

Article

Feasibility Study of Using Excess Sludge Fermentation Broth as a Co-Metabolic Carbon Source for 2,4,6-Trichlorophenol Degradation

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Abstract: Excess sludge fermentation is a commonly employed method for carbon sources in wastewater treatment plants, but its use as a carbon source for chlorophenol removal has been relatively underexplored. In this study, a laboratory-scale sludge fermentation SBR (FSBR) was integrated with a 2,4,6-trichlorophenol (2,4,6-TCP) degradation SBR (DSBR), resulting in a stable removal of 2,4,6-TCP without the need for external carbon sources. In this coupled system, the concentrations of volatile fatty acids in FSBR remained constant, with acetic acid, propionic acid, butyric acid, and valeric acid concentrations reaching 322.04 mg COD/L, 225.98 mg COD/L, 274.76 mg COD/L, and 149.58 mg COD/L, respectively, and the acid production efficiency increased to 88.40%. Throughout the 110-day operational period, the activated sludge concentration in the DSBR was consistently maintained at 3021 ± 110 mg/L, and the sludge SVI remained stable at 70 mL/g. The maximum amount of 2,4,6-TCP removed reached 240.13 mg/L within a 12 h operating cycle. The use of excess sludge fermentation can completely replace commercial carbon sources for 2,4,6-TCP removal, leading to cost savings in chlorophenol treatment and broadening the applicability of this technology.

Keywords: 2,4,6-trichlorophenol; co-metabolism; excess sludge fermentation; carbon source; coupling process



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1. Introduction

2,4,6-Trichlorophenol (2,4,6-TCP)-containing wastewater is a common form of organic effluent originating from the paper, printing, and dyeing industries. This wastewater is highly hazardous and accumulative, necessitating stringent treatment before discharge into the environment. Failure to do so could lead to substantial harm to aquatic ecosystems [1,2]. Currently, advanced oxidation techniques, including the Fenton method, ozone-catalyzed oxidation, and electrochemical catalytic oxidation, are employed to address persistent organic contaminants in wastewater [3–5]. Nevertheless, the widespread application of advanced oxidation methods is constrained by their rigorous treatment conditions, limited capacity, and substantial energy consumption. 2,4,6-TCP wastewater is categorized as a persistent contaminant due to its low five-day biochemical oxygen demand (BOD₅) to chemical oxygen demand (COD) ratio, which is less than 0.3 [6]. Additionally, the pronounced toxicity of 2,4,6-TCP exacerbates the challenges associated with its biological treatment. Consequently, the concept of biological co-metabolism was introduced by Stirling, D.I. et al. [7]: the addition of readily degradable organic compounds can stimulate the microbial degradation of recalcitrant organic compounds that cannot directly serve as carbon and energy sources. From an enzymatic metabolism perspective, the presence of easily degradable substrates induces the production of a broad-spectrum non-specific enzyme by microorganisms, which can facilitate the metabolism of recalcitrant organic compounds [8,9]. As a result, readily degradable carbon sources have been extensively investigated, as they play a pivotal role in influencing the effectiveness of biological co-metabolic treatment.

Previous research has revealed that readily degradable carbon sources offer more significant benefits compared to refractory carbon sources in enhancing the removal of 2,4,6-TCP [10]. Commonly employed biodegradable carbon sources, such as sodium acetate, glucose, and sucrose, have proven to be effective in removing various chlorophenol compounds, including trichlorophenol, dichlorophenol, and monochlorophenol [11–14]. Nevertheless, these readily degradable organic substances are typically obtained through artificial extraction or synthesis, making them expensive for large-scale applications in treating chlorophenol wastewater. Furthermore, various degradation devices used in chlorophenol treatment, such as Sequencing Batch Reactors (SBR) and Upflow Anaerobic Sludge Blanket reactors, still rely heavily on external carbon sources to enhance the biochemical characteristics of phenol-containing wastewater. Hence, the challenge remains to reduce treatment costs and enhance treatment sustainability when utilizing readily degradable organic compounds as the carbon source in biological co-metabolism processes.

The anaerobic digestion of excess sludge is a widely adopted approach for reducing sludge volume and harnessing resources in wastewater treatment plants (WWTP) [15,16]. By controlling the digestion process, it is possible to steer it toward the acidogenic phase, resulting in the production of short-chain fatty acids (SCFAs), including acetic acid, propionic acid, butyric acid, and valeric acid [17,18]. SCFAs, as high-quality carbon sources, have found application in municipal WWTP to address the shortage of carbon sources in managing domestic sewage [19]. Currently, research on sludge fermentation primarily focuses on the impact of the fermentation liquid as a carbon source on denitrification and phosphorus removal efficiency, as well as the study of fermentation conditions' influence [20,21]. Hu, C. et al. [22] investigated the impact of hydrolyzed acidification liquids from various residual sludges as carbon sources on denitrification. They found that the removal efficiency of nitrate nitrogen reached over 99% at the optimal hydraulic retention time (HRT). Specifically, the utilization rate of VFAs reached 90.6% at an HRT of 4 h, indicating that VFAs outperformed other hydrolyzed liquid components. Chen, Y. et al. [23] investigated the influence of surfactants (sodium dodecylbenzene sulfonate, SDBS) and alkaline conditions on VFA (volatile fatty acid) production. The results revealed that, at a hydraulic retention time of 10 h and under pH 10, VFAs production reached its peak at 2056 mg COD/L. However, there is limited research that explores the use of digestion products as a carbon source for co-metabolizing toxic pollutants.

