EFFECT OF SLUDGE AGE ON THE DIGESTION OF THE LIQUOR OBTAINED AFTER ENDOGENOUS ENZYMATIC HYDROLYSIS

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Abstract

Endogenous enzymatic pretreatment applied to sludge with different ages (1.5 days, S1.5D and 10 days, S10D) enhanced the digestion of the sludge liquor in UASB reactors. The optimum HRT for mesophilic digestion were 1 day (S1.5D) and 2 days (S10D), yielding 2.31 and 1.17 $m^3CH_4/m^3_{reactor}$·day, respectively. The optimum HRT for thermophilic digestion were 3 days (S1.5D) and 2 days (S10D), yielding 0.57 and 0.59 $m^3CH_4/m^3_{reactor}$·day, respectively. Adding glycerin to the sludge liquor (1% v/v) increases the methane yield between 19.5% (S1.5D) and 38% (S10D) under mesophilic conditions and between 59.6% (S1.5D) and 29% (S10D) under thermophilic conditions.

Keywords: enzymatic pretreatment, inverted phase fermentation, methane production, co-digestion, glycerin, sludge age

INTRODUCTION

The use of methane as an energy source is an incentive for anaerobic digestion at sewage plants and constitutes a solid process line in sludge treatment [1]. Its peculiarity resides, however, in its generally poor methane yield [2]. Hydrolysis is the limiting step in the anaerobic digestion of sewage sludge, resulting in the production of biogas and a more manageable digestate compared to the initial substrate. During this initial stage, macromolecules, cellular structures and organelles are broken up and transformed into monomers and low molecular weight chemical species, ready to be taken from the environment by bacteria. Once the nutrients are bioavailable after hydrolysis, biochemical reactions continue among bacterial communities following the well-known sequence: acidogenesis, acetogenesis and methanogenesis.

Pretreatments seek to enhance the hydrolysis stage of sludge digestion, ultimately accelerating its stabilization. Accelerating the hydrolysis step will result in the acceleration of the process [3]. There are a number of physical, chemical and biological methods available for this purpose, which are chosen to suit the demands of the wastewater treatment operator [4]. All pretreatments destroy structures until achieving a marked upgrade in the nutrients feeding the acidogenic microorganisms. This evolution may be observed chemically through an increase in indicative parameters such as the soluble chemical oxygen demand (sCOD) and ammonium nitrogen (NH_4^+-N) [5].

Whichever method is employed, the aim is to obtain the highest yield from hydrolysis while minimizing energy and time costs [6]. Enzymatic pretreatment takes advantage of the use of enzymes to catalyse the hydrolysis of different substrates. Enzyme implementation may be achieved by direct addition to sludge or by endogenous promotion of the enzymes naturally present in sludge [7,8]. In turn, this promotion of hydrolases requires the enhancement of their microbial producers. Le et al. presented the "Inverted Phase Fermentation" (IPF) technology, which consists in increasing the endogenous enzymatic activity of sludge

by heating to 42°C for 48 hours [8]. This process exploits the nascent bubbles generated in the sludge to thicken it, at the same time as performing hydrolysis. At the end of the process, a "solid phase, SP" floats above a very clarified "liquid phase/liquor, LP". As a result of IPF, the SP and the liquor present a higher content in soluble organic matter than the sludge. The liquor, highly hydrolised, is rich in soluble species such as volatile fatty acids (VFA)[5].

Besides pretreating the substrate prior to introducing it into the reactor, an upgrade in the methane yield may be achieved by co-digestion with crude glycerin from biodiesel production plants [9]. Glycerin as a by-product of biodiesel production is a magnificent co-substrate for sludge, as it presents C/N ratios of around 250. Another advantage of this industrial by-product is that it often contains methanol; for instance, Castrillón et al. reported contents of around 5-8% [9,10]. Methanol is a carbon source that is readily assimilated by microorganisms whose conversion to methane is very thermodynamically favourable [11].

The operating temperature of anaerobic digesters has a direct effect on methane production and digestate composition. It influences the process at a number of levels: the rate and thermodynamics of the reactions, bacterial sensitivity to xenobiotics, equilibrium displacement towards toxic compounds (e.g. NH_3), energy consumption by bacteria or digestate stabilization achieved after digestion.

Apart from temperature, a parameter that will influence methane production is the age of the sludge, young sludge being potentially more biodegradable [12].

