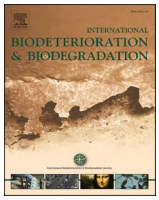
International Biodeterioration & Biodegradation 191 (2024) 105809

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod



Enhancing volatile fatty acid production in batch test reactors by modulating microbial communities with potassium permanganate

Ylenia Di Leto [a,1], Fanny Claire Capri [a,1], Giuseppe Gallo [a,c], Alida Cosenza [b], Antonio Mineo [b], Giorgio Mannina [b], Rosa Alduina [a,c,*]

[a] Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Viale delle Scienze, Bldg. 16-17, 90128, Palermo, Italy
[b] Engineering Department, University of Palermo, Viale delle Scienze, Bldg. 8, 90128, Palermo, Italy
[c] NBFC, National Biodiversity Future Center, piazza Marina 61, 90133, Palermo, Italy

ARTICLE INFO

Keywords:
Acidogenic fermentation
Metataxonomic analysis
Microbiota
Potassium permanganate
Sewage sludge
VFA

ABSTRACT

The shift from conventional wastewater treatment plants to biorefineries is one of the most environmentally and economically sustainable pathways for extracting valuable compounds from waste. Besides chemical-physical processes, microorganisms within sewage sludge utilize organic and inorganic pollutants in the wastewater as nutrients, leading to effective water purification. However, the remaining solid sludge residue, typically destined for specific landfills or incineration, could undergo microbial fermentation to produce volatile fatty acids (VFA), metabolic precursors for biopolymers. Increasing attention has been directed towards optimizing operational parameters to enhance VFA production during sewage sludge fermentation. This study examined the impact of potassium permanganate (PP) on microbial communities during the sewage sludge's acidogenic fermentation. The results highlighted the positive effect of PP treatment, which increased COD production and VFA yield up to 1263.5 mg/L and 664.2 mg COD/L, respectively. The presence of PP significantly enhances VFA yield promoting bacteria positively linked to VFA production.

1. Introduction

Wastewater comprises domestic, industrial, urban, and agricultural water collected through urban drainage systems, flowing into wastewater treatment plants (WWTP). Within WWTP, a series of purification processes, combining physical and microbiological procedures, are employed to eliminate organic and inorganic pollutants posing risks to human health and the environment, such as genotoxicity, cytotoxicity, tumorigenesis, and ecotoxicity (Choudri et al., 2020). The initial purification involves physical methods like sandblasting, de-oiling, and grilling to remove coarse particles, followed by microbial degradation of organic matter in the sludge. Clarification and decantation then separate solid components from the water, with subsequent disinfection marking the final stages (Newhart et al., 2019). Purified water is eventually discharged into receiving water bodies like lakes, rivers, and seas. However, sewage sludge produced in this process is typically disposed of in solid waste landfills or incinerators, contributing to environmental pollution alongside the greenhouse gas emissions produced by conventional WWTP (Hao et al., 2019).

In Italy alone, 395,000 tons of dry sewage sludge are produced annually from WWTPs, with only 9.9% used in agricultural applications. (Mininni et al., 2019). Thus, reclaiming and recycling valuable compounds (both materials and energy) remaining in the sludge poses a significant challenge, necessitating the transformation of traditional WWTP into Water Resource Recovery Facilities or Wastewater Bio-Refineries. This shift aims to safeguard water quality, the environment, and human health (Mannina et al., 2021a,b).

For instance, semiconductors can remove phenolic compounds from industrial discharges before the sewage sludge enters the WWTP. These semiconductors serve multiple purposes, including phenol removal from wastewater, CO_2 conversion, hydrogen generation from water splitting, and organic matter degradation (Al-Nayili and Alhaidry, 2023). Industrial wastewater contaminated by carcinogenic dyes can be effectively treated through adsorption using activated carbon, the most efficient,

* Corresponding author. Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Viale delle Scienze, Bldg. 16-17, 90128, Palermo, Italy.
E-mail address: valeria.alduina@unipa.it (R. Alduina).
[1] These two authors contributed equally to this work.

https://doi.org/10.1016/j.ibiod.2024.105809
Received 21 March 2024; Received in revised form 30 April 2024; Accepted 8 May 2024
Available online 11 May 2024

straightforward, and cost-effective natural treatment method for eliminating these organic compounds (Salman and Alshamsi, 2022). Undoubtedly, one potential application of organic matter usage is biopolymer production. Sewage sludge is particularly rich in compounds like biopolymers or their precursors produced by microbial metabolism. In the last years, an increased demand for alternatives safer than plastics that can replace non-biodegradable plastic-based materials has surged (Gomaa et al., 2018; Piacenza et al., 2022). Plastics are more durable also in harsh environments and can spread antibiotic resistance bacterial strains (Sucato et al., 2021). Among biodegradable polymers, polyhydroxyalkanoates (PHAs) stand out as environmentally and economically sustainable thermoplastic biodegradable polyesters of microbial origin, suitable to replace petroleum-derived plastics (Tarrahi et al., 2020).

Microorganisms play a significant role in breaking down and removing organic and xenobiotic contaminants to purify wastewater in WWTP and generate volatile fatty acids (VFA), H_2, and CO_2 (Sarkar et al., 2018). CO_2 and H_2 can be recovered from a wastewater treatment plant.

H_2 is a valuable energy source to mitigate finite resource exploitation. The yield may vary based on the type of sewage sludge, concentration, and operating conditions and can be collected using different techniques (*e.g.,* gas separation, membrane technologies, bioreactors). CO_2 is a pollutant greenhouse gas that can be recovered using different types of activated carbon synthesized from phenol-formaldehyde resins or zeolites and exploiting different adsorption-desorption dynamics (Gil et al., 2015). VFAs serve as excellent precursors for PHAs, accumulated in granular form as an energy reserve (Shahid et al., 2021). The production of VFAs starting from sewage sludge is significant for several reasons. In fact, VFAs play a crucial role as substrates for microbial processes like anaerobic digestion or fermentation, leading to biopolymer generations. VFA production from sewage sludge allows waste valorisation into a valuable resource. Furthermore, VFAs enhance biological nutrient removal and overall treatment efficiency in wastewater treatment processes. Thus, VFA generation from sewage sludge offers economic advantages and advances sustainability.

Thus, understanding the interplay between microbial communities and different WWTP operating conditions is crucial to improving fermentation processes aimed to VFA production.

Previous studies demonstrated the positive effect of $KMnO_4$ (potassium permanganate or PP), basic pH, and temperature on VFA production (Xu et al., 2021; Di Leto et al., 2022; Mineo et al., 2024). However, the impact of these conditions on microbial components remains relatively unexplored (Di Leto et al., 2022; Mineo et al., 2023). The Mn, contained in $KMnO_4$, is a contaminating heavy metal. Following the positive effect of potassium permanganate on the sludge microbiota and subsequent recovery of VFAs through suitable filtration, the Mn released in the sewage sludge must be recovered. Leaching the sludge with HCl followed by hydrothermal treatment at 270 °C enables the retention of up to 98% of the Mn in the sewage sludge, ensuring environmental safeguarding against this heavy metal (Liu et al., 2021). This study aims to provide valuable insights into the microbial community shift during sewage sludge fermentation, especially when a chemical pretreatment is involved. Sewage sludge's acidogenic fermentation was investigated in batch tests, and sludge was sampled from two different WWTP sources pretreated with PP addition. A metataxonomic-based analysis was carried out on the sewage sludge microbiota in view to assess the microorganisms involved in the process, their metabolism, and diversity change after the oxidant treatment.

2. Materials and methods

2.1. Experimental set-up

Two experiments were conducted to assess the impact of PP on sewage sludge fermentation. Two sludge samples were collected from the pilot plant at the Water Resource Recovery Facility of Palermo University and a full-scale WWTP located in Marineo (Italy), indicated as A and B, respectively (Mannina et al., 2021, 2022). T0-A and T0-B sewage sludge were previously characterised (Mineo et al., 2023) in terms of pH, Total Suspended Solids (TSS), Volatile Suspended Solids (VSS), total Chemical Oxygen Demand (COD), soluble COD (sCOD), ammonium (NH_4^+-N), and phosphate (PO_4^{3-}P) concentrations (Table 1). sCOD, TCOD, VSS, TSS, NH_4^+-N, and PO_4^{3-}P concentrations were analysed according to standard methods suggested by APHA.

Table 1
Details of the fermentation batch tests.

