INFLUENCE OF TRANSIENT CHANGE OF WATER TEMPERATURE ON PILOT-SCALE ANAEROBIC-ANOXIC-OXIC PROCESS UNDER PLATEAU ENVIRONMENTAL FACTORS

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Abstract. The objective of this study was to explore the effect of transient changes of Pilot-Scale Anaerobic-Anoxic-Oxic Process (A2O). The effects of transient changes in Tibet, China of water temperature on water quality indicators such as chemical oxygen demand (COD), the total phosphorous (TP), total nitrogen (TN) and ammoniacal nitrogen (NH_4^+-N), as well as microorganism and mixed liquor suspended solids were analyzed. The A2O system had a hydraulic retention time of 21 h, a mixture of the reflux ratio of the mixed liquor of 200%, a reflux ratio of the sludge of 100%, a temperature fluctuation range of 8-25 °C, and a water sampling interval of 3 h. The results showed that the optimal removal rates of different water quality indicators were different. The removal of COD, TN and NH_4^+-N mainly occurred in anaerobic tank and TP mainly in anaerobic tank and oxic tank. There was no obvious relationship between microorganism density and indicator organisms in anoxic tank, oxic tank and anaerobic tank. The concentrations of COD, TP, TN and NH_4^+-N in the effluents did not meet the first grade A standards, and the mixed liquid suspended solids did not show a significant growth trend with increasing temperature.
Keywords: *nitrogen and phosphorus removal, dissolved oxygen, microorganism, A2O, Tibet*

Introduction

Currently, the Anaerobic-Anoxic-Oxic (A2O) process, improved from the traditional activated-sludge process, has become the most popular sewage treatment method in Tibet, China (Chen et al., 2018). The sewage temperature in Tibet has obvious inter-annual and diurnal changes, the daily temperature difference can reach about 10 °C, and the inter-annual temperature difference can reach about 15 °C, which is mainly caused by drastic change of environmental temperature and centralized of water consumption time. Frequent change of water temperature could influence microbial growth and community structure, and adsorption performance and sedimentation performance of activated sludge, which ultimately leads to large fluctuations in wastewater treatment efficiency.

It is widely agreed that the effect of A2O is influenced by the unique environment factors of the plateau, namely, water temperature and atmospheric pressure. At present, according to the on-site measurement, the lowest temperature of sewage in Linzhi City is -1 °C, the highest is 14 °C (Zong et al., 2018). According to the comprehensive analysis of the survey, the reason for the sewage temperature of -1 °C in Linzhi City is mainly due to the following two reasons: firstly, the sewage pipeline in Linzhi City is too long, and the sewage flow is too small, resulting to a large heat loss in the sewage flow; secondly, the

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groundwater level is too high, and some groundwater seeps into the sewage pipeline, causing its average temperature to be too low (Zong, 2017).

Our previous study has shown that the traditional activated-sludge process cannot satisfactorily remove nitrogen and phosphorus from domestic sewage in Tibet (Zong, 2017). Temperature is a main influencing factor of sewage treatment efficiency (Ai et al., 2018). In the process of the phosphorus removal of sludge denitrification, the release and absorption rates of phosphorus are changed under excessively high or low temperatures, and the proportion of denitrifying phosphorus accumulating organisms in the activated sludge is greatly affected by temperature variation (Zhang et al., 2016). In addition, the nitrification capacity of the sewage treatment system is obviously weakened when the water temperature falls below 15 °C, and even lost totally when the temperature drops below 4 °C (He et al., 2010; Li et al., 2013). Thus, the nitrogen removal is severely inhibited under a low temperature (Li et al., 2014).

As for the A2O process, the nitrification/denitrification is weakened at a low temperature and a high sludge load. In this case, nitrite nitrogen will accumulate in the system, and denitrifying phosphorus removal will happen in the anoxic tank. The phosphorus removal efficiency of the system could decline due to the sludge bulking induced by non-filamentous bacteria (Li et al., 2014), and the lack of small molecular carbons required for phosphorus release by phosphorus-concentrating bacteria (as the hydrolysis of macromolecular organic matter is suppressed under a low temperature) (Shen et al., 2017).

