Correlation analysis of sewage removal rate and microbial quantity in plateau environment

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Abstract. Based on A\textsuperscript{2}O process in plateau environment, the correlation between the removal rate of chemical oxygen demand (COD), the total phosphorous (TP), total nitrogen (TN) and ammoniacal nitrogen (NH\textsubscript{4}+-N) in anaerobic tank, anoxic tank and aerobic tank and the quantity of indicator microorganisms was analyzed with temperature, dissolved oxygen (DO) and the hydraulic retention time (HRT) as working conditions, and the curve regression model of water removal rule and the optimal working conditions were obtained through SPSS analysis. The results showed that the removal rates of COD, TN, TP and NH\textsubscript{4}+-N were positively and negatively correlated with some indicator microorganisms. The three sets of curve regression models passed the significance test. The DO and COD were cubic models, HRT and COD were quadratic models, HRT and NH\textsubscript{4}+-N were S curve models. When DO is 2~3 mg/L, the removal rate of COD can reach 87%. When HRT is 30~40 h, the removal rate of COD can reach more than 90%, and the removal rate of NH\textsubscript{4}+-N can reach more than 50%.

1. Introduction
Tibet is located in the plateau zone of the world, with an average elevation of more than 4000 meters, and is in a cold region with a harsh environment such as strong ultraviolet light, low temperature, and low dissolved oxygen. Although the air quality is excellent all year round, the water environment is increasingly suffering damage and is irreversible, and the treatment of wastewater in Tibet is not mature and perfect [1-3]. Different conditions of water temperature, DO and HRT have different effects on wastewater treatment efficiency. In particular, it affects the rate of P uptake and P release, the ability of nitrification and denitrification, the removal effect of nitrogen and phosphorus, and the total number and activity of ammonia-oxidizing bacteria [4-7].

However, there is little research on this in the plateau environment, so the traditional A\textsuperscript{2}O process was used to analyze the correlation between the removal rate of COD, TN, TP and NH\textsubscript{4}+-N and the number of microorganisms in the anaerobic, anoxic and aerobic tanks under the conditions of temperature, DO and HRT in the plateau environment, and the curve regression model and the optimal conditions of effluent removal were obtained through SPSS analysis.

2. Materials and methods
The experiment adopts the traditional A\textsuperscript{2}O process, and the effective volume of anaerobic tank, anoxic tank, aerobic tank and secondary sedimentation tank are 35L, 58L, 117L and 39L respectively. Influent flow is 10L/h, DO of aerobic tank is 3.0 mg/L, water temperature is 20 \degree C, pH adjustment is about 7.5, sludge reflux ratio is 20~50%, and mixture reflux ratio is 100%~300%.
COD was measured by potassium dichromate method, TP was measured by spectrophotometry, TN was measured by potassium persulfate-ultraviolet spectrophotometry, and NH$_4^+$-N was measured by Nessler reagent spectrophotometry. Microbiological observation methods use water droplet counting methods, and the type and quantity of microorganisms are recorded by the microscope and the computer imaging system.

The water quality index of COD, TN, TP and NH$_4^+$-N and the quantity of indicator microorganism in anaerobic tank, anoxic tank, aerobic tank and effluents of each working condition were measured, and the best working condition was obtained.

3. Results and analysis

3.1. Correlation analysis of wastewater removal efficiency and microbial quantity

Pearson correlations of COD, TP, TN, NH$_4^+$-N removal rates in anaerobic, anoxic, and aerobic tanks with the number of indicator microorganisms such as rotifers, rotifers, bell insects, rotifers, nematodes, sun worms, aculeate caterpillars, cumulus, and paramecium, respectively, were analyzed by SPSS, and the results of microorganisms with good and excellent significance are shown in Table 1: (In the table, * indicates that the significance is less than 0.05, which is considered significant; ** indicates that the significance is less than 0.01, which is extremely significant)

| contaminant | anaerobic tank | anoxic tank | aerobic tank |
|-------------|----------------|-------------|--------------|
| COD         | point caterpillar | point caterpillar | rotifer |
|             | tractor         | paramecium  | nematode    |
|             | -0.436*         | -0.371*     | 0.423*      |
|             | -0.459*         | 0.441*      | 0.407*      |
| TP          | sun worm        | sun worm    | paramecium  |
|             | 0.430*          | -0.381*     | 0.409*      |
| TN          | caterpillar     | caterpillar | caterpillar |
|             | point caterpillar | point caterpillar | nematode |
|             | 0.579**         | -0.514**    | 0.385*      |
|             | -0.515**        | 0.389*      | -0.461*     |
| NH$_4^+$-N  | caterpillar     | caterpillar | caterpillar |
|             | point caterpillar | point caterpillar | point caterpillar |
|             | 0.491**         | 0.655**     | 0.618**     |
|             | -0.535**        | -0.732**    | -0.629**    |

