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Synergic Effect of Adsorption and Biodegradation by Microsphere Immobilizing *Bacillus velezensis* for Enhanced Removal Organics in Slaughter Wastewater

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Abstract: Bacterial cell immobilization offers considerable advantages over traditional biotreatment methods using free bacteria. *Bacillus velezensis* was underwented isolation and genetic identification as COD-degrading bacteria in slaughter wastewaterand immobilized on the surface of polyvinyl alcohol (PVA) microsphere with the adhesion to bio-carrier through direct physical adsorption. The removal CODMn rates of microsphere (PVA) immobilized cells were 16.99%, increased 9.38% from a 50% concentration of slaughter wastewater within 24 h at 37 °C, pH 7.0, and 120 rpm, which was about 2.2 times that of the free bacteria. A significant difference was found in two groups ($p < 0.01$ p value less than 0.01 means statistical significance), and the COD degradation rate of the microsphere immobilized *Bacillus velezensis* strain was higher than the control group (PVA: control vs 20.08: 10.81), with the processing time reaching 36 h ($p < 0.05$). Additionally, similar results were obtained from a 20% concentration of slaughter wastewater within 24 h and 36 h. Moreover, the starch and protein digestibility of the immobilized *Bacillus velezensis* strain was higher than that of the free bacteria (20.1%: 42.2% vs. 17.5%: 37.2%). These findings revealed that the PVA-bacteria system was a simple, green, and inexpensive process, as well as a promising method. The research goal is aimed to synergize the effects of adsorption and biodegradation, as it can enhance organic removal by immobilized *Bacillus velezensis* in slaughter wastewater. Moreover, it may be possible that more potential materials can be used as biological carriers for the immobilization of bacterial cells later, which is beneficial for the recycling of resources.

Keywords: PVA microsphere; *Bacillus velezensis*; immobilization; organic matter; green process

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1. Introduction

The continuous drive to increase meat processing plants for the meat production of the ever-increasing world population has some pollution problems attached [1]. Contaminants are produced during meat production activities due to failure to comply with good manufacturing practices and good hygiene practices [2]. For hygienic reasons, large amounts of water are used during slaughter and cleaning, which produces large amounts of wastewater. The main environmental problems associated with slaughter wastewater are large amounts of suspended solids and liquid, and waste and odor generation, as blood, fat, feces, urine,

and meat tissue are lost in the wastewater during slaughterhouse processing [3–5]. Blood is one of the main dissolved pollutants in the slaughterhouse wastewater, and has the highest COD of any effluent from slaughterhouse operations. The main characteristics of the slaughterhouse wastewater are high organic matter concentration, adequate nutrition, sufficient alkalinity, and no toxic substances. Emissions from untreated or treated imperfect wastewater are the main cause of most problems related to human and aquatic life health. If pollutants in the wastewater are discharged into natural waters, the water will become contaminated and toxic, posing a major threat to humans and aquatic life [6]. Therefore, pollutants in the wastewater must be properly disposed of or removed before discharged into the environment [7].