Parameters	T0-A	T0-B	T18PP-A	T18PP-B
$KMnO_4$ dosage (g/g TSS)	–	–	0.1	0.1
TSS (g/L)	4.7	4.8	4.7	4.8
VSS (g/L)	3.9	2.9	3.6	3.5
TCOD (mg/L)	5016.3	3329.4	4985.3	2972.4
sCOD (mg/L)	106.1	59.2	115.2	8.8
NH_4^+-N (mg N/L)	4.7	2.3	3.7	1.9
PO_4^{3-}P (mg P/L)	2.3	3.6	8.5	2.1

Two fermentation tests were conducted for each sewage sludge sample: control tests (T18-A and T18-B) and PP dosing tests (T18PP-A and T18PP-B). The fermentation tests were executed in 1.1 L reactors at room temperature, with uncontrolled pH. PP was added at a concentration of 0.1 g per 1 g of TSS, as previously reported by Quixiang et al. (2021). The details of the batch fermentation tests are reported in Table 1.

The tests were carried out for 18 days, during which the analysis of sCOD, VFA, NH_4^+-N and PO_4^{3-}–P were carried out. The VFAs were assayed by using gas chromatography (GC) after the extraction with dimethyl carbonate (DMC-OEI) as reported by Ghidotti et al., (2018). An Agilent Technologies 7820A GC with a flame ionisation detector (FID) and a DB FFAA column (30 m × 0.25 x mm x 0.25 μm) was used to analyse VFAs samples following the GC protocol described by Montiel-Jarillo et al. (2021). The VFA concentration (expressed as mg/L) was converted into COD concentration (mg COD/L) by applying the conversion factors reported in the literature (Yuan et al., 2011).

2.2. Microbiota analysis

Six sewage sludge samples were analysed: T0-A and T0-B, corresponding to samples taken at the start of the fermentation from plant A and plant B, respectively; T18, representing samples collected after 18 days of the fermentation process, and T18PP, indicating samples collected from the test with PP addition. Samples were collected on the 18th day of fermentation, which aligned with the decline in sCOD values following the peak phase. The procedures outlined by Mineo et al. (2023) were used. Metagenomic DNA extraction was performed using 1 g of samples. DNA quality and quantity were assessed through 1% agarose gel electrophoresis analysis, supplemented with 0.5 μg/mL ethidium bromide for visualization under a UV lamp, and by measuring absorbance ratios (260/280 and 260/230 nm) using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific) to detect protein, organic compound, or chaotropic agent contamination. Amplification of the V3–V4 regions of the 16S gene was conducted using the primers PRO 341F CCTACGGGNBGCASCAG and PRO 805R GACTACNVGGGTATCTAATCC, with DreamTaq DNA Polymerase (ThermoFisherScientific) following the manufacturer's protocol. The thermal profile comprised 95 °C for 2 min; 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, elongation at 72 °C for 30 s; and a final elongation at 72 °C for 5 min. Amplification products were sequenced in a single-300-bp paired-end run on an Illumina MiSeq platform at BMR Genomics (Padova, Italy). Raw 16S rDNA data were processed using QIIME2 software (https://qiime2.org/) for denoising, where overlapping paired-end reads were processed with the DADA2 plug-in. Unique Amplicon Sequence Variants (ASVs) were assigned and aligned to the

Table 2
sCOD, VFA, ammonium and phosphate concentration during the fermentation tests.

Parameters	Test	Time (days)					
		3	5	7	10	12	14
sCOD (mg/L)	**T18-A**	208.5	348.2	757.4	827.1	1119.2	880.9
	T18-B	10.2	50.3	120.1	150.8	284.4	90.2
	T18PP-A	667.1	768.2	954.8	1032.7	1226.4	1080.5
	T18PP-B	630.4	955.2	1263.5	922.7	890.2	758.3
VFA (mg COD/L)	**T18-A**	23.2	37.4	69.0	230.1	410.7	223.3
	T18-B	0.0	0.0	0.0	25.5	11.9	2.3
	T18PP-A	207.4	224.4	288.2	393.1	473.0	262.7
	T18PP-B	267.2	403.9	664.2	292.9	185.0	103.4
NH_4^+-N(mg N/L)	**T18-A**	4.7	16.2	49.5	61.3	70.4	60.2
	T18-B	2.8	11.2	15.8	18.5	19.3	27.8
	T18PP-A	10.2	37.8	55.2	68.4	78.7	63.9
	T18PP-B	15.2	36.8	87.6	84.3	78.5	77.1
PO_4^{3-}P(mg P/L)	**T18-A**	9.9	16.9	26.1	30.5	31.3	28.1
	T18-B	7.8	10.2	12.3	14.1	16.7	15.4
	T18PP-A	10.3	9.2	13.3	14.9	22.1	21.3
	T18PP-B	2.7	3.9	5.7	7.4	7.7	6.8

Greengenes reference database at 99% sequence similarity (https://greengenes.secondgenome.com/). For each sample, the count of ASVs and the relative abundance percentages of phyla, orders, classes, families, and genera were determined. HeatMap generation was conducted via the online web server (http://heatmapper.ca/expression/), employing a "complete linkage" calculation based on Pearson correlation and focusing on families. METAGENassist (http://www.metagenassist.ca (accessed on March 5, 2024) was used to distinguish the microbial species based on their metabolic activity. The sequences were deposited under the number PRJNA1078840.

3. Results and discussion

3.1. Effect of potassium permanganate addition

The monitoring results carried out during the fermentation are reported in Table 2. The addition of PP significantly enhanced sCOD production of tests T18PP-A and T18PP-B by up to 1226 mg COD/L and 1263 mg COD/L, respectively, on days 12 and 7. For T18-A and T18-B, sCOD peak values were observed at 1119 mg COD/L and 284 mg COD/L on the 12th day, respectively. The comparison of the control tests with those treated with the oxidant addition showed that the treatment increased the sCOD concentration at its peak by + 9.6% for Plant A,

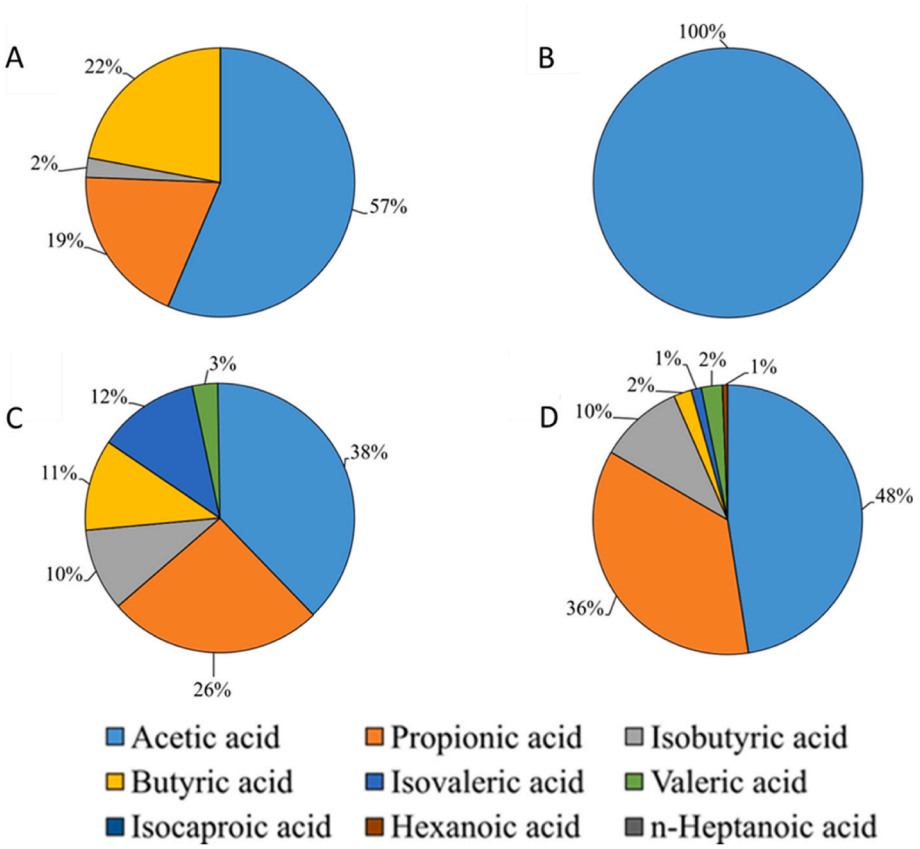


Fig. 1. Average composition of acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, hexanoic, and n-heptanoic acids in T18-A (A), T18-B (B), T18PP-A (C) and T18PP-B (D) samples after 18 days of fermentation.

