fermentation

Communication

Research on the Promotion of Sludge Anaerobic Fermentation with Sodium Citrate under Low Concentrations of Polyaluminum Chloride

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Abstract: Polyaluminum chloride (PAC) is used widely and increasingly in wastewater treatment plants, resulting in its inevitably high production in sludge. Previous studies have indicated that the production of short-chain fatty acids (SCFAs) is inhibited by the existence of PAC in sludge anaerobic fermentation, so it is necessary to study how to promote sludge anaerobic fermentation under low concentrations of PAC. In this study, sodium citrate (SC) was first used to improve the efficiency of anaerobic fermentation under low concentrations of PAC. The results showed that the production of SCFAs increased with SC, especially when the ratio of PAC to SC was 1:2, and the maximum production of SCFAs reached 2890 mg/L, which is 2.5 times more than when PAC only exists. The mechanism studies showed that SC could remove the Al^{3+} in the sludge floc, which led to deflocculation of the sludge floc, accelerated the destruction of extracellular polymers (EPS), and released soluble substances in the sludge. At the same time, the key enzymes that were bound and hidden originally in the sludge were also released, which promoted the further degradation of organic matter and shortened the anaerobic fermentation period. However, the higher concentration of SC was not conducive to sludge dehydration. Therefore, the optimal distribution ratio of PAC to SC should be 1:1. This study provides a new idea for the research and practical application of sludge anaerobic fermentation.

Keywords: sludge; anaerobic fermentation; polyaluminum chloride; sodium citrate; short-chain fatty acids

Citation: Zhu, P.; Wang, Y.; Bai, H.; Feng, J.; Zhang, R.; Bu, D.; Dan, Z.; Li, W.; Lu, X. Research on the Promotion of Sludge Anaerobic Fermentation with Sodium Citrate under Low Concentrations of Polyaluminum Chloride. *Fermentation* **2023**, *9*, 776. https://doi.org/10.3390/fermentation9080776

Academic Editor: Yang Wu

Received: 20 July 2023
Revised: 15 August 2023
Accepted: 16 August 2023
Published: 21 August 2023

1. Introduction

With the growth of the world population and the acceleration of urbanization, the biological treatment capacity of municipal wastewater has been strengthened, and the subsequently produced sludge has been increasing [1]. Sludge contains complex organic matter, making it a rich and cheap source of energy and resource recovery [2]. Resource recovery can be achieved through thermochemical processes or biological conversion techniques, including anaerobic fermentation, anaerobic digestion, and composting [3]. Anaerobic fermentation is an effective and environmentally friendly technology for sludge treatment and recycling, and is widely used [4]. The products of sludge anaerobic fermentation include short-chain fatty acids (SCFAs), hydrogen, and methane. Compared with methanogenesis, SCFAs have higher added value and practicability and have received more attention from scholars [5].

Polyaluminum chloride (PAC), as an inorganic flocculant, inevitably accumulates in sludge. According to the literature and previous studies by our research team, PAC could inhibit sludge anaerobic fermentation [6,7]. Mainly because the Al^{3+} can interact with the negative sites on extracellular polymers (EPS) (such as polysaccharides and proteins), which increase the size and strength of the flocs, the organic substances cannot be fully dissolved [8]. Therefore, it is very necessary to study the strategy of promoting sludge anaerobic fermentation under low concentrations of PAC.

Sodium citrate (SC) as a cationic binder can remove the bridged ions (such as Al^{3+}) in the floc, leading to deflocculation of the sludge floc [9,10]. So we tried adding SC to improve the efficiency of sludge anaerobic fermentation under low concentrations of PAC. SC is a hydroxycarboxylate compound. It is a white crystalline particle or crystalline powder that is easily soluble in water but insoluble in ethanol. SC, as the most important citrate, has the following excellent properties [11–13]: (1) metal ion complexing ability; (2) biodegradability; (3) excellent dissolution performance; (4) safety and non-toxicity; (5) pH adjustment and buffer performance; and (6) excellent retarding and stability performance. At present, there is no report mentioning that SC has been used to destroy a sludge flocculation structure in order to promote sludge anaerobic fermentation under low concentrations of PAC.