The microbial treatment method is a method that has low cost, no environmental pollution, and thorough purification compared with chemical treatment and physical treatment methods in the treatment of slaughterhouse wastewater, and will be the main method to solve the slaughterhouse wastewater pollution in the future. Slaughter wastewater with the previously describing characteristics is well-suited for microbial degradation treatment [8,9]. *Bacillus velezensis* demonstrates broad-spectrum antimicrobial activity against pathogenic bacteria, such as *Aeromonas hydrophilis*, *Aeromonas veronii*, *Vibrio parahaemolyticus*, *Escherichia coli*, etc. The safety evaluation showed that the strain was non-toxic with no hemolytic activity, and was sensitive to most antibiotics [10]. As in vitro study showed that *Bacillus velezensis* was viable at pH 2–7, and that slaughterhouse wastewater contains high concentrations of nitrate, it indicated that *Bacillus velezensis* can be applied to the recycling of slaughter wastewater during the biological processes [9–12]. Biological treatment has been proven to be relatively harmless and more environmentally friendly and energy efficient if the process can be ensured to be well controlled [13–16]. Bio-carriers, one of the key components used as a reactor in biological treatment, can enrich and immobilize high-density microorganisms and have good properties [17]. Microbial carriers have received increasing attention in the study of immobilized microorganisms, and have also had some advantages, including microbial cell stability [18], the good diffusion between substrates and products, the separation of biomass and water, and the protection of microbes from the rugged environmental conditions [19–21]. Compared to the traditional inactivation method, it avoids adding chlorine, which deals with the problems known in chemical chlorination, such as the transport and the storage of the chlorine in the case of may causing safety problems and the generation of potentially toxic disinfection by-products [22]. Moreover, electrochlorination is a better method to deal with slaughter wastewater through in situ generating of the disinfectant species. In the presence of oxygen, including ozone, hydrogen peroxide, and hydroxyl radical, the electrolysis using appropriate electrodes can produce a variety of oxidants (free chlorine and chlorine dioxide). Although it improves the inactivation efficiency, the utilization of the slaughter wastewater is still low because of the presence of the oxidants [23]. Therefore, utilizing *Bacillus velezensis* as the biological treatment method is better than the chemical method because of its low pollution, high cleanliness, and low energy consumption. Polyvinyl alcohol (PVA) is a good water-soluble material and has been widely used to immobilize bioactive materials [24]. There is great scope for designing biological carriers with PVA as a raw material to fix microorganisms and develop new technologies for wastewater biological treatment [25,26]. In addition, the most important element is the species with high degradation properties in the microbial treatment process [27]. We screened and isolated a new degradation strain (*Bacillus velezensis*) from slaughter wastewater that can be immobilized on the surface of polyvinyl alcohol (PVA) microsphere with the adhesion to bio-carrier through direct physical adsorption, which suggests a potential and ideal method for dealing with slaughter wastewater. The PVA material also prolongs the duration time of the presence of *Bacillus velezensis* in the slaughter wastewater, which can lead to a better and more stable treatment. Therefore, a high efficiency and stability microsphere (PVA)-immobilized *Bacillus velezensis* cell system was constructed to remove the chemical oxygen demand (COD) based on simultaneous adsorption and biodegradation (SAB) for the treatment of slaughter wastewater. The nov-

elty of this research is that it addresses an environmentally important problem: purifying slaughter wastewater. The idea is to improve the efficiency by immobilizing the bacteria instead of suspending them free in the wastewater.

2. Materials and Methods

The slaughter wastewater used in this study was collected from a livestock slaughter plant in Hunan Tangren Shen Co., Ltd (Zhuzhou city, China). Chemicals and media used were of analytical grade. All other chemicals and media used were of analytical grade and were obtained from various commercial sources.

2.1. Source and Identification of Strains

The activated sludge in the secondary sedimentation tank of the slaughter wastewater treatment plant of Hunan Tangren Shen Co., Ltd. was taken. A COD-degrading strain was screened by enrichment, separation and purification, and the COD degradation rate was determined by potassium permanganate method. Genetic identification of the isolated bacteria was done using 16S rDNA [19]. Basic Local Alignment Search Tool (MEGA) analysis of the nucleotide sequence of the 16S rDNA revealed the bacterial species [28,29].

2.2. Preparation of Carrier Microspheres

The PVA was dissolved in deionized water and stirred at 85 °C for 40 min until completely dissolved, and PVP was dissolved in DMF (DMF:PVP = 20:0.05, mass ratio). Subsequently, the two solutions were mixed and stirred evenly with a syringe, and gradually dropped into the acetone solution to form microspheres and which were soaking for 8 h. The microspheres were filtered and immersed in a glutaraldehyde crosslinker (glutaraldehyde: hydrochloric acid: acetone = 5:1:94) for cross-linking. The microspheres were separated and washed with deionized water, and then dried to obtain PVA microspheres. Optimized synthesis conditions were determined by adjusting the concentration of PVA in this experiment.

2.3. Microsphere Characteristics

2.3.1. Infrared Spectroscopy

The characteristic functional groups contained in the microspheres were analyzed by Fourier transform infrared spectroscopy. The microspheres sample was ground into a powder together with a small amount of potassium bromide, and subjected to a tableting test at a scanning speed of 20 min^{-1} and a resolution of 4 cm^{-1}, and was measured in a wave number range of 400~4000 cm^{-1}.

2.3.2. SEM Characterization

The carrier was rinsed with distilled water and vacuum dried to remove moisture. The PVA microsphere samples were subsequently cut with a sterile scalpel, sprayed with gold, and examined using a scanning electron microscope (S-520 SEM, Hitachi, Japan).

