Screening and Characterization of Polyacrylamide Degrading Fungi

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Keywords: Polyacrylamide, Degrading Fungi, Growth Characteristics, Degradation Conditions.

Abstract: In the study, soil samples around the oil concentration station of Yanchang Oilfield were selected for the experiment. With 10^{-2} as the soil dilution, the strains were screened by the plate stress enrichment culture method. Finally, three strains of high polyacrylamide degrading fungi were determined, which were respectively recorded as HPAMF1, HPAMF2 and HPAMF9. The rDNA-ITS assay showed that strains HPAMF1, HPAMF2 and HPAMF9 were Trichoderma alba, Fusarium and Merimbla, respectively. The growth characteristics and degradation conditions of the polyacrylamide degrading fungi were investigated and optimized. It was found that the degradation capacity of the three strains reached the strongest when the polyacrylamide was used as the sole nitrogen source.

1 INTRODUCTION

Polyacrylamide has flocculation (Haveroen, 2005); (Owen, 2002), adsorption (Szögi, 2007); (Sojka, 2006), thickening, resistance reduction and other properties, which has been widely used in petroleum exploitation, water treatment, papermaking, textile printing and dyeing, agriculture, medicine, sugar, building materials, aquaculture, construction and other fields (Shu, 2021); (Panova, 2021); (Yang, 2020).

Polyacrylamide is non-toxic, but it will undergo physical, chemical, and biological degradation in natural environment, and monomer acrylamide will be produced after degradation. Acrylamide may cause neurotoxicity, reproductive toxicity, genotoxicity and carcinogenicity (Huang, 2018). Therefore, the degradation of polyacrylamide wastewater has become a focus of attention in recent years.

Biodegradation technology is low cost, non-polluting and mature, which could be used in the degradation of polyacrylamide (Wijngaarden, 2016). In the exploration of polyacrylamide degrading microorganism, the study of bacteria is much more than that of fungi. Because the species of known polyacrylamide degrading fungus are few and the research is not in-depth, this will become a new direction of microbial polyacrylamide degrading.

2 MATERIAL AND METHODS

2.1 Sampling

The soil samples were taken from the surrounding soil samples of an oil selection station in Yanchang Oilfield.

Polyacrylamide purchased from Xi'an Lanxiang Chemical Co., LTD., which is anion polyacrylamide, with molecular weight of 5×10^8.

2.2 Medium

2.2.1 Martin Medium

$KH_2PO_4\cdot3H_2O$ 1 g, $MgSO_4\cdot7H_2O$ 0.5 g, peptone 5 g, glucose 10 g, constant volume to 1000 mL. Prepare rose-bengal with a concentration of 1% (m/m), and add 3.3 mL to the culture solution. Stir thoroughly until the mixture is evenly mixed, then weigh 15~20 g agar in the culture medium and heat to melt at natural pH.

2.2.2 Basal Culture Medium

Sucrose 30 g, $KH_2PO_4\cdot3H_2O$ 0.4 g, $K_2HPO_4\cdot3H_2O$ 1.6 g, $MgSO_4\cdot7H_2O$ 0.06 g, NaCl 0.5 g, $Fe_2(SO_4)_3$ 0.03 g, $CaCl_2$ 0.01 g, $CuSO_4$ 0.05 g, $ZnSO_4$ 0.05 g, with distilled water to 1000 mL at natural pH.

Cao, Y., Zhang, Q., Zhao, M., Bai, C. and Wang, T.
Screening and Characterization of Polyacrylamide Degrading Fungi.
DOI: 10.5220/0011508800003443
In *Proceedings of the 4th International Conference on Biomedical Engineering and Bioinformatics (ICBEB 2022)*, pages 1296-1301
ISBN: 978-989-758-595-1

2.3 Screening of Degrading Fungi

2.3.1 Selection of Soil Dilution

Soil samples around the oil concentration station of Yanchang Oilfield were taken and stored at 4 ℃. 2.0 g of soil sample was weighed and put into a conical flask, which was placed in a constant temperature gas bath oscillator at 34 ℃ and 140 rpm for 3 h. Then dilute the soil suspension to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}, and spread them evenly on Martin medium. Make three parallel samples for each dilution. The test was repeated three times for each strain. Observe the growth of the colony in the culture medium and select the appropriate soil dilution for subsequent experiments.