The atmospheric pressure has a direct bearing on the oxygen partial pressure, and the oxygen partial pressure controls the dissolved oxygen (DO) in water. When partial oxygen pressures was below 40 kPa, the removal efficiencies of chemical oxygen demand (COD), and ammoniacal nitrogen (NH_4^+-N) increased with the increase of DO, meanwhile, nitrification was the main control process in simultaneous nitrification and denitrification (SND). However, partial oxygen pressures above 40 kPa did not affect their removal efficiencies, and denitrification became the main control process in SND (Xing et al., 2013). The nitrification can be enhanced by extending the aeration time, while the denitrification can be stimulated by prolonged exposure to anaerobic conditions (Yoo and Lee, 2015). Under low aeration, the nitrogen and phosphorus removal were greatly hindered in the A2O system (Li et al., 2013). The DO could affect the conversion of polyhydroxy alkyl ester and glycogen (Chen et al., 2014), as well as the activity of desulfurase and polyphosphatase, and affect phosphorus removal subsequently. The total number and activity of ammonia oxidizing bacteria are respectively determined by the hydraulic retention time (HRT) of solids and the DO concentration (Limpiyakorn et al., 2005).

Transient and non-steady state of domestic sewage is relatively common, and the temperature of sewage varies significantly during a day in Tibet. The present study used A2O system as a typical sewage treatment process to investigate the removal rates of nitrogen and phosphorus in daily responding to water temperature change, analyze the characteristics of the microbes in anaerobic tank, anoxic tank and oxic tank, and explore the mechanisms of nitrogen and phosphorus removal and microbial transient response under plateau environmental factors.

Materials and methods

Description of the anaerobic-anoxic-oxic process system and wastewater

A pilot-scale A2O sewage treatment device was designed and fabricated with plexiglass in Tibet, China. With an effective volume of 210 L, the device consists of 8 segments: the

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first 2 are anaerobic tanks with an effective volume of 35 L, the middle 2 are anoxic tanks with an effective volume of 58 L, and the last 4 are oxic tanks with an effective volume of 117 L. Besides, the effective volume of the sedimentation tank is 210 L. In both anaerobic and anoxic sections, each tank has a 50 rpm stirring device at the bottom; in each oxic tank, there is an aerator for oxygen supply. Inflow, return sludge and nitrifying liquid are controlled by a peristaltic pump. To maintain a constant temperature, the water temperature was regulated by a constant temperature circulator. In each tank, a sampling hole was opened on the tank wall. Before the experiment, the activated sludge was cured for 32 d. The temperature, MLSS, and volume percent of MLSS after settling for 30 min (SV30) were set to 22.5 °C, 4,787 mg/L and 35%, respectively.

The urban domestic sewage in Linzhi was directly adopted for our experiments. The main water quality indices of the sewage are given in *Table 1*.

Table 1. *Quality indicators of sewage*

Potential of hydrogen	Dissolved oxygen (mg/L)	Temperature (°C)	Chemical oxygen demand (mg/L)	Total nitrogen (mg/L)	Total phosphorous (mg/L)	Ammoniacal nitrogen (mg/L)
6.20~8.70	1.87~4.68	8.05~23.10	217.17~526.36	23.12~112.51	2.59~7.28	12.09~45.15

Operation of the anaerobic-anoxic-oxic process

The operation of the A2O device was studied under transient change of water temperature, in order to disclose the law of removal rates under different water temperatures. The control plans for the three parameters are specified below. On water temperature control, the inlet water flow was designed as 10.0 ± 0.1 L/s, HRT as 21.0 ± 0.2 h (the HRT ratio between anaerobic tank, anoxic tank and oxic tank = 35:58:117), DO as 3.0~4.0 mg/L, the reflux ratio of the mixed liquor R_i = 200%, and the reflux ratio of the sludge R = 100%. Both the mixed liquor and the sludge were continuously refluxed. Temperature fluctuation range is set to 8~25 °C. The temperature was controlled with an error of or less than 0.1 °C. The experiment was replicated three times. Water quality sampling interval was 3 h (*Fig. 1*).

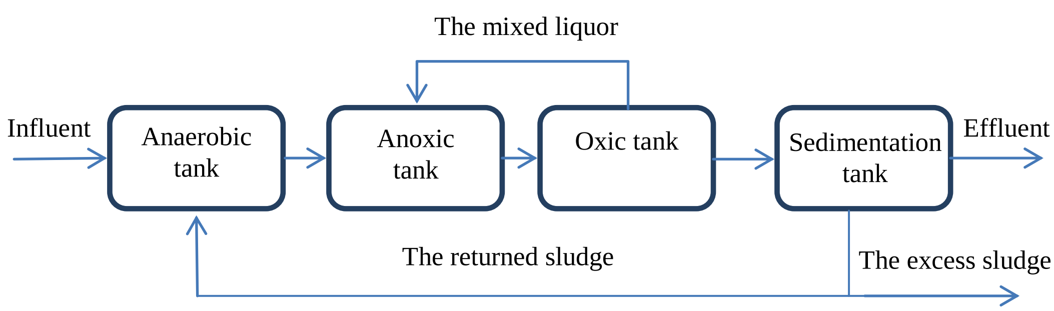

Figure 1. *Schematic of anaerobic-anoxic-oxic process*

Analytical methods

The measurement methods of the test indicators are selected according to the existing standards (State Environmental Protection Administration of China, 2002).

