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Intermittent Microaeration Technology to Enhance the Carbon Source Release of Particulate Organic Matter in Domestic Sewage

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Abstract: Domestic sewage treatment plants often have insufficient carbon sources in the influent water. To solve this problem, the commonly used technical means include an additional carbon source, primary sludge fermentation, and excess sludge fermentation, but these methods are uneconomical, unsustainable, and not applicable to small-scale wastewater treatment plants. Intermittent microaeration technology has the advantages of low energy-consumption, ease of application, and low cost, and can effectively promote anaerobic digestion of municipal sludge; however little research has been reported on its use to enhance the carbon sources release of particulate organic matter (POM) from domestic wastewater. Therefore, the effect of intermittent microaeration on the carbon source release of POM was evaluated in this study, with POM as the control test. The results showed that the release concentration of soluble chemical oxygen demand (SCOD) was the highest on day 4 under microaerobic conditions, and the concentrations of SCOD, NH_4^+-N, and PO_4^{3-}-P in the liquid phase were 1153, 137.1, and 13 mg/L, respectively. Compared with the control group, the SCOD concentration increased by 34.2%, and the NH_4^+-N and PO_4^{3-}-P concentrations decreased by 18.65% and 17.09%, respectively. Intermittent microaeration can effectively promote the growth of *Paludibacter*, *Actinomyces*, and *Trichococcus* hydrolytic fermentation functional bacteria. Their relative abundances increased by 282.83%, 21.77%, and 23.47%, respectively, compared with the control group. It can simultaneously inhibit the growth of acetate-type methanogenic archaea, *Methanosaeta* and *Methanosarcina*, with a decrease in relative abundances of 16.81% and 6.63%, respectively. The aforementioned data show that intermittent microaeration can not only promote the hydrolysis of POM, but can also reduce the loss of acetic acid carbon source, which is a cost-effective technical way to enhance the release of a carbon source of particulate organic matter in domestic sewage.

Keywords: microaeration; domestic sewage; particulate organic matter; carbon source release; hydrolysis

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1. Introduction

Currently, the influent of domestic sewage treatment plants often suffers from insufficient carbon sources in China, resulting in poor denitrification and phosphorus removal [1]. To solve the problem of insufficient carbon sources in the sewage treatment plant, external carbon sources, primary sludge fermentation, and excess sludge fermentation are currently commonly used [2,3]. However, these methods are uneconomical, unsustainable, and unsuitable for small-scale wastewater treatment plants. Therefore, finding technology suitable for the development of carbon sources in small-scale sewage treatment plants is necessary.

Microaeration is a promising method for anaerobic digestion improvement and is constructed by dosing small quantities of air or O_2 into the anaerobic bioreactor [4]. Xu et al. [5] found that an appropriate microaeration rate can improve the hydrolysis efficiency of carbohydrates and proteins, and the optimal aeration rate can promote the solubilisation of solid organic waste without adverse carbon loss and 258 L air/kg aeration of TS/day, which is

beneficial for kitchen waste. Montalvo et al. [6] conducted an anaerobic digestion study using mixed sludge from urban sewage plants as substrates (including 60% and 40% of primary and secondary sludge, respectively), and compared it with traditional anaerobic systems. The cumulative concentration of protein, total sugar, and soluble chemical oxygen demand (SCOD) in the system is higher under aeration conditions. The optimal process conditions for enhancing the hydrolysis rate under the 3 °C condition are aeration intensity (0.3 vvm) and aeration gas time (48 h). Lim et al. [7] conducted the effect of microaeration on the cofermentation of grey water and food waste. Compared with pure anaerobic conditions, the microaeration conditions were more diverse in the bacterial community structure in the system. The proportion of bacterial clones belonging to Firmicutes is higher, and the system has higher hydrolysis efficiency. Yu et al. [8] found that the hydrolysis efficiency of black water was highest when the microaeration intensity was 5 mg O_2/L-reactor/cycle. Microaeration technology had been applied to various substrates (e.g., corn stover, sludge, vegetable wastes, black water, brown water, and food waste), and could effectively improve the hydrolysis efficiency of organic matter [9–12]. However, no relevant report exists on the use of microaeration to enhance the carbon source release of particulate organic matter (POM) in the influent of small-scale sewage treatment facilities.

Previous studies of microaeration have mostly been concerned with improving the efficiency of methane production and few have analysed the release characteristics of carbon sources when used to enhance the hydrolysis of particulate organic matter. This study aims to find out the mechanism of intermittent microaeration in the process of enhancing the POM carbon source release. By combining the reactor performance and microbial community analyses, new insights on intermittent microaeration-enhanced POM carbon source release and suggestions for future promotion and application are provided.

2. Materials and Methods

2.1. Characteristics of Collected POM

The particulate organic matter is collected from the inlet of a small-scale domestic sewage treatment station in Yixing City, Jiangsu Province, China, after filtering through a 60-mesh screen. The inoculated sludge with high degradation capacity of organic matters was sampled from the anaerobic fermentation tank in Yixing City, Jiangsu Province, China, which is treated at a constant temperature of 80 °C for 1 h before use. The basic physical and chemical indicators of particulate organic matter are shown in Table 1.

Table 1. Physicochemical indexes of particulate organic matter (POM).

Soluble COD mg/L	Soluble TN mg/L	NH_4^+-N mg/L	Soluble TP mg/L	PO_4^{3-}-P mg/L	SS g/L	pH
450 ± 5	128 ± 2	67.3 ± 0.5	9.32 ±0.02	8.65 ± 0.01	38.9 ± 0.6	7.18 ± 0.03

2.2. Bioreactor Setup and Operation

The experiment was repeated three times using a sequential batch experimental design, and the mean data were obtained to ensure that the experimental data were authentic and reliable. The substrate and seed mud were mixed at a volume ratio of 9:1, placed in a 250 mL fermentation flask, and intermittent microaeration (10 min/6 h) was conducted following the intensity of 0.3 vvm using constant temperature magnetic stirring. A stirrer (30 °C, 150 rpm) was used for continuous culture for 8 days. A control group was set up in the experiment, and 10 mL of sludge samples were taken every day. After centrifugation, they were filtered with a 0.45 μm filter membrane to determine the concentrations of soluble chemical oxygen demand (SCOD), NH_4^+-N, and PO_4^{3-}-P in the filtrate. Sludge samples were taken every 2 days, and they were filtered through a 0.22 μm filter membrane after centrifugation, and the fluorescent substances in the filtrate were determined. Then, 10 mg (dry solid) of sludge samples were taken on day 4 for microbial community structure analysis.

