



# Assessment of crude glycerol for Enhanced Biological Phosphorus Removal: Stability and role of long chain fatty acids



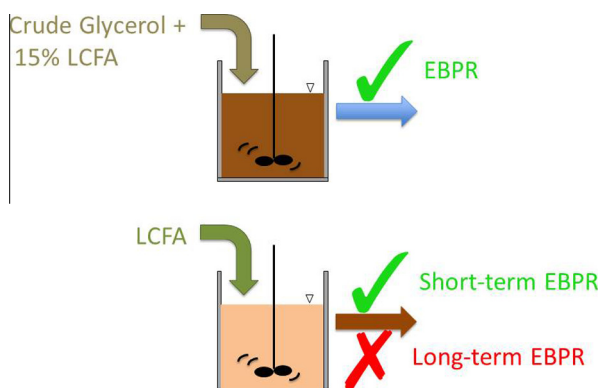
Carlota Tayà, Javier Guerrero, María Eugenia Suárez-Ojeda, Albert Guisasola\*, Juan Antonio Baeza

GENOCOV, Departament d'Enginyeria Química, Escola d'Enginyeria, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain

## HIGHLIGHTS

- Crude glycerol with low content of LCFA is a suitable carbon source for EBPR.
- EBPR activity with LCFA as sole carbon source is demonstrated for the first time.
- High LCFA content leads to its adsorption on the biomass surface and EBPR failure.
- Cell hydrophobicity increases with long-term utilization of LCFA.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Enhanced Biological Phosphorus Removal (EBPR) of urban wastewaters is usually limited by the available carbon source required by Polyphosphate Accumulating Organisms (PAO). External carbon sources as volatile fatty acids (VFA) or other pure organic compounds have been tested at lab scale demonstrating its ability to enhance PAO activity, but the application of this strategy at full-scale WWTPs is not cost-effective. The utilization of industrial by-products with some of these organic compounds provides lower cost, but it has the possible drawback of having inhibitory or toxic compounds to PAO. This study is focused on the utilization of crude glycerol, the industrial by-product generated in the biodiesel production, as a possible carbon source to enhance EBPR in carbon-limited urban wastewaters. Crude glycerol has non-negligible content of other organic compounds as methanol, salts, VFA and long chain fatty acids (LCFA). VFA and methanol have been demonstrated to enhance PAO activity, but there is no previous study about the effect of LCFA on PAO. This work presents the operation of an EBPR SBR system using crude glycerol as sole carbon source, studying also its long-term stability. The effect of LCFA is evaluated at short and long-term operation, demonstrating for the first time EBPR activity with LCFA as sole carbon source and its long-term failure due to the increased hydrophobicity of the sludge.

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## 1. Introduction

Enhanced Biological Phosphorus Removal (EBPR) is considered the most sustainable technology for P-removal from urban wastewater to meet the stricter discharge requirements of wastewater treatment plant (WWTP) (Broughton et al., 2008).

\* Corresponding author.

E-mail addresses: [carlota.taya@uab.cat](mailto:carlota.taya@uab.cat) (C. Tayà), [franciscojavier.guerrero@uab.cat](mailto:franciscojavier.guerrero@uab.cat) (J. Guerrero), [mariaeugenia.suarez@uab.cat](mailto:mariaeugenia.suarez@uab.cat) (M.E. Suárez-Ojeda), [albert.guisasola@uab.cat](mailto:albert.guisasola@uab.cat) (A. Guisasola), [juanantonio.baeza@uab.cat](mailto:juanantonio.baeza@uab.cat) (J.A. Baeza).

EBPR is based on the enrichment of activated sludge with polyphosphate accumulating organisms (PAO), which can take up carbon sources under anaerobic conditions, storing them as poly- $\beta$ -hydroxyalkanoates (PHA) for its utilisation when an electron acceptor as oxygen, nitrate or nitrite is available. Under anoxic or aerobic conditions, PAO are able to accumulate phosphorus (P) in excess, which is removed through the sludge waste. The effectiveness of EBPR depends on the nature of the carbon source used, being the presence of volatile fatty acids (VFA) a key factor to obtain a high P removal capacity (Chu et al., 1994; Randall et al., 1997a; Guerrero et al., 2011). Unfortunately, EBPR is often limited by the available organic carbon in the urban wastewater and hence, the utilisation of waste materials fermentable to VFA is a very attractive alternative to overcome this deficiency.

