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Delving into the Impacts of Different Easily Degradable Carbon Sources on the Degradation Characteristics of 2,4,6-Trichlorophenol and Microbial Community Properties

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Abstract: In chlorophenol wastewater treatment, adding easily degradable carbon sources, such as methanol, ethanol, sodium acetate, and sodium propionate, significantly improves the chlorophenol removal efficiency. This study systematically compares these conventional carbon sources in different sequencing batch reactors to understand their specific effects on both 2,4,6-trichlorophenol (2,4,6-TCP) degradation efficiency and microbial abundance. In a 35-day experiment, as a carbon source, ethanol exhibited a lower 2,4,6-TCP degradation concentration (77.56 mg/L) than those of methanol, sodium acetate, and sodium propionate, which achieved higher degradation concentrations: 123.89 mg/L, 170.96 mg/L, and 151.79 mg/L, respectively. As a carbon source, sodium acetate enhanced extracellular polymeric substance production (200.80 mg/g·VSS) by microorganisms, providing protection against the toxicity of chlorophenol and resulting in a higher 2,4,6-TCP removal concentration. Metagenomics identified crucial metabolic genes, including *PcpA*, *chqB*, *Mal-r*, *pcaI*, *pcaF*, and *fadA*. The abundance of genera containing the *chqB* gene correlated positively with the metabolic capacity for 2,4,6-TCP. Moreover, small molecular carbon sources such as methanol, sodium acetate, and sodium propionate promoted the enrichment of genera with functional genes.

Keywords: 2,4,6-trichlorophenol; easily degradable carbon sources; functional gene; microbial community

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1. Introduction

2,4,6-trichlorophenol (2,4,6-TCP) is a crucial chemical raw material that is widely utilized in various industrial sectors, such as synthesis, papermaking, printing and dyeing, and plastic production [1]. However, industrial wastewater containing 2,4,6-TCP is discharged in large quantities due to inadequate treatment, posing a new threat to aquatic ecosystems. The hazard of 2,4,6-TCP arises from its high toxicity and bioaccumulation characteristics, making it an environmentally high-risk compound [2]. It is particularly carcinogenic to humans and animals, leading it to be classified as a priority pollutant in many countries [3,4].

In comparison to physical and chemical methods, biological methods exhibit advantages in the treatment of chlorophenol wastewater, including strong applicability, high processing capacity, and minimal secondary pollution. However, biological methods, particularly the activated sludge method, face two major challenges in the degradation of toxic organic compounds: firstly, the slow growth and metabolism of microorganisms due to the effects of toxicity, and secondly, the lack of microbial growth and metabolism-requiring carbon sources in wastewater with toxic compounds. Due to the constraints of toxicity factors, the inflow concentration of chlorophenol wastewater is not likely to be excessively high. This results in chlorophenols and their intermediates not providing

sufficient carbon sources for microbial growth, leading to continuous loss of activated sludge biomass and a decline in biomass, which makes it challenging for the reactor to operate [5]. The bioco-metabolism method can effectively address this issue. Many wastewater streams containing chlorophenols, including monochlorophenols, dichlorophenols, and trichlorophenols, have indeed implemented biological co-metabolism degradation approaches [6–12]. This method not only provides necessary carbon sources and energy for microbial growth but also reduces the toxic inhibition of chlorophenols on activated sludge bacteria [13–15].

Currently, literature reports predominantly focus on studying the impact of the addition of a single carbon source on the microbial degradation of chlorophenolic pollutants and the microbial community. For example, studies have investigated the effects of sodium acetate or glucose as a sole carbon source on the degradation of phenol, 4-chlorophenol, and 2,4-dichlorophenol [11,12,16–20]. However, there were few attempts to compare the influences of different organic molecules on the degradation of chlorophenols, especially those of easily degradable carbon sources, such as methanol, ethanol, and sodium acetate. Furthermore, in previous studies, there has been a lack of research from the perspective of the conditioning of activated sludge and the operation of reactors on the mechanism of the impact of small molecular carbon sources on the cultivation of bacteria for degrading 2,4,6-TCP. Therefore, further research is needed to explore the effects of carbon sources with different molecular weights on the enrichment of functional bacteria and the stability of reactor operation during the 2,4,6-TCP degradation process.

Different wastewater qualities could significantly alter microbial communities and abundance [21]. The phyla *Proteobacteria*, *Actinobacteria*, and *Firmicutes* are considered the main phyla responsible for degrading chlorophenols [22]. However, it is currently unclear whether the type of carbon source can promote the growth of such chlorophenol-degrading bacteria in the process of 2,4,6-TCP degradation.

Against this background, the main content of this study includes the following: (1) studying the impacts of carbon sources with different molecular weights on the microbial degradation of 2,4,6-TCP; (2) exploring the changes in microbial diversity and the influence on functional microbial communities after adding different carbon sources during the conditioning process; (3) evaluating the potential correlation between the characteristics of activated sludge and the type of carbon source. This study conducted long-term conditioning experiments using conventional carbon sources with different molecular weights, such as methanol, ethanol, sodium acetate, and sodium propionate. High-throughput and metagenomic techniques were employed to analyze the potential relationship between the microbial community in activated sludge and the type of carbon source. The findings of this study can offer valuable data support for the selection of carbon sources during the treatment process of phenolic wastewater in practice.

