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Comparison of the acidogenic and methanogenic potential of agroindustrial residues

Anastasios Perimenis *, Thomas Nicolay, Matthieu Leclercq, Patrick A. Gerin

Université catholique de Louvain, Earth and Life Institute – Applied Microbiology, Unit of Bioengineering, Place Croix du Sud, 1348 Louvain-la-Neuve, Belgium

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ABSTRACT

The methanogenic and acidogenic potentials of six different agroindustrial residues, i.e. of fruit pulps and brewery residues, were determined. For all substrates, the methanogenic conversion yield was systematically higher than the acidogenic one in Chemical Oxygen Demand (COD) terms, ranging from 0.46 to 0.87 $g_{COD\_CH4}/g_{COD\_substrate\_fed}$ and from 0.24 to 0.56 $g_{COD\_tVFA}/g_{COD\_substrate\_fed}$, respectively. During methanogenic conversion, brewery trub exhibited the highest methane potential (304 $ml_{CH4}/g_{COD\_substrate}$). Trub also exhibited the highest total volatile fatty acids (tVFA) concentrations in the mixed liquor (ML) during acidogenic conversion (29.7 g_{COD_tVFA}/kg_{ML}). Acetic, butyric and caproic acids were the main carboxylates produced by the different substrates. Despite the lower conversion yields, the economic value of the acidogenic product (carboxylate streams) is higher than that of methanogenic conversion (methane) due to the higher value of carboxylates and their potential use in finer applications (e.g. bio-based products) compared to energy production form methane.

1. Introduction

Agroindustrial residues like fruit, vegetable or brewery waste have attracted increasing attention during the last decade owing to the potential of valorising their organic fraction for various energetic or material applications, thus bringing added value to the entire process from which they are generated (Oreopoulou and Tzia, 2007). According to Eurostat, the amount of the waste categories (i) animal & mixed food waste and (ii) vegetal waste (classification W091 and W092, respectively) generated by the economic activity "Manufacture of food products, beverages and tobacco products (activity C10–C12 according to NACE-Rev2 classification) amounted to 22.1 Mt in 2014 in the EU-28 (Eurostat-Waste Statistics, 2017). The valorisation potential of these residues is, therefore, attractive as suggested by several authors. Indicatively, for the type of waste that are of interest in this study, Ravindran and Jaiswal (2016) and Federici et al. (2009) both highlighted the potential of food processing waste and commented on the technological challenges that need to be overcome to increase product yield and decrease operating costs for their conversion. Mussatto (2009) and Mathias et al. (2014) also reviewed the biotechnological potential of the brewing industry by-products and their possible applications.

Among other options for the treatment of agroindustrial waste, anaerobic digestion has been widely implemented at industrial scale, typically, for the production of methane (biogas), a substitute of natural gas (Angelidaki et al., 2011). Anaerobic digestion is a sequence of four naturally occurring and interdependent processes i.e. hydrolysis, acidogenesis, acetogenesis and methanogenesis. Methane has been the main focus in literature, as the final product of anaerobic digestion. Nevertheless, short chain carboxylic acids deriving from the intermediate acidogenic phase like acetic, propionic, butyric and caproic acids, otherwise called volatile fatty acids (VFA) or carboxylates, are also of high industrial interest. Acidogenesis (or acidogenic fermentation) has been mostly studied in view of increasing biogas (e.g. two-stage anaerobic digestion systems) or biohydrogen (i.e. dark fermentation) yields, but it can be envisaged as a stand-alone process with a product of higher added value (Singhania et al., 2013; Alkaya and Demirer, 2011; Zacharof and Lovitt, 2013). VFA have a variety of potential end-uses, for example as monomers for polyhydroxyalkanoates (PHA) production, for bio-based solvent production, for biological nutrient removal, etc. Interest in VFA has increased during the last years in the context of the carboxylate platform, a term used in the biorefinery context highlighting the multitude of production processes and potential applications of VFA (Jang et al., 2012; Agler et al., 2011).

* Corresponding author at: Bioengineering Group, Croix du Sud, 2 Box L7.05.19, B-1348 Louvain-la-Neuve, Belgium.
E-mail address: aperimenis@outlook.be (A. Perimenis).

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Each phase in the sequence of anaerobic digestion is realised by a different group of microorganisms (i.e. acidogenic bacteria, acetogenic bacteria, methanogenic archaea) working in synergy but often also competition (Angelidaki et al., 2011). While methane production occurs naturally under anaerobic conditions, separating acidogenesis requires a more careful control of the process conditions. Acetogenesis and methanogenesis must be avoided to increase the net conversion yield of the substrate into VFA. This control is especially challenging for mixed culture fermentations, i.e. fermentation by natural microbial consortia, not pure cultures (Arslan et al., 2016; Rodríguez et al., 2006). Some advantages of using mixed cultures are: the ability to treat various types of complex substrates owing to the microbial diversity, no need for sterile conditions, lower costs of production and potential for a continuous process (Kleerebezem and van Loosdrecht, 2007). On the other hand, the lack of product specificity and the complexity of the medium are still a bottleneck in terms of product yields and recovery and prevent mixed culture fermentation from wide implementation at industrial scale.