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Statistical analysis

All data were initially verified for normality using the Shapiro-Wilk method. The differences between different treatments were evaluated by one-way analysis of variance in SPSS, followed by Tukey’s test. All differences were considered significant at $p < 0.05$. All these statistical analyses were performed using the SPSS statistical package (ver. 22.0, SPSS Company, Chicago, IL, USA).

Results

Removal rate at temperature transients

Figures 2–5 provide the removal rates of COD, TP, TN and NH_4^+-N in anaerobic, anoxic and Oxic tanks at different temperatures.

As shown in *Figure 2*, the COD removal effect in the anaerobic tank maintains a good removal efficiency under the temperature from 8 °C to 25 °C. The removal efficiency of individual temperatures is low, and the average removal rate is 67.18%, which is lower than 75.69% (Huang et al., 2017); COD is mainly removed in the anaerobic tank with a maximum removal rate of 89.09% and a minimum removal rate of 34.11% corresponding to the temperature of 18.2 °C and 22.3 °C, respectively. The COD removal effect in the anoxic tank is generally acceptable, and the COD removal rate in the anoxic tank is only increased by 4.44%, which is lower than 12.72% (Huang et al., 2017), which increases the maximum value by 15.91% and the minimum value of -8.13% corresponding temperature are 22.3 °C and 15.1 °C, respectively; the maximum removal rate of 87.48% and the minimum value of 50.02% corresponding temperatures are 18.2 °C and 22.3 °C. The COD in the oxic tank can be removed as a whole due to the removal of the anaerobic tank and anoxic tank, but the average value of the relative anoxic removal rate is 10.07%, which is larger than 5.98% (Huang et al., 2017), the removal rate increases by a maximum of 27.69% and the minimum value of -0.90%, the corresponding temperatures are 8.98 °C and 18.2 °C; the average COD removal rate is 81.69%, and the maximum value of 91.15% and the minimum value of 70.06% corresponding temperature are 8.45 °C and 17.50 °C. The above trend is also consistent with the low temperature and room temperature COD treatment effects, but the effect is poor (Li et al., 2012); according to the COD removal rate effect, the test temperature is 18.2 °C, the removal rate can reach 92.19%, the measured COD effluent can reach the first grade A standards (GB18918-2002), but other temperatures are difficult to reach this standard.

As shown in *Figure 3*, the TP removal effect in the anaerobic tank changes obviously between 3.54% and 83.41% at the temperature of 8~25 °C. The removal efficiency of individual temperatures is low, and the average removal rate is 30.15%, which is lower than 69% (Bao et al., 2012). The highest TP removal rate of 63.58% and the lowest value of 5.56% corresponding temperatures are 12.00 °C and 22.40 °C, respectively. The anoxic tank contributes very little to the TP removal rate which is only increased by only 2.50%, which was lower than 56.00% (Zhou et al., 2013), which increased the maximum value by 20.19% and the minimum value by -13.86% corresponding to temperatures of 14.70 °C and 8.05 °C, respectively; the maximum removal rate of 71.95% and the minimum value of 6.06% corresponding temperatures are12.00 °C and 22.4 °C. The TP in the oxic tank is not obvious due to the poor removal rate of the anaerobic tank and anoxic tank, but the average value of the relative anoxic removal

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rate is 11.90%. Considering the dilution of the anaerobic section, the effect of removing TP is more significant, but the overall TP removal rate is still low (Zhou et al., 2013), the removal rate increases by 46.93% and the minimum value by -3.50%, and the corresponding temperatures are 8.49 °C and 22.3 °C, respectively; The average removal rate was 44.55%, and the maximum values of 83.41% and the minimum value of 3.54% corresponding to the temperature of 12.00 °C and 22.40 °C, respectively. When the test temperature is 12.00 °C, the removal rate of TP can reach 83.41%, and the sedimentation tank effluent regarding the TP can reach the grade 1 B standard (GB18918-2002), but TP removal rate can not reach this standard at the other temperatures. Overall, the removal rate of TP has two critical points of 9.54 °C and 14.70 °C.

