

Morphology and physiology of anaerobic granular sludge exposed to organic solvents

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Abstract The use of quantitative image analysis techniques, together with physiological information might be used to monitor and detect operational problems in advance to reactor performance failure. Industrial organic solvents such as white spirit are potentially harmful to granular sludge. In preliminary batch assays, 33 mg.L⁻¹ of solvent caused 50% relative biomass activity loss. In an Expanded Granular Sludge Blanket reactor fed with 40 mg.L⁻¹ of solvent, during 222 h, the reactor performance seemed to be unaffected, presenting COD removal efficiency constantly above 95 %. However, in the first hours of exposure the specific acetoclastic and the specific hydrogenotrophic methanogenic activities decreased by 29 and 21 %, respectively. The % of projected aggregates area with equivalent diameter (D_{eq}) higher than 1 mm decreased from 81 to 53 % and consequently the % of projected area of small aggregates increased. The average equivalent diameter of the aggregates larger than 2 mm decreased as well as the settling velocity, showed that the granules experienced fragmentation phenomenon caused by the solvent shock load. The ratio between total filaments length and total aggregates projected area (LfA) increased 2 days before effluent volatile suspended solids, suggesting that LfA could be an early-warning indicator of washout events.

Keywords Anaerobic granular sludge; hydrocarbons; methanogenic activity; quantitative image analysis; shock load; solvent.

Introduction

Organic solvents comprise a group of various liquid hydrocarbons obtained from the intermediate products of the distillation of crude. They are used in the chemical industry as solvent for extraction, cleaning and degreasing, and, in aerosols, paints, wood preservatives, lacquers, varnishes, and asphalt products. The destiny of petroleum type pollutants in the environment has been investigated in many studies (Antic et al., 2006) and the biodegradation of many components of petroleum hydrocarbons has been reported in a variety of terrestrial and marine ecosystems (Margesin and Schinner, 2001). Due to the hydrophobic character, these compounds tend to accumulate in solid matter such as sludge (El-Hadj et al., 2006). It is nowadays undeniable that various toxic organic compounds, such as surfactants and hydrocarbons, like the organic solvent White Spirit (McGovern et al., 2002) are found in sewage and industrial wastewaters.

The common use of organic solvents leads to high risks of wastewater contamination, and, can be toxic to biological treatment systems (Enright et al., 2005). Together with the capability of adsorption into the biomass, the hazard to anaerobic digesters is considerable. It has been reported studies about the degradation of hydrocarbons in anaerobic systems (Schink, 2002; Christensen et al., 2004). However, although all the knowledge about the process itself, little is known about the effects caused by solvents in anaerobic granular sludge.

Combination of morphological analysis at micro and macro structure levels with physiological activity of anaerobic granular sludge and reactor performance may supply pertinent information about stability of high rate anaerobic reactors in the presence of potential disturbances. Similar techniques were already applied in surfactants shock loads (Costa et al., 2007). This work focuses on the study of the effects caused by the exposure of anaerobic granular sludge to the organic solvent White Spirit, which is the most widely used solvent in the paint industry.

Methods

Solvent characterization

The solvent tested is a paraffin-derived clear, transparent liquid which is a common organic solvent used as an extraction, cleaning and degreasing industrial processes. It is a mixture of saturated aliphatic and alicyclic C7 to C12 hydrocarbons with a maximum content of 25% of C7 to C12 alkyl aromatic hydrocarbons. The solvent present a relative density (15°C) of about 0.785 kg.m⁻³.

Toxicity assays

Toxicity batch experiments were performed by adding acetate (30 mM) and increasing solvent concentrations (1.6, 7.9, 39.3, and 78.5 mg.L⁻¹) to the sludge, in batch vials. Working volume was 12.5 ml and total volume 25 ml. Fifty percent inhibition concentration (IC₅₀) was defined as the solvent concentration that caused 50% relative acetoclastic activity loss. All batch experiments were performed in triplicate assays.

