Biorefinery of cellulosic primary sludge towards targeted Short Chain Fatty Acids, phosphorus and methane recovery

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Abstract

Cellulose from used toilet paper is a major untapped resource embedded in municipal wastewater which recovery and valorisation to valuable products can be optimized. Cellulosic primary sludge (CPS) can be separated by upstream dynamic sieving and anaerobically digested to recover methane as much as 4.02 m^3/capita·year. On the other hand, optimal acidogenic fermenting conditions of CPS allows the production of targeted short-chain fatty acids (SCFAs) as much as 2.92 kg COD/capita·year. Here propionate content can be more than 30% and can optimize the enhanced biological phosphorus removal (EBPR) processes or the higher valuable co-polymer of polyhydroxyalkanoates (PHAs). In this work, first a full set of batch assays were used at three different temperatures (37, 55 and 70 °C) and three different initial pH (8, 9 and 10) to identify the best conditions for optimizing both the total SCFAs and propionate content from CPS fermentation. Then, the optimal conditions were applied in long term to a Sequencing

Batch Fermentation Reactor where the highest propionate production (100-120 mg COD/g TVS_{fed}·d) was obtained at 37°C and adjusting the feeding pH at 8. This was attributed to the higher hydrolysis efficiency of the cellulosic materials (up to 44%), which increased the selective growth of *Propionibacterium acidopropionici* in the fermentation broth up to 34%. At the same time, around 88% of the phosphorus released during the acidogenic fermentation was recovered as much as 0.15 kg of struvite per capita·year. Finally, the potential market value was preliminary estimated for the recovered materials that can triple over the conventional scenario of biogas recovery in existing municipal wastewater treatment plants.

Keywords: cellulosic primary sludge; acidogenic fermentation; propionate; resource recovery; struvite

Highlights

- Separation and fermentation of cellulosic primary sludge enable wastewater-based biorefinery
- Initial pH (8) and T (37 °C) selected *Propionibacterium acidopropionici* which optimized the propionate recovery
- Struvite recovery from CPS fermentation liquid best integrates the biorefinery concept
- The CPS-based biorefinery could triple the economic value of wastewater

1. Introduction

Primary sludge (PS) usually contains a large quantity of biodegradable organic compounds such proteins, carbohydrates, cellulose and other organic materials. Among them, cellulose represents approximately 30-50% of the influent suspended solids in wastewater treatment plants (WWTPs) of Western European countries (STOWA report, 2012) where toilet paper is flushed into the sewers system. In these countries, the average per capita consumption of toilet paper was estimated around 15 kg per year which is 3 times more than the global average consumption (4.4 kg/capita·year) and 10 times more if compared with the consumptions of developing countries (Pulp and Paper Industry Intelligence, 2011). Due to the flushing of toilet paper in public sewers, in Western European countries cellulose usually enters municipal wastewater treatment plants (WWTPs) and is only partially degraded and valorised. However, flushing toilet paper may probably be considered more environmentally friendly practice compared to disposal in toilet trash and following transportation to landfills or incinerators, that is implemented in countries where sewers infrastructure can have clogging problems (Genty et al. 2013).

Usually, the rate-limiting step of cellulose degradation is the hydrolysis process (Noike et al., 1985), which makes difficult its degradation during the conventional biological treatments in WWTPs.

Verachtert et al., (1982) reports that 60% of the cellulosic material is degraded during 4-5 weeks of aerobic conditions, while 40% persists undegraded in the excess sludge. However, if anaerobic digestion of excess sludge is accomplished, additional 50% of the present cellulose could be degraded.