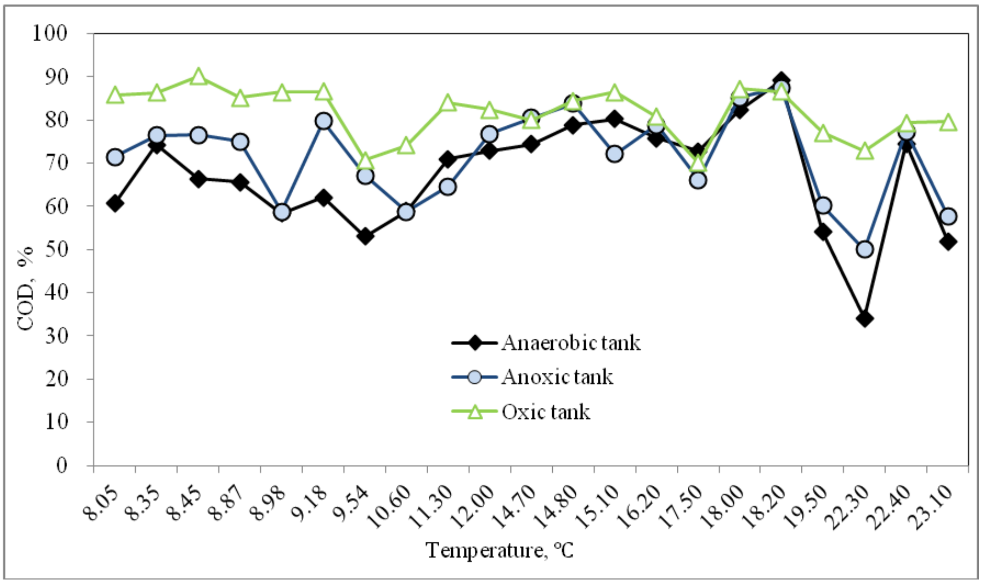

Figure 2. *Removal rate of chemical oxygen demand at different temperatures*

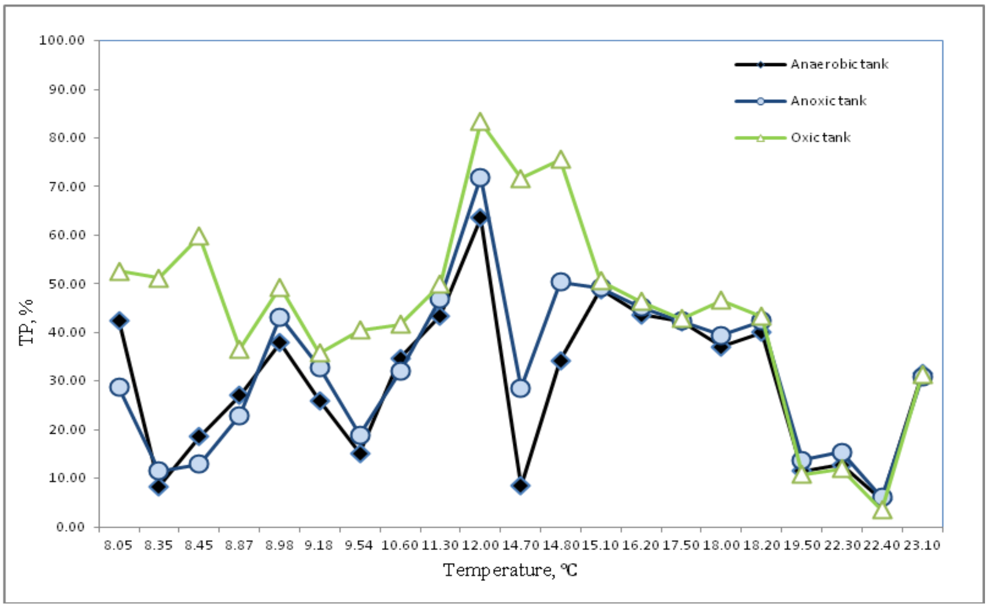

Figure 3. *Removal rates of total phosphorous at different temperatures*

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As shown in *Figure 4*, the TN removal rates in the anaerobic tank changes obviously between 1.34% and 95.37% when the temperature changed from 8 °C to 25 °C. The removal rate of TN is higher than other studies (Zhou et al., 2013). The average removal rate is 51.56%, which is higher than the result obtained by Zhou et al. (2013). The anoxic tank contributes very little to the TN removal rate which is only increased by 5.53%. The values corresponding to the value of 93.20% and the minimum of 20.23% corresponding to the temperatures were 9.18 °C and 8.35 °C. The TN in the oxic tank is not obvious due to the poor removal rate of the anaerobic tank and anoxic tank, but the average value of the relative anoxic removal rate is 4.53%, and the removal rate increases by 48.22% and -27.22%. The corresponding temperatures are 22.40 °C and 19.50 °C respectively; the average TN removal rate is 61.62%, and the maximum values of 95.37% and the minimum values of 23.16% correspond temperatures are 9.54 °C and 16.2 °C, respectively. According