2.4. Adsorption Ability of Microspheres to Degrading Bacteria

In the fermentation system, microorganisms adhere to the surface of the carrier microspheres, thereby improving the stability of the cell body. The effect of microspheres on cell body immobilization is most obvious in the logarithmic phase of microbial growth, which contributes to cell division and proliferation and stability. The microbial concentration is high after entering the growth stable phase, although the immobilization rate of the carrier microspheres to the cell body is not easy to measure. Therefore, the immobilization efficiency was selected when the *Bacillus velezensis* strain grew for 5 h. The *Bacillus velezensis* strain (100 μL) were added to a 100 mL conical flask containing 50 mL LB medium and PVA microspheres (80 particles) and cultured 5 h at 37 °C, 120 rpm. Each experimental group was sampled and the OD value was measured with an ultraviolet spectrophotometer at the wavelength of 600 nm. The decrease in the OD value in the experimental group can

be approximated as the bearing portion relative to the control group, and the calculation formula of the carrier microsphere immobilization rate is as following supplementary Equation (1). At the same time, 30 microspheres were randomly taken for vacuum drying, and a scanning electron microscope was used to observe the surface structure of the carrier.

$$\text{Carrier load ratio} = \frac{\text{Control group's OD} - \text{Experimental group's OD}}{\text{Control group's OD}} \times 100\% \quad (1)$$

Equation (1): The calculation formula of the carrier microsphere immobilization rat.

2.5. Slaughter Wastewater for COD Degradation

Slaughter wastewater samples were collected from a meat slaughterhouse in Hunan Province, China. *Bacillus velezensis* broth (200 µL) was inoculated into a 250 mL erlenmeyer flask containing 100 mL of 30% and 50% concentration of slaughter wastewater separately, adding PVA microspheres (80 particles) and cultivated at optimum pH (7.0), rotation speed (120) and temperature (37 °C). The degradation ability of immobilized degrading bacteria on slaughter wastewater was tested after shaking for 24 h and 36 h [30]. COD was determined by potassium permanganate method with diluting the appropriate amount of culture solution to 100 mL, which was calculated as the following supplementary Equation (2).

$$\text{KMnO}_4 \text{ index } (\text{O}_2,\ \text{mg/L}) = \frac{\{[(\ 10 + \text{V}_1)\text{K} - 10] - [(10 + \text{V}_0)\text{K} - 10]\text{C}\}\text{M} \times 8 \times 1000}{\text{V}_2} \quad (2)$$

Equation (2): Determination of chemical oxygen demand by potassium permanganate method. K-$KMnO_4$ solution correction factor, $K = \frac{10.00}{V}$; V1-The consumption of $KMnO_4$ solution (mL) when titrating the water sample; V_2-Water sample (mL); C-Ratio of water in the diluted water sample.

2.6. Amylase and Protease Activity Assay

Amylase activity was determined by measuring the release of reducing sugar from soluble starch. The soluble starch was diluted to 0.25%, 0.5%, 1.0%, 1.5%, and 2.0% for preparation of a standard curve of starch concentration. The control and PVA group of degrading bacteria culture fluids were filtered and used directly as a crude enzyme solution. Exactly 2 mL of the 0.2% starch solution was incubated for 5 min at 40 °C with 1 mL of Na_2HPO_4-Citrate buffer. The enzyme reaction was interrupted by the addition of 10 mL of 0.5 mol/l acetic acid, and the optical density of the solution containing the product was determined photometrically at 660 nm. The enzyme activity was measured in milligrams of starch broken down per milliliter of crude enzyme solution at 40 °C, pH 6.0.

The casein solution was diluted to 0.1%, 0.2%, 0.3%, 0.4%, and 0.5%, and the absorbance of each sample was measured by using a protein nucleic acid automatic analyzer (Eppendorf BioPhotometer D30) and a standard curve was prepared. It is known that anhydrous ethanol can destroy the surface hydrated membrane of protein molecules, and that the protein undergoes reversible denaturation and precipitation. After sufficient removal of absolute ethanol, the protein can be re-dissolved in a phosphate buffer. Exactly 1 mL of a 0.5% casein solution was incubated with 1 mL of the crude enzyme solution for 10 min at 40 °C. The undigested protein was denatured by the addition of 3 mL of cold absolute ethanol. The mixture was centrifuged at 8000 rpm for 5 min and the supernatant was removed, then 5 mL of phosphate buffer was added to re-dissolve the protein and shake evenly, and then the absorbance was measured using a nucleic acid protein analyzer. The amount of residual protein was calculated from the standard curve and the protein digestibility was calculated.

