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Full-scale application of microbial agent on in-situ sludge reduction

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Abstract: The full-scale application of Tx-1, a multifunctional microbial agent, was carried out for 8 months in an anoxic/oxic (A/O) municipal wastewater treatment process. The results show that the Tx-1 dosed system can obtain good effluent characteristics while minimizing sludge production and energy consumption. The total phosphorus (TP) is lower than 0.5 mg/L in effluent without any chemical regent added. The discharged dry sludge per 10 000 m^3 wastewater D_{wat} decreases from 1.4 to 0.5 t. For per cubic meter wastewater, the air supply decreases from 6.0 to 5.1 m^3 and the electricity consumption decreases from 0.412 - 0.425 kW · h to 0.331 kW · h. The addition of Tx-1 can improve the substrate removal constant and decrease the microorganism growth yield coefficient of activated sludge. At the same time, the structure of the microbial community changes and the biodiversity increases by adding Tx-1. The abundance of polyphosphate accumulating organisms (PAO), *Comamonadaceae* and *Tetrasphaera*, increased. Effective microbial agent is a potential way to combine in-situ sludge minimization with contaminants removal.

Key words: wastewater treatment; dosed anoxic/oxic (A/O) process; diversity of microbial community; kinetic parameters

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Activated sludge process is widely used for biological treatment around the world, however, it also generates a large amount of excess sludge. In fact, treatment and disposal of excess sludge from wastewater treatment plants (WWTPs) has been a great challenge[1]. Current legal constrains, rising costs and public sensitivity to sludge disposal necessitate new development strategies for the reduction of excess sludge production[1-4]. Among various processes of minimizing the production of sludge, the in-situ sludge reduction technology appears to be an ideal one by reducing sludge production in the biological wastewater treatment process instead of post-treating the waste sludge[1,5].

It has been reported that microbial agents have wide applications in the fields of deodorization, bioremediation and the enhancement of biological treatment of wastewater[6-9]. There are some commercial products such as effective microorganisms (EM), Novozymes products and bio-form BTZ® products. However, compared with the application and research of microbial agents on improving the effluent, only few reports on reducing sludge production were carried out, especially for full-scale application[7-9]. Lack of full-scale application data and mechanism research has hindered their application and improvement[10-11]. Several pilot test cases reported in China showed that sludge reduction can be achieved by introducing microbial agents into the wastewater treatment line. Li et al.[12] screened two kinds of microbial agents and achieved 53.1% sludge in-situ reduction in an A^2/O reactor (4.2 m^3). However, a concomitant decrease in biological phosphorus removal occurred[11-12].

In the present study, a full-scale study on application of Tx-1, a kind of multifunctional microbial agent, is carried out in a WWTP with an average capacity of 23 408 m^3/d. By introducing Tx-1 in the anoxic/oxic (A/O) activated sludge system and optimizing the operational conditions, the innovative system (Tx-1 dosed system) guarantees good effluent characteristics, particularly in the biological removal of total phosphorus (TP). At the same time, in-situ sludge reduction is achieved. The kinetic-parameters of the Tx-1 dosed system and the structure of a new microbial community are measured in the laboratory. The results prove that Tx-1 is an effective microbial agent for reducing sludge in-situ and enhancing phosphorous removal.

1 Materials and Methods

1.1 Materials

Tx-1 is produced by Shanghai Eco-Well Bioscience Co., Ltd in a pilot-scale fermentation system. The product is a light-yellow powder and consists of several kinds of facultative and aerobic stains. At the same time, the product also contains some enzymes and small molecules, such as organic acid, secreted in the fermentation process. Tx-1 has passed the safety-assessment on the application in environmental protection and obtained a secu-

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rity acceptance letter in Shanghai, China. Tx-1 is activated on site before using. Briefly, 20 g Tx-1 is added into 1 L influent, and the mixture is kept stirring in an activating tank at 30 ℃ for 12 h.

1.2 Full-scale pilot experiment

There are two A/O biological treatment lines (marked 1# and 2#) in the pilot plant. Swirling flow and micro-porous aeration equipment are equipped in two oxic zones, respectively, and the supplied air is adjusted by controlling the amount of aeration equipment. The water indices of effluent comply with the Grade Ⅱ standards in the national discharge standard of pollutants for the municipal wastewater treatment plant (GB 18918—2002, China).