to the TN removal rate, the better temperature is 9.54 °C, and the removal rate can reach 95.37%, which is better than 80% (Wang et al., 2011). However, the dissolved oxygen value is as high as 3.70 mg/L, which is much higher than 1.20 ± 0.2 mg/L (Liu et al., 2011). Other temperatures are difficult to reach the Grade 1 B standard (GB18918-2002). Overall, the removal rate of TN has two critical points of 10.60 °C and 16.20 °C, and it is possible that 10.60 °C and 16.20 °C are the extreme temperatures of certain low temperature denitrifying microorganisms and these require additional attention in future research.

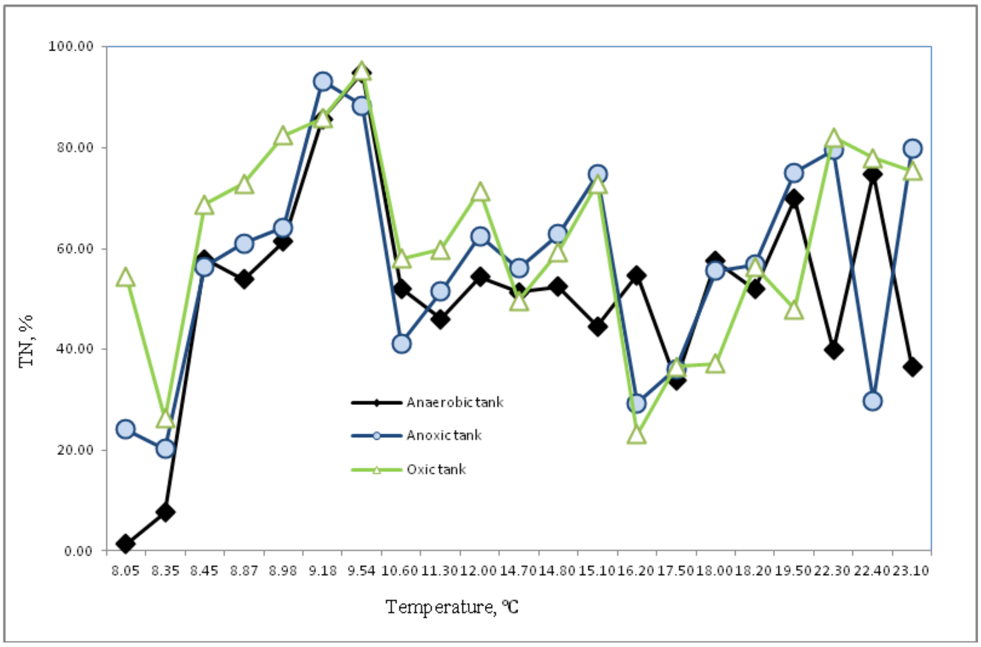

Figure 4. *Removal rates of total nitrogen at different temperatures*

As shown in *Figure 5*, the removal rate of NH_4^+-N in the anaerobic tank changes obviously between 9.36% and 97.23% under the fluctuation of temperature between 8 °C and 25 °C, the removal rate of NH_4^+-N changes obviously. The average removal rate is 61.46%, which is lower than 67.0% (Sohsalam and Sirianuntapiboon, 2008); the highest NH_4^+-N removal rate of 95.87% and the lowest value of 6.16% correspond temperatures are 22.30 °C and 8.35 °C, respectively. The anoxic tank contributes very little to the NH_4^+-N removal rate which is only increased by 4.04%, which is lower than

