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Application of Enzyme Immobilization Technology in Hydrolytic Enzyme Sludge Reduction System

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The excess sludge has become one of the most serious burdens of urban sewage treatment plants, because of their huge production, high processing costs and the serious pollution to the ambient environment. In this work, aiming to reduce the production of excess sludge, we choose the hydrolases, which have excellent catalytic hydrolysis and can efficiently hydrolyze bacterial cells, to analyze the sludge from the activated sludge system during the operational process. This research can broaden the sludge reduction field, deepen the mechanism of the reduction technology, and develop a reliable method to apply in the sludge reduction engineering. First, the effects of several typical hydrolases on lysing the sludge from the activated sludge system were analyzed, whose results showed that compared to a-amylase and protease, lysozyme is the most effective for the hydrolysis of the sludge with the same dosage of enzyme. Subsequently, the selected lysozyme was added in the sludge from the SBR system by static test reactor. And the most suitable hydrolysis conditions were obtained at the 0.072-0.250 g lysozyme /g MLSS, 30-60min, 30-40 °C.

1. Introduction

The large production and high disposal cost of sludge for urban sewage treatment plants has become one of the problems urgently to be solved in wastewater treatment. It is imperative to find an efficient and economical sludge reduction technology. According to the disadvantages of sludge digestion, one of the most widely used techniques of sludge reduction, like low efficiency, long processing cycles, high running costs and brarely normal operations, biocatalysis technology is applied to enhance thesludge reduction in this topic. Immobilized Biological Catalyst (IBC), compounded with complex enzymes and efficient microorganisms, is added to the aerobic digestion and mesophilic aerobic digestion respectively. It examines the influencing factors, and determines the optimum operation conditions (Ye et al., 2012; Parawira, 2012; Wu et al., 2016; Yu et al., 2013). Also, it compares the reduction effect and dewatering of supernatant respectively. Further, this paper analyzed production and the change characteristic, evaluated of the stability of the system, and made preliminary discussion on the mechanism of the IBC in the sludge digestion (Lage et al., 2013; Goel et al., 2008; Karn et al., 2015).
Nitrilase is an enzyme that can convert nitrites into carboxylic acid or ammonium. Compared with the harsh condition of chemical synthesis, such as high pressure, high temperature, poisonous heavy metal catalysis and alkali, catalysis through enzyme brings about high value and wide application under mild condition in organic synthesis and industrial production. In this paper, extraction, purification immobilization and application of nitrilase were studied (Nabarlatz et al., 2010; Jiang S., Chen et al., 2006; Guo et al., 2014).

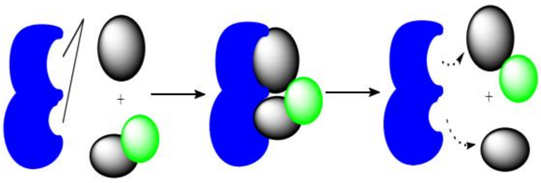

Figure 1: Lock and key model.

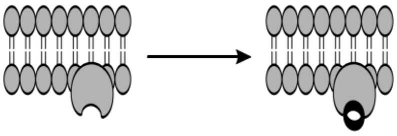

Figure 2: Induced fit model

Phenylglycinonitrile were prepared by chemical synthesis. Recombinant nitrilase was purred by Ni-NTA affinity chromatography, and displayed a single band in SDS-PAGE, which indicates the purification of nitrilase. Then, the properties of recombinant nitrilase were studied, including optimum reaction temperature, pH and the tolerance to organic reagents. Considering the application of methanol as cosolvent in improving dissolution of subtracts, we also researched the tolerance of recombinant nitrilase to methanol.
The activated sludge process is the most popular method in wastewater treatment plants, which will result in a large number of by-products-excess sludge. Excess sludge contains pathogens, heavy metals and residual organisms, harmful to people and the environment. What's more, it could cause secondary pollution if not being treated properly. The extremely high disposal expense of sludge also becomes one difficult problem for the municipal wastewater treatment plants and the urban development. Thus, developing an economic and efficient technology for sludge reduction is of highly practical significance.