Experimental set-up

A Plexiglas Expanded Granular Sludge Blanket (EGSB) reactor with a height of 1.95 m and internal diameter of 21 mm was used. The working volume was 1.15 L and the superficial velocity was 4.0 m.h⁻¹. Temperature was kept at 37 ± 1 °C by means of an external jacket for water circulation.

Inoculum and substrate

400 mL of granular sludge was used as the inoculum of the EGSB reactor. The biomass was characterised in terms of specific methanogenic activity with acetate (212 ± 27 mLCH_{4@STP}.gVSS⁻¹.d⁻¹) and H₂/CO₂ (910 ± 85 mLCH_{4@STP}.gVSS⁻¹.d⁻¹) as substrates. The morphological characteristics assessed by quantitative image analysis were: Filaments length/total area of aggregates, LfA = 24 mm⁻¹; total filament length/VSS = 1800 m.gVSS⁻¹; VSS/total projected area = 13 g.m⁻². The volatile suspended solids were 26.5 g.L⁻¹ and settling velocity was 26 ± 14 m.h⁻¹.

The reactors were fed with ethanol at a concentration of 1.5 gCOD.L⁻¹. Sodium bicarbonate was added as the alkalinity source (2 g.L⁻¹). Micro and macronutrients were added according to Zehnder et al. (1980). When the reactor was operating in steady-state, the solvent (40 mg.L⁻¹) was mixed with the feeding, with constant agitation, during 222 hours. The recovery phase was followed through 7 days.

Routine analysis

The COD and VSS were determined according to Standard Methods (Standard Methods, 1998). Biogas flow rate was measured by a *Ritter Milligascounter* (Dr. Ing. Ritter Apparatebau GmbH, Bochum, Germany). Methane content of biogas was determined by gas chromatography using a *Porapak Q* (100 - 180 mesh) column, with Helium as the carrier gas at 30 mL/min and thermal conductivity detector. Temperatures of the detector, injector and oven were 110, 110 and 35 °C, respectively. Volatile fatty acids (VFA) and ethanol were determined by high performance liquid chromatography using an *HPLC* (Jasco, Japan) with a *Chrompack column* (6.5 x 30 mm²); sulfuric acid (0.01 N) at a flow rate of 0.7 mL.min⁻¹ was used as mobile phase. Column temperature was set at 60 °C. Detection of VFA and ethanol was made sequentially with an UV detector at 210 nm and a RI detector, respectively.

Sludge Sampling and Dilution

The methodology and procedures for sludge sampling and dilution are described in Araya-Kroff et al. (2004). All the sludge samples were characterized by image analysis, SMA assays, VSS content and settling velocity. In these experiments, the optimal dilution was 1:5.

Image acquisition, processing, and analysis

Procedures for image acquisition of filaments, micro-aggregates (D_{eq} < 0.2 mm), and macro-aggregates (D_{eq} ≥ 0.2 mm), images, are described in Costa et al. (2007). Image processing and

analysis was accomplished by means of three programmes developed in Matlab (The Mathworks, Inc., Natick), for filaments, micro, and macro-aggregates. Descriptions of the programs can be found in Amaral (2003).

Morphological parameters:

Morphological parameters representing the dynamic evolution of filaments and aggregates inside the reactor were calculated as: $TL/VSS = L_{spec} / VSS$ and $VSS/TA = VSS / (A_{spec(<0.2mm)} + A_{spec(\geq 0.2mm)})$, respectively. Where, VSS are the volatile suspended solids present in each sample, $A_{spec(<0.2mm)}$ and $A_{spec(\geq 0.2mm)}$ are the specific aggregate area ratio for aggregates of equivalent diameter $<$ and ≥ 0.2 mm respectively, and, L_{spec} is the specific filaments length. A morphological parameter based on the ratio of total filament length to total aggregates projected area (LfA) is determined as:

$$LfA = L_{spec} / \left(A_{spec(<0.2mm)} + A_{spec(\geq 0.2mm)} \right)$$

Specific Methanogenic Activity (SMA) assays

The SMA assays were performed using a pressure transducer technique (Colleran et al., 1992). The Specific Acetoclastic Activity (SAA) was measured in the presence of acetate (30 mM) and the Specific Hydrogenotrophic Methanogenic Activity (SHMA) was measured in the presence of H_2/CO_2 80:20v/v, at 1 bar. No trace-nutrients were added. Methane was measured by gas chromatography with helium as the carrier gas and a TCD detector.