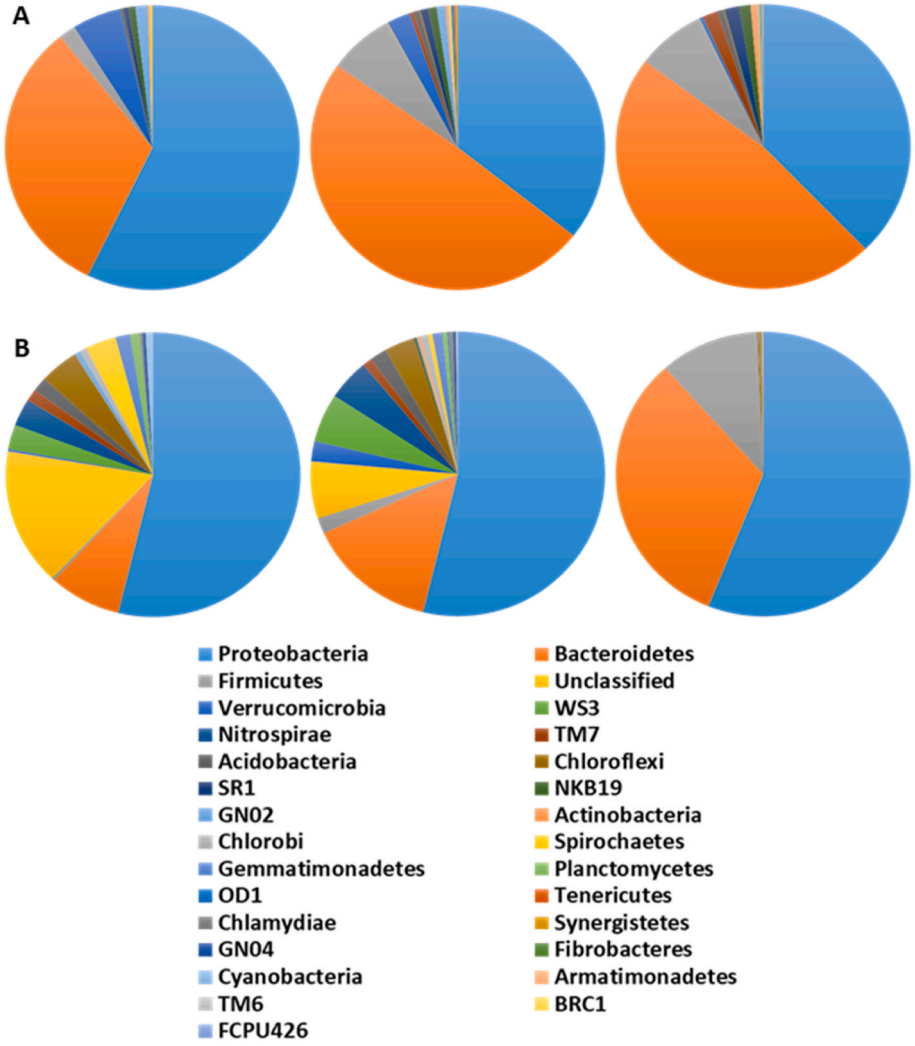


Fig. 2. Pie charts reporting the taxonomic composition of samples T0-A, T18-A, T18PP-A (A) and T0-B, T18-B, T18PP-B (B). The relative abundance indicated the percentage of each phylum compared to all the identified phyla.

while a higher increase of +344% was registered for Plant B. Additionally, T18PP-B achieved its peak of sCOD production five days earlier than T18PP-A, likely due to enhanced hydrolysis, as previously reported (Xu et al., 2019; Yu et al., 2008). PP considerably improved the breakdown of complex organic material, with a maximum effect observed in sludge from full-scale WWTP. The dosage of PP improved COD solubilization by nearly 30% for Plant B sludge and by only 2% for T18PP-A. Furthermore, PP favored the release of NH_4^+ to supernatant because of protein hydrolysis, as reported by the high ammonium concentrations on the sCOD production peak day for T18PP-B (87 mg N/L) compared to T18PP-A (78 mg N/L), conforming to sCOD trends. Peak day ammonium concentration in T18-A and T18PP-A showed no remarkable difference (70 mg N/L and 78 mg N/L, respectively). Furthermore, the addition of PP affected the concentration of phosphates, whereby its concentration was higher due to the promotion of precipitation in an alkaline condition, as reported in Table 2 (Banister et al., 1998). The VFA production followed the sCOD production trend across all the trials, clearly showing an increment in VFA production, more prominently in T18PP-B, where the VFA concentration increased to 664 mg COD/L at day 7, composing around 55% of sCOD. The T18PP-A peak (474 mg COD/L at day 12) gave around 38% of sCOD. This result is thus evidence of PP effectiveness also in the acidogenic phase of fermentation, by a noticeable improvement in sCOD production, COD solubilization, and VFA yield in T18PP-B as reported in other studies (Li et al., 2015, 2020; Yu et al., 2013).

As reported in Fig. 1, acetic acid was the most prevailing VFA, accounting for 57% in T18-A, 38% in T18PP-A, 100% in T18-B, and 48% in T18PP-B. PP addition mainly increased the production of propionic, isobutyric, and butyric acids in T18PP-A (26%, 10%, and 11%, respectively) and T18PP-B (35.8%, 10.1%, and 2.1%, respectively). The quantity of iso-valeric acid in T18PP-A was 12.2%, whereas other acids collectively contributed less than 4% in all tests. Regarding T18PP-B, butyric to n-heptanoic acid accounted for less than 7%. Generally, PP addition favored the production of different VFAs, suggesting a more efficient breakdown of organic materials, as reported above.

3.2. *Taxonomic composition of prokaryotic communities of sewage sludge at the phylum level*

Microbiota analysis showed that both the sludge samples contained microorganisms belonged to the bacterial kingdom, while no Archaea was detected. Similar results were found in a few previous studies on sludge, in which bacteria represented almost the totality of the prokaryotic community of the sewage sludge (98.8%) to Archaea (only 1.2%) (Yu and Zhang, 2012; Zhang et al., 2012; Xia et al., 2010; McLellan et al., 2010; Mathur et al., 2023). The bacterial composition of the sludge samples from plants A and B was comparatively analysed considering the relative abundances at the phylum level.

Proteobacteria represented the most abundant phylum accounting

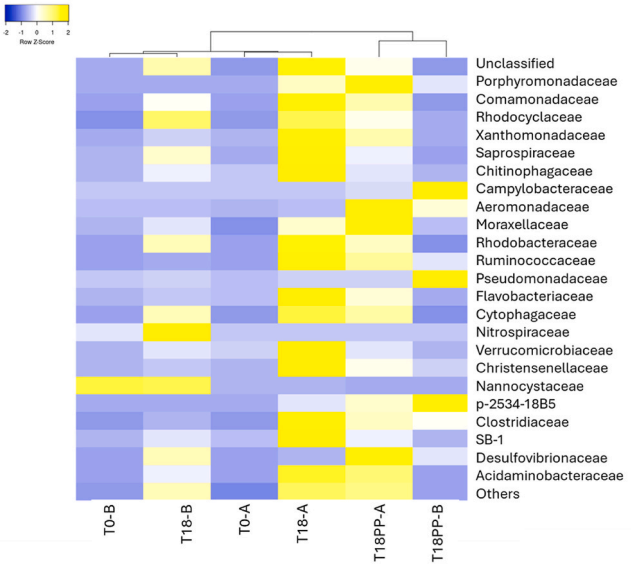


Fig. 3. Heatmap of 25 most abundant prokaryotic families' composition profiles in T0-A, T18-A, T18PP-A, and T0-B, T18-B, T18PP-B samples as inferred by HeatMapper bioinformatic analysis. The color intensity in each sample was normalized to represent its relative ratio in the two groups. Colors from blue to yellow indicated the relative values of microbiota.

for 57.24% in T0-A and 53.84% in T0-B. In metropolitan WWTP, Proteobacteria (21–65%) represent the utmost prevalent phylum and are primarily responsible for nutrient and organic removal; subdominant phyla include Chloroflexi, Acidobacteria, and Bacteroidetes (Mathur et al., 2023; Wang et al., 2012).