The work presented in this paper seeks to:

- Optimize mesophilic (37ºC) and thermophilic (55ºC) anaerobic digestion of liquor after applying IPF pretreatment to sewage sludge with different ages
- Optimize mesophilic and thermophilic co-digestion of liquor + crude glycerin

MATERIALS AND METHODS
Materials
Sludge samples

Two sludge samples from different sewage plants were studied. The first sludge is produced at a facility characterized by a maximum flow rate of 3,210 m^3/h and a high load of wastewater of industrial origin. It only produces one type of sludge, which comes from biological treatment without previous primary settling. The SRT is very short, around 1.5 days (S1.5D), while removal efficiencies are >75% SS and >65% BOD_5.

The other sludge comes from a sewage plant with an average flow rate of 900 m^3/h, with removal efficiencies of 87% SS and >90% BOD_5. It operates in a conventional manner with production of mixed sludge as a mixture of primary and secondary sludge. The sludge age is around 10 days (S10D).

Crude glycerin

Crude glycerin was obtained from an industrial plant which produces biodiesel from used vegetable oil. The main components were glycerol (49.4%, w/w), soap (27.4%, w/w) and methanol (5.6%, w/w). The water content and the C/N ratio were 0.3% (w/w) and 248, respectively.

Analytical methods

Both total (tCOD) and soluble (sCOD) chemical oxygen demand were determined following Method 5220 of the Standard Methods for the Examination of Water and Wastewater on a Perkin Elmer Lambda 35 Visible-UV spectrophotometer [13]. Samples were centrifuged (3500 rpm, 15 minutes) and filtered through 1.2 μm pore filter paper for sCOD determination. NH_4^+-N was determined using an Orion 95-12 selective electrode for ammonium. Solids (total and volatile) were determined following Method 2540 of the Standard Methods for the Examination of Water and Wastewater [13]. pH was determined using a Crison 25 pH-meter. Volatile fatty acids (VFA) were analysed by gas chromatography using an Agilent 7890A system, equipped with a Nukol column (30 m length, 0.25 mm diameter) manufactured by Supelco. Volatile acidity (VA) was determined following Method 805 described in Degrémont [14]. The

analytical composition of the crude glycerin was determined by an external laboratory. All analytical determinations were performed in triplicate.

Biogas composition was monitored on the aforementioned chromatograph using a Thermal Conductivity Detector (TCD) and a Porapack N packed column plus a molecular sieve, as described elsewhere [15]. Biogas volume was measured on a HI-TEC F101D thermal effect mass gas flow apparatus equipped with an electronic totalizer or with a rotameter and a pump after collection of the biogas in Tedlar bags. All the gas volumes in this paper have been converted to 273.15 K and 101.3 kPa.

Experimental procedure

IPF was performed with samples of sludge, promoting endogenous enzymes at 42°C for 48 hours under anaerobic conditions [8]. Samples of both separated phases (bottom liquid phase and upper solid phase) were taken after 48 hours. The liquor was then chemically characterised and kept refrigerated at 4°C until being used to feed 5 L UASB reactors. The mixture was similarly analysed when crude glycerin was added as a co-substrate (1% v/v).

The reactors were fed by means of a peristaltic pump at low velocitiy to avoid biomass washout and experiments were carried out under mesophilic and thermophilic conditions for both sludge types as mono-substrates and also co-digested with glycerin.

The organic loading rate (OLR) was increased progressively, thus decreasing the hydraulic retention time (HRT), until reaching a maximum value of 21 kg tCOD/m^3·day or until the efficiency dropped or the reactors became destabilized. Experiments were carried out at 37°C and 55°C.