Settling velocity

The settling velocity was measured by depositing several biomass samples in the top of a column filled with water. Afterwards the time that each particle takes to cover the 320 mm of the column was measured using a chronometer that automatically registers that time in an Excel spreadsheet. Then the average of all individual sedimentation velocities was determined. More than 150 particles were considered in the calculation.

Results And Discussion

Toxicity Test

Previously to exposure in a lab-scale reactor, a series of batch assays was performed to determine the concentration of solvent that caused 50% relative activity loss (IC_{50}). It was observed a decrease of methanogenic activity of the biomass proportional to the increase of solvent concentration, and, it was found that the IC_{50} was 33 mg.L^{-1} .

Reactor Performance

The EGSB reactor fed with $4.8 \text{ kgCOD.m}^{-3}.\text{d}^{-1}$ and hydraulic retention time of 8 hours, presented COD removal efficiency higher than 95 %. When the reactor showed stable performance it was exposed to 40 mg.L^{-1} of solvent. During the exposure phase the OLR increased to $6 \text{ kgCOD.m}^{-3}.\text{d}^{-1}$ (Fig. 1a). The reactor performance seemed to be unaffected by the shock load, since the COD removal efficiency remain, constantly, above 95 %. This fact may be explained by the low OLR fed to the reactor. The pH was 7.8 ± 0.1 throughout the exposure phase. In the recovery phase, the COD removal efficiency dropped during the first 3 days, reaching a minimum of 33 % (Fig. 1a). Simultaneously, the effluent volatile fatty acids increased, mainly acetate that presented concentrations of 500 mgCOD.L^{-1} (data not showed). The petroleum hydrocarbons tends to bind into the biomass (Christensen et al., 2004) being expected a continuous morphological deterioration of the granules. However, 90 hours after the end of the exposure time, the reactor performance returned to a COD removal efficiency higher than 95%.

Specific Methanogenic Activity (SMA)

The solvent shock load caused a decrease of 21% in the SHMA, during the first 24 hours of exposure (Fig 1b). Relatively to the SAA it was observed a continuous decrease during the first 73 hours of shock load, with 29% of inhibition (Fig 1b). The acetoclastic bacteria, which were protected inside the out layer of granules, became more vulnerable to the effects of solvent, because of their disintegration (Fig. 2c). Afterwards the SHMA and SAA stabilised around 760 and 150 $\text{mLCH}_4@_{\text{STP}}\cdot\text{gVSS}^{-1}\cdot\text{d}^{-1}$, respectively, until the end of exposure phase. In the recovery phase, a sudden increase in the SMA was observed. Afterwards, the SMA decreased again.

