Isolation of Oil-degrading Bacteria and Treatment of Oil Wastewater

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Abstract. With the improvement of people's living standards, the pollution caused by oil-rich kitchen wastewater has attracted people's attention. In order to screen efficient soybean oil-degrading bacteria, six strains that could degrade soybean oil were isolated from the soil with soybean oil as the sole substrate. Among them, the strain CR5 and CR6 had strong ability to degrade soybean oil. After 10 days of culture, the degradation rates of strain CR5 (pH 2.42) and CR6 (pH 3.84) on soybean oil were 65.25%, 66.04% respectively. In order to study the salt tolerance of the strains, the degradation rates of soybean oil by CR5 were 16.36%, 3.85% and 7.36% after 5 days of cultivation at different salt concentrations (1.0%, 1.5%, 3.5%) and those of CR6 were 5.52%, 8.45%, 16.98%.

1. Introduction

The richness and development of the catering industry have improved people's living standards and improved the quality of life, but it also brings some environmental problems. Specifically, the environmental impacts that are brought by the catering industry mainly include: soot, noise, food waste, emission of catering wastewater and so on [1]. At present, noise and soot pollution has attracted people's attention and taken corresponding measures to a certain extent because they have a direct impact on the surrounding residents. Relatively speaking, the treatment of kitchen wastewater was relatively lagging, and a large amount of kitchen waste water was directly discharged into the environment, which caused serious environmental pollution. If untreated kitchen wastewater is discharged directly, it will cause many hazards. Firstly, the discharge of kitchen wastewater directly into the water body will deteriorate the water quality. The kitchen wastewater contains animal and vegetable oil and floating on the surface of the water will hinder the exchange of oxygen between the air and water. However, the part of the oil dissolved in the water is degraded to consume oxygen due to the action of microorganisms, which reduces the oxygen in the water and causes the deterioration of water quality to endanger the survival of aquatic plants and fish [2]. Secondly, the direct discharge of kitchen waste water into the soil will destroy the soil layer structure.

Kitchen wastewater refers to the comprehensive wastewater produced during the operation of the catering industry and kitchen. It has the characteristics of large discharge, scattered emissions,
complex water quality and high organic content. The pollution characteristics of kitchen wastewater are mainly manifested in high concentration of organic pollution. According to data reports [3], the discharge of kitchen wastewater accounted for about 3% of the urban domestic sewage discharge, but the contents of its BOD\textsubscript{5} and COD accounted for 1/3 of the total load. The oil in the wastewater forms an oil film on the surface of the soil, which not only affects the aeration of the soil and makes the soil anoxic, but also is not conducive to the growth of microorganisms in the soil. Thus that destroys the particle condition of the soil layer. Thirdly, it will affect the normal metabolic process of activated sludge and biofilm and increase the load on the urban sewage treatment plants because of the oil is difficult to degrade if the kitchen waste water enters the urban sewage treatment plant without treatment [4]. Since direct discharge of kitchen waste water has many hazards, it is extremely urgent to find a fast, economic and convenient treatment method.

At present, there are many reports on the treatment methods of kitchen wastewater, mainly including biological treatment, physical treatment and chemical treatment. However, the physical and chemical treatment methods are rarely used in practice because their processing effect is not ideal, and the emission standards are not met after treatment. The biological treatment method has been used for treating sewage for hundreds of years. It mainly uses the oil and fat as a carbon source and energy substance during the process of microbial growth and metabolism, and the oil is degraded to reduce pollution by absorption, utilization and conversion. The biological treatment method has the advantages of small land occupation, low cost, no special equipment, and no secondary pollution. Therefore, the biological treatment method has been highly favored in recent years, and it has become a promising kitchen wastewater treatment method [5]. However, the ability of different microorganisms to degrade oil is not the same, which depends mainly on the type and metabolism of microorganisms. Therefore, it is important for the treatment of kitchen wastewater to find more strains with oil degradation ability.

For the treatment of oil wastewater, in this study soybean oil was used as the sole substrate to screen a high-efficiency soybean oil-degrading bacteria from the soil and study its characteristics and to do its basic research for the application of microbial treatment of oil wastewater in the future.

2. Materials and methods

2.1 Culture medium

Mineral salt medium was prepared and the formula was as follows: \( \text{K}_2\text{HPO}_4 \ 2 \text{ g}, \ (\text{NH}_4)\text{SO}_4 \ 1.4 \text{ g}, \ \text{MgSO}_4 \cdot 7\text{H}_2\text{O} \ 0.3 \text{ g}, \ \text{CaCl}_2 \ 0.3 \text{ g}, \ \text{CoCl}_2 \ 2 \text{ mg}, \ \text{FeSO}_4 \cdot 7\text{H}_2\text{O} \ 5 \text{ mg}, \ \text{MnSO}_4 \cdot \text{H}_2\text{O} \ 1.6 \text{ mg}, \ \text{ZnSO}_4 \ 1.7 \text{ mg}, \ \text{DW} \ 1 \text{ L}. \)

2 ml (about 1.50 g) of soybean oil was added to 100 ml of the medium to prepare an enrichment medium for screening strains with soybean oil degradation ability and the degradation rate of soybean oil was determined by using an enriched medium to culture strain.

