Bioresource Technology 198 (2015) 141–149

Contents lists available at ScienceDirect

Bioresource Technology

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

Microbial ecology overview during anaerobic codigestion of dairy wastewater and cattle manure and use in agriculture of obtained bio-fertilisers

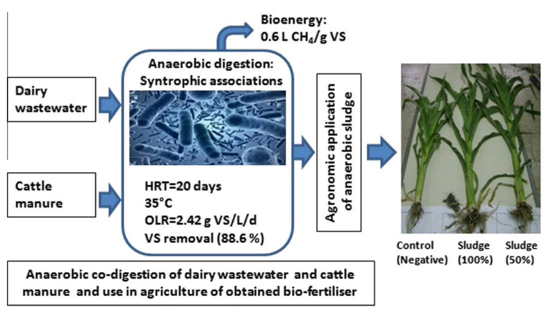
Jihen Toumi [a,b], Baligh Miladi [b], Amel Farhat [b], Said Nouira [a], Moktar Hamdi [b], Maher Gtari [c], Hassib Bouallagui [b,*]

[a] UR-Biodiversity and Biology of Populations, FST, University of Elmanar, Tunis, Tunisia
[b] LR-Microbial Ecology and Technology, INSAT, University of Carthage, Tunis, Tunisia
[c] Laboratoire Microorganismes et Biomolécules Actives, Université de Tunis El Manar, Tunisia

HIGHLIGHTS

- The anaerobic co-digestion of dairy wastewater and cattle manure.
- The associated microbial community's were described by DGGE.
- The volatile solids removal and biogas yield reached 88.6% and 0.87 L/g VS.
- Syntrophic associations were essential to keep low H_2 pressure.
- The digestate showed beneficial effects on the plants growth and crops.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 8 July 2015
Received in revised form 3 September 2015
Accepted 5 September 2015
Available online 9 September 2015

Keywords:
Dairy wastewater
Manures
Anaerobic digestion
Microbial ecology
Agronomic application

ABSTRACT

The anaerobic co-digestion of dairy wastewater (DW) and cattle manure (CM) was examined and associated with microbial community's structures using Denaturing Gradient Gel Electrophoresis (DGGE). The highest volatile solids (VS) reduction yield of 88.6% and biogas production of 0.87 L/g VS removed were obtained for the C/N ratio of 24.7 at hydraulic retention time (HRT) of 20 days. The bacterial DGGE profile showed significant abundance of Uncultured Bacteroidetes, Firmicutes and Synergistetes bacterium. The *Syntrophomonas* strains were discovered in dependent association to H_2-using bacteria such as *Methanospirillum* sp., *Methanosphaera* sp. and *Methanobacterium formicicum*. These syntrophic associations are essential in anaerobic digesters allow them to keep low hydrogen partial pressure. However, high concentrations of VFA produced from dairy wastes acidification allow the growth of *Methanosarcina* species. The application of the stabilised anaerobic effluent on the agriculture soil showed significant beneficial effects on the forage corn and tomato plants growth and crops.

* 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.2015.09.004
0960-8524/

1. Introduction

In Tunisia, the milk processing sector consists of nine central dairies producing drinking milk and fresh milk products. The quantities of milk received by these factories approached 619 million litres in 2012 compared to 536 million liters in 2011 with a daily processing capacity of about 2.5 million litres. Therefore, the dairy industry generates about 0.2–10 L of effluent per litre of processed milk with an average value of about 2.5 L (Gelegenis et al., 2007).

The continuous non controlled discharge of this effluent presents serious groundwater pollution problems because it is highly chemical oxygen demand (COD) of 60–80 g/L (Mockaitis et al., 2006; Passeggi et al., 2009). Several possibilities were assayed for dairy wastewater (DW) recycling and treatment. However, its high organic content renders the application of anaerobic digestion an excellent alternative in terms of both energy recovery and pollution removal. Various types of anaerobic digesters were used in laboratories to treat DW such as anaerobic sequencing batch reactor (ASBR) (Mockaitis et al., 2006), anaerobic sludge blanket (UASB) reactor, anaerobic filters (Gannoun et al., 2008). However, the anaerobic digestion of DW is not wide spread in the dairy industry. This is largely due to the poor process stability.

Toumi et al. (2010) showed that anaerobic co-digestions of DW and CM are a suitable solution for reducing their instability caused by their high biodegradable organic content. Particularly, the agriculture by-products already contain the anaerobic micro-flora of the animal's intestinal tract. They also present a buffer effect which is better for anaerobic digestion (Marcato et al., 2009). Therefore, the anaerobic co-digestion could be a practical alternative for the simultaneous recycling of different types of organic wastes. Its benefits include improved balance of nutrients, synergistic effect of micro-organisms, increased load of biodegradable organic matter and better biogas yield (Bouallagui et al., 2009).