Crude glycerol is a by-product of biodiesel fuel production: 1 L of glycerol is produced for every 10 L of biodiesel fuel (Johnson and Taconi, 2007). This by-product has many impurities that together with the increase of its production have transformed it in a waste material with associated disposal costs (Yazdani and Gonzales, 2007; Escapa et al., 2009). Pure glycerol has been reported for wastewater treatment as a proper external carbon source for denitrification (Grabinska-Loniewska et al., 1985; Akunna et al., 1993; Bodík et al., 2009), denitritation (Torà et al., 2011) and EBPR (Guerrero et al., 2012a). Although these uses of pure glycerol seem promising, the utilization of crude glycerol with impurities as salts, methanol and long chain fatty acids (LCFA) faces new operational problems.

Methanol is the most common external carbon source used for denitrification, but its utilization for EBPR has conducted to numerous failures (Puig et al., 2008; Randall et al., 1997b) before being successfully reported in a recent work (Tayà et al., 2013). PAO-enriched biomass in a conventional anaerobic-aerobic sequencing batch reactor (SBR) was unable to survive using methanol as sole carbon source when a direct replacement for propionic acid in the work of Tayà et al. (2013). However, methanol-driven EBPR (P-removal using methanol as sole carbon source) was obtained when using a syntrophic consortium between methanol-degrading acetogens and PAO. The first would be in charge of converting methanol to by-products (mainly VFA), which could be used by PAO as carbon source (Tayà et al., 2013).

Regarding LCFA treatment, wastewaters with a high LCFA content are not effectively treated by high-rate anaerobic technology, mostly by two phenomena: (1) acute toxicity of LCFA against methanogens and acetogens, the two main trophic groups involved in LCFA degradation, and (2) LCFA adsorption onto the sludge. LCFA adsorbed on the surface of microorganisms causes steric hindrance which increases the resistance to mass transfer, decreasing nutrient release and uptake rates between microorganisms and media (Roy et al., 1986; Rinzema et al., 1994). Another widely reported problem of LCFA adsorption is sludge flotation, which usually leads to biomass washout (Alves et al., 2009). However, sludge flotation and washout occurs at concentrations far below the toxicity limit in anaerobic sludge. This might suggest that complete washout of sludge would occur prior to inhibition (Alves et al., 2001a,b). Fortunately, inhibition caused by LCFA seems to be a reversible process on anaerobic digestion reactions, which increases its activity in a short time after LCFA washout from the reactor (Pereira et al., 2003, 2005).

Therefore, the objective of this work was to study the feasibility of using crude glycerol as sole carbon source in EBPR, evaluating its long-term stability and the problems associated to the presence of LCFA. Step-wise increase of crude glycerol in a propionic fed PAO-enriched sludge was conducted in an anaerobic/aerobic sequencing batch reactor (SBR). The aim was obtaining a microbial community able to ferment crude glycerol to propionic acid for PAO uptake to achieve stable EBPR operation. The effect of LCFA was also studied as a sole carbon source in the same SBR configuration.