LB solid medium was separated and purified of a single strain and the formula was as follows: Tryptone 10 g, Yeast extract 5 g, NaCl 10 g, Agar 15 g, DW 1 L.

2.2 Screening of soybean oil-degrading bacteria

2 g of the soil contaminated with petroleum was weighed and directly added to a 500 ml flask containing 100 ml of enriched medium (with 2 ml of soybean oil) and cultured at 30\textdegree \text{C}, 120 rpm shaking incubator. 5 ml of each enrichment solution was inoculated into the same fresh medium (with 2 ml of soybean oil) after observing the apparent decomposition of soybean oil in the medium and subculture was continued under the same conditions. Three days later, 1 ml was taken from the subculture enrichment fluid and added each to 100 ml of an enrichment medium (with 2 ml of soybean oil) and subcultured at the same temperature and speed. Two days later, the cultivation were used by the inoculating loop, and each was streaked on the surface of the LB agar plate. Then the flats were sealed with parafilm and placed in a 30\textdegree \text{C} incubator. After the bacteria grew, a single colony of
different forms was picked up by the inoculating loop and was streaked respectively on the surface of the LB to separate the different strains. Also that was sealed and placed in a 30°C incubator.

2.3 Determination of pH and soybean oil degradation rate
The rate of soybean oil degradation was analysed by the gravimetric method. 1 ml of the strain culture solution was inoculated into a 500 ml flask containing 100 ml of enriched medium supplemented with 2 ml of soybean oil and shaking culture (120 rpm) at 30°C. After 10 days, the pH of the cultivation was measured by a pH meter. The cultivation was extracted with n-hexane as an organic solvent, and the organic layer was collected. In addition, the organic solvent was removed by heating in a 70°C water bath. Then it was placed in a 70°C incubator to dry and weigh [6]. Finally, the rate of soybean oil degradation was calculated.

2.4 Determination of degradation rate under different salt concentrations
The salt concentration of inorganic culture solution was determined as 0.5% by a salt concentration meter. Then NaCl was separately added to the inorganic medium to cause the salt concentration of 1.0%, 1.5%, and 3.5% respectively. 1 ml of the strain cultivation was taken and seeding into 500 ml flask containing 100 ml of different salt concentration enriched culture medium. The degradation rate was measured after culture for 5 days and 10 days at 30°C and 120 rpm.

2.5 Determination of the number of colonies
The number of colonies was measured by CFU plate counting method, and the growth characteristics were evaluated at different salt concentrations. The initial bacterial solution was made with 100 μl of the culture solution and 900 μl of sterile water. Then, it was diluted and 100 μl of the diluted bacterial solution was applied to LB agar plate and cultured in a 30°C incubator for 24 to 48 hours. The growth was observed and the colonies that grew were counted. The cultivation was measured every 2 days.

3. Results and discussions

3.1 Screening of strains
Six strains that degraded soybean oil were isolated from petroleum-contaminated soil and named as strain CR1, CR2, CR3, CR4, CR5, and CR6. After 5 days and 10 days of culture, the measured degradation rate of soybean oil is shown in Figure 1. The degradation rates of all strains after 10 days of culture was higher than those after 5 days. Among them, the degradation rate of soybean oil of CR5 was 53.85% after 5 days, and that was 65.25% after 10 days of culture. The degradation rate of strain CR6 was 59.57% at 5 days of culture and that was 66.04% at 10 days. This indicated that the strain CR5 and CR6 were both high-efficiency soybean oil degrading bacteria.

3.2 Determination of pH
The pH of the medium was determined after the isolated six strains were cultured at 30°C and 120 rpm for 10 days. Figure 2 shows that the pH of the strain CR5 and CR6 are 2.42 and 3.84 respectively after 10 days of culture.

Soybean oil is a kind of oil and fat and its main component is a triglyceride. Simultaneously, it also contains a small amount of diacylglycerols, monoglycerides and fatty acids. Studies of Gao Gui et al. [7] have shown that triglycerides were degraded under the action of enzymes to produce fatty acids and diacylglycerols, which would keep the pH down. Soybean oil produced more fatty acids during biodegradation, so the pH of CR5 and CR6 was lower than other strains.
3.3 Effects of different salt concentrations on the growth of strains

Different salt concentrations in the medium result in different osmotic pressures and osmotic pressure has an effect on the metabolism and growth of the microorganisms. The kitchen wastewater has a high salt concentration and it is of great significance to research the degradation characteristics of the strains CR5 and CR6 at different salt concentrations. The degradation rate of soybean oil was measured at different salt concentrations and the number of live bacterial colonies was also measured.

