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Acclimating activated sludge with co-metabolic substrates for enhancing treatment of low-concentration polyether wastewater

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HIGHLIGHTS

- Efficient biological treatment of LCPW was achieved.
- LCPW effluent after treatment by acclimated activated sludge can meet standard.
- Activated sludge acclimated by CAPW showed good EPS content and dehydrogenase activity.
- Higher bacterial diversity acclimated by CAPW led to efficient LCPW treatment.

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ABSTRACT

Polyether wastewater from chemical industries is difficult to be degraded directly by microorganisms even at low concentration. The feasibilities and differences of using complex and single carbon sources as co-metabolic substrates to acclimate activated sludge for enhancing treatment of low-concentration polyether wastewater (LCPW) were comparatively analyzed. The complex carbon source was produced from citric acid production wastewater (CAPW), and the sodium acetate (NaAc) was selected as single carbon source. Results indicated that the LCPW could be effectively treated with acclimated activated sludge, and satisfying the wastewater discharge standard (COD ≤ 50 mg/L, TN ≤ 15 mg/L, TP ≤ 0.5 mg/L). Importantly, the activated sludge acclimated with CAPW (AS-CAPW) presented larger biomass, better treatment performance, and higher extracellular polymeric substance content, dehydrogenase activity and bacterial diversity than that acclimated with NaAc (AS-NaAc). Moreover, high-throughput sequencing revealed that the relative abundance of dominant families associated with nutrient removal in AS-CAPW or AS-NaAc (e.g., *Nitrosomonadaceae* and *Rhodocyclaceae*) was similar. In contrast, the dominant families involved in macromolecular pollutions degradation had significant differences. *Phycisphaeraceae* (10.3%) and *Lentimicrobiaceae* (14.3%) were mainly enriched in AS-CAPW and AS-NaAc, respectively. This comprehensive work provides a feasible biological process for enhancing treatment of LCPW.

1. Introduction

Polyether polyol, a macromolecular compound formed by polymerization of ether or epoxides [1], is widely applied in many fields, such as detergents, cosmetics, coatings, textiles, and architecture, thereby leading to a great market demand [2]. Refractory high-concentration polyether wastewater (COD > 100,000 mg/L) will be produced in the extraction process of polyether polyol, and directly

Abbreviations: ACE, Abundance-based coverage estimator; BOD_5, Five-day biochemical oxygen demand; CAPW, Citric acid production wastewater; CK, Control blank; COD, Chemical oxygen demand; DHA, Dehydrogenase activity; EPS, Extracellular polymeric substance; LCPW, Low-concentration polyether wastewater; MLSS, Mixed liquor suspended solids; MLVSS, Mixed liquor volatile suspended solids; NH_4^+-N, Ammonia nitrogen; NO_2^--N, Nitrite nitrogen; NO_3^--N, Nitrate nitrogen; OTU, Operational taxonomic unit; PO_4^{3-}-P, Phosphorus; NaAc, Sodium acetate; SBR, Sequencing batch reactor; SS, Suspended solids; TN, Total nitrogen; TP, Total phosphorus

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concentrated and combusted via adding combustion aids. Low-concentration polyether wastewater (LCPW; COD < 300 mg/L), derived from condensate water and equipment cleaning water, is difficult to handle via direct combustion because of the expensive concentration cost.

According to our survey of typical polyether manufacturers, about 7.0 million tons of LCPW is generated every year in China. The inappropriate treatment of LCPW poses a threat to human health and environment because LCPW contains refractory and toxic pollutants, such as ethers, amides, alcohols, esters, and so on. Currently, treatment methods for refractory wastewater mainly focus on specific industrial wastewater, including cooking, pharmaceuticals, papermaking, printing and dyeing, aniline, and so on [3]. However, the treatment of LCPW has not attracted sufficient attention in China. Thus, seeking an efficient LCPW treatment method is necessary. At present, physical, chemical, and biological methods are widely used to treat industrial wastewater. Physical methods, including coagulation, adsorption, and membrane filtration [4,5]; and chemical methods, including catalytic oxidation and electrolytic flocculation [6,7], can achieve pollutants to be removed in a short time, but the operation costs are expensive. Biological methods have higher degradation efficiency and lower operating cost than the other two methods [8,9]. Applying biological methods directly to treat refractory wastewater will be challenging task because the toxic organics will damage the metabolic system of activated sludge.

In our preliminary tests, we attempted to treat directly LCPW with activated sludge, but the treatment performance was not satisfied. Recently, co-metabolism has been applied widely as an efficient and economic method for enhancing treatment of refractory wastewater [10], which makes the biological treatment of LCPW possible. The mechanism of co-metabolism involves using biodegradable organic matter as co-metabolic substrates to induce the secretion of nonspecific enzymes for the degradation and detoxification of pollutants [11]. Single carbon sources (e.g., sodium acetate [NaAc], methanol, glucose, etc.) are generally selected as a co-metabolic substrate for the treatment of refractory wastewater, whereas complex carbon sources are rarely mentioned. Currently, large amounts of organic wastewater containing high levels of nontoxic and biodegradable organic matter are produced in the fermentation industry and lead to serious pollution problems and expensive treatment costs. In our previous research, we developed a commercially viable process to alleviate the pollution problem of citric acid production wastewater, which converted it into a complex carbon source for enhancing the treatment of domestic and industrial wastewater [12].

The objectives of the present work were (1) to investigate the feasibility of using a complex carbon source (CAPW, derived from citric acid production wastewater) and a single carbon source (NaAc as control) as co-metabolic substrates for LCPW treatment and (2) to comparatively analyze the variations of microbial activity and bacterial community structure for activated sludge before and after acclimation.