Apart from the similar percentages of Proteobacteria, comparing the microorganisms identified in T0-A and T0-B samples showed that the two samples contain different phyla or the same phylum with different relative abundance percentages. As seen in Fig. 2, the T0-B sample displayed a greater bacterial diversity than T0-A. In the T0-A sample, Bacteroidetes (32.01%) were predominant, followed by Verrucomicrobia (5.38%) and Firmicutes (1.86%). All the other phyla were present in a low percentage (less than 1.86%). In the T0-B sample, 15.60% of bacteria were unclassified, followed by Bacteroidetes (7.89%), Chloroflexi (4.28%), and Spirochaetes (3.41%). Also, a modest percentage of Nitrospirae (3.10%) and WS3 (2.77%) was found. This first result underscores the importance of bacterial composition that relies on the operational conditions but also on the initial bacterial load of the sewage sludge (Zhang et al., 2022).

Despite the high variability of WWTP microbial communities that can contain members of various taxonomic, physiological (*e.g.,* aerobic, anaerobic, heterotrophic, phototrophic), and biochemical (*e.g.,* nitrification, N_2-fixation, sulfur oxidation, and denitrification) groups, some phyla are frequently found, such as Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, and Verrucomicrobia that can be considered as the core microbiota of the sludge samples, coherently with other reports(Yu and Zhang, 2012).

Proteobacteria and Bacteroidetes are recognized for their pivotal roles in VFA production during fermentative processes (She et al., 2020). Verrucomicrobia are commonly found in wastewater and have recently been associated with VFA production via fermentation from substrates like glucose (Spring et al., 2016). Spirochaetes are also acknowledged for their proficiency in producing VFAs under alkaline conditions, around pH 8 (Slezak et al., 2021). Nitrospirae predominantly produce acetic and propionic acids (Yang et al., 2016), with an additional function of transforming nitrites into nitrates when VFA are present. Despite the WS3 phylum has been detected in Marineo sludge and polluted aquifers (Presti et al., 2021), their capability to produce VFA remains unproven.

T18-A and T18PP-A exhibited a comparable microbial composition in terms of phyla, with four predominant phyla: Bacteroidetes (increased to 49.14% and 47.43%, respectively), Proteobacteria (decreased to 35.63% and 37.80%, respectively), Firmicutes (increased to 7.27% and 7.60%), and Verrucomicrobia (decreased to 2.54% and 0.43%, respectively). Thus, PP addition to T0-A did not substantially influence the sludge's bacterial composition. Conversely, the addition of PP dramatically impacted bacterial abundance percentages. T18-B closely resembled the T0-B sample regarding Proteobacteria, except for the increased of Bacteroidetes (14.35%) and decreased of the unclassified bacteria (6.46%). Particularly noteworthy, the high bacterial diversity observed in T0-B was significantly reduced in T18PP-B, leaving only three dominant phyla: Proteobacteria (slightly increased to 56.09%), Bacteroidetes, and Firmicutes (increased to 32.34% and 10.90%, respectively). Collectively, these three phyla represent 99.29% of the entire bacterial community.

3.3. Clustering analysis of microbial community dynamics

According to the linkage of ASVs (Fig. 3), the bacterial communities of the six samples were clustered into two large groups: Group I contained the sewage sludge inocula (T0-A and T0-B) and the control samples (T18-A and T18-B); Group II contained the two samples of sewage sludge treated with PP (T18PP-A and T18PP-B) indicating that, although the effect of PP on VFA is different, the impact on bacteria is similar. According to weighted Pearson distances, significant position differences occurred between the inocula samples and control samples of both experiments. The T18PP-A and T18PP-B were close in cluster analysis, showing similar bacterial diversity but different bacterial family abundance. PP treatment in sample T18PP-B resulted in a drastic decrease in the abundances of many bacterial families, maintaining high percentages only of three families: *Campylobacteraceae, Pseudomonadaceae,* and p-2534-18B5 (indicated in yellow, Fig. 3). We hypothesized that the increase of Gram-negative bacteria could rely on the development of resistance systems of these bacteria towards oxidizing agents such as PP (da Cruz Nizer et al., 2020, 2021).

3.4. Taxonomic composition of prokaryotic communities of sewage sludge at the family level

In bacterial families' analysis, a notable contrast between samples of Plant A and Plant B became even more pronounced (Fig. 4). Sample T18PP-A was found enriched of Porphyromonadaceae (32.64%) towards 11.34% (T18-A). In T18PP-A, the modulation of unclassified bacteria (from 22.46% in T0-A and 29.21% in T18-A to 16.28% in T18PP-A) and the decrease of Moraxellaceae (from 5.94% of T0-A to 1.23% in T18-A and 3.26% in T18PP-A) was also evident. The percentage abundance of Saprospiraceae (7.80% in T0-A, 6.13% in T18-A and 1.99% in T18PP-A), Chitinophagaceae (7.40% in T0-A, 5.46% in T18-A and 1.03% in T18PP A), and Campylobacteraceae (4.38% in T0-A, 0.12% in T18-A and 0.70% in T18PP-A) decreased after the fermentation process, mainly in presence of PP.

In T18PP-B, there was a significant decrease in the presence of unclassified bacteria, from 39.43% of T0-B and 38.35% in T18-B to just 3.31%. The most abundant families in T18-PP-B were Campylobacteraceae (28.00% in T18PP-B towards 1.41% in T0-B and 0.48% in T18-B), Porphyromonadaceae (24.86% in T18PP-B towards 0.09% in T0-B and 0.12% T18-B), Pseudomonadaceae (12.27% in T18PP-B towards 0.97% in T0-B and 0.43% in T18-B) and Aeromonadaceae (8.19% in T18PP-B towards 1.44% in T0-B and 0.33% in T18-B). Contrarily, in T18PP-B Saprospiraceae were not present (0.00%) in respect to T0-B (4.17%) and T18-B (5.54%) and Comamonadaceae were very low (0.30%) in respect to T0-B (5.58%) and T18-B (7.01%).