Therefore, the aim of this study was to promote sludge anaerobic fermentation under low concentrations of PAC. First, the effect of SCFAs from sludge fermentation was studied after adding SC. Then, its effects on material dissolution, sludge floc stability, key enzyme activities, and sludge dewatering performance were evaluated. This is the first study about adding SC to promote sludge anaerobic fermentation under low concentrations of PAC, with a view to providing a reference for the research and practical applications of sludge treatment technology.

2. Materials and Methods

2.1. Sludge and Reagents

The sludge used in this study was collected from the secondary sedimentation tank of the sewage treatment plant in Tianjin without being treated with PAC. The sludge was first precipitated under gravity in the laboratory for 24 h, and the supernatant was drained off. The concentrated sludge was stored in a refrigerator at 4 °C. The main characteristics of sludge are listed in Table 1.

Table 1. Main characteristics of sludge.

Parameters	Sludge
pH	6.9 ± 0.1
Total solids (TS)	25,100 ± 370 mg/L
Volatile solids (VS)	16,800 ± 210 mg/L
Total chemical oxygen demand (TCOD)	19,800 ± 450 mg/L
Soluble chemical oxygen demand (SCOD)	165 ± 10 mg/L
Total protein	9740 ±330 mg/L
Total polysaccharide	2200 ± 120 mg/L

The PAC in the test was analytical-grade and was produced at Tianjin Damao Chemical Reagent Factory. The SC in the test was analytical-grade and was produced at the Tianjin Recovery Institute of Fine Chemicals. All the reagents used were analytical-grade, unless otherwise specified.

2.2. Experimental Design

Six 500 mL anaerobic bottles were set up for the experiment. According to the ratio of materials, each anaerobic bottle was filled with 400 mL of sludge and 50 mL of inoculated sludge, and then the SC and PAC solutions prepared in advance (shown in Table 2) were added to reach the predetermined dose. Then, the bottles were marked as T1 (control group), T2 (PAC to SC was 1:0), T3 (PAC to SC was 2:1), T4 (PAC to SC was 1:1), T5 (PAC to SC was 1:2), and T6 (PAC to SC was 0:1), respectively. All bottles were flushed with N_2 for approximately 2 min to remove oxygen, and then the bottles were sealed with a rubber stopper so that the fermentation bottle was in an anaerobic environment. The sealed fermentation bottles were placed in an air bath shaker at a stirring speed of 120 rpm (revolutions per minute) and a temperature of 37 °C for 7 days without pH control. During this period, all other anaerobic operating conditions were the same as described above, unless otherwise stated.

Table 2. The prepared solutions of SC and PAC.

Anaerobic Bottles	PAC (mg/g TS)	SC (mg/g TS)
T1	0	0
T2	70	0
T3	70	35
T4	70	70
T5	70	140
T6	0	70

2.3. Sludge Extraction

The sludge sample was centrifuged at 8000 revolutions per minute (rpm) (TGL16M, Changsha Xiangyi Centrifuge Co., Ltd., Changsha, China) for 10 min and immediately filtered through a 0.45 µm cellulose membrane. The supernatant obtained was used to determine SCOD, SCFAs, soluble proteins, and polysaccharides [14]. EPS was extracted using thermal extraction [15]. A total of 50 mL of sludge was centrifuged at 4000 rpm for 5 min, and the supernatant was used for measurement of soluble extracellular polymer (S-EPS). The remaining sludge particles in the tube were diluted to the original volume of 50 mL with a 0.05% NaCl solution, heated to 70 °C, and then the sludge suspension was cut with a magnetic stirrer for 1 min and centrifuged at 4000 rpm for 10 min. Organic matter in the supernatant was used to determine the loose extracellular polymer (L-EPS). The remaining sludge particles in the tube were suspended again with a 0.05% NaCl solution until the volume was 50 mL and heated in a water bath to 60 °C for 30 min, and then the sludge mixture was centrifuged at 4000 rpm for 15 min. The supernatant was collected and treated as tight extracellular polymers (T-EPS).