3. Results and Conclusions

3.1. Growth Characteristics and Identification of Strains

The suitable condition of the growth of strain was 37 °C, pH 7.0 in the previous experiment, and the major characteristics of slaughter wastewater were high temperature, sufficient organic nutrition, moderate alkalinity, and non-toxic substances, which are more suitable for the growth conditions of the strain. The 16S rDNA gene obtained from the isolates was amplified via PCR using a universal bacterial primer set and sequenced at Sangon Biotech. Phylogenetic analysis of cloned sequences was performed in MEGA version 5 based on 16S rDNA sequencing results. The obtained COD-degrading bacteria have the highest similarity with *Bacillus velezensis* [29]. The phylogenetic tree of the strain is shown in Figure S1.

3.2. Preparation of PVA Microspheres

3.2.1. Effect of PVA Concentration on Microsphere Morphology

The microspheres of the PVA foams were researched by adding different amounts of PVA. The proportions of 6%, 8%, 10%, 12%, and 14% PVA solution were mixed with the same amount of DMF solution and dropped into acetone solution to form the PVA microspheres. The sizes and shapes of the microspheres were strikingly different. The microspheres were wrinkled without cross-linking after drying, and their morphologies were unstable while the cross-linked microspheres remained intact. The optimum concentration of PVA was 10% in Table S1.

3.2.2. Characterization of PVA Microsphere

Figure 1 is an infrared spectrum of PVA powder and PVA sphere after chemical crosslinking. The strong absorption peak near 3342 cm^{-1} is a characteristic peak of a hydroxyl group caused by O-H stretching vibration, and the absorption peaks at 2943 cm^{-1} and 1427 cm^{-1} are a symmetric stretching vibration peak and an in-plane bending vibration peak of C-H, respectively, and the absorption peak at 1608 cm^{-1} belongs to the C=C stretching vibration peak of PVA. Compared with the infrared spectra of PVA powder and microspheres, the charge density of PVA flexible macromolecule decreased during the preparation of microspheres, which weakens the electrostatic interaction between molecular chains and the hydrogen bonding between PVA molecules, and it can be seen that the aggregation effect of the PVA microspheres formation process can be effectively prevented, which is conducive to the formation of microspheres independently [31]. The C-O characteristic peak of the PVA microsphere at 1135 cm^{-1} was enhanced, which indicates that the hydrolysis reaction may be promoted from the side, and that a network structure having a certain amount of hydroxyl groups was formed on the chain of the carrier [18].

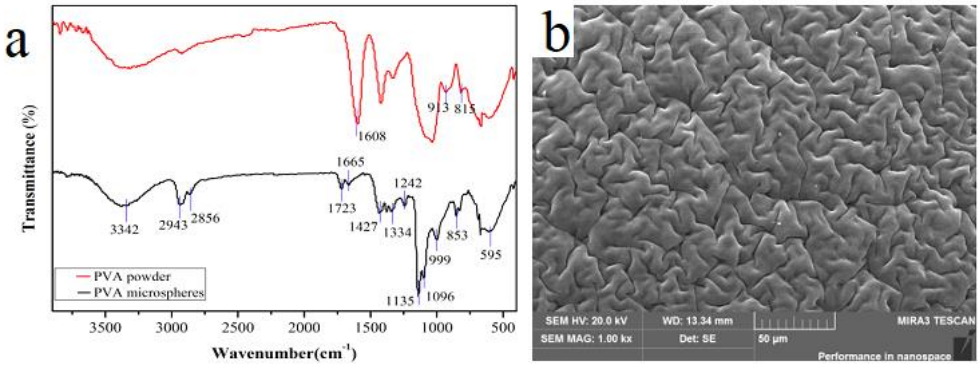

Figure 1. Characterization of PVA microsphere: ((**a**): the infrared spectrogram of PVA powder and PVA microsphere; (**b**): scanning electron microscope of PVA microsphere).