2.3. Chemical Analyses

Soluble COD(SCOD), NH_4^+-N, and PO_4^{3-}-P were regularly measured following the standard method [13]. pH was monitored using a portable multiparameter meter (HQ40d, Loveland, CO, USA). The total polysaccharides and total protein concentrations in the liquid phase were quantified by Dubois and Lowry methods, respectively [14].

2.4. EEM Fluorescence Spectroscopy Analysis

The extracted filtrate of the sample was measured by EEM fluorescence spectroscopy using a Hitachi F-7000 spectrofluorometer (Tokyo, Japan). Each plot was generated by scanning excitation wavelengths from 200 to 400 nm and emitting fluorescence between 280 and 500 nm with 10 nm steps. The slits for excitation and emission were set to 10 nm and the scan speed was 12,000 nm/min [15].

2.5. Microbial Analyses

The samples on day 4 in each group were collected to extract DNA by an E.Z.N.A.® soil DNA kit (Omega Bio-tek, Norcross, GA, USA). The hypervariable region V3-V4 of the bacterial 16S rRNA gene was then amplified with the bacterial (i.e., 341F and 805R)- and archaea (i.e., 524F10extF and Arch958RmodR)-fused primers by an ABI GeneAmp® 9700 PCR thermocycler (ABI, Vernon, CA, USA). The genomic DNA and PCR products were analysed through electrophoresis in 1% agarose gel. Finally, qualified samples were sequenced on an Illumina MiSeq platform at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China).

3. Results and Discussions

3.1. Variation Characteristics of SCOD, NH_4^+-N, and PO_4^{3-}-P Concentrations

The variation characteristics of SCOD concentration under microaerobic conditions are shown in Figure 1. With the increase in reaction time, the SCOD concentration first increased and then decreased, reaching a maximum value of 1153 mg/L on the fourth day, which was 2.56 times the initial SCOD concentration. Compared with the control group, the microaerobic condition promoted the release of more SCOD from particulate organic matter, and its concentration increased by 34.2%. This may be because microaerobic conditions stimulate the growth of anaerobic microorganisms, accelerate the metabolic rate, and release more enzymes to improve the hydrolysis efficiency of particulate organic matter.

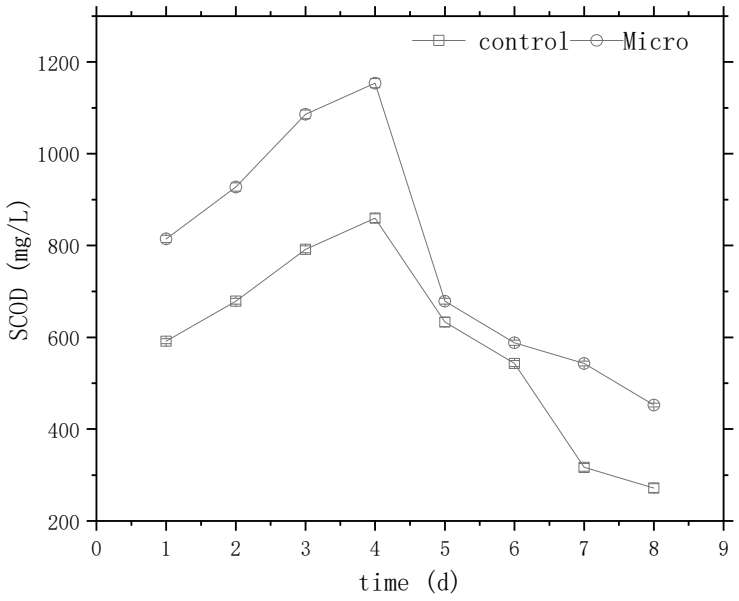


Figure 1. Variation of SCOD concentration under the microaerobic condition.

The variation characteristics of NH_4^+-N concentration under microaeration conditions are shown in Figure 2. The NH_4^+-N concentration, as observed in Figure 2, rapidly increased in the first 3 days under the microaeration condition, which may be because this condition can stimulate the microorganisms to release more enzymes, thereby depositing more POM to be hydrolysed. It fluctuates from 3 to 8 days, and the NH_4^+-N concentration decreases as a whole with the increase in reaction time, which may be caused by the following reasons: (1) the substrate hydrolysis rate of different components in POM is different, resulting in fluctuating changes of NH_4^+-N in the liquid phase; and (2) the pH value of the solution increases, which leads to an increase in the conversion of NH_4^+-N to NH_3 with the increase in reaction time, and, simultaneously, intermittent microaeration may take away a certain amount of NH_3 in the liquid phase.

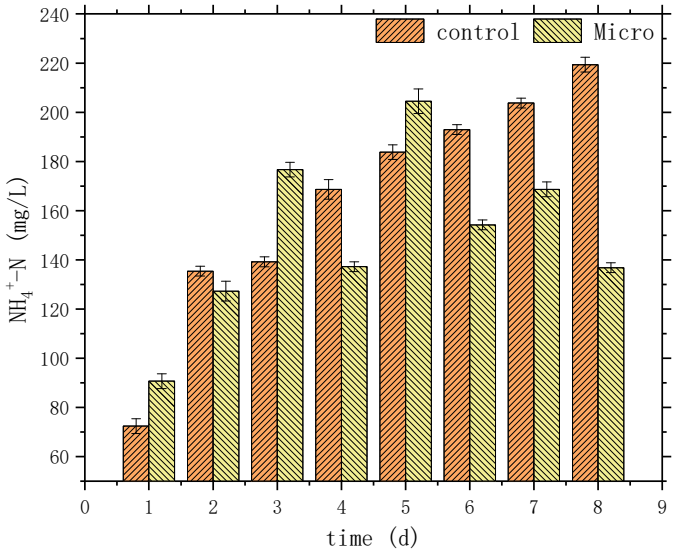


Figure 2. Variation of NH_4^+-N concentration under the microaerobic condition.