According to Table 1, the removal rate of COD is negatively correlated with the caterpillars in the anaerobic and anoxic tanks, and negatively correlated with the tractor in the anaerobic and aerobic tanks, and positively correlated with the rotifers and nematodes in the aerobic tank. The removal rate of TP is negatively correlated with sun worms in anaerobic and anoxic tanks, and positively correlated with paramecia in the anaerobic and aerobic tanks. The removal rate of TN is positively correlated with the caterpillars in the three tanks, negatively correlated with the point caterpillars in the anaerobic and anoxic tanks, positively correlated with the bell worms in the anoxic tank, and negatively correlated with the nematodes in the aerobic tank. The removal rate of NH$_4^+$-N is positively correlated with the caterpillars and negatively correlated with the point caterpillars in the three tanks.

When DO was high, rotifers appeared in the activated sludge, indicating a better treatment effect. Rotifers are higher metazoans, suitable for growing in environments with high DO concentrations, low pollutant concentrations, and pH around 6.8, and belong to indicator microorganisms with good microbial treatment effects and stable treatment systems [8]. Nematodes thrive under anoxic conditions and are indicative of poorly treated water bodies. The removal rate of TN in the above aerobic tank was negatively correlated with nematodes, indicating that the fewer the nematodes, the better the TN treatment. Paramecium bursaria has a strong ability to withstand pollution, mostly in
anoxic or anaerobic environments, and has poor purification [9]. When bell bugs and cumulants occur, they indicate better water quality and have the efficacy to reduce pollutant concentrations [10,11].

3.2. Regression analysis of effluent removal rate curve

Temperature, DO and HRT were used as independent variables, COD, TP, TN and NH$_4^+$-N removal rate were used as dependent variables to establish regression equation by SPSS22.0.

3.2.1. Curve regression analysis with temperature as independent variable. SPSS22.0 was used to fit the curve with temperature as independent variable and COD, TP, TN and NH$_4^+$-N removal rates as dependent variables, respectively, and the curve models that passed the significance test were screened to obtain the equation of temperature and COD curve. Summary and parameter estimation of the model were shown in Table 2:

| Equation   | Model Summary | Parameter Estimates |
|------------|---------------|---------------------|
|            | R Square | F | df1 | df2 | Sig. | Constant | b1   |
| Linear     | .676     | 6.250 | 1   | 3   | .088  | 88.606   | -.203 |
| Logarithmic| .720     | 7.729 | 1   | 3   | .069  | 95.707   | -3.813 |
| Inverse    | .751     | 9.063 | 1   | 3   | .057  | 80.875   | 63.435 |

It can be seen from Table 2 that the significance of the inverse model is the most, but it greater than 0.05, indicating that the statistical significance of the model is not significant. Therefore, this model equation is no longer discussed.

3.2.2. Curve regression analysis with DO as independent variable. With DO as the independent variable and COD, TP, TN and NH$_4^+$-N removal rates as the dependent variables respectively, the curves were fitted and the curve models that passed the significance test were screened to obtain the curve equations of DO and COD. The total model and parameter estimates are shown in Table 3:

| Equation   | Model Summary | Parameter Estimates |
|------------|---------------|---------------------|
|            | R Square | F | df1 | df2 | Sig. | Constant | b1  | b2 | b3 |
| Logarithmic| .555     | 9.981 | 1   | 8   | .013  | 80.662   | 6.467 |
| Inverse    | .701     | 18.723 | 1   | 8   | .003  | 93.129   | -14.448 |
| Cubic      | .877     | 14.220 | 3   | 6   | .004  | 47.584   | 41.947 | -13.549 | 1.383 |
| Power      | .566     | 10.442 | 1   | 8   | .012  | 80.508   | .078  |
| S          | .717     | 20.226 | 1   | 8   | .002  | 4.538    | -.174 |

When the significance reaches 0.05, it means that the comparison of statistical significance for model establishment is significant; when the significance reaches 0.01, it means that the statistical significance for model establishment is very significant. In Table 3, the conforming models are the inverse model, cubic curve model and S-curve model. The model with the largest R square is cubic curve model. Therefore, the optimal model of DO and COD is cubic curve model. The formula is:

\[
COD = 41.947 \times (DO) - 13.549 \times (DO)^2 + 1.383 \times (DO)^3 + 47.584
\] (1)
When the removal rate of COD reaches 100%, DO should be 5.58 mg/L. However, the actual removal rate cannot reach 100%, so the actual DO setting value should be less than 5.58 mg/L.