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16.12% (Sahariah et al., 2015), which increases the maximum value by 85.65% and the minimum value of -1.38% corresponding to the temperatures of 9.18 °C and 8.05 °C, respectively; the maximum removal rate of 95.08% and the minimum value of 9.36% corresponding temperatures are 95.02 °C and 8.05 °C. The NH_4^+-N in the oxic tank is not obvious due to the poor removal rate of anaerobic tank and anoxic tank, but the average value of the relative anoxic removal rate is 11.29%, which is larger than 11% (Peng et al., 2005), the removal rate increases the maximum value 57.14% and the minimum value of -2.50%, the corresponding temperatures were 8.05 °C and 17.50 °C, respectively; the average removal rate of NH_4^+-N was 77.42%, and the maximum value of 98.12% and the minimum value of 34.83% corresponding temperatures are 22.30 °C and 8.35 °C, respectively. According to the removal rate of NH_4^+-N, the better temperature is 22.30 °C, the removal rate can reach 98.12%, and the measured NH_4^+-N effluent in the sedimentation tank can reach the first grade A standards (GB18918-2002), and the initial concentration is low so other temperatures can also reach this standard. In general, the two critical temperatures of 10.60 °C and 17.50 °C divide the curve into three parts. 8.05~9.54 °C is the first part, which is a significant increase in the NH_4^+-N removal rate. 10.60~17.50 °C is the second part, which is not obvious for the NH_4^+-N removal rate. 17.50~23.10 °C is the third part, which is a significant change in the TP removal rate. And it is possible that 10.60 °C and 17.50 °C are the extreme temperatures of certain low temperature denitrifying microorganisms.

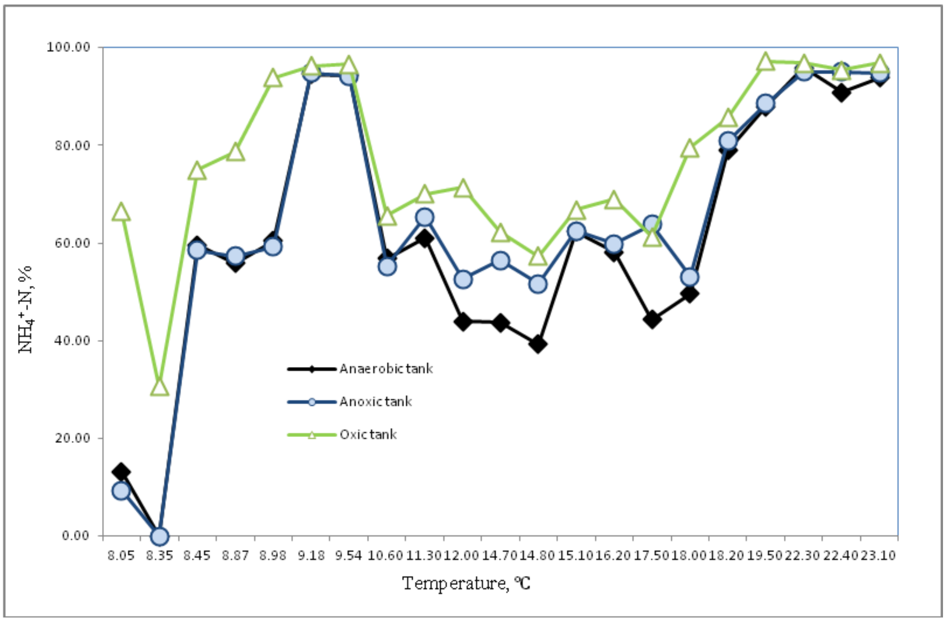

Figure 5. *Removal rates of ammoniacal nitrogen at different temperatures*

Microorganisms

The response of microorganisms in anaerobic tank, anoxic tank and oxic tank under temperature fluctuation of 8–25 °C is shown in *Figure 6.*

It can be seen from *Figure 6a* that the microbial density of anaerobic tanks generally shows a process of gradually decreasing from the high level and then increasing. The microbial density of the anoxic tank and oxic tank is flatter. The experiment showed that the microbial density in each tank was from 8 °C to 12 °C and 12 °C to 23.1 °C.

