer DR600 (HACH LANGE).

2.1.1. Phenolic compounds and total antioxidant activity

For phenols measurement, all samples were previously treated in order to extract them according to El-Abbassi et al. [29]. The extracts were brought to dryness in a rotary evaporator (Rotavapor R-114 from BÜCHI) at 40 °C and the residue was dissolved in methanol. The extracts obtained were used for total phenols and UPLC-PDA analysis. Total phenols (simple phenolic and polyphenolic compounds) were measured spectrophotometrically according to the Folin–Ciocalteu method [30]. Results were expressed as ppm equivalent of tyrosol (mg TY/L). Phenolic profile was measured by liquid chromatography. UPLC-PDA analysis were carried out on Waters Acquity UPLC system (Milford, MA, USA) equipped with a binary solvent manager, sample manager, column compartment, and 2996 PDA detector, connected to Waters Masslynx 4.1 software. The separation was carried out using a Waters BEH C18 column (2.1 × 100 mm, 1.7 μm) at 40 °C. The optimal chromatographic conditions were established: solvent system, phase A, 1% formic acid in acetonitrile, and phase B, 1% formic acid in water; gradient conditions were as follows: 100% B at 0 min for 1 min to 55% A in 25 min, then 100% A at 30 min, held for 5 min, returned to 100% B in 2 min, and equilibrated for 3 min before the next injection; flow rate of 0.4 mL min^{-1} and injection volume of 5 μL. Results were expressed as ppm of phenolic compound tested.

Total antioxidant activity (TAA) was determined by the modified version of ABTS assay reported by Cassano et al. [31]. Results were expressed in terms of mM trolox equivalents.

2.1.2. Toxicity

The Microtox® was used for the estimation of the toxicity [32]. The light emission reduction of microorganisms *Vibrio fischeri* in contact with FTOP was measured. The effective concentration of contaminant (mg/L) which reduces a 50% of the intensity of light bacteria emission, after 15 min contact, is named EC_{50}. The toxicity results have been expressed in toxicity units (TU). This parameter