Anaerobic digestion of vegetable tannery and municipal sludge

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Abstract
The possibility of treating vegetable tannery industry primary sludge (VTPS) through anaerobic digestion was investigated. A preliminary test was carried out using municipal anaerobic sludge as inoculum and VTPS as substrate in a continuously fed CSTR (Continuous-flow Stirred-Tank Reactor) under mesophilic conditions but no methane production was observed. As a consequence, different microbial community was used as inoculum. The process efficiency in two reactors (R1 and R2) was compared with the aim of evaluating microbial community adaptation towards the inhibiting character of the matrix. R1 was fed with a mixture of an increasing amount of VTPS and conventional domestic primary sludge (CDPS) and R2 with CDPS only as control.
Results showed that it is possible to co-digest VTPS:CDPS up to 30:70 before COD and VSS removals decrease and acetate starts to accumulate. A COD and a VSS removal efficiency of 48.7% and 53.2% were obtained with a CDPS/VTPS ratio of 30/70.
Through batch test, a specific methane productivity (SMP) of 0.27 NL CH_4 g-1 VSS_{fed} was estimated for R1 with a CDPS/VTPS ratio of 30/70, three times higher than the SMP obtained in batch test with CDPS as single substrate (0.09 NL CH_4 g^{-1} VSS_{fed}).

INTRODUCTION
Tannery industry generates a large amount of both solid and liquid wastes: more than 594.000 tons per year of solid wastes and 15-30 m^3 of water per ton of finished product are produced in the Italian context.
The Tuscan tannery district is the second largest in Europe and is divided into two different sectors were chrome and vegetable tanning processes are alternatively operated. This offers the opportunity of treating vegetable and chrome tannery wastewaters in separated wastewater treatment plants (WWTP).
In this context, in the Cuoiodepur WWTP (San Miniato, Pisa-Italy) are treated almost exclusively vegetable tannery wastewaters characterized by high COD concentration (12-23 gO_2 L^{-1}), Suspended Solids (6-31 gSS L^{-1}), Ammonium (0.12-0.25 gN-NH_4^- L^{-1}), Chlorides (0.3 to 8 g L^{-1}) and Sulphate (1.7-2.7 $gSO_4^=$ L^{-1}) (Mannucci et al., 2010).
In the last two decades, the potential benefits of the anaerobic treatment of tannery wastewater are confirmed by the increasing attention dedicated to the investigation of the anaerobic process as a technological solution to treat this particular industrial wastewater (Boshoff et al., 2004; Rajesh Banu and Kaliappan, 2007).

However, the presence of inhibiting compounds such as polyphenols (Vijayaraghavan and Murthy, 1997), metals and sulphide (Shin et al., 1997; Roy et al., 2013), limited the application of anaerobic processes to tannery wastewater at full-scale.
Moreover, tannins are commonly recognized as biorefractory compounds and their presence potentially affect anaerobic processes (Munz et al., 2009).
In this context, sulphate reduction remains a major issue due to higher kinetics of sulphate reducing bacteria (SRB) respect to methanogens; sulphide produced by SRB is, moreover, toxic to both SRB and methanogens. Two stages of inhibition exist as a result of sulphate reduction (Wang et al., 2013). Primary inhibition is due to competition for common organic and inorganic substrates from sulfate reducing bacteria (SRB), which suppresses methane production. Secondary inhibition results from the toxicity of sulphide to various bacteria groups. The inhibitory sulphide levels reported in the literature were in the range of 100–800 mg L^{-1} of dissolved sulfide or approximately 50–400 mg L^{-1} of undissociated H_2S (Chen et al., 2008).
Primary sludge obtained from the sedimentation of particulate matters contained in vegetable tannery wastewaters are still characterized by high concentration of inhibiting compounds for anaerobic processes. High organics and nutrients concentration allows the use of primary trannery sludge as a source for fertilizer production: in the Cuoiodepur WWTP context, the sludge is used to produce a high nitrogen and phosphorous content fertilizer after thermal drying and mixing with by-product of industrial tannery process.
Since European regulation on land application is becoming increasingly stringent, despite the presence of tannins and their effect on the outcome of the competition between sulphate reducing and methanogens (Mannucci et al., 2014), it is important to evaluate alternative options for sludge disposal such as anaerobic digestion.
Literature on anaerobic digestion of tannery sludge shows considerable variation in the inhibition/toxicity levels reported for most substances. The major reason for these variations is the complexity of the anaerobic digestion process where mechanisms such as antagonism, synergism, acclimation, and complexing could significantly affect the phenomenon of inhibition.
Although several works have been performed for tannery wastewater anaerobic treatment (Daryapurkar et al. 2001, Lefebvre et al. 2004), only few works on anaerobic digestion of tannery sludge have been published (Dhayalan et al. 2007; Thangamani et al., 2010; Zupancic et al., 2010; Sri Bala Kameswari et al., 2012) and no information about the application of anaerobic processes on the sole vegetable tannery primary sludge are present in literature.
Dhayalan et al. 2007 confirms the possibility to treat, in batch conditions, untanned solids leather wastes, chrome and vegetable tanned samples obtaining higher performance from the digestion of chrome tanning wastes than vegetable tanning ones.
Zupancic et al., 2010 investigated the potential of the anaerobic digestion of different types of tannery waste: chrome tannery sludge, waste fleshing and waste skin trimmings. Used tannery sludge is a mixture of primary chrome tannery sludge and biological sludge from an industrial WWTP treating tannery wastewaters. Batch tests have been conducted to estimate the BMP (Biochemical Methane Potential) of the individual substrates and co-digestion process have been tested using a semi-continuous and anaerobic sequencing batch reactor (ASBR).
Batch tests are generally used to evaluate the biodegradability and methane production potential of organic substrates and provide guidance to continuous study (Li et al., 2014). Continuous studies allow to assess the stability and performance of reactors, which is more useful for industrial application.
Regardless of the treated solid waste and the used reactor technology, when conducting