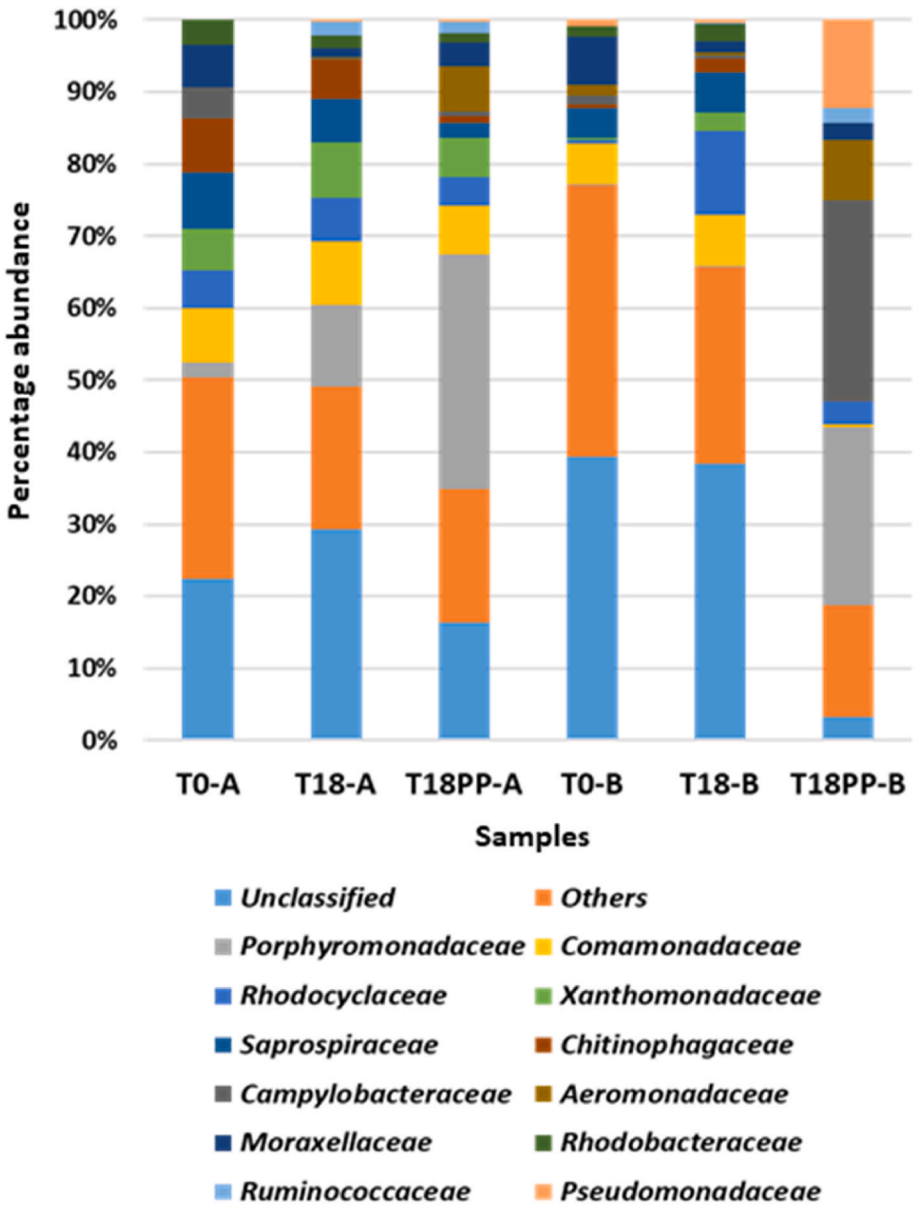


Fig. 4. Pie charts reporting the taxonomic composition of samples T0-A, T18-A, T18PP-A (A) and T0-B, T18-B, T18PP-B (B). The relative abundance was the percentage of each family related to all the identified families.

3.5. Predicting microbial metabolism

To analyse how PP can influence microorganisms capable of synthesizing VFA, bacteria were categorized based on their metabolic pathways. Metabolic profiling indicated the presence of dehalogenating bacteria, nitrite reducers, sulfate oxidizers, and ammonia oxidizers (Fig. 5), as already reported in Di Leto et al. (2022). Although the prokaryotic communities in Plants A and B displayed similar metabolic profiles, some interesting differences can be highlighted.

T18PP-B showed the abundance of ammonia oxidizers, sulfate reducers, sulfide oxidizers and nitrite reducers was higher than in T18PP-A. Bacteria capable of ammonium oxidation, referred to as nitrifying bacteria, can indirectly impact the production of VFA within wastewater systems. While nitrifying bacteria utilize ammonium as an energy source, they do not directly produce VFA as a metabolic byproduct (Luo et al., 2019). Sulfide oxidizer and sulfate reducer bacteria can influence the redox state of the environment and the availability of organic substrates for other bacterial species. For instance, sulfur oxidation can diminish the concentration of hydrogen sulfide, a compound toxic to numerous bacterial strains, thus fostering the proliferation of bacteria capable of VFA production during the fermentation of organic compounds within the wastewater (Odriozola et al., 2019; Samanides et al., 2020).

Interestingly, an increase of anaerobic bacteria was registered after PP treatment in both plants, confirming the activation of the fermentation process leading to VFA production in both T18PP- A and T18PP-B.

3.6. Bacterial signatures for VFA high production

When the microbial components were analysed for the presence of bacterial species, most bacteria belonged to unknown species; however, the species of *Macellibacteroides fermentans* was predominantly detected in samples PP-treated. After fermentation, the percentages of this species increased from almost zero in T0-A and T0-B, to 4.54% in T18-A, 29.30% in T18PP-A and 21.59% in T18PP-B, while it was reduced only in T18-B (0.04%) Also, *Pseudomonas veronii* increased to 9.66% in the T18PP-B sample. Finally, *Proteocatella sphenisci* was present higher in T18PP-B (1.51%) than in other samples, where the percentage was

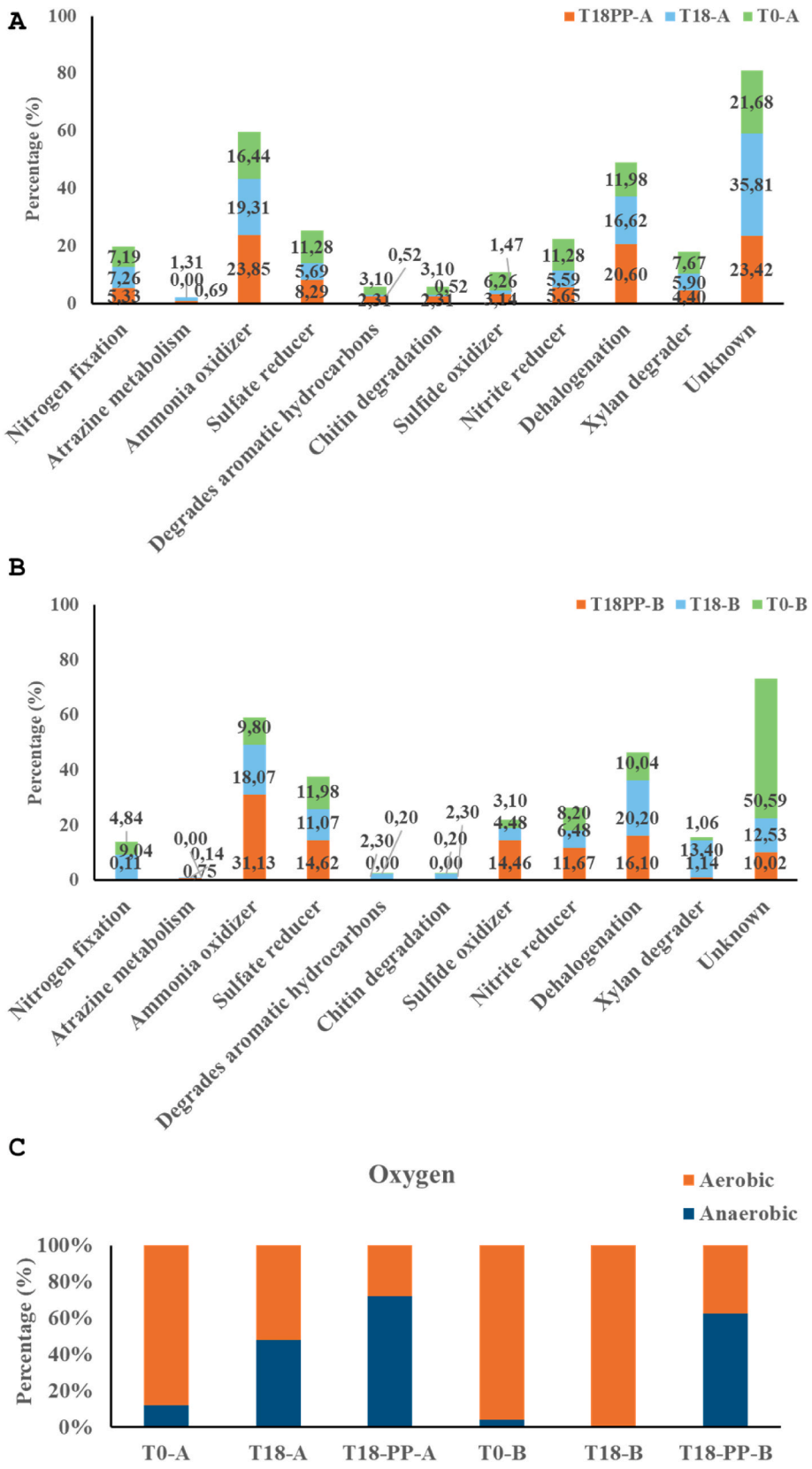


Fig. 5. Putative metabolic requirements of the bacterial communities residing in the samples T0-A, T18-A, T18PP-A (A) and T0-B, T18-B, T18PP-B (B) as inferred by METAGENassist bioinformatic analysis. Oxygen requirements of bacterial communities (C). The abundance percentage of bacteria having a specific metabolism is indicated.