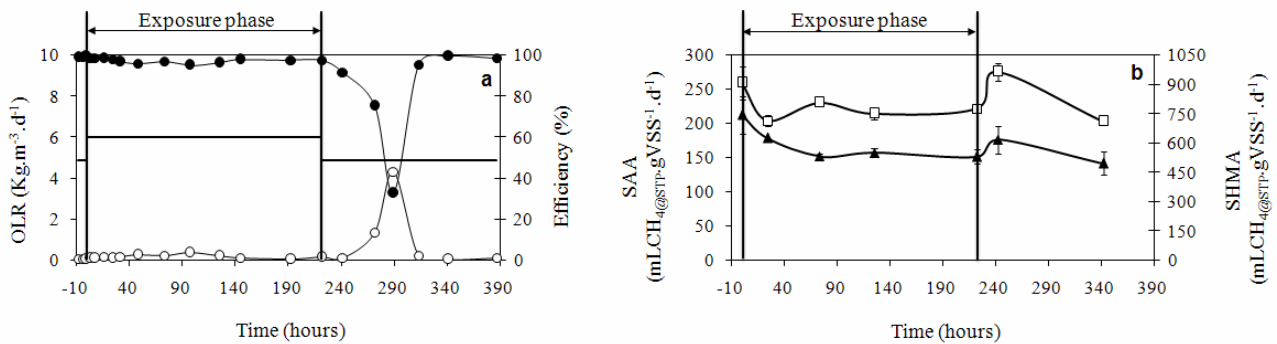


Figure 1 – Time course of: (a) influent organic loading rate (—), effluent organic loading rate (o), and, COD removal efficiency (●); and, (b) specific methanogenic activity (SMA) in presence of acetate (▲) and H_2/CO_2 (□) as substrates.

Image Analysis

The main changes caused by the shock load were observed in the granules distribution sizes (Fig. 2c). The % of projected area of aggregates with equivalent diameter (Deq) higher than 1 mm decreased from 81 to 53%, and, consequently, the % of projected area of small aggregates ($0.1 \leq \text{Deq} (\text{mm}) < 1$) increased from 18 to 46%. The average Deq of macroflocs decreased consistently from 0.9 to 0.6 mm (Fig. 2d). These results indicate that the bounding of the hydrocarbons to granules caused its fragmentation/erosion with a consequent decrease of Deq and filaments release. The increase of TL/VSS from 1300 to 2000 $\text{m}\cdot\text{g}^{-1}$ 40 hours after the beginning of the exposure (Fig. 2b) and the high dependence of LfA with filaments, allow us to previously identify an increase in free and protruding filamentous forms in the bulk before being washed out from the reactor. As already reported in Amaral et al. (2004) and Costa et al. (2007), the LfA parameter was also in this case, able to be an early-warning indicator of washout events during the solvent shock load. Effectively, during this experiment the LfA increased 35% between hours 24 and 73 (Fig. 2a), reaching the value of 29 mm^{-1} . In the same figure, it is showed that the effluent volatile suspended solids (VSS), only achieved its maximum value at 125 h, *i.e.* 52 hours after LfA . Until the end of shock load the TL/VSS and LfA parameters decreased to 1100 $\text{m}\cdot\text{g}^{-1}$ and 14 mm^{-1} , respectively. Relatively to effluent VSS after a decrease it was observed a new peak of 82 $\text{mg}\cdot\text{L}^{-1}$ at the end of exposure phase. These results suggest a washout of small aggregates at this time. This hypothesis was enhanced by the increase of the % of projected area of aggregates with $\text{Deq} \geq 1$ mm (Fig. 2c) during the first hours of recovery phase. A decrease in the average of settling velocity (v_s) from 26 to 14 $\text{m}\cdot\text{h}^{-1}$ was observed (Fig. 2d). However, the apparent granules density (VSS/TA) was almost constant, around 13 $\text{g}\cdot\text{m}^{-2}$ (Fig. 2b). Therefore, the granules fragmentation/erosion may have caused the v_s decrease, as observed by the similar trends of the averages of settling velocity and macroflocs Deq in Fig. 2d.

