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Optimization of Microbial Degradation of Polyvinyl Alcohol (PVA) Wastewater and Its Potential for Application in Public Health

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Abstract: In recent years, the application of biodegradable materials in the medical field has grown rapidly, covering bone scaffolds, vascular bridging, drug delivery carriers, implants, surgical instruments, and biochemical signal detection sensors. Compared with traditional materials, biodegradable materials are more environmentally friendly and have good biocompatibility, durability, and non-toxicity. However, the development of biodegradable materials in medicine still faces many challenges. The degradation time of the material is too long or too short, which will affect the therapeutic effect. At the same time, the degradation of the material is affected by external factors such as temperature, pH value, and pressure, and it is difficult to control. The complex degradation behavior in the body is difficult to predict, and the degradation of some materials may produce toxic substances or induce inflammatory reactions. Therefore, this article summarizes the types of medical biodegradable materials and systematically introduces the current application status of biodegradable materials in medical implants, tissue engineering, drug delivery, disease treatment, and biochemical electronic sensors. The application of biodegradable polymers in disposable medical treatment is also discussed. Specific medical application cases are studied to better explain the uses and benefits of these materials. Finally, the existing problems and future developments are discussed to provide a comprehensive reference for future research.

Keywords: polyvinyl alcohol, microbial degradation, public health.

1. Introduction

Polyvinyl Alcohol (PVA) has a wide range of biological and public health applications, and its unique physical and chemical properties make it ideal for drug delivery materials, medical protection, and biomaterials [1]. PVA has good biocompatibility and biodegradability, making it an ideal material for drug delivery systems. PVA nanofibers prepared by electrostatic spinning technology have high specific surface area and high porosity, which are favorable for drug loading and controlled release. This material can be used as an antimicrobial dressing or drug carrier to achieve precise delivery and sustained release of drugs and improve therapeutic effects. PVA can also be combined with other materials (e.g., peptides, graphene, etc.) to form composite materials to further enhance its antimicrobial activity and drug delivery capability [2,3].

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PVA also has important applications in medical protection, as it can be used as a material for medical anti-adhesive films for tissue separation and protection during surgery to reduce postoperative adhesions and complications. It can also be used to make medical devices such as highly absorbent infusion patches, which effectively protect wounds and promote the healing process [4]. PVA, as a synthetic water-soluble polymer, is widely used in biomedical fields such as bone, cartilage, artificial cornea, etc. because of its non-toxicity, good film-forming properties, good biocompatibility and biodegradability. Through blending and modification with other materials, PVA can also develop new biomaterials with special functions, such as waterproof and breathable, anti-bacterial and anti-odor, etc., to meet different medical needs.

However, PVA has poor biochemistry (BOD/COD = 0.07, high chemical oxygen consumption, long biodegradation cycle, biochemical wastewater contains a large amount of PVA, the direct discharge of the environment will bring serious pollution, greatly endangering the ecological environment [5,6]. In 1973, the Japanese scientist Suzuki for the first time to isolate the degradation of PVA can be individually degradation of Pseudomonas O-3 [7], followed by decades of domestic and international screening, enzyme gene cloning, and other work. In 1973, Suzuki isolated Pseudomonas O-3, which can degrade PVA alone [8]. Some PVA-degrading strains have been isolated and purified, among which Pseudomonas spp. are more frequent, and Bacillus and Fusobacterium are less frequent [8]. The results show that PVA-degrading bacteria are not widely distributed in nature, but mainly exist in some specific environments [9]. Compared to microorganisms capable of degrading other aliphatic polymers, microorganisms capable of producing PVA degrading enzymes are much less in number in terms of species and distribution, and most of these bacteria are in symbiotic relationships. Therefore, obtaining efficient degrading bacteria is the basis for the biological degradation of PVA, and the screening of microorganisms capable of producing PVA-degrading enzymes is a prerequisite for the biodegradation of PVA.

The purpose of this experiment is to screen and domesticate efficient PVA degradation strains, and to optimize the degradation process through the identification of PVA degradation bacteria and the optimization of degradation conditions.