RESULTS AND DISCUSSION

Mesophilic conditions

Digestion of Liquor

The experiments started operating at a HRT of 3 days, corresponding to an OLR of 6.2 and 6.9 g tCOD/L·day for liquor S1.5D and liquor S10D, respectively. It was possible to reduce the HRT to 1 day (OLR 18.5 g tCOD/L·day) for S1.5D and to 1.75 days (11.7 g tCOD/L·day) for S10D without destabilizing the process. The most favourable process data were obtained for liquor S1.5D (Figure 1). Results are shown in Figure 2 (a) and Table 1. As regards specific methane production, the maximum value obtained was 0.18 m^3 CH_4/kg tCOD (for HRT = 2 days) for S1.5D versus 0.15 m^3 CH_4/kg tCOD (for HRT = 3 days) for S10D. The methane production rate increased when lowering the HRT to 1 day (2.31 m^3 CH_4/$m^3_{reactor}$·day) for S1.5D and to 2 days (1.17 m^3 CH_4/$m^3_{reactor}$·day) for S10D. Despite having higher VA, the liquor from the younger sludge (i.e. the more microbiologically active sludge) could be digested at higher OLR without destabilizing the process.
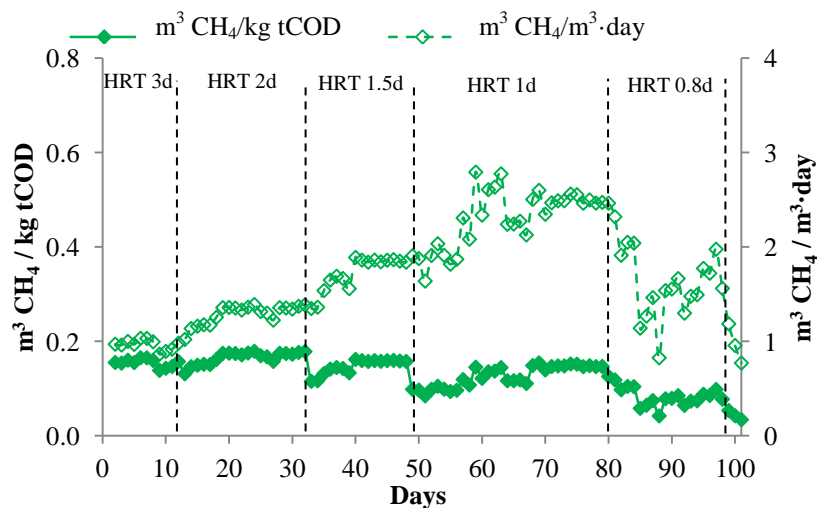

Figure 1: Methane production in the mesophilic digestion of liquor S1.5D in a 5 litre UASB reactor

When applying IPF to S1.5D, 60-75% of the sludge volume was transformed into liquor, while the percentage for S10D was 55-65%. The larger volume transfer in the younger sludge was also associated with greater microbiological activity in the sludge. In fact, the higher degree of hydrolysis in S1.5D resulted in a lower pH in its liquor (5.0-5.4) compared to that of S10D (7.0-7.2). This indicates that the transformation of macromolecules into organic acids was more pronounced in the former case. In spite of the low pH of the former liquor, it was not necessary to raise the pH of the substrate to 6.5-7.5 for anaerobic digestion to be successful [16].

Comparing the results with other enhanced digestion processes (e.g. pre-treated sludge, supernatants or liquors obtained by physical processes for the separation of sludge), the performance is exceptionally good, as it is unusual to find HRT below 3 days [17].

Table 1. Operating conditions and biogas production in the mesophilic digestion of liquors S1.5D and S10D and co-digestion with crude glycerin (1% v/v): optimum performance

	Liquor S1.5D		Liquor S10D		Liquor S1.5D + 1% (v/v) glycerin		Liquor S10D + 1% (v/v) glycerin	
	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
HRT (days)	1		2		2		2	
OLR (g tCOD/L day)	18.46		12.85		13.68		15.66	
tCOD (g/L)	18.46		5.51		25.71		9.80	
sCOD (g/L)	10.61		2.07		7.69		2.51	
Volatile acidity (g/L)	1.85	0.12	0.43	0.12	10.65	3.98	15.41	3.29
Acetic acid (mg/L)	203	81	125	71	9.16	2.22	10.81	2.45
Propionic acid (mg/L)	126	24	109	n.d.	1.33	0.07	1.61	0.12
Isobutyric acid (mg/L)	125	18	102	n.d.	185	75	126	68
Butyric acid (mg/L)	117	n.d.	99	n.d.	150	n.d.	116	n.d.
Isovaleric acid (mg/L)	34	n.d.	68	n.d.	90	n.d.	106	n.d.
Valeric acid (mg/L)	n.d.	n.d.	60	n.d.	111	n.d.	100	n.d.
pH	4.99	7.31	7.01	7.18	76	n.d.	71	n.d.
NH_4^+-N (mg/L)	301	296	853	828	90	n.d.	65	n.d.
m^3 biogas/kg tCOD	0.20	0.16	0.25	0.15	6.01	7.52	7.10	7.26
m^3 biogas/ $m^3_{reactor}$ day	3.35	1.95	3.77	2.47	318	208	798	423
% CH_4	69.0		60.2		73.2		65.3	
H_2S (ppm)	127		42		100		45	

n.d., not detected

Under high organic load operating conditions, destabilization is explained by the accumulation of VFA in the digestate. The pH itself did not provide evidence of instability, as its value in the digestates was moderately basic.

