Screening and degrading characteristics of high efficiency 1, 2, 4-trichlorobenzene-degrading bacteria under low temperature conditions

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Three strain of high efficiency 1, 2, 4-trichlorobenzene-degrading bacteria was screened out from the activated sludge under low temperature which was named a, b, c. According to morphological and physiological observation, strain a was identified as Achromobacter sp., b was Acetobacter sp., c was Archrobacter sp..The optimum condition of the mix-strain for degrading 1, 2, 4-trichlorobenzene was as follows: assistant carbon source is glucose, assistant nitrogen source is sulphur-urine, pH is 7, salinity is 2%, and volume is 100ml. The degradation rate of 1, 2, 4-trichlorobenzene reached 81.2% under this condition.

Keywords: Low Temperature; 1, 2, 4-Trichlorobenzene; Screening; Degradation Characteristics.

1. Introduction

1, 2, 4-TCB is one of the three isomerides of trichlorobenzene, in the category of chlorobenzene, which is common in industrial application, and has "three genicity" toxin. Chlorobenzene pollutant is easy to penetrate common water pollution control project shield to enter natural environment, stay and gather in organism for a long time, as a threat to human body health and ecological environment [1]. Since 1990s, a lot of domestic and foreign scholars have researched much on the bio-degradation of chlorobenzene, and now have separated to the degrading bacteria of monochlorobenzene, 1, 4-dichlorobenzene, 1, 3-dichlorobenzene, 1, 2-dichlorobenzene, 1, 2, 4-TCB and 1, 2, 4, 5-tetrachlorobenzene[2]. In which, there is few domestic and foreign report on suitable bacteria of 1, 2, 4-TCB, especially the research under low temperature condition. This research aims at the actual environmental condition of low-temperature groundwater in the northeast of China, 3 strain of low temperature and high efficiency 1,2,4-trichlorobenzene-degrading bacteria

areobtained from the slurry of sewage factory as the research object, the environmental condition that affects the degradation effect of the strain is confirmed through experiment, the strain is preliminarily identified to provide theoretical support for the subsequent research on the construction and engineering application of high efficiency engineering bacteria.

2. Materials and Experiment Method

2.1. *Reagent and medium*

1, 2, 4-trichlorobenzene, analytically pure.

The inorganic salt contains 1, 2, 4-TCB medium: Na H_2PO_4 5g, KH_2PO_4 2 g, $(NH_4)_2SO_4$ 0.5g, $MgSO_4 \cdot 7H_2O$ 0.2 g, $FeSO_4 \cdot 7H_2O$ 0.1 g, H_2O 1000ml and appropriate amount of 1, 2, 4-TCB, pH 7.0~7.2;

Enrichment medium: agar 15 g, NaCl 5g, beef extract 5g, peptone 10g, H_2O 1000ml, pH 7.0~7.2[3].

2.2. *Strain culturing and separation*

The bottom mud collected from Jilin and Changchun FAW sewage treatment factory is mixed with active slurry, 10ml mixed active slurry is vaccinated to the inorganic culturing medium with 1,2,4-TCB the only carbon source for domestication and culturing in 120r/min table at 10°C. 5~6d is a domestication period, later, the concentration in the medium is gradually increased under the same condition for domestication, after three domestication periods, the concentration of 1,2,4-TCB in the medium is increased from 5mg/L to 10mg/L. At the end of every domestication period, the medium is moved to fresh medium, after continuous enrichment and transfer for several times, in the dilution plate method, the strain obtained through repeated streaking separated on the enriched enrichment medium.

2.3. *Research on degrading characteristics of strain*

2.3.1. *Confirmation of influential factor*

5 factors that affect the growth and metabolism of microbe are selected in the experiment as research object: carbon source, nitrogen source, pH value, salinity and loaded liquid.

2.4. *Strain identification*

Eight physiological-biochemical tests including catalase test, sugar fermentation test, methyl red test, V-P test, amylolysis, cellulose hydrolysis, ammonia

producing test, H_2S producing test are conducted for the separated strain.

3. Result and Discussion

3.1. *Strain separation identification*

Through repeated domestication enrichment culturing streaking and separation, 3 low temperature high efficiency 1, 2, 4-TCB degrading strains are obtained. According to the form observation of the strain, the strain is further identified through physiological-biochemical test. a is preliminarily identified as Achromobacter sp., b is Acetobacter sp., c is Archrobacter sp [4].

Table 1. Physiological-biochemical characteristic of strains.