2. Experimental method and procedure

2.1. Experimental materials and equipment

Experimental Materials:

Soil samples, mixed strains, PVA, yeast powder, LB liquid medium, color developers: including H3BO3, KI, I2, filter paper sheets, distilled water

Lab equipment:

Shaker, centrifuge, UV spectrophotometer, volumetric flasks pipettes, petri dishes,microscope, constant temperature incubator, pH meter, aseptic operation

2.2. Experimental procedure

2.2.1. Isolation and purification of PVA-degrading bacteria

1) Enrichment culture: The collected soil samples are mixed with mixed strains of bacteria and enriched under certain conditions. PVA and yeast powder were used as carbon sources in the enrichment medium.

2) Screening culture: Prepare a liquid medium with PVA as the only carbon source, inoculate the bacterial solution into the PVA medium at a ratio of 1:100, and take 1% of the bacterial solution to repeat the domestication three times.

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3) Isolation of single colonies: Gradient dilution of the domesticated culture solution, diluted and coated on the plate with PVA as the only carbon source, analyzed according to its size, color, transparency, edge flatness, and surface wetting, and selected strains that were not identical for successive streak culture to isolate and obtain single colonies.

4) Transparent circle screening: the bacterial solution of each strain was coated on a filter paper sheet, which was placed in a PVA plate, and a transparent circle was formed after color development by Finley's method[9]. Under the same culture conditions, the formation of transparent circle with larger size and clear interface can initially indicate that the strain has better PVA degradation ability.

2.2.2. Determination of PVA degradation rate

1)A 5 g/L PVA masterbatch was prepared and then diluted to 0 g/L, 0. 004 g/L, 0. 008 g/L, 0. 012 g/L, 0. 016 g/L, and 0. 020 g/L of PVA solution, respectively.

2) Prepare the colorant and measure the absorbance at the wavelength of 690 nm: Take H3BO3 20.00 g, KI 12.50 g, I2 6.35 g, respectively, dissolved in 500 mL of distilled water. In the volumetric flask containing the concentration of PVA standard solution, were added to an equal amount of configured H3BO3 10 mL and I2 - KI solution 2 mL, add distilled water to the volume, shake well and let stand. The absorbance was measured at 690 nm with only equal amount of H3BO3-I2-KI solution and the solution diluted with distilled water as blank control.

3) Plotting PVA concentration-absorbance standard curve: take PVA concentration as the horizontal coordinate and the corresponding absorbance as the vertical coordinate to make a graph, which can get the PVA standard curve.

A modified Finley method was applied. The preserved strain was taken and added to the PVA enrichment medium. It was placed in a shaker for shaking incubation and samples were taken every 24 hours to determine the PVA degradation. After centrifugation of 1 ml of fermentation broth, the supernatant was aspirated and diluted, and H3BO3-I2-KI colorant was added and shaken well and reacted for a certain time away from light, then the absorbance of the solution was measured by UV spectrophotometry at a wavelength of 690 nm. The fermentation supernatant was replaced with water as a blank control, and the pre-fermentation medium was used as a standard control, and the absorbance was measured at 690 nm in 1 cm light range, and the PVA concentration was obtained by comparing with the standard curve, so as to evaluate the degradation of PVA by this bacterium.

$$\frac{C_0 - C_1}{C_0} \tag{1}$$

Where, C_0 - initial culture solution PVA concentration, mg/L

C_1 - degradation culture solution PVA concentration, mg/L

2.2.3. Effect of temperature on PVA degrading bacteria

1) Inoculate each strain into LB liquid medium for master preparation.

2) After 16 h of incubation, take out the bacterial solution with strong growth and reproduction for dilution, and measure the absorbance of the solution at a wavelength of 600 nm with a UV-visible photometer, and dilute it until the OD600 is 0.01.

3) The diluted bacterial solution was inoculated into new LB medium and incubated at 39°C, 42°C, 45°C, 48°C, and 51°C, respectively, and samples were taken every hour to measure the OD600, 12 times in total.

4) Organize the data and plot the growth curves of each strain at different temperatures as a way to infer the effect of temperature on PVA-degrading bacteria.

DOI: 10.54254/2753-8818/68/2025.20144

2.2.4. Effect of pH on PVA-degrading bacteria

1) Inoculate each strain into LB liquid medium for master preparation.

2) After 16 h of incubation, take out the bacterial solution with strong growth and reproduction for dilution, and measure the absorbance of the solution at the wavelength of 600 nm with a UV-visible photometer, and dilute it until the OD600 is 0.01.