While methane production is already established as an industrial process, acidogenic fermentation is still under research. Research is focusing mainly on (i) the influence of process parameters of batch or continuous operation (pH, substrate concentration, HRT, OLR) on the concentration and composition of VFA (Arslan et al., 2016; Lee et al., 2014; Gameiro et al., 2016; Jiang et al., 2013) and (ii) downstream processing for the concentration, recovery, separation and purification of VFA streams (Zacharof and Lovitt, 2014; López-Garzón and Straathof, 2014; Arslan et al., 2017).

Keeping in mind the difference in maturity between the two processes, the purpose of this study is to highlight the interest of mixed acidogenic fermentation as an alternative option for organic solid waste treatment. For that we determined and compared both the acidogenic and methanogenic potential of a number of organic residues of agroindustrial interest. The present paper presents the results and discusses the observed differences. The decision over the one or the other process will ultimately depend on the product yields, the economic potential deriving thereof and the investment required to harness it. To the best of our knowledge, this is the first study where these two processes are systematically examined as two separate processes for the same waste fraction.

2. Materials and methods

2.1. Substrates

Two types of agroindustrial residues were investigated: (i) fruit pulps after juice extraction and water washing, more specifically date, pear and apple pulp from a syrup producing industry (Aubel, Walloon Region, Belgium), (ii) brewery residues, more specifically hot trub, spent yeast, and spent grain from a small-scale brewery (Ath, Walloon Region, Belgium). The properties of the substrates are resumed in Table 1.

2.2. Concentrated activated sludge

Activated sludge from the municipal wastewater treatment plant in Mont-St-Guibert, Belgium was used as primary inoculum for both methanogenic and acidogenic conversions. The sludge was left to settle in the dark at 4 °C for two days and the supernatant was removed. Average properties of the concentrated sludge were: Total Solids (TS) in the fresh matter (FM) at 0.024 ± 0.005 g_{TS}/g_{FM} (8 samples from 4 experiments), Volatile Solids (VS) at 0.40 ± 0.08 g_{VS}/g_{TS} (10 samples from 4 experiments) and COD at 20.7 ± 1.2 g_{COD}/kg_{FM} (13 samples from 4 experiments. The treatment of the inoculum prior to each conversion is presented in 2.3.1 and 2.4.1, respectively.

2.3. Methanogenic conversion

2.3.1. Inoculum preparation

For the preparation of the methanogenic inoculum, the primary inoculum was maintained at 35 °C under anaerobic conditions for at least 4 weeks and fed every week with fresh activated sludge, concentrated as described in Section 2.2, at a ratio of 0.1 $g_{COD\_concentrated\_sludge}/g_{COD\_primary\_inoculum}$. The container of the primary inoculum was closed with a rubber cap. One extremity of a PVC tube was inserted through the rubber cap into the headspace of the container, while the other extremity was immersed into Erlenmeyer flask filled with water. This allowed the release of biogas while preventing air contact with the container (Angelidaki et al., 2011). Ten days before the start of the experiment, the inoculum received a last feed at a ratio of 0.3 $g_{COD\_concentrated\_sludge}/g_{COD\_primary\_inoculum}$ (Donoso-Bravo et al., 2015).

2.3.2. Bioreactor preparation

The anaerobic digestion took place in 1 l Schott Duran GL45 bottles with a lateral glass tube of 4 cm long placed at an angle of 45° upwards. A 2-way Luer polycarbonate valve (Fisher Scientific) was connected via a short PVC tube to the extremity of the glass tube. Gas sampling and pressure measurements were achieved through this valve. The bioreactor-bottle was closed with a PBT screw-cap, containing a PTFE coated silicone seal.

At the start of the experiment, the methanogenic inoculum was introduced in the reactors and the substrate was subsequently added. Experiments were conducted in triplicates. The quantities of each substrate added in the reactors are shown in Table 2. Three reactors contained the inoculum and water instead of substrate and served as a control. After adding the inoculum and the substrate, the headspace of the reactor was flushed with N_2 for one minute before closing the reactor, in order to remove oxygen and establish anaerobic conditions. The volume of each reactors' headspace was determined by comparing the weight of each reactor (i) empty, (ii) full of water, (iii) filled with the final volume of mixed liquor.

2.3.3. Monitoring of methanogenic conversion

Depending on the substrate, the anaerobic digestion lasted between 20 and 50 days. At regular intervals, the pressure of the reactor's headspace was measured with a manometer (UNIK 5000 PTX5072-TA-A3-CA-H0-PA, GE Measurement & Control Solutions) in order to determine the quantity of biogas produced, through the ideal gas law. The manometer was connected to the reactor with a 3-way valve, permitting the connection of a 50 ml polypropylene syringe to sample the gas phase. After sampling, the reactor was depressurized to atmospheric pressure that was also recorded. The gas phase sample was immediately analysed by GC-TCD (see Section 2.4) to determine its composition in CH_4, CO_2, H_2, O_2 and N_2.