2.4. Analysis Method

TS and VS were determined using the weighing method, and SCOD was measured using dichromate titration [16]. The soluble proteins were measured by the Lowry method (Lowry Protein Assay Kit, Solarbio PC0030, Beijing Soleibo Technology Co., Ltd., Beijing, China) according to the manufacturer's instructions with BSA as a standard substance, and the polysaccharides were determined using the anthrone–sulfuric method with glucose as a standard substance [17]. SCFAs were determined using a high-performance liquid chromatography (HPLC, Waters, Milford, MA, USA, e2695) unit with ultraviolet (UV) detection (λ = 210 nm) equipped with a C18 chromatographic column [18]. The injection volume was 10 µL, and the mobile phases used were phosphate-buffered saline (10 mmol/L, pH = 2.5) and methanol (78:22, v/v) circulated at 1.0 mL/min at 30 °C. The appearance of sludge was measured using scanning electron microscopy (SEM) [19]. The method for measuring the dewatering performance of sludge is the specific resistance (SFR) method of sludge [20]. Key enzyme activities were evaluated using the Lowry-Folin kit, alpha-amylase kit, and acetate kinase kit (Solarbio, Beijing Soleibo Technology Co., Ltd., Beijing, China) according to the manufacturer's instructions.

3. Results and Discussion

3.1. Effects of SC on SCFAs Produced in Sludge Anaerobic Fermentation

SCFAs production is an important parameter in the anaerobic fermentation process that can intuitively reflect the performance of the anaerobic fermentation, and it is an important basis for judging the quality of an anaerobic fermentation [21]. Figure 1 reflects SCFAs production with time under different concentrations of SC. It can be seen that the maximum SCFAs production when PAC alone existed (T2) was 1164.31 mg/L. When SC was added, SCFAs production increased. When the ratio of PAC to SC was 1:2 (T5), the production of SCFAs reached 2891.6 mg/L, which is 2 times greater than the control group (T1) and 2.5 times more than when PAC alone existed (T2) during the same period. As time increased, the SCFAs reached their maximum value, then gradually decreased. This is because the methanogens gradually adapted to the environment, and the SCFAs were utilized to produce methane. The above experimental results showed that the presence of SC increased SCFAs production and achieved the purpose of promoting sludge anaerobic fermentation under low concentrations of PAC.

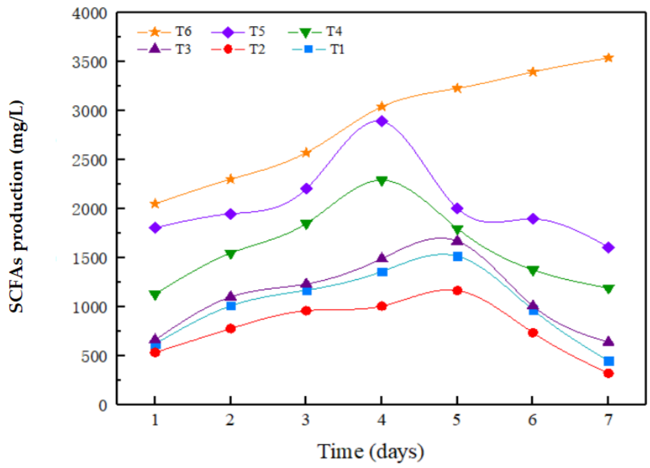

Figure 1. Variation of SCFAs accumulation with different dosages of SC.

3.2. Effects of SC on Sludge Dissolution in Sludge Anaerobic Fermentation

As is known, the hydrolysis efficiency during fermentation greatly affects the production of subsequent SCFAs [22]. Therefore, to explore the mechanism of SCFA production by adding SC, we must analyze hydrolysis efficiency. SCOD, soluble polysaccharides, and proteins can show the dissolution and output of organic substrates [23], and they have been confirmed to be closely related to the production of SCFAs [24].

The concentration of SCOD is depicted in Figure 2. It can be seen that the concentration of SCOD showed an increasing trend after adding SC, which indicated that the organic matter in the sludge was increasingly being converted into soluble matter. In particular, when the ratio of PAC to SC was 1:2 (T5), the maximum amount of SCOD was 9251 mg/L. It can be judged that there was more soluble organic matter in the fermentation broth at this time.