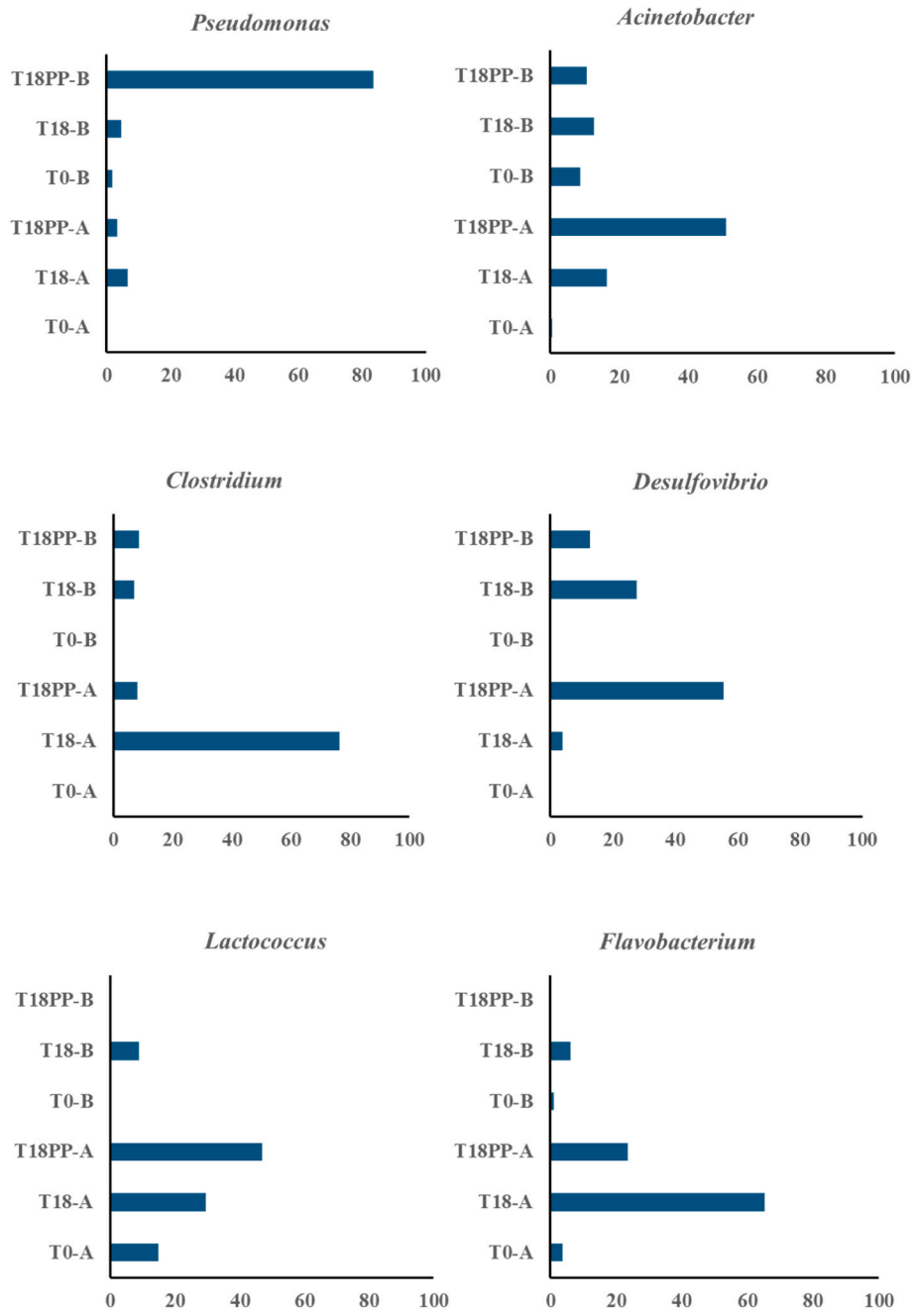


Fig. 6. Detection of potential harmful bacteria (*Pseudomonas, Acinetobacter, Clostridium, Desulfovibrio, Lactococcus* and *Flavobacterium*) in T0-A, T18-A, T18PP-A, and T0-B, T18-B, T18PP-B wastewater samples. The percentage of relative abundance of each potential pathogenic genus was indicated.

approximately zero.

M. fermentans is a species belonging to the *Porphyromonadaceae* family, isolated in recent years, which has the exceptional ability to degrade isosaccharinic acids (sugary acid typical of nuclear waste deposits). *M. fermentans* is capable of growing only in anaerobiosis, in the presence of sugars or the presence of isosaccharinic acid, it is capable of autonomously producing acetic acid, consequently causing a lowering of pH (Rout et al., 2017). It is also able to produce butyrate and isobutyrate (Jabari et al., 2012). *P. veronii*, belonging to the *Pseudomonas* genus, is among the most important producers of VFA. Previous studies confirmed that at any pH it can excellently produce VFA (Jie et al., 2014).

An interesting point that deserves to be further investigated concerns the mechanisms that allow this specie to survive despite the oxidizing action of the latter. Other studies have shown the ineffectiveness of PP treatments administered at 0.015% in *Pseudomonas* genus and Gram negative cultures (Hansson et Faergemann, 1995). As far as we know, there are no studies on the resistance of *Macellibacteroides* to PP or other antimicrobials. Being both Gram-negatives, we could speculate that these cells have developed resistance in the cell wall, which is the target of potassium permanganate (da Cruz Nizer et al., 2020; da Cruz Nizer et al., 2021).

3.7. Detection of potential harmful bacteria

Pathogenic bacteria can compete with other environmental bacteria for resources, such as organic substrates and nutrients. This competition can influence the growth and activity of bacteria involved in VFA

production, thus altering the quantity and composition of VFA produced.

Among the 108 bacterial genera, six are considered potentially harmful bacteria: *Pseudomonas* spp., *Acinetobacter* spp., *Clostridium* spp., *Desulfovibrio* spp., *Flavobacterium* spp., and *Lactococcus* spp. (Fig. 6). All detected potential harmful bacteria genera except *Pseudomonas* spp. had lower relative abundance in the T18PP-B sample compared to the other samples.

A greater abundance of pathogenic bacteria in Plant A, especially after PP treatment, may have caused the decreasing presence of bacteria capable of producing VFA. Some pathogenic bacteria can directly or indirectly use the organic compounds present in wastewater as a substrate for their metabolism and influence the structure and composition of the microbial community. Pathogenic bacteria that degrade organic matter can consume substrates that would otherwise be available to VFA-producing bacteria. This may reduce the availability of substrates for VFA production and thus affect their production, as already found (Zhang et al., 2022; Guo et al., 2020; Liu et al., 2021).

The presence of pathogenic bacteria can pose a risk to human and animal health (Vitale et al., 2019; Alduina 2020; Gargano et al., 2021; Gambino et al., 2022). Some pathogenic bacteria can cause disease if not treated properly, while others can produce toxins or harmful compounds. Therefore, the management and adequate treatment of wastewater are essential to mitigate the potential risks associated with the presence of pathogenic bacteria and to ensure the safety of water intended for human consumption and release into the environment.

4. Conclusions

This research represents a pioneering effort investigating how the microbial community in sewage sludge responds to potassium permanganate during fermentation to boost VFA production. Potassium permanganate, known for its antimicrobial properties, stimulated VFA yields by promoting the survival of VFA-producing bacterial species, belonging to Proteobacteria, Firmicutes, and Bacteroidetes phyla, and leading to a reduction of VFA degraders. Due to the complex system, further multidisciplinary studies are needed to define the bacterial signatures leading to specific production in a real WWTP.

Funding

This research was funded by the European Union's Horizon 2020 Research and Innovation Programme, (grant agreement number: 869283).

CRediT authorship contribution statement

Ylenia Di Leto: Writing – original draft, Methodology, Investigation, Data curation. **Fanny Claire Capri:** Writing – original draft, Methodology, Investigation, Data curation. **Giuseppe Gallo:** Writing – original draft, Investigation, Data curation, Conceptualization. **Alida Cosenza:** Writing – original draft, Methodology, Investigation, Data curation. **Antonio Mineo:** Writing – original draft, Methodology, Investigation, Data curation. **Giorgio Mannina:** Writing – review & editing, Funding acquisition, Conceptualization. **Rosa Alduina:** Writing – review & editing, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Giorgio Mannina reports financial support was provided by EU Framework Programme for Research and Innovation Euratom. If there are other authors, they 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

Data will be made available on request.

Acknowledgments

This work was funded by the project "Achieving wider uptake of water-smart solutions—WIDER UPTAKE" (grant agreement number: 869283) financed by the European Union's Horizon 2020 Research and Innovation Programme, in which Giorgio Mannina, is the principal investigator for the University of Palermo. The Unipa project website can be found at: https://wideruptake.unipa.it/.

References

Alduina, R., 2020. Antibiotics and environment. Antibiotics 9 (4), 202. https://doi.org/10.3390/antibiotics9040202. Apr 23.

Al-Nayili, A., Alhaidry, W.A., 2023. Batch to continuous photocatalytic degradation of phenol using nitrogen-rich g-C3N4 nanocomposites. Res. Chem. Intermed. 49, 4239–4255. https://doi.org/10.1007/s11164-023-05099-z.

