Bioresource Technology 142 (2013) 269–277

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

Microbial monitoring by molecular tools of an upflow anaerobic filter treating abattoir wastewaters

Hana Gannoun [a,b,c], Eltaief Khelifi [a,*], Ilhem Omri [a], Linda Jabari [a,c], Marie-Laure Fardeau [c], Hassib Bouallagui [a], Jean-Jacques Godon [d], Moktar Hamdi [a]

[a] Université de Carthage, Laboratoire d'Ecologie et de Technologie Microbienne, INSAT, B.P. 676, 1080 Tunis, Tunisia
[b] Université de Tunis El Manar, ISSBAT, 9, Avenue Zouhaïer Essafi, 1006 Tunis, Tunisia
[c] Universités de Provence et de la Méditerranée, Laboratoire de Microbiologie, UMR 180, IRD, 163, Avenue de Luminy, 13288 Marseille Cedex 9, France
[d] INRA U050, Laboratoire de Biotechnologie de l'Environnement, Avenue des Étangs, F-11100 Narbonne, France

HIGHLIGHTS

- We study the anaerobic digestion of abattoir wastewaters in different conditions.
- Increasing temperature will affect the dynamics of the sludge microbial community.
- Fermentative bacteria were the prominent members of the sludge microbial community.
- Three strains were identified as involved in the treatment of this wastewater.

ARTICLE INFO

Article history:
Received 16 March 2013
Received in revised form 8 May 2013
Accepted 9 May 2013
Available online 18 May 2013

Keywords:
Anaerobic digestion
Abattoir wastewater
Mesophilic
Thermophilic
Microbial diversity

ABSTRACT

The performance of anaerobic digestion of abattoir wastewaters (AW) in an upflow anaerobic filter (UAF) was investigated under mesophilic (37 °C) and thermophilic (55 °C) conditions. The effects of increasing temperature on the performance of the UAF and on the dynamics of the microbial community of the anaerobic sludge were studied. The results showed that chemical oxygen demand (COD) removal efficiency of 90% was achieved for organic loading rates (OLRs) up to 4.5 g COD L^{-1} d^{-1} in mesophilic conditions, while in thermophilic conditions, the highest OLRs of 9 g COD L^{-1} d^{-1} led to the efficiency of 72%. The use of molecular and microbiological methods to recover microbial populations involved in this process showed that fermentative bacteria were the prominent members of the sludge microbial community. Three novel strains were identified as *Macellibacteroides fermentans*, *Desulfotomaculum peckii* and *Defluviitalea saccharophila*.

1. Introduction

Abattoirs and meat processing plants demands large amounts of water for their processes and generated large amounts of wastewater. Such typical organic effluent is composed of the water generated from cleaning operations and the untreated blood, containing high amount of biodegradable organic matter, mostly from fats, proteins, sufficient alkalinity, and adequate phosphorous, nitrogen and micronutrient concentrations for bacterial growth (Del Nery et al., 2007).

Several industrial effluents such as textile wastewater, optoelectronic wastewater and microcrystalline cellulose wastewater, etc. contain recalcitrant organics colours, toxicants, nitrogen compounds, surfactants, chlorinated compounds and other molecules causing potential harm to the environment and human health. Many studies, have been proposed to treat successfully these industrial wastewaters, underlined the need of the presence of specialized and selected microflora which are very sensitive to the change of the operating conditions (Ji et al., 2012; Cirik et al., 2013).

In comparison with these effluents, the composition and the treatability of AW had been discussed in detail by many research groups. They indicated that is well suited to anaerobic treatment because it provides high biochemical oxygen demand (BOD) and COD removal. Although anaerobic process produces a recoverable source of energy in the form of methane, with the generation of very low quantity of sludge and does not require aeration (Cassidy and Beliz, 2005).

Generally, the success of this technology depends on the process configuration, operational conditions and microorganisms. Various types of high loading anaerobic reactors have been em-

* Corresponding author. Tel.: +216 73465405; fax: +216 73465404.
E-mail address: khelifi.eltaief@yahoo.fr (E. Khelifi).

0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.05.036

ployed for the treatment of abattoir effluent using high-rate anaerobic systems. Compared to mesophilic digestion, thermophilic anaerobic digestion has additional benefits including an improvement in process efficiency and methane gas production and destruction of pathogenic organisms (Gannoun et al., 2009). In spite of these benefits, the long start-up required and stability problems still prevent anaerobic digestion of AW due to the accumulation of inhibitors substances, mainly ammonia and long chain fatty acids (LCFAs) which may cause reactor upset, as indicated by reduced biogas production and/or biogas methane content, and possible reactor failure. Consequently, the failure of many anaerobic bioreactors to operate reliably and with constant performance has underlined the need for more basic information on the biological aspects of the anaerobic digestion ecosystem. In order to better understand the functions of the microbial community, a full description of the microbial ecosystem is required. The identification of microorganisms by conventional methods requires the isolation of pure cultures followed by laborious characterization experiments. Moreover, most culture media tend to favour the growth of certain groups of microorganisms, whereas others that are important in the original sample do not proliferate. These methods are therefore inadequate for study of the biodiversity of a natural or engineered ecosystem. One suitable solution to this problem is to use molecular biology approaches (Godon et al., 1997; Plumb et al., 2001).

Acquisition of DNA sequences is a fundamental component of most phylogenetic and molecular ecological studies. Single-stranded conformation polymorphism (SSCP) offers a simple, inexpensive and sensitive method for detecting whether or not DNA fragments are identical in sequence, and so can greatly reduce the amount of sequencing necessary (Plumb et al., 2001). This method has been applied to study microbial communities, e.g. in water, in the compost of organic agricultural substrate, and in anaerobic digesters (Khelifi et al., 2009).