The total volume of biogas produced and its composition permitted to calculate the amount of CH_4 produced. The amount of methane produced in the control reactors was subtracted from the amount of methane produced in the reactors with the substrate, in order to provide the net amount of methane produced by the substrate. The conversion yield of the substrate to methane (methanogenic potential) is determined by Eq. (1).

$$R_{CH4} = \frac{V_{CH4} * F}{Q_{substrate}}, \quad (1)$$

Table 1
Composition of the residues from brewery and fruit syrup activities, used as substrates. FM: Fresh Matter; TS: Total Solids; VS: Volatile Solids; COD: Chemical Oxygen Demand.

		Trub	Spent yeast	Spent grain	Pear pulp	Date pulp	Apple pulp
Total solids	% FM, w/w	23.8	13.8	25.0	32.4	24.5	22.2
Volatile solids	% TS, w/w	98.1	93.2	96.5	99.2	98.1	99.1
COD	g_{COD}/g_{FM}	0.294	0.289	0.316	0.427	0.317	0.360
COD	g_{COD}/g_{VS}	1.26	2.24	1.31	1.33	1.32	1.64
Cellulose	% TS, w/w	8.2	1.0	19.4	32.7	24.7	35.2
Hemicellulose	% TS, w/w	5.6	14.7	28.8	22.2	11.4	14.8
Lignin	% TS, w/w	8.0	0.3	4.2	17.3	30.6	14.3
Pectin	% TS, w/w	–	–	–	2.1	2.8	4.3
Sugar	% TS, w/w	57.4	38.6	12.4	7.5	10.3	10.2
Proteins	% TS, w/w	15.0	35.9	18.0	5.6	11.0	7.1
Lipid	% TS, w/w	3.0	3.9	10.0	1.6	1.0	2.9
Ash	% TS, w/w	2.5	5.6	5.0	0.8	1.9	1.2

TS, VS, COD values were determined by the authors for all substrates. For the other parameters i.e. cellulose, hemicellulose, lignin, sugar, lipid, protein and pectin content we refer the reader to (Aguedo et al., 2012) in the case of fruit pulps, since they used the same substrates as this study. For the respective parameters of brewery residues, the results have been delivered by Celabor, Belgium whom we gratefully acknowledge.

Table 2
Initial COD concentrations in anaerobic digestion experiments. Mean values and standard deviation of three reactors. ML: mixed liquor; COD; Chemical Oxygen Demand.

Substrate	Substrate (g_{COD})	Inoculum (g_{COD})	Reactor concentration (g_{COD}/kg_{ML})
Trub[1]	0.75 ± 0.00	5.00 ± 0.01	15.50 ± 0.02
Spent yeast[2]	1.00 ± 0.00	5.32 ± 0.03	16.17 ± 0.52
Spent grain[2]	1.00 ± 0.00	5.20 ± 0.08	16.15 ± 0.27
Pear pulp[3]	1.20 ± 0.00	7.50 ± 0.00	15.31 ± 0.00
Date pulp[1]	0.75 ± 0.00	5.00 ± 0.01	15.48 ± 0.02
Apple pulp[3]	1.50 ± 0.00	7.50 ± 0.00	15.81 ± 0.00

[1,2,3] Experiments took place at different times, due to seasonal substrate availability. Substrates sharing the same superscript were tested in the same experiment.

Table 3
Initial COD concentration in acidogenic fermentation experiments. Mean values and standard deviation or deviation from the mean of three or two reactors, respectively; ML: mixed liquor; COD; Chemical Oxygen Demand.

Substrate	Substrate (g_{COD})	Inoculum (g_{COD})	Reactor concentration (g_{COD}/kg_{ML})
Trub	4.81 ± 0.00	1.16 ± 0.01	60.72 ± 0.16
Spent yeast[1]	4.80 ± 0.00	1.22 ± 0.00	60.41 ± 0.02
Spent grain[2]	8.00 ± 0.02	2.00 ± 0.00	80.97 ± 0.27
Pear pulp[1]	4.80 ± 0.00	1.20 ± 0.00	59.64 ± 0.18
Date pulp[2]	8.01 ± 0.02	2.00 ± 0.00	80.57 ± 0.19
Apple pulp[2]	8.01 ± 0.01	2.00 ± 0.00	82.84 ± 0.48

[1,2] Experiments took place at different times, due to seasonal substrate availability. Substrates sharing the same superscript were tested in the same experiment.

where

R_{CH4}: yield of substrate conversion to methane ($g_{COD\_CH4}/g_{COD\_substrate}$)
V_{CH4}: Cumulated methane production at normal conditions (l_{CH4})
F: stoichiometric COD factor of methane (2.86 g_{COD_CH4}/l_{CH4})
$Q_{substrate}$: Initial COD quantity of the substrate fed to the reactor ($g_{COD\_substrate}$).