The removal efficiency of the target pollutant is influenced by the type and addition level of the carbon source [24]. Anaerobic digestion products serve as mixed carbon sources, primarily composed of SCFAs, ethanol, butanol, and a variety of other organic materials [25]. SCFAs, being readily biodegradable, play a beneficial role in enhancing the removal rate of chlorophenols. To improve the removal rate of chlorophenols, it is essential to control fermentation conditions, thereby increasing the SCFAs level in the FB. Furthermore, the concentration of the carbon source also impacts co-metabolism efficiency and the growth of functional microbes [24,26]. The feasibility of substituting the single organic source with a complex FB as the co-metabolism carbon source warrants further investigation in the field of wastewater treatment.

In light of the background and the challenges identified, this study's primary objectives are as follows: to investigate the viability of utilizing excess sludge FB as the co-metabolism carbon source for the removal of 2,4,6-TCP, and to establish a coupled system integrating excess sludge FB and co-metabolism to achieve the prolonged, stable, and efficient removal of 2,4,6-TCP. This experiment marks the pioneering introduction of sludge FB into the co-metabolic system, holding significant implications for the sustained and cost-effective operation of biological co-metabolism processes. Moreover, the stability of the integrated system offers a novel avenue for the resource utilization of excess sludge in wastewater treatment. These findings have the potential to drive advancements in both wastewater treatment technology and the responsible management of excess sludge resources.

2. Materials and Methods

2.1. System Setup and the Reactor Operation

The operation system employed in this study consisted of two sequencing batch reactors (SBR), each with a working volume of 3 L, as illustrated in Figure 1. The Fermentation SBR (FSBR) served as the dedicated fermentation reactor, while the Degradation SBR (DSBR) was utilized as the co-metabolism reactor for the degradation of 2,4,6-TCP. Both the FSBR and DSBR were meticulously controlled through automated timers to ensure the coupling of these reactors and enable their prolonged and stable operation.

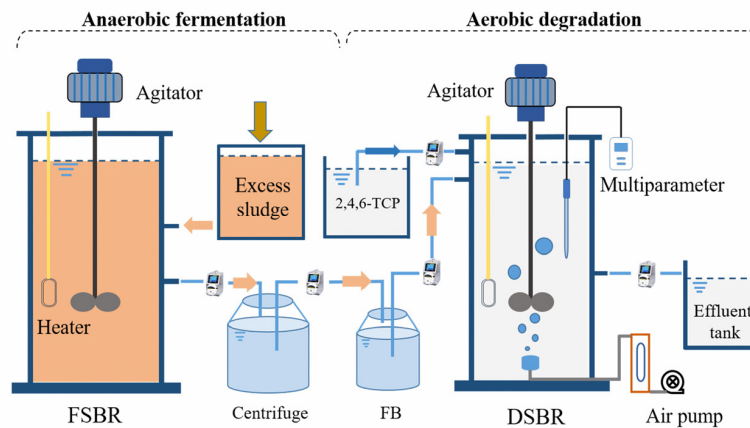


Figure 1. The schematic diagram of the experimental setup for this study is presented.

2.1.1. Long-Term Operation of FSBR

During the 180-day operation of the FSBR, as shown in Figure 2b, the FSBR operated for 24 h per cycle for the initial 110 days of operation, as depicted in Figure 2a. Each typical operation cycle was divided into three stages, comprising a 15 min discharge of FB, 15 min feeding of excess sludge, and a 23.5 h period of anaerobic fermentation. Throughout the entire cycle, a mechanical stirrer maintained a constant mixing rate of 200 revolutions per minute (rpm). The FSBR was held at a consistent temperature of 30 °C through the use of a heater. In each cycle, 300 mL of mixed FB was extracted from the reactor using a peristaltic pump to maintain a solid residence time (SRT) of 10 days. Subsequently, an equivalent volume of excess sludge was introduced into the reactor. Material exchange was carried out through the peristaltic pump to preserve an anaerobic environment within the FSBR. The Mixed Liquor Suspended Solids (MLSS) was consistently maintained at a level of 10,000 mg/L throughout the entire operation.