2. Materials and Methods

2.1. Reactor and Operation

The sequencing batch reactor (SBR), which served as a biochemical reaction apparatus, comprised a total of five SBRs in this study. Among these, four SBRs were supplemented with distinct carbon sources (methanol, ethanol, sodium acetate, and sodium propionate) (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China), while one SBR operated without the addition of any carbon sources (only 2,4,6-TCP(Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). The effective volume for each SBR was consistently maintained at 3 L. Operating under constant temperature conditions of 28 ± 0.5 °C, the SBRs underwent two cycles per day. This cycle encompassed several phases: a 5-min period for feeding synthetic wastewater, followed by a 600-min aerobic reaction phase with dissolved oxygen maintained at 5 ± 0.5 mg/L. Subsequently, a 60-min settling phase was followed by a 5-min discharge of supernatant, with a volumetric exchange ratio of 50%. Finally, a 50-min idling phase concluded the cycle. The solid residence time of 30 days was upheld by discharging 50 mL of mixed activated sludge from each SBR in every cycle.

2.2. Seed Sludge and Water

The activated sludge used for the experimental inoculation was sourced from the secondary sedimentation tank of the Gaobeidian Wastewater Treatment Plant in Beijing.

The experimental setup involved the use of artificially prepared influent, comprising 2,4,6-TCP, carbon sources, and trace nutrients. Methanol (Met), ethanol (Eth), sodium acetate (Sa), and sodium propionate (Sp) were employed as carbon sources, with an influent concentration of 300 mgCOD/L. The influent concentration of 2,4,6-TCP was incrementally elevated from low concentrations, with the dosing concentration slightly exceeding the degradable concentration of the activated sludge to maintain a certain level of selective pressure. The composition of trace nutrients primarily adhered to the components detailed in the work by Wang, J. et al. [7].

2.3. Batch Experiment

The activated sludge used in the batch experiments was obtained from the stable phase of a long-term experiment in the SBR. Batch experiments were conducted under constant temperature at 28 °C, with dissolved oxygen at 4–5 mgO_2/L and a magnetic stirrer set at a speed of 200 rpm.

The specific steps of the batch experiments involved studying the degradation characteristics of 2,4,6-TCP by activated sludge cultivated with different carbon sources. Parameters such as the 2,4,6-TCP removal efficiency, dechlorination capability, and mineralization ability were investigated. To eliminate interference from chloride ions in the nutrient elements, $CaCl_2 \cdot 2H_2O$ was removed from the water and replaced with ultrapure water. The addition of each of the four carbon sources was set at 300 mgCOD/L, and the influent concentration of 2,4,6-TCP ranged from 44 to 47 mg/L.

Samples were collected at 60-min intervals, with each sample consisting of 10 mL of mixed sludge. After low-speed centrifugation at 4000 rpm, the samples were filtered through a 0.22 μm polyethersulfone membrane. The treated samples were then analyzed using liquid chromatography, ion chromatography, and a TOC analyzer to determine the concentrations of 2,4,6-TCP, chloride ions, and TOC, respectively.

2.4. Analytical Methods

2.4.1. The Analysis of the Microbial Community

This investigation involved assessing variations in EPS, PN, and PS content under different carbon source conditions, as described in Reference [10].

During the long-term cultivation period in the SBR, mixed activated sludge samples were collected for an in-depth analysis of the microbial community, as described in Reference [23]. The raw sequence data generated through high-throughput sequencing were duly submitted to the NCBI under the sequence read accession number PRJNA707254.

2.4.2. Water Quality Testing

The water quality indicators encompassed 2,4,6-TCP, total organic carbon (TOC), and chloride ions, with their quantification being performed through distinct methodologies. High-performance liquid chromatography (HPLC, Waters 1525, Milford, MA, USA) was employed for the analysis of 2,4,6-TCP, while TOC was measured using a TOC analyzer (Elementar vario TOC, Frankfurt, Germany). The detection of chloride ions was conducted through ion chromatography (Metrohm 883 Basic IC plus, Herisau, Switzerland), as detailed in Reference [23].

The conventional water quality parameters, including COD, mixed-liquor suspended solids (MLSSs), SV_{30} (settling velocity in 30 min), and the sludge volume index (SVI), were assessed using the standard methods outlined in Reference [24].

In this study, water quality sampling was conducted using triplicate samples for each sampling point.

3. Results and Discussion

3.1. Long-Term Acclimatization Characteristics of Activated Sludge

The carbon source could alter the microbial community, thereby influencing the capacity for the degradation of target pollutants [21]. Figure 1 illustrates the long-term degradation characteristics of activated sludge in different carbon source conditions when degrading 2,4,6-TCP. It describes the concentrations of influent, effluent, and degradable 2,4,6-TCP. The SBR had been inoculated with activated sludge lacking any chlorophenol degradation capabilities before operation. This sludge had initially been used for nitrogen and phosphorus removal in municipal wastewater treatment, exhibiting rich population diversity and microbial communities conducive to the selection and acclimatization of trichlorophenol-degrading bacteria [7].