APHA, 1999. Standard Methods for the Examination of Water and Wastewater.

Banister, S., Pitman, A., Pretorius, W., 1998. The solubilisation of N and P during primary sludge acid fermentation and precipitation of the resultant P. WaterSA 24 (4), 337–342.

Choudri, B.S., Al-Awadhi, T., Charabi, Y., Al-Nasiri, N., 2020. Wastewater treatment, reuse, and disposal-associated effects on environment and health. Water Environ. Res. 92, 1595–1602. https://doi.org/10.1002/wer.1406.

da Cruz Nizer, W.S., Inkovskiy, V., Versey, Z., Strempel, N., Cassol, E., Overhage, J., 2021. Oxidative stress response in Pseudomonas aeruginosa. Pathogens 10 (9), 1187. https://doi.org/10.3390/pathogens10091187. Sep. 14.

da Cruz Nizer, W.S., Inkovskiy, V., Overhage, J., 2020. Surviving reactive chlorine stress: responses of gram-negative bacteria to hypochlorous acid. Microorganisms 8 (8), 1220. https://doi.org/10.3390/microorganisms8081220. Aug11.

Di Leto, Y., Capri, F.C., Mineo, A., Cosenza, A., Gallo, G., Alduina, R., Mannina, G., 2022. Initial pH conditions shape the microbial community structure of sewage sludge in batch fermentations for the improvement of volatile fatty acid production. Microorganisms 10 (10), 2073. https://doi.org/10.3390/microorganisms10102073.

Gambino, D., Savoca, D., Sucato, A., Gargano, V., Gentile, A., Pantano, L., Vicari, D., Alduina, R., 2022. Occurrence of antibiotic resistance in the mediterranean sea. Antibiotics 11 (3), 332. https://doi.org/10.3390/antibiotics11030332, 2022 Mar 3.

Gargano, V., Sciortino, S., Gambino, D., Costa, A., Agozzino, V., Reale, S., Alduina, R., Vicari, D., 2021. Antibiotic susceptibility profile and tetracycline resistance genes detection in Salmonella spp. strains isolated from animals and food. Antibiotics 10 (7), 809. https://doi.org/10.3390/antibiotics10070809. Jul 2.

Ghidotti, M., Fabbri, D., Torri, C., Piccinini, S., 2018. Determination of volatile fatty acids in digestate by solvent extraction with dimethyl carbonate and gas chromatography-mass spectrometry. Anal. Chim. Acta 1034, 92–101. https://doi.org/10.1016/j.aca.2018.06.082.

Gil, M.V., Álvarez-Gutiérrez, N., Martínez, M., Rubiera, F., Pevida, C., Morán, A., 2015. Carbon adsorbents for CO2 capture from bio-hydrogen and biogas streams: breakthrough adsorption study. Chem. Eng. J. 269, 148–158. https://doi.org/10.1016/j.cej.2015.01.100.

Gomaa, M., Fawxy, M.A., Hifney, A.F., Abdel-Gawad, K.M., 2018. Use of the brown seaweed Sargassum latifolium in the design of alginate-fucoidan based films with natural antioxidant properties and kinetic modeling of moisture sorption and polyphenolic release. Food Hydrocolloids 82, 64–72. https://doi.org/10.1016/j.foodhyd.2018.03.053.

Hansson, C., Faergemann, J., 1995. The effect of antiseptic solutions on microorganisms in venous leg ulcers. Acta Derm. Venereol. 75 (1), 31–33. https://doi.org/10.2340/00015555753133.

Hao, X., Wang, X., Liu, R., Li, S., Van Loosdrecht, M.C.M., Jiang, H., 2019. Environmental impacts of resource recovery from wastewater treatment plants. Water Res. 160, 268–277. https://doi.org/10.1016/j.watres.2019.05.068.

Jabari, L., Gannoun, H.J., Cayol, J.L., Hedi, A., Sakamoto, M., Falsen, I., et al., 2012. Macellibacteroides fermentans gen. nov., sp. nov., a member of the family Porphyromonadaceae isolated from an upflow anaerobic filter treating abattoir wastewaters. Int. J. Syst. Evol. Microbiol. (Pt 10), 2522–2527. https://doi.org/10.1099/ijs.0.032508-0.

Jie, W., Peng, Y., Ren, N., Li, B., 2014. Volatile fatty acids (VFAs) accumulation and microbial community structure of excess sludge (ES) at different pHs. Bioresour. Technol. 152, 124–129. https://doi.org/10.1016/j.biortech.2013.11.011.

Li, L., He, Q., Ma, Y., Wang, X., Peng, X., 2015. Dynamics of microbial community in a mesophilic anaerobic digester treating food waste: relationship between community structure and process stability. Bioresour. Technol. 189, 113–120. https://doi.org/10.1016/j.biortech.2015.04.015.

Li, W., Lou, Y., Fang, A., Feng, K., Xing, D., 2020. Potassium permanganate (KMnO4)/sodium sulfite (Na2SO3) rapidly disintegrates waste activated sludge by reactive Mn (III) species and shapes microbial community structure. J. Chem. Eng. 394 https://doi.org/10.1016/j.cej.2020.124920.

Liu, C., Han, Q., Chen, Y., Zhu, S., Su, T., Qu, Z., Gao, Y., Li, T., Huo, Y., Huo, M., 2021. Resource recycling of Mn-rich sludge: effective separation of impure Fe/Al and