2. Materials and methods

2.1. Wastewater and co-metabolic substrates

LCPW was collected from a polyether production plant in Wuxi City, China. The samples were stored in a 4 °C refrigerator for less than one week prior to use. The main characteristics of LCPW and CAPW are shown in Table 1. The analysis of pollutant compositions in LCPW was performed by using gas chromatography mass spectrometer (GC-MS) (Supplementary data, Fig. S1). NaAc was analytical reagent grade.

2.2. Setup and operation of SBR

Two experimental groups were set up using CAPW (E_{CAPW}) and NaAc (E_{NaAc}) as co-metabolic substrates and another experimental group without co-metabolic substrate (E_{CK}) to comparatively analyze the performance of LCPW treatment. Each group was set with two parallel sequencing batch reactors (SBRs) with a load volume of 1.0 L, and equipped with dissolved oxygen (DO) probe, gas regulating valve, mechanical stirrer, and air diffuser. The seed sludge of the SBRs was obtained in the aerobic tank of the A^2/O (anaerobic–anoxic–oxic [AAO]) process in the Shuofang Wastewater Treatment Plant (Wuxi, China). The initial mixed liquor suspended solids (MLSS) in SBRs were adjusted to about 5.5 g/L. The cycle for each SBR lasted 12 h, and two cycles were conducted per day. Each cycle included anaerobic (6 h), anoxic (2.5 h), and aerobic (2.5 h) sections; settlement (0.5 h); and decanting (0.5 h). The groups of E_{NaAc} and E_{CAPW} were run for 100 days, and the group of E_{CK} was run for 75 days. The detailed operating parameters of different reactors are listed in Table 2. The samples were collected from two parallel running SBRs and mixed homogenously for experimental analysis.

Table 1
Characteristics of LCPW and CAPW

Parameters	LCPW	CAPW	Unit
pH	7.2 ± 1.2	4.7 ± 0.3	-
Chemical oxygen demand (COD)	213 ± 18.5	18870 ± 220	mg/L
Biochemical oxygen demand (BOD_5)	15.6 ± 3.6	12270 ± 162	mg/L
BOD_5/COD	0.07 ± 0.02	0.65 ± 0.01	-
Total nitrogen (TN)	33.1 ± 4.1	263 ± 17	mg/L
Total phosphorus (TP)	3.2 ± 0.6	13.9 ± 0.11	mg/L
Ammonia nitrogen (NH_4^+-N)	18.1 ± 3.1	77.8 ± 3.2	mg/L
Nitrate nitrogen (NO_3^--N)	2.2 ± 0.41	18.3 ± 1.3	mg/L
Nitrite nitrogen (NO_2^--N)	1.3 ± 0.06	1.3 ± 0.3	mg/L

LCPW: Low-concentration polyether wastewater;
CAPW: Complex carbon source derived from citric acid production wastewater.

2.3. Analytical method

The concentrations of COD, TN, TP, NH_4^+-N, NO_3^--N, NO_2^--N, MLSS, mixed liquor volatile suspended solids (MLVSS) and pH were analyzed according to standard methods [13]. The concentrations of proteins and carbohydrates were measured using Coomassie Brilliant Blue G250 [14] and phenol–sulfuric [15], respectively. The analysis of extracellular polymeric substance (EPS) components was carried out in accordance with the methods developed by Bourven et al [16]. The concentration of DO was detected by a portable DO probe (WTW Company, German).

2.4. Analysis of enzyme activity

The dehydrogenase activity (DHA) was determined as follows: 2 mL of activated sludge samples were separately extracted from seed sludge, and homogenously mixed sludge samples from two parallel E_{CAPW} (90th day), E_{NaAc} (90th day) and E_{CK} (75th day). Four sludge samples were separately added into tubes, and then 2 mL of 2,3,5-triphenyl tetrazolium chloride (0.5%, w/v), 2 mL of tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) buffer (pH = 7.6), and 2 mL of glucose (0.1 M) were added successively. The tubes were placed in a shaking bed at 37 °C for 24 h. Thereafter, the reaction was stopped by adding 2 mL of formaldehyde. Then, 5 mL of dichloromethane was further added to extract the red triphenyl formazen (TF). The organic phase was collected by centrifugation at 8000 rpm for 10 min, and the absorbance of the TF solution was measured at 485 nm. Finally, the DHA was calculated from the calibration curve of TF and described as per mg-TF/(g-MLVSS·h).

2.5. Analysis of microbial communities

The total genomic DNA samples were separately extracted from

Table 2
Detailed operating parameters of E_{NaAc}, E_{CAPW}, and E_{CK}.

Groups	Parameters	Periods						
		I	II	III	IV	V	VI	VII
E_{NaAc}	COD-NaAc (mg/L)	500	450	400	300	100	0	0
	COD-LCPW (mg/L)	0	50	100	200	200	220	220
	DO range (mg/L)	0-5.5	0-5.5	0-5.5	0-5.5	0-5.5	0-5.5	0-3.5
	Operation time (d)	0-10	11-20	21-30	31-40	41-50	51-80	81-100
E_{CAPW}	COD-CAPW (mg/L)	500	450	400	300	100	0	0
	COD-LCPW (mg/L)	0	50	100	200	200	220	220
	DO range (mg/L)	0-5.5	0-5.5	0-5.5	0-5.5	0-5.5	0-5.5	0-3.5
	Operation time (d)	0-10	11-20	21-30	31-40	41-50	51-80	81-100
E_{CK}	COD-LCPW (mg/L)	50	100	-	-	-	-	-
	DO range (mg/L)	0-5.5	0-5.5	-	-	-	-	-
	Operation time (d)	0-10	11-75	-	-	-	-	-