As a result of the formation of cross-linked network structures, the physicochemical stability of the PVA carrier was increased and the hydroxyl groups created a hydrophilic

micro-environment for the metabolism of the immobilized microorganisms. The microspheres have a uniform particle size of about 2 mm, which have a fluffy foam shape and a relatively stable structure. It can be seen from Figure 1 that there are a lot of wrinkles on the surface of the microspheres, and that the surface is rough, which greatly increases the surface area of the microspheres, whichis beneficial to the growth and reproduction of the degrading bacterial.

3.3. Ability of PVA Microspheres to Immobilize Bacillus Velezensis Strain

Precisely 80 particles of PVA vector microspheres and 100 µL of *Bacillus velezensis* medium were added to each 50 mL of LB medium at 37 °C with shaking at 120 rpm. The reduction of the OD value can be regarded as the ability of the carrier microsphere-immobilized *Bacillus velezensis* strain between in the experimental group and the control group. The immobilization ability of the PVA carrier microspheres is shown in Table 1. It was found that the PVA microspheres had obvious carrying capacity on the *Bacillus velezensis* strain. The adsorption capacity of *Bacillus velezensis* reached 36.99% in 5 h with PVA microsphere, and the surface of the PVA microspheres is densely packed and porous, and has a large specific surface area, which is favorable for the adhesion of degrading bacteria. PVA microspheres are feasible as a carrier matrix, which are suitable for the initial rapid colonization of microbes.

Table 1. Ability of carrier microspheres for *Bacillus velezensis*.

Group	Initial Value	OD Value after 5 h	Bearing Rate
Control group	0.205 ± 0.029	0.546 ± 0.047	None
PVA microspheres	0.211 ± 0.021	0.413 ± 0.039	36.99%

(N = 3, n = 3, $p < 0.001$).

The degrading bacterium growth and adsorption were observed microscopically in PVA microspheres. Figure 2 shows samples of the carriers used for COD-degrading bacterium immobilization in a low vacuum SEM image. The cocci growing on the surface of PVA skeleton is considered to be the COD-degrading bacteria, and the porous PVA microspheres could provide sufficient space for microbial growth.

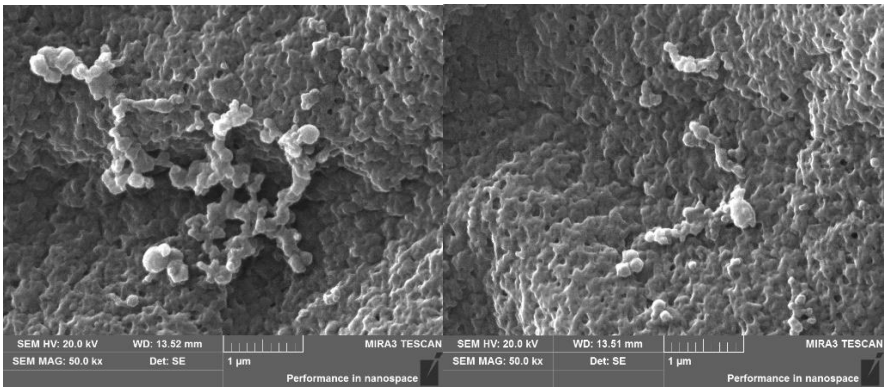

Figure 2. SEM images of the immobilized bacterium in PVA microsphere.

3.4. The COD Degradation of Microspheres-Immobilized Bacillus velezensis Strain

The experimental results are shown in Tables 2 and 3, as well as in Figure 3. It can be seen from these results that the immobilized *Bacillus velezensis* strain in the groups of PVA microspheres have a better degradation effect than the free bacteria after 24 h and 36 h. The PVA microsphere immobilized cells completely removed 2.976 mg/L of CODMn from 50% concentration slaughter wastewater within 24 h at 37 °C, pH 7.0, and 120 rpm, whereas the freely suspended cell system removed 1.24 mg/L of CODMn. tThe COD degradation rate of the microsphere immobilized *Bacillus velezensis* strain was 16.99%, increasing by 9.38%,

which was about 2.2 times that of the free bacteria, meaning that a significant difference was found in two groups ($p < 0.01$). Moreover, the COD degradation rate of the microsphere immobilized *Bacillus velezensis* strain was higher than the control group (PVA: control vs 20.08: 10.81), with the processing time reaching 36 h ($p < 0.05$). Additionally, similar results were obtained from 20% concentration slaughter wastewater within 24 h and 36 h.

Table 2. The degradation of microspheres-immobilized *Bacillus velezensis* strain treating 50% concentration slaughter wastewater.

