Screening and Evaluation of the Biodegradation of Glass Fiber by Bacillus Mucilaginosus

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Keywords: Bacillus mucilaginous; glass fiber; microorganism degradation; potassium degradation ability.

Abstract. This paper, 9 strains of bacillus producing capsule were isolated from soil by potassium shale medium. Glass fibers with similar structure of the ore were used as potassium sources. And the potassium degradation ability of the selected strain was analyzed by the weight loss method. The bacillus with the obvious degradation effect was screened out and named as X SC-6. According to the analysis of physiological characteristics and 16S drank sequence, the fungus was confirmed to be Bacillus mucilaginous. To optimize the degradation condition, the fermentation medium also discussed. The results showed that the potassium degradation effect of potassium shale medium was better than the other. Attractively, the K^+ content in the supernatant was increased by 12.97% and the degradation rate reached 2.44%. In addition, scanning electronic microscope was used to measure the microstructure of the glass fiber before and after degradation. The results indicated that the bacteria had good ability to decompose the potassium. This work provides supporting theories for the degradation of solid wastes (such as glass fiber) by microorganism.

1. Introduction

During the producing process of glass fiber, the existence of discarded glass fiber scraps is unavoidable. The waste of glass fiber occupied 15% of the entire production process [1]. Due to its unique product performance and composition, glass fiber cannot be normally degraded in the normal environment. Traditional handling method was heated to ultra-high temperatures for recycling use or applied as simple asbestos products. All of these methods resulted in exhaust emission and caused extremely high cost [2-3]. In view of environmental protection and cost control. High-temperature recycling method had become unpopular. Simple asbestos products are mostly used for temporary convertible and decorative application, Due to the addition of other chemical components, they existed the disadvantages of carcinogenicity and how safety factor. Therefore, this method had been banned internationally. It is particularly tempting to find a new way to dispose of waste glass fibers.

As we know, *Bacillus mucilaginosus* is regard as silicate bacteria or potassium bacteria, which is different from the common Bacillus bacteria. It is a special kind of soil Bacillus, which deserved the ability to decompose and transform silicate minerals [4]. Lots of literatures have reported that *Bacillus mucilaginosus* was applied in the degradation of K^+. For glass fiber, it displayed certain similarity in composition with ore, such as SiO_2, Al2O3, Cano, KO2 and so on [5]. Thus, it is possible to degrade waste glass fibers by *Bacillus mucilaginosus*. All of the results provide a theoretical basis for seeking a new environment-friendly degradation method.

2. Materials and Methods

2.1 Sample.

In this paper, soil samples and glass fibers were respectively taken from Changqing District and Taishan Glass Fiber Co., Ltd in Shandong Province.

2.2 Medium.

Medium (g/L)	Component	pH
Potassium powder medium	Sucrose (5g), $MgSO_4.7H_2O$ (0.5g), $FeCl_3$ (0.005g), $CaCO_3$ (0.1g), NaH_2PO_4 (1g), PotashShale Powder (1g), Agar (17g)	7.2.
Slope and preservation medium	Sucrose (5g), yeast (0.5g), NaH_2PO_4 (1.5g), NaCl (1g), $MgSO_4$ (0.5g), $FeCl_3$ (0.005g), Agar (17g)	7.0
Potassium medium 1	Molasses (15 g), Soybean meal (7g), NaH_2PO_4 (1.5 g), NaCl (1g), $MgSO_4$ (0.5g), $FeCl_3$ (0.005g)	7.0
Potassium medium 2	sucrose (5g), yeast (0.5g), NaH_2PO_4 (1.5g), NaCl (1g), $MgSO_4$ (0.5g), $FeCl_3$ (0.005g), Agar (17g)	7.0

2.3 Instrument.

Ultraviolet Clean Bench, optical microscope, refrigerator, direct-reading pH meter, Autoclave, electronic balance, Muffle furnace, high speed centrifuge, constant temperature incubator, PCR amplification.