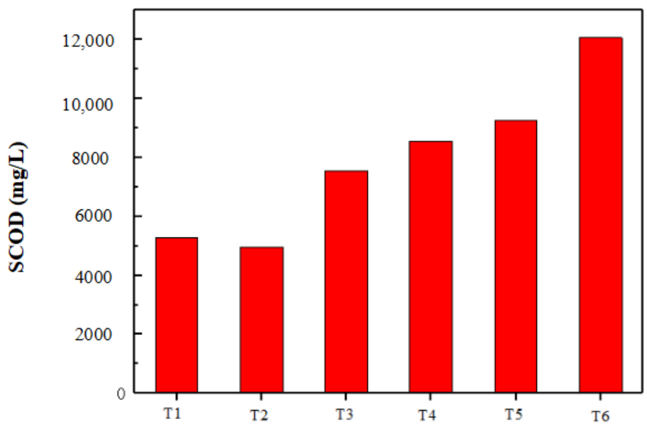

Figure 2. Variation of SCOD with different dosages of SC.

Proteins and polysaccharides can also show the dissolution effect of sludge. The production of proteins and polysaccharides with time is described in Figures 3 and 4. It can be seen that the dissolution of soluble proteins and soluble polysaccharides increased after adding SC. The maximum soluble protein concentrations in T1 and T2 were 1420 mg/L and 1055 mg/L, respectively. The maximum soluble polysaccharide concentrations in T1 and T2 were 50.7 mg/L and 36.2 mg/L, respectively. However, when the ratio of PAC to SC was 1:2 (T5), the maximum values of soluble proteins and soluble polysaccharides were 1650 mg/L and 69.2 mg/L, respectively. It was further proven that the addition of SC promoted sludge anaerobic fermentation under low concentrations of PAC.

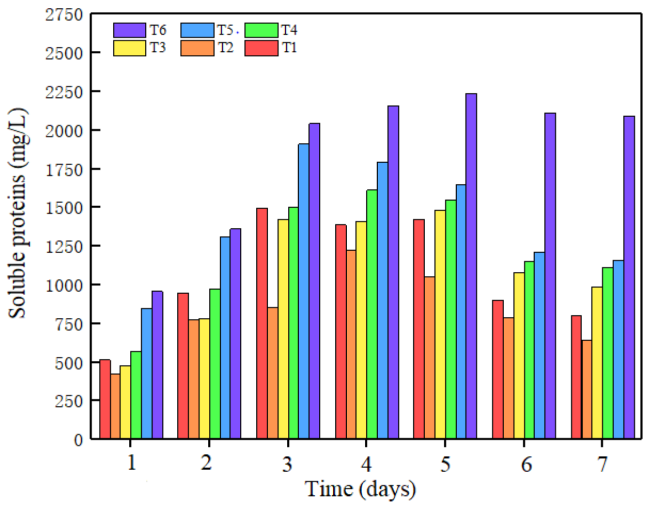

Figure 3. Variation of soluble proteins accumulation with different dosages of SC.

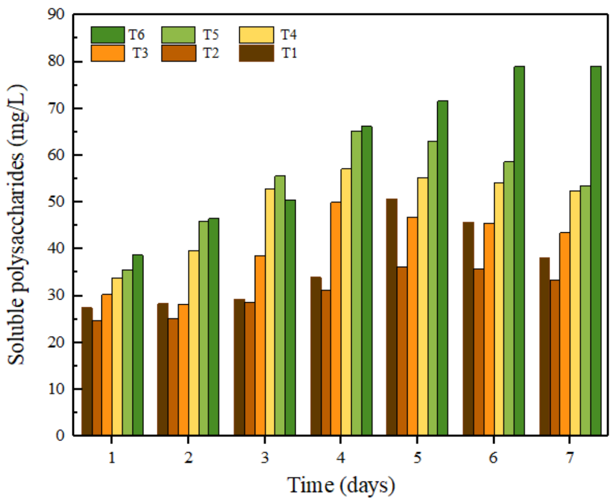

Figure 4. Variation of soluble polysaccharides accumulation with different dosages of SC.

3.3. Effect of SC on the Structure of Sludge Floc

3.3.1. Effect of SC on the Appearance of Sludge

SEM was used to observe the effect of SC on the appearance of sludge. The changes on the appearance of sludge floc under different concentrations of SC are shown in Figure 5. It can be seen that, compared to T1, the sludge structure of T2 became compact and dense, and the size of the sludge particles became larger, which made the organic matter difficult to dissolve. However, after adding SC (T4), the surface structure of the sludge changed greatly. The sludge floc became dispersed, and the size of the sludge particles became smaller, which increased the specific surface area of the sludge particles and made organic matter dissolve more easily. This is because SC can remove bridging ions (such as Al^{3+}) to deflocculate the sludge floc, thereby promoting sludge anaerobic fermentation under low concentrations of PAC.