3.3.1 Effects of different salt concentrations on degradation rate

The degradation rates of soybean oil by strain CR5 and CR6 were determined for 5 and 10 days of culture at 1.0%, 1.5% and 3.5% respectively. It can be seen from Figure 3 that the degradation rate of soybean oil by CR5 after 5 days of culture is very low at different salt concentrations. However, the degradation rate is the highest at 1.0% salt concentration, that is, 16.36%. Similarly, the degradation rate is the highest after 10 days of culture at 1.0% salt concentration and it’s 55.97%.
Figure 3. Degradation rate of strain CR5 at different salt concentrations

Figure 4 shows that the degradation rate of soybean oil is the highest, that is, 16.98% when the strain CR6 is cultured for 5 days and the salt concentration is 3.5%. When it’s cultured for 10 days and the salt concentration is 1.0%, the degradation rate is the highest at 78.21%. The degradation rate of soybean oil for 10 days of culture was higher than that for 5 days of culture. The degradation rate was all increased significantly at different salt concentrations when the salt concentration was 1.0% after 10 days of culture. The strain CR5 and CR6 require an acclimation period after inoculation into the environment of high salt concentration, so the degradation rate is not high after 5 days of culture. After being adapted to the environment, the degradation rate will be improved. As it could be seen from Figure 1, the degradation rate of the strain CR5 at high salt concentration is lower than that at the low salt concentration, but the degradation rate of the strain CR6 is increased.

Figure 4. Degradation rate of strain CR6 at different salt concentrations

3.3.2 Effect on the number of colonies

The number of viable cells in the culture solution was measured every 2 days and the results were shown in Figure 5-6. The number of colonies reaches the maximum after 4 days when the salt concentration is 1.0% and 3.5%. They are $2.16 \times 10^6$ cfu/ml and $2.77 \times 10^6$ cfu/ml respectively and then decrease gradually. When the salt concentration is 1.5%, the number of colonies of the strain CR5 is always increased. Figure 5 shows that the number of colonies of the strain CR5 is the least at $1.94 \times 10^6$ cfu/ml when the salt concentration is 1.0% after 6 days. At that time the salt concentration is 1.5%, the number of colonies of the strain CR5 is the largest, which is $3.4 \times 10^6$ cfu/ml.
Figure 5. Number of colonies of strain CR5

Figure 6 shows that the number of viable cells reaches the highest value in the three salt concentrations for 4 days. The highest number of viable cells is $20 \times 10^6$ cfu/ml when the salt concentration is 3.5%. It is $11.4 \times 10^6$ cfu/ml at 1.5% of salt concentration. When the salt concentration is 1%, the number is $1.49 \times 10^6$ cfu/ml. The number of viable bacteria began to decrease at the three salt concentrations after the culture time was more than 4 days, the decrease was the largest when the salt concentration was 3.5%. The viable cell count of the strain CR6 doesn’t change much at 1.0% of salt concentration. As shown in Figure 5-6, the salt tolerance of the strain CR6 was higher than that of the strain CR5.

Figure 6. Number of colonies of strain CR6

4. Conclusions

In this study, six kinds of soybean oil degrading strain were isolated, and the degradation ability of the strains CR5 and CR6 were higher. After 5 days of culture, the degradation rates were 53.85% and 59.57% respectively, and the pH of the culture medium was both reduced to below 4. At different salt concentrations (1.0%, 1.5%, 3.5%), the degradation rates of the strains CR5 and CR6 were the highest at 1.0%. Among them, the strain CR6 to salt concentration was more resistant than the CR5. In addition, it grew in the salt concentration of 3.5% better than other low salt concentration conditions. Therefore, it was more suitable for the treatment of kitchen wastewater containing grease.

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References
[1] P. Gao, Y.H. Wang, X.M. Dai, et al. Discussion on the treatment technology of kitchen wastewater. Jiangsu Environmental Technology, 20:90-92 (2007)
[2] Wakelin N G, Forsterc.f. Aerobic treatment of grease-containing fast food: Restaurant wastewater. Process safety and Environmental Protection Transactions of the Institution of Chemical Engineers, 76, 1: 55-61 (1998)
[3] H.D. Wang. Current status and prospects of kitchen wastewater treatment methods. Sichuan environment, 23, 2:14-16 (2004)
[4] X.G. Jiang. Study on treatment technology and equipment of kitchen wastewater and method of oil recovery. Tianjin University, Tianjin (2013)
[5] Loperena L, Ferrari M D, Diaz A L, et al. Isolation and selection of native microorganisms for the aerobic treatment of simulated dairy wastewater. Bioresource Technology, 100, 5:1762—1766 (2009)
[6] H.M. Yan, D.M. Wang, J. Gao, et al. Screening and Degradation Performance of a Highly Efficient Oil Degrading Bacteria. Journal of Gansu Agricultural University, 48, 4:176-180 (2013)
[7] G. Gao, L.Y. Zheng, S.P. Han, et al. Monitoring the progress of lipase-catalyzed hydrolysis of soybean oil by acid value. Journal of Jilin University (Science Edition), 43, 6:853-857 (2005)