3. Experiment

3.1 Glass Fiber Pretreatment.

The glass fiber was baked in a muffle furnace at 550 °C for 4 hours to remove the impregnating compound that stick on the glass fiber surface. Then cooled to room temperature and pulverized into powder.

3.2 Screening of Strains.

Preliminary screening.Ore (5 g) was dissolved in 45 mL of normal saline, and the solution was shaking thoroughly with a shaker for 40 min to fully homogenize. Then the sample was kept at 50°C water bath for 15-20 min, and attenuate according to 10-2, 10-3, 10-4, 10-5 and 10-6, respectively. The bacterial suspension (100 μL) was coated on a potassium shale mineral flour medium plate, cultured with a constant temperature incubator at 30 °C for 2 to 3 days. The single colony having a large single transparent colony characteristic of Bacillus mucilaginosus was picked and numbered. The preliminary screening strains were numbered as X SC1-9 [6-8].

Rescreen.After baking in a muffle furnace at 550 °C for 4 hours, the glass fiber was ground into powder. Adding with the ratio of 60 g/L, primary strains were cultured in shake flasks and cultured in potassium shale ore powder medium with the volume of 50 mL/250 mL. Two rings of primary screening strains were selected in the inoculation loop to enter the culture medium and cultured on a shaker at 30° C at 150 r/min [9]. Three parallel groups were set in each group. After 72 hours of cultivation, they were taken out and centrifuged at 10000 r/min for 10 min. The supernatant was removed. The precipitate was dried in muffle furnace at 550 °C for 4h to remove ash. After drying, the mass difference was weighed. Comparing with the spore morphology of the primary screening strains, the strain with the best differential weight was selected as the starting strain.

Morphological species.The colony morphology of the strain and the individual morphology were observed under the microscope.

Molecular biology identification of bacterial species.According to the recordation of Common Bacterial System Identification Manual, Bacillus species were identified by morphological, physiological, biochemical characteristics and 16S rDNA sequence analysis [10].

3.3 Determination of Potassium Release Capacity.

Flame photometric determination of K+ concentration in supernatant solution.The identified strains were rejuvenated for 2 or 3 times and then stored at 4 °C. The superfine pulverized glass fiber was added to the potassium-dissolving medium 1 and the potassium-removing medium 2 according to the ratio of 60 g/L. The two mediums were sterilized at 121°Cand 115°C for 30 mins.

Two or three loops of well-activated bacteria were inoculated into two mediums, and cultured at 30° C, 150 r/min for 3 days. Each group had 3 replicates. Potassium shale ore powder medium has a high viscosity after liquid culture. In order to reduce the viscosity, it was sealed and heated at 90°C. The supernatants were obtained after centrifuged at 10,000 r/min for 20 min for each medium. Flame photometer was used to measure K content with 4 mL supernatants.

The blank group was set without the Bacillus mucilaginosus, and the others were consistent with the above-mentioned methods.

SEM analysis.The pre-degraded glass fibers and the degraded glass fibers were measured by scanning electron microscopy (SEM) to verify whether they played a role in degradation by comparing the smoothness of the surface of the glass fibers.

4. Results and Discussion

4.1 Screening of Strains.

In the processing of growth and metabolism, *Bacillus mucilaginosus* releases extracellular organic acids, such as acetic acid and lactic acid. These acids can destroy the crystal lattice of silicate minerals and release potassium, silicon and other elements [11]. Glass fiber contains a large number of mineral substance. Its main components are silica, alumina, calcium oxide, potassium dioxide. According to the degradation rate of glass fiber in Table 1, it can be found that *Bacillus mucilaginosus* has played an important role in the degradation of glass. The overall degradation rate of the selected strain X SC-6 reached 3%, whereas X SC-4 and X SC-7 are less than 1% and the other strains have reached more than 1%. Therefore, X SC-6 strain with the highest degradation rate was selected as the starting strain.