3) The LB medium was adjusted to pH 4, pH 4.5, pH 5, pH 5.5, and pH 6 using NaOH and HCl, respectively, and the diluted bacterial solution was inoculated into the new LB medium and incubated at the optimal pH, respectively, and samples were taken every hour to measure the OD600 , 12 times in total.

4) Organize the data and plot the growth curves of each strain at different pH as a way to infer the effect of pH on PVA-degrading bacteria.

3. Experimental results

3.1. Identification of transparencies

The isolated and frozen strains were resuscitated and cultured. In boric acid medium, PVA can react with iodine-potassium iodide solution to form a stable blue-green complex. Poorly soluble PVA was infiltrated into the solid medium to form a turbid, opaque medium background. PVA-degrading bacteria will form a transparent circle around the colony after utilizing PVA, and the size of the transparent circle reflects the strength of the strain's ability to degrade PVA.

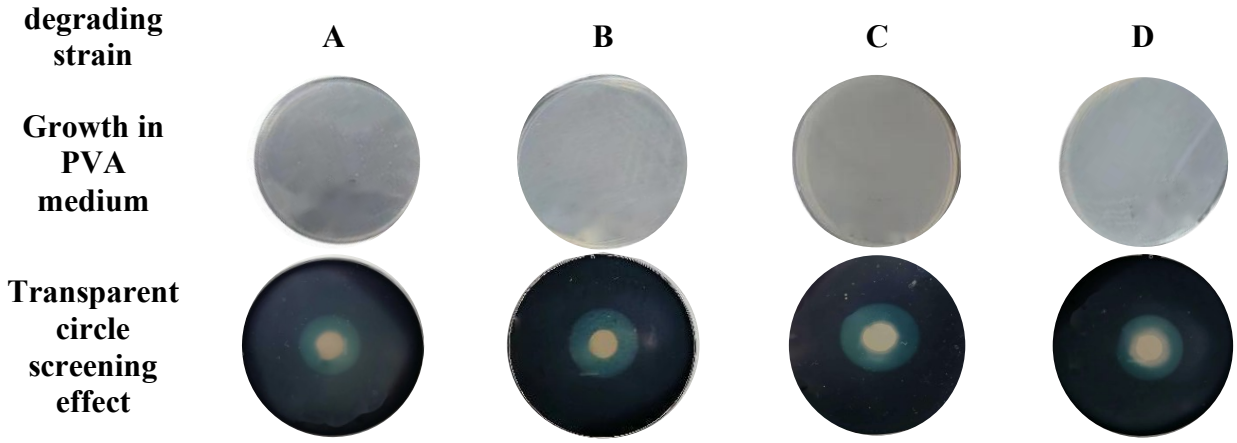

Figure 1: Results of transparent circle screening for each strain of bacteria

After observing the presence or absence and size of the transparent circle (Figure 1), single colonies of strains A, B, C and D were picked and delineated and preserved in PVA plates, and then transferred to a 4°C refrigerator for preservation and reserve after 24 hours of incubation in a 45°C constant temperature incubator.