2. Hydrolase Screening

Recombinant nitrilase was separately immobilized on both amino-support and epoxy-support. VVe optimized the immobilized conditions, such as immobilized bufFer pH, immobilized temperature and immobilized time, etc. Under optimized conditions, we successfully prepared the immobilized nitrilase, and then studied the optimum reaction temperature, pH, and stability of temperature and pH, compared with those of purified recombinant nitrilase. The temperature and pH stability of nitrilase were enhanced after immobilization on amino-support or epoxy-support.

$$U^T = \frac{D_{485}V}{\mathrm{k}_T W_t} \tag{1}$$

Table 1: Sludge characteristics (mg/L)

MLSS	MLVSS	TCDO	SCOD	TN
6958	5343	5702	8	0.12

In this paper, bioaugmentation was used in the sewage treatment system with immobilized biocatalysts to achieve sludge reduction at the sources. The catalyst through the immobilization technology will be able to make multi-functional microbial agents by fixing on wheat bran by the amylase, lipase, protease, cellulose enzyme, lactose enzyme and lactic acid bacteria and yeast, bacillus, etc. Adding IBC to the sewage treatment system could optimize the microbial colonies structure, promote the rapid mineralization and degradation of organic matters in the sewage treatment, and make it harmless substance as C02, H20, and N2. Because of the low concentration of organic matters, most of the sludge in the system was in the endogenous respiration state with a high oxidation rate, producing less excess sludge and achieving the sludge reduction at the sources.

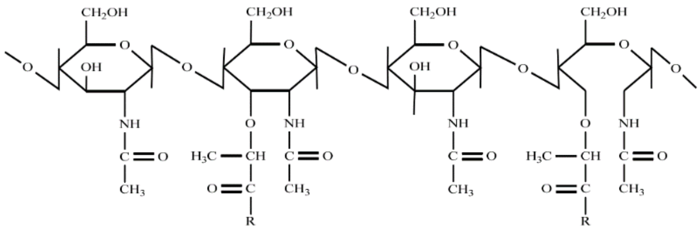

Figure 3: Lysozyme molecular structure

Take 7 250 mL Erlenmeyer flask, and add 100 mL of mixed test sludge in it. Then add in turn 0.005, 0.025, 0.05, 0.10, 0.15, and 0.25 g lysozyme, with the constant temperature of 35 ˚C. Stir them for reaction of 30min.

After the reaction, 20 mL of the muddy water mixture was centrifuged at 10,000 r / min for 15 minutes, and the supernatant was filtered through a 0.45} m filter to measure the COD C SCOD in the filtrate. The TP test results were shown in Figure 3.8. The COD and SCOD in the sludge were subtracted from the COD of the corresponding lysozyme in the aqueous solution, and the calculated SCOD / TCOD was shown in Figure 3.5. The TN in the sludge supernatant was subtracted from the TN of the corresponding lysozyme in the aqueous solution, with the calculated TN shown in Fig. The test also determined MLVSS for each sludge sample at the end of the reaction, and the calculated sludge MLVSS dissolution rate was shown in Fig. 3.

3. Study on Lysozyme-based Hydrolysis Conditions of Activated Sludge

3.1 Reagents and Materials

The results of IBC applied to single-phase aerobic digestion indicated that the optimal activation time of IBC was 8h, with the minimum dosing capacity as 20 ppm, the most economical aeration as 2.0 mg/L, the most suitable mud concentration as 3.46%,and the maximum sludge dosing rate as 9%. The treatment efficiency increased by adding IBC. Under these conditions, the comparing experiments further validated that IBC has a strengthening effect on aerobic sludge digestion process. It took 8 days to make the VSS removal rate exceed 38% for the dosing group, 4 days shorter than that of the control group. 12 days later, it entered the platform stage, and VSS, TSS, and TCOD removal rates respectively reached 54.41%,31.88%,77.20%, higher than that of the control group by 11.74%, 6.52%, and 9.90%. The effect of sludge reduction increased significantly, while the processing cycle declined. After the aerobic digestion, sludge resistance would rise, but the increase of dosing group was slighter than that of the control group, with the dewaterability improved. The pH of the supernatant declines after the rise, and variation of the dosing group was less than that of the control group, maintaining at6.8-7.2; COD, turbidity, and TP rose rapidly and then slowed down, with the values of the dosing group lower than those of the control group. TN showed a rising trend, and the dosing group was significantly lower than the control group. The indicators of supernatant did not sharply fluctuate, indicating that IBC will not have an obvious negative impact on the nature of the digestive juices when the treatment efficiency of aerobic sludge digestion is improved.