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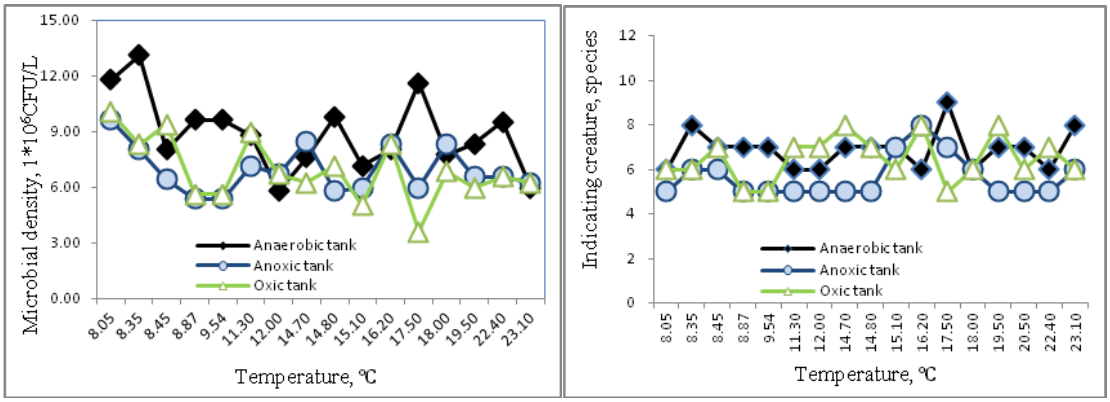
The microbial density of 8~12 °C showed a downward trend, which may be related to the higher temperature of sludge culture and domestication. The trend appeared in density; the microbial density of each tank showed a oscillating upward trend at 12 °C to 23.1 °C. Overall, the microbial density of anaerobic tanks was higher than that of anoxic tank and oxic tank. There was no significant difference in microbial density between anoxic tank and oxic tank, but the experimental microbial density was lower than 10^8 CFU/L (Yang, 2017).



a b

Figure 6. *Microbial response at different temperatures*

It can be seen from *Figure 6b* that the relative changes of indicating creature are not significant in the three reaction cells, and the anaerbic tank and oxic tank indicate that the indicating creature are significantly better than the anoxic tank. Microscopic observations showed that the indicated microorganisms in each tank were pointed caterpillars, bell worms and trichinella, and the number of pointed caterpillars and bell worms decreased gradually while the trichinella showed an increasing trend. It has also been observed that Surticae has an increasing trend, which may be related to DO, and DO is greater than the minimum limit of 2.5 mg/L (Bernot and Wilson, 2012).

Mixed liquid suspended solids and volume percent after settling for 30 min

It can be seen from *Figure 7* that MLSS shows a certain increasing trend with increasing temperature in the range of 8.05 °C to 11.3 °C. In the range of 11.3 °C to 23.1 °C, MLSS shows a certain downward trend with increasing temperature; MLSS is less than 3,000 mg/L (Whang and Lim, 2008). SV30 shows a slow downward trend with increasing temperature.

Discussion

The removal contributions of anaerobic tank, anoxic tank, oxic tank and secondary sedimentation tank at the average removal rate of COD, TN, NH_4^+-N and TP are shown in *Figure 8*.

In addition to TP, the removal of COD, TN, and NH_4^+-N mainly occurs in anaerobic tank, while TP mainly occurs in anaerobic tank and oxic tank. The COD removal effect in each tank is consistent with the microbial density in the corresponding tank; the TP removal effect in each tank is consistent with the change trend of MLSS, and the two

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also exhibit a certain positive correlation, which is related to MLSS was key factors, which had a significant impact on Anaerobic phosphorus release and anoxic phosphorus uptake in the biological phosphorous removal process. Wang et al. (2007) are more consistent; TN and NH_4^+-N removal effects have a more consistent trend.