E_{NaAc} and E_{CAPW}: NaAc and CAPW were separately used as co-metabolic substrates.
E_{CK}: Without co-metabolic substrate.
Periods I-VII for E_{NaAc} and E_{CAPW}: Concentration of LCPW was gradually increased while concentrations of co-metabolic substrates were gradually decreased.
Periods I–II for E_{CK}: Concentration of LCPW was gradually increased without co-metabolic substrates.
COD-NaAc, COD-CAPW and COD-LCPW: COD concentrations in the influent of SBR were separately provided by NaAc, CAPW and LCPW.

seed sludge, and homogenously mixed sludge from two parallel E_{CAPW} (90th day), E_{NaAc} (90th day) and E_{CK} (75th day) by E.Z.N.A. Soil DNA isolation kit. The four DNA samples were sent to Meiji, Inc. (Shanghai, China) for high-throughput sequencing. The primers were 338 F (5′-ACTCC TACGG GAGGC AGCAG-3′) and 806R (5′-GGACT ACHVG GGTWT CTAAT-3′) for PCR amplification of the V3–V4 region of 16S rRNA genes [17]. Pyrosequencing was conducted on Illumina HiSeq platform using the MOTHUR program, and the similar sequences were clustered into operational taxonomic units (OTUs) at the threshold of 97% identity. The alpha diversity indexes of Sobs, Shannon, Chao 1, abundance-based coverage estimator (ACE) and Coverage were separately analyzed to evaluate microbial diversity.

2.6. Statistical analysis

The samples were analyzed in triplicate, and the results were presented as mean ± standard deviation. The one-way analysis of variance was used to test the significance of the results, and $p < 0.05$ was considered as a statistical criterion.

3. Results and discussion

3.1. Performance of LCPW treatment

The MLSS and the ratio of MLVSS/MLSS during the treatment of LCPW are presented in Fig. 1. The observed decrease in the concentration of MLSS and the ratio of MLVSS/MLSS in each group at the beginning of the reactors' operation was evident in E_{CK}. The MLSS and the ratio of MLVSS/MLSS were separately decreased from the initial 5.5 g/L and 57.3% to 1.8 g/L and 33.2% by the 30th day, and then remained stable. By contrast, the downward trends of E_{CAPW} and E_{NaAc} were moderate and comparable to each other. The average concentrations of MLSS and the average ratios of MLVSS/MLSS after stabilization were 4.1 g/L and 59.3% for $E_{CAPW;}$ 3.2 g/L and 55.3% for E_{NaAc}, respectively. The result is due to the toxic pollution in LCPW exerting a damaging effect on the metabolic system of the activated sludge to an extent and reducing microbial activity, whereas adding NaAc or CAPW during LCPW treatment process could promote the degradation of toxic substances to ensure the normal metabolic function of activated sludge [18].

Fig. 2 shows the variations and trends in the concentrations of COD, TN, and TP during the treatment of LCPW. With the LCPW concentration increasing from 50 mg/L to 100 mg/L and the run of E_{CK} lasting up to 75 days, the improvement in the treatment of LCPW was not evident,

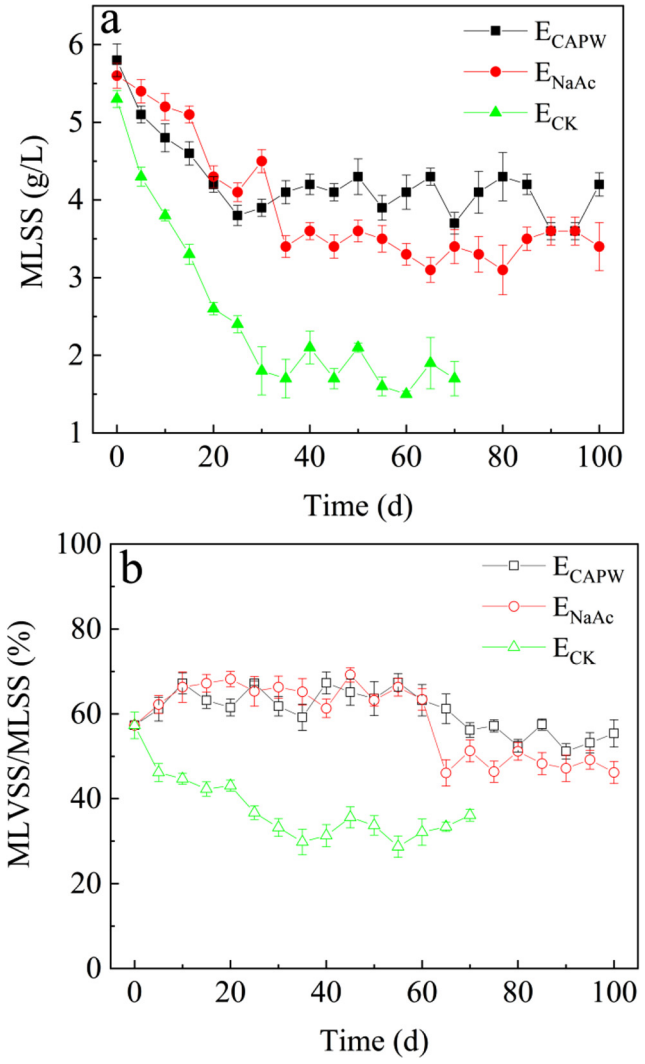

Fig. 1. Concentration variations of MLSS (a) and MLVSS/MLSS (b) in E_{CK}, E_{NaAc}, and E_{CAPW}.

as demonstrated in Fig. 2a. Without the assistance of co-metabolic substrates, the refractory and toxic organics presented in LCPW might inhibit microorganisms from degrading organic matter for a long time.

