

THE PERFORMANCE OF ORIENTED LACTIC ACID FERMENTATION BROTH FROM FOOD WASTE AS EXTERNAL CARBON SOURCE FOR DENITRIFICATION

Feng Liu^{1, 2}, Ming Gao³, Beiping He², Qunhui Wang^{3, *}, Leiyu Feng¹, Yinguang Chen^{1, *}

1 State Key Laboratory of Pollution Control and Resources Reuse, School of Environmental Science and Engineering, Tongji University, 1239 Siping Road, Shanghai 200092, China

2 Tus-Environmental Technology Development Co., LTD. Haidian District, Beijing 100084, China

3 Department of environmental engineering, University of Science and Technology Beijing

30 Xueyuan Road, Haidian District, Beijing 100083 P. R.China

*Corresponding author: yinguangchen@tongji.edu.cn (Yinguang Chen); wangqh59@ustb.edu.cn (Qunhui Wang)

INTRODUCTION

The problem of water environment pollution has been attached of great importance all over the world. In 2019, the Ministry of Housing and Urban-Rural Development and other ministries and commissions issued the ‘Three-year Action Plan for Improving the Quality and Efficiency of Urban Sewage Treatment (2019-2021)’. The pollutant discharge standards of China's urban sewage treatment plants were gradually raised from Grade I B standard to Grade I A standard, and even proposed to meet the grade IV water standards of the Surface Water Environmental Quality Standard (GB3838-2002). The introduction of these policies put forward higher requirements for the total nitrogen concentration of effluent from sewage treatment plants. At the same time, with the proposal of carbon peak, and carbon neutrality in China, new challenges have been put forward for exogenous carbon source and other drugs in the sewage treatment process. At present, commercially available carbon sources for enhancing biological denitrification efficiency were expensive and had many drawbacks. For example, methanol was a class A dangerous chemical commonly used, which had toxic effects and great potential safety risks in the process of storage and appliance. Glucose was easy to cause the microbe growth and reproduction drastically, resulting in sludge expansion. The COD equivalent of sodium acetate was low, whose dosage was relatively high with great mud yield, etc^[1-3]. Therefore, alternative carbon sources, cheaper and more efficient, were extremely urgent for sewage treatment. The high content of organic matter in food waste (FW) could theoretically be used as raw material for alternative carbon source^[4,5]. However, due to the complex composition of FW and various fermentation products, it was necessary to investigate the denitrification effect and stability of the carbon source generated from FW.

Therefore, in this paper, by comparing with the traditional chemical carbon sources, we investigated the denitrification effect of the oriented lactic acid (LA) fermentation broth from FW as a denitrification supplementary carbon source (FW-LA) in the SBR reactor. Further, Microbial community analysis in SBR system was performed. Here, we would like to provide theoretical support for an efficient liquid composite carbon source for sewage treatment, which was obtained from oriented LA fermentation of FW.

MATERIALS AND METHODS

Feedstock and inocula

FW was obtained from a kitchen waste treatment plant in Anhui Province, China. The collected FW was pretreated as follows: high-pressure extrusion→impurity removal→boiling for oil removal→three-phase separation. In detail, the original FW with total solids (TS) 15%, was firstly treated with high-pressure extrusion (12 MPa) to remove the impurities and mix slurry, with an extruder filter hole diameter of 8 mm, and with 10% dry impurities (TS 50%) removed; after the stock was sieved through the screening machine with hole diameter 5 mm, and 5% small slag (TS 40%) was removed; then, the organic material was mixed with 160°C saturated steam within the boiling tank, where the temperature was kept at 75-80°C for 1.5 h. The boiled material was extracted by a three-phase separator to extract crude oil, and the remaining organic slurry was the FW substrate taken in this experiment. Sodium acetate (NaAc), chemical grade, was directly purchased.

Experimental Methods

Preparation of FW-LA: *Enterococcus mundii* was used as inoculum, and the fermentation temperature was carried out at 43°C with continuously pH control at 6.8

by using 10 M NaOH as a neutralizer in an open system for 7 days. Subsequently, the fermentation broth was filtered through a 0.45 μm membrane to produce FW-LA.

Denitrification and nitrogen removal effect test: Two SBR reactors with a working volume of 8 L were operated in AOA mode, and the operation cycle was 8 h (anaerobic 50 min, aerobic 120 min, anoxic 180 min, and static sedimentation 60 min). The initial sludge concentration MLSS in SBR reactors was 3800-4200 mg/L, the temperature was 23-25°C, the influent pH was 6.5-7.0, and the aeration volume was 3.5 L/min. The influent ammonia nitrogen concentration was 35 mg/L, and the initial COD concentration was adjusted to 200, 300, and 400 mg/L, respectively. Carbon sources were NaAc and FW-LA prepared above.