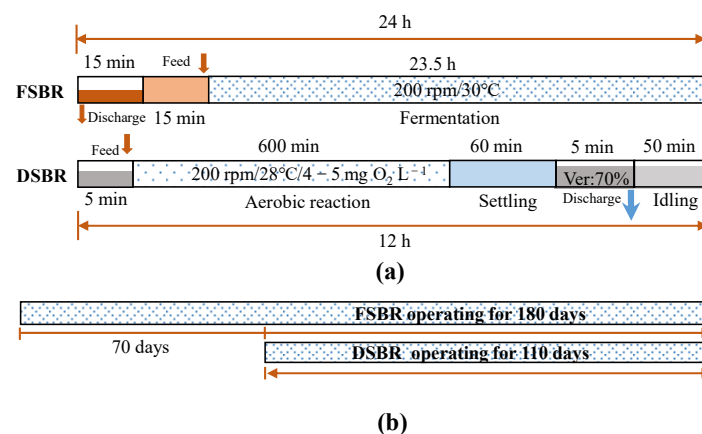


Figure 2. The typical operation of the system: (a) the operating mode of FSBR and DSBR, (b) the operation timeline of the FSBR-DSBR coupling process.

2.1.2. Long-Term Operation of DSBR

The DSBR was operated for a total of 110 days. DSBR was initiated after the FSBR had been in operation for 70 days and had reached a point where it could consistently produce a constant concentration of FB. The FB from FSBR was utilized as the co-catabolic carbon source and was introduced into DSBR along with the 2,4,6-TCP. DSBR operated with a cycle duration of 12 h, and a typical cycle, as illustrated in Figure 2a, included five stages (Figure 2a): a 5 min period for feeding synthetic wastewater; a 600 min period of aerobic reaction, maintaining an oxygen concentration of 4–5 mgO₂/L; a 60 min settling phase; a 5 min discharge phase with a volumetric exchange ratio of 70%; and a 50 min idling phase. The hydraulic retention time was consistently held at 24 h, and the SRT was maintained at 120 days by discharging 25 mL of mixed sludge per day. An agitator was used to thoroughly mix the activated sludge at a stirring speed of 200 rpm. Throughout the entire operation, the temperature was maintained at 28 °C using a constant heater. In our previous research, it has already been demonstrated that under conditions without the addition of a carbon source, activated sludge cannot attain a high concentration 2,4,6-TCP degradation capacity [24].

2.2. Seeding Sludge and Influent Wastewater Composition

The inoculum used in this study was derived from aerobic activated sludge collected from the aeration tank of the Xiaohongmen municipal WWTP located in Beijing, China. It is important to note that this activated sludge primarily serves in the treatment of municipal domestic wastewater and does not possess the capability to degrade 2,4,6-TCP. Before inoculation, the freshly acquired sludge underwent a washing process with tap water to eliminate impurities and contaminants. Subsequently, the initial concentration of this inoculum, amounting to 3000 mg/L, was introduced into both the FSBR and the DSBR for the study.

The feed for the FSBR comprised pre-treated excess activated sludge, sourced from the WWTP at Xiaohongmen, Beijing, along with the sludge discharged from the DSBR. The excess sludge had undergone hydrothermal treatment at 100 °C. This treatment is aimed at disintegrating the sludge cells, releasing extracellular polymeric substances, and making intracellular organic matter more accessible. This results in the generation of abundant substrates to support the anaerobic fermentation process. The treated sludge utilized in the FSBR had a concentration of 10,000 mg/L. The specific compositions and details of this treated sludge are outlined in Table 1.

Table 1. Compositions of added excess sludge and effluent fermentation broth (mg/L).

Components	TCOD	SCOD	SS	Proteins	Polysaccharide	VFAs (mg COD/L)	NH ₄ ⁺	PO ₄ ³⁻	NO ₃ ⁻
Influent	11,770	4984	10,830	1130	155	-	75.7	23.6	-
Effluent	1223.5	1120.2	-	-	-	974.5	634.4	193.4	5.2

Note: “-” represents values falling below the detection limit.

The influent of the DSBR included centrifugal-fermentation broth, 2,4,6-TCP within the concentration range of 0–200 mg/L, and trace elements. The composition of the trace elements is shown in Table S1 of the Supplementary Material. The centrifugal-fermentation broth was used as the co-metabolism carbon source in this experiment (Table 1).

2.3. The Batch Experiment

2.3.1. Mineralization and Chlorine Ion Removal Experiments of 2,4,6-TCP

The influent concentration of 2,4,6-TCP was set at 100 mg/L, and to eliminate any interference from chlorine ions in the influent, ultrapure water was used for preparation. The influent concentration of the FB was the same as that of DSBR, which is 150 mg COD/L. Dissolved oxygen was maintained at 4–5 mg/L. The experiment lasted for 10 h, with sampling intervals of 60 min. Mixed sludge samples were centrifuged, passed through a

0.22 µm filter membrane, and the clear liquid was analyzed for chloride ions, 2,4,6-TCP, and Total Organic Carbon (TOC) values.