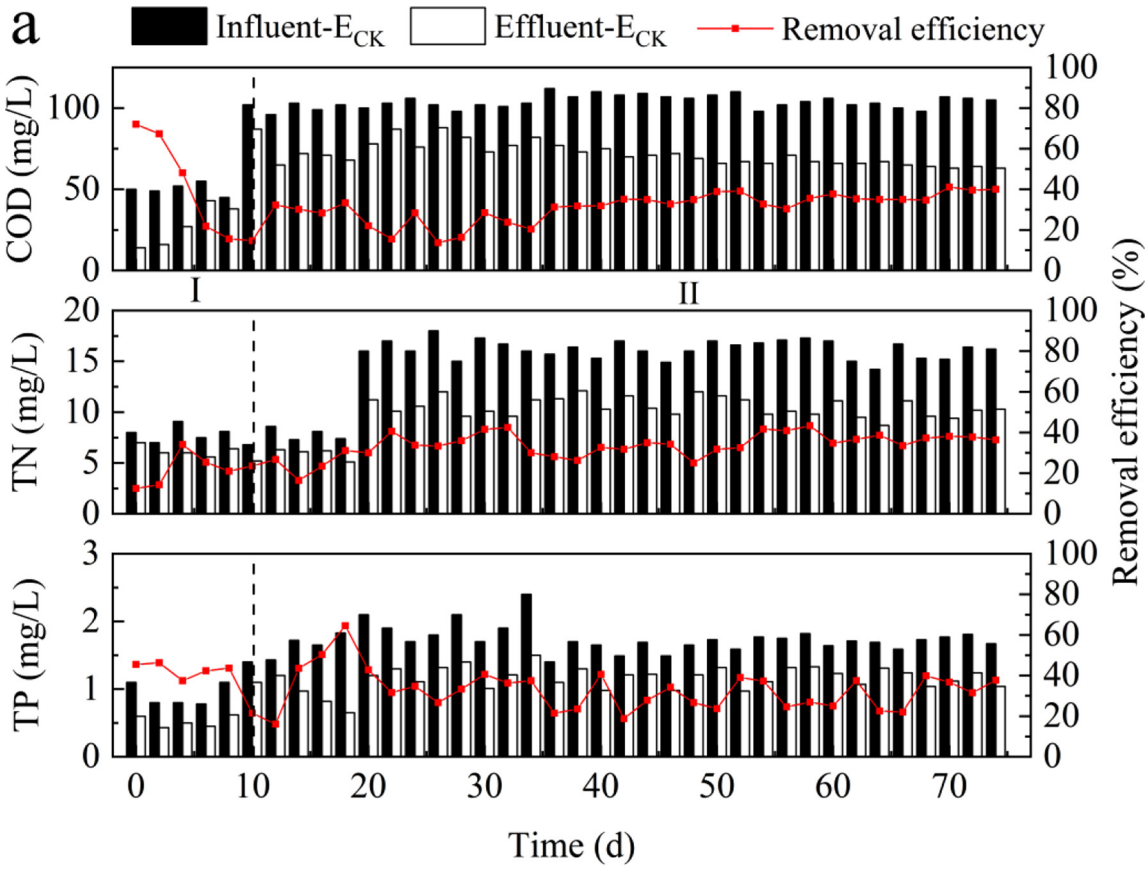

Fig. 2. Concentration variations of COD, TN, and TP in E_{CK} (a), E_{NaAc} (b), and E_{CAPW} (c).

The average removal efficiencies of COD, TN, and TP were only 36.1%, 36.2%, and 30.7%, respectively. The reasons for these efficiencies could be the observation that the residual microorganisms could only adapt to the toxicity but that their metabolic system for the degradation of pollutants might be damaged. Incomplete degradation of the toxic organics in E_{CK} would inhibit the hydrolysis of organic nitrogen and phosphorus and the supply of external carbon source, thereby resulting in inefficient biochemical processes of nitrogen and phosphorus removal. The data presented in Figs. 1 and 2a provide reliable evidence that LCPW could not be directly and effectively treated with activated sludge to satisfy the discharge standard under the operating conditions of SBRs.

Figs. 2b and c showed the performance in the runs of E_{NaAc} and E_{CAPW}. Although the influent concentrations of LCPW and co-metabolic substrate were gradually increased and decreased, respectively, the performance of LCPW treatment remained stable and efficient continuously from the 1 st day to the 50th day. Given that the concentrations of LCPW and co-metabolic substrates were changed slowly during the runs of E_{CAPW} and E_{NaAc}, these results could be interpreted as the microorganisms having sufficient time to adapt to the LCPW quality changes. Meanwhile, the concentrations of COD, TN, and TP in the effluents of E_{NaAc} and E_{CAPW} satisfied the discharge standard (COD ≤ 50 mg/L, TN ≤ 15 mg/L, and TP ≤ 0.5 mg/L) except for the COD concentration in the effluent of E_{CAPW} from the first 10 days. The observation indicated that the adaptation period of microorganisms to CAPW was about 10 days because some macromolecular organics matter (such as polysaccharides and proteins) required to be hydrolyzed before being directly utilized by microorganism. The removal performance of COD in E_{NaAc} was better than that in E_{CAPW} from the 1 st day to the 50th day ($P < 0.05$). It was observed that the utilization efficiency of NaAc by microorganisms was faster than that of CAPW, which would further accelerate the non-specific enzymatic reaction of detoxification and degradation of pollutants [19]. Moreover, a small number of refractory organics contained in CAPW could have increased the co-metabolic burden. The concentrations of COD and TP in the E_{NaAc} and E_{CAPW} effluents can still satisfy the discharge standards without co-metabolic substrate addition after the 50th day. The average removal efficiencies of COD and TP for E_{NaAc} and E_{CAPW} all exceeded 85% and 90%, respectively. It could be speculated that the microorganisms had been acclimated by adding NaAc or CAPW in the previous 50 days, and may utilize directly hydrolyzed small molecule organics as growth substrates to mineralize and degrade the non-growth substrates. Furthermore, the removal efficiency of COD in E_{CAPW} was higher than that in E_{NaAc} from 76th day to 100th day ($P < 0.05$). The microbial diversity would be higher in E_{CAPW} than that in E_{NaAc} because the organic composition of CAPW is more complex [9]. Hence the satisfactory performance of LCPW treatment in E_{CAPW} might be the more complex metabolic pathways that led to thorough pollution degradation effect.