Ruiken et al, 2014 carried out batch experiments to investigate the mechanism of toilet paper under anaerobic conditions. The authors found 100% of removal after 8 days at 30°C of temperature, confirming that the cellulose degradation is indeed a slow process. On the other hand, the presence of cellulose in activated sludge and digested sludge calls for more in-depth studies on the conversion processes of these fibres (Ruiken et al., 2014; Rusten et al., 2006). When properly separated and refined, the cellulose can be used as raw material to make paper products or adhesion binders for asphalts (STOWA report 2012; Godow et al., 2013). In addition, cellulose can be used to produce valuable chemicals or biofuels, such as short-chain fatty acids (SCFAs), poly lactic acid, bioethanol (Van der Hoek et al., 2015; Honda et al., 2002). The recovery of cellulosic primary sludge (CPS) in a water resource recovery facitity (WRRF) can be performed by fine-mesh sieves (<500 μm) and the resulting primary sludge where the fraction cellulose achieves 79% of the total mass and 84% of the organic mass (STOWA report 2012; Ruiken et al., 2014; Rusten et al., 2006). Currently, only few studies have investigated the best pathways to valorise the CPS (Ruiken et al., 2014; Honda et al., 2002; Ghasimi et al., 2016), while recovery of propionate-rich SCFAs have never been studied within a wider biorefinery concept. In this regard, carbon upgrading to SCFAs, mainly acetate, propionate and butyrate, etc, is a cost-effective strategy to produce intermediates which can be processed to (bio)products with higher potential market value than methane (CH_4) from biogas (Kleerebezem et al., 2015; Holtzapple et al., 2009). Moreover, recent studies suggest that propionate can best enhance the biological phosphorus removal (BPR) processes in biological nutrients removal systems (Chen et al., 2004; Oehmen et al., 2006). On the other hand, higher propionate/acetate ratio promotes the selective growth of polyphosphate accumulating organisms compared to the glycogen accumulating organisms in enhanced biological phosphorus removal systems (Oehmen et al., 2006). In addition, SCFAs with higher propionate/acetate ratio promote the production of co-polymers characterised by less stiffness and brittleness, higher flexibility (higher elongation to break), and higher tensile strength and toughness (Laycock et al., 2014; Frison et al., 2015). Consequently, the selective production of SCFA from sewage sludge under optimized acidogenic fermenting conditions have become an emerging research field that enables wastewater-based biorefineries (Lee et al., 2014; Basset et al., 2016).

Other authors (Zurzolo et al., 2016) studied the SCFAs production from the fermentation of conventional primary and secondary sludge, while the potential of SCFAs production and nutrients recovery (e.g. struvite) from CPS is still unknown. In this regard, the rates and the yields of CPS fermentation could be influenced by key operating parameters such as pH, sludge retention time (SRT) and temperature. pH affects the hydrolysis and the subsequent acidification step during the fermentation. Alkaline conditions (pH >9) promotes the fermentation of primary sludge and inhibits the methanogenic activity, to achieve higher conversion to SCFAs (Wu et al., 2010). On the other hand, it is reported that the optimum pH range for the hydrolysis and acidogenesis of cellulose is between 5.6 and 7.3 (Hu et al., 2004).

The fermentation of sewage sludge is usually performed at mesophilic (30-40 ºC) or moderate thermophilic (50-55 °C) conditions. Thermophilic condition may increase the substrate degradation rate, but this is unfavourable for the both energy balance and for the process stability. Therefore, mesophilic conditions are still recommended to achieve a robust and stable sludge fermentation (Yu et al., 2002). However, the combined effect of operating temperature, pH and SRT on the production of SCFAs from CPS fermentation is still unknow. In addition, the fermentation of sewage sludge involves relevant orthophosphate release in the liquid phase, which can be effectively recovered through struvite crystallization (Tong et al., 2009; Zhang et al., 2009).

In this paper, the optimization of the SCFAs production was explored through the fermentation of CPS at different temperature (37, 55 and 70 ºC) and initial sludge pH (uncontrolled pH, 8, 9 and 10) to maximize: (1) the production of propionate (Pr) in SCFAs, (2) the recovery of phosphorus (PO_4-P) as struvite from the fermentation liquid; (3) the final biogas production from the fermentation solid. The resulting optimized parameters were then used to set-up and study the long-term operation of a Sequencing Batch Fermentation Reactor (SBFR), that provided the results for the forthcoming scale-up for WRRFs. Finally, the market added value of the recovered materials from CPS fermentation was estimated based on the experimental results. Based on the rates and mass flows obtained in the SBFR, the scale-up of this scheme will be integrated in the real wastewater treatment plant of Carbonera (Treviso) within the European Horizon2020 Innovation Action "SMART-Plant".

