Bioresource Technology 201 (2016) 337–346

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Microbial community structure associated with the high loading anaerobic codigestion of olive mill and abattoir wastewaters

Hana Gannoun [a,b], Ilhem Omri [a], Rakia Chouari [d], Eltaief Khelifi [a], Sajiaa keskes [a], Jean-Jacques Godon [c], Moktar Hamdi [a], Abdelghani Sghir [e,f], Hassib Bouallagui [a,*]

[a] Université de Carthage, LR: LETMi, INSAT, B.P. 676, 1080 Tunis, Tunisia
[b] Université de Tunis El Manar, ISSBAT, 9, Avenue Zouhaïer Essafi, 1006 Tunis, Tunisia
[c] INRA U050, LBE, Avenue des Étangs, F-11100 Narbonne, France
[d] Faculté des Sciences de Bizerte, Unité de Toxicologie Végétale et Biologie moléculaire des micro-organismes (UR11-ES-32), Tunisia
[e] Université d'Evry Val d'Essonne (UEVE), Evry, France
[f] CEA, Institut de Génomique, Genoscope, Evry, France

HIGHLIGHTS

- The anaerobic digestion of olive mill and abattoir wastewaters was investigated.
- Effects of temperature and loading on the microbial structure were examined.
- PCR-SSCP showed significant dynamic of both bacteria and archaea.
- The high COD removal and biogas yield were achieved at 55 °C.
- *Methanobacteriales* and *Thermoplasmales* are predominant archaea.

ARTICLE INFO

Article history:
Received 4 October 2015
Received in revised form 17 November 2015
Accepted 18 November 2015
Available online 2 December 2015

Keywords:
Olive mill wastewater
Anaerobic digestion
Upflow anaerobic filter
Temperature
Microbial diversity

ABSTRACT

The effect of increasing the organic loading rates (OLRs) on the performance of the anaerobic codigestion of olive mill (OMW) and abattoir wastewaters (AW) was investigated under mesophilic and thermophilic conditions. The structure of the microbial community was also monitored. Increasing OLR to 9 g of chemical oxygen demand (COD) L^{-1} d^{-1} affected significantly the biogas yield and microbial diversity at 35 °C. However, at 55 °C digester remained stable until OLR of 12 g of COD L^{-1} d^{-1} with higher COD removal (80%) and biogas yield (0.52 L g^{-1} COD removed). Significant differences in the bacterial communities were detected between mesophilic and thermophilic conditions. The dominant phyla detected in the digester at both phases were the *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, *Synergistetes* and *Spirochaete*. However, *Verrucomicrobia*, *Proteobacteria* and the candidate division *BRC1* were only detected at thermophilic conditions. The *Methanobacteriales and the Thermoplasmales* were found as a high predominant archaeal member in the anaerobic sludge.

1. Introduction

Anaerobic codigestion of organic matter in wastewaters, coming from animal and vegetable agro- industries, to produce biogas is an alternative solution for decentralized waste management (Misi and Forster, 2002). However, in several cases, the application of anaerobic digestion for the treatment of these wastewaters may be hindered by various factors. Therefore, the biodegradation rate and the biogas production may be directly affected by inadequate nutrient balances and the presence of toxic compounds (Chen et al., 2008).

The nature of animal and vegetable agro-industry wastewaters, having complementary characteristics, can be also overcome by co-digestion. It will be an advantageous way to dilute toxicants and to improve the nutrient balance (Yen and Brune, 2007). Moreover, co-digestion contributes to improve plant profitability and to increase methane yield. Several studies have shown that multi-component mixtures of agro-industrial wastewaters can be digested successfully, although with some mixtures a degree of both synergism and antagonism were occurred (Misi and Forster, 2002).

* Corresponding author. Tel.: +216 22 524406; fax: +216 71 704329.
E-mail address: hassibbouallagui@yahoo.fr (H. Bouallagui).

http://dx.doi.org/10.1016/j.biortech.2015.11.050
0960-8524/

The successful application of anaerobic technology depends on several factors such as the process configuration, operational conditions and microorganisms. Previous study suggested that by adjusting the substrate concentration and the initial solid loading rate, improved the performances of anaerobic digestion (Fernandez et al., 2005). Mixing different kinds of wastes also improved the digestion of organic wastes. In addition, numerous environmental factors affect the performance of anaerobic digesters, such as low pH, ammonia inhibition, and the accumulation of volatile fatty acids (VFAs). They could inhibit the activity of anaerobic microorganisms (Chen et al., 2008).

Knowledge of the microbial aspects of the co-digestion of various co-substrates is still incomplete despite several studies focused on bacteria and methanogenic archaea. In order to better understand the functions of microbial community, a full description of the microbial ecosystem is required. The possibility of identifying specific populations of microorganisms without the need to isolate them is revolutionizing microbial ecology. Several works have been made to analyze the bacterial community structure of anaerobic sludge by molecular tools based on the analysis of 16S rRNA gene sequences. (Chouari et al., 2005; Gannoun et al., 2013; Toumi et al., 2015).

This study investigates the codigestion of two typical agro-industry wastewaters in Tunisia: olive oil mill and abattoir wastewaters. They are totally unexploited and in some cases dangerous for the environment. OMW is becoming a serious environmental problem especially for its high COD, which is in the range of 100 g L^{-1}. It is generally acknowledged that the high toxicity of OMW is entirely ascribable to phenols (Pérez et al., 1992).