Table 1. Degradation ratio of different strains

Strain/X SC	1	2	3	4	5	6	7	8	9
Time/3d	3	3	3	3	3	3	3	3	3
Number of Bacteria/(×108cfu/mL)	1	1.1	1.1	0.9	1.2	1	1.1	1	1
Difference/g	0.07	0.04	0.05	0.01	0.08	0.09	0.02	0.04	0.06

4.2 Morphological Characteristics of Strains.

As shown in Figure 1. A, on a fresh medium incubated at 30°C for 36 hours, the colonies were smooth and transparent. Meanwhile, the edges were neat, the middle uplifted, and the threads were drawn into a line and a clear glass color. As shown in Figure 1.b, a single colony, stained by ammonium oxalate crystal violet, displayed the individual morphology of the bacterium Bacillus spp under oil microscopy. *Bacillus mucilaginosus* as a special gram-negative *Bacillus mucilaginosus* exhibited rod-like or fusiform organisms with 3-5 μm long and 0.8-1.5 μm wide. It contains one to several fat particles in cells. In addition, the surface of bacteria showed hypertrophic decidua and several bacteria was easily to form bacteria group [12].

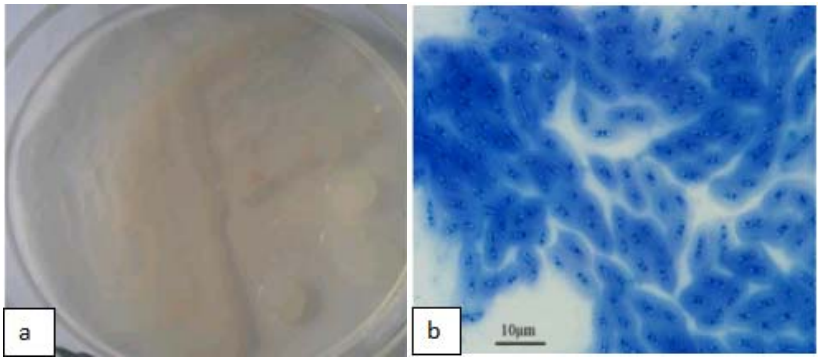

Figure 1. Colony morphology and individual morphology of Bacillus mucilaginosus

4.3 Molecular Biology.

The 16S rDNA sequence of *Bacillus mucilaginosus* were obtained from sequencing company and were BLAST by DNAStar. It was identified as a genus. The phylogenetic tree of this strain was

established. From the phylogenetic tree in Figure 2, this strain is the same as *Bacillus mucilaginosus*. The interspecific similarity with 2 *Bacillus mucilaginosus* was 100%, and the homology was greater than 99% with *Bacillus mucilaginosus* strain AHZ1 and *Bacillus mucilaginosus* AC2. The strain can be initially identified as *Bacillus mucilaginosus* and named as X SC-6.

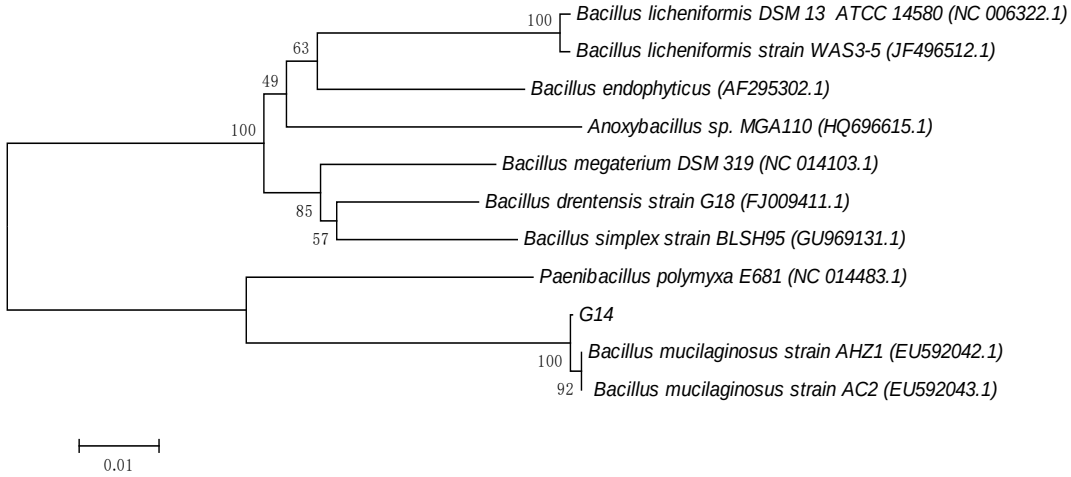

Figure 2. Phylogenetic Tree of 16S rDNA Sequences of Bacillus mucilaginosus X SC-6 and Related Strains

