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Enhancing volatile fatty acid production from sewage sludge in batch fermentation tests

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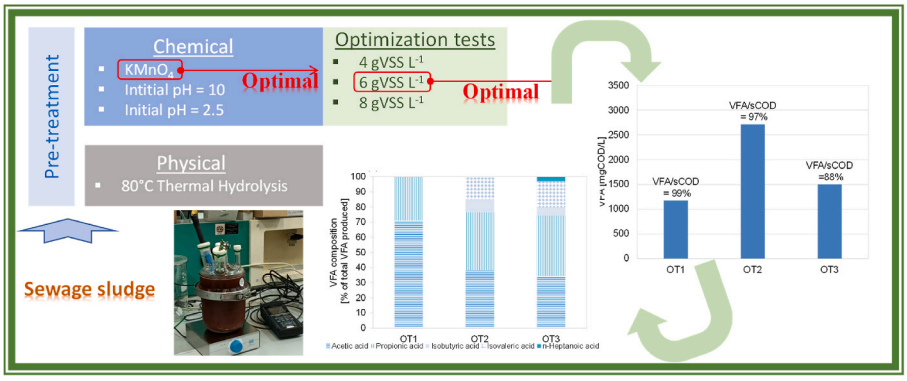
HIGHLIGHTS

- Sewage sludge acidogenic fermentation was performed in batch scale reactors.
- Potassium permanganate, pH and thermal hydrolysis were applied as pre-treatments.
- Potassium permanganate increased VFA production by 45200% compared to control test.
- VFA accounted for 95 ± 4 % of the soluble COD produced.
- Comamonadaceae and Chlamydiae percentage abundance was reduced by permanganate.

GRAPHICAL ABSTRACT



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ABSTRACT

Volatile fatty acids (VFA) from sewage sludge represent an excellent recovered resource from wastewater treatment. This study investigated four sludge pre-treatments (namely, potassium permanganate - $KMnO_4$, initial pH = 10, initial pH = 2.5 and low-temperature thermal hydrolysis) by operating batch reactors under acidogenic fermentation conditions. Results revealed that 0.1 g $KMnO_4$/g of total suspended solids represents the best pre-treatment obtaining up to 2713 mgCOD L^{-1} and 452 mgCOD/g of volatile suspended solids. These results also paralleled metataxonomic analysis highlighting changes in prokaryotic microbial structures of sewage sludge of the batch fermentations subjected to the different pre-treatments.

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1. Introduction

Volatile fatty acids (VFA) can be a valuable recovered resource recovered from wastewater treatment in view of a circular economy approach. The change of paradigm from a linear to a circular economy model favoured the spread of new biotechnologies with the aim to use the waste activated sludge (WAS) to recover resources, treating the biosolids as resource instead of waste. VFA popularity increased in the past years, mostly because they are produced by WAS acidogenic fermentation and are one of the more valuable resources that can be recovered (Li et al., 2018). VFA are used as building blocks from several microorganisms to produce biopolymers, such as the poly-hydroxyalkanoates (PHA) (Liu et al., 2020).

However, VFAs production under acidogenic conditions still represents a challenge due to several limitations in this process: low biodegradability of the substances contained in the sewage sludge, low hydrolysis rate during the fermentation and the VFAs separation from the fermentation broth are some of the main difficulties that need to be overcome to improve the process (Bruni et al., 2021; Parchami et al., 2020). The fermentation hydrolysis rate still represents one of the key challenging issues since despite the huge effort performed in literature still requires to be optimized (Valentino et al., 2021). Several operating factors such as pH and temperature are critical for the fermentation hydrolysis rate. For example, pH may strongly affect the microorganism's activity (Wang et al., 2019). Recent studies have demonstrated that under hydrothermal treatment conditions at 80 °C the fermentation acid production is higher than that obtained without sludge pre-treatment (Penghe et al., 2020). Wu and co-authors have investigated the effect of pre-treating sludge under acid or alkaline conditions finding that the acid pre-treatment improves VFA production (Wu et al., 2017). Guo and co-workers used the polyoxometalates (POM) addition as pre-treatment to enhance the short-chain fatty acids (SCFAs) production from WAS (Guo et al., 2021). They found that at 0.05 g POM/g Total Suspended Solids (TSS) the SCFAs production was enhanced by 6.18 times. Wang and co-wokers have investigated the potential of coconut shell ash addition to promote VFAs production from algae fermentation obtaining VFAs concentration 1.4 folds higher than without treatment (Wang et al., 2021). Zheng and co-workers investigated the effects of polystyrene (PS) microplastics (MPs) on VFA production (Zheng et al., 2021). It was found that at 30 particles/g total solid the VFAs production was promoted compared to the control, with a peak of 1493 mg COD/L. Chen and co-workers used roxithromycin (ROX) during WAS anaerobic fermentation (Chen et al., 2020). This pre-treatment increased the maximum amount of VFAs accumulated from 295 to 610 mg COD/L. Very recently, Xu and co-workers have investigated the adoption of a strong green oxidant such as potassium permanganate ($KMnO_4$) in view of improving SCFAs production and fermented quality by using sewage sludge at 14 g Volatile Suspended Solids (VSS)/L concentration (Xu et al., 2021). The work of Xu and co-workers was mostly focused on selecting the effective $KMnO_4$ dosage finding optimal results by dosing 0.1 mg $KMnO_4$/g VSS as pre-treatment. However, as authors are aware the performance of acidogenic fermentation (in terms of SCFAs production) obtained by dosing $KMnO_4$ has never been compared with that of other pre-treatments.