Activated Tx-1 solution is added into the inflow of oxic zones continuously. The dissolved oxygen (DO) is controlled at 0.9 to 2.2 mg/L.

1.3 Analysis and data collection

During data collection, both the on-line and off-line monitoring indices are used. The on-line data of influent and effluent are recorded every day. Off-line indices of water (COD_{Cr}, BOD_5, SS, NH_3-N, TN and TP) and sludge (MLSS, SV_{30} and MLVSS) are measured every week in the plant laboratory.

The data are collected for one month (Stage 2) before the application of Tx-1 as the background data. After the start-up period of Tx-1 addition (15 d), the data is collected for eight months (Stage 3). The data from the same months (Stage 1) in the previous year is analyzed as the historical data. The influent water volume at different stages is shown in Tab. 1.

Tab. 1 Influent water volume at different stages m^3/d

Stage	Maximum	Minimum	Average
1	25 562	12 777	22 663
2	23 575	21 841	22 713
3	27 977	14 174	23 408

1.4 Kinetic parameters

The kinetic parameters are determined based on the Lawrence-McCarty model and the Monod model[13-14] in our laboratory. The activated sludge is collected in the pilot plant and cultivated in two identical bioreactors (signed as Tx-1 dosed and Blank) under the following conditions: Temperature is 20 ℃; DO is 2 mg/L; and MLSS is 1.5 to 3.5 g/L.

1.5 Structure of microbial community

The structure of microbial community is analyzed by extraction, propagation and sequencing of DNA.

2 Results and Discussion

2.1 Effluent characteristics

The most important task for the pilot WWTP is to ensure the effluent quality, so the influent and effluent indices are monitored through the experiment and they are listed in Tab. 2.

Tab. 2 Statistical results of influent and effluent indices with mass fraction at different stages mg/L

Stage	Value	Influent indices						Effluent indices					
		BOD_5	COD_{Cr}	SS	TN	NH_3-N	TP	BOD_5	COD_{Cr}	SS	TN	NH_3-N	TP
1	Maximum	256.0	637.8	292.0	78.4	63.6	5.0	14.1	49.5	30.0	34.2	16.9	2.8
	Minimum	117.6	205.8	142.0	43.7	12.3	2.6	6.5	19.2	12.0	11.8	0.3	0.1
	Average	172.3	386.0	199.4	57.2	42.8	3.6	10.3	32.7	21.8	21.5	5.6	0.9
2	Maximum	199.5	376.0	264.0	69.4	54.6	5.0	12.5	44.1	24.0	24.1	12.5	1.4
	Minimum	155.0	264.6	186.0	54.9	42.8	3.9	8.8	29.1	18.0	17.9	5.4	0.5
	Average	171.8	320.3	218.1	60.7	48.2	4.3	10.8	34.5	21.3	20.8	8.3	0.9
3	Maximum	267.0	510.0	294.0	71.7	56.7	5.0	28.6	49.0	28.0	33.6	26.9	1.6
	Minimum	112.0	192.0	132.0	28.6	30.3	3.0	6.4	19.0	1.8	1.5	1.1	0.1
	Average	160.6	316.5	207.8	58.4	42.9	3.9	9.9	33.5	21.0	22.8	12.8	0.4

Compared to the historical and background values, the application of Tx-1 results in a significant increase of TP removal efficiency. Without adding any chemical phosphorus removal agent after biological treatment, the average TP content in effluent is less than 0.5 mg/L, the standard value of Grade IA in GB 18918—2002, China.

In terms of organic pollutants COD_{Cr} and BOD_5, the removal efficiencies are similar to those in historical and background stages, and all the values are better than the drainage standard. It should be noted that similar removal efficiency is obtained with less air supply than the original process, which implies that the lower energy cost is available in the Tx-1 dosed system.

For nitrogen nutrients, it is found that the removal efficiency of TN and NH_3-N is clearly affected by temperature. In July and August, the average water temperature is 27.2 ℃, and the average NH_3-N and TN content in effluent are 6.4 and 17.4 mg/L, respectively, which can comply with the standard values of Grade I B in GB 18918—2002. However, when the average temperature decreases to 12.9 ℃ in February and March, the average NH_3-N and TN content in effluent is 20.3 and 28.6 mg/L, respectively, which can only comply with the standard value of Grade Ⅱ. The nitrogen removal in A/O process is not so ideal in practical operation[15], so it is necessary to update the process in this plant in order to improve nitrogen removal. For microbial agents, adding some specially-selected strains with high nitrogen removal capacity at low temperatures should be considered.