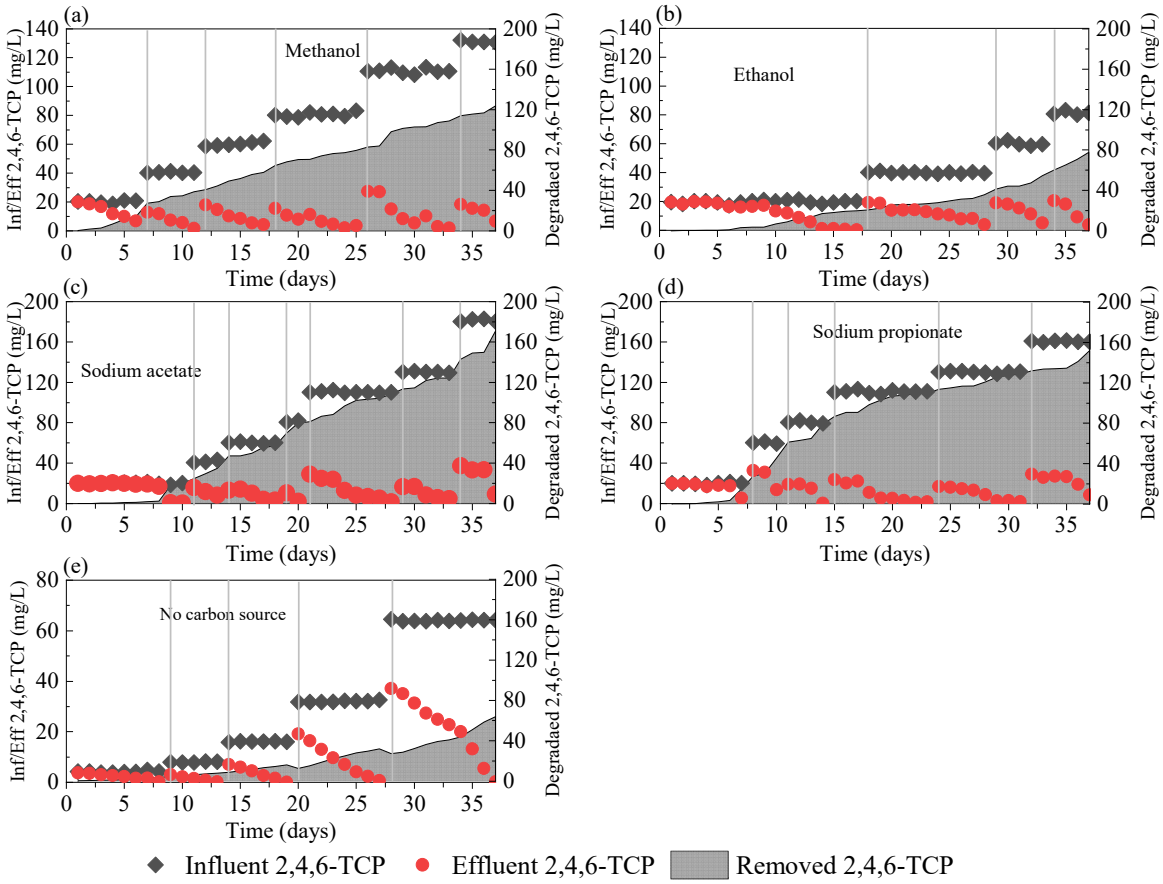

Figure 1. The domesticated performance of 2,4,6-TCP with the addition of different carbon sources ((**a**) Methanol; (**b**) Ethanol; (**c**) Sodium acetate; (**d**) Sodium propionate; (**e**) No carbon source).

Considering the biological toxicity of 2,4,6-TCP, a gradient-increasing dosing approach was adopted. When the 2,4,6-TCP concentration was 10 mg/L or 20 mg/L, except for the SBR utilizing ethanol as a carbon source, which had a longer adaptation period, the other SBRs achieved the degradation of low-concentration chlorophenols in a relatively short time. The 2,4,6-TCP was gradually increased until each SBR reached the maximum degradation concentration. During the stable operational phase, the maximum degradation concentrations of 2,4,6-TCP were as follows: 123.89 mg/L (methanol), 77.56 mg/L (ethanol), 170.96 mg/L (sodium acetate), 151.79 mg/L (sodium propionate), and 58.72 mg/L (no carbon source). With the exception of ethanol, small organic molecules as carbon sources all achieved relatively high 2,4,6-TCP degradation concentrations, especially in the cases of sodium acetate and sodium propionate as carbon sources. The SBR that did not receive additional carbon sources removed 58.72 mg/L 2,4,6-TCP in the short term. However, the degradable 2,4,6-TCP gradually decreased with the SBR's operation. This decline could

potentially be attributed to the reduction in the activated sludge concentration, leading to a decrease in the concentration of 2,4,6-TCP degradation. In conclusion, the use of sodium acetate as a carbon source is likely to yield higher concentrations of 2,4,6-TCP degradation compared to other carbon sources.

Figure 2 illustrates the changes in the MLSSs and settling characteristics within the SBRs. It was observed that the SBR without additional carbon sources experienced a rapid reduction in initial MLSSs from 3000 mg/L to 1000 mg/L. In contrast, the SBRs with added carbon sources maintained a higher microbial biomass level and exhibited a gradual upward trend.