Therefore, this study has the aim to: i. compare several pre-treatments ($KMnO_4$ dosing, initial pH = 10, initial pH = 2.5 and low temperature thermal hydrolysis) and identify the best one in view of improving the VFA production by sewage sludge acidogenic fermentation; ii. evaluate the influence of VSS for the selected best pre-treatment; iii. perform microbial community analysis to reveal the mechanism involved in the process.

Table 1
Details of the performed pre-treatment and VSS evaluation (OT) batch fermentation tests.

Batch test	Pre-treatment	Reference
T1	Untreated	–
T2	Potassium permanganate	Xu et al. [13]
T3	Initial pH = 10	Presti et al. (Presti et al., 2021); Wu et al. [8]
T4	Initial pH = 2.5	Wu et al. [8]; Devlin et al. (Devlin et al., 2011)
T5	Low-Temperature Thermal Hydrolysis	Penghe et al. [7]
OT1	0.1 g $KMnO_4$/g TSS VSS: 4 g/L	–
OT2	0.1 g $KMnO_4$/g TSS VSS: 6 g/L	–
OT3	0.1 g $KMnO_4$/g TSS VSS: 8 g/L	–

Table 2
Sewage sludge features.

Parameters	Inoculum pre-treatment sewage sludge (T1-T5)	Inoculum optimisation sewage sludge (OT1- OT3)
pH	6.93 ± 0.35	7.88 ± 0.55
Total Suspended solids, TSS (g/L)	6.84 ± 0.03	3.1 ± 0.01
Volatile Suspended Solids, VSS (g/L)	3.80 ± 0.02	1.58 ± 0.01
Total Chemical Oxygen Demands, TCOD (mg/L)	4365 ± 33.00	2760.00 ± 35.00
Soluble Chemical Oxygen Demands, sCOD (mg/L)	141 ± 9.52	66.23 ± 4.21
Protein (mg/L)	651 ± 2.35	243.15 ± 7.69
Carbohydrate (mg/L)	98 ± 1.35	45.70 ± 2.91
Ammonium, NH_4^+-N (mg/L)	3.40 ± 0.21	2.60 ± 0.15
Phosphate, PO_4^{3-}-P (mg/L)	2.41 ± 0.52	1.76 ± 0.23

2. Material and methods

2.1. Batch fermentation tests

Batch fermentation tests have been performed in 1100 mL magnetic stirred glass bottles, equipped with two sampling ports for liquid and gas sampling and two electrode ports at the Water Resource Recovery Lab of Palermo University (Mannina et al., 2021a). The activities are related the EU Project Wider-Uptake and include, among others, poly-hydroxyalkanoate production from fermentation of domestic sewage sludge (Mannina et al., 2021b; Mannina and Mineo, 2023). Table 1 summarise the batch test details. Specifically, 5 batch tests (T1 to T5) were performed to investigate the influence of four pre-treatments: i. $KMnO_4$ dosing – T2; ii. initial pH = 10 – T3; iii. initial pH = 2.5 – T4; iv. low temperature thermal hydrolysis at 80 °C before starting the test – T5. T1 without pre-treatment was the reference test. Afterwards, 3 more batch tests were performed to evaluate the influence of VSS on KMnO4 pre-treatment which provided the best results during the "pre-treatment" tests. Specifically, three VSS conditions under the same $KMnO_4$ dosing (0.1g $KMnO_4$/g TSS) were investigated: i. VSS 4 g/L - OT1; ii. VSS 6 g/L - OT2; VSS 8 g/L - OT3. All the tests have been performed at room temperature.

The batch tests were monitored for 15 and 24 days respectively. During the tests, soluble Chemical Oxygen Demand (sCOD), VFA andammonium (NH_4^+-N) and phosphate (PO_4^{3-}-P) concentrations were analysed in the fermented liquid. pH, temperature and redox potential have been continuously monitored by us a WiFi - Multi 3630 IDS "WTW and related probes.