As can be seen from Figure 1, the cubic model had the best goodness of fit and outperformed the other models. The trend of cubic model is to increase first and then decrease and then increase. When the removal rate of COD reaches more than 87%, DO is between 2~3 mg/L, so it is reasonable to set DO at 2~3 mg/L, which is similar to the results of Li Shuang [12] and inconsistent with the results of Zhao Weibing [13].

Performing a coefficient test on Equation 1, and the regression coefficient test of the cubic model is less than 0.05, so the regression coefficient of the cubic model is significant, indicating that the cubic curve model of DO and COD passed the coefficient test.

### 3.2.3. Curve regression analysis with HRT as independent variable

With HRT as the independent variable and COD, TP, TN and NH$_4^+$-N removal rates as the dependent variables, respectively, the curve curves were fitted and the curve models that passed the significance test were screened. The curve equations of HRT and COD and HRT and NH$_4^+$-N were obtained. The total number of HRT and COD models and parameter estimation were shown in Table 4:

| Equation | Model Summary | Parameter Estimates |
|----------|---------------|---------------------|
|          | R Square      | F       | df1 | df2 | Sig.   | Constant | b1    | b2    | b3    |
| Quadratic| .991          | 110.452 | 2   | 2   | .009   | 39.094   | 3.492 | -.056 |       |
| Cubic    | .984          | 60.208  | 2   | 2   | .016   | 48.914   | 2.097 | .000  | -.001 |

According to Table 4, the relationship between HRT and COD conforms to the quadratic curve model, R square is 0.991, and the significance level is extremely significant, which can reach 0.009. Other curvilinear models were not analyzed in the table, and the models with better fit were quadratic and cubic equations, which were not presented in the table because of the poor significance level of the other models. The curve equation of HRT and COD is thus:

\[
COD = 39.094 + 3.492 \times (HRT) - 0.056 \times (HRT)^2
\]  

(2)
Figure 2. Fit regression line of COD and HRT models.

It can be seen from Figure 2 that the fit of the quadratic curve is better than that of the cubic curve, and the removal rate of COD can reach the maximum value when the water conservancy residence time is from 30 h to 40 h. COD removal rate increases at first and then decreases with the increase of water retention time, so the HRT setting of A²O unit is still very important for COD removal rate. HRT ranged from 10.5 h to 26.25 h. The removal rate of COD increased with the increase of HRT, which was consistent with the findings of Liu Rui et al.[14].

Perform coefficient test on Equation 2, and obtain the regression coefficients of the quadratic models were all less than 0.5, and the regression coefficients of the squared models were significant. It is thus shown that the squared model of Equation 2 passes the significance test and has applied research value.

The model totals and parameter estimates for HRT versus NH₄⁺-N are shown in Table 5:

### Table 5. HRT and NH₄⁺-N model summary and parameter estimates.

| Equation   | Model Summary | Parameter Estimates |
|------------|---------------|---------------------|
|            | R Square | F | df1 | df2 | Sig. | Constant | b1 |
| Logarithmic | .859     | 18.247 | 1    | 3    | .024  | -52.693  | 31.266 |
| Inverse    | .877     | 21.476 | 1    | 3    | .019  | 79.317   | -666.911 |
| Power      | .858     | 18.077 | 1    | 3    | .024  | 3.609    | .774 |
| S          | .907     | 29.246 | 1    | 3    | .012  | 4.566    | -16.785 |

According to Table 5, the S-curve model had the best fit, followed by the inverse model, and the logarithmic model and the power model are the worst. This result was analyzed according to the R-square and significance level. Thus, the model of HRT and NH₄⁺-N is S-curve model, and the curve equation is:

\[ NH_4^+-N = e^{(4.566-16.785/HRT)} \]  

The fitted regression line of the S-curve model of HRT versus NH₄⁺-N was derived from Equation 3 and shown in Figure 3.
According to Figure 3, the slope of water conservancy residence time increases from 10 h to 30 h, and the growth rate becomes smaller; the slope of water conservancy residence time increases from 30 h to 60 h, and the growth rate becomes larger. Since the actual experiment cannot only ensure the removal rate, the HRT should be set at 30 h to 40 h in actual operation to ensure that the removal rate of NH$_4^+$-N is above 50%, which is similar to the results of Liu et al [14].

Perform coefficient test on Equation 3, and the regression coefficient test of the S-curve model is less than 0.5, indicating that the regression coefficient of the S-curve model is significant, which has application value and significance for this study and subsequent studies through coefficient test.

3.3. Discussion
In this paper, SPSS22.0 is used to analyze the effluent removal rate, and the partial curve model and the optimal operating condition range are obtained, which is more systematic and specialized than the conventional analysis, but the scientific reliability remains to be demonstrated.