2.3.2 Isolation of Polyacrylamide-degrading Fungi

A certain amount of polyacrylamide was prepared and added to the sterilized Martin culture medium by means of plate stress enrichment method, where the concentration of polyacrylamide was 100, 200, 500, 1000 and 1500 $mg \cdot L^{-1}$. A drop was added to Martin medium containing polyacrylamide and coated evenly. Three parallel samples were made for each concentration and incubated at 34 ℃ for 72 h. fungi with good growth and fast growth rate were selected and further enriched and separated on Martin medium containing polyacrylamide until pure colonies were obtained.

2.3.3 Screening of Polyacrylamide Degrading Fungi

The spores on each fungal body were washed with phosphate buffer solution, and the spore suspension was diluted to 10^6 $CFU \cdot mL^{-1}$, and stored in the refrigerator at 4℃ for future use. The polyacrylamide was added to the basal culture medium at a concentration of 500 $mg \cdot L^{-1}$, and the spore suspension were added to the medium at a volume ratio of 2%. Then the suspensions were cultured in a shaking table at 34 ℃ and 140 rpm for 10 days, and the uninoculated solution was used as the control. After the culture medium was diluted to an appropriate concentration after being treated by each fungus for 10 days, the absorbance was measured by starch-cadmium iodide spectrophotometry, and the change of polyacrylamide concentration was obtained, and the degradation rate was calculated, so as to screen out the polyacrylamide efficient degradation fungi.

2.4 Identification of Efficient Degradation Strains

1μL spore suspension was added to Martin medium, and each fungus was repeated for three times. After uniform coating, the bacteria were cultured in 34 ℃ incubator for 5 days, and the characteristics of each colony were observed. The morphology of fungus was observed by scanning electron microscope. The strains were identified by rDNA-ITS assay.

2.5 Method for Determination of Degradability of Each Fungus Strains

The growth of polyacrylamide degraded fungus was determined by dry cell weight. The polyacrylamide concentration was determined by starch-cadmium iodide method, and its removal rate was calculated.

2.6 Growth and Degradation Characteristics of Single Each Fungus

2.6.1 Growth with Polyacrylamide as the Only Carbon Source

Three basal culture medium was prepared to remove sucrose and added 500 $mg \cdot L^{-1}$ NH_4Cl, and then inoculated with the three dominant strains which can degrade polyacrylamide, respectively. Then the three dominant strains were cultured at 34 ℃ in constant temperature incubator, and the growth of the degrading fungus was observed to explore whether the three dominant strains could grow with polyacrylamide as the only carbon source.

2.6.2 Growth with Polyacrylamide as the Only Carbon and Nitrogen Source

Prepare three 500 $mg \cdot L^{-1}$ basal culture medium with polyacrylamide as the only carbon and nitrogen source, and the three dominant fungi which can degrade polyacrylamide isolated to basal culture medium respectively, and then arrange them in a 34 ℃ constant temperature incubator for culture, and observe the growth of the degrading fungi. To explore whether polyacrylamide can be used as the only carbon and nitrogen source for the growth of the three dominant strains.

2.6.3 Single Growth Curve and Polyacrylamide Degradation Rate Curve

Three 500 mg·L^{-1} basal culture medium containing polyacrylamide were prepared and 2% (v/v) sporospora suspensions of the three strains were added, respectively. Three parallel groups were set in each group, and they were placed in a 34 ℃ 140 rpm shaking table for 10 days. The growth amount of the fungi and the degradation rate of polyacrylamide were measured every day.

3 RESULTS

3.1 Screening of Polyacrylamide Degrading Fungi

After incubating in a constant temperature incubator for 72 h, the plate with soil dilution of 10^{-2} had more types of colonies and was suitable for separation. Different fungus was selected from the plate and inoculated in Martin medium containing different concentrations of polyacrylamide for stress enrichment culture. The polyacrylamide concentration was 100 mg·L^{-1}, 200 mg·L^{-1}, 500 mg·L^{-1}, 1000 mg·L^{-1}, 1500 mg·L^{-1}. The growth of the strains was observed in the incubator at 34 ℃ for 72 h, and the growing strains were selected for isolation and purification. Finally, 8 strains of different tolerance fungi were obtained.