This paper aims to study the diversity of the microbial community present in the sludge of an upflow anaerobic filter treating AW under mesophilic and thermophilic conditions and to identify the most dominant bacteria involved in this process.

2. Methods

2.1. Bioreactor design and operational condition

The experiments were carried out in a 5 L continuous 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%, specific surface of 230 $m^2\ m^{-3}$) as a media support entities for the growth of microorganisms. The bioreactor was inoculated with mixed non defined cultures obtained from an anaerobic sludge from a (i) the microflora from the cow rumen and (ii) a mixed sludge obtained from an anaerobic reactor treating agro-industrial effluents. Firstly the system was operated batch-wise with circulation for one month until biofilm formation was established. Continuous feeding with the AW (pH = 7–7.2, alkalinity = 300–400 mg L^{-1}, total COD = 5800–6100 mg L^{-1}, soluble COD = 4100–4500 mg L^{-1}, total solids (TS) = 3200–3400 mg L^{-1}, total volatile solids (TVS) = 1920–2240 mg L^{-1}, total suspended solids (TSS) = 300–400 mg L^{-1}, volatile suspended solid (VSS) = 220–312 mg L^{-1}, N-ammoniacal = 130–280 mg L^{-1}, protein content = 1044 mg L^{-1}, phosphorous = 10–15 mg L^{-1}) was started at low organic loading rates (OLR) of 0.9 COD g L^{-1} d^{-1}.

The anaerobic filter was initially operated during 140 days at the optimal mesophilic temperature range (37 ± 1 °C) and then, during 140 days at the optimal thermophilic temperature range (55 ± 1 °C). The bioreactor was maintained at constant at each condition by running water through its outer mantle. The mesophilic UAF was fed initially with an OLR of 0.9 g COD L^{-1} d^{-1} and at hydraulic retention time (HRT) of 5 days. Then, the OLR was increased gradually by varying the HRT, from 2.5 days (OLR = 1.8 g COD L^{-1} d^{-1}) to 0.75 day (OLR = 6 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 to 55 °C in a single step with a simultaneous decrease of the OLR from 6 to 0.9 g COD L^{-1} d^{-1}. The OLR was increased gradually by varying the HRT, from 2.5 days (OLR = 1.8 g COD L^{-1} d^{-1}) to 0.75 day (OLR = 6 g COD L^{-1} d^{-1}) at thermophilic condition. Bioreactor operating conditions are presented in Table 1.

2.2. Physico–chemical analyses

The pH was measured using a digital calibrated pH-meter (HANNA pH 210). The total and soluble COD were measured by dichromate method. Measured COD values were used for calculation of biodegradation efficiencies. TS, TVS, TSS, VSS, alkalinity, phosphorous and Nitrogen-Ammonium (N–NH_4^+) were determined according to the procedure listed in (Standard Methods for the Examination of Water and Wastewater, 1995). Free ammonia (FA) concentrations were estimated based on pH and total ammonia (Cuetos et al., 2008). The total soluble proteins were determined by the standard method (Bradford, 1976). The produced gas was measured by gas meter, and time to time the methane content was estimated using an ORSAT apparatus. Total volatile fatty acids (tVFA) were measured by HPLC (Waters) equipped with a polypore H column (250 mm by 7.8 mm [inside diameter]) connected to a detector (RI-401 Waters). The mobile phase was 0.02 N H_2SO_4 at a flow rate of 0.6 ml min^{-1}. It was centrifuged 15 m at 13,000 rpm and filtered through 0.22 μm filter (Millipore) before use. The volume of injection was 20 μl.

2.3. DNA extraction, PCR and SSCP analysis of the digestor sludge

Anaerobic sludge samples were collected from an UAF treating AW (OLR = 6 g COD L^{-1} d^{-1}, HRT = 0.75 d) in mesophilic and thermophilic conditions. DNA extractions were performed on samples collected from the bioreactor sludge at the start up and the end of the treatment. Four millilitres of samples were centrifuged at 6000 rpm for 10 min. Pellets were resuspended in 4 ml of 4 M guanidine thiocyanate-0.1 M Tris pH 7.5 and 600 μl of N-lauroyl sarcosine 10%. 250 μl of treated samples were transferred in 2 ml tubes and stored at −20 °C.

Extraction and purification of total genomic DNA was implemented according to the protocol developed by Godon et al. (1997). This protocol is based on mechanical cell disruption by heat treatment (70 °C for 1 h) and cells being shaken in the presence of zirconia beads. Nucleic acids are recovered after several washes with polyvinylpolypirolidone to remove PCR inhibitors, followed by alcohol precipitation. Concentration and size of DNA were estimated by electrophoresis on a 0.7% agarose gel and viewed by ethidium bromide with ultra violet (UV) emission.

Highly variable V3 regions of bacterial 16S rRNA genes were amplified by PCR using either bacterial (w49–w34) or archaeal (w36–w31) primers (Table 1). Samples were treated according to the protocol previously described by Delbes et al. (2001). For each reaction, the solution with 5 μl of the enzyme *Tampon Red Taq* 5 U μl^{-1} and dNTP 2.5 mM in amounts of 4 and 2 μl of each primers (bacteria (w49, 100 ng μl^{-1} – w34, 100 ng μl^{-1}) and archaea (w36, 100 ng μl^{-1} – w31, 100 ng μl^{-1})), was added to 1 μl of DNA diluted in water. PCR conditions were as follows: an initial denaturation step at 94 °C for 2 min, followed by 25 cycles of a three-stage program with 1 min at 94 °C, 1 min at 61 °C for bacteria and 51 °C for archaea, then 1 min at 72 °C; and a final elongation step run for 10 min at 72 °C. PCR products were purified with the QIAamp kit