2.3.2. Ammonia Nitrogen Concentration Influence Experiment

The experiment commenced with the addition of 100 mg/L of 2,4,6-TCP, and the influent concentration of the FB was maintained at 150 mg COD/L. In order to assess the influence of ammonia nitrogen (NH_4^+ -N) concentrations, levels of 40 mg/L, 200 mg/L, 400 mg/L, 800 mg/L, and 1200 mg/L were tested. The experiment lasted for 10 h, maintaining the same sampling intervals and sample processing procedures as described in the previous test. The focus was on measuring the concentration of 2,4,6-TCP.

2.4. Analytical Methods

In this study, a comprehensive set of parameters was analyzed, including Total Chemical Oxygen Demand (TCOD), Soluble Chemical Oxygen Demand (SCOD), Ammonium Nitrogen (NH_4^+ -N), Nitrate Nitrogen (NO_3^- -N), Phosphate (PO_4^{3-}), Suspended Solids (SV), Sludge Volume Index (SVI), and Mixed Liquor Suspended Solids (MLSS). These measurements were conducted following the standard procedures outlined in the standard method [27].

The quantification of 2,4,6-TCP and SCFAs was carried out using high-performance liquid chromatography (HPLC, Waters 1525, Milford, MA, USA) and gas chromatography (Agilent 7890A, Santa Clara, CA, USA), respectively. The specific testing conditions were based on the methodology described by Wang, J. et al. [24] and Wang, B. et al. [18].

2.5. Calculation

The calculation of $\text{COD}_{\text{SCFAs}}$ (mg/L) and the efficiency of SCFAs production (%) was performed using Equations (1) and (2):

$$\text{COD}_{\text{SCFAs}} \text{ (mg/L)} = C_{\text{SCFAs}} \times n_x \quad (1)$$

$$\text{SCFAs production efficiency (\%)} = \text{COD}_{\text{SCFAs}} / \text{COD}_{\text{Eff}} \times 100\% \quad (2)$$

C_{SCFAs} (mg/L) represents the concentration of SCFAs as determined through liquid chromatography testing. The conversion factor, denoted as “ n_x ”, was defined based on the methodology described by Wang, B. et al. [18]; COD_{Eff} stands for the COD of the effluent FB.

3. Results and Discussion

3.1. The Performance of FSBR in the Long-Term Operation

The stable acid production process in FSBR plays a crucial role in facilitating the efficient degradation of 2,4,6-TCP in DSBR. Overall, anaerobic sludge fermentation comprises four distinct stages: particulate organic matter dissolution and release, hydrolysis of soluble organic matter, acidogenesis, and methane production. Of these stages, converting particulate organic matter into soluble organic matter is a crucial step in anaerobic acidogenesis [28]. Consequently, several researchers have aimed to improve the solubilization of particulate organic matter by implementing various techniques including ultrasound, microwave treatment, heat treatment, the supplementation of surfactants, and alkaline treatment, as evidenced by previous studies [29–33].

The primary objective was to investigate the feasibility of utilizing sludge fermentation byproducts for the co-metabolic degradation of 2,4,6-TCP. To achieve this aim, sludge fermentation was conducted under optimal conditions with the intention of obtaining stable fermentation byproducts. Additionally, a thermal treatment method involving heating at 100 °C for 3 h was employed as a pretreatment for the residual sludge. A comparison of the sludge composition before and after pretreatment revealed a significant increase in the concentration of soluble organic substances in the mixed liquor, as shown in Table 1.

As shown in Figure 3, during the initial 10 days of the first run, VFAs production was low. In the subsequent period, from day 10 to day 30, the production of acetic acid and propionic acid initially increased, reaching 256.93 mg COD/L and 160.38 mg COD/L, respectively. However, at this point, the production of butyric acid and valeric acid remained low, resulting in an overall acid production efficiency of 55.95%. During days 30 to 70, VFAs gradually increased and stabilized, with concentrations of acetic acid, propionic acid, butyric acid, and valeric acid reaching 322.04 mg COD/L, 225.98 mg COD/L, 274.76 mg COD/L, and 149.58 mg COD/L, respectively. The acid production efficiency increased to 88.40%, which is significantly higher than the average acid production efficiency reported in the literature ($55 \pm 10.3\%$) [34]. After day 70, VFAs production remained stable.