In the basal medium containing 500 mg·L^{-1} polyacrylamide, the suspensions of each sporozoa were added at 2% (v/v). After 10 days of shaking culture at 34 ℃ and 140 rpm, the absorbance was measured to determine the concentration of polyacrylamide, so as to obtain the degradation rate of polyacrylamide by each fungus.

Table 1: Degradation ability of eight tolerant fungi to polyacrylamide.

Strains	1#	2#	3#	5#	6#	9#	10#	11#
Degradation efficiency (%)	27.36	27.99	14.75	18.30	22.76	27.58	9.51	23.77

As can be seen from Table 1, the degradation rate of polyacrylamide of each degradation fungus were different after 10 days, and 1#, 2# and 9# with higher degradation rate than other strains were selected as the dominant degradation fungus, which were denoted as HPAMF1, HPAMF2 and HPAMF9.

3.2 Morphological Observation and Identification of Polyacrylamide Degrading Fungi

3.2.1 Colony Characteristics of Polyacrylamide Degrading Fungi

The colony morphology was observed, and the colony morphology was shown in Fig. 1. Observation showed that HPAMF1 mycelia grew rapidly on the medium, and the surface color of the colony changed from white to yellowish green and then to dark green. The HPAMF2 colony was grapefruit red, with abundant velour shaped aerial mycelia, and the mycelia were white, while the mycelia of HPAMF9 colony was light yellow, and the surface was nearly smooth.

Figure 1: Cultural characteristic of HPAMF1, HPAMF2 and HPAMF9.

3.2.2 Characteristics of Polyacrylamide-degraded Fungi

Three strains of fungi were placed under scanning electron microscopy for observation, and the scanning electron microscopy results of each strain were shown in Fig. 2.

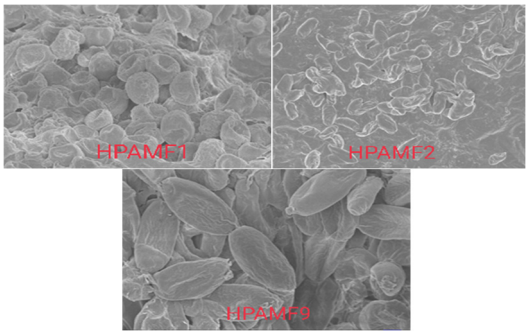

Figure 2 SEM image of HPAMF1, HPAMF2 and HPAMF9(×5000).

Under the scanning electron microscope, the strain HPAMF1 was oblate with depressions; the strain HPAMF2 was rod-shaped; and the strain HPAMF9 was ellipsoidal with fine wrinkles on the surface.

3.2.3 rDNA-ITS Determination

Through BLAST comparison, it is found that the similarity between HPAMF1 and Trichoderma aspen had reached 100%, the similarity between HPAMF2 and Fusarium sequence had reached 99.82%, and the similarity between HPAMF3 and Merimbla sequence had reached 99.48%. Therefore, HPAMF1 was preliminarily identified as Trichoderma aspen, HPAMF2 was preliminarily identified as Fusarium, and HPAMF9 was preliminarily identified as Merimbla through the determination of the colony morphology, cell morphology and rDNA-ITS of polyacrylamide-degrading fungi.

3.3 Growth and Degradation Characteristics of Single Fungus

3.3.1 Growth of Degrading Fungi using Polyacrylamide as the Sole Carbon Source

HPAMF1, HPAMF2 and HPAMF9 were cultured in a constant temperature incubator at 34 °C for 5 days. It could be seen from Fig. 3 that HPAMF1 and HPAMF9 have hyphae growth but HPAMF2 didn't grow. From this we could conclude that HPAMF1 and HPAMF9 grow with polyacrylamide as the sole carbon source, but HPAMF2 didn't.