Microbial community analysis: In this paper, 16SrRNA high-throughput gene sequencing molecular biology technology was used to analyze the community of the initial sludge of WWTP, the sludge with FW-LA as carbon source (COD=200 mg/L), and the sludge with NaAc as carbon source (COD=200 mg/L).

Analysis method

The fermentation broth was sampled periodically and centrifuged, then the supernate was filtered through a 0.45 μm membrane for COD analysis regarding 'HJ/T399-2007', and further filtered through a 0.22 μm membrane for LA and acetate measurement by High-Performance Liquid Chromatography (HPLC, LC-20A, Shimadzu, Japan). TN 'HJ636-2012', $\text{NH}_4^+\text{-N}$ 'HJ535-2009', $\text{NO}_3^-\text{-N}$ 'HJ/T346-2007', $\text{NO}_2^-\text{-N}$ 'GB7493-1987', and MLSS were determined by standard methods. Microbiological analysis was performed based on 16SrRNA high-throughput gene sequencing.

RESULTS AND DISCUSSION

The performance of SBR reactors with different carbon sources

By comparing the changes of COD and nitrides in two different systems, we found that when NaAc was used as the carbon resource, the COD removal rate was 63%-80% (average 74.0%), and the TN removal rate was 55%-73% (average 64.7%), 78%-88% (average 77.8%), 50%-60% (average 57.0%) under different influent COD 100, 200, 300 mg/L, respectively (Fig.1). The COD removal rate of the reactor with FW-LA as carbon source ranged from 65% to 82%, with an average removal rate of 72.13%. The TN removal rate was 45.2%-68.1% (average 52.3%), 75.1%-91.9% (average 83.8%), and 74.3%-84.1% (average 78.2%) under different intake COD 100, 200, 300 mg/L, separately (Fig.2). On the whole, the performance of denitrification with FW-LA as carbon source was better

than that with NaAc, especially at higher influent COD concentration.

Furthermore, we focused on the variation of sludge settling performance during each cycle in the SBR system and got some interesting findings. In the reactor with FW-LA as the carbon source, MLSS was 4209, 4460, 4216 mg/L, separately under the influent COD 200, 300, and 400 mg/L. In NaAc reactor, MLSS was 4129, 4223, 4339 mg/L respectively under influent COD 200, 300, 400 mg/L. With the growth of sludge, the phenomenon of sludge swelling gradually occurred, especially in the FW-LA system, accompanied by sludge floating. Under inlet COD 400 mg/L, the sludge of both reactors could swell and rise above obviously. After the reaction tended to be stable, SV30 of the FW-LA reactor ranged from 47% to 51%, which was 43%-48% in the NaAc reactor, and the sludge settling performance decreases greatly. So, slashes with FW-LA as carbon sources showed obvious swelling and sludge upwelling. This reminded us that we should pay much more attention to the system stability and sludge bulking when using FW-LA as a carbon source in practical engineering.

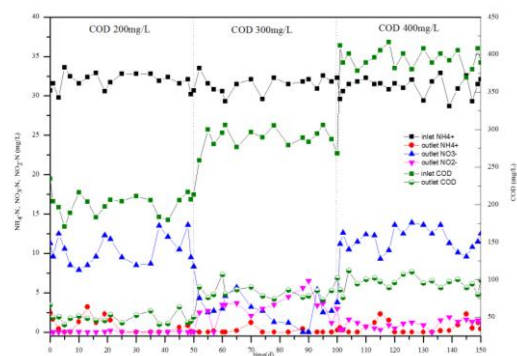


Fig. 1. The variation of COD and nitrides of the SBR reactor with NaAc as carbon source

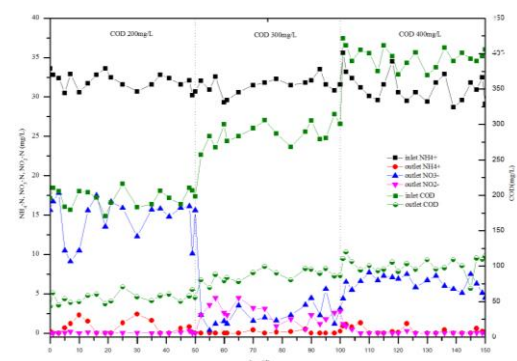


Fig. 2. The variation of COD and nitrides of the SBR reactor with FSFW-lactic acid as carbon source