The variation characteristics of PO_4^{3-}-P concentration under microaerobic conditions are shown in Figure 3. The concentration of PO_4^{3-}-P, as seen in the figure, fluctuated under microaerobic conditions, which may be because the microaerobic conditions promoted the growth of phosphatidyl bacteria and absorbed more phosphorus at the beginning of the reaction, resulting in a decrease in PO_4^{3-}-P concentration in the first 3 days. However, with the increase in reaction time, the hydrolysing bacteria convert more organophosphorus to PO_4^{3-}-P, which leads to an increase in its concentration. The concentration of PO_4^{3-}-P decreased continuously from 5 to 8 days, which may be caused by the following three reasons: (1) phosphorus-accumulating bacteria continue to increase in value, converting more PO_4^{3-}-P into ATP, PHB, and so on; (2) PO_4^{3-}-P is adsorbed by extracellular polymers produced by microbial metabolism; (3) PO_4^{3-}-P is converted into other forms of phosphorus (e.g., HPO_4^{2-}, $H_2PO_4^-$, and HPO_3^{2-}) under biochemical action.

3.2. C/N and pH Variations

The change in C/N and pH characteristics in the solution under microaerobic conditions are shown in Figure 4. The pH increases with time under microaerobic conditions and is higher than that of the control group. This may be because the microaerobic conditions promote the release of more enzymes by microorganisms to hydrolyse proteins into NH_4^+-N, thereby causing the rise of pH. On day 4 under microaerobic conditions, the C/N value was increased by 48.59% compared with the control group, indicating that microaerobic conditions were more favorable for POM to release more effective carbon sources.

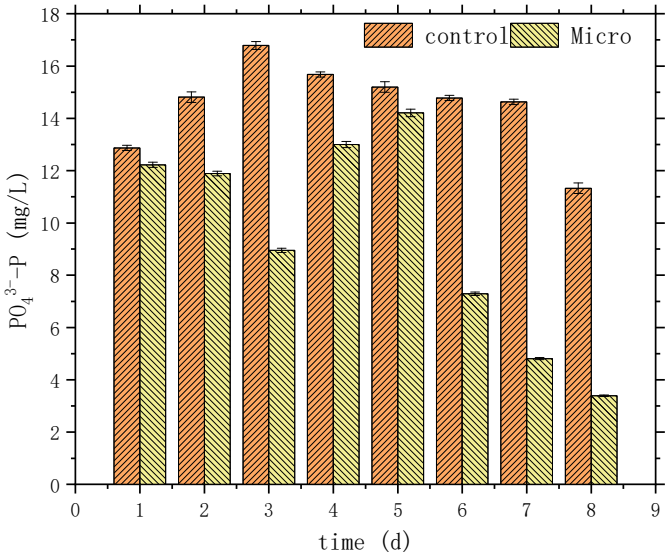


Figure 3. Variation of PO_4^{3-}-P concentration under the microaerobic condition.

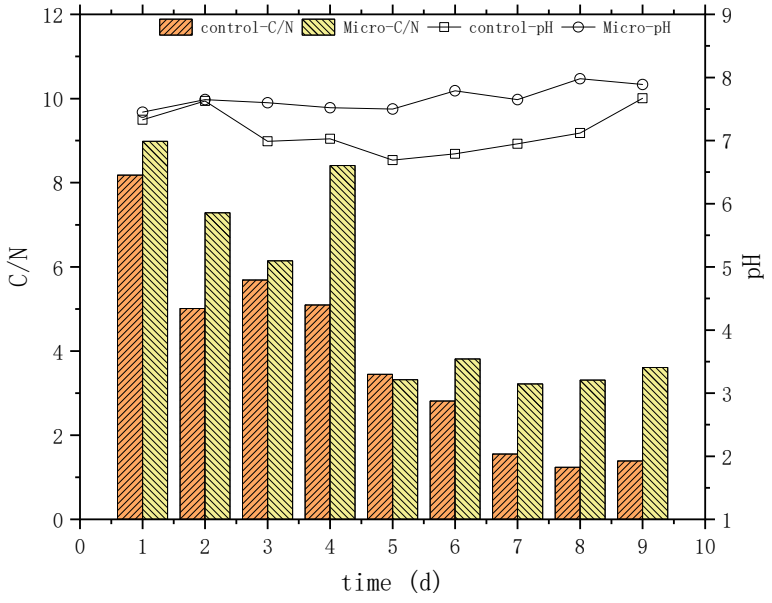


Figure 4. Variation characteristics of C/N and pH under microaerobic condition.

3.3. Variation of Protein, Polysaccharide, and Fluorescent Substance Components

The variation characteristics of protein and polysaccharide concentrations under microaerobic conditions are shown in Figure 5. The concentration of polysaccharides increases with the increase in time, indicating that microaerobic conditions can effectively promote the hydrolysis of POM to release more polysaccharides. With the increase in reaction time, the concentration of protein increases rapidly in the first 3 days and fluctuates in a small range later. It may be because the microaerobic conditions not only promote the hydrolysis of particulate organic matter to release more water-soluble proteins, but also promote the growth of protein-fermenting bacteria to further convert more soluble proteins into volatile fatty acids (VFAs), NH_4^+-N, and so on, resulting in fluctuations in the concentration of soluble proteins. Compared with the control group, microaerobic conditions were more conducive to promoting the action of POM in hydrolysing bacteria to release more polysaccharides and proteins, indicating that microaerobic conditions could more effectively improve the biological activity of protein and polysaccharide hydrolysis

and fermentation bacteria and release more hydrolase, resulting in the release of more proteins and polysaccharides from the particulate organic matter into the liquid phase.