anaerobic digestion, start-up and biomass adaptation procedures are pivotal (Zupancic et al., 2010).
The presence of high concentration of recalcitrant and inhibitory compounds and the lack of adaptation of the biomass, are regarded as the main cause of the low efficiency, as solids reduction and biogas production, of the anaerobic digestion applied to vegetable tannery WWTP sludge.
An appropriate management of the acclimatation process could allow the application of the anaerobic digestion process to vegetable tannery primary sludge reducing the inhibitory effects on methanogenic bacteria.
The aim of this work is to evaluate the efficiency of the anaerobic digestion process of vegetable tannery industry primary sludge (VTPS). The technical feasibility of the process was investigated through continuously fed bench scale reactors and specific batch tests. The role of a gradual adaptation of anaerobic biomass to VTPS was investigated through the feeding of a mixture of tannery and civil wastewater sludge with an increasing fraction of industrial sludge.

MATERIALS AND METHODS

Anaerobic batch test were conducted using an OxiTop® OC110 system (WTW Ltd, Germany) while continuous tests were performed using two (R1 and R2) identical bench scale continuously fed 3.8 L CSTR (Continuous Stirred Tank Reactor).
Control reactor (R2) was fed with CDPS from a municipal WWTP (Poggibonsi, Siena - Italy) for more than 230 days and have been used as control reactor. R1 was fed with a mixture of CDPS and VTPS. The CDPS/VTPS ratio was decreased in time in order to acclimate the biomass. Four volumetric CDPS/VTPS ratios have been tested (90/10; 80/20; 70/30; 60/40) during the experimentation that lasted more than eight months. VTPS were collected daily from the primary settler of the Cuoiodepur WWTP.
Temperature and pH were continuously measured trough a pH probe (LZX546, Hach Lange, Germany) connected to a control unit (SC1000, Hach Lange, Germany) that allowed the dosage of a buffer solution (NaOH 0.1M). Temperature control was obtained by a cryo-thermostat and liquid recirculation in the external shell of the reactors. A rubber seal ensures hermetic closure of the reactors. Sludge mixing was ensured by three shovels installed on a vertical shaft.
The process was operated at 35±0.5°C, pH value was maintained at 7±0.02 and solids retention time (SRT) was controlled at 15 days. Feeding and wasting pumps worked simultaneously and with the same flow to avoid changes in pressure inside the reactors. Feeding and effluent were analyzed twice a week and the following parameters were monitored according to IRSA-CNR (Italian Institute of Water Research-National Research Council) methods: COD, TSS and VSS. Sulphates were evaluated through ionic chromatography (ICS1000, Dionex, U.S.A) while Sulphides and Acetate were measured through colorimetric analysis using cuvette test (Hach-Lange, Germany).
Methane fraction in the produced biogas was evaluated through gas chromatography (MicroGC3000, Pollution, Bologna - Italy).
As inoculums, for both R1 and R2, a mixture of anaerobic sludge from a municipal anaerobic digester (54 %) (San Colombano, Florence – Italy), anaerobic biomass acclimated to VTPS (15%), bovine manure (22%) and primary tannery sludge from Cuoiodepur WWTP (VTPS, 9%) have been used to obtain an high biodiversity of the microbial consortia.
Acclimated biomass to VTPS was obtained in batch conditions after 100 days of operation at 37 °C. Bovine manure (80%) and VTPS (20%) have been used as inoculum. Every 7 days, 33% of the total volume were replaced with a mixture of bovine manure and VTPS. The ratio between VTPS and bovine manure have been