2.4. Acidogenic conversion

2.4.1. Inoculum preparation

The concentrated sludge (see Section 2.2) was incubated under anaerobic conditions at 35 °C for five days. The pH of the concentrated sludge was then adjusted to 5.5 with HCl and the sludge was maintained for 1 h at 70 °C in a shaking water bath to select spore-forming acidogenic species (Arslan et al., 2016). It was left to cool down to room temperature and the pH was re-adjusted to 5.5 with HCl just before use as acidogenic inoculum.

2.4.2. Bioreactor preparation

Acidogenic fermentation was performed in the same type of reactors as for methanogenic conversion (Scott Duran GL 45 bottles), though smaller in size (250 ml). The substrate and the inoculum were introduced in the reactors (triplicate for spent grain, apple pulp and date pulp; duplicate for spent yeast and trub) at a COD ratio of 4 $g_{COD\_substrate}/g_{COD\_inoculum}$ (Perimenis et al., 2016) and water was added to reach a mixed liquor volume of 100 ml (Table 3). The substrate to inoculum ratio (S/I) was higher than in the methanogenic conversion, to ensure the presence of sufficient digestible fraction allowing acidogenic bacteria to rapidly produce VFA and inhibit development of methanogens (van Aarle et al., 2015). Control reactors, where either the inoculum or the substrate was replaced by deionised water, were also prepared. Before hermetic closure, the bioreactor headspace was flushed for 1 min with N_2.

2.4.3. Monitoring of acidogenic conversion

The batch incubation was conducted at 35 °C in the dark. When necessary, the pH of the mixed liquor was adjusted to the desired value of 4.5–5.5 with the addition of 1 M HCl or 1 M NaOH solution. Depending on the substrate, fermentation lasted between 22 and 66 days. Samples of the gas phase and the mixed liquor were taken at regular intervals for analysis. Flushing with nitrogen took place each time the reactors were opened. The parameters monitored were: pH, soluble COD (CODs), biogas production and composition and the concentration of metabolites (e.g. VFA, ethanol).

Monitoring of the gas production and composition were performed in the same way as described for the methanogenic conversion. The samples of the mixed liquor were firstly centrifuged at 15,300 × g for 10 min. The supernatant was separated and stored at −20 °C for the metabolic analysis. The solid remainder was also stored at −20 °C.

It was assumed that the inoculum has the same contribution in the inoculated reactors as in the control reactors. The contribution of the inoculum to the final tVFA concentration was then subtracted from the results of the inoculated reactors. The acidogenic potential was then determined by Eq. (2):

$$R_{tVFA\_substrate} = \frac{C_{tVFA\_inoculated} - C_{tVFA\_inoculum}}{C_{substrate}}, \quad (2)$$

where

R_{tVFA}: conversion yield of substrate fed to tVFA ($g_{COD\_tVFA}/g_{COD\_substrate\_fed}$)
$C_{tVFA\_inoculated}$: tVFA concentration in inoculated substrate series (g_{COD_tVFA}/kg_{ML})
$C_{tVFA\_inoculum}$: tVFA concentration in inoculum control series (g_{COD_tVFA}/kg_{ML})
$C_{substrate}$: Initial COD concentration of the substrate fed in the reactor ($g_{COD\_substrate}/kg_{ML}$).

2.5. Analytical methods

Total solids (TS), volatile solids (VS) and total COD (CODt) were measured according to Standard Methods (APHA, 1998). Soluble COD (CODs) was measured with the COD Cell Test method (Spectroquant® kits 1.14541.0001 and 1.14555.0001, Spectroquant® ThermoReactor 620, Photometer SQ200, Merck Germany) according to the provider's instructions.

Analysis of brewery residues was based on the hypothesis that the sample was composed of fibres (cellulose, hemicellulose and lignin), proteins, fat, ash and sugars. Fibre content was determined according to [AOAC]. Proteins were determined according to [ISO 1871:2009], lipid content according to [ISO 1443:1973] and ash content according to [ISO 936]. Sugar content was calculated by difference.

The composition of the biogas was analysed using a gas chromatograph (compact GC, Interscience) with a thermal conductivity detector (GC-TCD). The chromatograph combined two channels. The front channel was equipped with a Rt-QBond column (10 m × 0.32 mm), which separates CO_2 from the other gases. The back channel includes two columns in series: one similar to the front channel (Rt-QBond, 2 m × 0.32 mm) which retains CO_2, followed by a Molsieve 5A column (7 m × 0.32 mm) that separates the other gases. A valve between the two columns, allows to back-flush CO_2 to the injection port so that it doesn't enter in the Molsieve column. The elution was performed under isotherm conditions at 60 °C with helium as carrier gas at 20 ml/min and a pressure of 80 kPa for the front channel and at 70 °C with argon as carrier gas at 10 ml/min and a pressure of 70 kPa for the back channel. The detector was heated at 90 °C and the filament at 170 °C.

