Change the original microbial community structure in the hydrolysis acidification tank to enhance the COD removal performance of oily wastewater

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ABSTRACT

The hydrolysis acidification tank mainly relies on microorganisms to treat oily sewage, but in many cases the chemical oxygen demand (COD) of the effluent from the hydrolysis acidification tank does not decrease or even increase. In this work, about 50 L of oily wastewater is treated in a facultative anaerobic hydrolysis acidification tank with a temperature of 29 °C, pH 6, high-throughput sequencing technology analyzes found that after long-term operation of the hydrolysis and acidification tank, the dominant bacterial *Pseudomonas* accounted for only 2.87%, at this time, the effluent COD of the hydrolysis and acidification tank was 450 mg/L. *Pseudomonas stutzeri* LH-42 a strain screened in the laboratory, was domesticated and colonized in the hydrolysis acidification tank. High-throughput sequencing and bioinformatics analysis showed that the proportion of *Pseudomonas* in the hydrolysis acidification tank reached 5.89%, the effluent COD of the hydrolysis and acidification tank was 200 mg/L. The above results indicate the importance of the proportion of *Pseudomonas* in the hydrolysis and acidification tank for the COD degradation of oily wastewater.

Key words: COD, hydrolysis acidification, oily sewage, *Pseudomonas stutzeri* LH-42

HIGHLIGHTS

- After the bacteria intensification, the COD removal rate of the oily wastewater by the hydrolysis acidification reactor increased by 50%.
- The biodegradability of wastewater has also been correspondingly improved.
- The microbial community structure in the hydrolysis acidification reactor showed obvious changes.
- Provide a practical way to improve the efficiency of hydrolysis and acidification.
- The role of *Pseudomonas*.

INTRODUCTION

There is a large amount of oil, suspended solids, heavy metals and other substances in oily sewage. If they are not treated, they can be discharged arbitrarily, which is extremely harmful to soil, aquatic organisms, human health and crop growth. (Cabezas *et al.* 2020), the treatment of oily wastewater has become an urgent environmental issue in China (Desilva 2015).Various oily wastewater treatment technologies have been developed, the hydrolytic acidification–aerobic biological treatment process is considered an important link in the treatment of oily wastewater, which converts the insoluble organic matter in the original oily wastewater into dissolved organic matter, the soluble organic matter is absorbed and utilized by the hydrolytic acidifying bacteria, which ultimately improves the biodegradability of oily sewage to facilitate subsequent aerobic treatment (Koo *et al.* 2016; Wang *et al.* 2017). The diversity and structure of the microbial flora in the hydrolysis acidification tank has a significant impact on the conversion of the refractory organic matter in the oily sewage into the easily degradable organic matter.

Due to the biological toxicity of polycyclic aromatic hydrocarbons, long-chain hydrocarbons, and heavy metals such as iron, manganese, zinc and the biological tolerance of the system in oily sewage will cause poisoning and loss of the dominant bacteria in the hydrolysis acidification tank. Also the lost bacteria micelles will show an increase in chemical oxygen demand (COD) in the effluent test, which will cause a serious load on the downstream aerobic treatment (Yu *et al.* 2017). Therefore, the optimization of the microbial flora in the hydrolysis and acidification tank is getting increasing attention, in the past, the optimization of the microbial flora structure in the hydrolysis acidification tank involved mainly in two aspects: co-substrate

enhancement and bacterial agent enhancement (Cai *et al.* 2019). Compared with the co-substrate, the addition of microbial agents has a greater impact on the changes in the microbial community structure in the hydrolysis acidification tank. At present, the dominant bacteria belonging to the phyla Proteobacteria and Firmicutes. These are used as intensifying bacteria, and they are added to the hydrolysis and acidification reactor by means of fed bacteria. After the enhancement of bacteria, the COD removal rate of the effluent from the hydrolysis acidification tank is significantly higher than before the enhancement. At the same time, the biochemical oxygen demand (BOD)/COD value of oily wastewater is also significantly increased, its biodegradability is significantly improved, and the species richness in the hydrolysis acidification tank is increased, the microbial community structure has changed significantly, and the dominant bacteria in the hydrolytic acidification system, Bacteroidetes, can maintain the advantage. Proteobacteria and Firmicutes bacteria in the system have been significantly strengthened (Zhang *et al.* 2010), these superior microorganisms can fully degrade the long-chain hydrocarbons and insoluble organics in the oily wastewater and then complete the intracellular biochemical reaction, which reduces the organic content in the wastewater and the COD of the wastewater. Although the effect of the bacteria is obvious in a short time, but due to the effect of organic matter such as polycyclic aromatic hydrocarbons, long-chain hydrocarbons and heavy metals such as iron, manganese, zinc, the biological toxicity tolerance is poor. The microbial community structure is unstable after the system runs for a long time, and the performance of treating oily sewage is also reduced. Compared with ordinary strains, *Pseudomonas* shows high tolerance to oxidative stress of metal ions in the environment and can degrade aromatic hydrocarbons in petroleum and remove organic sulfur (Bosch *et al.* 2000; Lanfranconi *et al.* 2009; Hu *et al.* 2019a; Zhang *et al.*). Therefore, it is a feasible method to use this bacteria to treat oily wastewater.