As mentioned previously, the VA of liquor S1.5D is higher than that of liquor S10D, the removal of this acidity was also higher, pointing to the robustness of the process. The appearance of propionic acid began to indicate acidogenic activity unmatched by the syntrophic oxidation of propionate from HRT ≤ 1 day in liquor S1.5D. The accumulation of this acid did not affect methanogenesis, as the percentage of CH_4 was maximal (69%). pH actually affects all stages of anaerobic digestion, although Siegert and Banks found that the accumulation of VFA inhibited hydrolysis/acetogenesis [18]. That is, if the substrate is already hydrolysed, inhibition will affect acetogenesis, thus limiting the production of CH_4 to the hydrogenotrophic pathway.

◆ S1.5D specific production   ■ S10D (s.p.)
△ S1.5D+1% (v/v) gly (s.p.)   ● S10D+1% (v/v) gly (s.p.)
◇ S1.5D volumetric production   □ S10D (v.p.)
△ S1.5D+1% (v/v) gly (v.p.)   ○ S10D+1% (v/v) gly (v.p.)

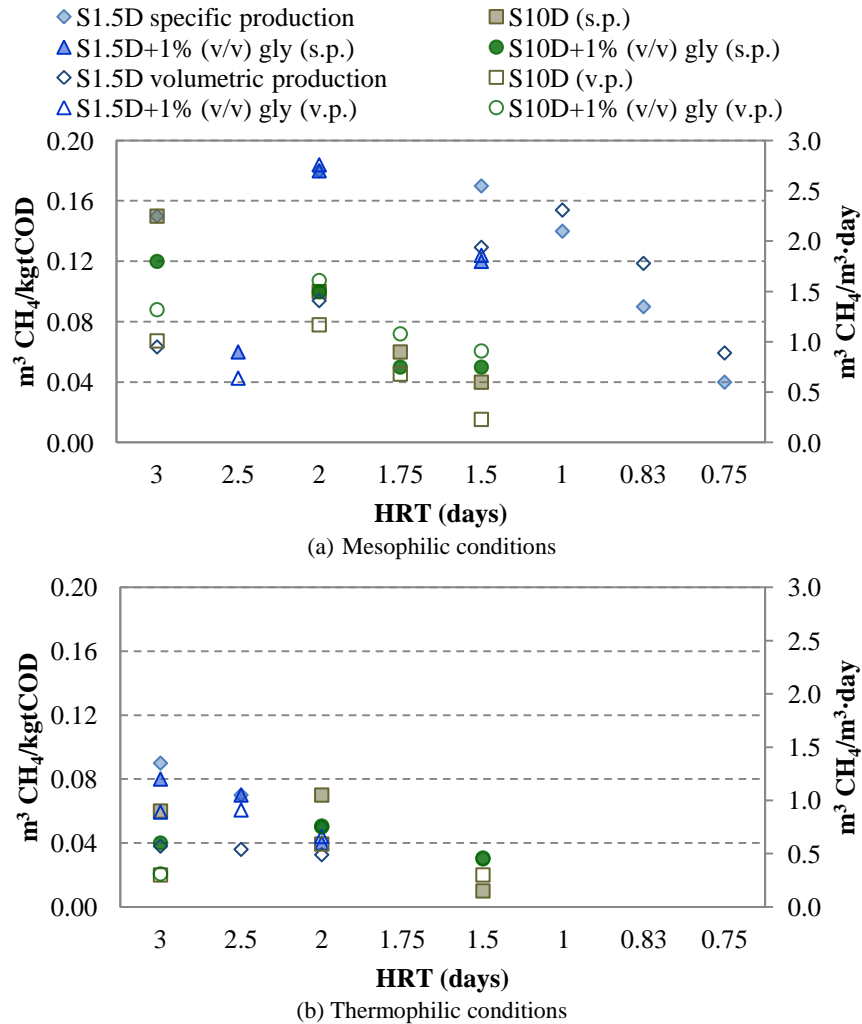(a) Mesophilic conditions

(b) Thermophilic conditions

Figure 2: Methane production of liquors S1.5D and S10D and co-digestion with crude glycerine (1% v/v)
a) Mesophilic conditions b) Thermophilic conditions

Liquor co-digested with crude glycerin

Sludge liquor was co-digested with 1% v/v crude glycerin. Due to the high content in organic matter of the glycerin, the OLR for similar HRT to those applied in the digestion of the liquor were higher. The values ranged from 11.3 to 19.0 tCOD/L·day for S1.5 D and from 10.4 to 21.0 tCOD/L·day for S10D.