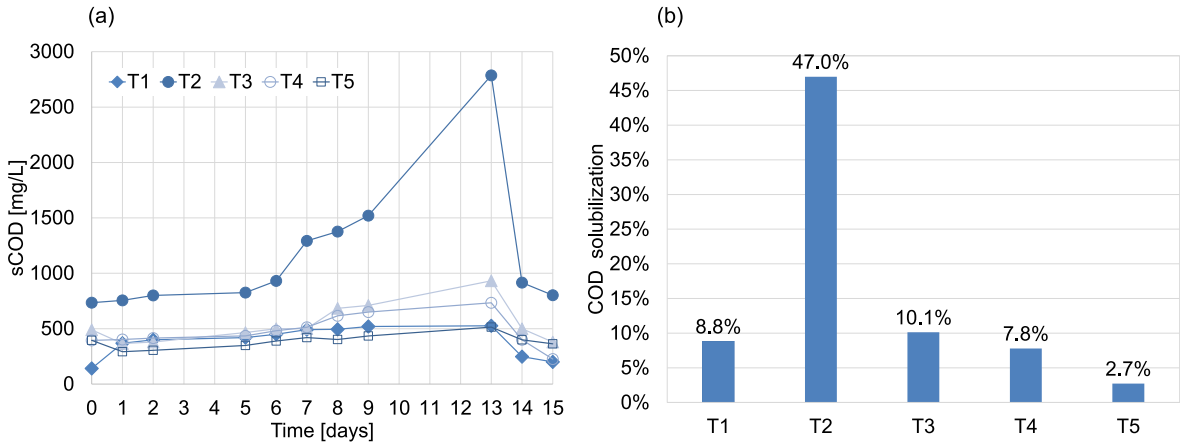

Fig. 1. Trend of sCOD (a) and COD solubilisation on day 13 (b).

2.2. Sewage sludge features

The sewage sludge was collected from a real WWTP sludge recycle line (Marineo, Italy). Table 2 reports the adopted sewage sludge features and optimisation batch tests.

2.3. Analytical methods

Standard methods (APHA, 1999) were followed to analyse COD, VSS, TSS, NH_4^+- N and PO_4^{3-}- P concentrations. VFAs were measured according to the VFA extraction with dimethyl carbonate (DMC-OEI) method proposed in the literature (Ghidotti et al., 2018). Specifically, for measuring VFAs, it was employed an Agilent Technologies 7820A gas chromatograph equipped with a flame ionization detector (FID) and a DB FFAA column (30 m x 0.25 x mm x 0.25 μm). Specifically, the following VFAs were measured: formic, acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, hexanoic and n-heptanoic. The GC protocol proposed by the literature was used to analyse the samples (Montiel-Jarillo et al., 2017). VFA concentration was converted into COD concentration using the conversion factors of the literature (Yuan et al., 2011).

The proteins and carbohydrates extracellular polymeric substances (EPSs) soluble and bound have been analysed according to the literature (Mannina et al., 2016).

Once the batch tests were finished, COD solubilisation was calculated according to equation (1) (Mohammad Mirsoleimani Azizi et al., 2021).

$$COD\ solubilization = \frac{sCOD_t - SCOD_0}{TCOD_0} \quad (1)$$

where (t) and (0) refer to the generic and initial time, respectively.

2.4. Metagenomic analysis

Metagenomic analysis was performed using Next Generation Sequencing (NGS) according to the procedures described in the literature (Presti et al., 2021). The DNA extractions were evaluated by 1% agarose gel electrophoresis analysis, adding 0.5 μg/mL ethidium bromide for visualisation using an UV lamp. The DNA concentrations, extracted from 1 g of activated sludge samples and the corresponding ten-fold serial dilutions were measured by reading absorbance at 260 nm with NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific). The purity of the extracted DNA was evaluated by measuring the absorbance ratios (260/280 and 260/230 nm) in view of finding contamination due to proteins, organic compounds or chaotropic agents. To perform a qualitative evaluation, metagenomic DNA and the diluted DNA aliquots were used as a template in a PCR protocol to amplify the region V3–V4 of the 16S rDNA gene (Taki and Kibayashi, 2014). Amplicons (464 bp in size) were visualised by 1.5% agarose gel electrophoresis and stained with ethidium bromide.

Table 3
Sewage sludge and final total proteins and carbohydrates concentration for each batch test.

Sewage sludge	Proteins mg/L	Carbohydrates mg/L
	651 ± 2.35	98.34 ± 1.35
Final test		
T1	850 ± 5.21	120.33 ± 3.21
T2	1600 ± 1.68	400.17 ± 7.36
T3	720 ± 6.54	125.22 ± 2.89
T4	1125 ± 7.89	287.08 ± 6.12
T5	710 ± 6.71	148.14 ± 7.98

3. Results and discussion

3.1. Organic matter solubilisation and VFA production

3.1.1. Pre-treatment

Fig. 1 reports the trends of sCOD (a) concentration during the pre-treatments batch tests and the maximum COD solubilisation (b). As reported in Fig. 1a, an increasing trend of the sCOD concentration was obtained during the batch tests for all the used pre-treatments. All the investigated pre-treatments have provided the maximum results in terms of COD during the 13th fermentation day. Specifically, the initial sCOD concentration under un-treatment (T1) was 141 mg L^{-1} and increased to a maximum of 526 mg L^{-1} (day 13th). Under the $KMnO_4$ (T2) pre-treatment condition the initial sCOD concentration was 735 mg L^{-1} and increased to the maximum value of 2736 mg L^{-1}(day 13th). Under the initial alkaline pH of 10 (T3) and acid pH of 2.5 (T4) the maximum sCOD concentration was 932 mg L^{-1} and 734 mg L^{-1}, respectively (day 13th). At the same time, the maximum sCOD concentration under low temperature thermal conditions (T5) was 514 mg L^{-1} (day13th). Therefore, the maximum COD solubilisation was obtained under $KMnO_4$ pre-treatment (T2).