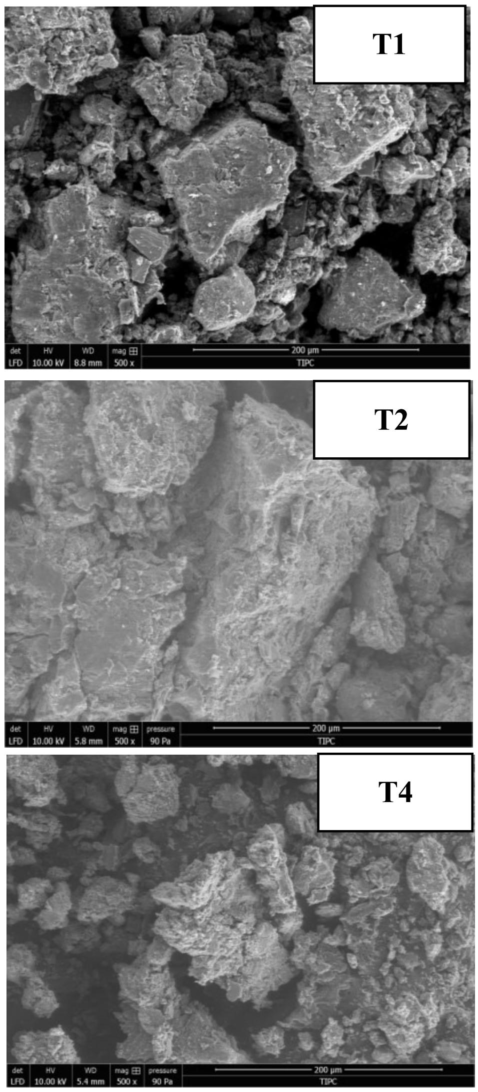

Figure 5. Variation of appearance of sludge floc with different dosages of SC.

3.3.2. Effect of SC on EPS Distribution

EPS surrounds bacterial cells and forms a stable floc structure [25]. The distribution of EPS in sludge under different concentrations of SC is shown in Figure 6. It can be seen that the T-EPS decreased and the L-EPS increased, which indicated that the stability of the sludge floc was greatly damaged, as the SC was added. The reason is that the addition of SC replaced the polyvalent cations in sludge, destroyed the structure of the EPS, and released the macromolecular organic substances. And the organic matter in the sludge diffused from the inner layer T-EPS to the outer layers L-EPS and S-EPS. L-EPS and S-EPS were more easily utilized for microorganisms, which further improved the efficiency of sludge anaerobic fermentation.

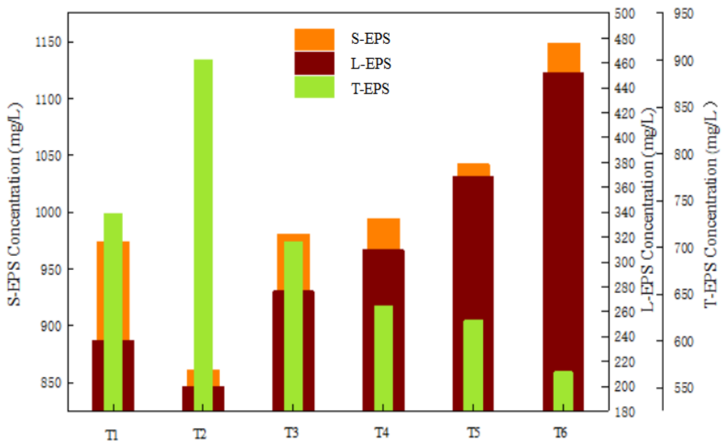

Figure 6. Distribution of EPS with different dosages of SC.

3.4. *Effect of SC on Key Enzyme Activities*

The extracellular enzymes are protease, a-amylase, and acetate kinase, which play a major role in anaerobic fermentation. Proteases can hydrolyze proteins into amino acids; α-amylase can catalyze the hydrolysis of carbohydrates into glucose and maltose; and acetate kinases are mainly responsible for converting monosaccharides and amino acids into acetate [26]. The relative activity of key enzymes after the addition of SC is shown in Figure 7. When the ratio of PAC to SC was 1:2, the relative activities of the three key enzymes were the highest. It can be seen that the key enzyme activities were significantly enhanced after the addition of SC. This is because the deflocculation of SC made the sludge loose, which released the enzymes that were originally bound and hidden in the sludge, thereby promoting the further degradation of organic matter.