increased in time reaching manure/VTPS = 4 at the end of the acclimatation phase.
VTPS fraction in the R1 feeding mixture have been increased stepwise to encourage the adaptation of the inoculum to the tannery primary sludge:

- Phase I: CDPS/VTPS = 90/10, from day 0 to day 69;
- Phase II: CDPS/VTPS = 80/20, from day 70 to day 127;
- Phase II: CDPS/VTPS = 70/30, from day 128 to day 251;
- Phase IV: CDPS/VTPS = 60/40, from day 251 to day

Batch tests were conducted with an inoculum:substrate ratio of 1:1, using the R1 effluent as inoculum and a mixture of VTPS and CDPS with a volumetric ratio of 30:70. Batch tests using R2 effluent as inoculums and CDPS as substrate were conducted to estimate the specific methane productivity (SMP) in R2. Batch tests were carried out in triplicate, at 35 ± 0.5 °C for 15 days, in a closed temperature-controlled anaerobic digester (Pitk et al. 2012). Cumulative biogas production, COD and VSS removal efficiency, biogas production and CH_4 percentage in the produced biogas were used as key parameters for the estimation of the anaerobic process efficiency.
To solve the carbon mass balance Eq. 1 and Eq. 2 have been used:

$$\mathrm{CH_{4_{COD}}} = (\mathrm{COD_{in}} - \mathrm{COD_{out}} - \mathrm{COD_{SO_4}} - \Delta\mathrm{COD_s}) * 0.35\,\frac{\mathrm{NLCH_4}}{\mathrm{gCOD}} \qquad \text{(Eq. 1)}$$

$$\mathrm{CH_{4_{VSS}}} = (\mathrm{VSS_{in}} - \mathrm{VSS_{out}}) * 1.42 * 0.35\,\frac{\mathrm{NLCH_4}}{\mathrm{gCOD}} \qquad \text{(Eq. 2)}$$

Where:

- COD_{in} is the influent COD;
- COD_{out} is the effluent COD;
- COD_{SO_4} is the COD used int the sulphate reduction process ($COD_{SO_4} = \Delta SO_4 * 0.67\ gCOD\ g^{-1}SO_4$)
- ΔCOD_s is the removed soluble COD;
- 1.42 $gCODg^{-1}VSS$ is the conversion between COD and SSV.

Adopted Oxitop® reactor are equipped with a pressure transducer for the measurement of the pressure variation in the headspace due to biogas production. The volume (in normal conditions) of the produced biogas was estimated using Eq 3.

$$\mathrm{V_{Biogas}} = \frac{(\mathrm{V_{hs}} * \mathrm{P_R})}{\mathrm{T_R}}\frac{\mathrm{T_N}}{\mathrm{P_N}} \qquad \text{(Eq. 3)}$$

Where:

- V_{hs} is the volume of the headspace;
- P_R is the recorded pressure;
- P_N is pressure in normal conditions = 1 atm
- T_R is temperature inside reactor = 35°C = 308 K;
- T_N is temperature in normal conditions = 20°C = 293 K.

RESULTS AND DISCUSSIONS

A preliminary continuous test was carried out using anaerobic sludge from a municipal WWTP as inoculum and 100% VTPS from Cuoiodepur WWTP as the only substrate in R1. No methane production and a COD removal efficiency of 13% have been observed after 30 days of operation.
A new inoculum was performed for both R1 and R2.
In R2 average COD and VSS removal during the whole experimentation were

22.4±5.1% and 25.3±4.6%, respectively.
Referring to R1, COD and VSS removal efficiency in the last 20 days of phase I were 37.6% and 37.0%, respectively, and increased when the percentage of VTPS in the feed increased from 10% to 20%, reaching removal efficiencies of 61.3% and 54.6%, respectively, in the last 20 days of phase II. At the end of phase III, COD and VSS removals dropped to 48.7% and 53.2%, respectively. The COD and VSS removals trend during the whole experimentation are reported in Figure 2 and 3, respectively.