VFA and ethanol were analysed using a gas chromatograph (Thermo Finnigan, Trace) with a flame ionisation detector (GC-FID). The column was packed with Carbowax (2m × 1/8′, 4% Carbowax 20 M on Carbograph 1-DA, 80/120 mesh, Alltech). The temperature of the injector and the detector was 250 °C. The sequence for the temperature of the oven started at 140 °C at injection, rising to 180 °C at 4 °C/min and maintained for 10 min, rising to 190 °C at 10 °C/min and maintained for 3 min and decreasing to 140 °C at 10 °C/min and maintained for 1 min. The carrier gas was helium at 1.5 ml/min.

The stoichiometric COD factors for soluble metabolites are 2.087 $g_{COD}/g_{ethanol}$, 1.067 g_{COD}/g_{acetic_acid}, 1.512 $g_{COD}/g_{propionic\_acid}$, 1.813 $g_{COD}/g_{butyric\_acid}$, 1.813 $g_{COD}/g_{iso\text{-}butyric\_acid}$, 2.036 $g_{COD}/g_{valeric\_acid}$, 2.036 $gCOD/g_{iso\text{-}valeric\_acid}$ and 2.207 $g_{COD}/g_{caproic\_acid}$.

3. Results and discussion

3.1. Methanogenic conversion

Fig. 1 presents the production of methane. Fruit residues produce less methane than brewery residues most likely due to their higher content in less easily digestible fibres (i.e. lignin and cellulose combined content of 50, 50 and 55%TS for apple, pear and date pulp, respectively). The highest production is observed for trub (304 $ml_{CH4}/g_{COD\_substrate}$), most likely due to its high sugar content (59% of TS). Indeed, this trub (hot trub) is formed through the precipitation of insoluble substances (e.g. polyphenols and proteins) during wort boiling and thus contains a substantial amount of free sugars. Large scale breweries have systems in place to recover wort from the trub and further ferment it to increase beer yield, but these techniques are not economically viable for micro-breweries.

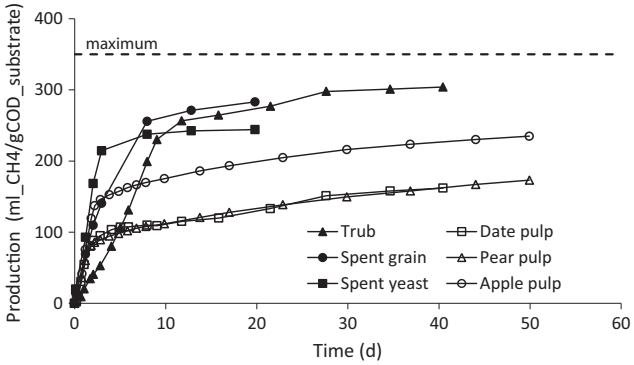

Fig. 1. Methane production. The dashed line indicates the stoichiometric methane production from 1 g_{COD}. Coefficients of variation between triplicates (relative standard deviation) at the end of experiment: 9.3% for date pulp; 1.6% for pear pulp; 3.3% for apple pulp; 1.0% for trub; 6.2% for spent yeast; 2.0% for spent grain.

As indicated by the steep curves of Fig.1, the kinetics of methanogenic conversion are fast as there is sufficient readily digestible substances to be converted. A small delay was observed in the case of trub that can be attributed to the high sugar content that was readily digested in the acidogenic phase, leading to acidification of the broth, which in turn prevented methanogens from developing as fast as in the case of the other substrates (Labatut et al., 2011). In all cases, more than 75% of the final methane production has been reached within the first 17 days of the experiment; in the case of brewery residues this percentage increased to 85% in the first 10 days.

3.2. Acidogenic conversion

Fig.2 shows the evolution of the concentration of tVFA for the substrates tested. The first experiments (date, spent grain and apple pulp) showed that about 60–90% of the highest concentration is obtained within 15–35 days of fermentation, therefore the subsequent experiments had a shorter duration. Similar to the methanogenic conversion, trub exhibits the highest tVFA concentration of approximately 30 g_{COD_tVFA}/kg_{ML}, followed by spent yeast

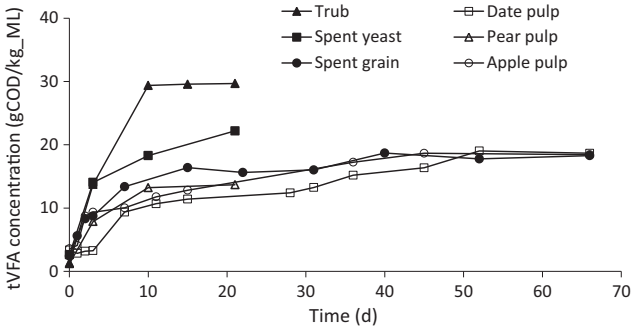

Fig. 2. Total VFA concentrations. Coefficients of variation between reactors at the day of the highest concentration: 8.8% for date pulp at day 52; 4.0% for pear pulp at day 21; 18.4% for apple pulp at day 45; 6.1% for trub at day 21; 3.0% for spent yeast at day 21; 3.9% for spent grain at day 40.

at approximately 22 g_{COD_tVFA}/kg_{ML}. Fruit pulps resulted in lower tVFA concentration values than brewery residues. The highest tVFA concentrations in the control series are presented in Table 4.

