Water Research 134 (2018) 181–191

Contents lists available at ScienceDirect

Water Research

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

Performance and genome-centric metagenomics of thermophilic single and two-stage anaerobic digesters treating cheese wastes

Alessandra Fontana [a, c], Stefano Campanaro [b], Laura Treu [c], Panagiotis G. Kougias [c, *], Fabrizio Cappa [a], Lorenzo Morelli [a, 1], Irini Angelidaki [c, 1]

[a] Department for Sustainable Food Process – DiSTAS, Catholic University of the Sacred Heart, 29122 Piacenza, Italy
[b] Department of Biology, University of Padua, 35131 Padua, Italy
[c] Department of Environmental Engineering, Technical University of Denmark, 2800 Kongens Lyngby, Denmark

ARTICLE INFO

Article history:
Received 8 November 2017
Received in revised form
31 January 2018
Accepted 1 February 2018

Keywords:
Thermophilic anaerobic digestion
Dairy wastes
Acidogenesis
Metagenomics
Syntrophisms
Methanogens

ABSTRACT

The present research is the first comprehensive study regarding the thermophilic anaerobic degradation of cheese wastewater, which combines the evaluation of different reactor configurations (i.e. single and two-stage continuous stirred tank reactors) on the process efficiency and the in-depth characterization of the microbial community structure using genome-centric metagenomics. Both reactor configurations showed acidification problems under the tested organic loading rates (OLRs) of 3.6 and 2.4 g COD/L-reactor day and the hydraulic retention time (HRT) of 15 days. However, the two-stage design reached a methane yield equal to 95% of the theoretical value, in contrast with the single stage configuration, which reached a maximum of 33% of the theoretical methane yield. The metagenomic analysis identified 22 new population genomes and revealed that the microbial compositions between the two configurations were remarkably different, demonstrating a higher methanogenic biodiversity in the two-stage configuration. In fact, the acidogenic reactor of the serial configuration was almost solely composed by the lactose degrader *Bifidobacterium crudilactis* UC0001. The predictive functional analyses of the main population genomes highlighted specific metabolic pathways responsible for the AD process and the mechanisms of main intermediates production. Particularly, the acetate accumulation experienced by the single stage configuration was mainly correlated to the low abundant syntrophic acetate oxidizer *Tepidanaerobacter acetatoxydans* UC0018 and to the absence of aceticlastic methanogens.

1. Introduction

The dairy industry consists of several production divisions, each one of them generating considerable amounts of effluent wastewater streams. Especially, during the cheese making process, different types of residues are discarded at various steps of the production chain. Cheese whey permeate (WP) is a by-product originating from the cheese manufacturing process during the step of proteins recovery by ultrafiltration and/or diafiltration. It mainly contains lactose and therefore is often used to standardize the nutritional composition and taste of milk. However, in most cases, WP is not exploited and thus is considered as high strength wastewater (i.e. BOD_5/COD ratio is usually higher than 0.5) (Prazeres et al., 2012). It is estimated that the annual production of WP at global scale can be over 10^8 tons per year (Grba et al., 2002). A less known waste derives from the portioning and shaving phases of hard-cheese manufacturing process, and it consists in a cheese powder waste (CP), which mainly contains proteins and fats. Especially, in Italy, the production of two Protected Designation of Origin (PDO) hard-cheeses, Grana Padano and Parmigiano Reggiano, counted more than 182,000 t and 137,000 t, respectively in 2015 (ISMEA, 2016). These volumes suggest a considerable amount of waste derived from each cheese mould. From the above, it is obvious that the residues of dairy industry require an effective treatment before their disposal to the final recipients.

Several biological treatments have been proposed to process WP

Abbreviations: WP, whey permeate; CP, cheese powder; AD, anaerobic digestion; VFA, volatile fatty acid; CSTR, continuous stirred-tank reactor; TRS, total random sequencing; PG, population genome; R1, single stage reactor; R2, acidogenic reactor of the two-stage configuration; R3, methanogenic reactor of the two-stage configuration.

* Corresponding author.
E-mail address: panak@env.dtu.dk (P.G. Kougias).
[1] Shared last authors.

https://doi.org/10.1016/j.watres.2018.02.001

including anaerobic digestion (AD), lactose hydrolysis, ethanol, hydrogen or lactic acid fermentations, enzyme production, and microbial fuel cells (Cota-Navarro et al., 2011; Prazeres et al., 2012; Schirru et al., 2014). Among them, AD for biogas production is considered as a sustainable solution for waste valorization and energy recovery. AD is a complex biological process involving different microbial consortia to break down organic matter into several by-products and finally to biogas, which is mainly composed by methane and carbon dioxide. This overall process consists of four steps namely hydrolysis, acidogenesis, acetogenesis and methanogenesis (Batstone et al., 2008); the resulting methane can be used for electricity and thermal energy generation or by performing additional purification steps biomethane can substitute natural gas (Kougias et al., 2017).

Nevertheless, the high sodium content, acidic pH and low alkalinity of WP hampers its treatment by biological processes (Backus et al., 1988; Ghaly, 1996; Castelló et al., 2009). In order to overcome such technical challenges, different reactor configurations (Stamatelatou et al., 2014) were tested or co-digestion strategies were employed in order to efficiently degrade wastewater from cheese-making processes (Gelegenis et al., 2007; Hagen et al., 2014). The majority of these studies have been performed under mesophilic conditions and the few works reporting thermophilic reactor operation are focusing on simultaneous production of H_2 and CH_4 in two steps (Fernandez et al., 2015; Kisielewska et al., 2014). However, it is well known that thermophilic conditions, even if they are more sensitive to inhibitors, pose several advantages in biogas production, such as higher methane production rates and shorter hydraulic retention times (Harris and Dague, 1993; Wiegant et al., 1986; Zinder et al., 1984). To the best of our knowledge, information regarding thermophilic operation of anaerobic reactors fed exclusively with cheese wastewater and by-products for biogas production is missing.