Nitrogen accumulated in the effluents of E_{NaAc} and E_{CAPW} to the extent from 50th day to 60th day, so that the concentration of TN exceeded the discharged standard (Figs. 2b and 2c). The ratio of COD/TN in LCPW was only 6.1 (Table 1), and the level of DO concentration was high (0–5.5 mg/L). These conditions may lead to insufficient carbon sources used in the denitrification process. Thereafter, exactly 30 mg/L of NaAc and CAPW (calculated as COD) were separately added into anoxic sections of SBRs from 62th day to 80th day to enhance nitrogen removal. Subsequently, the concentration of TN in the effluent was gradually decreased and then satisfied the discharge standard. The concentrations of COD in the effluents of E_{NaAc} and E_{CAPW} were observed to occasionally exceed the wastewater discharge standard (e.g., days 58, 62 and 75). It might be due to the carbon source addition (30 mg/L) to the anoxic section that was not fully utilized, and simultaneously the nitrogen removal process of SBRs was post-

denitrification, thereby making the residual organics matter difficult to be further removed.

Optimizing the addition amount of carbon source may be one of the solutions to the problems mentioned above, although it may not be the best option. The maximum DO concentrations of E_{NaAc} and E_{CAPW} were adjusted to not exceed 3.5 mg/L starting from the 80th day to decrease the organic matter consumption and facilitate nitrite accumulation [17]. The accumulation efficiencies of nitrite for E_{NaAc} and E_{CAPW} were separately up to 58.5% and 42.9% on the 90th day of reactor operations (Supplementary data, Fig. S2). The low level of DO is contribute to enhance the shortcut nitrification and denitrification reaction, which had faster nitrogen removal efficiency and lower carbon source consumption compared with the traditional denitrification reaction [17]. Overall, a standardization treatment of LCPW was ultimately achieved without adding external carbon sources.

3.2. Analysis of EPS content and DHA

EPS, which mainly accumulates on the cell surface, plays an important role in maintaining the physical and chemical characteristics of activated sludge, and protecting microorganisms from damage of toxic pollutants [20,21]. The organic carbon concentrations of proteins and polysaccharides usually account for 70%–80% of EPS [22]. Here, the EPS content was represented by the sum of the contents of proteins and polysaccharides. Dehydrogenase is involved in all energy metabolic pathways of microorganisms and related to the oxidation of organic matter [23].

To further explain the performance differences of LCPW treated with acclimated activated sludge, the EPS content and DHA per unit mass of activated sludge in seed sludge and homogenously mixed sludge samples from two parallel E_{CAPW} (90th day), E_{NaAc} (90th day) and E_{CK} (75th day) were separately analyzed (Fig. 3). The results demonstrated that the EPS content and DHA in E_{CAPW}, E_{NaAc}, and E_{CK} decreased relative to the seed sludge. The sludge from E_{CAPW} and E_{NaAc} could retain most EPS content and DHA levels, whereas the EPS content and DHA in E_{CK} decreased steeply by 3.10- and 22.3-fold, respectively. The EPS formation and dehydrogenase expression may be related to the degradation and utilization of co-metabolic substrates and pollutants. As aforementioned, the activated sludge had been completely acclimated during the first 50 days of E_{CAPW} and E_{NaAc} operations, whereas the activated sludge in E_{CK} was hard to be directly acclimated without the assistance of co-metabolic substrates; as a result, the microorganisms enriched in E_{CAPW} and E_{NaAc} could directly and effectively degrade toxic pollutions into low–molecular organics, which could be assimilated by microorganisms to satisfy self-growth. Some microbial metabolites (e.g., polysaccharides or proteins) as energy storage substances would be secreted outside the cell to form EPS. In contrast, the toxic pollutions were degraded insufficiently in E_{CK} owing to the damage of microbial metabolic system. In this harsh living environment, these residual microorganisms could only assimilate limited carbon and energy sources as growth substrate, thereby resulting in the low capacity of EPS production and dehydrogenase expression [9,24]. Also, the higher EPS content and DHA in E_{CAPW} than E_{NaAc} could be elucidated by the following reasons. Compared with the CAPW, the NaAc is a more easily biodegradable carbon source that can be utilized quickly between 6 and 9 hours, leading to mutual competition and endogenous respiration between microorganisms to increase EPS production [25]. However, given that the sampling time on activated sludge from E_{CAPW} and E_{NaAc} was 90th day, and the co-metabolic substrates were not added after the 50th day. The contribution of co-metabolic substrates on the EPS production and dehydrogenase expression might have been insignificant, whereas the primary factor might be related to the degradation degree of pollutions. The more thoroughly the pollutants were degraded, the more small molecular carbon sources could be assimilated by microorganisms. Activated sludge acclimated with complex carbon sources exhibits higher bacterial diversity, complex metabolic pathways and complex organics utilization efficiency compared with that acclimated by single carbon sources [26]. Given that E_{CAPW} had higher COD removal efficiency than E_{NaAc} from 76th day to 100th day (see Figs. 2b and c), it could be deduced that the microorganisms in E_{CAPW} could obtain more carbon sources derived from the pollutions degradation, thereby improving the EPS content and DHA.