(1) Using SPSS analysis, get the corresponding model curve and formula, more intuitive than conventional analysis, operating space is larger, but the data may be distorted, larger than the original data changes, persuasion needs to be strengthened.

(2) The effective combination of experiment and theory provides the direction for future research, and readers can demonstrate or publish different conclusions.

(3) Some curve models were not successfully fitted, and further study is needed. The curve fitting is of practical value and significance for this study and subsequent studies.

4. Conclusion
Through the two-tailed test of Pearson correlation of SPSS, the removal rates of COD, TN, TP and NH$_4^+$-N were positively and negatively correlated with some indicator microorganisms in the three tanks.

Using the curve estimation method in SPSS 22.0, using temperature, DO and HRT as independent variables, using the effluent removal rate of COD, TP, TN and NH$_4^+$-N as the dependent variables to establish the regression equation, and 12 sets of curve regression equations were obtained. Among them, three groups determined the appropriate curve model through R square and significance level, and further proved the applicability of the curve model through coefficient test, and finally determined the appropriate operating condition range of A'O device according to the curve model diagram.

Figure 3. Fit regression line of S curve model of HRT and NH$_4^+$-N.
When the model of DO and COD is obtained as a cubic model, the equation is: \[ \text{COD} = 41.947 \times (\text{DO}) - 13.549 \times (\text{DO})^2 + 1.383 \times (\text{DO})^3 + 47.584. \] When DO was at 2~3 mg/L, the removal rate of COD was over 87%.

The relationship between HRT and COD was found to fit a quadratic model with the equation: \[ \text{COD} = 39.094 + 3.492 \times (\text{HRT}) - 0.056 \times (\text{HRT})^2. \] The maximum removal rate of COD was over 90% when HRT was from 30h to 40h. The curve model of HRT and \( \text{NH}_4^+ - \text{N} \) is S curve model, the equation is \[ \text{NH}_4^+ - \text{N} = e^{(4.566 - 16.785/\text{HRT})}. \] When the removal rate of \( \text{NH}_4^+ - \text{N} \) is above 50%, HRT should be set as 30h~40h.

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References
[1] Xiangyu C, Kaiyue H, Dong S, et al. 2018 Characteristic Study on Wastewater Treatment in High Altitude Area by A2/O Process [J] TECHNOLOGY OF WATER TREATMENT
[2] Kaiyue H, Xiangyu C, Yuanwei L, et al. 2018 Correlative Analysis of Ultraviolet Radiation and Air Quality in Linzhi[J] Environmental Science & Technology
[3] Yongchen Z, Yongheng Z, Guanghua L, et al. 2018 Study on Process Characteristics of High Altitude A2/O Process Based on Principal Component Analysis[J] TECHNOLOGY OF WATER TREATMENT
[4] Li Si Min, Du G S, Tang F B 2013 Nitrogen and phosphorus removal of modified A2/O process on low-carbon domestic sewage under low temperature[J] Advanced Materials Research 777 187-191
[5] Si-Min LI, Tong H, Ruo-Bing W, et al. 2014 Operation of Modified A~2/O Process at Low Temperature and Different Sludge Loadings[J] China Water & Wastewater 2014(13) 64-68
[6] Shen N, Chen Y, Zhou Y 2017 Multi-cycle operation of enhanced biological phosphorus removal (EBPR) with different carbon sources under high temperature[J] Water Research 114 308-315
[7] Yoo H S, Lee B 2015 A study on adjustment of operational factor in A2O process[J] Korea Organic Resource Recycling Association 23(3) 33-41
[8] SalvadóH, Gracia M P 1993 Determination of organic loading rate of activated sludge plants based on protozoan analysis[J] Water Research 27(5) 891-895
[9] Haitao K, Wemping C, Tianyi M, et al. 2018 Functions Analysis of Protozoans and Metazoans in Wastewater Bio-chemical Treatment System [J] Industrial Safety and Environmental Protection 44(05) 42-44+56
[10] Fang M, Jixian Y, Li W, et al. 2010 Environmental microbial map [M] China Environmental Science Press 2010
[11] Shenghua Z 2005 Water treatment microbiology [M] Chemical Industry Press 2005
[12] LI Shuang 2013 DO Research on A~2O System Nitrogen and Phosphorus Removal and Microbial Community Structure [D] Shenyang Jianzhu University 2013
[13] Weibing Z, Tianhu C, Qiang Z, et al. 2014 Effect of dissolved oxygen on removal of nitrogen and phosphorus in Biolak/A~2O process[J] Acta Scientiae Circumstantiae 2014
[14] Rui L, Yanmei G, Xiaohui W, et al. 2017 Effects of hydraulic retention time on MFC coupled A2/O progress for domestic wastewater treatment[J] Acta Scientiae Circumstantiae 37(2) 680-685