Indeed, the COD solubilisation for T0-T5 at day 13th was 8.8%, 47%, 10.1%, 7.8% and 2.7%, respectively (Fig. 1b). This result was mainly debited to the $KMnO_4$ features as a strong oxidant. Indeed, $KMnO_4$ can break down large molecules into substances with lower molecular weight, thus promoting cell desegregation and the consequent organics solubilisation in the mixed liquor (Wang et al., 2020).

This statement can also be corroborated by the increase of soluble proteins and carbohydrates concentration between the initial and final fermentation day. Indeed, according to the literature the sludge organic

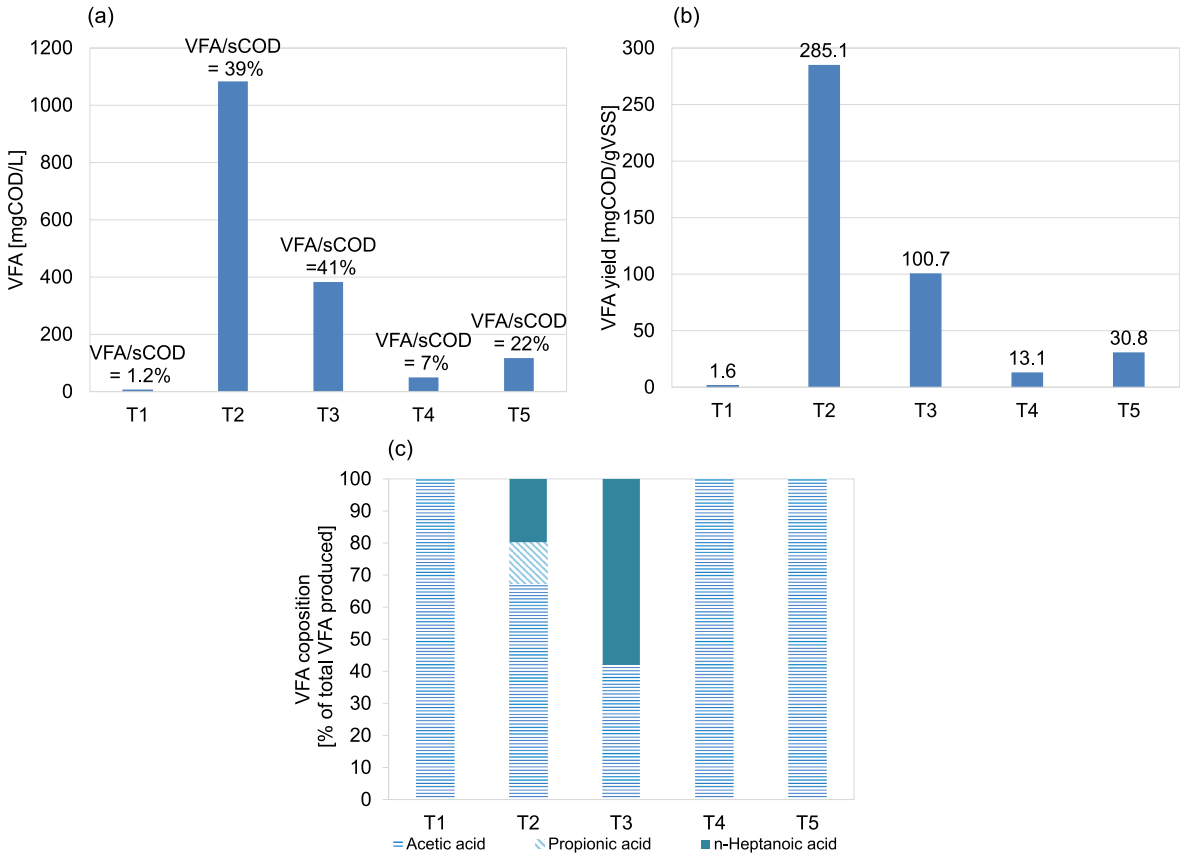

Fig. 2. VFA production on day 13 (a), VFA yield on day 13 (b) and VFA composition on day 13 (c).

matter is ordinarily protected by the EPS released under the $KMnO_4$ action (Wu et al., 2014; Xu et al., 2021). Table 3 summarises the final total proteins and carbohydrates concentration for each batch test. As reported in Table 3 the maximum proteins and carbohydrate concentration was obtained for the batch test T2.