Physiological-biochemical test		Bacteria a	Bacteria b	Bacteria c
Methyl red test		-	-	-
V-P test		-	-	-
Amylolysis		-	-	-
Cellulose hydrolysis		-	-	-
Ammonia producing test		+	+	+
H2S producing test		+	+	+
Sugar fermentation test	Glucose fermentation	-	-	-
	Lactose fermentation	-	-	-
	Cane sugar fermentation	-	-	-
Catalase test		+	+	+

Note: + is positive reaction; - is negative reaction.

3.2. *Confirmation of the Best Degradation Condition for 1,2,4-TCB Degrading Bacteria*

3.2.1. *Selection of optimum assistant carbon source for degrading*

Figure 1 shows that when glucose is used as the assistant carbon source, the degrading rate of 1, 2, 4-TCB is relatively the highest, reaching 69.0%. Glucose is easy to be utilized by mixed strain, therefore, the growth of mixed strain is vigorous, which also improves utilization efficiency to 1, 2, 4-TCB.

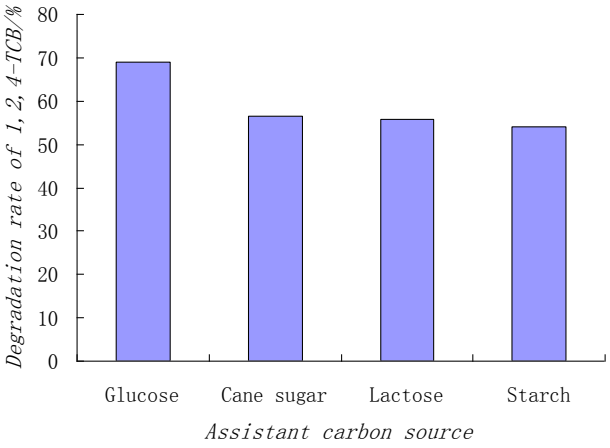

Fig. 1. Selection of Optimum Assistant Carbon Source for Degradation.

3.2.2. *Selection of the optimum nitrogen source for degradation*

Figure 2 shows that it is ideal to select thiourea as the nutrition nitrogen source of mixed strain, whose 1, 2, 4-TCB degradation rate is relatively higher, reaching 70.0%. Thiourea is organic nitrogen source, can be directly utilized and transformed by microbe into its own nutrient; therefore, it is rational to select thiourea as the nitrogen source.

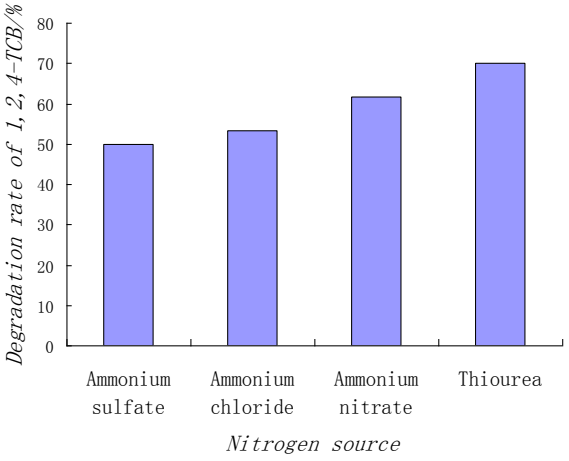

Fig. 2. Selection of Optimum Assistant Nitrogen Source for Degradation.

3.2.3. *Selection of the optimum pH value for degradation*

Figure 3 shows that when the initial pH value is less than 7.0, the degradation rate of mixed strain to 1, 2, 4-TCB is slowly increased with the increase of pH value, when pH=7.0, 1, 2, 4-TCB degradation rate is the highest, reaching 66.0%. After pH value is more than 7.0, especially when pH value is 7-8, with the increase of pH value, 1, 2, 4-TCB degradation rate is rapidly reduced. Therefore, in the acid and alkaline environment, the degradation effect is not as good as that in the neutral environment. The enzyme activity of mixed strain in neutral environment is high; in addition, no acid or alkaline accumulation will be produced in the growth and metabolism, which inhibits the growth and breeding of mixed strain.