## 2. Material and methods

### 2.1. Equipments

Two 10 L SBRs (named SBR<sub>GLY</sub> and SBR<sub>LCFA</sub>) were used in this study. They were monitored for dissolved oxygen (DO) (Hamilton, Oxyferm 120 probe), pH (Hamilton, polilyte Pro120 probe), ORP and temperature. Both SBR contained a PAO-enriched sludge developed with propionic acid as sole carbon source and operated with 4 cycles per day with configuration of 2 h anaerobic phase, 3.5 h aerobic phase, 25 min of settling and 5 min to extract 5 L of the supernatant. Temperature was controlled at  $25 \pm 1$  °C. HCl (1 M) and NaOH (1 M) were added to control the pH at  $7.5 \pm 0.1$ . A constant nitrogen gas flow was sparged during the anaerobic phase to maintain strict anaerobic conditions. DO was maintained from 2.5 to 3.5 mg L<sup>-1</sup> in the aerobic phase to avoid oxygen limitations. A volume of 5 L (synthetic wastewater + concentrated carbon solution) was added during the first 5 min of the cycle, resulting in a HRT of 12 h. The synthetic wastewater solution (4.97 L) consisted of (mg L<sup>-1</sup> in reverse osmosis water): 110.5 KH<sub>2</sub>PO<sub>4</sub>, 83.7 K<sub>2</sub>HPO<sub>4</sub>, 100 NH<sub>4</sub>Cl, 43.9 MgSO<sub>4</sub>·7H<sub>2</sub>O, 160 MgCl<sub>2</sub>·6H<sub>2</sub>O, 42 CaCl<sub>2</sub>·2H<sub>2</sub>O, 50 allylthiourea (ATU) to inhibit nitrification and 30 mL of trace element solution. The trace element solution (g L<sup>-1</sup>) used consisted of: 1.5 FeCl<sub>3</sub>·6H<sub>2</sub>O, 0.15 H<sub>3</sub>BO<sub>3</sub>, 0.03 CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.18 KI, 0.12 MnCl<sub>2</sub>·4H<sub>2</sub>O, 0.06 Na<sub>2</sub>MoO<sub>4</sub>·4H<sub>2</sub>O, 0.12 ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.15 CoCl<sub>2</sub>·6H<sub>2</sub>O and 68.5 mL EDTA 0.5 M (Smolders et al., 1994). The initial phosphorus concentration was fixed at 20 mg P-PO<sub>4</sub><sup>3-</sup> L<sup>-1</sup> and COD concentration at 225 mg COD L<sup>-1</sup> (50% as acetic acid and 50% as propionic acid) for PAO-enrichment. The reactors were enriched for more than 90 days reaching a pseudo-steady state with successful biological P removal. The sludge residence time was kept at 15 days by periodic wastage at the end of the aerobic phase.

After the enrichment period, SBR<sub>GLY</sub> was fed with crude glycerol (100 mg COD L<sup>-1</sup>) and VFA (112.5 mg COD L<sup>-1</sup> as acetic acid and 112.5 mg COD L<sup>-1</sup> as propionic acid) during 26 days. Then, it was operated from day 27 to day 42 with crude glycerol (300 mg COD L<sup>-1</sup>) as sole carbon source. The crude glycerol used was provided by *Stocks del Vallés S.A.* (Montmeló, Barcelona, Spain) a company producing biodiesel from recycled animal fats and vegetables oils. The crude glycerol contained around 15% of LCFA and 5–7% of methanol. From day 43 until the end of the experiment the crude glycerol concentration was reduced to 265 mg COD L<sup>-1</sup>. The rest of conditions of SBR<sub>GLY</sub> were kept constant along the operation.

Regarding SBR<sub>LCFA</sub>, after the 90 days of enrichment period, it was fed with 260 mg COD L<sup>-1</sup> of mixed carbon source containing LCFA (50 mg COD L<sup>-1</sup> as propionic acid, 50 mg COD L<sup>-1</sup> as acetic acid and 160 mg COD L<sup>-1</sup> as LCFA) during 27 days. The LCFA composition was 50% myristic acid and 50% palmitic acid in terms of mg COD L<sup>-1</sup>. After this acclimation period, SBR<sub>LCFA</sub> was fed with LCFA as sole carbon source (325 mg COD L<sup>-1</sup>) for 34 days (from day 27 to day 61). The EBPR activity of the reactor was progressively lost during the operation with LCFA. Then, it was fed during 20 days (from day 62 to day 82) with 200 mg COD L<sup>-1</sup> of VFA (50% as acetic acid and 50% as propionic acid) to recover its PAO activity. From day 82 on, SBR<sub>LCFA</sub> was switched again to LCFA although at lower concentration (200 mg COD L<sup>-1</sup>).

### 2.2. Chemical and microbiological analyses

Propionic and acetic acid concentrations in 0.22  $\mu$ m filtered samples were analysed by using a gas chromatograph (GC Agilent Technologies 7820 A) equipped with a BP21 SGE column (30 m 0.25 mm 0.25 mm; length internal diameter film thickness)

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