In this study, we used the *Pseudomonas stutzeri* LH-42 strain that was screened from the oil field soil in the early stage as an intensifying microbial agent. After being adapted by oily sewage, it is planted in a hydrolysis acidification tank, which can not only increase the abundance of hydrolysis acidification pond species from a more accurate level of microbial classification (genus level), but also change the microbial community structure, and significantly enhance the COD removal rate of oily wastewater. The BOD/COD value of oily wastewater was obvious improved, biodegradability has also been greatly improved, and because the strain has a good desulfurization effect and can produce surfactants, it plays an important role in degrading long-chain hydrocarbons in oily sewage. Therefore, it has strong tolerance in the environment to organic substances such as polycyclic aromatic hydrocarbons, long-chain hydrocarbons, and heavy metals such as iron, manganese and zinc, which are biologically toxic, *Pseudomonas stutzeri* LH-42 strain as an intensifier will not be poisoned and lost, so the hydrolysis acidification tank can maintain long-term and efficient oily wastewater treatment performance. This provides new, more effective and stable microbial intensifiers for sewage treatment plants in the future, which can not only better improve the performance of the hydrolysis acidification tank, but also save many costs for enterprises.

MATERIALS

EZNA® water DNA kit; EZNA® Gel Extraction Kit; 6× loading DNA Buffer; Ethidium bromide (EB); Agarose (Agarose); Dl2000 Plus DNA Marker (Thermo Scientific); 50× TAE buffer; 1 mol/L $K_2Cr_2O_7$ solution; $HgSO_4$ solution; $AgSO_4$ solution; 0.2 mol/L NaOH solution; 0.2 mol/L HCl solution; 1 mol/L $CaCl_2$ buffer solution; 1 mol/L $MgSO_4$.

METHODS

Sample collection and analysis

The water samples came from Changling Sewage Treatment Plant in Yunxi District, Yueyang City, Hunan Province, China. The plant uses CAST treatment technology, 10 kilograms of water samples were collected from two sewage mixed influent (TSM), a contact oxidation pond effluent (COP), a hydrolysis acidification tank (HRT), and an oxidation ditch effluent (ODP). The water samples were collected and sent to the Modern Analysis and Test Center of Central South University, and the water samples were analyzed using inductively coupled plasma-emission spectroscopy at the test center (Table S4).

After analyzing the elements of the four water samples, a rapid digestion spectrophotometric method was used to detect the COD of the four water samples. The relevant reagents need to be prepared before detection: D reagent and E reagent, the preparation of reagent D requires weighing 2.4544 g of the basic substance potassium diloxate that has been dried at 120 °C for 2 hours in advance, adding 5.7143 g mercury sulfate, then adding 6.2 mL concentrated sulfuric acid to dissolve, then cool to room temperature, and dilute to 100 mL. The preparation of reagent E is relatively simple, add 2.5 g of silver sulfate to 250 mL of concentrated sulfuric acid and leave it for 1–2 days. After matching the relevant testing reagents, we

used the laboratory's 5B-3A COD rapid tester (Shanghai Reunion Scientific Instrument Co., Ltd) to detect the COD of the four water samples (Yang *et al.* 2017).

The BOD of the hydrolysis acidification tank was tested, before testing we needed to prepare relevant reagents: 0.2 mol/L sodium hydroxide solution, 0.2 mol/L hydrochloric acid solution, and dilution water. Among them, the dilution water is the most important. In a 1,000 mL volumetric flask, 500 mL of water was added in advance, then 1 mL of calcium chloride solution, magnesium sulfate solution, and phosphate buffer solution were added respectively, and then diluted to 1,000 mL with water and mixed well. Keeping the solution at a constant temperature of about 20 °C, a small oil-free air pump was used for aeration. The bottle cap was covered with two layers of washed and dried gauze. The 5-day biochemical oxygen demand of this diluted water was less than 0.2 mg/L. After all the reagents were prepared, we used a BODTrak® II BOD analyzer (American Hach Company) to detect the BOD of the hydrolysis acidification tank (Kundu & Mishra 2013), the results are shown in Tables S1, S2, and S3 in Supplementary Information.

The hydrolysis acidification system is simulated and constructed

The hydrolysis and acidification system was simulated through two UASB reactors and continuous circulation aeration device (Figure S1 in Supplementary Information). With reference to the existing equipment of the sewage treatment plant, we simulated the construction of the tank body, filler, water distribution device and aeration device in the contact oxidation tank, and set the filler in the aeration tank as the carrier of the biofilm. After being oxygenated, the oily sewage flows through the filler at a certain flow rate, and contacts the biofilm, which interacts with the suspended activated sludge. The hydrolysis and acidification tanks are also equipped with a tank body, a water distribution system, an effluent collection device, and a mud discharge system. Finally, the oxidation ditch is designed as a circulating flow aeration ditch, connected end to end, so that the activated sludge can circulate with the water flow (Lotito *et al.* 2012). After the system is built, according to the hydraulic residence time of the oily sewage treatment plant, the HL-2S constant flow pump controls the hydraulic residence time in the range of 10–40 h, the water flow rate is 0.3–0.5 m/s, and the MEMS gas mass flowmeter controls the hydrolysis. The dissolved oxygen in the acidification tank is within 4%, and the inactive sludge in the hydrolysis acidification tank is regularly discharged.

