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Performance and microbial communities of a continuous stirred tank anaerobic reactor treating two-phases olive mill solid wastes at low organic loading rates

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Abstract

A study of the performance and microbial communities of a continuous stirred tank reactor (CSTR) treating two-phases olive mill solid wastes (OMSW) was carried out at laboratory-scale. The reactor operated at a mesophilic temperature (35 °C) and an influent substrate concentration of 162 g total chemical oxygen demand (COD) L^{-1} and 126 g volatile solids (VS) L^{-1}. The data analyzed in this work corresponded to a range of organic loading rates (OLR) of between 0.75 and 3.00 g COD L^{-1} d^{-1}, getting removal efficiencies in the range of 97.0–95.6%. Methane production rate increased from 0.164 to 0.659 L CH_4 $L_{reactor}{}^{-1}$ d^{-1} when the OLR increased within the tested range. Methane yield coefficients were 0.225 L CH_4 g^{-1} COD removed and 0.290 L CH_4 g^{-1} VS removed and were virtually independent of the OLR applied. A molecular characterization of the microbial communities involved in the process was also accomplished. Molecular identification of microbial species was performed by PCR amplification of 16S ribosomal RNA genes, denaturing gradient gel electrophoresis (DGGE), cloning and sequencing. Among the predominant microorganisms in the bioreactor, the Firmicutes (mainly represented by Clostridiales) were the most abundant group, followed by the Chloroflexi and the Gamma-Proteobacteria (*Pseudomonas* species as the major representative). Other bacterial groups detected in the bioreactor were the Actinobacteria, Bacteroidetes and Deferribacteres. Among the Archaea, the methanogen *Methanosaeta concilii* was the most representative species.

Keywords: Two-phases olive mill solid waste; Anaerobic digestion; Performance; Microbial community

1. Introduction

Olive oil manufacturing has a great importance in the Mediterranean basin. This industry has undergone important changes in the last years (Alba et al., 1990).

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Lately, a two-phases centrifugation process, separating the olive oil fraction from the vegetable solid material and vegetation water has been introduced (Borja et al., 1996). This improvement reduces water consumption (Alba, 1993). Currently, more than 90% of the Spanish olive oil is extracted with the two-phases system.

The aqueous solid waste from a primary centrifugation, also called olive mill solid waste (OMSW), has an average composition of: 60–70% water, 13–15% lignin, 18–20% cellulose and hemicellulose, 2.5–3% olive oil retained in the pulp and about 2.5% mineral solids. Among the organic components, the following components can be highlighted: sugars (3%), volatile fatty acids (C2–C7) (1%), polyalcohols (0.2%), proteins (1.5%), polyphenols (0.2%) and other pigments (0.5%) (Borja et al., 2002).

The high pollution potential and large volumes of solid wastes pose serious environmental problems. Only in Spain an average of 978,800 tonnes of olive oil are produced per year (IOOC, 2004), yielding around 4 million tonnes of two-phases OMSW per year.

The anaerobic digestion is a biological process in which a complex community of microorganisms work in a stable, self-regulating steady state converting waste organic matter into a mixture of carbon dioxide and methane gases (Kaspar and Wuhrmann, 1978; Zeikus, 1980; Gujer and Zehnder, 1983; Speece, 1983; Sterling et al., 2001).

Anaerobic digestion of vegetable liquid, semisolid and solid wastes is an alternative to other treatments. Several agro-industrial residues, including wastewaters (Tsonis and Grigoropoulos, 1993; Harndi, 1996; Ubay and Ozturk, 1997; Ugoji, 1997; Borja et al., 2001, 2004), semisolid and solid wastes (Weiland, 1993; Lastella et al., 2002; Rani and Nand, 2004) can be anaerobically treated obtaining efficient stabilization of solids and energy recovery. Anaerobic digestion has a great number of advantages: low nutrient requirements, energy savings, generation of low quantities of sludge, excellent waste stabilization, production of biogas (methane) without the requirement of pre-treatments of the residues (Kang and Weiland, 1992; Weiland, 1993; Yadvika et al., 2004).

A previous study (Borja et al., 2002) has shown the anaerobic digestibility of two-phases olive mill solid waste operating at four different influent substrate concentrations ranging from 34.5 to 150.3 g COD L^{-1} corresponding to 20–80% OMSW. The two-phases OMSW utilized in these anaerobic experiments derived from ripened olives processed at the end of an annual campaign. Chemical oxygen demand (COD) and volatile solids (VS) removal efficiencies of 88.4 and 90.9%, respectively, were achieved at an organic loading rate (OLR) of 12.02 g COD L^{-1} d^{-1} for the most concentrated substrate (OMSW 80%) with a maximum CH_4 production rate of 2.12 L CH_4 L^{-1} d^{-1} for this OLR with a hydraulic retention time (HRT) of 12.5 days. The CH_4 yield coefficients ranged from 0.30 to 0.20 L CH_4 at standard temperature and pressure conditions (STP) per gram of COD removed for the OMSW concentrations tested (Borja et al., 2002).

The aim of this work was to carry out an evaluation of the substrate utilization and methane production rates in the anaerobic digestion of two-phases OMSW using low organic loading rates. Simultaneously a molecular characterization of the microbial communities involved in this anaerobic digestion process of two-phases OMSW was also carried out by using a stirred tank reactor operating at mesophilic temperature (35 °C). Moreover, given that the anaerobic digestion process of high strength waste can suffer a destabilization and a decrease in the overall efficiency when high OLR are processed, affecting simultaneously to the activity and metabolism of bacteria involved in the system, the present study was focussed in the study of the operational parameters and microbial communities corresponding to low levels of OLR, for which the high stability and good performance of the process are assured.

2. Material and methods

2.1. Equipment

Experiments were carried out in an anaerobic stirred tank reactor (2 L working volume) with a thermostatic jacket set at 35 °C. The reactor was fed daily by means of an external feeder and liquid effluent removed daily through a hydraulic seal, comprising 25 cm liquid column, designed to prevent air from entering the reactor and biogas from leaving it.

The volume of CH_4 produced in the process was measured using a 5 L Mariotte reservoir (Martín et al., 1991) fitted to the reactor. CO_2 produced in the process was collected by bubbling the gas mixture through a NaOH solution (3 M).