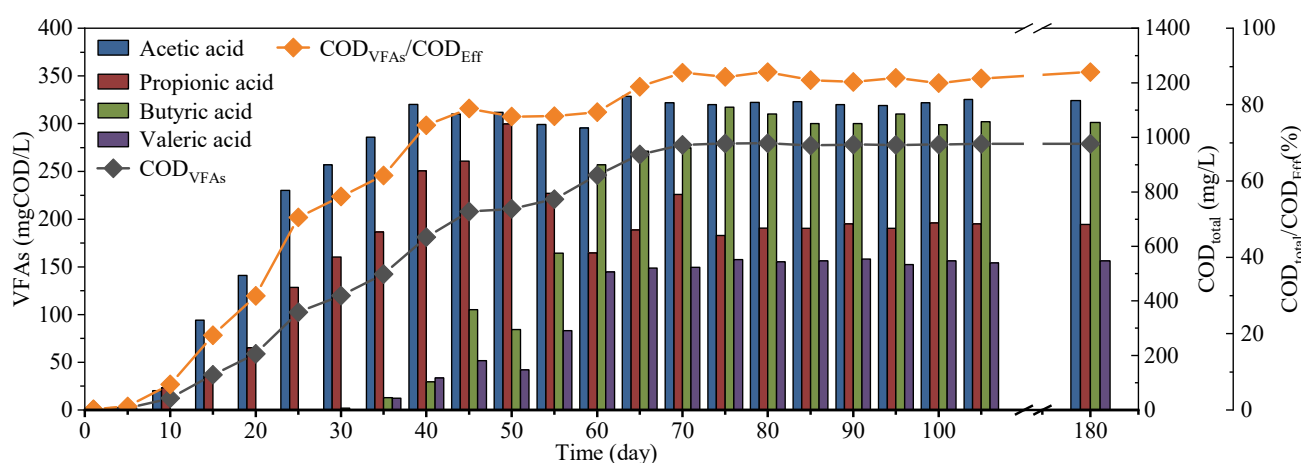


Figure 3. Long-term variation in VFA production in FSBR.

3.2. DSBR Performance in Long-Term Acclimation

3.2.1. 2,4,6-TCP Degradation Performance

The influent sludge fermentation liquid in the DSBR was consistently maintained at 150 mg COD/L, utilizing the activated sludge control method with a constant sludge concentration as described in Wang, J. et al. [24]. As shown in Figure 4a, a gradient increment approach was used for adding 2,4,6-TCP with influent ranging from 10 to 250 mg/L. The effluent 2,4,6-TCP was carefully monitored during each additional concentration. If a notable quantity of 2,4,6-TCP was detected in the effluent, the influent concentration was reduced or maintained to avoid impeding the activated sludge metabolism with excess inhibition from the 2,4,6-TCP. Over a lengthy operational period and with adjustments to the reactor, beneficial bacteria in the activated sludge were steadily enriched, enabling the decomposition of elevated 2,4,6-TCP concentrations. After a 110-day acclimation period, the influent 2,4,6-TCP was maintained at 250 mg/L, while the effluent 2,4,6-TCP remained consistently at 9.87 mg/L. This indicates that the functional bacteria achieved the maximum degradation concentration of 2,4,6-TCP, which is 240.13 mg/L.

Wang, J. et al. [35] used domestic wastewater as a substitute for commercial carbon sources and achieved a maximum degradation concentration of 2,4,6-TCP at 208 mg/L. When using glucose, sucrose, or starch as single carbon sources, they obtained 2,4,6-TCP degradation concentrations of 65 mg/L [10]. In this study, the obtained 2,4,6-TCP degradation concentration of 240.13 mg/L was significantly higher than the scenarios mentioned in the literature. The primary components of sludge fermentation liquid were volatile fatty acids (VFAs), and the FSBR achieved an acid production efficiency of 88.40%. VFAs were more easily biodegraded and utilized by microorganisms compared to domestic wastewater or single carbon sources. This may have explained why using sludge fermentation liquid as a carbon source yielded superior results compared to other carbon sources.