Co-digestion raised the methane production rate of liquor S1.5D to 2.76 m^3 $CH_4/m^3_{reactor}$·day, for 2 days HRT (13.7 g tCOD/L·day) achieving very high COD removal efficiencies, up to 89%. Decreasing the HRT from 2 to 1.5 days resulted in poorer performance which, however, cannot be explained by increased acidity, neither NH_4^+-N nor H_2S. As regards specific methane production, the maximum value was also obtained for 2 days HRT (0.18 m^3 CH_4/kg tCOD).

For liquor S10D, the optimal HRT was also 2 days, obtaining a methane production rate of 1.61 m^3 $CH_4/m^3_{reactor}$·day and a specific methane production of 0.10 m^3 CH_4/kg tCOD.

The enhancement is not so much in terms of the degradation of organic matter as in the methane production rate and the methane content of the biogas. In fact, the crude glycerin promotes the removal of organic matter in the case of liquor S1.5D, but there is no such trend in the case of liquor S10D. That is, the fraction of refractory organic matter in the older sludge, liquor S10D, did not decrease as a result of the occurrence of synergies in co-digestion.

Thermophilic conditions
Digestion of Liquor
Experiments commenced at HRT = 3 days (6.4 g tCOD/L·day for S1.5D and 5.3 g tCOD/L·day for S10D). Without destabilizing the process, it was possible to reduce the HRT to 2.5 days for liquor S1.5D and to 2 days in the case of liquor S10D. Results are shown in Figure 2 (b) and Table 2.
In thermophilic digestion, the highest methane production rate (0.57 m^3 $CH_4/m^3_{reactor}$·day) was obtained at HRT = 3 days, 6.4 g tCOD/L·day, for liquor S1.5D and at HRT = 2 days, 8.2 g tCOD/L·day, for the liquor S10D (0.59 m^3 $CH_4/m^3_{reactor}$·day).
Major differences were observed with respect to the digestion of these substrates under mesophilic conditions. In the case of liquor S1.5D, the optimum values led to a 50% decrease in specific methane production, and a reduction of more than 75% in the methane production rate, while tCOD removal efficiencies decreased from 75% to 55%. In liquor S10D, the optimal values led to a 53% decrease in specific methane production and a 50% reduction in the methane production rate, tCOD removal efficiencies decreasing from 62% to 46%.

Table 2. Operating conditions and biogas production in the thermophilic digestion of liquors S1.5D and S10D and co-digestion with crude glycerin (1% v/v): optimum performance

	Liquor S1.5D		Liquor S10D		Liquor S1.5D + 1% (v/v) glycerin		Liquor S10D + 1% (v/v) glycerin	
	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
HRT (days)	3		2		2.5		2	
OLR (g tCOD/L day)	6.36		8.19		13.07		14.21	
tCOD (g/L)	19.07		9.47		16.38		8.82	
sCOD (g/L)	13.64		4.61		8.52		4.35	
Volatile acidity (g/L)	1.75	0.17	0.77	0.07	15.49	6.93	14.31	3.19
Acetic acid (mg/L)	194	100	176	73	10.61	5.16	9.34	2.21
Propionic acid (mg/L)	124	43	170	n.d.	1.42	0.12	0.67	0.10
Isobutyric acid (mg/L)	106	n.d.	120	n.d.	190	95	161	69
Butyric acid (mg/L)	103	n.d.	115	n.d.	123	n.d.	158	n.d.
Isovaleric acid (mg/L)	94	n.d.	89	n.d.	106	n.d.	112	n.d.
Valeric acid (mg/L)	91	n.d.	87	n.d.	107	n.d.	110	n.d.
pH	5.25	7.15	6.90	7.28	95	n.d.	93	n.d.
NH_4^+-N (mg/L)	757	1074	903	856	92	n.d.	86	n.d.
m^3 biogas/kg tCOD	0.15	0.12	0.11	0.08	6.40	7.25	6.25	7.25
m^3 biogas/ $m^3_{reactor}$ day	0.94	0.97	1.47	1.16	516	636	801	795
% CH_4	60.8		61.1		61.7		65.1	
H_2S (ppm)	62		106		98		140	