4.4 Potassium Dissolving Ability Analysis

Determination of K+ concentration.From Figure 3, the linearity of the K+ standard curve (y=4.9317x+0.24 R2=0.9997) indicated that the test conditions were good and stable. By measuring the K+ concentration of the two potassium-solubilizing medium supernatants, the blanks also contain trace amounts of potassium (displayed in Figure 4) [13]. The blank potassium ion contents of the two media are different. It may be due to chemical groups inside the reaction, or the medium itself contains a certain amount of potassium. Both of the two Medias showed an increasing trend of potassium ion concentration. And the potassium concentration in the supernatant of the potassium decontamination medium 1 was increased by an average of 0.887 mg/mL. The K+ concentration in the supernatant of the potassium solution media 2 was increased by an average of 0.836 mg/mL.

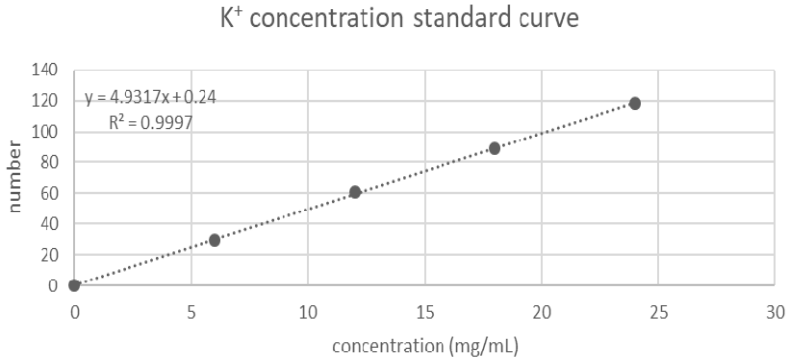

Figure 3. K+ concentration standard curve

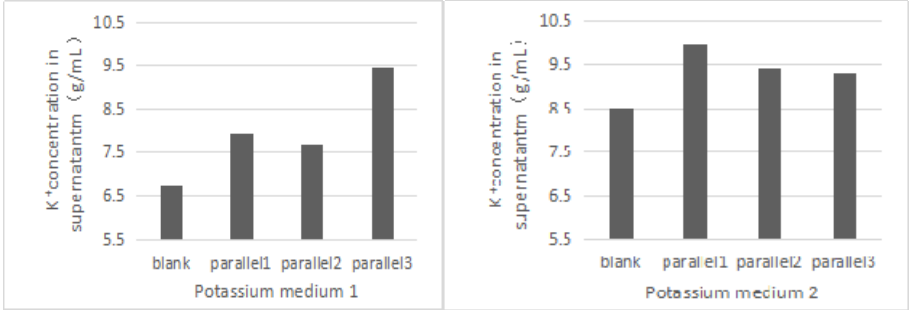

Figure 4. K+ Concentration in Supernatant from Two Culture Media

Comparison of two media degradation.Based on the above mentioned, we can conclude that the average increase rate of medium 1 in the solution of potassium is 12.97%, and the average increase

rate of medium 2 is 10.86%. It indicated that both of the Medias exhibit derivative effect. The effect of the 1 is better than the 2. The potassium mass of the two culture media accounts for 2.44% and 2.12% of the glass fiber, respectively. It may be attribute to the fact that the structure between the glass fibers is too tight and the force between the molecules is too strong. The actual degradation rate caused of the fibers is not particularly high, which needs further research.