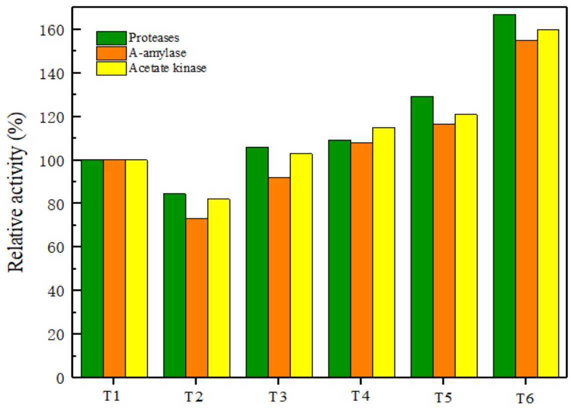

Figure 7. Effect on key enzyme activities with different dosages of SC.

3.5. *Effect of SC on Sludge Dewatering Performance*

The sludge dewatering performance also determines the sludge anaerobic fermentation performance [27]. Generally, the specific resistance (SRF) of sludge is used as a key indicator for studying dewatering performance [28]. The effect of different concentrations of SC on the dewatering performance of sludge is shown in Figure 8. It can be seen that the SRF of the sludge increased as the concentration of SC increased. When the ratio of PAC to SC was 1:2 (T5), the SRF of the sludge was 4.1×10^{12}, which shows that the addition of SC made the sludge dewatering more difficult. Because SC made the large organic particles in the sludge break down, the particles in the sludge were greatly reduced, which resulted in SRF increasing. Therefore, it can be considered that the optimal ratio of PAC to SC was 1:1.

At this time, the dehydration ability was not too bad, and it could achieve the purpose of promoting sludge anaerobic fermentation under low concentrations of PAC.

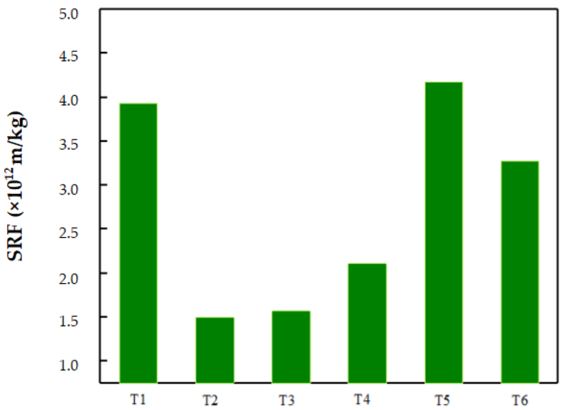

Figure 8. Effect on the dewatering performance of sludge with different dosages of SC.

4. Conclusions

In this study, SC was added to promote sludge anaerobic fermentation under low concentrations of PAC. First, the effect of SCFAs from sludge fermentation was studied after adding SC. Then, its effects on material dissolution, sludge floc stability, key enzyme activities, and sludge dewatering performance were evaluated. The results showed the SCFAs production was increased, more organic matter was dissolved, and the activity of key enzymes were improved by SC added, which achieved the purpose of promoting sludge anaerobic fermentation under low concentrations of PAC. However, the higher concentration of SC was not conducive to sludge dehydration. Therefore, the optimal distribution ratio of PAC to SC should be 1:1. This study provides a new idea for the research and practical application of sludge anaerobic fermentation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation9080776/s1, Table S1: Raw data-SCFAs.

Author Contributions: Methodology, P.Z. and X.L.; validation, P.Z.; analysis, P.Z., J.F. and H.B.; resources, X.L.; data curation, P.Z., R.Z. and D.B.; writing—original draft preparation, P.Z., Y.W. and X.L.; writing—review and editing, P.Z., Z.D. and W.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Tibet University 2018, 2019 Central Financial Support Special Funds for Local Colleges and Universities (grant number: [2018] No. 54; [2019] No. 19), Cultivation Foundation of Tibet University (grant number: ZDTSJH18-04), and The Seed Foundation of Tianjin University (grant number: [2018] XZC-0059).