$$\frac{dV}{dt} = \frac{pa^2}{r\mu(vV + V_E)} \tag{2}$$

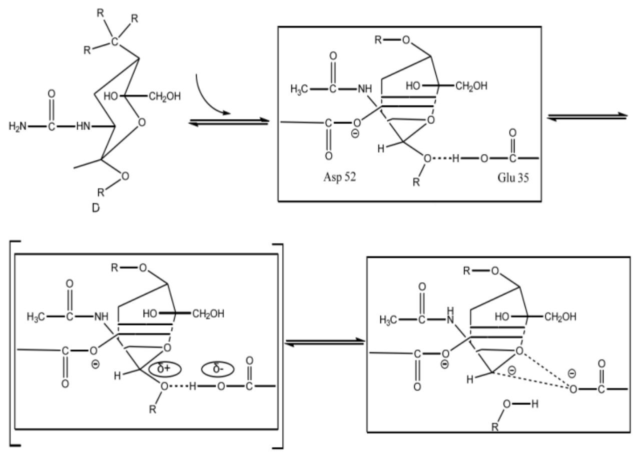

Figure 4: Lysozyme action steps

3.2 Instruments and Devices

The results of IBC applied to mesophilic aerobic digestion indicate that the minimum dosing capacity was 30 ppm, while the most appropriate mud concentration was 3.63%, with the maximum sludge dosing rate as 7%.The treatment efficiency increases after IBC was added. Under these conditions, the comparing experiments of 60 days further validated that IBC could improve the efficiency of sludge mesophilic anaerobic digestion. VSS removal rate of the dosing group reaching a plateau required 21 days, 6 days shorter than that of the control group. VSS, TSS, and TCOD removal rates respectively reached 51.35%, 31.45%, 53.00%, higher than the control group by 10.60%, 7.30%, and 8.91%. Meanwhile, the daily gas production 3.633 L/d of the dosing group was higher than that of the control group, respectively reaching 12.931 L/d and 9.298 L/d. And the cycle was shorter, and the effect of sludge reduction and gas production were improved. In addition, the rising range of sludge resistance of the dosing group was smaller than that of the control group after mesophilic anaerobic digestion, which were 2.56 times and 2.07 times as before, with the dewater ability being improved. Lastly, pH of the dosing group was valued at 7.0-7.3, with the alkalinity as 1500-2000 mg/L, VAF as 250-350 mg/L, and VFA/alkalinity as 0.1-0.2. Comparing with the control group, the stability of the system was enhanced, instead of being weakened.

$$\varpi = \frac{W}{V_Y} \tag{3}$$

Figure 5 shows the changes in TN in the sludge suspension. It was found that the TN content increased gradually with the sludge hydrolysis, especially in the first 30 min after the addition of lysozyme, and the concentration increased from 0.13 mg / L to 42.10 mg / L. Followed by a further extension of the hydrolysis, it slowed down with the passage of time, and reached 47.91mg / L at 60 min. It can be seen that as the hydrolysis went, the changes in the TN content in the sludge saw rapid growth at the initial reaction, and then after a certain time, the growth rate declined without an obvious trend. The changes in TP along the hydrolysis time passage in the sludge is shown in Fig. It can be found that the variation curve of TP along the reaction time can be divided into two stages. The slope of the curve at the first stage refers to the lysing enzyme during the first 30 minutes, which indicates that TP increases with the passage of reaction time. The second stage is the period after the first 30 min when although TP increases with time, the rate of improvement is significantly lower than that at the first stage.