In terms of VFA production, the highest concentration was obtained under $KMnO_4$ conditions (T2). Indeed, for T2 VFA concentration was 320 mg COD L^{-1} on day 1 and increased to the maximum value of 1083 mg COD L^{-1} on day 13 (corresponding to 39% of VFA/sCOD ratio and VFA yield of 285.1 mg COD g^{-1} VSS) (Fig. 2a and b). The values of VFA yield obtained during tests T1 and T5 were lower than 31 mg COD g^{-1} VSS. Conversely, during the test T4, VFA yield was 100 mg COD g^{-1} VSS. The result obtained for T4 slightly contrasts the literature. Indeed, VFA yield up to 480 mg COD g^{-1} VSS were obtained in the literature under alkaline conditions during co-fermentation of primary sludge with organic waste (Owusu-Agyeman et al., 2021) or up to 350 mg COD g^{-1} VSS during high sludge retention time sewage sludge fermentation (Presti et al., 2021). The contrasting results obtained here can be likely debited to the different fermented matter or the different environmental conditions (e.g., temperature) in terms of temperature. Indeed, the temperature increase favours VFA hydrolysis (Zhang et al., 2019). For example, Presti et al. (2021) performed their tests under room temperature which ranged between 27 and 34 °C, while here ranged between 20 and 24 °C.

The obtained VFA composition on day 13 showed that for tests T1, T2, T4 and T5 the acetic acid was dominant with n-heptanoic acid as the second most dominant VFA species followed by propionic acid (only for T2) (Fig. 2c). Specifically, for T1, T4 and T5 100% of VFA on day 13 was

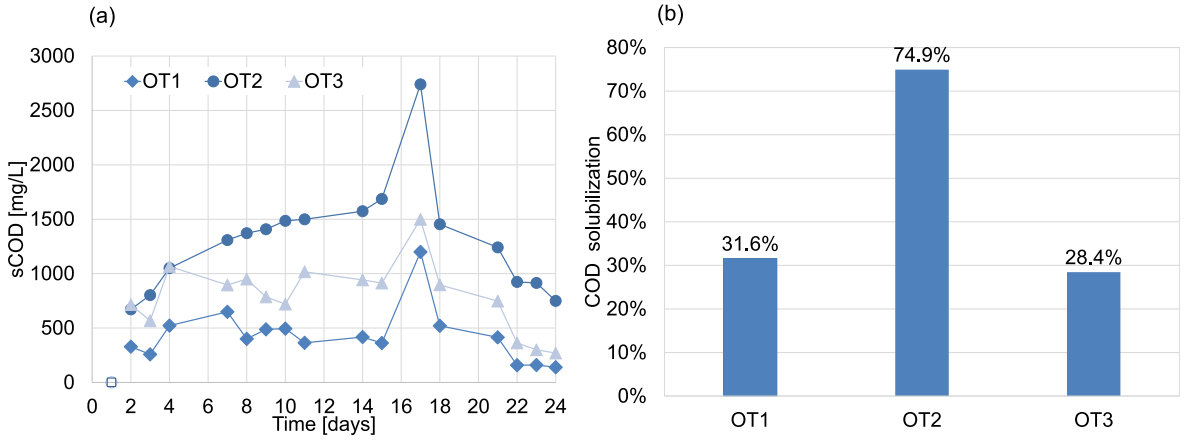

Fig. 3. Trend of sCOD (a) and COD solubilisation on day 17 (b).

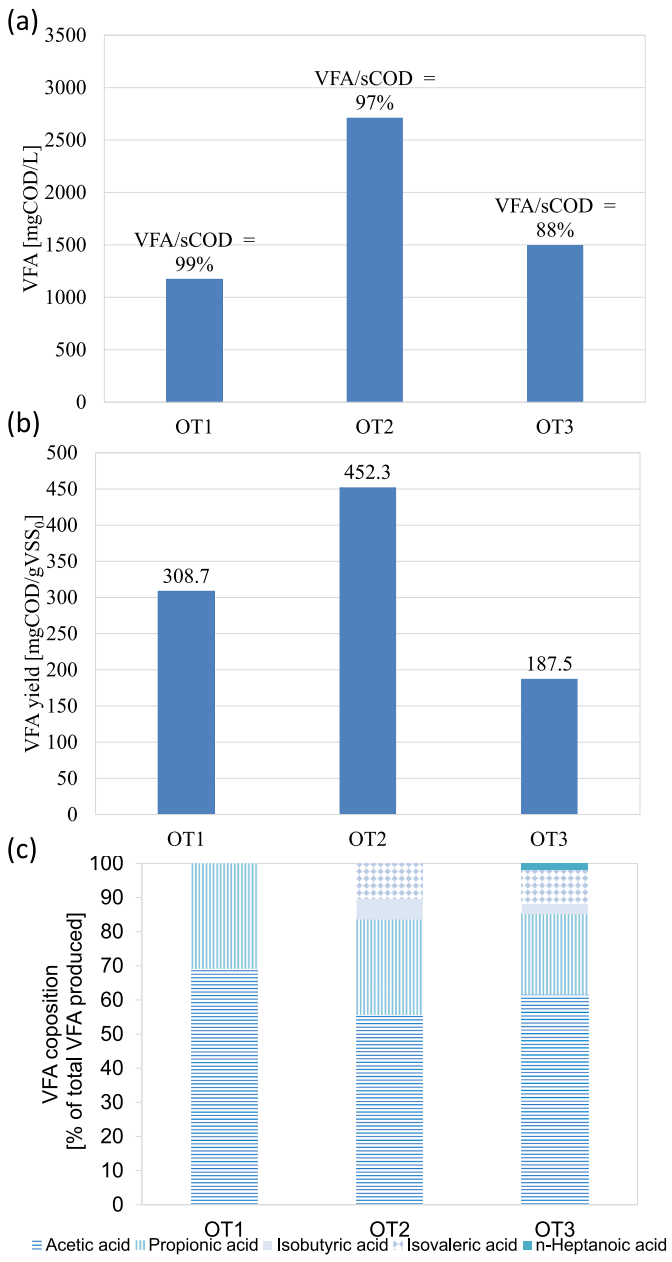

Fig. 4. VFA production on day 17 (a), VFA yield on day 17 (b) and VFA composition on day 17 (c).