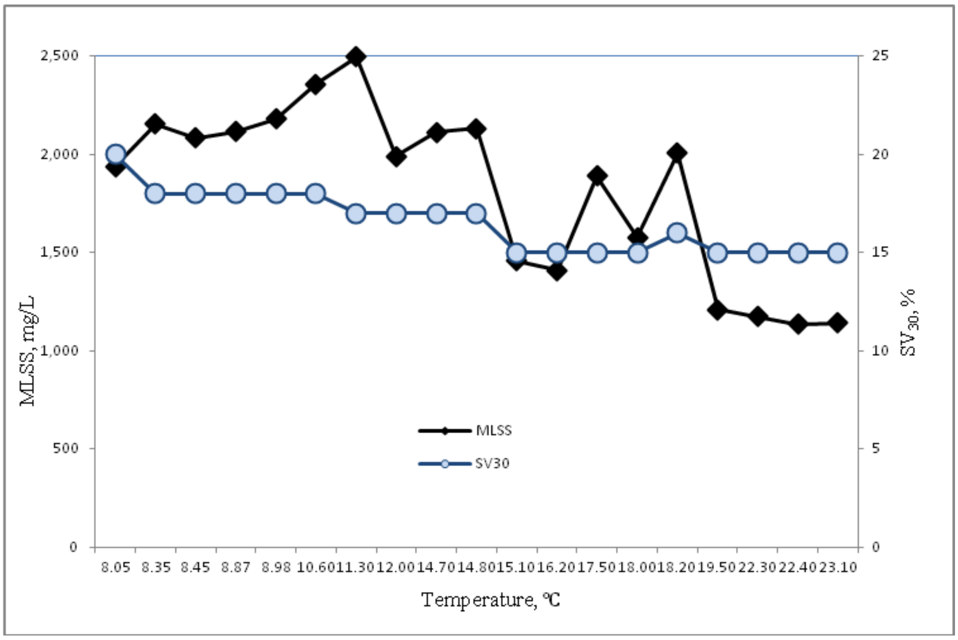

Figure 7. *Mixed liquor suspended solids and volume percent after settling for 30 min at different hydraulic retention times*

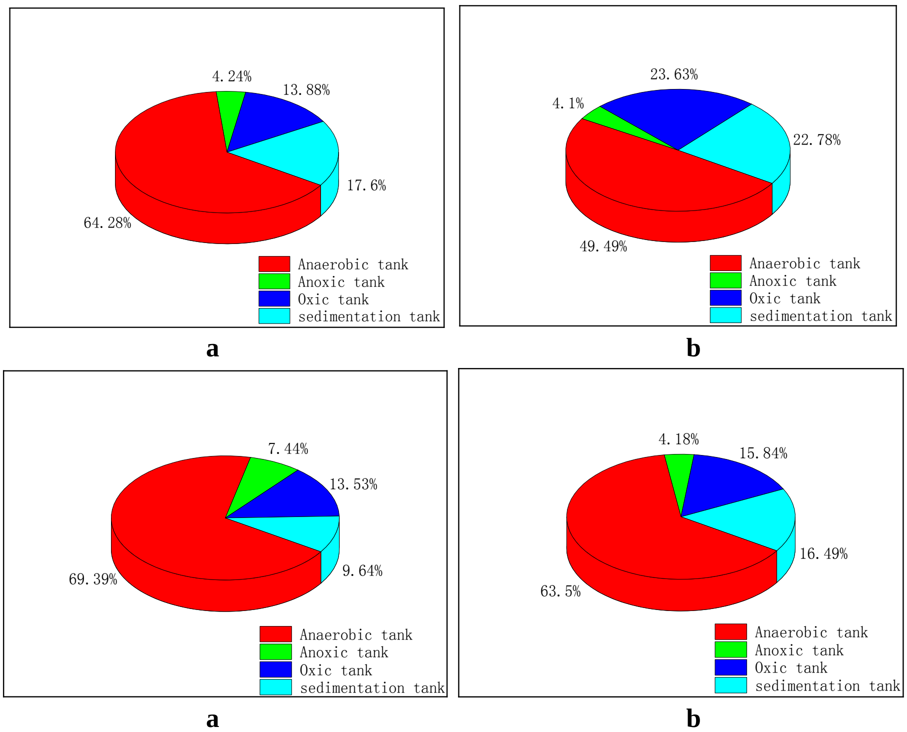

Figure 8. *Removal contribution ratios of **a** chemical oxygen demand, **b** total phosphorous, **c** total nitrogen and **d** ammoniacal nitrogen of each tank*

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Conclusion

Under the unique plateau environmental factors, the effects of temperature transients on COD, TP, TN, NH_4^+-N removal rate and microbial response were studied using a pilot-scale A2O process. The transient temperature changes show that the optimal removal rate of each water quality index is different. The COD removal rate maintains a good removal efficiency and the removal rate of TN, TP and NH_4^+-N in the anaerobic tank changes obviously under the temperature from 8 °C to 25 °C. The removal of COD, TN and NH_4^+-N mainly occurs in anaerobic tanks while TP mainly occurs in anaerobic and oxic tanks, anaerobic There is no obvious response relationship between microorganisms and indicator organisms in the tank, anoxic tank and oxic tank. The COD, TP, TN, NH_4^+-N in the effluent do not meet the Grade A standard, and the MLSS follows the increased temperature. The increase has not shown a significant growth trend. There are two distinct critical temperatures of 10.60 °C and 16.20 °C to the NH_4^+-N removal rate, of 10.60 °C and 17.50 °C to the TN removal rate and of 9.54 °C and 14.70 °C to the TP removal rate, and it is possible that two distinct critical temperatures are the extreme temperatures of certain low temperature denitrifying microorganisms. The above problems may be related to the long-term response time of temperature transient changes under high altitude environmental factors, and the corresponding mechanism needs to be studied later.

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