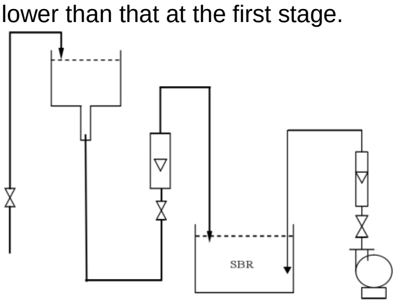

Figure 5: Process flow of SBR system test device

3.3 Experimental Method

Immobilized biocatalysts were used in Sequencing Batch Reactor (SBR) to study the sludge reduction. The results showed that sludge reduction of the dosing group was 55.28%, less than that of the contrast group. At the same time, the treatment effects of COD, NH3-N, TP of the dosing group were better than those of the contrast group. The average removal rate of TP is 7.21%, higher than that of the contrast group. From the 31st to the 80th days, MLUSS/MLSS of the dosing group was 8.50%, lower than that of the control group.

Immobilized biocatalysts were applied in a municipal wastewater treatment plant in Zhongshan for a test lasting 142 days. The outcomes showed that sludge production of the dosing pool was 76.79%, less than that of the contrast pool, and discharged 63.58% less sludge. After dosing IBC for a month, the sludge concentration reduced gradually. About 75 days later, sludge concentration was about 1600 mg/L, 50% lower than that of the contrast pool. From the second month, the sludge emission reduced gradually, and there was no sludge discharge in the 3rd and 4th months. From the 31th to 142th days, the average value of MLUSS/MLSS of the dosing pool was 6.74% lower than that of the contrast pool. Moreover, the effluent quality of the dosing pool was better than that of the control pool. The mean removal ratio of TP increased by 11.73%. The amount of TP in sludge of the dosing pool was 0.76% higher than that of the contrast pool, saving electricity consumption by 11.44%.

$$\mu = \frac{0.00178}{1+0.0337\text{t}+0.00022\text{t}^2} \tag{4}$$

250 mL conical bottles were labelled with 1, 2, 3, 4, 5, and 6 respectively. 100 mL mixed homogeneous sludge was added, and then O.OSg lysozyme was added at the same temperature of for 35 ˚C. Then the mechanical stirring reaction was carried out. In the stirring, the white powder in the20 mL bottles labelled with 1, 2, 3, 4, 5, and 6 disappeared after 15 min, 30 min, 60 min, 90 min, and 120 min respectively, out of a mixture with the speed of 10000 r/min. After the centrifugation of 15 min and supernatant with the filtration by 0.45 m, of the COD C SCOD, TN, and TP were measured. At the same time, the sludge suspension in the corresponding cone bottle was taken to determine the TCOD of sludge.

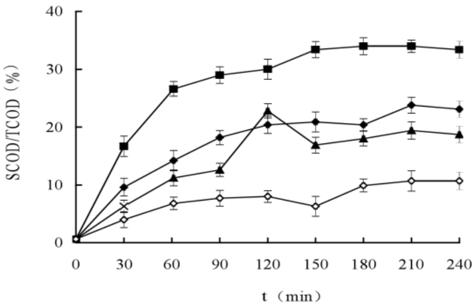

Figure 6: Changes of sludge SCOD/TCOD with time under different hydrolytic enzymes