composed of acetic acid (Fig. 2c). While, for T2 and T3 the SCFA also appeared. Specifically, for T2 13% and 20% of total VFA were composed of propionic and n-heptanoic acid, respectively (67% was acetic acid) (Fig. 2c). In T2 increased the amount of SCFA (propionic and n-Heptanoic) due to the capability of $KMnO_4$ to hydrolyze also recalcitrant organics in sludge (Xu et al., 2021).

The percentage of acetic and propionic acid in the VFA mixture from the alkaline test (T3) was 42% and 58%, respectively.

3.1.2. Evaluation of the effect of VSS concentration

Based on the best results obtained from the pre-treatment batch tests, other tests have been performed in view of evaluating the influence of VSS in the VFA production. Specifically, three VSS concentration (4 gVSS L^{-1}- OT1, 6 gVSS L^{-1}- OT2, 8 gVSS L^{-1}- OT3) have been investigated by dosing 0.1 g $KMnO_4$/g TSS.

According to previous results, the sCOD concentration for all tests increased from the initial to the final monitoring day. The maximum sCOD concentration was obtained on day 17 for all tests (Fig. 3a). Specifically, for T1 sCOD concentration was 1200 mg L^{-1}, for T2 was 2740 mg $L^{-1}$and for T3 was 1500 mg L^{-1} (Fig. 3a). The percentage of COD solubilisation for T1, T2 and T3 was 31.6%, 74.9% and 28.4% (Fig. 3b). COD solubilisation increases with VSS (till 6 gVSS L^{-1}). However, a further VSS increase reduces the COD solubilisation. This result could likely be because of the increase in VSS the amount of fermentation bacteria and the organics to be hydrolysed (Xiong et al., 2012). However, with the further increase of the VSS (and consequently TSS), especially under high sludge retention time conditions, the recalcitrant organics increase and the $KMnO_4$ dosing should increase to obtain similar results.

The results presented in Fig. 4 confirm that the hydrolysis process increased with the VSS increasing to VSS values equal to 6 gVSS L^{-1} and then decreased. Indeed, the VFA production on day 17 for T1 was 1172 mgCOD L^{-1}, for T2 was 2713 mgCOD L^{-1} and for T3 was 1499 mgCOD L^{-1} resulting in a VFA/sCOD percentage ratio of 99%, 97% and 88%, respectively (Fig. 4a). In terms of VFA yield, 308.7, 452.3 and 187.5 mgCOD/gVSS of VFA were produced during tests T1, T2 and T3, respectively (Fig. 4b). A statistical data analyis was carried out for veryfing the goodness of monitored data by ANOVA (Freni and Mannina, 2010).

The obtained VFA composition on day 17 showed that the acetic acid was dominant only for T1 (Fig. 4c). Specifically, for T1, T2 and T3 71%, 38% and 34% of VFA on day 17 was composed of acetic acid (Fig. 4c). While, for T2 and T3 propionic acid was dominant (39% and 40%, respectively). For T2 and T3 also the isovaleric acid was measured (14% and 16%, respectively). Finally, only for the T3 the n-heptanoic acid was also measured (3%). Results obtained in terms of VFA composition corroborates the fact that with the increase of VSS the increase of the complex organics increases thus producing VFA more complex acids (such as n-heptanoic and isovaleric).

3.2. Ammonium and phosphorus release

Fig. S1a shows the NH_4^+- N concentration pattern measured during the pre-treatment batch tests. At the VFAs peak (13th day), NH_4^+- N concentration for tests T1- T5 was 12.4, 42.9, 23.4, 34.3 and 16.9 mg L^{-1} respectively (Fig. S1a). These results show that the $KMnO_4$ pre-treatment was the most effective in enhancing biomass decomposition, as reported by the sCOD trend (see, Fig. 1a). During T3, NH_4^+- N concentration was 32% lower than in test T4. This result contrasts with literature suggesting an increase of NH_4^+- N concentration with the pH increase (Ye et al., 2020; Yu et al., 2013). However, important to be precise is that at the sCOD peak, T3 pH was 7.6, while T4 was 5.3 (Fig. S1b). Indeed, under pH 7.6 around 2% of the total ammonia (NH_3) and ionized-ammonia (NH_4^+) concentration is in the free ammonia form. Under pH 5.6 the free ammonia is around 0.02%, thus explaining why the NH_4^+- N concentration was higher in T4 than in T3.