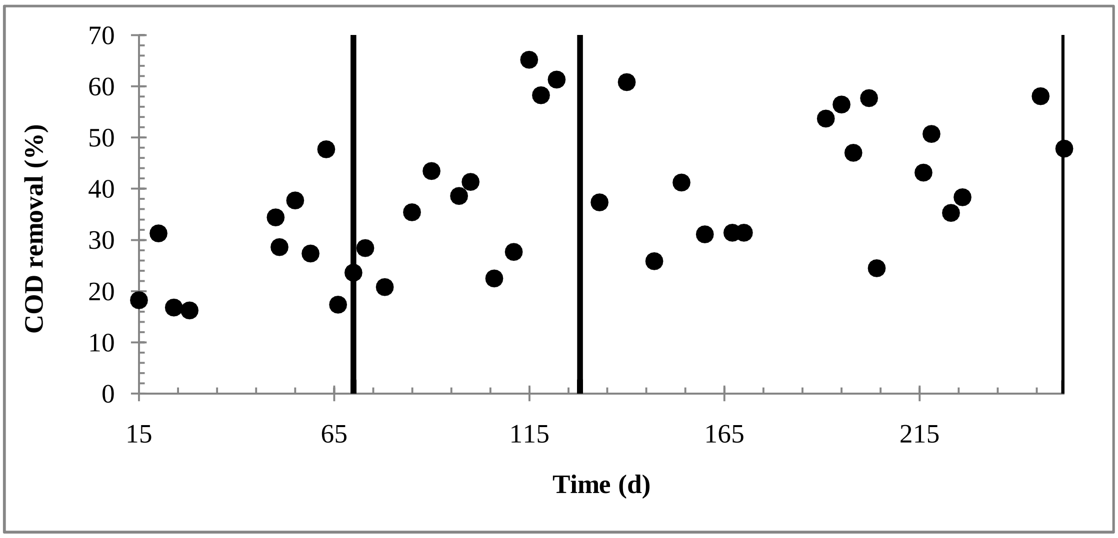

Figure 1 – COD removal efficiency in R1

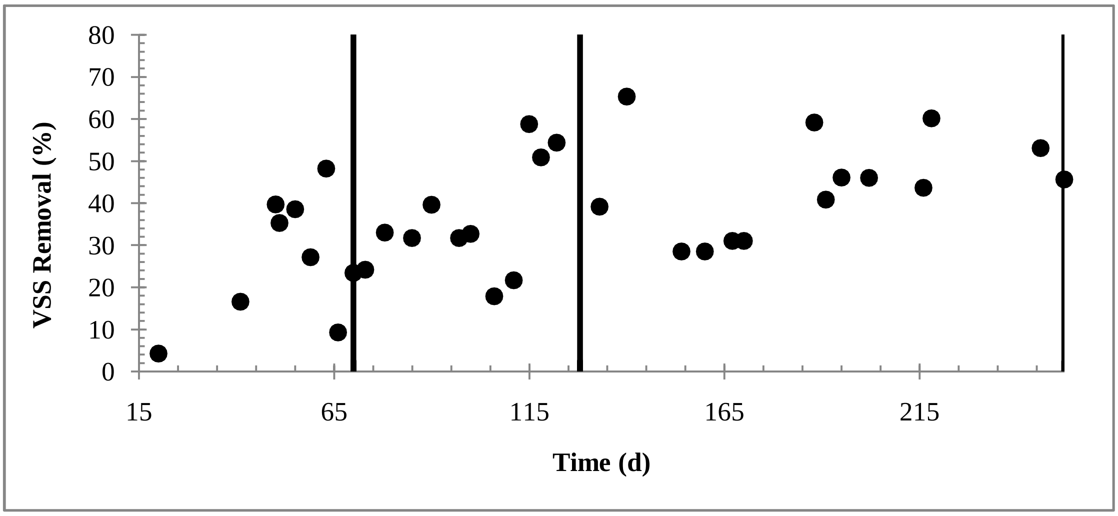

Figure 2 – VSS removal efficiency in R1

For both COD and VSS removal, changes in feeding composition causes rapid reductions that are more evident in the transition from phase II to phase III. Within a single phase, both COD and VSS increases reaching maximum values at the end of each phase maybe due for the acclimatation of the anaerobic biomass to the new feeding composition.
The reduction of the removal performances in R1 at the end of phase III respect to those in phase II could be due to the achievements of inhibiting condition after the increase of VTPS fraction in the feeding. VSS removal efficiencies are less influenced by feeding changes than COD removal efficiencies maybe due to higher inhibitory