Another crucial parameter, which determines the degradation efficiency of these wastes, is the microbial consortium involved in the AD process. Understanding the diversity and dynamics of such community will lead to process optimization by calibrating operational parameters and by enhancing preferred microbial pathways, which will result in higher CH_4 yields. A way to achieve this goal is via genome-centric metagenomics, which employs shotgun sequencing, *de novo* assembly of the obtained reads and binning of the scaffolds in population genomes (Campanaro et al., 2016).

This study aims to compare the efficiency of two thermophilic reactor configurations, single and two-stage continuous stirred tank reactors (CSTRs), on the anaerobic degradability of a mixture of cheese wastes, namely WP and CP. Furthermore, we analyzed the reactors' metagenomes via Total Random Sequencing (TRS) and metagenomic binning strategy. Functional analyses of the main population genomes (PGs) were also carried out, in order to highlight possible connections with the main intermediates produced along the process, such as volatile fatty acids (VFAs).

2. Materials and methods

2.1. Substrates characterization and feedstock preparation

The cheese whey permeate was obtained from Arla, Denmark, and stored at −20 °C, in 2 L bottles. The Grana Padano PDO cheese waste powder (from the portioning phase of manufacturing process) was obtained from Colla S.p.A., Italy, and stored in vacuum-sealed bags at 4 °C. Before usage, the whey permeate was thawed at 4 °C for 1–2 days. The feedstock was prepared by mixing the two substrates by hands and it was kept homogenized with a magnetic stirrer during the feeding times. The chemical composition of each substrate and mixed feedstock are shown in Table 1.

2.2. Reactors' configurations and process parameters

The setup consisted of a single (R1) and a two-stage CSTRs (R2 and R3, respectively); each setup had a total working volume of 3 L. The working volume of the two-stage configuration was split between the acidogenic reactor (R2; 0.6 L) and the methanogenic reactor (R3; 2.4 L). Each reactor was filled with inoculum, obtained from Snertinge thermophilic biogas plant (Denmark), which is mainly fed with livestock manure (pig and cattle) and wastes from food industry. The inoculum had a pH of 8.1, total solids (TS) and volatile solids (VS) content of 31.71 ± 0.04 and 21.45 ± 0.05 g/L, respectively. The total volatile fatty acids (VFAs), total Kjeldahl Nitrogen (TKN) and ammonium nitrogen (NH_4^+-N) concentrations were 0.13 ± 0.02, 3.78 ± 0.01, 3.15 ± 0.01 g/L, respectively. The reactors were mixed by magnetic stirrers (stirring intensity equal to 150 rpm) and were kept at thermophilic conditions (55 ± 1 °C) using thermal jackets. R1 and R2 were fed four times per day with a mixture of whey permeate (WP) and cheese powder (CP), while R3 was fed with the effluent from R2. Each time the reactors were fed with fresh substrate, equal volume of effluent digestate was removed from the reactors by pneumatic pressure. The hydraulic retention time (HRT) was set at 15 days, for both configurations (split in 3 and 12 days in R2 and R3, respectively). The organic loading rates (OLRs) tested were initially 3.6 g COD/L-reactor day (Phase I), then, due to acidification problems, 2.4 g COD/L-reactor day (Phase II). Sodium bicarbonate addition in R1 and R3 was applied whenever the pH dropped below 6.5.

2.3. Analytical methods

The daily biogas production of R1 (single stage reactor) and R3 (methanogenic reactor of the two-stage configuration) were measured by an automated gas meter (Angelidaki et al., 1992). Total Solids (TS), Volatile Solids (VS), Chemical Oxygen Demand (COD),

Table 1
Substrates and feedstocks physico-chemical characteristics.

Parameter	WP	CP	Feedstock (Phase I)	Feedstock (Phase II)
pH	6.30 ± 0.20	5.10 ± 0.20	5.50 ± 0.20	5.50 ± 0.20
TS (g/L)	36.62 ± 3.35	854.03 ± 6.43	46.01 ± 4.89	34.55 ± 3.90
VS (g/L)	33.37 ± 3.00	826.70 ± 19.37	42.46 ± 3.21	31.89 ± 2.41
COD (g/L)	33.98 ± 2.99	1545.95 ± 36.13	54.02 ± 3.39	36.01 ± 2.54
Total VFA (g/L)	0.05 ± 0.01	0.53 ± 0.17	0.05 ± 0.01	0.04 ± 0.01
Lactic acid (g/L)	0.41 ± 0.01	0	0.41 ± 0.01	0.31 ± 0.01
TKN (g/L)	0.26 ± 0.05	69.81 ± 0.10	1.03 ± 0.05	0.77 ± 0.04
NH_4^+-N (g/L)	0	9.37 ± 0.02	0.10 ± 0.01	0.08 ± 0.01
Lipids (g/L)[a]	0	290.00[a]	3.20[a]	2.40[a]

[a] Data estimated considering the lipid content established by the PDO regulation in 100 g of Grana Padano cheese (https://www.granapadano.it/public/file/tabNutriGPit-5067-20094.pdf), whose powder waste was used in the experiment as indicated in the "Materials and methods" section.