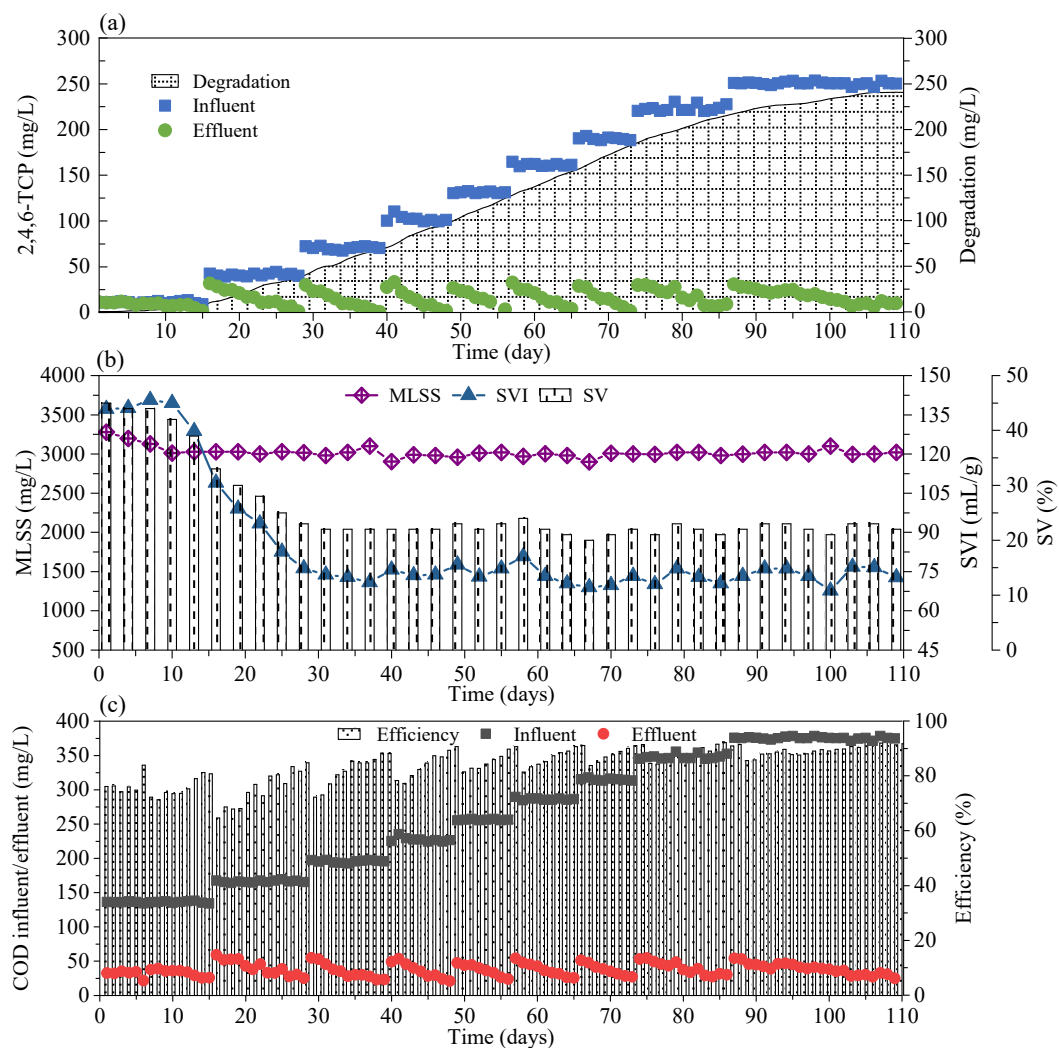


Figure 4. Operating characteristics of the DSBR during 110-day domestication: (a) the variation characteristics of influent and effluent of 2,4,6-TCP and degradable concentration; (b) the variations in activated sludge operating characteristics; (c) COD influent and effluent concentrations and removal efficiency.

3.2.2. COD Removal Performance

The influent and effluent COD, as well as the removal efficiency during the long-term operation of the DSBR, are depicted in Figure 4c. During the operation, COD was primarily contributed by the fermentation liquid and 2,4,6-TCP. It can be observed that the influent COD was correlated with 2,4,6-TCP, showing a gradient increase. Throughout the long-term operation of the reactor, maintaining effluent COD consistently below 50 mg/L signifies the stability of sludge properties and the absence of significant toxic inhibition.

However, the COD removal efficiency was relatively low during the early acclimation phase, ranging from 70% to 80%. In the stable operational phase, the removal efficiency can exceed 90%. Therefore, throughout the entire acclimation period, the DSBR demonstrates a substantial capability for COD degradation.

3.2.3. Sludge Characteristics

Before the start of the DSBR operation, the inoculated sludge concentration was 3280 ± 100 mg/L. After 110 days of operation, the sludge concentration slightly decreased but was maintained at 3021 ± 110 mg/L, with no significant fluctuations during this period. This indicates that a sludge fermenter with a concentration of 150 mg COD/L

can sustain the carbon source metabolism of activated sludge and maintain a constant sludge concentration.

Furthermore, the sedimentation performance of the sludge during DSBR operation can be reflected by calculating the SVI value, and a good sedimentation performance helps to reduce the loss of activated sludge during the DSBR drainage phase. In the initial 0–10 days, the sludge's SVI value was relatively high, averaging 137 mL/g, indicating slight expansion [36]. From day 10 to day 30, it rapidly decreased to a stable level of 70 mL/g. These results indicate that an influent concentration of 150 mg COD/L can maintain the normal metabolism of activated sludge and ensure the proper operation of the reactor.

3.3. Mineralization

The absence of 2,4,6-TCP in the long-term experiment effluent does not necessarily indicate complete microbial metabolism. Only when complete dechlorination and mineralization were achieved could it be confirmed that 2,4,6-TCP was thoroughly metabolized by microorganisms. Therefore, a batch test was established to demonstrate that the microorganisms in the DSBR possess the capability to completely degrade 2,4,6-TCP. The 2,4,6-TCP dechlorination was confirmed by comparing the Cl^{-1} in the effluent to its theoretical Cl^{-1} based on its molecular formula. Complete 2,4,6-TCP mineralization was indicated by the total organic carbon (TOC) value in the effluent.