2. Material and Methods

2.1 Source and physicochemical characteristics of the CPS

Real and raw thickened PS was collected once per week and for 3 months during spring season and dry weather from the municipal WWTP of Verona (Veneto Region, Italy). Primary sludge was settled in primary clarifiers after the removal of grit, sand particles and oil from the influent wastewater. Then, the PS was thickened up to around 4% total solids (TS) using gravity belt thickening (Klein Technical Solutions, Germany). After sampling the PS was mixed and homogenized with toilet paper obtaining a total concentration of cellulose around 70-75% in agreement with other literature studies (Ruiken et al., 2014). Before the preparation of CPS, the toilet paper was soaked in wastewater for 4 h to achieve a CPS with similar characteristics to those found in WWTPs. The main characteristics of CPS were as follows: total solids (TS) 56.0 ± 17.2 g TS/L, volatile solids (TVS) 48.2 ± 14.0 g TVS/L, pH of 6.3 ± 0.1, total chemical oxygen demand (tCOD) and soluble chemical oxygen demand (sCOD) concentrations of 949 ± 156 mg COD/g TVS and 48 ± 26 mg COD/g TVS, respectively, ammonium concentration (NH_4-N) 1.7 ± 0.4 mg N/g TVS and ortophosphate concentration 0.5 ± 0.1 mg PO_4-P /g TVS. The concentration of total SCFAs detected in the CPS was 26.2 ± 9.8 mg COD/g TVS.

2.2 Operating temperatures and pH of the CPS fermentation

In order to acquire full understanding of inputs and outputs being investigated, the complete matrix of batch fermentation experiments of CPS was performed at: (a) different temperatures, 37 ± 1 ºC (mesophilic), 55 ± 1 ºC (thermophilic) and 70 ± 1 ºC (hyperthermophilic); (b) different initial value of pH (8, 9 and 10) to investigate the effect of these operating parameters on the SCFAs production and composition. The batch fermentation experiments were performed in duplicates by using 1 L glass bottles, with a working volume of 0.6 L. The initial pH was adjusted using sodium hydroxide (NaOH). In addition, a batch experiment with uncontrolled pH was used as the reference fermenting conditions. The batch assays were kept at controlled temperature for 16 days, while pH was not controlled during the fermentation tests. The reactors were sealed with rubber stopper and opened for only approximately 1 min for sampling and to measure the pH using a pH sensor (Eutech pH 700). The samples were centrifuged, filtered through membrane filters (Whatman, 0.45 µm), then analysed for PO_4-P, NH_4-N, sCOD concentrations and SCFAs concentration and composition. Total solids (TS) and volatile solids (TVS) were determined at the beginning and end of the fermentation experiments.

The actual production of SCFAs was always calculated subtracting the initial SCFAs concentration of the raw sludge. The yield of SCFAs production was expressed as mg COD/L of SCFA per g TVS/L in the feed sludge (mg COD/g TVS_{fed}). Similarly, the released PO_4-P was determined as mg PO_4-P/gTVS_{fed}.

2.3 Optimization of the CPS fermentation to enhance propionate production

Based on the full set of batch experiments the response surface methodology (RSM) was applied to further advance the propionate production based on the fermenting temperature and initial pH value. The regression model used is shown in Equation 1 and the target responses were the production of SCFAs (Y_{SCFAs}) and the content of propionate over those SCFAs ($\%Pr$).

$$Y(z) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{1,2} x_1 x_2 + \beta_{1,1} x_1^2 + \beta_{2,2} x_2^2 \quad \text{(Eq. 1)}$$

where $Y(z)$ is the response variable (i.e. Y_{SCFAs} (mg COD/g TVS_{fed}) or $\%Pr$ (gCOD/gCOD x 100), x_1 is the initial pH and x_2 is the temperature (ºC), β_0 is the model constant, β_1 and β_2 are linear coefficients, $\beta_{1,2}$ is the cross-product coefficient, and $\beta_{1,1}$ and $\beta_{2,2}$ are the quadratic coefficients.