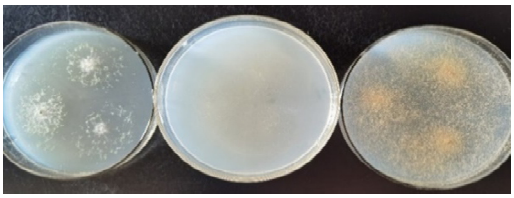

Figure 3: HPAMF1, HPAMF2 and HPAMF9 grew with polyacrylamide as the only carbon source.

Prepare basal culture medium with different initial concentrations of polyacrylamide, in which sucrose is removed and NH_4Cl was added, and then connected to the HPAMF1 and HPAMF9 spore fungus suspension respectively, and placed in a shaker at 34 ℃ 140 rpm for 10 days. After the measurement, the degradation rate of polyacrylamide is shown in Fig. 4.

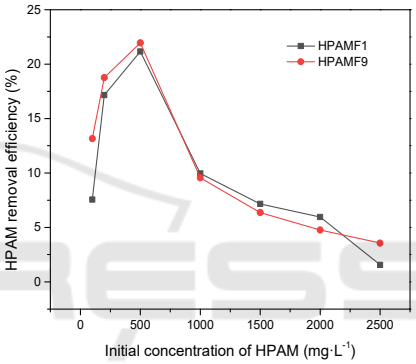

Figure 4: Degradation curve of HPAMF1 and HPAMF9.

It can be seen from Fig. 4 that within the range of the initial polyacrylamide concentration of 100~500 $mg \cdot L^{-1}$, the degradation rate of HPAMF1 and HPAMF9 on polyacrylamide will increase rapidly as the concentration increases, and both are at a concentration of the maximum value is reached at 500 $mg \cdot L^{-1}$: 19.16%, 20.91%. When it exceeds 500 $mg \cdot L^{-1}$, with the continuous increase of the concentration, the degradation rate presents an obvious decreasing trend.

3.3.2 Growth of Degrading Fungi using Polyacrylamide as the Only Carbon and Nitrogen Source

Strains HPAMF1, HPAMF2 and HPAMF9 were placed in a 34 ℃ constant temperature incubator for 5 days, and the experimental results were shown in Fig. 5. It was observed that HPAMF1 and HPAMF9 could grow with polyacrylamide as the only carbon and nitrogen source, while HPAMF2 could not.

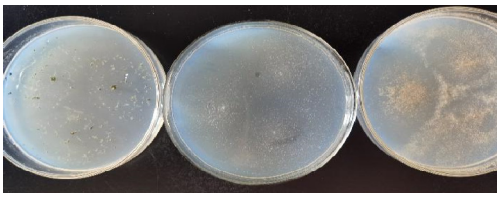

Figure 5: HPAMF1, HPAMF2 and HPAMF9 grow with polyacrylamide as the only carbon and nitrogen source.

In the basic culture medium with different initial concentrations of polyacrylamide and sucrose removed, two spore suspensions, HPAMF1 and HPAMF9, were added, respectively, and placed in a shaking table at 34 ℃ and 140 rpm for culture for 10 days. During the experiment, the degradation rate of polyacrylamide changed as shown in Fig. 6.

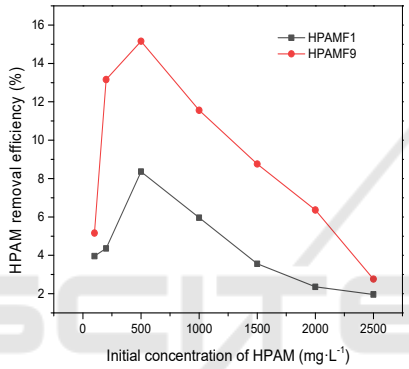

Figure 6: Degradation curve of HPAMF1 and HPAMF9.