AW contains a high concentration of biodegradable organics mostly in the form of fats and proteins, sufficient alkalinity, adequate nitrogen and micronutrients for bacterial growth. Then, OMW could be mixed with AW in order to dilute phenols and provide a source of nitrogen needed to achieve a favorable Carbon/Nitrogen ratio.

This paper reports for the first time, the effects of increasing the OLR and changing temperature from mesophilic to thermophilic conditions on dynamics and the microbial community structure during anaerobic co-digestion a mixture of olive mill and abattoir wastewaters (OMW/AW: 40%/60%) using an upflow anaerobic filter (UAF).

2. Methods

2.1. OMW and AW sampling

Fresh OMW used in the present study was obtained from an olive oil production plant located in the north of Tunisia, which uses a continuous process for extraction of olive oil. Because of the seasonal production and the instability of the waste, it was stored at −20 °C. The AW was collected from an abattoir factory (El Ouardia City, Tunis). Different mixtures of OMW: AW (v/v) (10/90; 20/80; 40/60; 60/40) were previously tested in order to decrease the toxicity of phenol compounds and to achieve a favorable TOC/N ratio, required for stable and better biological conversions during the anaerobic digestion. Kayhanian and Hardy (1994) reported that a C/N ratio between 25 and 30, corresponding in this case to OMW/AW ratio of 40%/60%, was optimal for anaerobic digestion process. Analysis of raw OMW, AW and the mixture of OMW/AW (40%/60%) were carried out and the average composition is shown in Table 1.

Table 1
Physico-chemical characteristics of the olive mill (OMW) and abattoir (AW) wastewaters and the mixture of OMW:AW (40%/60%).

Parameter	OMW	AW	OMW/AW (40%/60%)
pH	5.16 ± 0.09	7.5 ± 0.05	6.5 ± 0.3
Conductivity (ms/cm)	15.33 ± 3.5	7.26 ± 2	11.71 ± 1
TS (g L^{-1})	48 ± 2.5	5.44 ± 1.5	28 ± 3
TSS (g L^{-1})	6.5 ± 0.3	0.5 ± 0.1	1.8 ± 0.2
COD (g L^{-1})	110 ± 5	6 ± 1.5	41 ± 2
Total organic carbon (g L^{-1})	30.24 ± 2.5	3.42 ± 0.3	19.47 ± 1
Total Kjeldhal Nitrogen (g L^{-1})	0.1 ± 0.02	0.81 ± 0.04	0.69 ± 0.01
TOC/N	302.4	4.22	28.21
Total phenol (g L^{-1})	4.7 ± 2	–	2.04 ± 1

2.2. Bioreactor design and operational conditions

The anaerobic digestion of the OMW/AW (40%/60%) was carried out in a 2 L continuous upflow anaerobic filter (UAF) consisting of glass column of 30 cm in height and 20 cm in diameter. The UAF was filled with Flocor (Φ3L3, porosity of 95% and specific surface of 230 m^2 m^{-3}) as a media support entities for the growth of microorganisms. The used inoculum (pH = 6.93 and total solids of 4.15% w/v) was taken from an active mesophilic digester treating OMW. Firstly, the UAF was operated as a batch system. It was fed with a mixture of OMW/AW (40%/60%) and operated for one month with total recirculation of sludge. The total solids in the effluent of the UAF decreased until biofilm formation was established. The sludge issued from the batch fermentation was used to start the UAF.

The mesophilic UAF was fed initially with an OLR of 3 g COD L^{-1} d^{-1} and at hydraulic retention time (HRT) of 13.66 days. Then, the OLR was increased gradually by varying the HRT, from 10 days (OLR = 4.1 g COD L^{-1} d^{-1}) to 4.5 day (OLR = 9 g COD L^{-1} d^{-1}). The start up of the thermophilic UAF was brought by increasing the temperature of the mesophilic UAF from 37 °C to 55 °C in a single step with a simultaneous decrease of the OLR from 9 to 4.1 g COD L^{-1} d^{-1}. The OLR was increased gradually by varying the HRT, from 10 days (OLR = 4.1 g COD L^{-1} d^{-1}) to 3.33 days (OLR = 12 g COD L^{-1} d^{-1}) at thermophilic condition.

2.3. Analytical methods

The pH was measured using a digital calibrated pH-meter (HANNA pH 210). Chemical oxygen demand (COD) was measured using the dichromate method. Total organic carbon (TOC) was measured by catalytic oxidation on a TOC 1200 Euro glace analyzer. Total solids (TS), total suspended solids (TSS) and Total Nitrogen Kjeldhal (TNK) were determined according to the procedure listed in Standards Methods for the Examination of Water and Wastewater (APHA, 1995). The biogas produced was collected daily in plastic bags at room temperature. The total volume was later determined with a wet gas meter and time to time the methane content was estimated using an ORSAT apparatus.

Volatile fatty acids (VFA) were measured by HPLC (Waters) equipped with a polypore H column (250 mm × 7.8 mm of the inside diameter) connected to a differential refractometer (RI-401 Wates) and a CR-6A Shimadzu integrator. The mobile phase was 0.02 N H_2SO_4 at a flow rate of 0.6 mL/min. It was filtered through a 0.22 μm filter (Millipore) before use. The volume of injection was 20 μl. Total polyphenol content was determined using the Folin–Ciocalteu method.

2.4. DNA extraction, PCR – SSCP analysis of the anaerobic sludge

DNA extractions were performed on samples collected from the UAF sludge at the start up of the mesophilic anaerobic digestion of the mixture OMW/AW (40%/60%) (OLR = 3 g COD L^{-1} d^{-1}; HRT = 13.66 days), at the transition time between the mesophilic