Finally, a statistical analysis was carried out by means of the analysis of variance (ANOVA) to test the significance of predicted and experimental results, under a significance level of 0.05 (p). The regression model and the statistical analyses were performed using the software R 3.2.3 (The R Foundation for Statistical Computing).

2.4 Sequencing Batch Fermentation Reactor

A sequencing batch fermentation reactor (SBFR) with a reaction volume of 4 L was operated at 37±1 °C by a thermostatic bath, while the HRT was kept constant at 4 days by the daily exchange of 25% of the reactor volume between fermented and fresh CPS. The SBFR was equipped with a blade stirrer installed in the bottom. In this work, two representative experimental periods were carried out (period 1 and 2) where the steady-state conditions were considered when the calculated Relative Standard Deviation (RSD) of the propionate production was below 10% for at least 3 times the HRT of the SBFR (Ghasem et al., 2008). The Equation 2 reports how the RSD was calculated:

$$\text{RSD (\%)} = \frac{\sigma\,(\text{Pr production})}{\mu\,(\text{Pr production})} \times 100 \text{ (Equation 2)}$$

where σ (Pr production) is the standard deviation of the propionate production found during the period, while μ (Pr production) is the average of the propionate production found during the period.

The period 1 (0-18 days) was operated to determine the yield of Pr production without any adjustment of pH in the fed CPS. In the period 2 (19-96 days), every day the pH of the fresh CPS was adjusted to 8 before the feeding of the reactor. Steady-state conditions were achieved between days 4 and 16 for period 1, while during days 36-96 for period 2.

Samples were periodically taken from the effluent of the SBFR and analysed for PO_4-P, NH_4-N, SCFAs, chemical oxygen demand (total COD, soluble COD), total solids (TS), volatile solids (TVS) and pH. The composition of the SCFAs (i.e. acetate, propionate) were also investigated to determine the propionate/acetate ratio (g $COD_{propionate}$/g $COD_{acetate}$) as monitoring parameter during the experiment. Propionate/acetate between 0.25-0.75 g $COD_{propionate}$/g $COD_{acetate}$ is considered the optimal biological phosphorus removal processes (Broughton et al., 2008; Yuan et al., 2012). On the other hand, higher $COD_{propionate}$/g $COD_{acetate}$ ratios promote the production of polyhydroxyvalerate (PHV) instead of polyhydroxybutyrate (PHB) improving the mechanical and physical properties of the biologically recovered PHAs (Jiang and Chen, 2009).

Twice per week the "cellulosic materials" in the influent and effluent from the SBRF were quantified as the volatile fraction at 550°C of the solids recovered and washed after the sieving at mesh 54 μm. During the periods 1 and 2, samples of biomass from the SBFR were taken and characterized by the FISH quantification of the *Propionibacterium acidopropionici* using the Apr820 and DAPI probes, following the methodology described Nielsen et al. (2009). Forty images of each sample were taken using a fluorescence microscope (Leica DM2500) and then analysed with the Image J software.

During the steady-state conditions of the SBFR, struvite recovery tests were performed from the CPS fermentation liquid. The fermentation liquid from the supernatant was obtained after the centrifugation at 4000 rpm for 10 minutes of the effluent from the SBFR. The experiments started with the addition of 5 g/L of seed struvite crystals and magnesium hydroxide ($Mg(OH)_2$) according to a PO_4^{3-}: Mg^{2+} molar ratio of 1:1.5, and adjusting the initial pH at 8.5 with NaOH (0.1 M). Samples were collected at 5 min, 10 min, 15 min, 30 min and 60 min, filtered through cellulose membrane filters (Munketll Ahlstrom) and analysed to determine their PO_4-P concentration. After the experiments, the precipitated solids were washed with distilled water to remove impurities and soluble salts. The recovered solids were dried at 45 °C for 24 h to avoid thermal decomposition (Bhuiyan et al., 2008). The crystals produced were analysed according to Fattah et al. (2012) and the molar ratio between nitrogen and phosphorus was used to confirm the struvite formation.