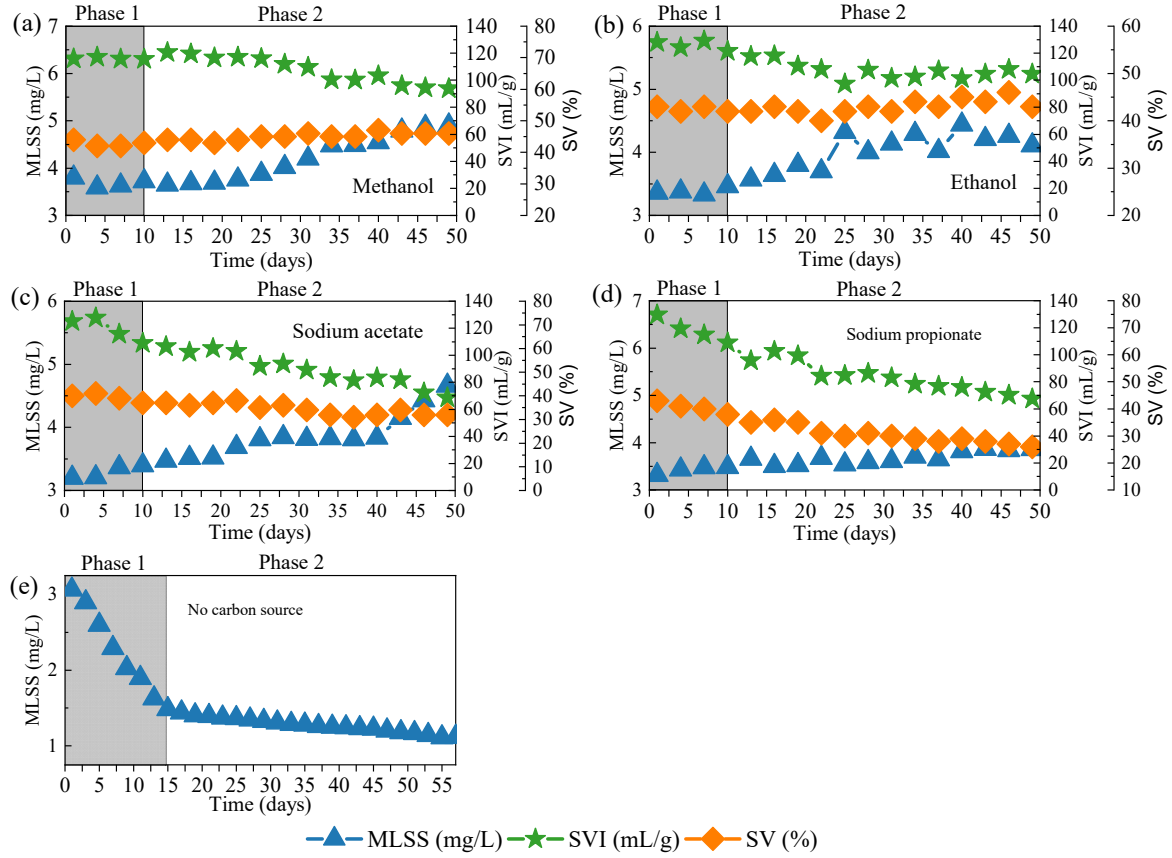

Figure 2. MLSSs and settling performance of each SBR in long-term operation ((**a**) Methanol; (**b**) Ethanol; (**c**) Sodium acetate; (**d**) Sodium propionate; (**e**) No carbon source).

The settling characteristics of activated sludge directly influenced the operational performance of the SBRs during the settling phase. As depicted in Figure 2, the addition of small molecular carbon sources contributes to maintaining favorable sludge settling. However, the use of starch as a carbon source results in inferior settling characteristics for an SBR, with an SVI close to 200 mL/g [7]. The 300 mgCOD/L carbon source leads to a gradual increase in sludge concentration in the SBR, but it has not yet reached a complete steady-state operation. Therefore, further optimization and adjustment of the carbon source dosage are needed to achieve optimal performance. The MLSS and SVI indicators can provide feedback on the operational status of an SBR. Based on the results obtained, it is observed that the use of sodium acetate and sodium propionate as carbon sources results in lower SVI values, which is beneficial for the sedimentation of activated sludge and the discharge of effluent from the SBR.

Additionally, the effluent COD did not exhibit significant fluctuations during the long-term operational process, as illustrated in Figure 3. The influent COD was primarily contributed by the added carbon sources, and the average effluent COD for each SBR was as follows: 37.8 mg/L (methanol), 36.2 mg/L (ethanol), 38.2 mg/L (sodium acetate),

40.7 mg/L (sodium propionate), and 10.5 mg/L (no carbon source). These values were consistently maintained at relatively low levels, indicating that different carbon sources had no significant impacts on the effluent COD from the SBRs.