effects on methanogenic bacteria than others as the acetate accumulation during phase III confirms (Figure 4). No acetate accumulation was observed in R2.

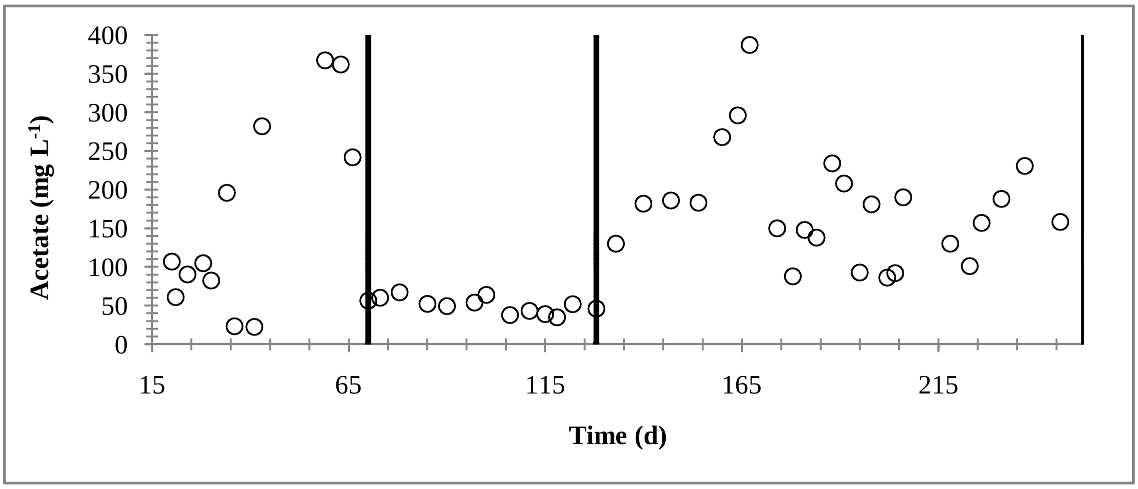

Figure 3 – Acetate concentration in R1

As COD and VSS removal, Sulphate removal efficiency was influenced by feeding changes. At the beginning of phase III there was a change in tannery primary sludge sulphate concentration that caused a reduction in sulphate removal efficiency also in favourable condition for SRB due to high acetate concentration (Figure 5). At the end of phase III with an influent sulphate concentration similar to influent sulphate in phase I and II, sulphate removal reached 84% similarly to what obtained in the previous phases. Sulphide concentration lower than 1 mg L^{-1} were maintained during the whole experimentation.