2.5 Biochemical methane potential tests

The BMP test were also investigated. The BMP test were carried out following the procedure defined by Angelidaki et al., (2009) at 37 °C, while parallel tests were carried out using raw primary sludge to compare biogas production and composition from CPS and PS BMP tests. More details of this method are reported in Supporting Information.

2.6 Analytical methods

Soluble COD, TSS and TVS were measured according to Standards Methods (APHA-AWWA-WPCF, 2012). NH_4-N concentration was measured by an ion selective electrode (Orion 9512). The concentration of SCFAs was determined by gas chromatography (Dionex ICS-1100 with

AS23 column). PO_4-P concentration was measured by ion chromatography (Dionex ICS-900 with AS14 column) and calibrated using a combined five anion standard (Thermo Scientific™ Dionex™ Ion Standards).

3. Results and Discussion

3.1 SCFAs production and composition from the batch fermentation experiments

No relevant lag-phase was observed before the production of SCFAs started. The concentration increased gradually until the peak and plateau values were reached (between the 9th and 13th day) at 37 and 55 ºC (Figures S1, Supplementary Material). On the other hand, the SCFAs production at 70 ºC increased up to day 2 and then decreased (Figure S1 in the Supplementary Material). The highest production yield of SCFA of 340.4 mg COD/g TVS_{fed} was observed at 37 ºC (Figure 1), while the lowest were 155.4 and 46.1 mg COD/g TVS_{fed} observed at 55°C and 70°C respectively. Therefore, the temperature had a major effect on the production of SCFAs. In particular, the effect the fermentation temperature had on the hydrolyses and the acidogenic process can clearly be detected from the pH profile over the batch experiments (SI Figure S2), since it was adjusted only at the beginning of the experiments. Moreover, higher variation of pH resulted in higher SCFAs production, thus pH seemed to act as a surrogate parameter for monitoring the fermentation process.

(Figure 1)

Although the increase of pH can positevely influence the fermentation efficiencies (Wu et al., 2010), in this study the highest SCFAs production were observed at pH 8, while at pH 9 and 10 the production of SCFAs were much lower at 37 °C (Figure 1). On the other hand, under thermophilic and hyper-thermophilic conditions, fermentation pH was almost stable and the production of SCFAs was comparable notwithstanding the initial pH condition.

Acetate and propionate were the most relevant SCFAs in the fermentation liquid for all the fermentation experiments at 37 ºC (acetate 55-80%, propionate 12-33%), with minor concentration of mainly butyrate and n-valerate (around 6-9% and 3-4% respectively) (Figures S3(a), S3(b), S3(c) in the Supporting Information). Propionate was mostly produced under mesophilic condition in a range of percentage between 25-33%, while under thermophilic and hyper thermophilic propionate contents below 20% were observed. The fermentation temperature increase led to propionate/acetate ratio decrease: the higher ratio of 0.6 gCOD/gCOD was observed at 37 °C and initial pH of 8.

3.2 Optimal key operating parameters for CPS fermentation

The 3D rensponse surface methodology (RSM) was applied to optimize both the total SCFAs production and the propionate percentage based on the initial pH and the fermentation temperature. The results of the regression model indicated that the increase of the fermentating temperature has a detrimental effect on total SCFAs and Pr productions than initial pH fixed value (SI Figure S2a and 2b). Figure 2a and 2b shows that SCFAs and Pr percentage productions decrease with the increase of fermentation temperature, obtaining higher SCFAs productions from CPS fermentation at 37 ºC. So, the response surface plot indicates that the most favourable operating conditions to maximize SCFAs production were 37 ºC of temperature and initial pH of 8 (Figure 2a), while the highest percentage of propionate can be obtained at lower initial pH ($7.5 < pH < 8.0$) and a temperature of 37 ºC. Under these operating conditions the production of SCFAs and propionate percentage were 281.5 mg COD/g TVS and 30.3%, respectively (Figure 2a and 2b).

(Figure 2a and 2b)

The statistical significance of total SCFAs production and propionate percentage models were evaluated by ANOVA (Table 1).