Cultivation and domestication of strains

The strain *P. stutzeri* LH-42 is a Gram-negative bacterium isolated in the Liaohe Oilfield in the early stage of the laboratory (Zhang *et al.* 2017; Hu *et al.* 2019b), PHE can be used as the sole carbon source. When culturing, first remove the bacterial solution from the seed tube and spread it on the LB solid medium plate, add a little PHE on the lid of the Petri dish to invert the culture at a temperature of 30 °C. After about two days, large numbers of colonies are picked from the solid plate medium and inoculated into the inorganic salt liquid medium containing PHE. After about one week of culture, using continuous culture method, the oily sewage in the hydrolysis and acidification tank was cultured in 150 mL and 100 mL of inorganic salt and 50 mL *P. stutzeri* LH-42 bacteria liquid for mixed culture, and regular samples were taken to detect the *P. stutzeri* LH-42 bacteria in the culture medium. Growth conditions, and then the best-growing *P. stutzeri* LH-42 strain was selected for enrichment culture, and after enrichment culture, *P. stutzeri* LH-42 strain was colonized into the hydrolysis acidification tank by immobilization technology.

DNA extraction and PCR amplification

Samples of the secondary sewage mixed influent (TSM), contact oxidation tank effluent (COP), hydrolytic acidification tank effluent (HRT), and oxidation ditch effluent (ODP) collected through bacterial 16SrRNA gene pyrosequencing analysis microbial community genomic DNA was extracted from TSM, COP, HRT, ODP samples using the ENZA® soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S. USA) according to manufacturer's instructions. The DNA extract was checked on a 1% agarose gel, and DNA concentration and purity were determined using a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA). The hypervariable region V3–V4 of the bacterial 16S rRNA gene was amplified with primer pairs 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) using an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA). The PCR amplification of the 16S rRNA gene was performed as follows: initial denaturation at 95 °C for 3 min, followed by 27 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 45 s, and single extension at 72 °C for 10 min, and end at 4 °C. The PCR mixtures contained 5× *TransStart* FastPfu buffer 4 μL, 2.5 mM dNTPs 2 μL, forward primer (5 μM) 0.8 μL, reverse primer (5 μM) 0.8 μL, *TransStart* FastPfu DNA Polymerase 0.4 μL, template DNA 10 ng, and finally ddH₂O up to 20 μL. PCR reactions were performed in triplicate. The PCR product was extracted from a 2% agarose gel and purified using the AxyPrep DNA

Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to manufacturer's instructions and quantified using a Quantus™ Fluorometer (Promega, USA) (Yu *et al.* 2010; Magoč & Salzberg 2011; Chen *et al.* 2018).

Illumina MiSeq sequencing

Purified amplicons were pooled in equimolar concentrations and paired-end sequenced on an Illumina MiSeq PE300 platform/NovaSeq PE250 platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd (Shanghai, China). The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database. the optimized bacterial community analysis (HRT-1) of the hydrolysis acidification tank is consistent with the above process (Stackebrandt & Goebel 1994).

Processing of sequencing data

The raw 16S rRNA gene sequencing reads were demultiplexed, quality-filtered using fastp version 0.20.0 and merged using FLASH version 1.2.7 with the following criteria: (i) the 300 bp reads were truncated at any site receiving an average quality score of <20 over a 50 bp sliding window, and the truncated reads shorter than 50 bp were discarded, reads containing ambiguous characters were also discarded; (ii) only overlapping sequences longer than 10 bp were assembled according to their overlapped sequence. The maximum mismatch ratio of overlap region is 0.2. Reads that could not be assembled were discarded; (iii) Samples were distinguished according to the barcode and primers, and the sequence direction was adjusted, exact barcode matching, and two nucleotide mismatch in primer matching was applied.

Operational taxonomic units (OTUs) with 97% similarity cutoffs were clustered using UPARSE version 7.1, and chimeric sequences were identified and removed. The taxonomy of each OTU representative sequence was analyzed using RDP Classifier version 2.2 against the 16S rRNA database (eg. Silva v138) using a confidence threshold of 0.7 (Wang *et al.* 2007).

Treatment of sewage after optimization of hydrolysis acidification tank

After the *P. stutzeri* LH-42 strain was placed in the hydrolysis acidification tank, the simulated sewage treatment plant operated the device for a period of time, and COD and BOD changes in the effluent from the hydrolysis acidification tank were resampled. The specific steps are as described above.

Statistical analysis

PAST software was used to perform box plots using the parameter values shown in Figure 1. For this reason, the values were divided into two sets of values, corresponding to the time period before and after revision. In a box diagram, the 25–75% quartiles are drawn using boxes, and the median is shown with a horizontal line inside the box. To determine the difference between sets of values obtained before and after modifying each reactor, a Mann–Whitney test was used to determine whether the difference between the medians of the two sets of values was significant. This is a non-parametric test and does not assume a normal distribution. The Mann-Whitney test was performed using PAST software. The data were analyzed on the free online platform of Majorbio Cloud Platform (www.majorbio.com).