3.3. Microbial community analysis

The diversity and abundance of microbial communities in seed sludge and homogenously mixed sludge samples from two parallel E_{CAPW} (90th day), E_{NaAc} (90th day) and E_{CK} (75th day) were separately analyzed by high-throughput sequencing. The rarefaction curves of the four samples tended to plateau, signifying that the bacterial communities were completely involved in this study. The Venn analysis of the OTUs of the four samples is displayed in Fig. 4. The observed numbers of OTUs of bacterial sequences were 1022, 764, 589, and 478 for seed sludge, E_{CAPW}, E_{NaAc}, and E_{CK}, respectively. Moreover, only 155 OTUs were shared by them. These data indicated the significant difference among the four samples.

Several indexes, such as OTUs, Good's coverage, Shannon, Chao1, and ACE at a cutoff level of 3%, were calculated as described in Table 3. The coverage of the four samples of activated sludge ranged from 99.5% to 99.6%, indicating that the microbial community was almost covered completely. The diversity of bacteria in E_{CAPW}, E_{NaAc}, and E_{CK} was gradually decreased relative to that of seed sludge, as evidenced by the indexes of Shannon, Chao1, and ACE. The diversity variation might be due to the toxic and refractory organic matters inhibited significantly the growth and metabolism of some microorganisms, whereas the addition of co-metabolic substrates could attenuate this inhibition. Correspondingly, microorganisms enriched by complex carbon source showed higher microbial diversity than those enriched by single carbon source [20], which is consistent with the results of the higher diversity in E_{CAPW} than E_{NaAc}.

The relative abundance of the top 30 identified bacteria in the phylum and family levels derived from seed sludge, E_{CAPW}, E_{NaAc}, and E_{CK} was analyzed on the basis of the OTU sequence alignments as illustrated in Fig. 5. *Proteobacteria* (39.3%), *Bacteroidetes* (14.1%), and *Chloroflexi* (12.6%) were the three main phyla in seed sludge (Fig. 5a), and the result was in accordance with the previous research of Tang et al. [27]. The total relative abundance of *Proteobacteria* and *Bacteroidetes* for E_{CAPW}, E_{NaAc}, and E_{CK} was increased to 63.3%, 87.2%, and 82.4%, respectively. By contrast, the proportions of *Chloroflexi* in E_{CAPW}, E_{NaAc}, and E_{CK} was decreased to 6.5%, 8.1%, and 0.9%, respectively. *Proteobacteria* are related to the removal of nitrogen and phosphorus and have the exceptional ability to degrade some refractory organics [9,27]. *Bacteroidetes* are widely distributed in soil, sea water, sediments, and so on, and they have the potential to degrade cellulose and polycyclic aromatic hydrocarbons [9]. Consequently, these properties might imply that these bacteria have better endurance and degradation capacity for some macromolecules and toxic organics, resulting in the increase of their proportions. *Chloroflexi* appear commonly in wastewater treatment systems for long sludge ages, feed on soluble microbial products, and can degrade low-concentration phenol [9,28]. Compared with *Proteobacteria* and *Bacteroidetes*, *Chloroflexi* have a lower adaptive capacity for the pollutants carried in LCPW. Hence, they might not be suitable for growth in the LCPW system.

Fig. 5b illustrates the variation of the bacterial community structure among four samples at the family level. *Rhodocyclaceae* (13.4%), *Gemmatimonadaceae* (8.6%), *Nitrosomonadaceae* (6.5%), *Burkholderiaceae* (6.3%), *Blastocatellaceae* (5.1%), and *Saprospiraceae* (5.0%) were the top six families in seed sludge. All of them are widely detected in municipal sewage treatment plants and are related to the removal of nitrogen, phosphorus, and COD [17,27,29], which indicates the normal physiological function of seed sludge. Compared with seed sludge, the