3.4 Items and Methods for Analysis

A pilot study of adding immobilized biological catalyst (IBC) in a municipal wastewater treatment plant in Guangzhou was carried out. The results showed that the sludge production of the dosing pool was 21.04% less than the contrast pool. And the sludge sedimentation of the dosing pool was improved. After 40 days of the experiment, the sludge concentration reduced gradually. The value of MLUSS/MLSS of the dosing pool was 6.24% less than that of the contrast pool. From the 40th days to the 106th days, the dosing tank was 8.33% lower than the control pool. What's more, water quality, embodied by pH, SS, COD, NHS-N, and TP, of the dosing pool, was superior to the quality of the control pool. The average removal rate of TP was 6.30% higher than that of the contrast pool. The dosing pool consumed 13.24% less electricity, and the sludge resistance was 47.14% lower, compared to the contrast pool. The results of the sludge microscopic experiment showed that the morphology of activated sludge in the dosing pool was significantly changed. The sludge flocculation was tighter and bigger than that of the contrast pool. Results also showed that the immobilized biological catalyst had an effect on the type and quantity of dominant species of the biochemical system. It strengthened the function of the activated sludge, and promoted the organic decomposition into CO_2 and H_2O, which reduced the excess sludge. The benefit analysis indicated that added immobilized biological catalyst is a kind of economic and efficient technology that can realize the sludge reduction from the source. What's more, it also produces economic, social and environmental values.

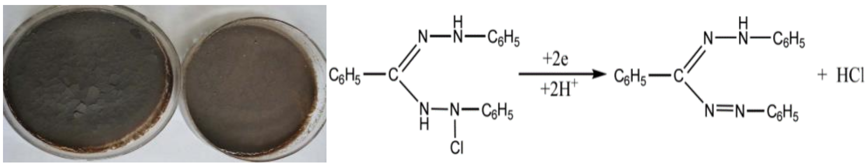

Figure 7: Activated sludge drying effect diagram

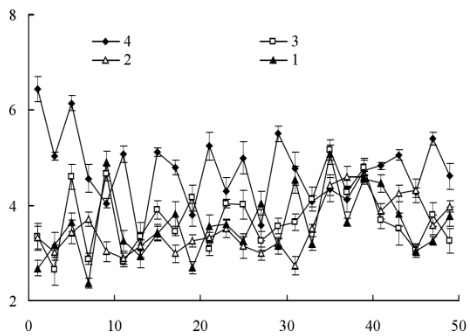

Figure 8: Variation of Ammonia Nitrogen in S - effluent System with Different Reflux Sludge

The results showed that the content of organic matters in sludge suspension increased with the passage of hydrolysis time, and the change trend of SCODiTCOD and MLVSS dissolution rate was consistent with the change trend. Analysis of the reasons is as follows. Mainly under the action of lysozyme, accompanied by the hydrolysis of sludge, sludge flocs in the solid phase organic matters, such as carbohydrates and protein substances were gradually released from the solid phase to the liquid phase; Due to the lysozyme break wall, the intracellular organic matters out of the cell wall rupture were also released into the liquid phase. The role of both in the sludge suspension led to the increase of SCOD, TN, and TP concentrations. In addition, the higher the dissolution rate of sludge, the more the organic matters entering the liquid phase and the higher sludge dissolution rate and the consistency of the organic matter content in the sludge.

4. Conclusion

The results about the reduction of sludge in the SBR system show that: (1) The sludge treated by lysozyme before being returned to the reactor shows the least amount of sludge yield. (2) The hydrolyzed sludge reach 10% of the total amount of the activated sludge in the SBR system, ensuring both the best reduction of the excess sludge and degradation efficiency of the organic pollutants. (3) The sludge operated by lysozyme 3 cycles per day show the best sludge reduction effect. The results about the efficient of effluent in the SBR system show that in the different operating cycles, the COD and TN with and without lysozyme addition are almost the same. The in-depth research found that compared with the reference system without lysozyme, the higher value of the protease activity, the TTC-ETS, and the ATP of the activated sludge with lysozyme indicate a better ability to digest organic pollutants. The reasons for sludge reduction under the SBR system may be ascribed to following points:(1) Decomposition of the dissolved sludge nutritional media is the predominant reason. (2) Enhanced endogenous metabolism of activated sludge especially in the anaerobic period (i.e. a stronger oxidizability) can result in a better ability of self-consumption. The DGGE fingerprints reveal that no significant differences of the microorganisms can be observed between the activated sludge systems with and without lysozyme.

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