Data Availability Statement: The data presented in this study are available in Supplementary Materials Table S1.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Zhang, Q.; Wu, L.; Huang, J.; Qu, Y.; Pan, Y.; Liu, L.; Zhu, H. Recovering short-chain fatty acids from waste sludge via biocarriers and microfiltration enhanced anaerobic fermentation. *Resour. Conserv. Recycl.* **2022**, *182*, 106342. [CrossRef]
2. Liu, X.; Du, M.; Yang, J.; Wu, Y.; Xu, Q.; Wang, D.; Yang, Q.; Yang, G.; Li, X. Sulfite serving as a pretreatment method for alkaline fermentation to enhance short-chain fatty acid production from waste activated sludge. *Chem. Eng. J.* **2020**, *385*, 123991. [CrossRef]
3. Tsui, T.H.; Van Loosdrecht, M.C.; Dai, Y.; Tong, Y.W. Machine learning and circular bioeconomy: Building new resource efficiency from diverse waste streams. *Bioresour. Technol.* **2023**, *369*, 128445. [CrossRef] [PubMed]
4. Tsui, T.H.; Zhang, L.; Zhang, J.; Dai, Y.; Tong, Y.W. Engineering interface between bioenergy recovery and biogas desulfurization: Sustainability interplays of biochar application. *Renew. Sustain. Energy Rev.* **2022**, *157*, 112053. [CrossRef]