Fig. S1bb shows the pattern of NH_4^+- N concentration measured during the optimisation batch tests. Under VFAs peak (17th day) conditions, NH_4^+- N concentration for OT1, OT2 and OT3 was respectively 39.9, 119 and 85 mg L^{-1}. The highest ammonium release was obtained for test OT2 (VSS = 6 g/L) (Fig. S1b).

PO_4^{3-}- P concentration increased throughout the batch test duration (Fig. S1c). Specifically, under VFAs peak the highest phosphate release was measured for test OT3 (18.2 mg L^{-1}). For tests OT2 and OT1 the pick of PO_4^{3-}- P concentration was 8.3 mg L^{-1} and 8.1 mg L^{-1}, respectively. These results suggest that high VSS concentration increases the PO_4^{3-}- P concentration, unlike ammonium release.

3.3. Prokaryotic community structures of sewage sludge

A metataxonomic approach was used to characterise the possible influence of batch fermentation conditions on the microbial composition of the collected sewage sludge and used as fermentation inoculum.

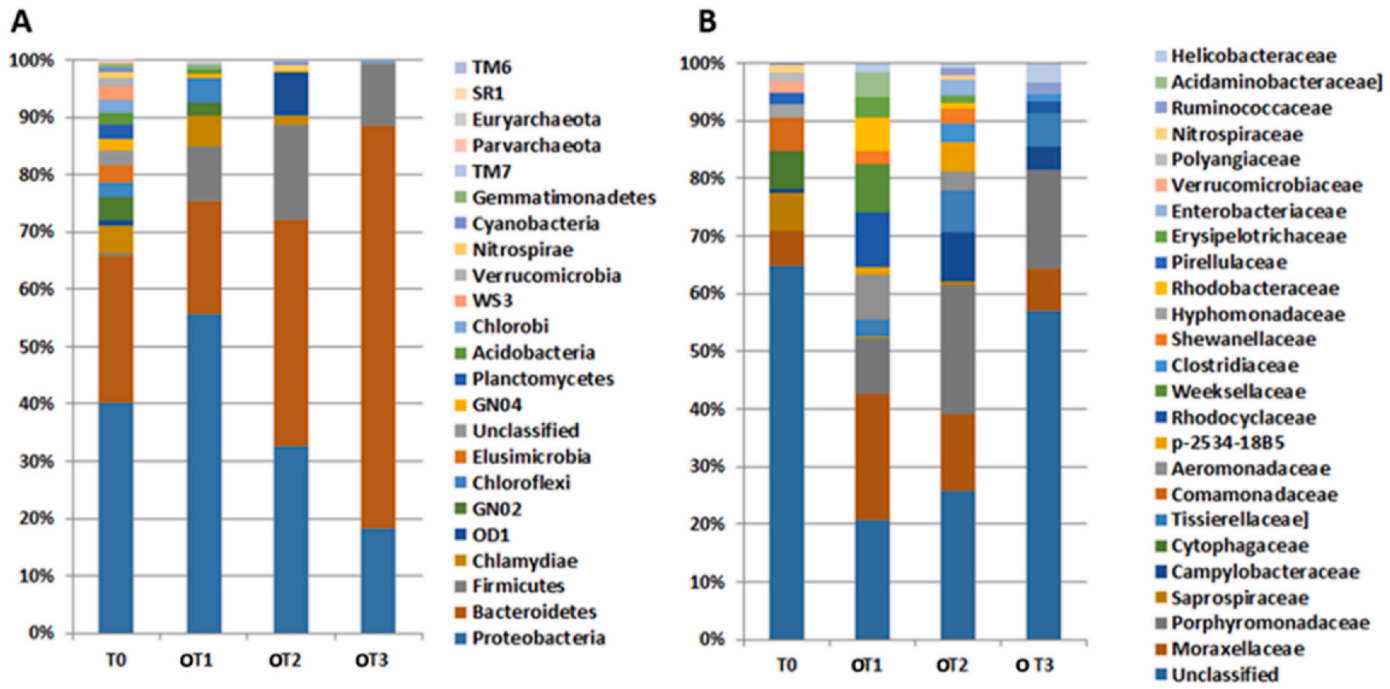

Fig. 5. Structure of microbiota residing in the sewage sludge of T0, OT1, OT2 and OT3 reactors. Relative abundances of the top 25 bacterial phyla (A) and top 25 bacterial families (B) were identified by a metataxonomic approach based NGS analysis of the V3–V4 region of the gene encoding the 16S rRNA.