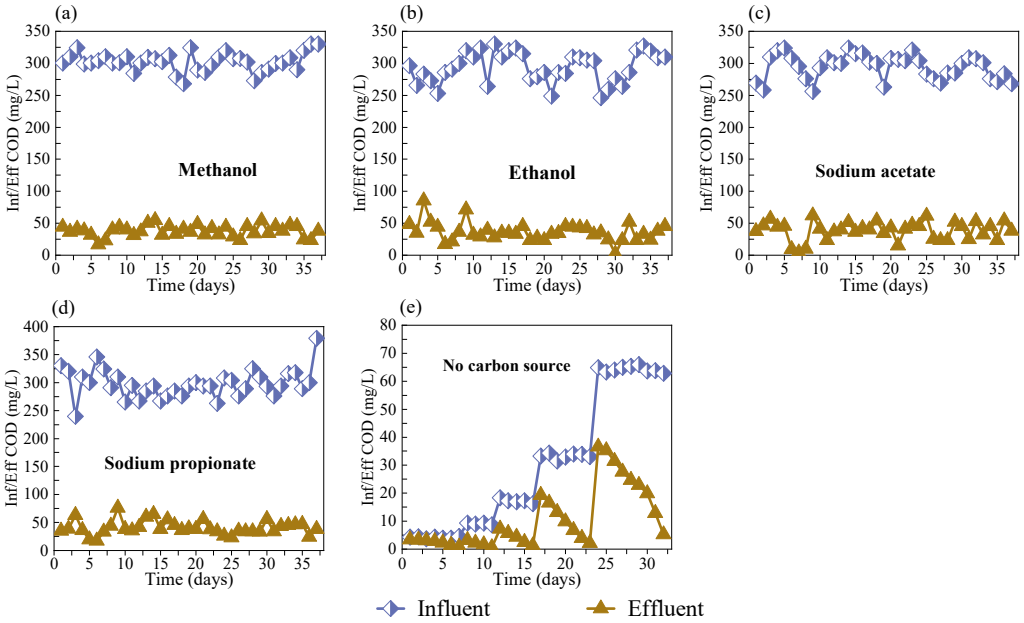

Figure 3. The average influent and effluent CODs of the SBRs in long-term operation ((**a**) Methanol; (**b**) Ethanol; (**c**) Sodium acetate; (**d**) Sodium propionate; (**e**) No carbon source).

3.2. The Mineralization and Dechlorination of 2,4,6-TCP

In Figure 4, the degradation, dechlorination, and TOC removal of 2,4,6-TCP are depicted over a typical cycle. At the end of the typical cycle, no 2,4,6-TCP was detected, indicating that the sludge acclimated with various carbon sources could effectively remove 2,4,6-TCP. The theoretical dechlorination concentration was calculated based on the chlorine content in the 2,4,6-TCP molecule. The detected chloride ion in the effluent was found to be almost identical to the theoretical chlorine concentration, suggesting complete dechlorination. A comparison revealed that the activated sludge acclimated with different carbon sources could achieve complete dechlorination of 2,4,6-TCP.

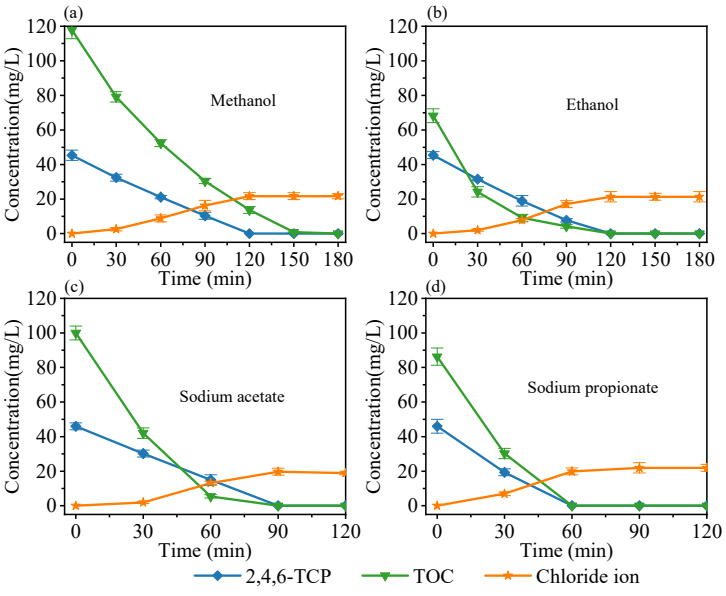

Figure 4. Batch tests of 2,4,6-TCP mineralization with different carbon sources ((**a**) Methanol; (**b**) Ethanol; (**c**) Sodium acetate; (**d**) Sodium propionate).

Furthermore, the removal efficiency of TOC in the effluent from the SBRs exceeded 95%. The inability to achieve a 100% removal rate might have been associated with suspended microorganisms in the effluent. The TOC removal rate and chloride ion data indicated that activated sludge acclimated with different carbon sources could effectively achieve the harmless degradation of 2,4,6-TCP.

3.3. The EPS Content of the Activated Sludge

In the biological treatment of toxic wastewater, EPS played a role in protecting microbial cells from external toxic substances while also enhancing the removal efficiency of activated sludge for toxic compounds [10]. The influent water quality was a key factor influencing the content and composition of EPS [25].

In Figure 5, it is evident that the activated sludge, after acclimatization (excluding the sludge without a carbon source), exhibited an increase in EPS. This suggested that activated sludge, by generating a higher amount of EPS, aimed to mitigate the toxic effects of 2,4,6-TCP on microbial cells. The activated sludge without the addition of a carbon source had the lowest total EPS, measuring only 59.69 mg/g·VSS. This was attributed to the lack of carbon sources in the system, leading to a reduction in EPS production, or to microbes utilizing EPS as a carbon source in the absence of an external carbon source. Wang, J. et al. [7] utilized starch as the carbon source for 2,4,6-TCP degradation, resulting in the production of a higher amount of EPS by activated sludge, reaching 285.81 mg/g·VSS.

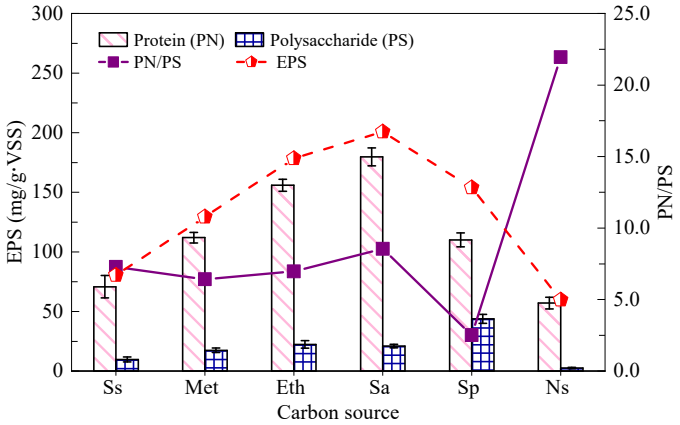

Figure 5. EPS concentration of 2,4,6-TCP-degrading sludge using different carbon sources (Ss: seed sludge; Met: methanol; Eth: ethanol; Sa: sodium acetate; Sp: sodium propionate; Ns: no substrate).