2.2 In-situ sludge reduction

The MLSS in the oxic zone is set to be 2.0 ± 0.5 g/L and DO 1.5 ± 0.5 mg/L for the experimental stage according to prior studies. The characteristics of activated sludge and sludge production at different stages are listed in Tab. 3.

Tab. 3 Characteristics of activated sludge in an oxic zone and sludge production at different stages

Stage	MLSS/($g\cdot L^{-1}$)	F	SVI/($mL\cdot g^{-1}$)	D_{wat}/t	D_{COD}/g
1	2.67	0.57	109.2	1.4	0.41
2	1.35	0.59	101.1	1.1	0.41
3	2.03	0.65	81.3	0.5	0.20

Note: SVI is the sludge volume index.

After the addition of Tx-1, the ratio F of MLVSS to MLSS increases from 0.57 to 0.65, which indicates that the microorganism fraction has increased. Thus, the degradation activity of activated sludge increases, which perhaps is one of the reasons for the good performance in the dosed Tx-1 system at the lower DO concentration.

SVI results indicate that the sludge in the dosed Tx-1 system shows much better settle-ability than that in the original process. One of the major operational problems in the pilot plant is due to the poor settle-ability of activated sludge before the application of Tx-1. When MLSS is higher than 1.8 g/L, the flotation of sludge in the secondary clarifier often occurs. However, no more flotation of sludge occurs by adding Tx-1, even though MLSS is higher than 2.0 g/L. Good settle-ability can also provide a high concentration of return sludge. The relationship of sludge age and the ratio of X_R/X in a steady state is[14]

$$\frac{1}{\theta_c} = \frac{Q}{V}\left[1 + R\left(1 - \frac{X_R}{X}\right)\right] \tag{1}$$

where θ_c is the sludge retention time (SRT) or the sludge age, d; Q is the flow rate, m^3/d; V is the volume, m^3; R is the recycling ratio; X is the solid concentration in the aeration zone, mg/L; X_R is the solid concentration in the return line, mg/L, and it can be roughly estimated as

$$X_R = \frac{10^6}{\mathrm{SVI}} \tag{2}$$

So, it is clear that θ_c increases correspondingly when SVI and X decrease in the Tx-1 dosed system. Then the excess sludge (ΔX, g/d) decreases according to the following equation:

$$\Delta X = \frac{XV}{\theta_c} \tag{3}$$

The statistical results in Tab. 3 show that the discharged dry sludge per 10^4 m^3 wastewater D_{wat} in the Tx-1 dosed system decreases by 64.3% and 45.5%, respectively, compared to the historical and background data. The sludge production per gram COD_{Cr} D_{COD} was only 48.8% of that at stages 1 and 2. The results show that effective in-situ sludge reduction is achieved in the Tx-1 dosed system.

2.3 Energy saving

Wastewater treatment is an energy-intensive process. In typical biological WWTPs, the aeration process accounts for more than 50% of the energy consumption[16-17] in order to supply DO for the microorganisms in sludge. So energy consumption can be significantly reduced when the oxygen requirement is reduced in the aeration process.

The aeration system in the pilot plant was designed to maintain DO at 2.0 mg/L, which is the average value in the activated sludge system[17]. However, the Tx-1 dosed system can be operated under a much lower DO concentration, which can be proved by the performance of line 2# in August at stages 1 and 3, respectively (see Fig. 1).

The highest temperature appears in August in the pilot plant. The water temperature is 20 to 28 ℃ (Avg. 22.9 ℃) in Stage 1 and 26 to 30 ℃ (Avg. 28.1 ℃) in Stage 3, respectively, as shown in Fig. 1. High temperatures lead to higher oxygen consumption due to the rapid biological reactions and the rapid oxygen depletion in the sludge[16], which means that more air supply is needed. Before Tx-1 application, another air supply fan (55 kW) was used to complement the DO during the high-temperature period from July to September in the pilot plant. However, it is not necessary at stage 3. So, at least approximately 1.0×10^5 kW·h electricity was saved in the summer.