recovery of high-purity hausmannite. ACS Omega 6 (11), 7351–7359. https://doi.org/10.1021/acsomega.0c05487.
Luo, K., Pang, Y., Yang, Q., Wang, D., Li, X., Lei, M., Huang, Q., 2019. A critical review of volatile fatty acids produced from WasteActivated sludge: enhanced strategies and its applications. Environ. Sci. Pollut. Res. 26, 13984–13998. https://doi.org/10.1007/s11356-019-04798-8.
Mannina, G., Alduina, R., Badalucco, L., Barbara, L., Capri, F.C., Cosenza, A., et al., 2021a. Water resource recovery facilities (Wrrfs): the case study of Palermo University (Italy). Water 13. https://doi.org/10.3390/w13233413.
Mannina, G., Badalucco, L., Barbara, L., Cosenza, A., Di Trapani, D., Gallo, G., et al., 2021b. Enhancing a transition to a circular economy in the water sector: the EU project WIDER UPTAKE. Water 13 (7), 946. https://doi.org/10.3390/w13070946.
Mannina, G., Badalucco, L., Barbara, L., Cosenza, A., Di Trapani, D., Laudicina, V.A., et al., 2022. Roadmapping the transition to water resource recovery facilities: the two demonstration case studies of corleone and Marineo (Italy). Water 14. https://doi.org/10.3390/w14020156.
Mathur, H., Joshi, N., Khaturia, S., 2023. Microbial community profiling in wastewater of effluent treatment plant. Genom. Approach Biorem.: Princ. Tool. Emerg. Techno. https://doi.org/10.1002/9781119852131.ch9 (Chapter 9).
McLellan, S.L., Huse, S.M., Mueller-Spitz, S.R., Andreishcheva, E.N., Sogin, M.L., 2010. Diversity and population structure of sewage-derived microorganisms in wastewater treatment plant influent. Environ. Microbiol. 12 (2), 378–392. https://doi.org/10.1111/j.1462-2920.2009.02075.x.
Mineo, A., Cosenza, A., Ni, B.J., Mannina, G., 2023. Enhancing the production of volatile fatty acids by potassium permanganate from wasted sewage sludge: a batch test experiment. Heliyon 9 (11), e21957.
Mineo, A., Di Leto, Y., Cosenza, A., Capri, F.C.C., Gallo, G., Alduina, R., et al., 2024. Enhancing volatile fatty acid production from sewage sludge in batch fermentation tests. Chemosphere 349–140859. https://doi.org/10.1016/j.chemosphere.2023.140859.
Mininni, G., Mauro, E., Piccioli, B., Colarullo, G., Brandolini, F., Giacomelli, P., 2019. Production and characteristics of sewage sludge in Italy. Water Sci. Technol. J. Int. Assoc. Water Pollut. Res. 79, 619–626. https://doi.org/10.2166/wst.2019.064.
Montiel-Jarillo, G., Gea, T., Artola, A., Fuentes, J., Carrera, J., Suárez-Ojeda, M.E., 2021. Towards PHA production from wastes: the bioconversion potential of different activated sludge and food industry wastes into VFAs through acidogenic fermentation. Waste Biomass Valorization 12, 6861–6873. https://doi.org/10.1007/s12649-021-01480-4.
Newhart, K.B., Holloway, R.W., Hering, A.S., Cath, T.Y., 2019. Data-driven performance analyses of wastewater treatment plants: a review. Water Res. 157, 498–513. https://doi.org/10.1016/j.watres.2019.03.030.
Odriozola, M., Abraham, E., Lousada-Ferreira, M., Spanjers, H., Van Lier, J.B., 2019. Identification of the methanogenesis inhibition mechanism using comparative analysis of mathematical models. Front. Bioeng. Biotechnol. 7, 93. https://doi.org/10.3389/fbioe.2019.00093.
Piacenza, E., Presentato, A., Alduina, R., et al., 2022. Cross-linked natural IntegroPectin films from citrus biowaste with intrinsic antimicrobial activity. Cellulose 29, 5779–5802. https://doi.org/10.1007/s10570-022-04627-1.
Presti, D., Cosenza, A., Capri, F.C.C., Gallo, G., Alduina, R., Mannina, G., 2021. Influence of volatile solids and pH for the production of volatile fatty acids: batch fermentation tests using sewage sludge. Bioresour. Technol. 342, 125853 https://doi.org/10.1016/j.biortech.2021.125853.
Quixiang, X., Fu, Q., Liu, X., Wang, D., Wu, Y., Li, Y., et al., 2021. Mechanisms of potassium permanganate pretreatment improving anaerobic fermentation performance of waste activated sludge. J. Chem. Eng. 406, 126797 https://doi.org/10.1016/j.cej.2020.126797.
Rout, S.P., Salah, Z.B., Charles, C.J., Humphreys, P.N., 2017. Whole-Genome sequence of the anaerobic isosaccharinic acid degrading isolate, *Macellibacteroides fermentans* Strain HH-ZS. Genome Biol. Evol. 9 (8), 2055–2059. https://doi.org/10.1093/gbe/evx151.
Salman, N.S., Alshamsi, H.A., 2022. Synthesis of sulfonated polystyrene-based porous activated carbon for organic dyes removal from aqueous solutions. J. Polym. Environ. 30, 5100–5118. https://doi.org/10.1007/s10924-022-02584-1.
Samanides, C.G., Koutsokeras, L., Constantinides, G., Vyrides, I., 2020. Methanogenesis inhibition in anaerobic granular sludge for the generation of volatile fatty acids from CO2 and zero valent iron. Front. Energy Res. 8, 37. https://doi.org/10.3389/fenrg.2020.00037.
Sarkar, O., Butti, S.K., Mohan, S.V., 2018. Acidogenic biorefinery: food waste valorization to biogas and platform chemicals. Waste Biorefinery 203–218. https://doi.org/10.1016/B978-0-444-63992-9.00006-9.
Shahid, S., Razzaq, S., Farooq, R., Nazli, Z.I.H., 2021. Polyhydroxyalkanoates: next generation natural biomolecules and a solution for the world's future economy. Int. J. Biol. Macromol. 166, 297–321. https://doi.org/10.1016/j.ijbiomac.2020.10.187.
She, Y., Hong, J., Zhang, Q., Chen, B.Y., Wei, W., Xin, X., 2020. Revealing microbial mechanism associated with volatile fatty acids production in anaerobic acidogenesis of waste activated sludge enhanced by freezing/thawing pretreatment. Bioresour. Technol. 302, 122869 https://doi.org/10.1016/j.biortech.2020.122869.
Slezak, R., Grzelak, J., Krzystek, L., Ledakowicz, S., 2021. Influence of initial pH on the production of volatile fatty acids and hydrogen during dark fermentation of kitchen waste. Environ. Technol. 42 (27), 4269–4278. https://doi.org/10.1080/09593330.2020.1753818.
Spring, S., Bunk, B., Spröer, C., Schumann, P., Rohde, M., Tindall, B.J., Klenk, H.P., 2016. Characterization of the first cultured representative of Verrucomicrobia subdivision 5 indicates the proposal of a novel phylum. ISME J. 10 (12), 2801–2816. https://www.nature.com/articles/ismej201684.
Sucato, A., Vecchioni, L., Savoca, D., Presentato, A., Arculeo, M., Alduina, R., 2021. A comparative analysis of aquatic and polyethylene-associated antibiotic-resistant microbiota in the mediterranean sea. Biology 10 (3), 200. https://doi.org/10.3390/biology10030200. Mar 6.
Tarrahi, R., Fathi, Z., Seydibeyoğlu, M.Ö., Doustkhah, E., Khataee, A., 2020. Polyhydroxyalkanoates (PHA): from production to nanoarchitecture. Int. J. Biol. Macromol. 146, 596–619. https://doi.org/10.1016/j.ijbiomac.2019.12.181.
Vitale, M., Galluzzo, P., Buffa, P.G., Carlino, E., Spezia, O., Alduina, R., 2019. Comparison of antibiotic resistance profile and biofilm production of Staphylococcus aureus isolates derived from human specimens and animal-derived samples. Antibiotics 8 (3), 97. https://doi.org/10.3390/antibiotics8030097, 2019 Jul 19.
Wang, X., Hu, M., Xia, Y., Wen, X., Ding, K., 2012. Pyrosequencing analysis of bacterial diversity in 14 wastewater treatment systems in China. Appl. Environ. Microbiol. 78 (19), 7042–7047. https://doi.org/10.1128/AEM.01617-12.
Xia, S., Duan, L., Song, Y., Li, J., Piceno, Y.M., Andersen, G.L., et al., 2010. Bacterial community structure in geographically distributed biological wastewater treatment reactors. Hermanowicz, S.W. Environ. Sci. Technol. 44 (19), 7391–7396. https://doi.org/10.1021/es101554m.
Xu, Z.X., Song, H., Deng, X.Q., Zhang, Y.Y., Xue-Qin, M., Tong, S.Q., et al., 2019. Dewatering of sewage sludge via thermal hydrolysis with ammonia-treated Fenton iron sludge as skeleton material. J. Hazard Mater. 379 https://doi.org/10.1016/j.jhazmat.2019.120810.
Xu, Q., Fu, Q., Liu, X., Wang, D., Wu, Y., Li, Y., et al., 2021. Mechanisms of potassium permanganate pretreatment improving anaerobic fermentation performance of waste activated sludge. Chem. Eng. J. 406, 126797 https://doi.org/10.1016/j.cej.2020.126797.
Yang, X., Liu, X., Chen, S., Liu, G., Wu, S., Wan, C., 2016. Volatile fatty acids production from codigestion of food waste and sewage sludge based on β-cyclodextrins and alkaline treatments. Archaea 2016. https://doi.org/10.1155/2016/1698163.
Yu, G.H., He, P.J., Shao, L.M., He, P.P., 2008. Toward understanding the mechanism of improving the production of volatile fatty acids from activated sludge at pH 10.0. Water Res. 42 (18), 4637–4644. https://doi.org/10.1016/j.watres.2008.08.018.
Yu, K., Zhang, T., 2012. Metagenomic and metatranscriptomic analysis of microbial community structure and gene expression of activated sludge. PLoS One 7 (5), e38183. https://doi.org/10.1371/journal.pone.0038183.
Yu, H., Wang, Z., Wang, Q., Wu, Z., Ma, J., 2013. Disintegration and acidification of MBR sludge under alkaline conditions. Chem. Eng. J. 231, 206–213. https://doi.org/10.1016/j.cej.2013.07.012.
Yuan, Q., Sparling, R., Oleszkiewicz, J.A., 2011. VFA generation from waste activated sludge: effect of temperature and mixing. Chemosphere 82, 603–607. https://doi.org/10.1016/j.chemosphere.2010.10.084.
Zhang, L., Yan, M., Tsui, T.H., Lee, J.T.E., Loh, K.C., Dai, Y., Tong, Y.W., 2022. Chapter 16 - functional microbial characteristics in acidogenic fermenters of organic wastes for production of volatile fatty acids. Biomass, Biofuels, Biochem. 367–394. https://doi.org/10.1016/B978-0-323-90633-3.00017-1.
Zhang, T., Shao, M.F., Ye, L., 2012. 454 pyrosequencing reveals bacterial diversity of activated sludge from 14 sewage treatment plants. ISME J. 6 (6), 1137–1147. https://doi.org/10.1038/ismej.2011.188.