Comparison of nitrogen removal effect between two carbon sources

As was shown in Table1, the nitrosation and nitrification rate with the FW-LA was much lower than that of NaAc. When the influent COD was 200 mg/L, the denitrification rate, TN remove rate, and Nitrogen removal load (NRR) with FW-LA as the carbon resource was inferior to that with NaAc. Whereas with the increase of COD concentration, the advantages of the FW-LA carbon source became more obvious, and it was worth stating that the denitrification yield, TN remove rate, and NRR in the FW-LA system was approximately 1.6, 1.4, 1.3 times higher than that in NaAc reactor when the COD inlet was 400 mg/L. In the

SBR system with FW-LA as carbon source, the denitrification efficiency of inlet COD 400 mg/L, on which high organic loading inhibited the nitrification process with NH_4^+ incompletely nitrated, was weaker than that of 300 mg/L. When the influent COD concentration was 300 mg/L, there was little difference in the TN removal rates between the two carbon sources. When COD was 400 mg/L, FW-LA had little influence on the nitrification process and was more easily utilized by denitrifiers, which displayed obvious advantages over NaAc in the TN removal rate. On the other hand, the SBR system supplementing NaAc as a carbon source was more likely to cause the accumulation of $\text{NO}_2\text{-N}$.

Table 1 Comparison of nitrogen removal effect between two carbon sources

Carbon source	Inlet COD (mg/L)	removal ratio COD (%)	nitrosation rate ($\text{NO}_2^-/\text{NH}_4^+$)	nitrification rate ($\text{NO}_x^-/\text{NH}_4^+$)	Denitrification yield ($\Delta\text{NO}_3^-/\text{NO}_3^-\text{max}$)	TN remove rate ($\Delta\text{TN}/\text{NH}_4^+$ inlet)	Nitrogen removal load (NRR) ($\Delta\text{TNg}/\Delta\text{CODg}$)
FW-LA	200	71.86	0.48	68.26	30.61	51.44	0.11
	300	66.73	9.61	68.70	86.82	85.73	0.15
	400	74.97	0.65	48.77	65.69	78.75	0.08
NaAc	200	75.32	15.37	82.93	49.61	64.45	0.14
	300	71.36	29.39	67.63	79.67	83.04	0.13
	400	74.77	6.51	51.09	40.66	56.51	0.06

Microbial community analysis

Based on the species-abundance curve analysis (Fig.3), the population diversity of the sludge with FW-LA addition was close to that of the sludge with stable operation and good reaction effect retrieved from the municipal sewage works. On the contrary, the diversity of the sludge population with NaAc as carbon source changed significantly. Therefore, the operation effect of FW-LA as carbon source was more stable. In Fig.4, the main microorganisms in the reaction system were *Gammaproteobacteria*, *Bacteroidia**Anaerolineae*, *Alphaproteobacteria*, *Actinobacteria*, etc. *Gammaproteobacteria* was the most abundant group in class level. The distribution of the raw sludge group, FW-LA group, and NaAc group were 33.08%, 31.97%, and 7.12% respectively. Some *Gammaproteobacteria* contained the *NosZ* enzyme gene in denitrification, which always played an important role in the nitrogen transfer process. *Bacteroidia* had the function of nitrogen and phosphorus removal, whose distribution in the original sludge group, FW-LA group, and NaAc group were 16.42%, 25.92%, and 15.84%,

respectively. The results indicated that FW-LA as carbon source was more conducive to the growth of this class of bacteria and the nitrogen cycle process. *Anaerolineae* was one of the main bacterial groups to remove COD and $\text{NH}_4^+\text{-N}$ in WWTP, and the distribution of the three groups were 19.87% (original), 17.95% (FW-LA), and 16.43% (NaAc) respectively, which was slightly affected by carbon sources. *NirK* sequences were found in some *Alphaproteobacteria*, and some *NirK*-type denitrifiers had both denitrification and DNRA pathways, which were highly correlated with nitrogen cycling. The distributions of *Alphaproteobacteria* in the three groups were 9.80% (original), 8.97% (FW-LA), and 6.64% (NaAc), respectively. From the second genus-related population abundance map (Fig.5), *Ca.competibacter* showed a significant difference between the two reactors with the intake of COD 200 mg/L was *Candida polygonatum*, which was a class of glycan bacteria, that could make the sludge easier to form condensed structures. However, the content of *Candida polygonatum* in the reactor with FW-LA as the carbon source was significantly lower than that in

the reactor with NaAc, which also explained the obvious phenomenon of sludge bulking in the reactor with FW-LA as the carbon source in the experiment. This issue should be addressed emphatically in actual application. Another species with notable differences in content was Rank-F-Saprospiraceae, the genus Saprospiraceae, a primary key contributor to sludge bulking in SBR reactor, which was obviously higher in FW-LA group than in NaAc. Ferruginibacter was shown in relevant studies that it could release relevant signaling factors to promote the self-cleaning of EPS, thus promoting the denitrification process. And fortunately, it was found the FW-LA reactor contained a high amount of functional microbes, which could bring about its better denitrification effect.