The efficiency of the anaerobic microbial activity depends to the type of reactor and operating conditions such as the temperature, the HRT and carbon/nitrogen (C/N) ratio (Gomez et al., 2006). However, our knowledge about the microbial consortia involved in this process is limited because of a lack of phylogenetic and metabolic data on these predominantly Uncultured microorganisms. As an alternative to culture techniques, several molecular inventories, based on the study of the 16S rRNA gene, were carried out on anaerobic environments and have shown the extent of the diversity in these complex ecosystems (Rivière et al., 2009; Zhang et al., 2011). Therefore, molecular inventories have increased significantly our knowledge of the understanding of the function and the metabolic role of some microorganisms in the anaerobic digestion of various pollutants. In this context, the performance of anaerobic co-digestion of DW and CM in ASBR was studied at different ration of DW/CM and an inventory of microbial ecology was achieved by the technique of DGGE.

The remaining anaerobic stabilised sludge may be used as a soil fertiliser. However, the addition of organic bio-fertiliser is beneficial for plants growth, since it will improves soil structure, increases water holding capacity, stimulates microbial activity (Ritz et al., 1997) and reduces nitrogen losses (Marcato et al., 2009). Sewage sludge and waste activated sludge were found to be an effective organic fertiliser causing increments in the biomass of many crops. However, their long term use may generate a problem of metal accumulation depending of the chemical characteristic of the sewage sludge (Singh and Agrawal, 2010).

There is still a lack of information about the agronomic benefits and disadvantages of the organic matter quality from anaerobic digested wastes. The second aim of this work is to investigate the use in agriculture of anaerobic sludge obtained from a pilot digester treating BW and CM. The efficiency of the treated wastes application on the seed germination, plants growth and crops was studied.

2. Methods

2.1. Feed stocks characterisation

The wastes used in this study were of two types: dairy effluent and cattle manure, coming from cheese factory located in Vocational Training Centre in Sidi Thabet (Tunis, Tunisia) which uses traditional technologies for cheese manufacture. The CM was collected from the cowshed in which the evacuations of cattle are mixed with the water of washing. It had an initial concentration of total solids (TS) of 27 g/kg with a percentage of volatile solids (VS) of 75.9% with respect to the TS content (Table 1). The DW showed an initial TS concentration of 68 g/kg with a percentage of VS of 83.5% with respect to the TS content.

Feedstocks were made up by using raw DW and by adding a percentage by volume of CM. These gave four feedstocks: F1 (80% DW/20%CM), F2 (70%DW/30%CM), F3 (60%DW/40%CM) and F4 (50%DW/50%CM), with average VS contents of 48 g/L, 42.3 g/L, 36.1 g/L and 33.7%, respectively. The feedstock F1, F2, F3 and F4 were used to load the reactors R1, R2, R3 and R4, respectively.

2.2. Experimental digestion processes

Four laboratory-scale anaerobic sequencing batch reactors (R1, R2, R3, R4) of 2 L effective volume were used. ASBR is a single vessel system that doesn't requires an integrated decanter, which simplifies its design to be used in farms. However, a settling step before withdrawn of the digested effluent is instrumental to facilitate high biomass levels and long sludge retention time. In addition, it permits to reduce sludge production and to increase methanogens concentration in the reactor. The temperature was controlled at 35 °C by a thermostatically regulated water bath. Peristaltic pumps were used to fill the reactors and to draw off the effluents after settling. Mixing in the reactors was done by a system of magnetic stirring (Bouallagui et al., 2009).

Each digester was initially inoculated with an acclimated anaerobic sludge obtained from an active mesophilic (35 °C) digester of agro-wastes treatment plant (Toumi et al., 2010). The initial TS and VS concentrations of inoculum were 18.4 g/L and 14.8 g/L, respectively. They were concentrated to obtain 20 g VS/L to increase the biomass content in the reactor for operating as ASBR. Besides developing a well settling biomass the concentration of methanogens in the reactor biomass may also need to be increased to obtain high volumetric loading rate. In the first 5–10 days of operating period the washout of sludge in ASBRs was observed and the biomass levels decreased. After that the settle ability of biomass in all

Table 1
Pysico-chemical characteristics of used wastes (DW: dairy wastewater; CM: cattle manure).

	Raw CM	Raw DW
TS (%)	2.7 ± 0.08	6.8 ± 0.38
VS (g/L)	20.5 ± 0.23	56.82 ± 2.3
TSS (g/L)	16.45 ± 0.6	8.6 ± 0.4
pH	6.93 ± 0.1	4.5 ± 0.2
CODt (g/L)	28.05 ± 0.4	80.3 ± 4.1
CODs (g/L)	11.1 ± 0.1	64 ± 2.1
TN (% of TS)	5.1 ± 0.2	1.4 ± 0.1
TC (% of TS)	56.5 ± 0.8	50.7 ± 1.1
P (% of TS)	0.34 ± 0.02	0.14 ± 0.01
K (% of TS)	0.18 ± 0.01	0.38 ± 0.02
C/N	11.1 ± 0.04	36.2 ± 0.1