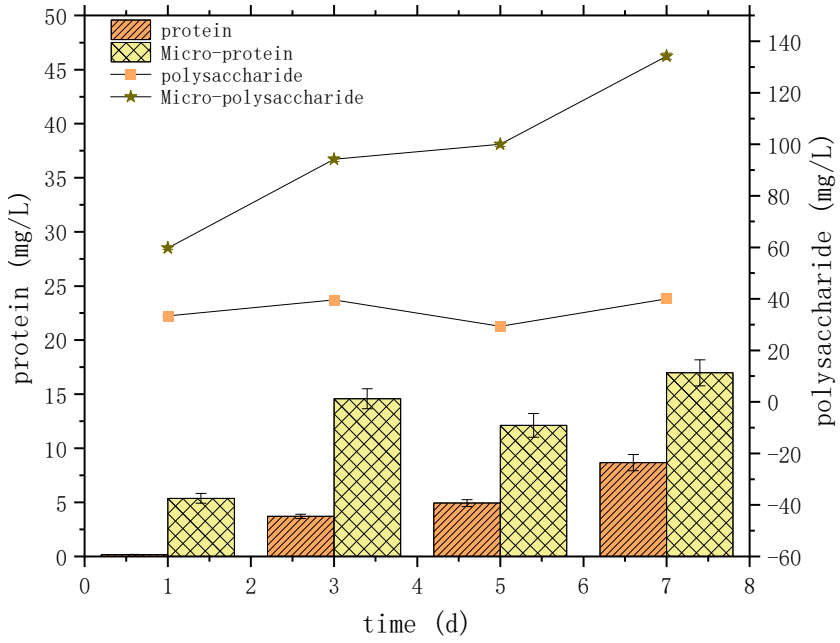


Figure 5. Effect of reaction time on protein and polysaccharide concentration.

Figure 6 shows the change characteristics of the components of fluorescent substances at different times. With the increase in time under microaerobic conditions, the peaks in region IV moved to the positive direction of the abscissa, and their fluorescence intensity was higher than that of the control phase, indicating that the metabolic activity of microorganisms was higher under microaerobic conditions. The fluorescence intensity of amino acid protein and tyrosine protein showed an increasing trend and decreasing trend, and a relatively low fluorescence intensity appeared on day 3, indicating that the protein may be converted into NH_4^+-N under the action of microorganisms, which is consistent with the phenomenon in Figure 2.

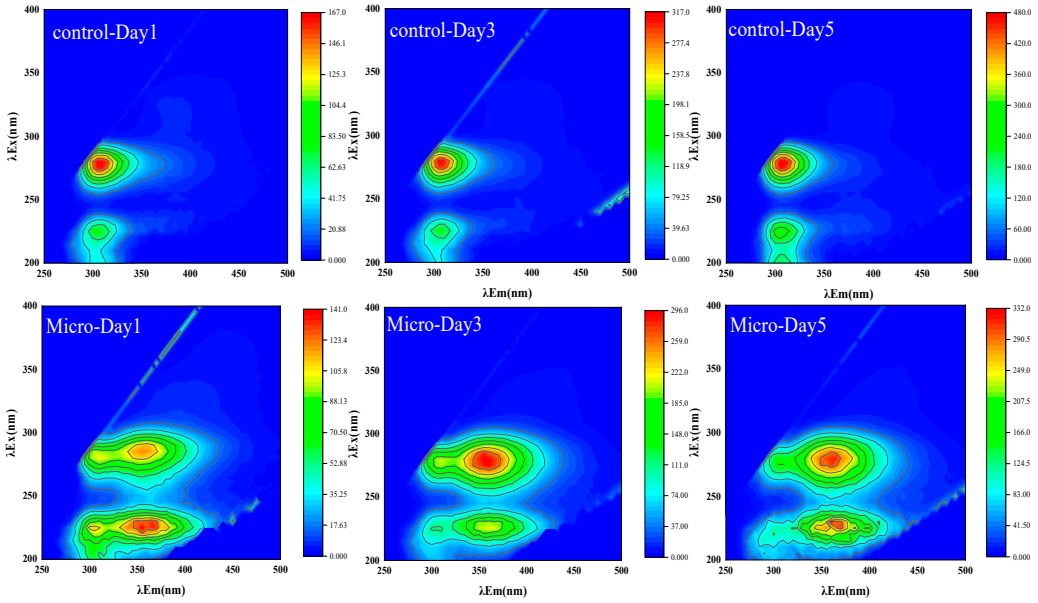


Figure 6. 3D-EEM at different time under microaerobic condition.

The variation characteristics of the corresponding percentages of fluorescent substances are shown in Figure 7. The corresponding percentages of tryptophan proteins gradually decreased with the increase in time, while the corresponding percentages of tyrosine proteins gradually increased, indicating that tryptophan proteins are more easily degraded by microorganisms under microaerobic conditions.

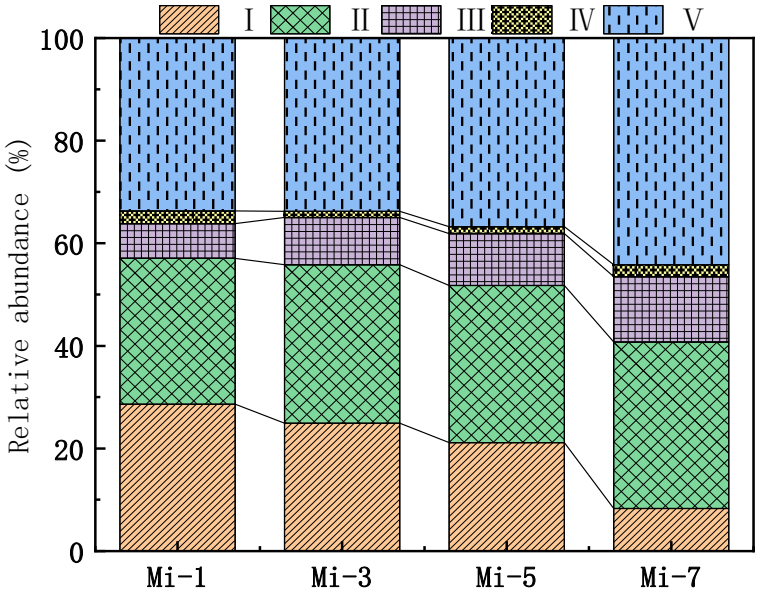


Figure 7. Percentage of sludge liquid phase fluorescence response at different reaction times.