n.d., not detected

In liquor S1.5D, in addition to acetic acid, other VFA already appear in the digestate at HRT = 2 days. This had not occurred when operating under mesophilic conditions until HRT = 1 day. The poorer performance should not be attributed to deficiencies in the inoculum, as the biomass was taken from a high performance IBR [10].
On reviewing the literature, it can be seen that many authors favour thermophilic digestion due to the higher methane yields obtained [20]. Hydrolysis is faster under thermophilic conditions. For the other stages, the kinetic parameters are not unique to either temperature regime. Ge et al., conducting experiments with primary sludge, concluded that "the improved performance in thermophilic-mesophilic temperature phase anaerobic digestion of primary sludge was due to increased hydrolysis rate, rather than overall degradability" [6]. They also rejected the need for a single thermophilic reactor to achieve similar results.

Liquor co-digested with crude glycerin

For liquor S1.5 D, the reactors were operated at OLR in the range of 10.6-15.9 g tCOD/L·day. The methane production rate rose to 0.91 m^3 $CH_4/m^3_{reactor}$·day, operating at HRT = 2.5 days while the specific methane production was similar to that obtained in the digestion of the liquor alone.

Although co-digestion leads to a 60% improvement in the methane production rate, the value is much lower than the values obtained when operating under mesophilic conditions.

The methane yield decreased when operating at the lowest HRT. Although the reactor was kept operating for several days without acidification under mesophilic co-digestion conditions, inhibition was observed when operating under thermophilic conditions due to the lower tolerance to VFA of thermophilic versus mesophilic methanogenic microorganisms [19].

As for mono-substrate digestion, the optimal HRT for co-digestion of liquor S10D was 2 days. Although the methane production rate increased up to 0.76 m^3 $CH_4/m^3_{reactor}$·day, the specific methane production was similar to that of mono-substrate digestion and lower than under mesophilic conditions.

CONCLUSIONS

Hydrolysed liquor (0.5-1.5 % TS) was obtained from two different sewage sludges by applying IPF. Using this method, it was possible to employ high organic loading rate anaerobic reactors (UASB) to digest this substrate. It was not necessary to raise the pH of the liquor (5.0-6.9) to carry out the digestion process.

Mesophilic anaerobic digestion of the liquor was optimized for the two types of sewage sludge, managing to decrease the HRT to 3-1 days, while increasing the OLR to 6.9-18.5 g tCOD/L day. These HRT were consistent with a specific methane production of 0.15-0.18 m^3 CH_4/kg tCOD, a methane production rate of 1.17-2.31 m^3 $CH_4/m^3_{reactor}$·day and tCOD removal efficiencies of 62-75%. The younger sludge yielded the best results.

Thermophilic anaerobic digestion of the liquor was not able to improve the values obtained under mesophilic conditions. The optimal HRT remained at 3-2 days, with OLR of 6.4-8.2 g tCOD/L day, yielding methane production rates of 0.57-0.59 m^3 $CH_4/m^3_{reactor}$·day and tCOD removal efficiencies of 46-55%.

The differences between the two temperature regimes are mainly attributed to the fact that the substrate is hydrolysed when introduced into the digesters, as hydrolysis is the limiting step when digesting sludge, and it is faster at 55ºC than at 37ºC. In our case, the substrate has been previously hydrolysed and therefore, no benefits are found for thermophilic operation, but rather drawbacks due to the reduced tolerance of thermophilic microorganisms to process inhibitors, such as VFA.

Co-digestion with crude glycerin not only brought stability to the process, but also improved the performance of the system. Under mesophilic conditions, digestion was optimized, enabling operation at OLR of 13.7-15.7 g tCOD/L day and achieving methane production rates of 1.61-2.76 m^3 $CH_4/m^3_{reactor}$·day and tCOD removal efficiencies of 59-89%. Poorer results were obtained for thermophilic co-digestion, achieving methane production rates of 0.76-0.91 m^3 $CH_4/m^3_{reactor}$·day and tCOD removal efficiencies of 48%, for OLR of 10.6-14.2 g tCOD/L day.

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