RESULTS AND DISCUSSION

The elements in the effluent of the pool, the effluent of the hydrolysis acidification pool, and the effluent of the oxidation ditch were analyzed,among them, 1#, 2#, 3#, and 4# respectively represent the mixed inflow of the two sewage, the effluent of the contact oxidation tank, the effluent of the hydrolysis acidification tank, and the effluent of the oxidation ditch. It can be seen from the analysis result table that a total of 36 elements was detected, among these elements. As (arsenic), B (boron), Ce (cerium), Cr (chromium), Cu (copper), Li (lithium), Na (sodium), S (sulfur), Se (selenium) are the total elements. There is little difference in quantity; Ag (silver) and Hg (mercury) elements were not found in the four water samples; Be (beryllium), Cd (cadmium), Co (cobalt), Sb (antimony), Sc (scandium), V (vanadium) and Zr (zirconium) elements were only found in the hydrolysis acidification tank. They were not detected in other water samples. La (lanthanum) and Sn (tin) were only detected in the hydrolysis acidification tank and oxidation ditch water samples; Mo (molybdenum) and Ti (titanium) were only detected in the oxidation tank and hydrolysis and acidification baths. In addition, like Al (aluminum), Ba (barium), Ca (calcium), Fe (iron), K (potassium), Mg (magnesium), Mn (manganese), the contents of Ni (nickel), P (phosphorus), Si (silicon), Sr (strontium), Y (yttrium), and Zn (zinc) were very different in several water samples. These elements were especially found in the hydrolysis acidification tank. The content was the most, of which the content of Ba (barium) in the hydrolysis acidification pool was 800 times that of the other three pools. For Al (aluminum), Fe (iron), Ni (nickel), Y (yttrium), Zn (zinc) the
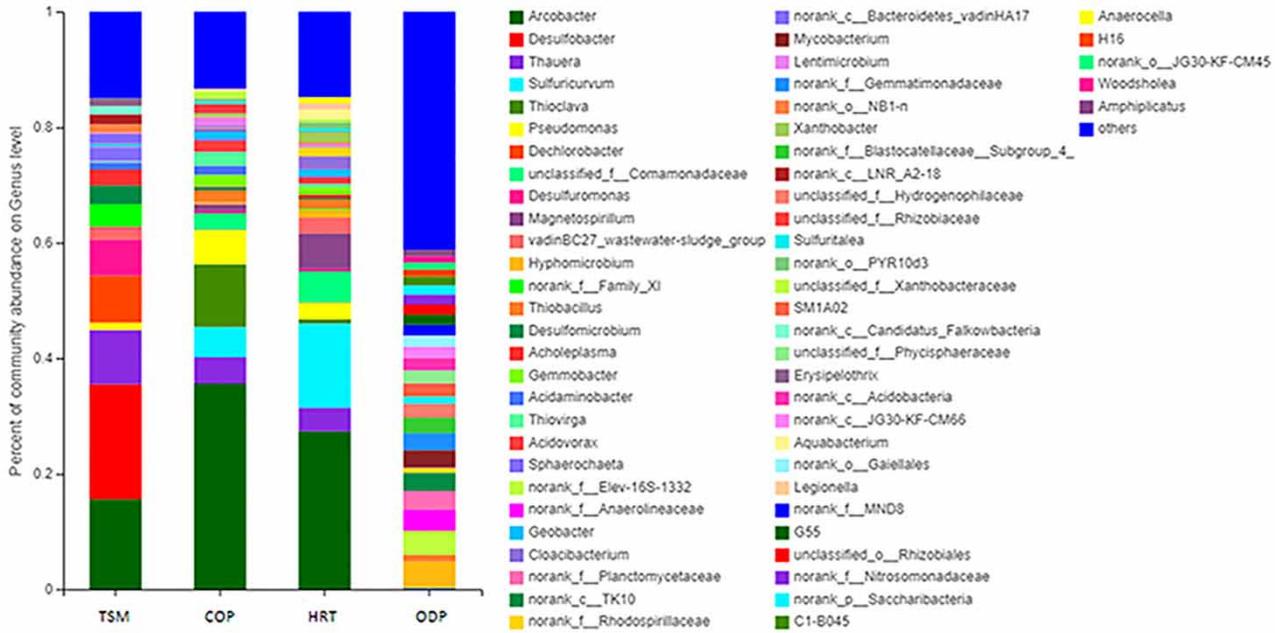
Figure 1 | From left to right are the TSM secondary sewage mixing tank, COP contact oxidation tank, HRT hydrolysis acidification tank, and ODP oxidation ditch. The microbial communities of the four water samples have abundance at the bacterial level. The ODP community has the highest abundance, followed by TSM and COP have the lowest community abundance. HRT has the lowest community abundance, and there is little difference between COP and HRT. Among them, the dominant genus *Pseudomonas* is obviously insufficient in HRT. Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wst.2021.348.

content in the hydrolysis acidification tank was more than 100 times that of the other three tanks. The content of Mn (manganese) and P (phosphorus) in the hydrolysis acidification tank was dozens of times that of the other three tanks K (potassium). The content of Mg (magnesium), Si (silicon) and Sr (strontium) in the hydrolysis acidification tank was several times that of the other three tanks. In general, the content of many elements in the hydrolysis acidification tank was too high, and the element content in the environment often had a great impact on the growth and metabolism of microorganisms, so the microorganisms in the hydrolysis acidification tank are bound to be affected by the environment and change. To further verify the failure of the dominant microbial strains in the hydrolysis acidification tank, we then tested the COD of several water samples.