DOI: 10.54254/2753-8818/68/2025.20144

3.2. Determination of PVA degradation rate

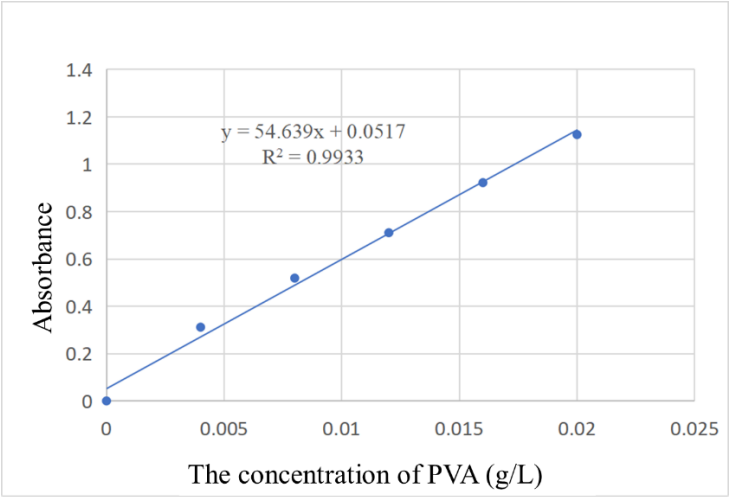

Figure 2: Standard curve of PVA concentration corresponding to absorbance value

A 5 g/L masterbatch of PVA was prepared, and then diluted to 0 g/L, 0.004 g/L, 0.008 g/L, 0.012 g/L, 0.016 g/L, 0.020 g/L of PVA solution, and then the absorbance was measured at 690 nm after treatment with H3BO3 -I2 -KI reagent. As shown in Fig. 2, the concentration of PVA was linearly related to the absorbance under certain conditions, and the correlation coefficient of this standard curve was R2 =0. 9933, which was of high confidence.

Take the preserved strains A, B, C and D respectively, activate them with 5 ml of PVA medium as seed solution, and then add 1 ml of the bacterial solution into 100 ml of PVA medium, and incubate them in a shaker at 45℃ with 150 rpm, and then take samples of 5 ml of the bacterial solution every 24 hours for a total of 6 days. The concentration of the bacterial solution at 690 nm was determined and the degradation rate was calculated.

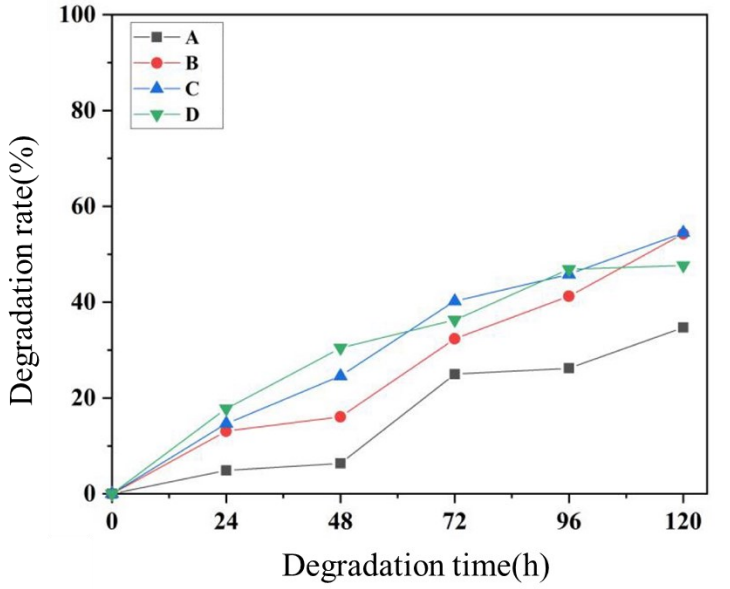

Figure 3: Degradation curves of PVA by strains of bacteria

DOI: 10.54254/2753-8818/68/2025.20144

As shown in Fig. 3, the bacterial solution was added for continuous incubation and the degradation curves of each strain on PVA were plotted. The degradation trend of PVA by the four strains A, B, C, and D was generally the same. The degradation rate in the first 5 days increased with the increase of incubation time, and the degradation rate reached the maximum in 5 days at 120 h, which was 34.73%, 54.25%, 54.5%, and 47.68%, respectively.

3.3. Effect of temperature on PVA degrading bacteria

The diluted bacterial solution was inoculated into new LB medium and incubated at 39°C, 42°C, 45°C, 48°C and 51°C, respectively, and samples were taken every hour to measure the OD600, for a total of 12 times. The growth curves of the four strains, A, B, C, and D, at different incubation temperatures are shown in the figure4