3.4. Microbial Community Characteristics

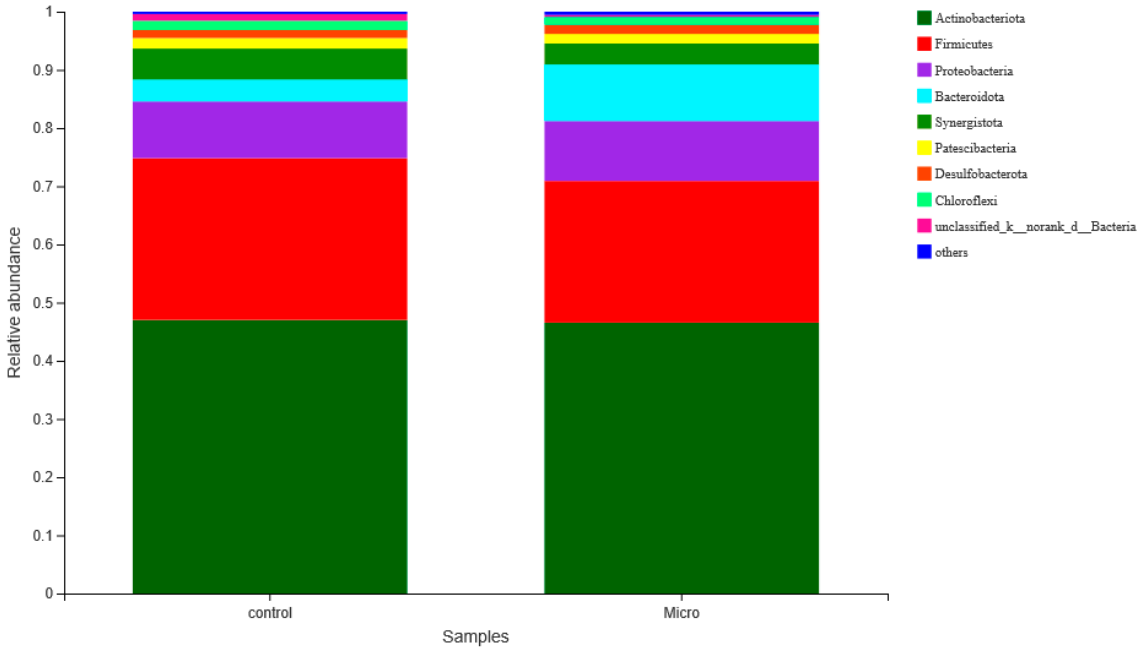
3.4.1. Bacterial Community Structure

For the characterisation of microbial community structure during intermittent microaeration by a high-throughput sequencing method, the bacterial abundance at the phylum level is shown in Figure 8a, and the main dominant bacterial phyla are Actinobacteriota, Firmicutes, Proteobacteria, Bacteroidota, Synergistota, Patescibacteria, Desulfobacterota, and Chloroflexi. Compared with the control group, the relative abundances of Proteobacteria, Bacteroidota, and Desulfobacterota increased by 4.66%, 158.11%, and 18.22%, respectively, under microaerobic conditions. Among them, Bacteroidota can convert proteins and carbohydrates to propionate and acetate in anaerobic sludge fermentation [16].

Bacterial abundance at the class level is shown in Figure 8b. The figure shows that the main dominant bacterial classes are Actinobacteria, Clostridia, Bacteroidia, Gammaproteobacteria, Alphaproteobacteria, Synergistia, Bacilli, Saccharimonadia, and Desulfobulbia. Compared with the control group, the relative abundances of Bacterodia, Gammaproteobacteria, Bacilli, and Desulfobulbia increased by 157.37%, 31.66%, 42.01%, and 21.18%, respectively, under microaerobic conditions.

The bacterial abundance at the genus level is shown in Figure 8c. The main genera under microaerobic conditions include norank_f__Actinomycetaceae, norank_f__Eubacteriaceae, *Paludibacter*, *Romboutsia*, *Brooklawnia*, *Propioniciclava*, *Clostridium sensu stricto* 1, *Christensenellaceae* R-7 group, CI75cm.2.12, *Corynebacterium*, *Actinomyces*, *Trichococcus*, *Tessaracoccus*, *Enterococcus*, *Gallicola*, *Desulfobulbus*, *Micropruina*, and *Lactivibrio*. Studies show that *Paludibacter* could consume soluble starch, glucose, and xylose to produce VFAs [17]. *Brooklawnia* belongs to fermentative bacteria [18]. Propioniciclava accounted for the most in the A/O process and was beneficial for carbohydrate consumption [19]. Actinomyces can effectively degrade cellulose [20]. *Trichococcus* could metabolically break down carbohydrates into lactate, formate, acetate, ethanol, and CO_2 [21]. *Tessaracoccus* were identified as abundant putative phosphate-accumulating organism (PAO) [22]. Gallicola is a non-saccharolytic anaerobic bacterium appearing on VFAs, H_2, and CO_2 formation [23]. *Desulfobulbus* can utilise pyruvates, propionates, and ethanol as a carbon source and oxidise organic matter to acetate incompletely [24].

Compared with the control group, the relative abundances of the main hydrolytic fermentative bacteria genera *Paludibacter*, *Brooklawnia*, *Actinomyces*, *Trichococcus*, *Gallicola*, and *Desulfobulbus* increased by 282.82%, 18.01%, 21.77%, 23.47%, 16.76%, and 19.37%, respectively, under microaerobic conditions. The relative abundance of *Tessaracoccus* increased by 19.41%, which was beneficial to absorb more PO_4^{3-}-P.