Metagenomic DNA was extracted from the sewage sludge of the recycle line used as inoculum (T0) and from the sewage sludge of three different batch fermentation reactors (each indicated as OT1, OT2, and OT3, respectively) after 20 days of incubation. Preliminarily, the metagenomic DNA samples were quantified and analysed by PCR amplifying the V3–V4 region of the 16S rRNA encoding gene for a qualitative evaluation. Then, the structures of the bacterial community on the sewage samples were elucidated at phylum and family levels based on NGS analysis of the amplicons covering the V3–V4 region of the 16S rRNA encoding gene (Fig. 5, Table S2). Looking at the microbial composition at the phylum level, represented in Fig. 5a it is possible to appreciate that the microbial diversity reduces from the inoculum T0 to OT3 reactor in parallel with the concentrations of added potassium permanganate increasing from OT1 to OT3. Table S1 not only shows the ASV and ACE values which indicate the richness of species present in the different samples, indices which therefore overlap, but also shows the alpha-diversity indices. In particular, the Shannon and Simpson indices define the relative abundance of the different taxa that make up the four analysed samples. The alpha-diversity indices show, coinciding with the histograms of Fig. 5, that there is an increase in diversity in OT1 (especially at the family level) while there is a gradual reduction in diversity in OT2 and OT3, as increases the concentration of added potassium permanganate.

The most represented phyla are Proteobacteria, Bacteroidetes and Firmicutes. In particular, Proteobacteria are 40.32 % in T0, a slight increase in OT1 (55.71 %) and a decrease in OT2 and OT3 (32.72 and 18.36 %, respectively). Bacteroidetes are present at 25.55% in T0 and, contrarily Proteobacteria, decrease in OT1 (19.78%) and increase in OT2 and OT3 (39.31 and 70.22 %, respectively). The Firmicutes are 0.46%, 9.60%, 16.62% and 10.78 % in T0, OT1, OT2 and OT3, respectively. Furthermore, in T0 there is a high percentage of Chlamydiae (4.79 %) which increases in OT1, decreases in OT2 and even zeroes in OT3 (5.30, 1.64 and 0 %, respectively). The microbial composition is related to the different yields of VFA, in fact Proteobacteria, Bacteroidetes and Firmicutes are excellent producers of VFA (She et al., 2020). Chlamydiae is a phylum whose abundance generally decreases as fermentation processes progress (Qin et al., 2021). Potassium permanganate has a good bactericidal effect against Chlamydiae, at high concentrations, which justifies the reduction of the percentage abundance in OT2 and OT3 (Bommana and Polkinghorne, 2019). The main families (Fig. 5b) present in the samples were *Moraxellaceae, Porphyromonadaceae, Saprospiraceae, Cytophagaceae* and *Comamonadaceae. Moraxellaceae* which are present at 5.66% in T0 have a maximum growth in OT1 (19.42%), average in OT2 (12.4%) and minimum in OT3 (7.06%). *Porphyromonadaceae* are present in T0 in minimal percentages, less than 0%; however, they undergo a notable growth during fermentation, as they are present at 8.42% in OT1, at 20.91% in OT2 and 16.69% in OT3. *Saprospiraceae* are drastically reduced during fermentation, in fact, they are abundant at 6.34% in T0 while in OT1, OT2 and OT3 they are respectively abundant at 0.39%, 0.53% and 0%. As for *Cytophagaceae* and *Comamonadaceae* present respectively at 6.04% and 6.97% in T0, during fermentation and after the addition of potassium permanganate, the percentages of both bacterial families are zero (0.00%). The first three families can ferment organic matter to produce VFA; therefore, the production of VFA can be delegated to these three families, especially *Moraxellaceae* and *Porphyromonadaceae* (Llamas et al., 2022). *Comamonadaceae* have a role in the degradation of VFAs to produce polyhydroxyalkanoates (Khan et al., 2002). The fact that adding different concentrations of potassium permanganate reduces the percentage abundance of this bacterial family can promote VFA accumulation.

4. Conclusions

Batch fermentation tests to maximise VFAs production using sewage sludge have been performed. Among the tested pre-treatments (potassium permanganate - $KMnO_4$, initial pH = 10, initial pH = 2.5 and low temperature thermal hydrolysis) the initial $KMnO_4$ dosing at 0.1 mg $KMnO_4$/gVSS was selected as the optimal. Further investigations about the role of VSS under $KMnO_4$ dosing have been performed in view of optimising the results.

Based on the results obtained here, the optimal conditions to maximise VFAs can be obtained under 6 gVSS/L coupled with 0.1 mg $KMnO_4$/gVSS dosing. The main bacterial phyla involved in the

fermentation process were Proteobacteria, Bacteroidetes, Firmicutes; the main bacterial families affected were *Moraxellaceae, Porphyromonadaceae, Saprospiraceae.*

Several pre-treatments have been compared, under the same operative conditions, in view of enhancing VFA production by sewage sludge acidogenic fermentation. The best pre-treatment has been further optimized, by evaluating the influence of VSS, in order to achieve the best performing conditions. Furthermore, metataxonomic analysis provided several insights into the process performance and future perspectives.

CRediT authorship contribution statement

Antonio Mineo: Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Ylenia Di Leto:** Writing – review & editing. **Alida Cosenza:** Writing – review & editing. **Fanny Claire Capri:** Writing – review & editing. **Giuseppe Gallo:** Writing – review & editing. **Rosa Alduina:** Writing – review & editing. **Bing-Jie Ni:** Writing – review & editing. **Giorgio Mannina:** Conceptualization, Resources, Writing – review & editing, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2023.140859.

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