In the inoculum controls, tVFA concentrations ranged between 2 and 3.6 g_{COD_tVFA}/kg_{ML}, which corresponds at maximum to 19% of the tVFA concentration of the inoculated series (for apple pulp). In the substrate controls, tVFA concentrations ranged between 2% (for trub) and 66% (for spent grain) of the tVFA concentration in the inoculated series. It can be concluded that the presence and conversion of the substrate contributes the most to the final tVFA concentration of the inoculated series; addition of the inoculum effectively enhances acidogenic fermentation performance.

Of the different VFA (Fig. 3), butyric and acetic acid were the two dominant products in almost all cases, a result that is in accordance with many acidogenic studies using similar types of substrates (Cysneiros et al., 2012; Han and Shin, 2002; Parawira et al., 2004; Rincón et al., 2008). Butyric acid ranges from 5.5 to 13.2 g_{COD}/kg_{ML} for date pulp and trub, respectively, while acetic acid ranges from 2.9 to 9.8 g_{COD}/kg_{ML} for trub and spent yeast, respectively. Interestingly, trub shows a considerable production of caproic acid (up to 13 g_{COD}/kg_{ML}), a result which coincides with the recent interest in production of caproic acid from biomass waste. Caproic acid has a higher carbon number and is easier to separate from water than acetic and butyric acid (Agler et al., 2014; Grootscholten et al., 2013; Kucek et al., 2016; Spirito et al., 2014; Vasudevan et al., 2014). Caproic acid is produced through the chain elongation process, a process where short-chain carboxylic acids like acetic and butyric are elongated with two carbons from a reduced molecule like ethanol (Angenent et al., 2016). In our experiments, ethanol was the main product of the non-inoculated trub series (up to 28.5 g_{COD}/kg_{ML}, see Table 4), which is an indication that production of ethanol could also have taken place in the inoculated reactors and permitted the natural chain elongation process without external ethanol addition.

3.3. Substrate conversion yield

The conversion yields of the substrates into product (methane or tVFA), calculated following Eqs. (1) and (2), are presented in Fig.4 in terms of COD.

Irrespectively of the type of anaerobic conversion, substrates with a higher lignocellulosic content (like fruit pulps) are more recalcitrant to conversion. This is reflected in the product/substrate COD ratio (conversion yield), which is systematically lower than the one of the other residues. The methanogenic conversion yield is significantly higher than the acidogenic potential, for all the substrates tested. Since (i) acidogenic fermentation is an intermediate stage of anaerobic digestion, i.e. methane production goes through VFA production and (ii) the processes do not involve an external

Table 4
tVFA and ethanol concentrations of control series in acidogenic fermentation experiments. tVFA: total volatile fatty acids; COD: chemical oxygen demand; ML: mixed liquor.

Series	tVFA Concentration (g_{COD_tVFA}/kg_{ML})	Ethanol concentration (g_{COD}/kg_{ML})
Inoculum control A	3.56 ± 0.95	
Inoculum control B	2.25 ± 0.03	
Inoculum control C	2.04 ± 0.09	
Apple pulp control	8.15 ± 0.67	
Date pulp control	4.36 ± 0.12	
Spent grain control	12.35 ± 0.48	
Trub control	0.69 ± 0.15	29.03 ± 4.17 (d27)
Spent yeast control	5.59 ± 0.27	20.85 ± 1.80

A: for substrates apple pulp, date pulp, spent grain; B: for substrate trub; C: for substrates spent yeast, pear pulp

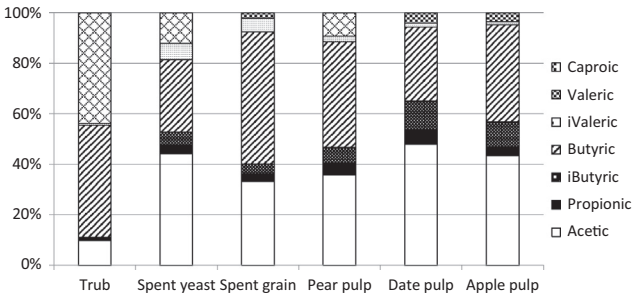

Fig. 3. Volatile fatty acid profile of acidogenic fermentation experiments.