The main components of EPS are proteins and polysaccharides, with proteins playing a crucial role in the extracellular hydrolysis process of microorganisms. For activated sludge with low-molecular-weight organic compounds as carbon sources, the protein in EPS was lower than that in cases where high-molecular-weight organic compounds served as carbon sources. Among them, the protein in the sodium acetate group was the highest. Considering that the sodium acetate group achieved the highest removal of 2,4,6-TCP as a carbon source and that sodium acetate could be directly utilized by activated sludge without undergoing hydrolysis, the proteins may have directly contributed to the 2,4,6-TCP degradation.

The content of polysaccharides in EPS is related to hydrophilicity, where a smaller PN/PS value indicates stronger hydrophilicity [26]. Higher hydrophilicity allowed for sufficient contact between microbial cells and 2,4,6-TCP, facilitating the degradation process. Activated sludge with better settling characteristics often accompanied a lower PN/PS. Under the no-addition condition, the PN/PS value of EPS reached 22, consistently with the poor settling performance. Simultaneously, the polysaccharide level in EPS was at a lower level (2.6 mg/g·VSS), leading to reduced cell aggregation capabilities. This was a significant factor contributing to the substantial loss of sludge in the SBR reactor without the addition of a carbon source during the later stages of cultivation.

3.4. Bacterial Abundance and Functional Gene Analysis

3.4.1. Influences of Different Carbon Sources on Bacterial Abundance

As depicted in Figure 6, the microbial community at the phylum level with different carbon sources is illustrated. At the phylum level, a total of 14 phyla were detected, and the proportions of these phyla varied significantly with the different carbon sources. According to literature reports, phyla such as *Proteobacteria*, *Actinobacteria*, *Firmicutes*, and *Saccharibacteria* have been found to have higher proportions in phenol- and 4-chlorophenol-degrading bacteria [18,22,27–31]. This study exclusively employed 2,4,6-TCP as the target pollutant and similarly observed higher proportions of the four aforementioned phyla: Under the no-addition condition, these four phyla collectively accounted for 93.36% of all detected phyla. Under different carbon source conditions, the abundances of these four phyla were 95.92% (methanol), 90.94% (ethanol), 71.89% (sodium acetate), and 81.25% (sodium propionate), respectively, which were significantly higher than those in the inoculated activated sludge. This indicated that at the phylum level, the chlorophenol-degrading microbial community was similar.

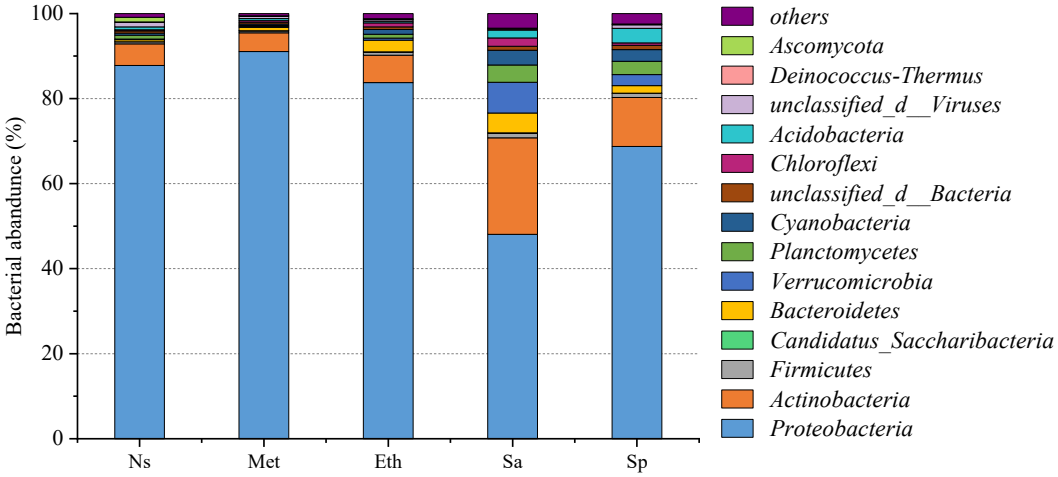

Figure 6. Bacterial community at the phylum level with different carbon sources (Ns: no substrate; Met: methanol; Eth: ethanol; Sa: sodium acetate; Sp: sodium propionate).

As shown in Figure 7, at the genus level, the dominant bacterial genera under different carbon source conditions were significantly different. The dominant genera and their abundances were 19.16% *Sphingomonas* (no carbon source), 20.40% *Ralstonia* (methanol), 18.68% *Sphingomonas* (ethanol), 16.53% *Mycobacterium* (sodium acetate), and 16.62% *Variovorax* (sodium propionate). The genus *Sphingomonas* is known for its ability to degrade 2,6-dichlorophenol, 2,4,6-TCP, 2,3,4,6-tetrachlorophenol, and pentachlorophenol [32,33]. With no carbon source or with ethanol as a carbon source, the enrichment of *Sphingomonas* as a dominant genus was promoted. *Ralstonia* is a common dominant genus in the metabolism of chlorophenolic compounds [34–36], and in this study, methanol increased its abundance to 20.4%. The dominant genus with the addition of sodium propionate (*Variovorax*) has not been reported to have relevant chlorophenol-degrading capabilities. *Mycobacterium*, the genus with the second-highest abundance after *Variovorax*, played a crucial role in the degradation of monochlorophenols [37,38], and sodium acetate promoted its dominance as a genus. Different carbon sources significantly altered the microbial community, and although some genera are commonly associated with chlorophenol metabolism, their high abundance can only suggest their potential for 2,4,6-TCP metabolism. To further verify the capabilities for 2,4,6-TCP metabolism of the mentioned genera, we conducted further analyses from a genetic perspective.