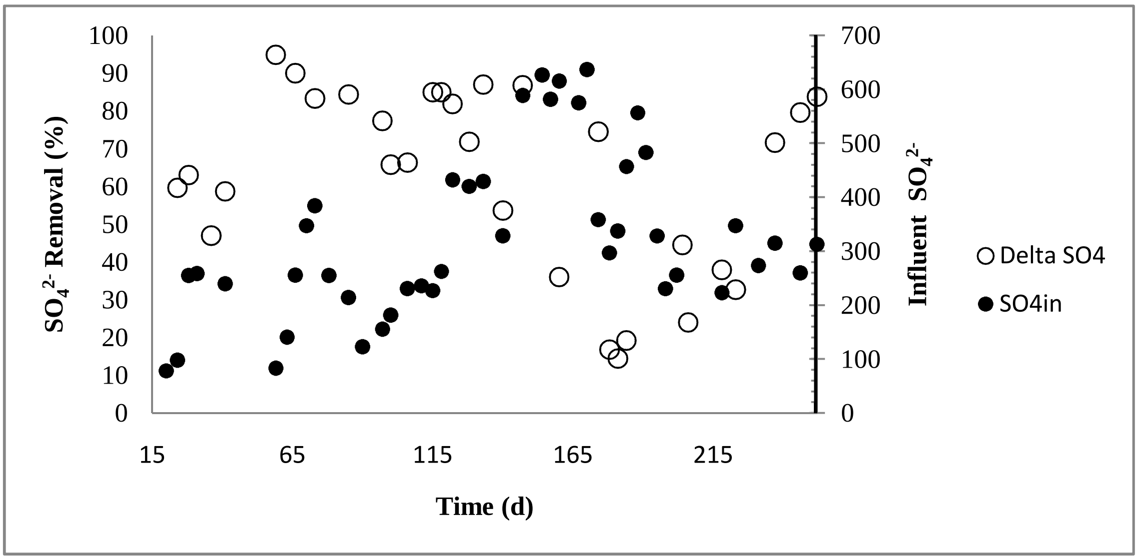

Figure 3 – R1 influent sulphate concentration and sulphate removal efficiency in R1

During phase III a percentage of 65±5.5 % of CH_4 in the produced biogas was obtained in three different sampling; batch tests on biogas production were made through Oxitop system to confirm the data and to estimate the ratio between removed COD and produced CH_4 with the operational conditions maintained in phase III and in R2.
An example of the trend of the overpressure in performed batch tests is reported in Figure 6.

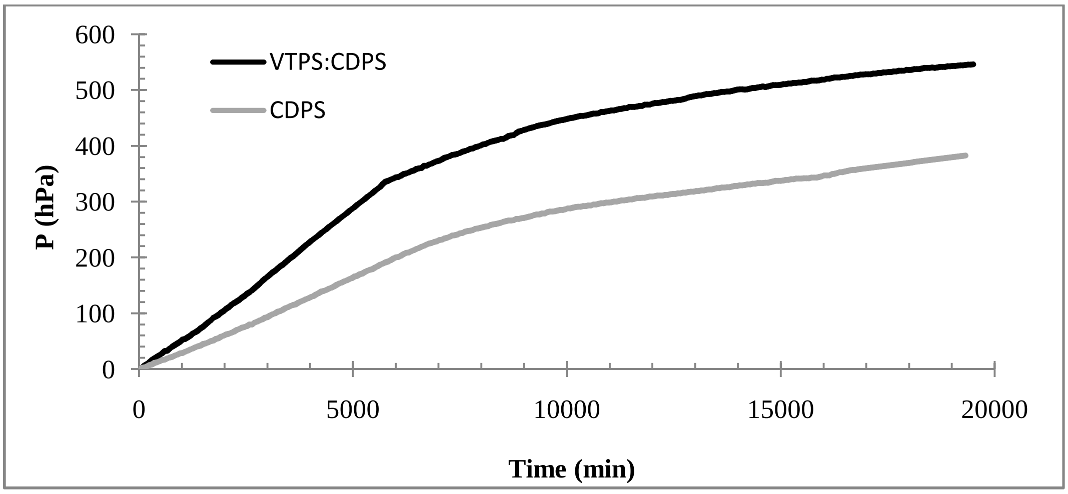

Figure 6 – Overpressure trend in batch tests

A biogas production of 0.25 NL was estimated from the COD removal (0.65±0.08) and a methane percentage of 65±2.2% and a methane production of 0.29 L CH_4 per g COD were obtained. The ratio between produced CH_4 and removed COD was used to estimate the SMP during phase III, that resulted 0.27 NL CH_4 g-1 VSS_{fed}, three times higher than the SMP obtained in batch test with CDPS as single substrate (0.09 NL CH_4 g^{-1} VSS_{fed}).

CONCLUSIONS

The study was carried out in laboratory scale reactors with the aim of evaluate the efficiency of the anaerobic digestion process of vegetable tannery industry primary sludge (VTPS). Continuously fed CSTR experiments shown that inhibitory effects on methanogenic biomass could starts with a CDPS/VTPS ratio of 30/70 when acetate started to accumulate in R1 and COD removal decreases. No significant variation of VSS removal efficiency were observed and VSS removal of 53.3% was maintained in steady state in all the tested conditions. The presence of up to 30% of primary sludge in the feeding resulted in an increase of 300% on specific methane production respect to the digestion of the sole civil primary sludge.

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