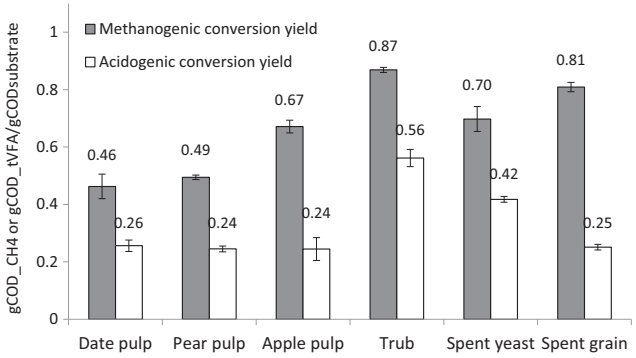

Fig. 4. Acidogenic and methanogenic conversion yield.

electron acceptor, i.e. the COD value of methane as a final product is equal to the COD value of its precursors, the difference in conversion yield is striking. It appears that when the natural process of anaerobic digestion is stopped at the stage of acidogenesis, i.e. inhibition of methanogenesis through the combination of inoculum pre-treatment, pH adjustment and high initial substrate concentration, no further conversion of the substrate to VFA takes place after a certain point, probably due to inhibition effects. On the opposite, when the anaerobic digestion process is naturally left to continue, the produced VFA are not accumulating but are further consumed until the majority of the digestible fraction of the substrate is converted into methane (Agler et al., 2014).

Arslan et al. (2016) suggest overloading (substrate concentration) and product inhibition as the major causes of limitations in acidogenic conversion. The mechanism of product inhibition is summarised in Batstone and Jensen (2011): in the bulk solution, acids are mainly in their undissociated form since their pKa (4.8) is similar to the acidic pH; in this form, they can pass through the cell membrane and dissociate in the cell where the pH is neutral. Cells are then forced to divert energy from growth and metabolism into maintaining homeostasis by exporting protons formed by acid dissociation. Maximum values reported in the comprehensive reviews of Lee et al. (2014) and Arslan et al. (2016) for batch acidogenic reactors are in line with the ones reported in this study. Indicatively, approximately 30 g_{COD_tVFA}/l for food waste (Kim et al., 2011a) and 19 g_{COD_tVFA}/l for rice waste (Dong et al., 2009). Furthermore, Veeken et al. (2000) report that high VFA concentration in the area of 40–50 g_{COD}/l lead to severe inhibition of hydrolysis, which is performed by the same acidogenic microorganisms. Findings of Jiang et al. (2013) confirm this threshold, as VFA production in their experiments on food waste reached a plateau at around 40 g/l. On the substrate concentration, Arslan et al. (2016) suggest that at a level of 40 g_{COD}/l, no further increase of carboxylate concentration is observed. In our study, initial substrate concentration was higher than this threshold and compara-

ble to the ones of Kim et al. (2011b) (approximately 56 g_{COD}/l of a mixture of food waste and sewage sludge leading to approximately 22 g_{COD}/l tVFA concentration) and Komemoto et al. (2009) (approximately 93 g_{COD}/l of food waste leading to 11 g_{COD}/l tVFA concentration). Overall, the range of tVFA concentrations in this study is of the highest reported in literature for stand-alone acidogenic batch reactors and it is plausible to assume that the lower conversion yield (compared to methanogenic conversion) can, indeed, be attributed to overloading and product inhibition.

Acidogenic conversion yields are comparable to the ones in the literature for similar type of substrates. Traverso et al. (2000) reported a COD conversion yield of total COD into tVFA of approximately 24% for mixed vegetable waste, while Espinoza-Escalante et al. (2008) reported a value of 16% for tequilla stillage. Higher values of approximately 55%, comparable to the ones of trub in this study, are reported from Shen et al. (2014) for waste molasses. Given that acetic and butyric acid are in all cases the dominant VFA products, there is no specific correlation between the distribution of VFA and the substrate composition.

Concerning the methanogenic conversion, a 24% substrate-to-methane COD conversion yield for spent yeast (corresponding to 74.2 $ml_{CH4}/g_{COD\_substrate}$) was reported by Sosa-Hernández et al. (2016), which is considerably lower that the approximately 70% yield reported in this study (see Fig. 4). A possible explanation is the high substrate-to-inoculum (S/I) ratio that they used (1.2 compared to 0.19 g_{COD}/g_{COD} in the present study) that could have led to a strong initial acidification of the mixed liquor that prevented methanogenic microorganisms from developing properly and producing methane; a delayed methane production of approximately 10 days, as reported by the authors, supports this interpretation.

Table 5 summarises the methane potential determined for the substrates in this study and compares it to other studies in literature, despite the differences in starting conditions, for example in terms of substrate-to-inoculum (S/I) ratio.