5. Li, H.; Wang, Y. The effect of propionic acid accumulation on methane production in dry mesophilic anaerobic fermentation. *IOP Conf. Ser. Earth Environ. Sci.* **2021**, *675*, 012208. [CrossRef]
6. Chen, Y.; Wu, Y.; Wang, D.; Li, H.; Wang, Q.; Liu, Y.; Chen, Y. Understanding the mechanisms of how poly aluminium chloride inhibits short-chain fatty acids production from anaerobic fermentation of waste activated sludge. *Chem. Eng. J.* **2018**, *334*, 1351–1360. [CrossRef]
7. Zhu, P.; Li, X.; Feng, J.; Zhang, R.; Bai, H.; Bu, D.; Dan, Z.; Li, W.; Lu, X. Short-Chain Fatty Acids Production from Anaerobic Fermentation of Sewage Sludge: The Effect of Higher Levels Polyaluminium Chloride. *Int. J. Environ. Res. Public Health* **2022**, *19*, 2806. [CrossRef]
8. Park, C.; Muller, C.D.; Abu-Orf, M.M.; Novak, J.T. The effect of wastewater cations on activated sludge characteristics: Effects of aluminum and iron in floc. *Water Environ. Res.* **2006**, *78*, 31–40. [CrossRef]
9. Ebenezer, A.V.; Kaliappan, S.; Adish Kumar, S.; Yeom, I.T.; Banu, J.R. Influence of deflocculation on microwave disintegration and anaerobic biodegradability of waste activated sludge. *Bioresour. Technol.* **2015**, *185*, 194–201. [CrossRef]
10. Park, C.; Novak, J.T. Characterization of activated sludge exocellular polymersusing several cation associated extraction methods. *Water Res.* **2007**, *41*, 1679–1688. [CrossRef]
11. Wawrzynczyk, J.E.; Szewczyka, O.; Norrlöw, E.; Dey, S. Application of enzymes, sodium tripolyphosphate and cation exchange resin for the release of extracellular polymeric substances from sewage sludge characterisation of the extracted polysaccharides/glycoconjugates by a panel of lectins. *J. Biotechnol.* **2007**, *130*, 274–281. [CrossRef] [PubMed]
12. Xie, B.X.; Luo, K.; Yang, Q.; Mo, C.R.; Li, X.M.; Yu, J. Influence of complexing agents on enzymatic hydrolysis of sludge during anaerobic digestion. *Acta Sci. Circumstantiae* **2011**, *31*, 1699–1705.
13. Brown, M.J.; Lester, J.N. Metal removal in activated sludge: The role of bacterial extracellular polymers. *Water Res.* **1979**, *13*, 817–837. [CrossRef]
14. Feng, L.; Chen, Y.; Zheng, X. Enhancement of Waste Activated Sludge Protein Conversion and Volatile Fatty Acids Accumulation during Waste Activated Sludge Anaerobic Fermentation by Carbohydrate Substrate Addition: The Effect of pH. *Environ. Sci. Technol.* **2009**, *43*, 4373–4380. [CrossRef] [PubMed]
15. Yu, G.; He, P.; Shao, L.; He, P. Stratification structure of sludge flocs with implications to dewaterability. *Environ. Sci. Technol.* **2008**, *42*, 7944–7949. [CrossRef]
16. Xu, Q.; Liu, X.; Wang, D.; Wu, Y.; Wang, Q.; Liu, Y.; Li, X.; An, H.; Zhao, J.; Chen, F.; et al. Free ammonia-based pretreatment enhances phosphorus release and recovery from waste activated sludge. *Chemosphere* **2018**, *213*, 276–284. [CrossRef]
17. Duan, X.; Wang, X.; Xie, J.; Feng, L.Y.; Yan, Y.Y.; Zhou, Q. Effect of nonylphenol on volatile fatty acids accumulation during anaerobic fermentation of waste activated sludge. *Water Res.* **2016**, *105*, 209–217. [CrossRef]
18. Zhang, L.; Zhang, Z.; He, X.; Zheng, L.; Cheng, S.; Li, Z. Diminished inhibitory impact of ZnO nanoparticles on anaerobic fermentation by the presence of TiO_2 nanoparticles: Phenomenon and mechanism. *Sci. Total Environ.* **2019**, *647*, 313–322. [CrossRef]
19. Chen, Y.; Liu, K.; Su, Y.; Zheng, X.; Wang, Q. Continuous bioproduction of short-chain fatty acids from sludge enhanced by the combined use of surfactant and alkaline pH. *Bioresour. Technol.* **2013**, *140*, 97–102. [CrossRef]
20. Pang, L.; Ni, J.; Tang, X. Fast characterization of soluble organic intermediates and integrity of microbial cells in the process of alkaline anaerobic fermentation of waste activated sludge. *Biochem. Eng. J.* **2014**, *86*, 49–56. [CrossRef]
21. Huang, X.; Shen, C.; Liu, J.; Lu, L. Improved volatile fatty acid production during waste activated sludge anaerobic fermentation by different bio-surfactants. *Chem. Eng. J.* **2015**, *264*, 280–290. [CrossRef]
22. Duan, Y.; Zhou, A.; Wen, K.; Liu, Z.; Liu, W.; Wang, A. Upgrading VFAs bioproduction from waste activated sludge via co-fermentation with soy sauce residue. *Front. Environ. Sci. Eng.* **2018**, *1*, 53–62. [CrossRef]
23. Lu, H.W.; Dai, R.H.; Liu, Y.; Song, A.N.; Lv, L.H. The influence factors and research progress in Anaerobic hydrolysis/acidification of sludge. *Chem. Bull.* **2012**, *75*, 489–495.
24. Tyagi, V.K.; Lo, S.L.; Rajpal, A. Chemically coupled microwave and ultrasonic pre-hydrolysis of pulp and paper mill waste-activated sludge: Effect on sludge solubilisation and anaerobic digestion. *Environ. Sci. Pollut. Res.* **2014**, *21*, 6205–6217. [CrossRef]
25. Park, C.; Abu-Orf, M.M.; Novak, J.T. Analysis of floc stucture and predicting sludge digestibility using different cation-associated EPS extraction methods. *Proc. Water Environ. Fed.* **2004**, *8*, 21–37. [CrossRef]
26. Xin, X.; He, J.; Li, L.; Qiu, W. Enzymes catalyzing pre-hydrolysis facilitated the anaerobic fermentation of waste activated sludge with acidogenic and microbiological perspectives. *Bioresour. Technol.* **2018**, *250*, 69–78. [CrossRef]
27. Wolski, P.; Zawieja, I. Hybrid conditioning before anaerobic digestion for the improvement of sewage sludge dewatering. *Desalin. Water Treat.* **2014**, *52*, 3725–3731. [CrossRef]
28. Yuan, H.Y.; Yang, Y.P.; Wang, Y.N.; Wang, T.; Du, Y.M. Effects of Different Oxidation Agents on Sludge Dewatering and Hydrolysis Performance. *Environ. Sci. Technol.* **2017**, *40*, 33–37.