CONCLUSIONS

When the oriented LA fermentation broth of FW pretreated with ‘high-pressure extrusion→impurity removal→crude oil removal→three-phase separation’ was used as the carbon source for sewage treatment, the denitrification efficiency was comparable to that of NaAc in SBR system. The FW-LA carbon source exhibited a higher denitrification capacity with increasing to a higher inlet COD. The denitrification and TN removal rates could reach over 87% and 85% respectively when the influent COD was 300 mg/L. Analysis of the microbial community indicated the operation was relatively stable with FW-LA as carbon source, illustrating that FW-LA was of great significance in the recycling of FW, energy saving, and consumption reduction of sewage. Certainly, since LA carbon source has not yet been applied in the projects, its long-time operating impact on the sewage treatment system needed to be further studied.

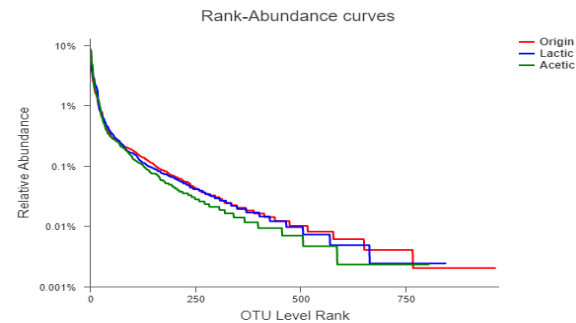


Fig. 3. Relative abundance curves of the sludge with FW-LA reactor, NaAc reactor, and origin sewage plant (COD inlet 200 mg/L)

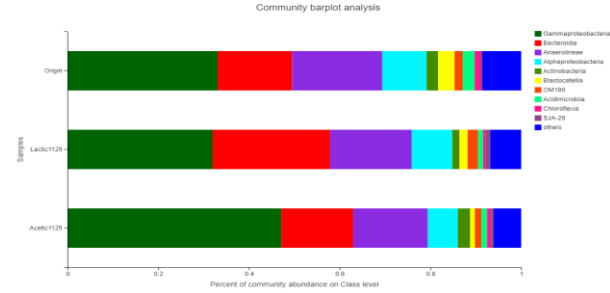


Fig. 4. Percent of community abundance on the class level in FW-LA group, NaAc group, and origin sewage plant (COD inlet 200 mg/L)

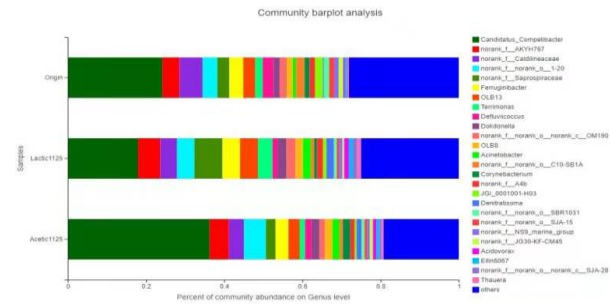


Fig. 5. Percent of community abundance on the genus level in FSFW-Lactic acid reactor, NaAc reactor, and origin sewage plant (COD inlet 200 mg/L)

REFERENCES

1. Wang D.M. Study on the characteristics and application of food waste fermentation with high lactic acid [D]. Xi 'an University of Architecture and Technology, 2016.
2. Yang Q.L, Xi XY et al. Enhanced nitrogen removal efficiency of external carbon source during abnormal influent in municipal waste water treatment plant[J]. China Water & Wastewater, 2011, 27(3):106-108.
3. Hu X.Y, Zhu JP. Biological denitrification efficiency of three single and mixed carbon sources[J]. Technology of water treatment, 2020, 46(01):57-61.
4. Tang J, Pu Y, Wang X. C, et al. Effect of additional food waste slurry generated by mesophilic acidogenic fermentation on nutrient removal and sludge properties during wastewater treatment[J]. Bioresource Technology, 2019, 294: 122218.
5. Hu H, Ma S, Zhang X, et al. Characteristics of dissolved organic nitrogen in effluent from a biological nitrogen removal process using sludge alkaline fermentation liquid as an external carbon source [J]. Water Research, 2020, 176: 115741.