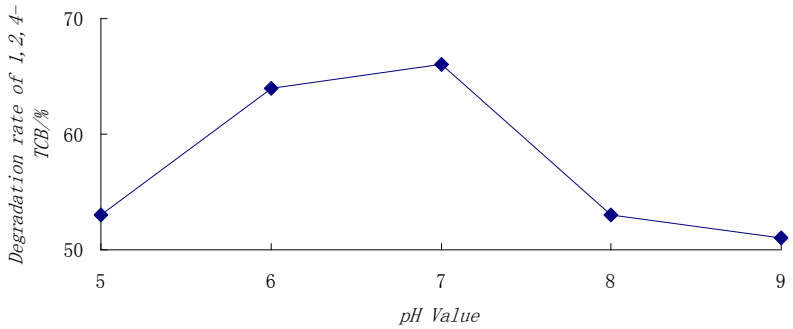

Fig. 3. Selection of Optimum pH Value for Degradation.

3.2.4. *Selection of the optimum salinity for degradation*

Figure 4 shows that when the salinity is less than 2.0%, the degradation rate of mixed strain to 1,2,4-TCB is suddenly increased with the increase of salinity; when the salinity is 2.0%, the degradation rate of mixed strain to 1,2,4-TCB is good, reaching 71%; when the salinity continues to rise, 1,2,4-TCB degradation rate presents slow reduction. When the salinity is low, it cannot meet the content of zwitterion required by the cell normal metabolism; when the salinity is high, it is easy to cause cell dehydration, and affect the normal physiological growth of cell.

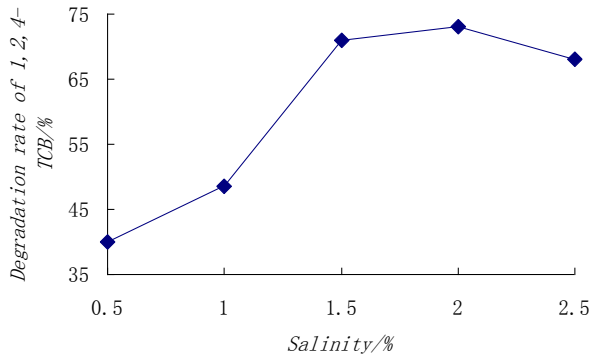

Fig. 4. Selection of Optimum Salinity for Degradation.

3.2.5. *Selection of the optimum loaded liquid for degradation*

Figure 5 shows that with the increase of loaded liquid, the degradation effect of 1, 2, 4-TCB is lower and lower. The increase of loaded liquid reduces the mass transfer effect in the flask culturing liquid, which is mainly reflected in the reduced collision between cell and bottom object and reduced oxygen mass transfer efficiency. Therefore, it is rational to select the optimum loaded liquid as 100mL.

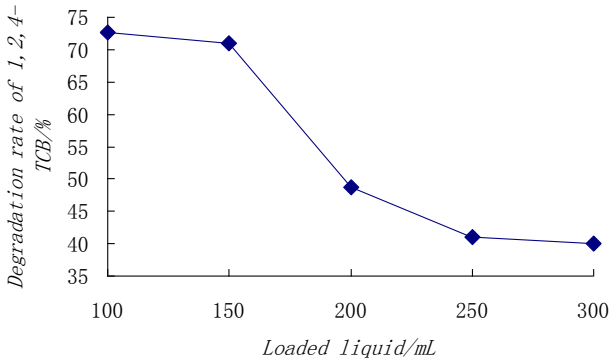

Fig. 5. Selection of Optimum Loaded Liquid for Degradation.

4. Conclusion

Under the low temperature condition of 10°C, three 1, 2, 4-TCB degrading strains with 1, 2, 4-TCB the only carbon source are screened from the mixed slurry of the sewage pipeline of some chemical factory in Jilin and Changchun FAW sewage treatment factory, respectively Achromobacter sp., Acetobacter. sp, Archrobacter. sp through preliminary identification.

Degrading characteristics research experiment is conducted for the mixed strain of the three strains, the optimum condition for mixed strain to degrade 1, 2, 4-TCB is confirmed from the experiment result: glucose as the assistant carbon source, thiourea as the nitrogen source, pH=7, salinity 2.0%, loaded liquid 100ml, under this condition, the degradation rate of the mixed strain to chlorobenzene can reach 81.2%.

Through domestication at low temperature 10°C, the strain conforms to the temperature of common groundwater, when the bacteria is added to the groundwater, it can adapt to the environment of the groundwater without inhibiting its breeding and activity due to low temperature, or affecting 1, 2, 4-TCB degradation effect, which can provide reference data and information for in-depth research on 1, 2, 4-TCB microbe degradation under low temperature condition. In the experiment, three high efficiency degrading strain are preliminarily identified, the further screening and identification of advantageous strain requires further research.

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