By detecting the COD of the mixed influent of the two sewage, the effluent of the contact oxidation tank, the effluent of the hydrolysis acidification tank, and the effluent of the oxidation ditch, we can calculate from the results that the COD removal rate of the contact oxidation tank was 45.24%, and the COD removal rate of the hydrolysis acidification tank was 15.23%. Ditch COD removal rate was 50.5% (Table S6), the COD removal rate of the hydrolysis acidification tank was obviously too low. This reflects the insufficient ability of the hydrolysis acidification tank to treat oily sewage. It may be that some functions of the hydrolysis acidification tank have gradually failed after so many years of operation, or it may be due to the hydrolysis acidification tank. Microbes have poor tolerance to biological toxicity of polycyclic aromatic hydrocarbons, long-chain hydrocarbons and other heavy metals in oily sewage, as well as heavy metals such as iron, manganese, and zinc. After the system has operated for a long time, the microbial community structure is unstable, and the performance of oily sewage treatment also declines. The test results for the BOD of the hydrolysis acidification tank are all around 90 mg/L (Table S7), the BOD/COD value of the hydrolysis acidification tank is about 20%. This result further shows that the ability of the hydrolysis acidification tank to treat sewage is indeed insufficient, and the biodegradability of the effluent is poor. This result has a great relationship with the microbial flora in the hydrolysis acidification tank.

Analyze the microbial community composition of four water samples by bacterial 16SrRNA gene pyrosequencing, and the effective sequences of the four water samples are shown in the additional materials (Figure S2). We extracted non-repetitive sequences from optimized sequences, removed single sequences without repetitions, and performed OTU clustering on non-repetitive sequences (excluding single sequences) according to 97% similarity to obtain representative sequences of OUT. Of

these the results were domain: 1; kingdom: 1; phylum: 49; class: 107; order: 191; family: 329; genus: 512; species: 819; OTU: 1383. Through the alpha diversity analysis of four water samples, it can be seen that the hydrolysis acidification pool is relatively low in terms of species diversity, and the series is only 404, and according to the heatmap chart of beta diversity analysis (Figure S3), it can be clearly seen that there is little difference between the hydrolysis acidification tank and the contact oxidation tank. At the genus level, the species abundance of the four water samples was counted, and the community composition of the four water samples was intuitive. The subordinate level can be very obvious for the analysis of the four water samples. For the difference in the microbial genus between the four water samples, comparing the contact oxidation tank and the hydrolysis acidification tank, we can easily see that in the hydrolysis acidification tank it is not the type of microorganisms or the proportion of each microbe. The difference between microorganisms in the oxidation tank was not large, even for some dominant bacteria, visualized as the yellow color represented by *Pseudomonas*, were significantly lower in the hydrolysis acidification tank. Specifically, *Pseudomonas* was found in the contact oxidation tank and accounted for 6.03%, while *Pseudomonas* only accounted for 2.9% in the hydrolysis and acidification tanks, thiosphere accounted for 5.89% in the contact oxidation tank, and only 1.2% in the hydrolysis and acidification tank (Figure 1). Based on this, we know that the loss of *Pseudomonas* and thiosphere in the hydrolytic acidification tank was the root cause of the insufficient sewage treatment HRT capacity of the hydrolytic acidification tank. For this reason, we will focus on the hydrolysis acidification tank in future optimizations.