As can be seen from Fig. 6, when the initial concentration of polyacrylamide is in the range of 100~500 mg·L^{-1}, the degradation rate of HPAMF1 and HPAMF9 on polyacrylamide will increase with the increase of the concentration, and the degradation rate of HPAMF1 will increase rapidly in the range of 100 mg·L^{-1} to 200 mg·L^{-1}. The degradation rate of HPAMF9 increased rapidly from 200 mg·L^{-1} to 500 mg·L^{-1}, and both strains reached the maximum degradation rate of 8.36% and 13.21% at 500mg·L^{-1}. When the initial concentration of polyacrylamide is greater than 500 mg·L^{-1}, the degradation rate decreases with the increasing of the concentration.

3.3.3 Based on the above Experimental Results

The degradation rates of HPAMF1 and HPAMF9 strains were 27.36% and 27.58%, respectively, when the initial concentration of polyacrylamide was 500 mg·L^{-1} as the only nitrogen source. When polyacrylamide was used as the only carbon source, the corresponding degradation rates were 19.16% and 20.91%, respectively. When polyacrylamide was used as the only carbon and nitrogen source, the corresponding degradation rates were 8.36% and 13.21%, respectively.

It was found that polyacrylamide was the only nitrogen source for HPAMF2 growth. For HPAMF1 and HPAMF9 strains, polyacrylamide can be used as the only nitrogen source, the only carbon source and the only carbon and nitrogen source. According to the data analysis, the degradation capacity of these three strains can reach the strongest when they grow with polyacrylamide as the only nitrogen source. Therefore, in the subsequent experiments, polyacrylamide was set as the only nitrogen source to study the growth and degradation characteristics of HPAMF1, HPAMF2 and HPAMF9.

3.3.4 Growth Curve and Degradation Rate Curve of Single Fungus

Three strains of HPAMF1, HPAMF2 and HPAMF9 were cultured. The changes of thallus growth and polyacrylamide degradation rate are shown in Fig. 7 and Fig. 8.

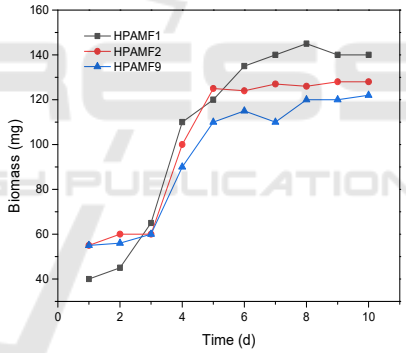

Figure 7: Growth curve of HPAMF1, HPAMF2 and HPAMF9.

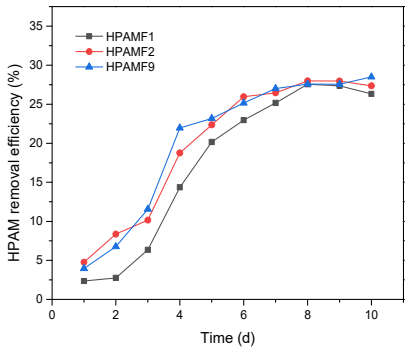

Figure 8: Degradation rate curves of HPAMF1, HPAMF2 and HPAMF9.

As shown in the Fig. 8, the growth curve of single fungus HPAMF1, HPAMF2 and HPAMF9 was s-type, in line with the microbial growth law. The strain first passed a relatively short retardation period and continuously adjusted itself to adapt to the new environment. Then it entered the logarithmic growth period, the fungus weight will show exponential growth, at this time the degradation rate of polyacrylamide will also increase; Then the strain continued to grow into a stable phase, at which the number of new cells and the number of dead cells were in a dynamic balance, and the polyacrylamide degradation rate tended to be stable. The maximum removal rates of HPAMF1, HPAMF2 and HPAMF9 were 27.36%, 27.99% and 27.58%, respectively.

4 CONCLUSION

8 strains of polyacrylamide degrading fungi were isolated from soil in this experiment. Moreover, the dominant degradation fungi were selected through the degradation effect of each strain on polyamide, which were respectively recorded as HPAMF1, HPAMF2 and HPAMF9. By observing the colony morphology and cell characteristics of the three fungus and identifying the three fungi, it was finally concluded that strains HPAMF1, HPAMF2 and HPAMF9 were Trichoderma alba, Fusarium and Merimbla, respectively. In addition, in the growth environment with polyacrylamide as the only nitrogen source, the degradation ability of the three fungi growth showed the strongest.

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