

Anaerobic digestion process for biogas and biomolecules production: microflora identification and characterization

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HIGHLIGHTS

The anaerobic process was efficient in organic matter removal. During the process, an interesting compound as quercetin was produced inside of reactor. Phylogenetic analysis showed the presence of phylotypes affiliated with gamma-*Proteobacteria*, *Chloroflexi*, and *Bacteroidetes*. Archaea were represented by phylotypes belonging to the genus *Methanosarcina* and *Methanosaeta*.

Keywords

Anaerobic digestion, olive mill wastewater, TGGE fingerprinting, phenolic compounds, microbiota characterization

INTRODUCTION

Several agro-industrial processes in Mediterranean countries generate effluents, often containing recalcitrant molecules, which are an environmental problem and have to be disposed. This is the case of olive mill wastewater (OMW). Raw effluent was digested into an up-flow anaerobic hybrid reactor, using the complementary substrate (piggery effluent) concept, to evaluate phenols removal capacity and the microbial consortium composition that occurred during the anaerobic process. OMW valorisation was maximized considering its ability to provide compounds of industrial interest, as phenolic compounds with antiradical activity.

The aim of this study was to correlate the phenols profile with diversity of microbiota and to evaluate bacterial and archaeal populations that occurred during the anaerobic digestion process, using temperature gradient gel electrophoresis (TGGE) of PCR amplified 16S rRNA gene fragments.

MATERIALS & METHODS

OMW was digested anaerobically in a 2L up-flow hybrid digester complemented with a piggery effluent (PE), working under different operational conditions. Samples were taken from the inlet and outlet of the hybrid digester when organic loading rate was increased to 3, 4, 6 and 8 kg COD m⁻³d⁻¹, respectively, for microbial and physicochemical characterization.

TGGE of PCR amplified 16S rRNA gene fragments was used to evaluate the composition of microbial populations. TGGE bands were reamplified and sequenced to identify the community members. Each sequence was submitted to a BLAST search to determine phylogenetic affiliations.

Quantitative and qualitative analyses of phenols were made by HPLC (La Cara *et al.* 2012), total phenols were evaluated by a modified Singleton and Rossi (1965) method and COD was determined using test kits (Merck).

RESULTS AND DISCUSSION

The reactor was operated at an OLR between $3.3 \text{ kg}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ and $8.0 \text{ kg}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$, for 300 days. A good biogas quality was detected throughout the experiment with a simultaneous increasing of biogas production, reaching $3.16 \text{ m}^3\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ (Gonçalves *et al.* 2012). The removed phenolic fraction was constant between 40% and 58% of total phenols. Piggery effluent is an interesting alternative to amend OMW stream and to enable its biodegradation (achieving 81% COD removal).

HPLC sample analyses showed several peaks corresponding to different phenols among which some compounds were identified: phenyl acids (gallic, caffeic and ferulic acid), phenyl alcohols (hydroxytyrosol, tyrosol), catechin, rutin, quercetin and oleuropein. Oleuropein was the main phenolic compound present in the substrate before and after anaerobic digestion. A general decrease in concentration of the identified phenolic compounds as gallic acid, hydroxytyrosol, and tyrosol was observed. The exception is related to the quercetin whose concentration was increased during anaerobic process in almost operational situations. The quercetin is a flavonoid widely distributed in nature with antioxidants properties that act as a scavenger substance against the free radical formation in the human body (Formica and Regelson, 1995). Thus, after the OMW treatment by anaerobic digestion to produce biomethane, the remaining flow yet contain useful compounds with antiradical activity.

TGGE bands were reamplified and sequenced in order to determine the composition of the microbial communities in the up-flow hybrid digester in close association with step-increases on OLR. TGGE fingerprinting analysis revealed a low diversity in bacterial and archaeal communities. The five nucleotide sequences obtained for bacterial populations affiliated with gamma-Proteobacteria, Chloroflexi and Bacteroidetes. The archaeal populations were mainly represented by four phylotypes belonging to the genus *Methanosarcina* and *Methanosaeta*.

The implemented process allows solving the environmental problem, obtaining an energy carrier gas and additionally selecting useful microorganisms able to perform a complex biotransformation leading to biomolecules of industrial interest.

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