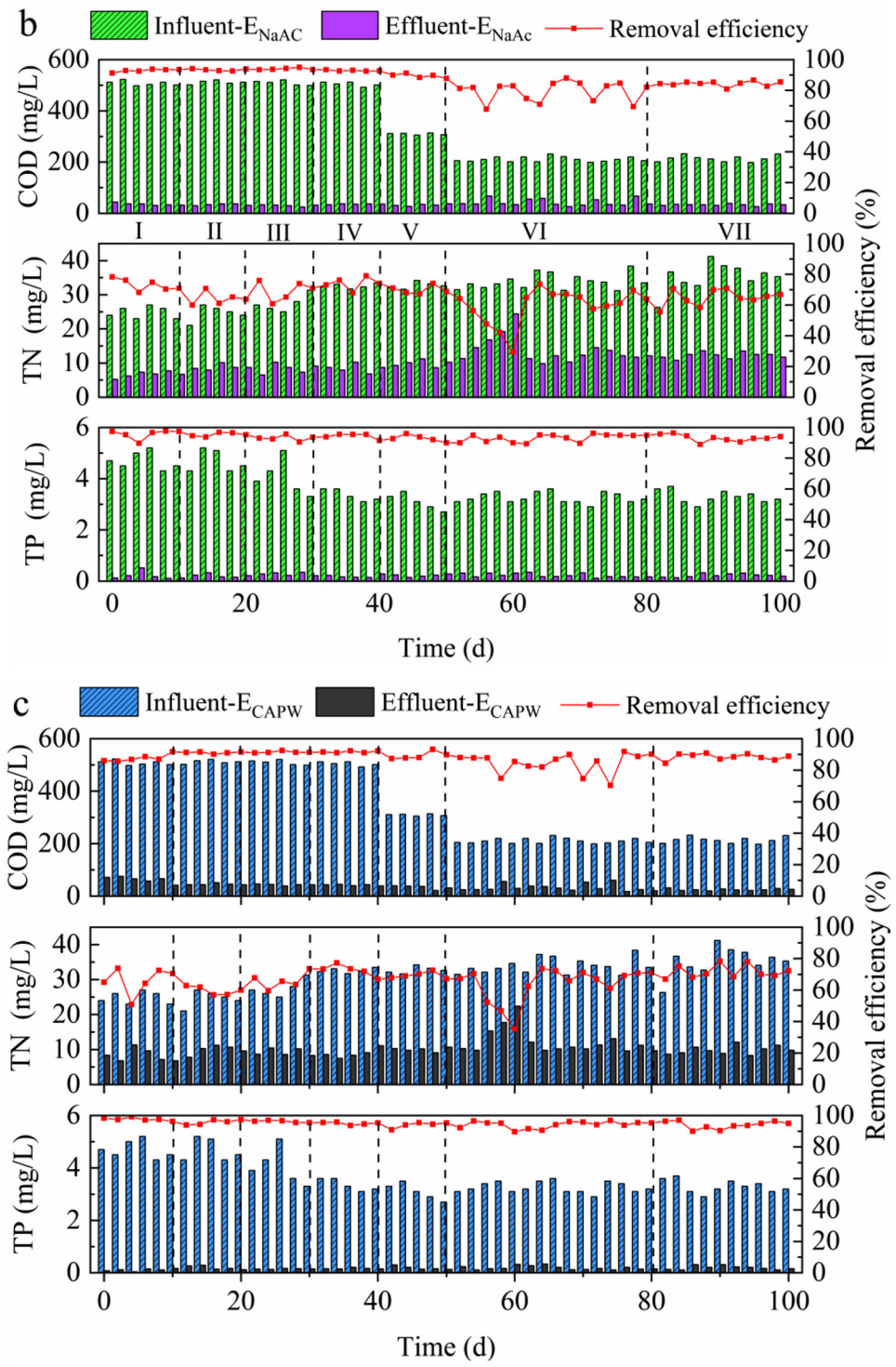

Fig. 3. Analysis of EPS content (a) and DHA (b) for unit mass activated sludge in seed sludge, $E_{CAPW\text{-}90d}$, $E_{NaAc\text{-}90d}$, and $E_{CK\text{-}75d}$.

dominant families in E_{CAPW}, E_{NaAc}, and E_{CK} were gradually changed, and the changes of E_{CK} were most obvious. It could be deduced that partial microorganisms in seed sludge could survive in E_{CAPW} and E_{NaAc} due to the toxic effects reduced by the addition of co-metabolic substrates.

The relative abundance of *Nitrosomonadaceae* and *Rhodocyclaceae* was 17.5% and 10.5% for E_{CAPW}; 18.5% and 8.7% for E_{NaAc}, respectively, whereas the total relative abundance of these families was only 1.2% for E_{CK}. It had been reported that *Nitrosomonadaceae* and *Rhodocyclaceae* are responsible for nitrogen removal by nitrification and denitrification processes, and could survive in petrochemical wastewater [30,31], which suggests that these families may have certain tolerance to toxicity. Thus, these families acclimated with co-metabolic substrates may be suitable for growth in LCPW. The total proportion of these families was decreased significantly in E_{CK}, which could be inferred as follows. Although, these families have a certain ability to degrade toxic substances, the toxic effects of LCPW may have exceeded the endurance capacity of these families without co-metabolic substrates. The abundance differences of these families were consistent with the higher biological nutrients removal efficiencies in E_{CAPW} and

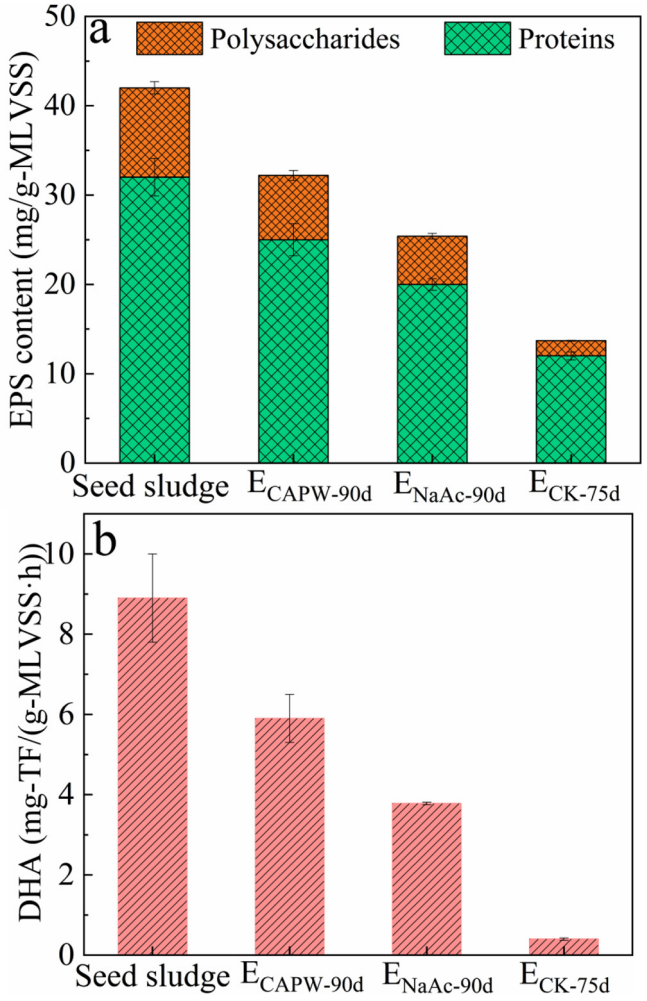

Fig. 4. Venn analysis of OTUs of sludge samples.