Samples	Blank Sample	Control Group	PVA Group	
			24 h	36 h
V1(mL)	6.80 ± 0.134	6.30 ± 0.122	5.60 ± 0.107	5.40 ± 0.084
V2(mL)	30.0	30.0	30.0	30.0
K	0.93	0.93	0.93	0.93
Potassium permanganate index (O_2, mg/L)	16.304	15.064	13.328	12.832
Reduction(O_2, mg/L)	0	1.240	2.976	3.224
Degradation rate (%)	0	7.61	16.99	20.08

Table 3. The degradation of microspheres-immobilized *Bacillus velezensis* strain treating 20% concentration slaughter wastewater.

Samples	Blan Sample	Control Group	PVA Group	
			24 h	36 h
V1(mL)	5.3 ± 0.124	4.9 ± 0.113	4.3 ± 0.103	4.2 ± 0.071
V2(mL)	60.0	60.0	60.0	60.0
K	0.93	0.93	0.93	0.93
Potassium permanganate index (O_2, mg/L)	6.012	5.51	4.77	4.648
Reduction(O_2, mg/L)	0	0.673	1.24	1.364
Degradation rate (%)	0	8.35	20.63	22.69

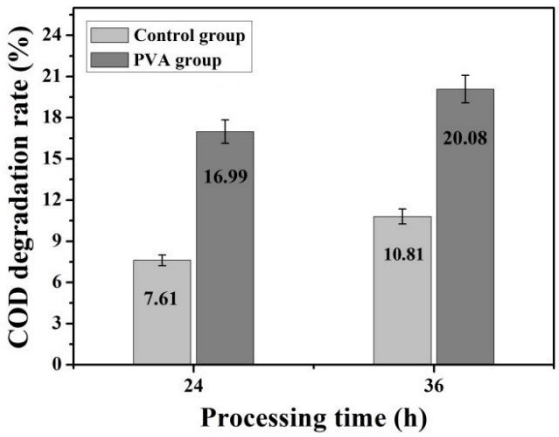

Figure 3. The degradation of microspheres-immobilized *Bacillus velezensis* strain treating 50% concentration slaughter wastewater.

3.5. Starch and Protein Degradation Assay

The slaughter wastewater contains animal blood and food residues, which contain a lot of protein and starchy substances. The rate of degradation of protein and starch was detected by immobilized *Bacillus velezensis* and free bacteria, respectively. The experimental results are shown in Tables 4 and 5, as well as in Figure 4. It can be seen from these results

that the immobilized *Bacillus velezensis* strain in the PVA group carrier microspheres have a better degradation effect to protein and starch than the free bacteria. The immobilized cell system crude enzyme extract digested 20.1% of the starch from the 2.0% concentration strength starch solution, whereas the crude enzyme of the freely suspended cell system digested 17.5% of the starch. In addition, similar results were obtained from the protease activity test group. The protein digestibility of the crude enzyme extract of the immobilized *Bacillus velezensis* strain was higher than that of the control group (PVA group: control group vs. 42.2%: 37.2%). The activity of amylase and protease in the crude enzyme solution obtained by immobilizing the PVA microspheres was increased.

Table 4. Protein degradation assay.

Groups	Absorbance	Starch Concentration (%)	Degradation Rate (%)
control	1.343 ± 0.0062	1.651 ± 0.0071	17.5
PVA	1.302 ± 0.0059	1.599 ± 0.0067	20.1

(N = 3, n = 3, $p < 0.001$).

Table 5. Protein degradation assay.

Groups	Absorbance	Casein Concentration (%)	Degradation Rate (%)
control	1.29 ± 0.0059	0.316 ± 0.0022	37.2
PVA	1.18 ± 0.0064	0.289 ± 0.0019	42.2

(N = 3, n = 3, $p < 0.001$).

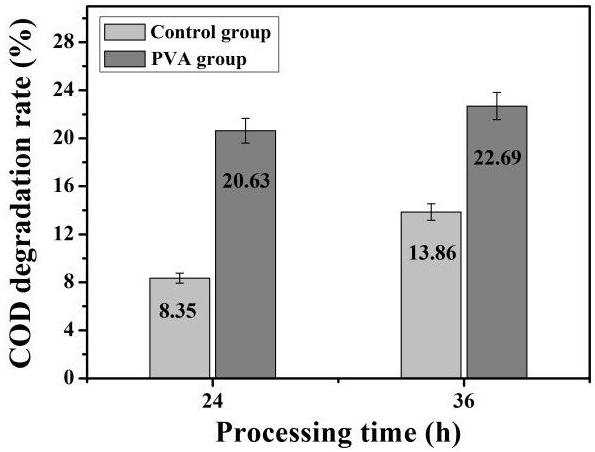

Figure 4. The degradation of microspheres-immobilized *Bacillus velezensis* strain treating 20% concentration slaughter wastewater.