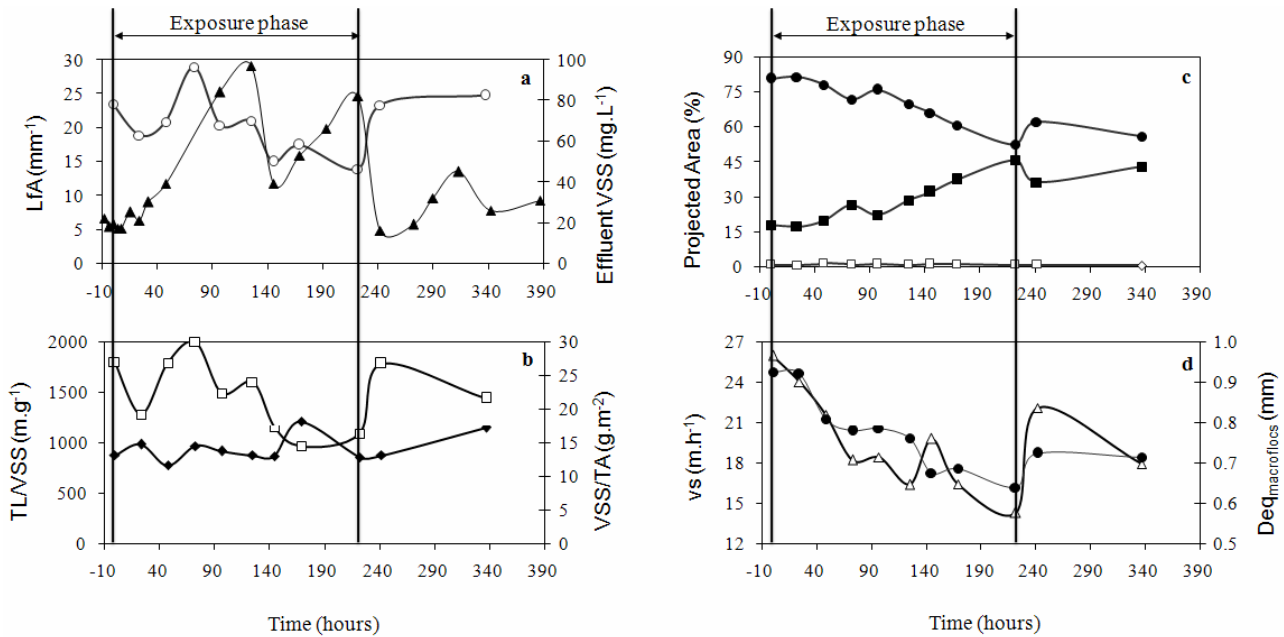


Figure 2 – Time course of morphological parameters: (a) dynamics between total filament length and total aggregates projected area (LfA, \circ) and effluent volatile suspended solids (effluent VSS, \blacktriangle); (b) total filament length per volatile suspended solids (TL/VSS, \square) and volatile suspended solids per total aggregates projected area (VSS/TA, \blacklozenge); (c) percentage of projected area for different size ranges of aggregates equivalent diameter ($Deq \geq 1$ mm, \bullet ; $0.1 \leq Deq$ (mm) < 1, \blacksquare ; $Deq < 0.1$, \diamond); and, (d) averages of the settling velocity (vs , Δ) and the equivalent diameter of macroflocs ($Deq_{\text{macroflocs}}$, \bullet).

Conclusions

The overall reactor performance was unaffected by a “White Spirit” Shock load of 9 days. The COD removal efficiency was regularly higher than 95%, possibly because the reactor was underloaded. During the recovery phase the efficiency decreased to 33 %, possibly due to the accumulation of solvent into the biomass. However, the reactor recovered rapidly. A decrease of 21% was observed in the SHMA whereas the SAA decreased 29% few hours after the exposure.

Phenomenon of granular erosion and/or fragmentation and filaments release were identified and quantified, during the exposure of anaerobic granular sludge to organic solvents. The % of projected area of aggregates with $Deq \geq 1$ mm decreased from 81 to 53%. The average settling velocity (vs) decreased from 26 to 14 m.h^{-1} , although the VSS/TA was almost constant through the exposure phase, symptomatic that the granules density remained approximately unchanged. The decrease in settling velocity was mainly caused by the decrease in size as a result of erosion/fragmentation of granules. The peak in effluent VSS was observed 52 hours after LfA increased 35 %, suggesting that it could be used as an early-warning indicator of washout events when anaerobic granular sludge is exposed to organic solvents used in industrial cleaning processes such as “white spirit”.

Acknowledgements

We grateful acknowledge the financial support to J.C. Costa and I. Moita through the grant SFRH/BD/13317/2003 and the project POCTI/AMB/60141/2001, respectively, from the Fundação para a Ciência e a Tecnologia (Portugal).

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