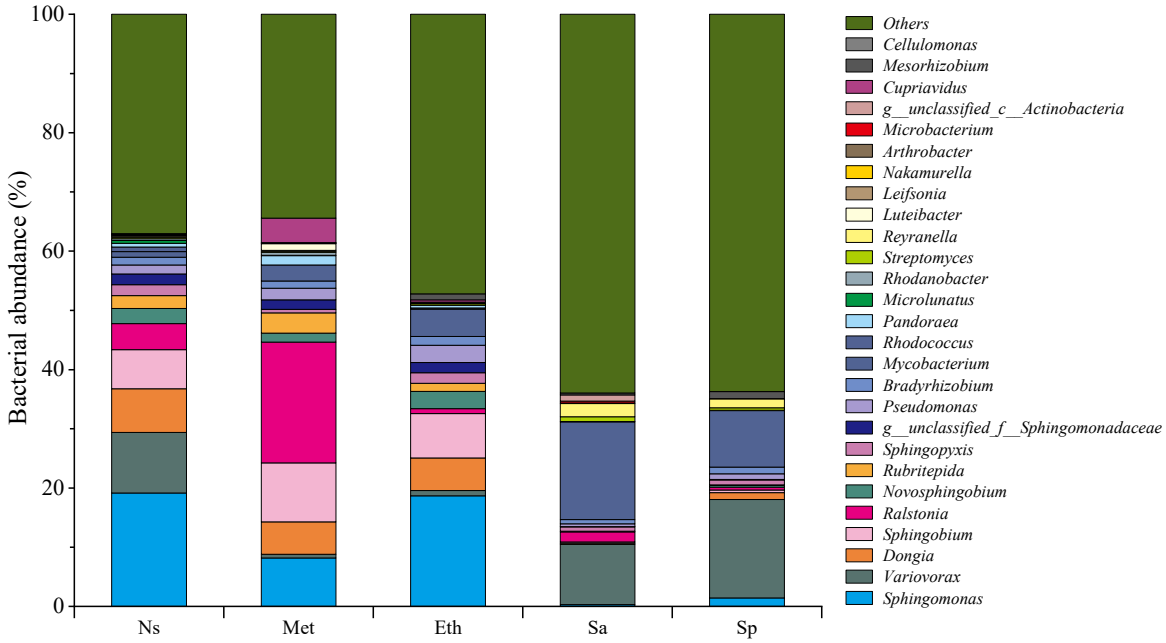

Figure 7. Bacterial community at the genus level with different carbon sources.

3.4.2. The Impacts of Different Carbon Sources on the Abundance of Functional Genes

As shown in Figure 8, the abundance of functional genes related to 2,4,6-TCP metabolism under different carbon source conditions is illustrated. The abundance of functional genes at the front end of the 2,4,6-TCP metabolic pathway was relatively low, while the abundance of functional genes closer to the back end of the metabolic pathway was higher. The abundance of *fadA* was consistently above 100,000 hits, indicating that at the front end of the metabolic pathway, where 2,4,6-TCP and its toxic intermediates are present, fewer microorganisms possess metabolic capabilities, resulting in significantly lower gene abundance. In contrast, at the back end of the metabolic pathway, where the toxicity of the intermediates is lower, they can serve as carbon sources for more microorganisms, leading to a higher abundance of functional genes.

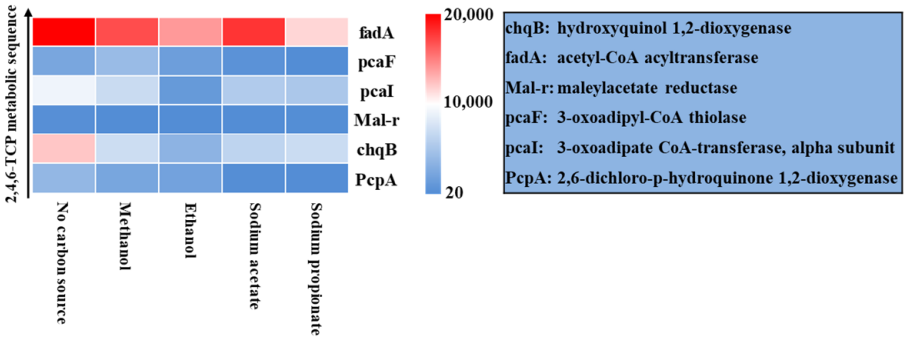

Figure 8. Heat map of gene abundance for 2,4,6-TCP degradation with different carbon sources.

As shown in Figure 9, the total abundance of functional genes under different carbon source conditions is depicted. Under the no-carbon-source condition, the gene abundance reached 42,858 hits, which was higher than that in the cases with added carbon sources. However, due to the low biomass under the condition without adding carbon sources, it was challenging to maintain a satisfactory 2,4,6-TCP degradation performance. Although adding carbon sources reduced the abundance of functional genes related to chlorophenol degradation, it increased microbial biomass. This indicates that both microbial abundance and the functional genes' abundance jointly determined the effectiveness of microbial degradation of 2,4,6-TCP.