In the previous optimization of the hydrolysis and acidification tanks, the two most methods were co-matrix enhancement and bacterial agent enhancement, and bacterial agent enhancement was the most economical and reasonable method. Our experiment also used bacterial agent enhancement to hydrolyze acidification. The microbial flora of the pond was optimized. Before the optimization of the hydrolytic acidification pond flora, according to field observations and literature reports, we simulated and constructed our own contact oxidation tank, hydrolysis acidification tank and oxidation ditch device in the Biology Building of Central South University. The contact oxidation tank and oxidation ditch were constructed to work with the hydrolysis acidification tank to facilitate our experiments (Figure S1); among them, the hydrolysis and acidification unit was replaced by a 1.5 LUASB reactor. However, unlike traditional bacterial intensification, we used the *Pseudomonas stutzeri* LH-42 strain, which was screened from oilfield soil in the early stage, as the intensifier. This strain was reported in the previous study. The continuous culture method was adopted after 12 days. After acclimatization, the number of strains of
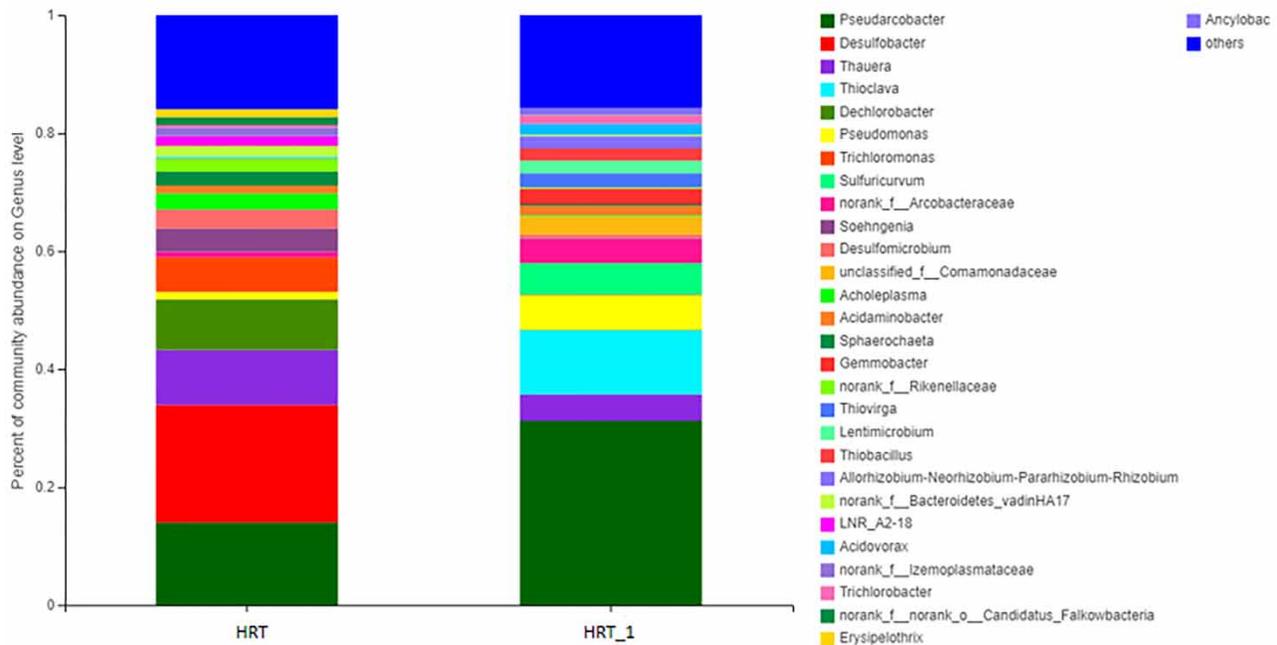

Figure 2 | The HRT on the left is the original hydrolysis and acidification pond microbial community, and the HRT_1 on the right is the optimized hydrolysis and acidification pond microbial community after colonization and domestication of *Pseudomonas stutzeri* LH-42. It can be seen that the proportion of *Pseudomonas* after optimization had increased significantly. Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wst.2021.348.

Pseudomonas stutzeri LH-42 in a mixed medium mainly composed of water from the hydrolysis acidification tank and inorganic salts can reach the level of 10^8 and the full loading of *Pseudomonas stutzeri* LH-42 can reach 5×10^9 (Table S5), this indicates that the *Pseudomonas stutzeri* LH-42 strain we independently screened can adapt well to the environment of the hydrolysis acidification pool, and will not be poisoned and inactivated by organics such as polycyclic aromatic hydrocarbons, long-chain hydrocarbons and heavy metal ions such as iron, manganese, and zinc in the water. This provides the feasibility for optimizing the bacterial flora of the hydrolysis and acidification pond. Then, the *Pseudomonas stutzeri* LH-42 strain was places in the hydrolysis and acidification pond by immobilization technology. After the hydrolysis and acidification system was running for 24 hours, we extracted the hydrolysis and acidification pond again. The effluent samples were analyzed by 16SrRNA gene pyrosequencing to measure the changes in the bacterial population of the hydrolysis acidified pool after optimization. It was found that the proportion of the bacterial population of *Pseudomonas* increased from 2.9% to 5.9%, and the entire hydrolysis acidification pond community had also undergone many changes, and the dominant bacteria like *Pseudarcobacter* had been greatly improved (Figure 2). This result was due to the fact that we planted the domesticated *Pseudomonas stutzeri* LH-42 as an intensifier in the hydrolytic acidification pool to enhance the diversity of its species and increase the overall microbial flora to the hydrolysis acidification pool for high metal ions and long-chain aromatics. This achieved tolerance so that it could maintain a good microbial community for a long time. This is different from the previously reported research on using dominant bacteria from the phyla Proteobacteria and Firmicutes as a strengthening agent.

