Study on the Treatment of Domestic Sewage by Lactococcus

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Abstract: As a commonly probiotics, lactococcus produces a large amount of lactic acid during the growth and metabolism process, which can regulate the pH value of the environment. At the same time, lactic acid can also transform the refractory organic matter in sewage into degradable or easily degradable substances, thus accelerating the degradation rate of pollutants. The removal rate COD, TP and TN were selected as the test indexes in the experiment. The single-factor experiment was carried out first. Orthogonal experiments were carried out according to the result of single-factor experiment. Experiments showed that the optimal reaction time of COD, TP and TN removal was 54h/42h/24h, the optimal dosage was 9.00mL/4.00mL/9.00mL, the optimal pH value was 6.00/6.00/8.00, and the removal rate was 68.41%/80.04%/32.54% respectively. It can be concluded that the degradation efficiency of lactococcus on TP and COD is better, while the degradation efficiency on TN is worse.

1. Introduction
With the acceleration of urbanization and the improvement of people's living standards, sewage discharge is increasing. Domestic sewage is the main source of pollution after industrial sewage, which contains a large number of carbohydrates, nitrogen, phosphorus and a variety of pathogenic microorganisms[1]. If domestic sewage is discharged untreated, it will seriously threaten people's health.

Compared with the traditional treatment methods, the microbial treatment method has the advantages of low energy consumption, efficiency, economically, simple process, easy operation, safety and environmental[2]. The lactic acid produced during the metabolism of lactococcus can regulate the pH value, inhibit the growth of harmful bacteria and reduce the release of odorous gases. Inoculation of Lactococcus into a certain amount of domestic sewage can degrade inorganic substances such as nitrogen and phosphorus in domestic sewage, and the degraded domestic sewage can be reused as reclaimed water to alleviate water pollution[3].

The removal rate COD, TP and TN were selected as the test indexes in the experiment[4]. Potassium dichromate method[5-6] was used for the determination of COD, Molybdenum-Antimony Anti-Spectrophotometry[7] was used for the determination of TP, and alkaline potassium persulfate digestion ultraviolet spectrophotometry[8-10] was used for the determination of TN.

2. Materials and Methods

2.1 Experimental Instruments and Materials
SG-300 optical microscope (Huizhou Mixian e-commerce Co., Ltd.); XFH-75CA vertical high-pressure steam sterilizer (Shanghai Precision Instrument Co., Ltd.); SW-CJ-1D single super clean workbench (Suzhou purification equipment Co., Ltd.); SHZ-82A thermostatic oscillator (Changzhou Guohua Electric Co., Ltd.); 101-1AB electric blast drying oven (Tianjin HongNuo Instrument Co.,
2.2 Experimental Method

2.2.1 Screening and domestication of lactococcus.
Lactobacilli and lactococcus were isolated from yoghurt by preliminary identification[11-12]. 1.00 mL of lactobacilli and lactococcus were respectively put into three test tubes, each of which was cultured in three tubes, and cultured at 43°C. Observe the condition of the test tube every 1 hour, mainly including the color, whey precipitation, bubble generation, etc., and measure the acidity of the fermentation liquid every 2 hours. Select the strains with good coagulating effect and fermentation performance for standby[13-14]. lactococcus is selected for the experiment according to the comparison results.

Figure 1. Lactococcus under microscope

10.00 mL domestic sewage was added in 250 mL conical bottle, then medium was added and solution diluted to to 100 mL. The conical bottle was sterilized in a high-pressure steam sterilizer. After cooling to room temperature, lactococcus was inoculated and cultured for 48 hours. If the lactococcus grows well, the dominant lactococcus was extracted from it. Continue to improve the sewage content in the culture medium, add 20 mL sewage in conical bottle, dilute it to 100 mL, and inoculate lactococcus after sterilization which are the dominant lactococcus in the culture medium with 10% sewage concentration. And so on, the proportion of sewage in the culture medium should be continuously improved until the required dominant bacteria can be obtained. The culture of the dominant bacteria was expanded to obtain the lactobacillus solution with a concentration of $7.5 \times 10^9$ cfu/mL.

2.2.2 Single factor degradation experiment.
Single factor degradation experiment was carried out to explore the effects of reaction time, lactococcus dosage and pH value of water on the removal rate of COD, TP and TN in domestic sewage. The experimental process is as follows: firstly, take 200.00 mL of sewage and add it into five 250mL
conical flasks. Secondly, add 5.00 mL of lactococcus liquid culture medium. Finally, Measure the concentration of COD, TP and TN before and after the reaction under different single factor conditions and calculate the removal rate, determine the best reaction time, dosage, pH value.

2.2.3 Orthogonal experiment.
In the actual sewage treatment process, each factor does not play a role alone, but a combination of many factors. Therefore, L_9(3^4) orthogonal experiment is designed with reaction time (A), dosage (B) and pH (C) as the investigation factors. The factor level table is shown in Table 1, and the determination method is the same as the degradation experiment.