4. Discussion

Slaughter wastewater contains a large amount of nitrogen-containing organic and harmful pathogen, presented a huge impact on the environment and even a threat to human health [1,2,4,7]. Currently, there are many methods for treating wastewater, but it is not possible to treat harmful microorganisms and degrade organic matter. In this study, we designed a strategy for increasing the degradation efficiency of bacteria in slaughter wastewater.

Bio-carriers are one of the core technologies of wastewater biofilm treatment [14,18]. Polyvinyl alcohol (PVA) hydrogel has a three-dimensional network structure, good biological affinity, developed pores, and specific gravity close to the specific gravity of granular sludge, and is usually used in microbial embedding agents. PVA can be made into a spherical biological carrier, which can give full play to its advantages, especially as a macroporous carrier [32]. At present, an efficient and stable microsphere (PVA/ZnO) immobilizing cell system with *Bacillus velezensis* has been constructed for the treatment

of slaughter wastewater. The results of wastewater treatment experiments showed that the microsphere-immobilized *Bacillus velezensis* cell system has improved the properties of *Bacillus velezensis* strain, such as COD degradation rate, amylase activity, and protease activity. This phenomenon may be caused by the following reasons: On the one hand, the PVA microspheres are structurally stable as a carrier and are not easily destroyed by the integrity of the carrier microspheres in a flowing environment. Moreover, another significant reason for choosing PVA as a carrier matrix is its high surface area and porosity, which are suitable for the initial rapid colonization of microbes. The carrier structure may be beneficial to the growth of *Bacillus velezensis* strain, thereby increasing the content of the strain and increasing the organic digestive enzymes and degradation rate of COD [24,25,32–35]. On the other hand, the strain *Bacillus velezensis* belongs to the genus *Bacillus*, PVA carrier microspheres could stimulate the relatd genes regulatory pathways of *Bacillus*, increasing the expression of the enzyme [25,27,33,36]. The experimental results showed that the immobilized degrading bacteria have great advantages in removing protein and starchy organic matter from slaughter wastewater.

5. Conclusions

As the research showed, COD-degrading bacteria strain were isolated from wastewater collected from the slaughterhouse wastewater treatment pond, and identified as *Bacillus velezensis*. The novelty of this research was that it addresses an environmentally important problem: purifying slaughter wastewater. The idea was to improve the efficiency by immobilizing the bacteria in the wastewater. Bacterial cells were successfully immobilized on the surface of PVA microsphere. The PVA-bacteria system was a simple, green, and inexpensive process as a promising method in the degradation of organic matter in slaughter wastewater. Synergic effect of adsorption and biodegradation enhanced organic removal in the slaughter wastewater. This study will give us some new inspiration for the facile construction of a high quality biological vector method and also provide a potential strategy for the disposal of slaughter wastewater treatment. Not only for this, but for more environmentally friendly materials can be composited for application on the system for slaughter wastewater treatment for higher efficiency and better effect later.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/pr9071145/s1, Figure S1: Neighbor-joining phylogenetic tree constructed from 16S rDNA gene sequence, Figure S2: Starch concentration standard curve, Figure S3: Protein concentration standard curve, Table S1: Effect of PVA concentration on microsphere molding.

Author Contributions: J.D. and W.L. conceived and designed the experiments; Q.C., M.J., Y.S. and W.L. performed the experiments; Y.S., B.H., S.X., J.B. and H.Y. performed statistical analyses; J.D., W.L. and M.J. wrote the paper. All authors have read and agreed to the published version of the manuscript.

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Abbreviations

PVP: Polyvinyl Pyrrolidone; DMF: N,N-Dimethylformamide; OD: optical density; LB: Luria-Bertani medium; COD: Chemical Oxygen Demand; PVA: Polyvinyl alcohol; SAB: simultaneous adsorption and biodegradation.

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