After improving the microbial flora in the hydrolysis and acidification tank, simulating the sewage treatment plant after operating the device for a period of time, re-sampling and detecting the degradation of COD in the hydrolysis and acidification tank (Figure 3), the results show that the COD of the effluent of the hydrolysis and acidification tank was significantly
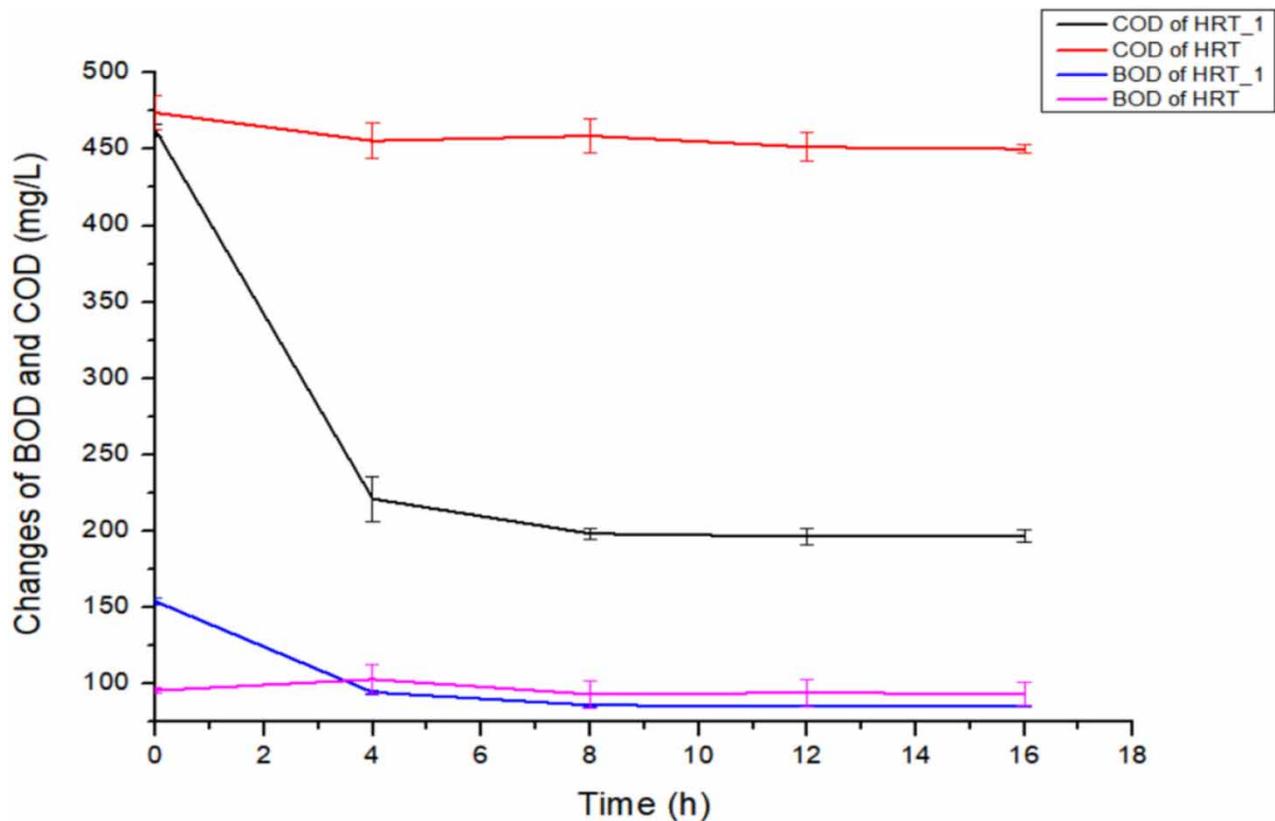

Figure 3 | The red line is the COD change curve in the original hydrolysis acidification tank, the black line is the COD change curve in the optimized hydrolysis acidification tank, the blue line is the BOD change curve in the optimized hydrolysis acidification tank, and the pink line is the BOD change curve in the original hydrolysis acidification tank. It can be seen from the figure that, compared with the original hydrolysis and acidification tank, the COD is basically unchanged, and the optimized hydrolysis and acidification tank has significantly improved COD removal performance, especially the most obvious change in 0–4 hours. After optimization, the BOD of the hydrolysis and acidification tank began to decrease from 150 to 90 mg/L after 4 hours and remained stable, while the BOD of the original hydrolysis and acidification tank was maintained around 100 mg/L. Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wst.2021.348.

lower than the COD value of the previously unoptimized hydrolysis and acidification tank flora, which was reduced from the previous 485.3 mg/L to about 200 mg/L; the degradation rate was increased by 58.79%. At this time, the BOD of the hydrolysis and acidification tank reached about 90 mg/L, and the BOD/COD increased from 20% to more than 40% (Figure 4). The biodegradability for the hydrolysis and acidification tank was significantly improved. This was different from the actual treatment of oily wastewater by the hydrolysis and acidification system. The effect once again verified the feasibility of using our self-screened *Pseudomonas stutzeri* LH-42 as an enhancer to improve the microbial community. Because *Pseudomonas stutzeri* LH-42 can adapt well to the environment of the hydrolysis and acidification tanks, this is in line with the previous studies that found that *Pseudomonas* has tolerance for metal ion oxidative stress and the ability to degrade long-chain hydrocarbons in petroleum and for desulfurization. The stability of *Pseudomonas stutzeri* LH-42 in the hydrolysis acidification tank provides a new, more effective and stable microbial enhancer for sewage treatment plants in the future, which not only can better improve the performance of the hydrolysis acidification tank but also save many costs for enterprises.