(Table 1)

The combination of low p value and high R^2 indicated that the model explains a lot of variation within the data and is significant. The models of F-value showed a low p-value (<0.016), which implied that both models were significantly affected by temperature and initial pH, and able to estimate total SCFAs production and %Pr. The predicted high R^2 (between 0.7 and 0.9) indicated both models were sufficiently high to show the significance of the fit of the models (Table 1). Table 2 reported the coefficients of the quadratic models for the responses of the Y_{SCFA} and $\%Pr$. For %Pr model, pH, temp and the interaction effect $\beta_{1,2}$ and $\beta_{2,2}$ were not significant ($p>0.05$), while only $\beta_{1,1}$ was found significant ($p<0.05$). For SCFAs production model, all the terms were found not significant ($p>0.05$) meaning that the effect on response is considerable.

(Table 2)

Figure 3 shows the comparison between the predicted and experimental values for the total SCFAs and the percent of propionate production. The experimental results are consistent with the regression model (R^2 >0.86) for both key parameters.

(Figure 3)

3.3 Long-term SCFAs production and phosphorus recovery in the SBFR

During the period 1 (0-18 days), the sCOD varied in the range 10-12 gCOD/L and lower yields of Pr production were observed (around 60 $mgCOD_{propionate}/gTVS_{fed}\cdot d$). In period 2, the sCOD higher and more stable in the range 14-18 gCOD/L (see Figure S4, Supporting Information). The increase of the sCOD was a result of the higher degradation of the cellulosic materials observed in period 2 (Table 3).

(Table 3)

The effect of the influent pH was clearly observed by the productivity of the SCFAs, which increased from 162.4±12.8 mg COD/g $TVS_{fed}\cdot d$ (period 1) to 253.8±26.1 mg COD/g $TVS_{fed}\cdot d$ (period 2). Moreover, the increase of the pH influent had effect on the propionate production, which gradually increased and reached a stable production of 100-120 mg COD/g $TVS_{fed}\cdot d$ during days 36-96 of period 2 (Figure 4). The latter is higher than the batch experiments, probably due to the speciation of the microbial community accomplished in the SBFR. In fact, the percentage of propionate to total SCFAs was up to 46%. This corresponded to propionate/acetate ratio of 0.9 gCOD/gCOD, which was higher than the period 1 (0.6 gCOD/gCOD).

Many authors reported that the fermentation of cellulosic compounds at relatively low pH, lactic acid could be produced (Abdel-Rahman et al., 2013). Indeed, despite the initial pH of the CPS in period 2 (19-96) was adjusted to 8, the average pH in the SBFR dropped to 5.1±0.1 due to simultaneous production of the SCFAs and alkalinity consumption (Figure 4). As a consequence, bacteria of the genus *Propionibacterium* may produce propionate from lactate as the end-product of their anaerobic metabolism (Liu et al., 2012). In period 2, FISH analyses (see Table S1 and Figure S6 in Supporting Information) confirmed selective growth of *Propionibacterium acidopropionici* that were at 33.8%, more abundant than period 1 (24.5%). So, the beneficial speciation of the microbial community in the SBFR seems to be related with the higher solubilization of the cellulosic materials achieved in period 2.

(Figure 4)

Furthermore, NH_4-N and PO_4-P were released in the CPS fermentation liquid of the SBFR as much as 1.6 ± 0. 5 mg P/g TVS_{fed}·d and 6.1± 1.4 mg N/g TVS_{fed}. d. As a consequence, the average concentrations of PO_4-P and NH_4-N in sludge fermentation liquid during steady conditions were 130 ± 23 mg P/L and 430 ± 29 mg N/L, respectively. Due to the high P and N content, the recovery of the released PO_4-P from CPS fermentation by struvite crystallization was examined and the average efficiency of phosphorus recovery was 88%.