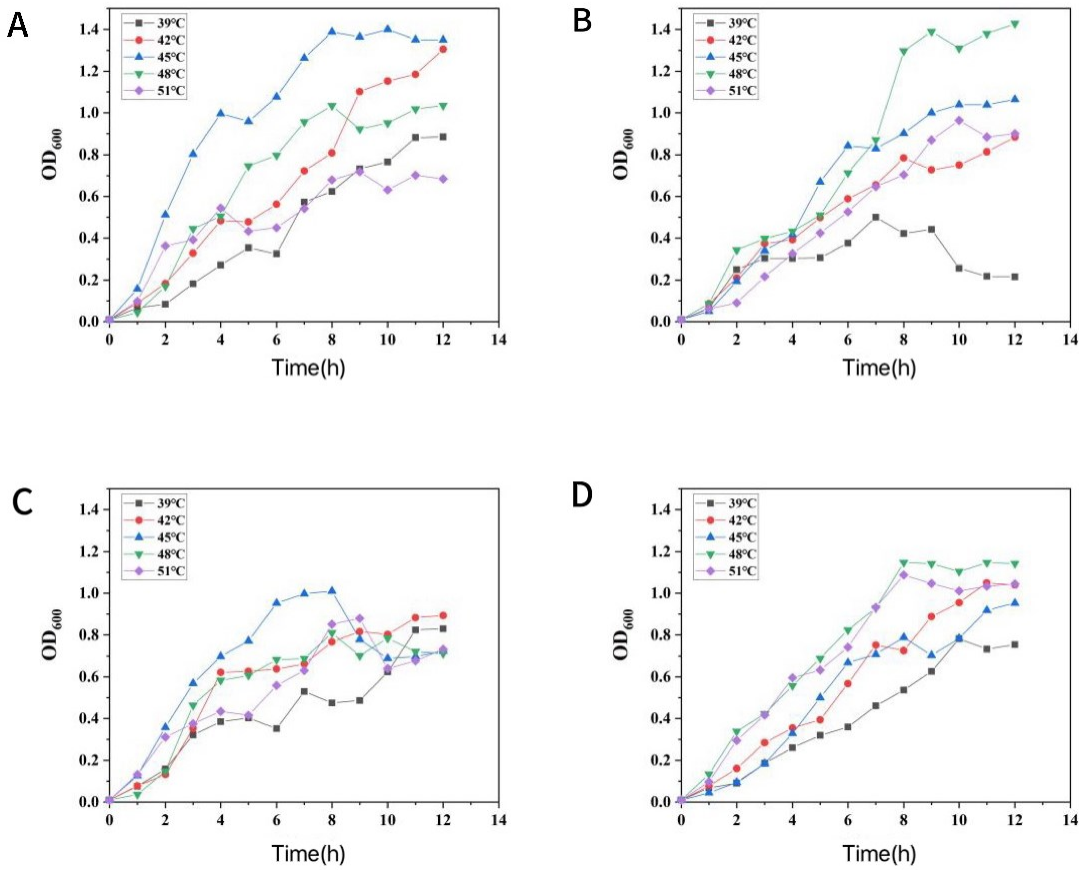

Figure 4: Growth curves of four bacteria A, B, C and D at different temperatures

Observing the growth curves of A, B, C and D bacteria at different temperatures, it can be seen that A and C bacteria all grow better under the culture conditions of 45 ℃, and it can be preliminarily determined that 45 ℃ is the optimal growth temperature for A and C bacteria; B and D bacteria grow better under the culture conditions of 48 ℃, and it can be preliminarily determined that 48 ℃ is the optimal growth temperature for B and D bacteria. Among them, the growth of B, C and D bacteria were more stable in the range of 42-51 ℃, indicating that the strains have a wide range of adaptation to temperature. The operation of the experimental process, it was thought that a possible problem was the short period of measurement (12 h), which failed to observe the decay period of the strains, and

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therefore could not effectively determine the growth status of each strain. Ideally, the data should be measured at 2 h intervals and continuously for 24 h, but it is more difficult to realize in practice.