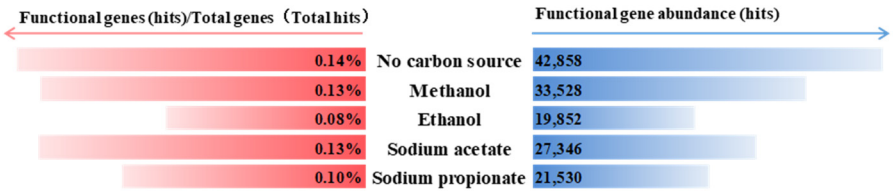

Figure 9. Comparison of the total gene abundance in 2,4,6-TCP degradation under different carbon sources.

3.4.3. Distribution Characteristics of Functional Genes in Different Microbial Communities

The abundance of functional genes did not exhibit a significant correlation with the effectiveness of 2,4,6-TCP degradation. Consequently, an analysis was conducted on the abundance of microbial communities containing specific genes, as illustrated in Figure 10. Several key genes in the metabolic pathway of 2,4,6-TCP, including *PcpA*, *chqB*, *Mal-r*, *pcaI*, *pcaF*, and *fadA*, were considered. Additionally, it was noted that the toxicity of products at the front end of the metabolic pathway was higher, gradually decreasing or becoming non-toxic as the reaction progressed. Combining the degradation concentrations of 2,4,6-TCP under different carbon source conditions, it was observed that the abundance of microbial communities containing *chqB* exhibited a significant positive correlation with the degradation rate of 2,4,6-TCP (correlation coefficient: 0.61, $p < 0.01$). The proportions of microbial communities containing other specific genes showed correlation coefficients with the degradation rate of 2,4,6-TCP of less than 0. Therefore, the abundance of microbial communities containing the *chqB* gene was positively correlated with the degradation rate of 2,4,6-TCP. Methanol, sodium acetate, and sodium propionate as carbon sources facilitated the enrichment of these microbial communities, enabling the removal of higher concentrations of 2,4,6-TCP.

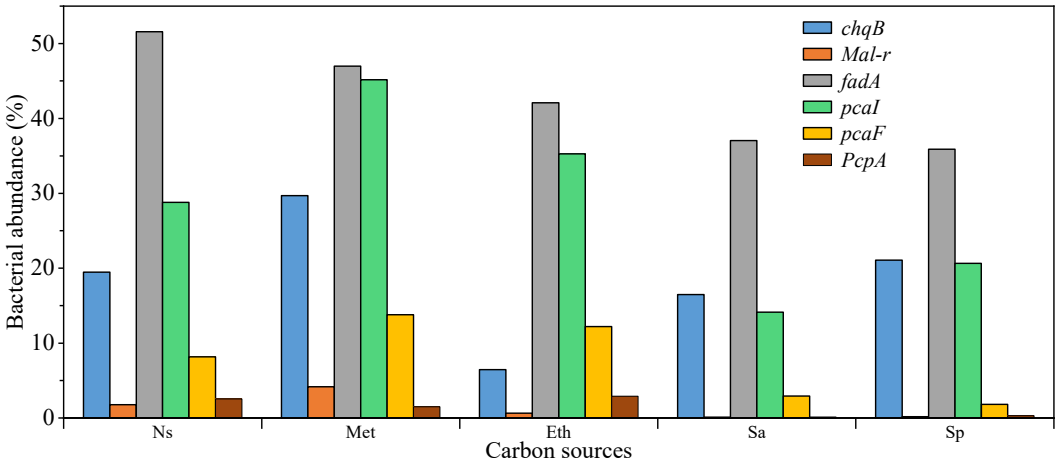

Figure 10. Abundance of bacteria containing specific functional genes.

4. Conclusions

Under different carbon source conditions, varying degrees of 2,4,6-TCP degradation were achieved by altering the microbial community. In comparison to hard-to-degrade carbon sources, easily degradable co-metabolic carbon sources demonstrated higher concentrations of 2,4,6-TCP removal. Specifically, activated sludge supplemented with methanol, sodium acetate, and sodium propionate as carbon sources achieved degradation concentrations of 123.89 mg/L, 170.96 mg/L, and 151.79 mg/L, respectively, surpassing those of other carbon sources. From the perspective of 2,4,6-TCP removal efficiency, sodium acetate appears to be the most suitable as a carbon source in the treatment of actual phenolic wastewater. Not only does it yield higher concentrations of 2,4,6-TCP removal, but it also enhances the overall performance of the SBR's operation. When compared to scenarios

without the addition of carbon sources, all four carbon sources were found to significantly increase the functional gene abundance related to 2,4,6-TCP degradation, including *PcpA*, *chqB*, *Mal-r*, *pcaI*, *pcaF*, and *fadA*. The abundance of genera containing the chqB gene demonstrates a strong correlation with the metabolic capacity of 2,4,6-TCP. Small molecular carbon sources such as methanol, sodium acetate, and sodium propionate can facilitate the enrichment of genera harboring functional genes. However, this study solely focused on the effects of adding different carbon sources on 2,4,6-TCP removal efficiency and microbial community abundance. The underlying mechanisms of different carbon sources' profound impacts require further exploration to provide guidance for the effective treatment of actual phenolic wastewater.

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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 potential conflicts of interest.

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