Statistic results show that the DO content was low at stage 3. Even under the low DO concentration, similar removal efficiency of COD_{Cr} and NH_3-N and the dramatic increase of TP removal efficiency were achieved. As shown in Tab. 4, with the decrease of DO concentration, the average air supply per cubic meter wastewater decreases from 6.0 to 5.1 m^3 in the oxic zone. Correspondingly, the aeration power requirements decrease. Also, the total electricity consumption per cubic wastewater decreases by 19.7% to 22.1%.

In this pilot plant, air supply was only adjusted by controlling the number of aeration equipment, so it was difficult to adjust the air supply volume according to the actual DO and designed parameters. Frequency conversion technology is strongly recommended to recast the operational model for the improvement of DO adjustment in the future.

2.4 Kinetic parameters

Two identical bioreactors were set up in our laboratory to compare the kinetic parameters of activated sludge with (Tx-1 dosed) and without (Blank) addition of Tx-1.

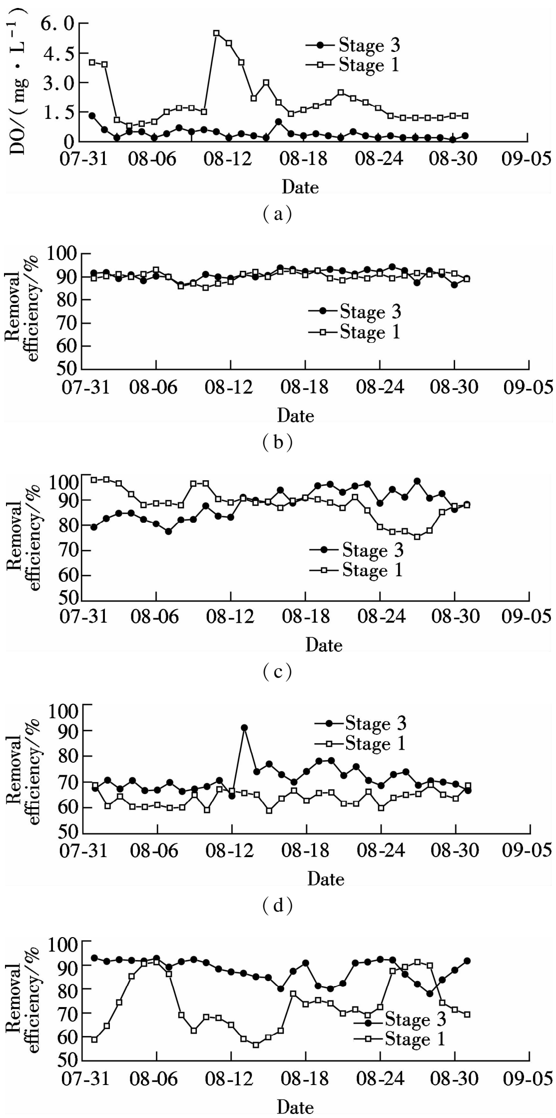


Fig. 1 Indices change in line 2# at Stage 1 and Stage 3. (a) DO; (b) COD_{Cr} removal efficiency; (c) NH_3-N removal efficiency; (d) TN removal efficiency; (e) TP removal efficiency

Tab. 4 Statistical results of DO, air supply and electricity consumption at different stages

Stage	Value	DO/(mg·L^{-1})		Air supply/m^3	Electricity consumption/(kW·h)
		1#	2#		
1	Maximum	5.5	5.7		
	Minimum	0.5	0.1		
	Average	2.3	2.3	6.0	0.412
2	Maximum	4.0	3.5		
	Minimum	1.0	0.7		
	Average	1.9	2.7	6.7	0.425
3	Maximum	5.2	5.5		
	Minimum	0.5	0.1		
	Average	2.1	1.7	5.1	0.331

The following equations are used to calculate the kinetic parameters[13-14]:

$$\frac{1}{\theta_c} = Yv - K_d = Y\frac{(S_0 - S_e)}{x_v t} - K_d \tag{4}$$

where Y is the microorganism growth yield coefficient, mg/mg; v is the specific substrate utilization rate, 1/d; S_0 and S_e are the substrate concentration in the influent and effluent, respectively, mg/L; X_v is the biomass concentration, g/L; t is the hydraulic retention time, d; K_d is the microorganism decay coefficient, 1/d.

$$\frac{1}{v} = \frac{1}{k}\frac{1}{S_e} + \frac{1}{v_{max}} = \frac{K_s}{v_{max}}\frac{1}{S_e} + \frac{1}{v_{max}} \tag{5}$$

where v_{max} is the maximum specific substrate utilization rate, 1/d; k is the first-order substrate removal constant, L/(mg·d); K_s is the half-saturation constant, mg/L.