3.4. Effect of pH on PVA-Degrading Bacteria

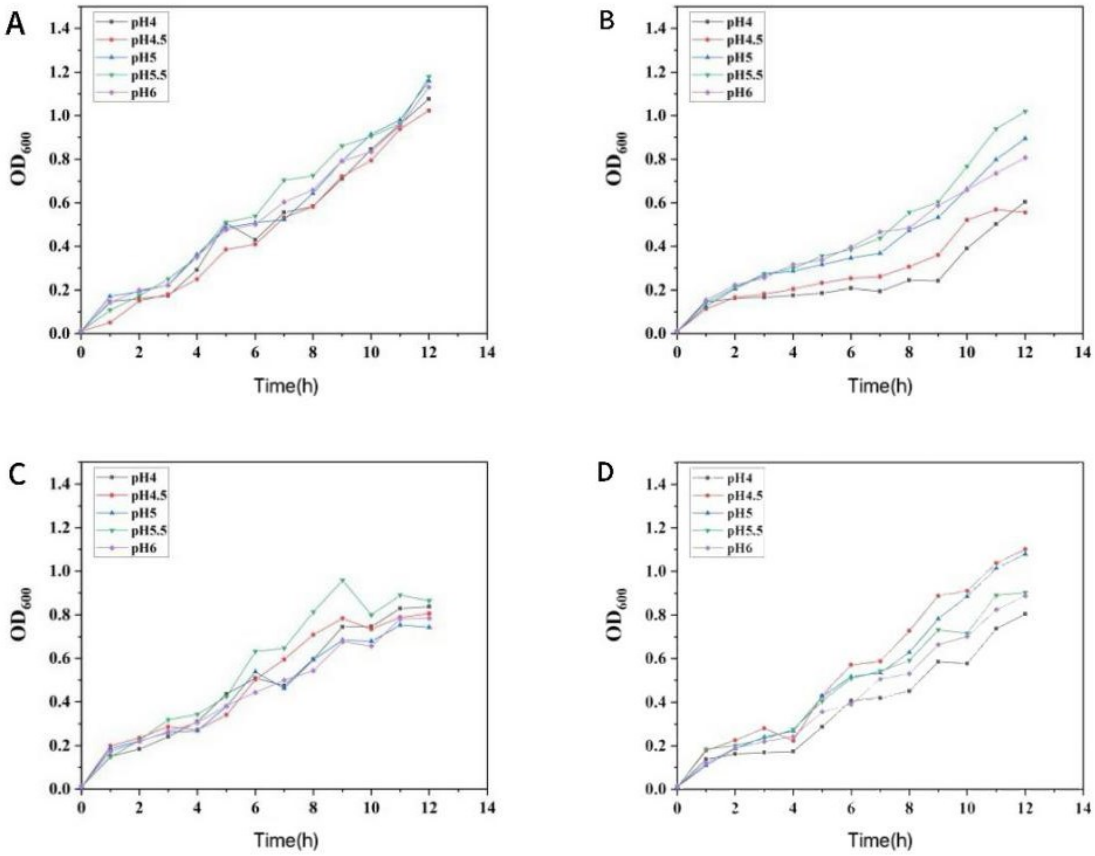

Figure 5: Growth curves of bacteria A, B, C and D at different pH.

Observing the growth curves of Bacteria A, B, C and D at different pH, it can be seen that Bacteria A, B and C all grew better under the culture condition of pH 5.5, and it can be preliminarily determined that pH 5.5 is the optimal growth pH for Bacteria A, B and C. Bacteria D grew better under the culture condition of pH 4.5, and it can be preliminarily determined that pH 4.5 is the optimal growth pH for Bacteria D (Figure 5). The growth of all the four strains at pH 4-6 was more stable, showing better adaptability. All four strains showed better adaptability with stable growth at pH 4-6.

4. Discussion

4.1. Discussion of experimental results

Through the screening and characterization of four PVA-degrading bacteria strains A, B, C and D, we found that these strains reached the maximum degradation rate of PVA on the fifth day, which was 34.73%, 54.25%, 54.5% and 47.68%, respectively (Figure 3). This result not only verified the findings of previous studies on the ability of PVA-degrading bacteria to degrade this polymer [9, 10] but also further revealed the significant differences in degradation efficiency among different strains. It is noteworthy that strain C exhibited the highest degradation rate, which may be related to its specific enzyme system or metabolic pathway and deserves to be explored in depth in future studies.

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Although we successfully screened strains with efficient degradation ability, we encountered some challenges during the experiments. For example, in the experiment to determine the effects of temperature and pH on PVA-degrading bacteria, we failed to observe the decay period of the strains due to the limitation of the measurement period (sampling and measuring OD600 every hour, 12 times in total), which may lead to a bias in the overall assessment of the growth status of the strains. In order to more accurately depict the growth curves of the strains, future studies should consider extending the measurement period or even achieving continuous monitoring.

4.2. Comparative analysis of different results

Comparing the degradation performance of the four strains A, B, C and D, it is found that strain C had the highest degradation rate, while strain A had a relatively low degradation rate. This difference may stem from the differences in genetic background, enzyme systems, and different adaptations to environmental conditions among the strains. For example, strain C may possess more efficient PVA-degrading enzymes, or its metabolic pathway may be more favorable for PVA degradation [11]. In addition, strains B, C, and D grew stably over a wide temperature range (42-51°C) and showed good temperature adaptation, whereas strain A had a more specific optimal growth temperature (45°C), which may be related to their ecological niche and originating environment.