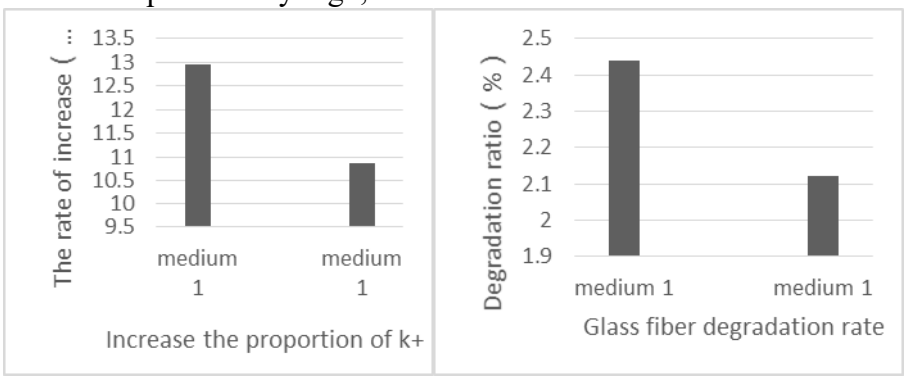

Figure 5. Comparison of two Medias

Treated glass fiber surface analysis.Through comparative observation of Figure 6, we found that the surface of the original (shown in Figure 6 (a)) is relatively smooth and clean. Whereas, after being degraded by the microorganism, the surface of the glass fiber (shown in Figure 6 (b)) turned to be rough. The existence of large amounts of ravines was ascribed to the formation of polysaccharides and organic acids during the growth procedure of *Bacillus mucilaginosus*. For the contact between polysaccharides and organic acids with the surface of the glass fiber, the glass fiber was eroded [14-16]. The coordinate bonds were generated by parts of the uronic acid and metal ions, thereby weakening the interaction force between oxygen and K^+. Hence, the balance of the reaction was changed. Ultimately, the reaction was carried out towards the equilibrium of dissolution.

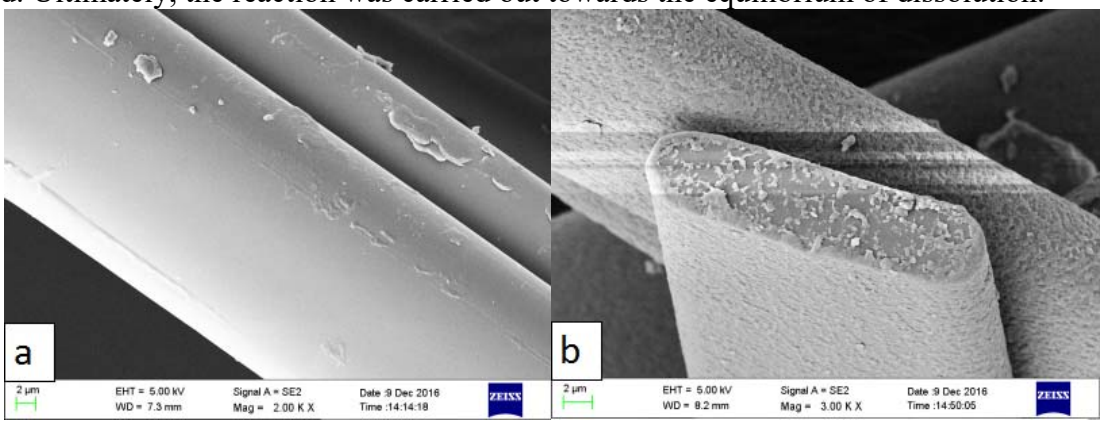

Figure 6. Scanning Electron Microscope of glass fiber surface: (a) Untreated glass fiber (b) Treated glass fiber

5. Conclusion

The XSC-6 strain, with the best degradation ability of glass fiber, was selected from the Changing district using the potassium shale mineral powder. It was identified as Bacillus mucilaginous by the sequence analysis of 16S drank and morphological characteristic. The Fire Wire Photometer and scanning electronic microscope were used to measure the K^+ content and microstructure. The results indicated that the strain exhibited the feasibility in the degradation of glass fiber. This paper provides a certain theoretical basis for the biodegradation of glass fiber, whether it can be carried out on a large scale and application still needs further study.

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