3.4 Specific per capita recovery of valuable resources from CPS and preliminary economics

The selective production of mixture of SCFAs through acidogenic fermentation was considered best available carbon source to enhance the nutrients removal in the mainstream or in the sidestream (Frison et al., 2013, 2016). Recently, Longo et., 2017 considered this practice an economic and environmentally friendly solution to reduce energy and chemical consumption for the removal of nitrogen and phosphorus. Moreover, a number of Horizon2020 Innovation Actions (such as SMART-Plant (www.smart-plant.eu) or INCOVER (www.incover.eu) were evaluated and funded to demonstrate the technical, economic and environmental long-term viability of further (bio)conversion of the SCFAs to biopolymers, such as PHAs, that could enable the recovery of high added value products by minor integration of existing WWTPs. Although the best technical and economical evaluation should always be referred to single WWTP, the specific economic advantages of alternative for CPS processing in comparison with the only biogas production is estimated below.

Every year, around 36-43 kg of COD are discharged in municipal wastewater by individuals (capita) (adapted Metcalf and Eddy, 2014). The observed average removal efficiency of COD by the sieving municipal wastewater is between 10-60% (Ruiken et, 2014) and 12-13 kg COD/capita·year could be recovered as suspended solids from municipal wastewater. In this study the BMP test (SI Figure S5) showed that CPS may produce up to 0.30-0.34 m^3 CH_4/kg COD_{fed} equivalent to 3.7-4.5 m^3 CH_4/capita·year (Figure 5a), which is in agreement with other studies (Ghasimi et al., 2016). Therefore, the convertible COD to CH_4 by anaerobic digestion in a current WWTP (Table 4) would be around 11 kg COD/capita·year, which represents around 25% of the total COD influent in a WWTP. In a CPS-based biorefinery scenario (Table 4), considering the observed SCFAs production rate, around 3.0 kg COD_{SCFA}/capita·year could be produced by the fermentation of CPS, where acetate and propionate represent 1.30 and 1.17 kg COD_{SCFA}/capita·year, respectively. However, around 2.7-3.3 m^3CH_4/capita·year of residual CH_4 could be further produced by the anaerobic digestion of CPS after fermentation (Figure 5b) Moreover, during the fermentation of CPS, nutrients are released and 88% of the phosphorus could be recovered in the form of struvite, that amounts to 0.07-0.15 kg struvite/capita·year.

Assuming CH_4 a market price of 0.11 €/m^3 (Energy Information Administration, 2017), the best valorisation of CH_4 from CPS can be as high as 0.46 €/capita·year. On the other hand, better value can derive from valorising CPS first to the suitable mix of SCFAs (mainly acetate and propionate) and struvite from the fermentation liquid, while CH_4 can be recovered after digestion of fermentation solids. Although the market price of the recovered materials is very volatile and often unknown because of the variable purity and quality, according to a recent review, acetate and propionate price can be as high as 0.45 and 1.01 €/kg respectively (Global Chemical Price, 2017; ICIS, 2017), while struvite can be sold up to 0.76 €/kg (Molinos-Senante et al., 2011; P-REX report, 2014). Therefore, the SCFAs and struvite route before the bio-methanization can increase the market value potential of CPS up to 1.55-1.95 €/capita·year (Table 4).

(Table 4)

(Figure 5)

4. Conclusions

This paper studied the maximum potential recovery of SCFAs, particularly propionate, struvite and CH_4 from the CPS. Based on the results of the RSM, the optimal production of propionate is obtained by the fermentation of CPS at mesophilic conditions (37 °C) and at initial pH between 7.5-8. By the long-term operation of a SBFR the observed production of propionate in the fermentation liquid was 100-120 mg COD/g TVS_{fed}·d, with a propionate/acetate ratio of 0.9 g COD/g COD. Best performances in the SBFR may be attributed to the observed enhanced growth of *Propionibacterium acidopropionici*. At the same time, 88% of the phosphate released in the fermentation liquid can be recovered as struvite. From a techno-economic point of view, the integration of the wastewater dynamic sieving to recover CPS in a WWTP may make existing units (e.g., gravity sludge thickener) redundant and available to be revamped to controlled fermenter to recover optimal mixture of SCFAs. In addition, the recovery of SCFAs and struvite before the bio-methanization can increase the market value potential of CPS up to 1.55-1.95 €/capita·year.

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