4.3. value of biodegradation research in public health

As a widely used polymer material, PVA has an important position in the field of public health. However, its characteristic of not being easily biodegradable imposes a heavy burden on the environment. Therefore, the development of efficient biodegradation methods for PVA not only contributes to environmental protection but also has important public health significance. For example, in medical waste disposal, biodegradation of PVA products can reduce long-term pollution of the environment and decrease ecological risks. In addition, PVA-based biomaterials prepared by biodegradation methods have potential applications in medical protection and drug delivery [12].

4.4. Various degradation methods and their advantages and disadvantages

At present, the degradation methods of PVA mainly include two kinds of methods: physical and chemical methods and biochemical methods. Although the physical method can realize the degradation of PVA to a certain extent, it is often accompanied by high costs and the risk of secondary pollution. Physical degradation of PVA there are three main ways: coagulation and precipitation method by adding coagulants to make the suspended and colloidal substances in the PVA wastewater coagulation into larger particles, and then precipitate to remove them. Easy to operate, but requires a large number of coagulants, and the resulting sludge needs further treatment. Activated sludge method using microbial adsorption and degradation to remove organic matter in PVA wastewater, but requires a longer period of domestication and strict control of microbial growth conditions. The catalytic oxidation method accelerates the oxidative decomposition process of PVA by means of a catalyst, but the selection and regeneration of the catalyst is a challenge. In this study, a biochemical method was used to realize the effective degradation of PVA by screening and identifying PVA-degrading bacteria with efficient degradation ability. However, the method still has some limitations. For example, PVA-degrading bacteria are not widely available in nature and the degradation efficiency varies among different strains, which limits the general applicability of the method. In addition, the incomplete degradation of PVA can lead to difficulties in the extraction of PVA-degrading enzymes, which further increases the difficulty of application. Nevertheless, this study provides some reference value for the research and practical application of biodegradation of PVA wastewater.

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5. Conclusion

From the results of previous researchers, PVA degrading bacteria are not widely existed in nature, and there is a certain difference in the efficiency of different strains in degrading PVA, and it is still difficult to achieve complete degradation of PVA after a long time of cultivation. The incomplete degradation of PVA will cause difficulties in the extraction of PVA-degrading enzyme, because PVA and protein will form a milky-white gel during the extraction process, which makes the extraction of PVA-degrading enzyme impossible. There are not many species of PVA-degrading enzyme-producing bacteria, and the long cultivation period and low enzyme activity, coupled with the difficulty in extraction, have hindered the use of PVA-degrading enzyme in practical production.

In this experiment, the collected soil samples were mixed with mixed strains, enriched and cultured under certain conditions to domesticate and isolate PVA degrading bacteria by using PVA as the sole carbon source culture. The PVA-degrading bacteria were screened by Finley's method after color development using transparent circles, and the PVA degradation rate of each strain of A, B, C and D was determined, and the degradation rate reached the maximum at the fifth day, which was 34.73%, 54.25%, 54.5% and 47.68%, respectively, to analyze the degradation performance of the screened strains. Through the exploration, it was preliminarily determined that 45°C was the optimal growth temperature for Bacteria A and C, and 48°C was the optimal growth temperature for Bacteria B and D. It was preliminarily determined that pH 5.5 was the optimal growth pH for Bacteria A, B, and C, and pH 4.5 was the optimal growth pH for Bacteria D. Among the tested strains, Bacteria C had the highest degradation rate of PVA, which demonstrated strong degradation performance.
Temperature and pH had significant effects on the growth and degradation efficiency of PVA-degrading bacteria, and the adaptability of different strains to temperature and pH varied.
Strain D, although slightly inferior to in overall degradation rate, still has some degradation ability under its optimal growth conditions (48°C, pH 4.5) and may be useful in specific application scenarios.

Although the genus identification of the isolated and purified strains needs to be further improved, and the current experiments are only based on the laboratory scale, which needs to be scaled up and explored. This project aims to optimize the degradation process of PVA degrading bacteria through the screening and identification of degrading bacteria and the optimization of degradation conditions. This project is intended to provide a certain reference value for the biodegradation research and practical application of PVA wastewater.

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