CONCLUSION

Recently, the optimization of the microbial community in the hydrolysis acidification tank has attracted more and more international attention. How to improve the biodegradability of the hydrolysis acidification tank and reduce the COD of the effluent of the hydrolysis acidification tank is still a key issue that needs to be solved urgently. Previous studies have reported that the dominant bacteria in the phyla Proteobacteria and Firmicutes were used as intensifiers for the hydrolysis and acidification pool. This significantly increased the proportion of dominant bacteria in the hydrolysis and acidification pool over a short period of time and improved the removal rate of COD. However, due to the poor tolerance of Proteobacteria and Firmicutes bacteria to long-chain hydrocarbons and heavy metals such as iron, manganese, zinc and other heavy metals in oily wastewater, the dominant bacteria in the hydrolysis and acidification tank were poisoned and inactivated over time, and the
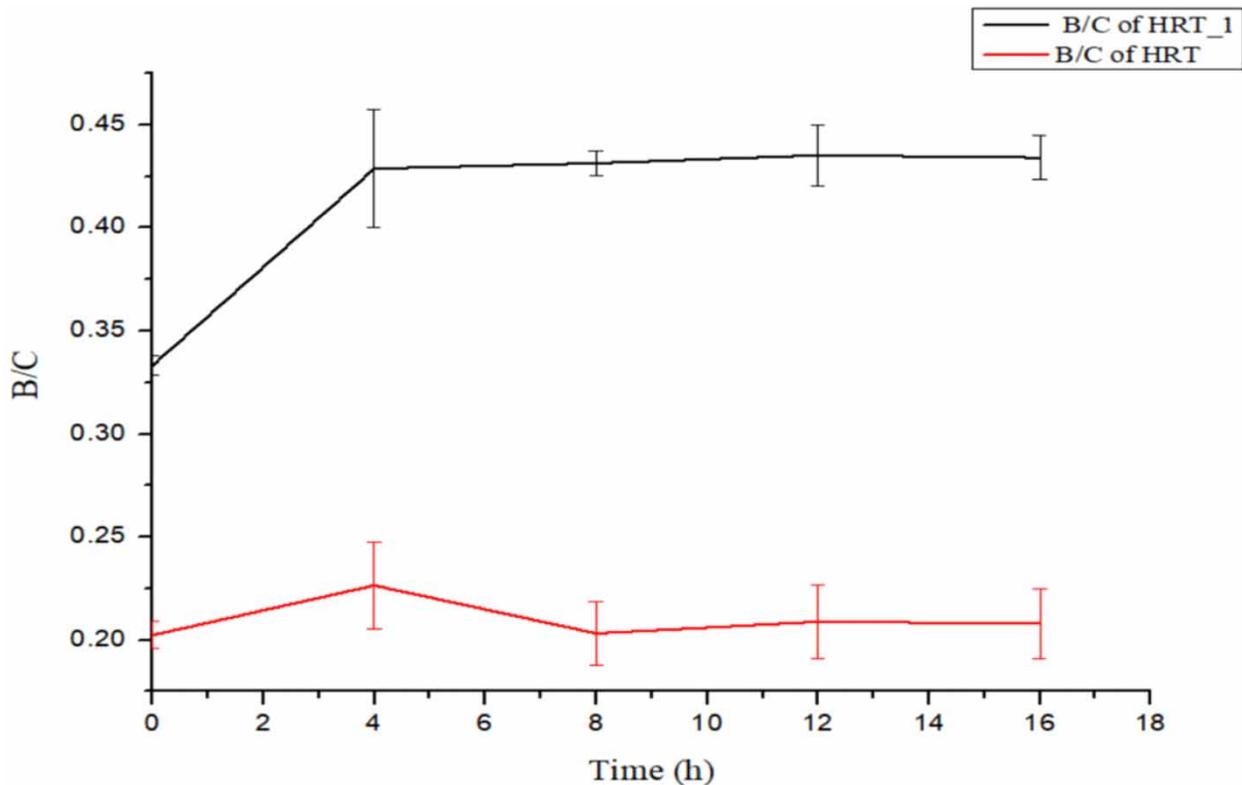

Figure 4 | The black line is the change of BOD/COD in the optimized hydrolysis and acidification tank, and the red line is the change of BOD/COD in the original hydrolysis and acidification tank. It can be seen from the figure that the BOD/COD in the hydrolysis and acidification tank has been improved significantly after optimization, from 0.33 to 0.43, and the biodegradability is significantly enhanced, while the BOD/COD of the original hydrolysis and acidification tank is basically unchanged at about 0.2. Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wst.2021.348.

performance of wastewater treatment deteriorated. We have optimized the intensified bacterial agent, using the *Pseudomonas stutzeri* LH-42 strain screened from the oilfield soil in the early stage as the intensifying bacterial agent. This had a desulfurization effect and could produce surfactants, which play an important role in degrading long-chain hydrocarbons in oily wastewater. It has certain resistance to biologically toxic heavy metals such as polycyclic aromatic hydrocarbons, long-chain hydrocarbons, and heavy metals such as iron, manganese, and zinc. However the content of many metal elements in the hydrolysis acidification tank can be too high, and the content far exceeds the national wastewater discharge standard value. The high content of metal elements in the environment may cause the loss of durability and stability of the *Pseudomonas stutzeri* LH-42. In general, addition of bacterial agents increased the proportion of *Pseudomonas* in the hydrolysis and acidification tank from 2.9% to 5.9%, and the proportion of other dominant strains was also significantly increased. The microbial community structure was significantly optimized, thereby enabling the removal of COD in the hydrolysis and acidification tank. The rate was increased by 58.79%, BOD/COD also increased from 20% to more than 40%, and the hydrolysis acidification tank could maintain a long-term and efficient oily wastewater treatment performance.

In the future, we will adapt *Dietzia* sp. DQ12-45-1b selected from the oil field according to the same method and then colonize it in the hydrolysis acidification tank to further enhance the dominant bacteria in the hydrolysis acidification tank and provide new sewage treatment plants in the future. This will produce more effective and stable microbial intensifier preparations.

ACKNOWLEDGEMENTS

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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