The plots based on the experimental data and Eqs. (4) and (5) are shown in Fig. 2, and the calculated kinetic parameters are shown in Tab. 5.

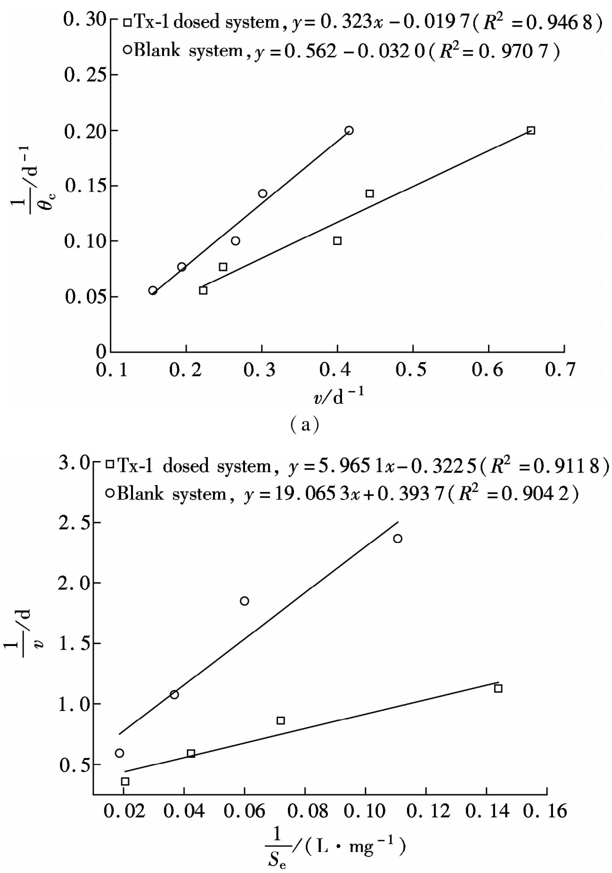


Fig. 2 Plots of the calculated kinetic parameters. (a) In the Tx-1 dosed system; (b) The Blank system

Tab. 5 Kinetic parameters in Tx-1 dosed and Blank systems

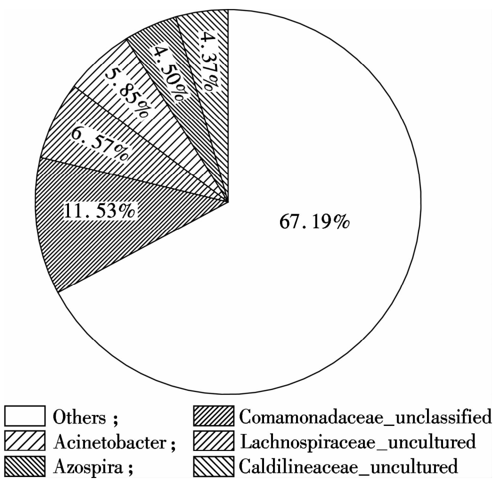
Parameter	Tx-1 dosed	Blank
Y	0.323	0.562
K_d/d^{-1}	0.019 7	0.032 0
v_{max}/d^{-1}	3.10	2.54
K_s/(mg·L^{-1})	18.51	48.43
k/(L·(mg·d)$^{-1}$)	0.168	0.052

Compared to the Blank, Y decreased by 42.8%, which indicated that excess sludge decreased when the same amount of substrate was degraded. At the same time, K_d