The influent 2,4,6-TCP was set at 100 mg/L, and the fermentation liquid was added at the same concentration as in the long-term DSBR process, which was 150 mg COD/L. As shown in Figure 5, the 2,4,6-TCP at 100 mg/L was completely degraded within 300 min, achieving a removal rate of 100%. In the reaction solution, the Cl^{-1} concentration reached its highest point at 360 min, measuring 53.90 mg/L, which was consistent with the theoretical value for 2,4,6-trichlorophenol. This indicates that 2,4,6-TCP achieved nearly 100% dechlorination. The delay of 60 min in reaching the maximum Cl^{-1} concentration in the solution was attributed to the fact that 2,4,6-TCP initially underwent degradation into intermediate products bearing Cl^{-1} . The initial TOC was 69.20 mg/L, and this value was contributed by 2,4,6-TCP, sludge fermentation liquid, and some of the membrane-bound bacteria. At 360 min, the TOC decreased to 10.67 mg/L and remained constant until the end of the reaction, resulting in a removal rate of 84.58%. The lowest TOC and the highest Cl^{-1} content both occurred at 360 min, indicating that 2,4,6-TCP achieved simultaneous mineralization and dechlorination.

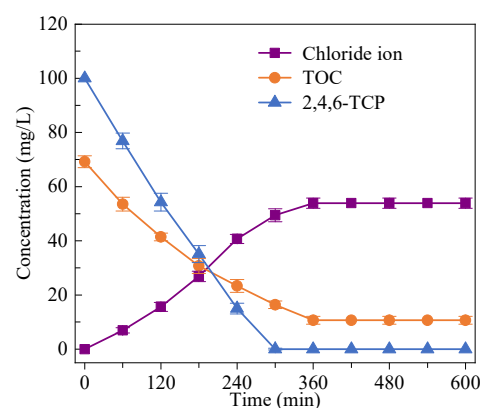


Figure 5. Mineralization characteristics of 2,4,6-TCP using fermentation broth as the carbon source.

3.4. The Impact of Ammonia Nitrogen Influent Concentration on the Degradation of 2,4,6-TCP

High concentrations of NH_4^+ -N are a significant characteristic of sludge fermentation. In this study, a heat pretreatment method was employed to process the influent sludge of the FSBR, which accelerated the release of nitrogen-containing compounds generated from extracellular polymeric substances and cell lysis. As a result, the NH_4^+ -N content in the FB produced by FSBR reached as high as 634.4 mg/L. This concentration is notably

higher than the 144.7 mg/L NH_4^+ -N content produced using alkaline sludge fermentation methods, as reported in the literature [37]. When using sludge FB as a carbon source in denitrification processes, to mitigate the impact of high NH_4^+ -N levels, it is common to employ a chemical method for NH_4^+ -N and phosphorus removal [38]. In this study, no pretreatment of the FB precipitate was conducted. Therefore, it is necessary to investigate the influence of different influent NH_4^+ -N concentrations on the metabolism of 2,4,6-TCP. Batch tests were conducted with a 2,4,6-TCP concentration set at 100 mg/L and influent NH_4^+ -N concentrations of 40 mg/L, 170 mg/L, 340 mg/L, 680 mg/L, and 1360 mg/L.

As depicted in Figure 6, NH_4^+ -N concentrations ranging from 0 to 680 mg/L exhibited no significant impact on the metabolism of 2,4,6-TCP. However, when the influent NH_4^+ -N was raised to 1360 mg/L, there was a noticeable inhibition of 2,4,6-TCP metabolism. Changes in sludge fermentation conditions can lead to fluctuations in effluent NH_4^+ -N, subsequently affecting the metabolism of 2,4,6-TCP. Therefore, when NH_4^+ -N levels are excessively high in the FB, it is essential to employ necessary pretreatment methods to reduce the influent NH_4^+ -N concentration. This is performed to prevent the inhibition of 2,4,6-TCP metabolism.

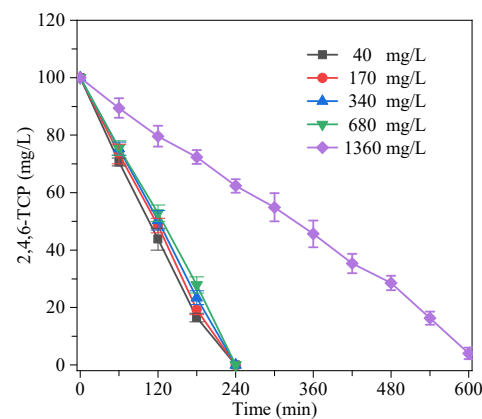


Figure 6. Degradation characteristics of 2,4,6-trichlorophenol under different ammonia nitrogen influent concentrations.

3.5. Novel Strategy for Degrading 2,4,6-TCP in a Pilot-Scale Application

2,4,6-TCP wastewater, characterized by its high toxicity, stands apart from general wastewater such as domestic sewage, imposing more stringent requirements on treatment methods. However, practicality and cost-effectiveness are paramount in engineering applications. Currently, microbial co-metabolism methods predominantly rely on artificially extracted or synthesized single organic compounds as carbon sources, thus incurring a substantial economic burden in practical operations.