### Table 1. Table of orthogonal experimental factors.

| Index | Factor | Level | Time(h) | Dosage(mL) | pH  |
|-------|--------|-------|---------|------------|-----|
|       | COD    | 1     | 42      | 9.00       | 5.00|
|       |        | 2     | 48      | 10.00      | 6.00|
|       |        | 3     | 54      | 11.00      | 7.00|
|       | TP     | 1     | 30      | 4.00       | 5.00|
|       |        | 2     | 36      | 5.00       | 6.00|
|       |        | 3     | 42      | 6.00       | 7.00|
|       | TN     | 1     | 18      | 9.00       | 7.00|
|       |        | 2     | 24      | 10.00      | 8.00|
|       |        | 3     | 30      | 11.00      | 9.00|

3. Results and Discussion

3.1 Single Factor Experiment Results
The optimum reaction time of COD, TP and TN treated by lactococcus was 48h/36h/24h, the optimum dosage was 10.00mL/5.00mL/10.00mL, and the optimum pH value was 6.00/6.00/8.00, respectively.

3.2 Orthogonal Experiment Results and Discussion
The reaction time, dosage and pH value that affect the removal rate were selected as the factors, and the removal rate of pollutants was taken as the index. The experiment was carried out according to L_9(3^4) orthogonal experiment. The orthogonal experiment design and results are shown in Table 2, and the visual analysis table is shown in Table 3.

### Table 2. Orthogonal experimental design and results of domestic sewage treatment by Lactococcus.

| Number | A | B | C | D(blank) | COD | TP | TN  |
|--------|---|---|---|---------|-----|----|-----|
| 1      | 1 | 1 | 1 | 1       | 61.02| 76.69| 29.79|
| 2      | 1 | 2 | 2 | 2       | 62.93| 75.54| 13.19|
| 3      | 1 | 3 | 3 | 3       | 60.65| 72.47| 11.81|
| 4      | 2 | 1 | 2 | 3       | 57.14| 73.62| 32.54|
| 5      | 2 | 2 | 3 | 1       | 61.88| 70.84| 30.58|
| 6      | 2 | 3 | 1 | 2       | 52.83| 50.90| 17.80|
| 7      | 3 | 1 | 2 | 2       | 68.41| 80.04| 25.66|
| 8      | 3 | 2 | 1 | 3       | 50.12| 63.94| 18.69|
| 9      | 3 | 3 | 3 | 1       | 57.32| 50.13| 15.84|
Table 3. Visual analysis table.

| Category | Index | A    | B    | C    |
|----------|-------|------|------|------|
| COD      | $K_1$ | 0.6153 | 0.6219 | 0.5466 |
|          | $K_2$ | 0.5728 | 0.5831 | 0.6283 |
|          | $K_3$ | 0.5862 | 0.5693 | 0.5995 |
|          | $R_1$ | 0.0292 | 0.0526 | 0.0817 |
|          | $K_2$ | 0.7490 | 0.7678 | 0.6384 |
| TP       | $K_1$ | 0.6512 | 0.7011 | 0.7640 |
|          | $K_2$ | 0.6470 | 0.5783 | 0.6448 |
|          | $K_3$ | 0.1020 | 0.1895 | 0.1256 |
|          | $R_1$ | 0.1826 | 0.2933 | 0.2209 |
|          | $K_2$ | 0.2697 | 0.2082 | 0.2380 |
|          | $K_3$ | 0.2006 | 0.1515 | 0.1941 |
|          | $R_1$ | 0.0871 | 0.1418 | 0.0268 |

Note: $K_1$, $K_2$, $K_3$ represent the mean value of experimental results, and $R$ represents the range.

The experimental results show that:

1. In the orthogonal experiment of COD removal, the order of factors affecting the removal rate was pH > dosage > reaction time. The removal rate of group 8 was the lowest (50.12%), and group 7 was the highest (68.41%). The best orthogonal experimental combination of the treatment of COD in domestic sewage by lactococcus is $A_3B_1C_2$, that is, the reaction time is 54h, the dosage is 9.00mL, and the pH value of the solution is 6.00.

2. In the orthogonal experiment of TP removal, the order of factors affecting the removal rate was dosage > pH > reaction time. The removal rate of group 9 was the lowest (50.13%), and group 7 was the highest (80.04%). The best orthogonal experimental combination of the treatment of TP in domestic sewage by lactococcus is: $A_3B_1C_2$, i.e. the reaction time is 42h, the dosage is 4.00mL, and the pH value of the solution is 6.00.

3. In the orthogonal experiment of TN removal, the order of factors affecting the removal rate was dosage > reaction time > pH. The removal rate of the third group was the lowest (11.81%), the fourth group was the highest (32.54%). The best orthogonal experimental combination of treating TN in domestic sewage by lactococcus is $A_2B_1C_2$, that is: reaction time 24h, dosage 9.00mL, solution pH 8.00.

4. Conclusions

As a common probiotic bacteria, lactococcus produces a large amount of lactic acid during its growth and reproduction, which can regulate the pH value of the environment and inhibit the growth of harmful bacteria. At the same time, it can transform the refractory organic matter in sewage into degradable or degradable substances, thus speeding up the degradation of pollutants. Through the results of experiment, it can be concluded that the degradation of TP and COD by lactococcus is better than that of TN.

5. References

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