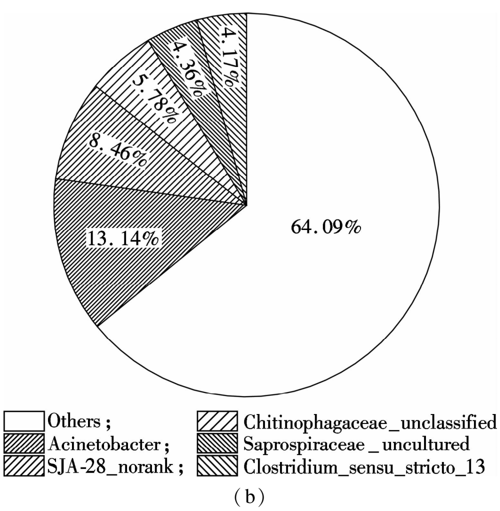
decreased by 38.2%, which means that the microorganism activity increased. The biochemical reactions in both systems followed the first-order kinetics and the constant in the Tx-1 dosed system was 3.2 folds of the Blank. The kinetic parameters proved the good performance of the Tx-1 dosed activated sludge system.

2.5 Structure of microbial community

A different structure of microbial community was developed and a new balance in microbial community was established in the Tx-1 dosed system due to the selective and competitive growth of foreign and indigenous strains. It was observed that *Acinetobacter*, as a kind of representative model microorganism in the activated sludge system[18], had the highest abundance in the Blank system. After the addition of Tx-1, the *Acinetobacter* also appeared in high abundance (5.85%), but a different structure of microbial community appeared, as shown in Fig. 3.



(a)



(b)

Fig. 3 Top five dominant microorganisms and their relative abundance. (a) Tx-1 dosed system; (b) Blank system

The abundance of polyphosphate accumulating organisms (PAOs) *Comamonadaceae*[19] and *Tetrasphaera*[20-21] increases from 3.24% and 1.31% to 11.53% and 3.39%, respectively, after the addition of Tx-1. The results show that more PAOs have colonized in the Tx-1 dosed system, and this may be one of the reasons for the enhancement of biological phosphorus removal.

The average Shannon indices of the TX-1 dosed and Blank systems are 4.58 and 4.32, respectively. According to the ecological principles, the higher the Shannon index, the greater the diversity, and the better stability and flexibility in the microbial community. That is to say, the Tx-1 dosed system has greater biodiversity, better stability and flexibility. So, the Tx-1 dosed system shows more resistance to the changes, including influent and operational conditions.

3 Conclusion

The multifunctional microbial agent Tx-1 is safe and convenient for the application in WWTPs. It can be introduced into the existing activated sludge process directly. Based on the full-scale study in A/O process, the addition of Tx-1 enhances the original A/O process. The Tx-1 dosed system obtains good effluent characteristics while minimizing sludge production and energy demand. The biological TP removal is enhanced in particular. The discharged dry sludge decreases by 64.3%; the air supply per cubic meter water reduces to 5.1 m^3; and the electricity consumption reduces to 0.331 kW·h/m^3. Based on the research in the laboratory, the addition of Tx-1 improves the first-order substrate removal constant and decreases the microorganism yield coefficient in the activated sludge process. The structure of the microbial community changes and the abundance of two kinds of PAOs increases. Tx-1 is a potential microbial agent in WWTPs for reducing excess sludge and enhancing phosphorous removal. In the future, a more effective microbial agent focusing on combining in-situ sludge minimization with pollutants removal should be developed.

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微生物菌剂在线污泥减量的生产性应用研究

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摘要:在 A/O 污水处理线上开展了为期 8 个月的投加多功能菌剂 Tx-1 的生产性应用试验. 结果表明:菌剂的投加明显提高了水处理效果,同时减少了污泥产量,降低了能量消耗. 在不投加化学除磷剂的情况下,出水总磷含量低于 0.5 mg/L;万吨水外排绝干泥量 D_{wat} 由 1.4 t 下降到 0.5 t;处理每立方米水所需曝气量由 6.0 m^3 下降到 5.1 m^3;处理每立方米水的电耗由 0.412 ~ 0.425 kW · h 下降至 0.331 kW · h. 投加 Tx-1 可以提高活性污泥系统的一级基质去除速率常数,降低污泥产率系数. 同时投菌体系中的活性污泥菌群结构发生了变化,生物多样性增加;其中 2 种聚磷菌 *Comamonadaceae* 和 *Tetrasphaera* 的丰度明显增加. 有效的微生物菌剂可为实现在线污泥减量及污染物去除提供解决途径.

关键词:污水处理;投菌 A/O 工艺;微生物菌落多样性;动力学参数

中图分类号:X703.1