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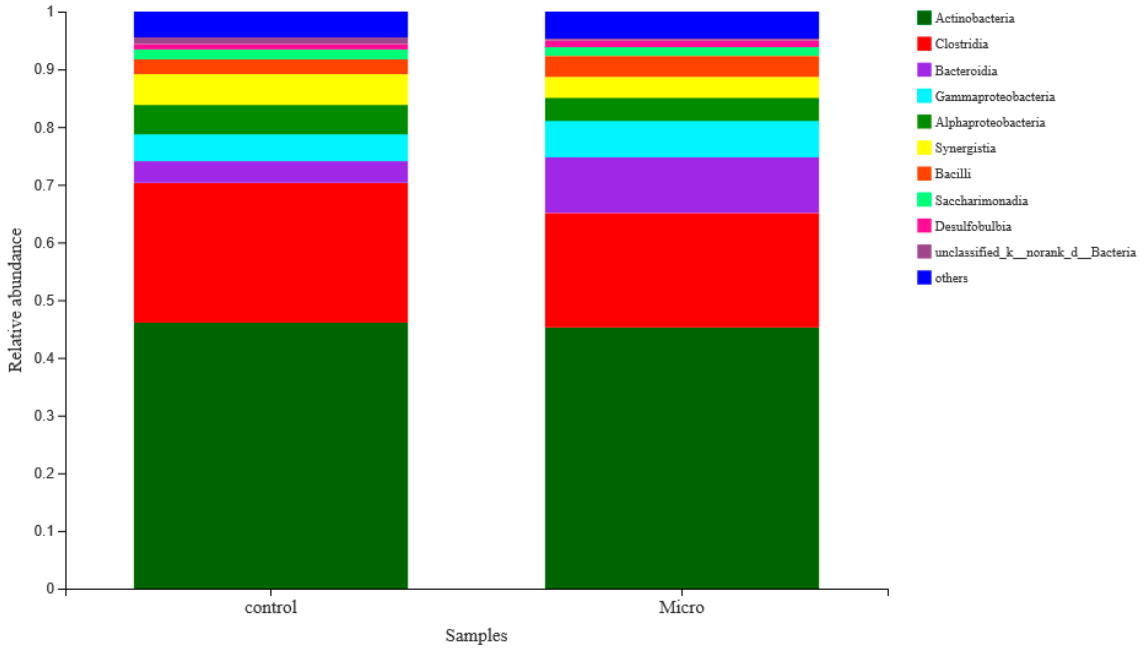

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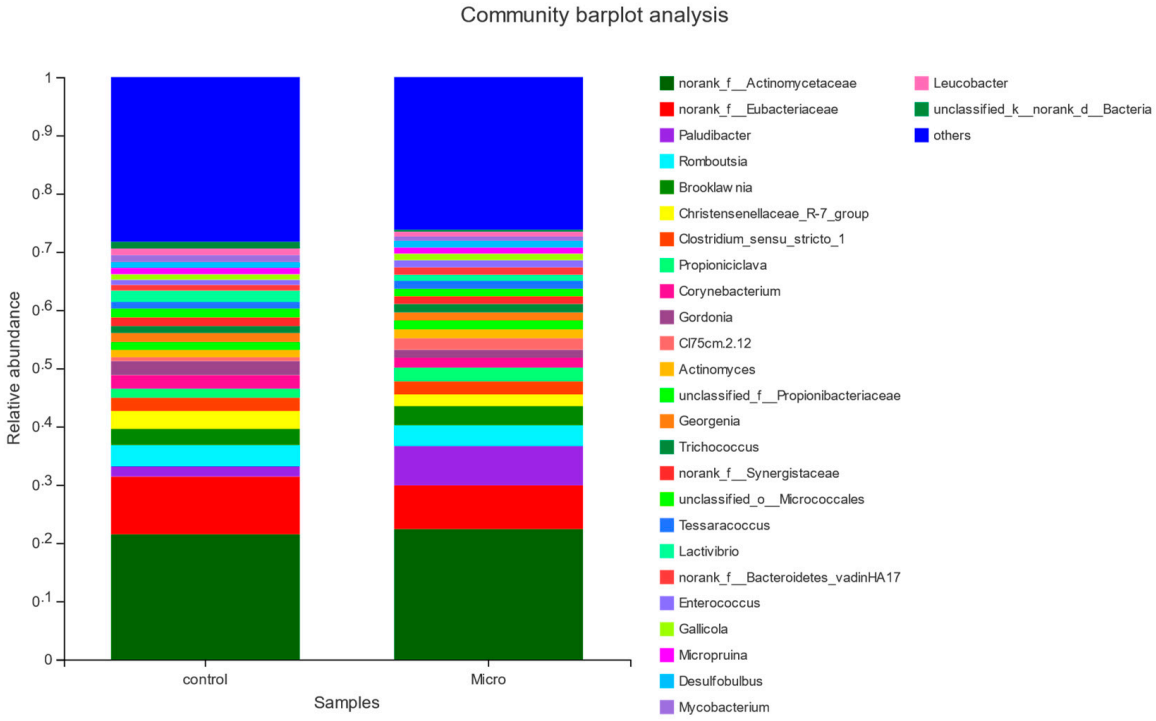


(**c**)

Figure 8. (**a**) Bacterial community phylum-level relative abundance; (**b**) bacterial community class-level relative abundance; (**c**) bacterial community genus-level relative abundance.