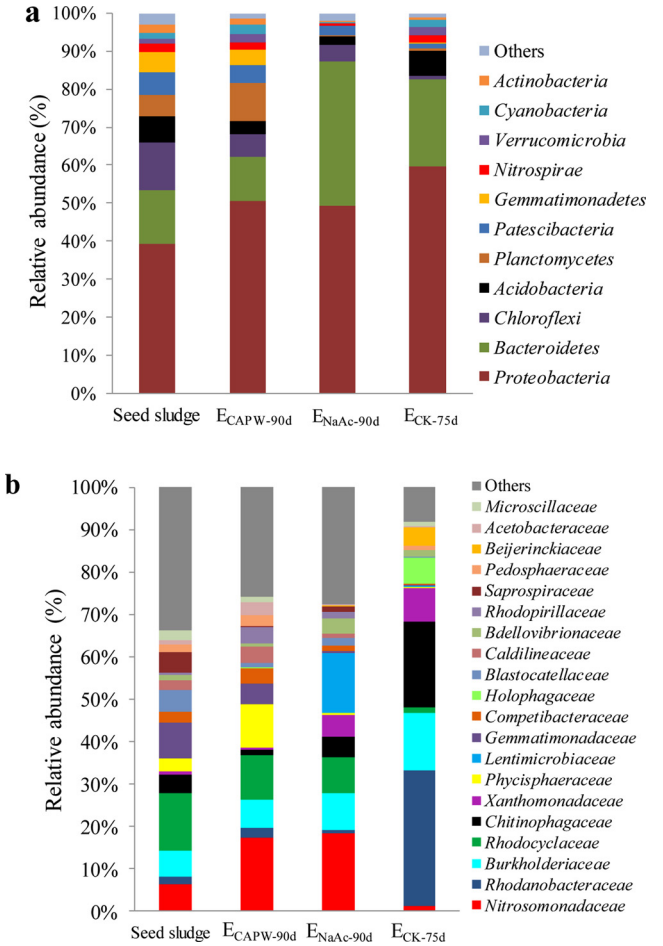

Fig. 5. Bacteria community structure in seed sludge, $E_{CAPW\text{-}90d}$, $E_{NaAc\text{-}90d}$, and $E_{CK\text{-}75d}$ at phylum (a) and family (b) levels.

Table 3
Parameters of microbial communities in seed sludge, $E_{CAPW\text{-}90d}$, $E_{NaAc\text{-}90d}$, and $E_{CK\text{-}75d}$.

Samples	OTU	Shannon	Sobs	Chao 1	ACE	Coverage (%)
Seed sludge	1022	5.68	1022	1066	1067	99.5
$E_{CAPW\text{-}90d}$	764	4.85	764	871	870	99.5
$E_{NaAc\text{-}90d}$	589	4.37	589	698	706	99.6
$E_{CK\text{-}75d}$	478	3.83	478	573	550	99.6

E_{NaAc} than in E_{CK} (see Figs. 2b and 2c).

Chitinophaga (20.1%) and *Rhodanobacteraceae* (32.1%) are responsible for denitrification [32,33], and were enriched in E_{CK}, but were rarely detected in E_{CAPW} and E_{NaAc}. It could be inferred from the results of insufficient nitrogen removal in E_{CK} (see Fig. 2a) that these families might not effectively perform denitrification functions. Although these families could survive in E_{CK}, the incomplete degradation of pollutions would limit the hydrolysis of organic nitrogen and phosphorus, thereby causing nitrogen and phosphorus hard to be removed through biological processes. Moreover, the higher proportions of these families in E_{CK} might also be attributed to the reduction of microbial diversity.

Phycisphaeraceae (10.3%) were mainly enriched in E_{CAPW} but rarely detected in E_{NaAc} (0.27%) and E_{CK} (0.25%), whereas *Lentimicrobiaceae* (14.3%) were mainly enriched in E_{NaAc} but rarely detected in E_{CAPW} (0.04%) and E_{CK} (0.07%). It had been reported that *Phycisphaeraceae* can participate in global nitrogen cycle and degrade the by-products of petroleum industry [34,35], and *Lentimicrobiaceae* was detected in a full-scale upflow anaerobic sludge blanket reactor to treat high-strength starch organic wastewater [36]. The properties of these bacteria may help degrade complex refractory substrates and promote the nitrogen removal. These families may perform similar functions, but were separately enriched in E_{CAPW} and E_{NaAc}, which was probably due to different carbon sources with different metabolic mechanisms, thereby leading to different microbial community structures [37]. Overall, the addition of co-metabolic substrates reduced the damage of toxic organics to activated sludge metabolic system, and facilitated the enrichment of functional microorganisms.

4. Conclusions

Activated sludge, which was acclimated separately by using NaAc and CAPW as co-metabolic substrates, could directly treat LCPW without the addition of co-metabolic substrates after acclimation. Activated sludge acclimated with CAPW showed better COD removal effect, higher EPS content and DHA, and higher bacteria diversity. The bacteria related to nitrogen removal and macromolecular degradation were enriched (e.g., *Nitrosomonadaceae*, *Phycisphaeraceae*, and *Lentimicrobiaceae*) after acclimation.

CRediT authorship contribution statement

Wenhao Liu: Validation, Software, Investigation, Writing - original draft, Methodology. **Zhiyong Zheng:** Conceptualization, Supervision, Methodology, Validation, Visualization, Writing - review & editing.

Fuxin Sun: Resources, Investigation. **He Liu:** Conceptualization, Supervision, Methodology, Validation, Visualization, Writing - review & editing. **Min-Hua Cui:** Writing - review & editing. **Yuan Ye:** Writing - review & editing. **Yan Zhang:** Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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