3.4. Economic potential

Table 6 presents the annual economic value of the residues, indicatively calculated for the case of brewery residues for both types of anaerobic conversion. For the acidogenic conversion the following were considered:

- the annual tVFA production which is calculated by multiplying the amount of residues from an artisanal brewery of approximately 6,200 hl/a beer production (Table 6) with the COD content of the residue (Table 1) and the respective acidogenic conversion yield (Fig.4).
- the annual economic value of the VFA streams based on the tVFA production previously calculated, the VFA distribution from Fig. 3 (we only consider acetic, butyric and caproic acid with COD values of 1.067, 1.813 and 2.207 g_{COD}/g_{VFA}, respectively) and commercial VFA prices from Zacharof and Lovitt (2013):

For the methanogenic conversion the following were considered:

- the annual methane production which is calculated by multiplying the amount of residues (Table 6) with the COD content of the residue (Table 1) and the respective methanogenic conversion yield (Fig.4).
- the annual energetic and economic value of methane based on the methane production previously calculated as well as the energy content (LHV: 9.97 kWh/m^3) and the residential D3 price (approximately 32 €/MWh; only considering energy price without transmission, distribution and taxes; D3 corresponds to consumption of 23.3 MWh/a for heating) of natural gas in the Walloon Region, respectively (CWAPE - Price Observatory, 2016).

Table 6 shows that the potential value of the residues of this small artisanal brewery amounts to around 8,600 €/a if they are valorised for the production of methane and approximately 29,250 €/a if they are valorised for the production of VFA. VFA are indeed a higher added value product (in this case more than three times higher) due to their potential use in finer applications (e.g. green chemistry) compared to energy production from methane.

This simplified calculation considers only the value of the final product and not the investment required to harness it in its usable form; on the other hand it also does not consider the price increase margin for bio-based products. In a biorefinery context, the residues' added value could increase even more if the non-converted COD of the substrate after acidogenic conversion is further digested for methane production.

Table 5
Biomethane potential in $ml_{CH4}/g_{VS\_substrate}$ and corresponding S/I on VS basis (values in parenthesis) of residues tested.

Trub	Spent yeast	Spent grain	Pear pulp	Date pulp	Apple pulp	Source[#]
382 (0.21)	548 (0.15)	370 (0.26)	230 (0.20) 426 (0.5)[§]	214 (0.20)	385 (0.22)	This study 1
					297 (0.21–1.50)	2
		429 (0.03–0.08)				3
	313[*] (0.53)					4

[#] 1: Ariunbaatar et al. (2014); 2: Nieto et al. (2012); 3: Colussi et al. (2016); 4: Sosa-Hernández et al. (2016).
[*] Own calculation based on information available in the study.
[§] For food waste composed primarily of fruit and vegetable waste.

Table 6
Economic value of brewery residues on an annual basis.

Residue	Production ($t_{residue}$/a)	Acidogenic conversion		Methanogenic conversion		
		tVFA production (kg_{COD_tVFA}/a)	Economic value (€/a)	CH_4 production (m^3_{CH4}/a)	Energetic value (MWh/a)	Economic value (€/a)
Trub	80	13,190	13,709	7,141	71	2,294
Spent grain	200	15,847	13,315	17,870	178	5,742
Spent yeast	24	2,900	2,228	1,694	17	544
Total	304	31,930	29,252	26,705	266	8,580

a: annual.

In the entire Walloon Region, Belgium, artisanal breweries like the one in this study have an annual production capacity of approximately 778,200 hl. Assuming, for simplification, a similar ratio of residue production, the value of residues could amount to up to 3.7 M€/a for the entire region in the case of VFA and 1.1 M€ in the case of methane. The respective values for the entire Belgium territory are approximately 2,488,600 hl production capacity, 11.7 M€/a VFA potential and 3.4 M€/a methane potential.

As a benchmarking value we refer to the PRODCOM annual data 2015 of Eurostat for two product categories that are the closest to the ones of this study, but still not fully representative: (i) product code 20143220: mono-, di- or tri-chloroacetic acids; *propionic, butanoic and pentanoic acids*; their salts and esters; and (ii) product code 20143271: *acetic acid*. The value of sold production for those product codes in Belgium amounts to 36.5 M€ (EUROSTAT, PRODCOM, Annual Data 2015, 2016).

4. Conclusions

This study highlighted the relevance of both conversion processes for organic waste valorisation of substrates with high solids content. For all substrates, acidogenic conversion yields are systematically lower than the ones in methanogenic conversion (from 35 to 65%), indicating an inhibition of the process of acidogenesis, probably as a result of substrate overloading and product concentration. Nevertheless, the product of acidogenic conversion (VFA streams) has a higher economic value, but also a more costly and technically demanding downstream processing requirement.

The tVFA concentrations and substrate-to-tVFA conversion yields presented here are among the highest reported in literature for batch experiments, with trub performing better than any other substrate. Particularly interesting in trub is the production of caproic acid, a VFA with a high economic value due to its potential applications. Therefore, trub is a substrate that merits further research and together with the other brewery residues, they exhibit a considerable valorisation potential, basing on product value.

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