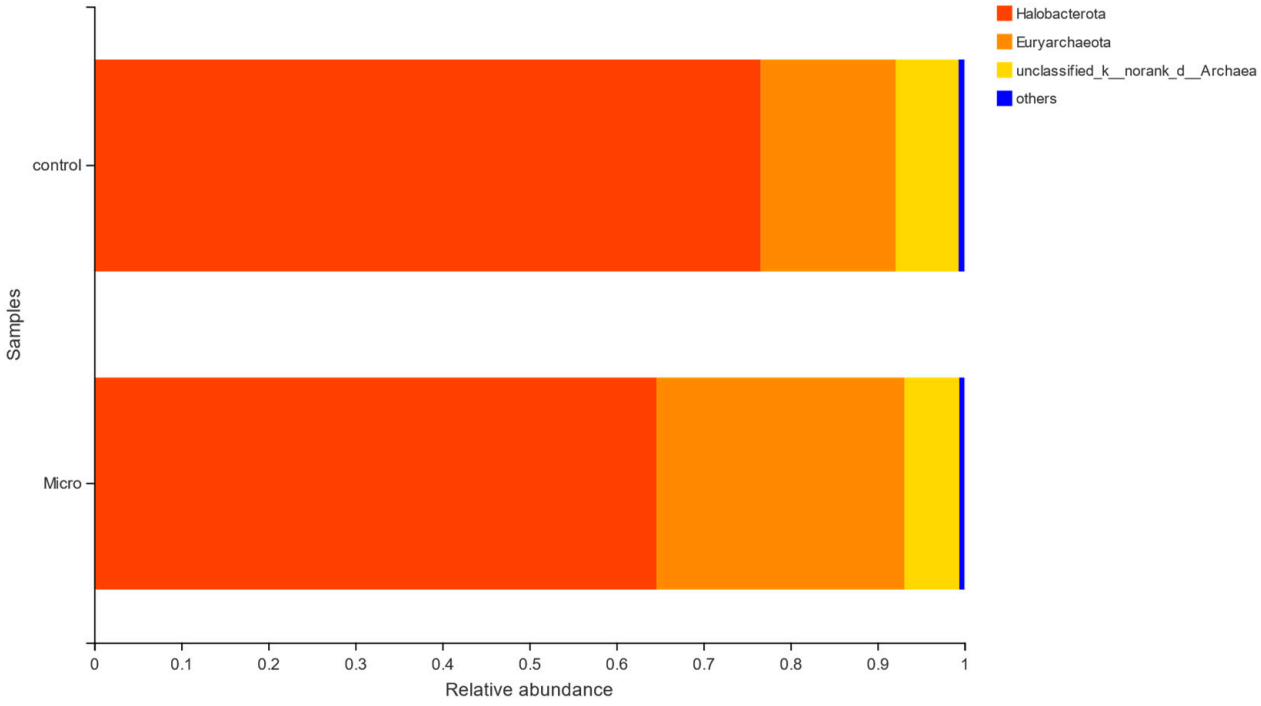
3.4.2. Archaeal Community Structure

The main phyla of Archaea under microaeration conditions included Halobacterota and Euryarchaeota (Figure 9a), accounting for 93.3% of the total abundance. The main classes of Archaea include Methanosarcinia and Methanobacteria (Figure 9b), accounting for 92.8% of the total abundance. Figure 9c shows that the dominant genera of Archaea include Methanosaeta, Methanobacterium, Methanosarcina, unclassified_k__norank_d__Archae and Methanobrevibacter, with relative abundances of 55.37%, 25.12%, 8.97%, 6.07%, and 1.79%, respectively. Methanosaeta is composed of acetoclastic species, capable of forming long and thin filaments [25]. Methanosarcina can use acetic and hydrogen to produce methane [26], and Methanobacterium is typical of hydrogen-utilising methanogens [27]. The relative abundance of Methanosaeta, Methanosarcina, and unclassified_k__norank_d__Archae decreased by 16.81%, 6.63%, and 12.98%, respectively, and the relative abundance of Methanobacterium increased by 90.45% under microaerobic conditions. Therefore, intermittent microaeration can inhibit the growth of Methanosaeta and Methanosarcina and reduce the depletion of acetic acid in the carbon source released by POM.

3.5. Discussion

The test results show that intermittent microaeration can promote the release of more carbon sources from POM under the action of hydrolytic fermentation bacteria, inhibit acetate-type methanogens, and reduce the loss of VFAs to achieve a better carbon source recovery effect. Combining reactor performance results and microbial community characterisation data as well as bioenergetics evaluations, a putative pathway of carbon source release from POM via intermittent microaeration was proposed, as illustrated in Figure 10. However, further metagenomic analyses are required to confirm the main functional microbes and their interactions in this complex microbial community to confirm the proposed pathway. The microaeration technology avoids the addition of carbon sources and alkaline agent, and only need some simple modifications before practical application.

Thus, it is a very promising technology to alleviate the problem of insufficient carbon sources in small-scale domestic sewage treatment facilities.



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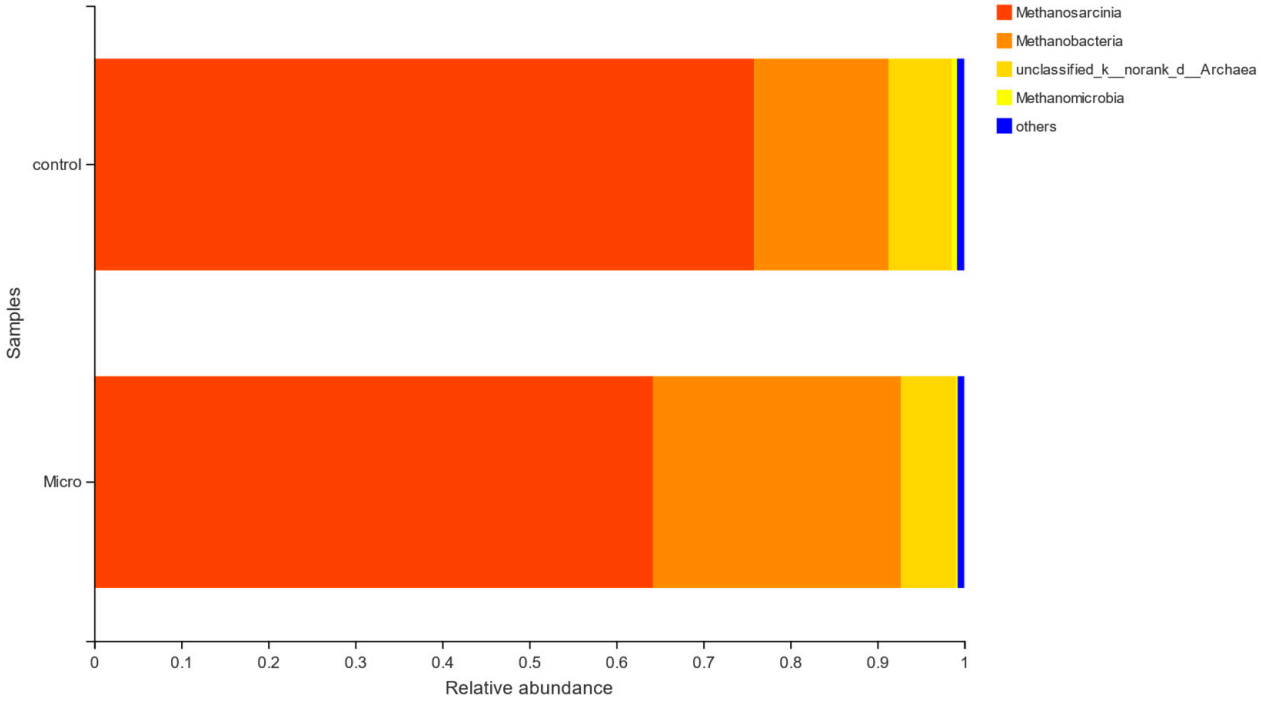