This study presents groundbreaking evidence that sludge fermentation liquor not only finds application in biological nitrogen and phosphorus removal processes, but also serves as an exceptional mixed carbon source for microbial co-metabolism. Furthermore, sludge fermentation contributes to the consumption of surplus sludge from wastewater treatment plants, reducing the residual sludge generated during the treatment of chlorophenol wastewater. Figure 7 depicts the pilot-scale application for degrading 2,4,6-TCP using sludge fermentation liquid as a carbon source.

During the pilot-scale operation, considering that the pre-treatment method of thermal hydrolysis for residual sludge may lack operational feasibility, it is advisable to replace the residual sludge fermentation with alkaline fermentation to enhance practical applicability. Alkaline fermentation is a more mature fermentation method, where the fermentation sludge can achieve higher acid production efficiency without the need for special treatment [34,39].

In practical applications, it is essential to determine the dosage of sludge fermentation liquid to ensure that its introduction does not lead to competition with the metabolism of

2,4,6-TCP. The control strategy employed can be achieved by maintaining a constant sludge concentration.

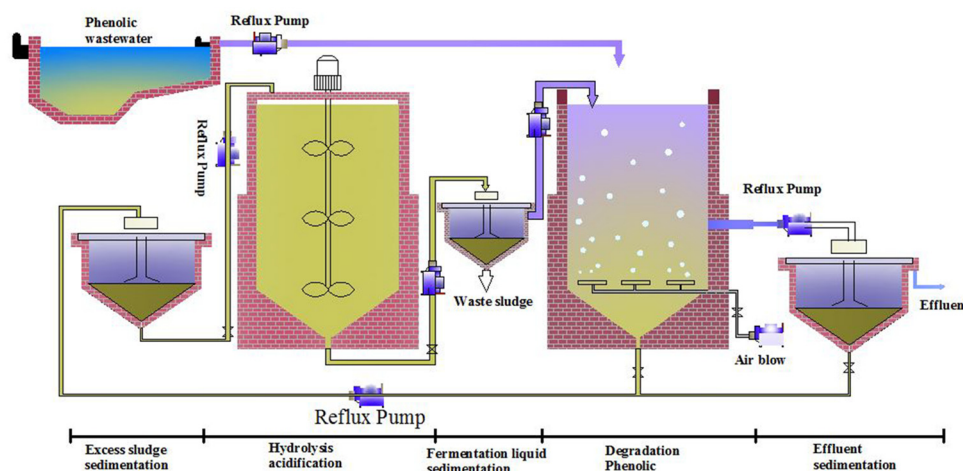


Figure 7. The schematic diagram of the pilot-scale conceptual apparatus for degrading 2,4,6-TCP using sludge fermentation liquid as a carbon source.

In the pilot-scale operation, the pre-treatment of sludge fermentation liquid will involve natural sedimentation, which differs from the centrifugation method used in small-scale studies. Sludge fermentation liquid's settling performance is poor, and natural settling will inevitably introduce fermentation bacteria into the DSB. Therefore, the pilot-scale operation requires further research into the acclimatization of 2,4,6-TCP degradation bacteria and operational parameters under the conditions of mixed fermentation liquid addition. The sludge retention time in the degradation unit was maintained at 120 days, and the resulting residual sludge was recirculated back to the fermentation unit as a fermentation substrate.

4. Conclusions

The utilization of sludge fermentation liquid as a carbon source for the degradation of 2,4,6-TCP presents a viable strategy to tackle the problem of exorbitant treatment expenses linked to the incorporation of external carbon sources. Throughout the 180-day operational period, the concentrations of VFAs in FSB remained constant, with acetic acid, propionic acid, butyric acid, and valeric acid concentrations reaching 322.04 mg COD/L, 225.98 mg COD/L, 274.76 mg COD/L, and 149.58 mg COD/L, respectively. The acid production efficiency peaked at 88.40%, further substantiating the significant improvement in acid production rates in sludge fermentation through the application of thermal pretreatment. The maximum removal of 2,4,6-TCP reached 240.13 mg/L within a 12 h operating cycle, significantly outperforming the use of single commodity carbon sources or other mixed carbon sources. However, the composition and dosage of sludge fermentation liquid will directly affect the removal of 2,4,6-TCP, which remains an aspect to be explored in future research.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/w15224008/s1>, Table S1: The DSB Inflow Trace Elements Concentrations.

Author Contributions: Conceptualization, J.W.; data curation, J.W.; formal analysis, J.W.; writing—original draft, J.W.; writing—review and editing, J.W., Z.S. and J.L. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data are contained within the article and Supplementary Materials.

Conflicts of Interest: Author Jianguang Wang was employed by the company PowerChina Huadong Engineering Corporation Limited. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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