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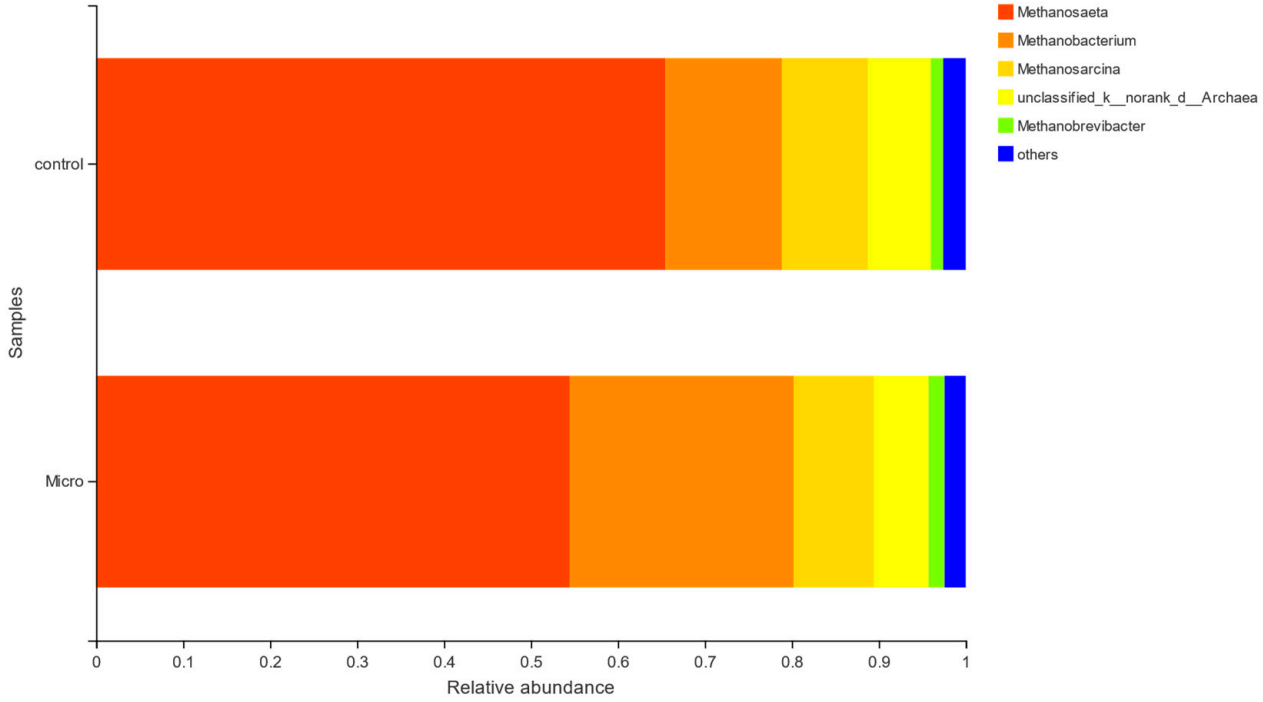

(**c**)

Figure 9. (**a**) Phylum-level relative abundance of archaeal communities; (**b**) class-level relative abundance of archaeal communities; (**c**) genus-level relative abundance of archaeal communities.

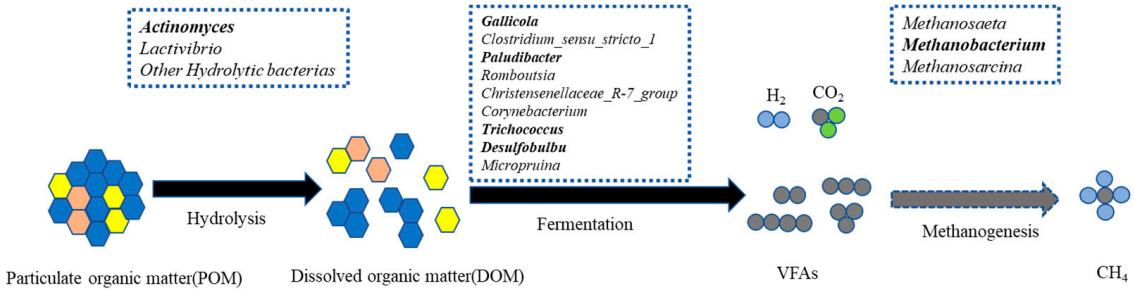

Figure 10. Proposed pathway of carbon source release from POM via intermittent microaeration.

4. Conclusions

Intermittent microaeration can effectively promote the hydrolysis efficiency of particulate organic matter (POM), with increased protein, polysaccharide, SCOD, and greater removal of NH_4^+-N and PO_4^{3-}-P compared with the control group. The SCOD concentration released by the hydrolysis of POM was the largest on day 4, and its C/N ratio was 48.59% higher than the control group. In addition, through high-throughput sequencing, microaeration was found to effectively promote the growth of *Paludibacter*, *Actinomyces*, *Trichococcus*, *Gallicola*, and *Desulfobulbus*, which may promote the hydrolytic fermentation of particulate organic matter, and the relative abundance increased by 282.82%, 21.77%, 23.47%, 16.76%, and 19.37% compared with the control group, respectively. Simultaneously, microaeration can inhibit the growth of acetate-consuming methanogens (e.g., Methanosaeta and Methanosarcina), thereby reducing the loss of acetic acid. Therefore, microaeration obtains more carbon sources by improving the hydrolysis efficiency of particulate organic matter and inhibiting the consumption of acetic acid by archaea.

Author Contributions: Conceptualisation, Y.L.; methodology, C.L. and L.Z.; investigation, C.L. and L.Z.; writing—original draft preparation, L.Z.; writing—review and editing, Y.L. and C.L.; supervision, G.L.; project administration, C.L. and L.Z.; funding acquisition, L.Z. and C.L. All authors have read and agreed to the published version of the manuscript.

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