Biodegradation of poly(aspartic acid-lysine) copolymers by mixed bacteria from natural water

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ABSTRACT

Poly(aspartic acid-lysine) copolymers (PAL), as an alternative product to traditional water treatment agents, was developed based on poly(aspartic acid) through microwave-assisted synthesis of aspartic acid and lysine. Its biocompatibility and performance in inhibiting scale and corrosion are determining factors in its application. In this paper, biodegradability of PAL was investigated by carbon dioxide evolution tests. The degradation ratio of PAL on day 10 and day 28 respectively reached 35.01% and 80.00%, which indicated that PAL was a degradable water treatment agent. PAL is superior to polyacrylic acid and phosphonic carboxylic acid in biodegradability. With the increase in the addition ratio of lysine in copolymerization process and the decrease in the molecular weight of copolymer, the biodegradability of PAL was significantly enhanced. In addition, the incubation period before the biodegradation was shortened. The result showed that Cu^{2+} could decrease the degradation rate through inhibiting enzymatic activities.

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1. Introduction

Polyaspartic acid (PASP) is a promising biopolymer. As a water treatment agent, it shows the superior performance in inhibiting scale and corrosion [1]. PASP has become a research hotspot in the field of water treatment chemicals since the 1990s and has been widely used in industrial circulating cooling water [2,3]. However, its scale inhibition performance was not better than that of organophosphorus agents under some conditions. In order to improve the scale inhibition performance of PASP, various PASP modification methods were studied. The chemical modification methods include crosslinking modification, open-loop modification, and copolymerization modification, which is the most widely used at home and abroad [4]. During copolymerization modification, hydroxyl, ether, hydroxyalkyl amine, carboxyl, sulfo group, and phosphoric acid group were introduced into the molecular structure of PASP to obtain PASP derivatives [5]. However, the majority of PASP derivatives showed the superior scale inhibition performance and the worse environmental friendliness, thus hampering the application of modified polyaspartic acid as the

substitute of PASP. In the paper, poly(aspartic acid-lysine) co-polymers (PAL) was manufactured by introducing −COOH into PASP through the copolymerization between lysine and aspartic acid. Then, the biodegradability of PAL was investigated by mixed bacteria from sewage discharge water in order to evaluate its environmental hazards.

The biodegradation performance evaluation methods of chemicals are divided into two types: aerobic and anaerobic methods. The former includes aerobic rate method, matrix removal method, microbial index method, and carbon dioxide evolution test. The latter includes gas production and volatile acid assays, volatile suspended solid assay, and radiolabeled compound assay [6]. At present, there are many evaluation methods on biodegradation performance of PASP and its derivatives. These methods are almost aerobic.

In the development of green/biodegradable polymers for water scaling applications, with malic acid (MA) and citric acid (CA) and through a bulk-melt condensation process, S. R. Popuri [7] synthesized green copolymers PMC, which were water-soluble, biodegradable, and non-toxic and hence had a good water descaling property without disturbing the taste, quality, and purity of water. S. Roweton [8] focused on the synthesis, biodegradation, and current application of poly(aspartic acid) and found that poly(-aspartic acid) was unique biodegradable and water-soluble

polymer with wide valuable potential applications as a homopolymer and a component in novel copolymers. In the study of the biodegradability performance of polyaspartic acid and its derivatives, with polysuccinimide, D.Y. Wang [9] synthesized four kinds of PASP derivatives respectively containing functional groups of one or two hydroxyl, carboxyl, or sulfonic groups and found that PASP and PASP-ASP including −COOH had excellent biodegradability and that polyaspartic acid-taurine(PASP-SEA), grafted single hydroxyl polyaspartic acid derivative (HPAP) and dihydroxyl polyaspartic acid derivative(DHPAP) were biodegradable through the biodegradability evaluation with the evaluation index of CODcr. In the investigation of the corrosion inhibition capacity and biodegradability of polyaspartic acid copolymers containing hydroxyl, with groundwater in Shijiazhuang City as testing water and COD as the evaluation index of biodegradability, R.H. Guo [10] found that with the increase in the hydroxyl group, the corrosion inhibition capacity of polyaspartic acid copolymers containing hydroxyl increased and the biodegradability decreased. For the Poly(aspartic acid-lysine) copolymers, the ratio of Asp to Lys and the molecular weight of copolymers significantly affected their scale inhibition performance and the copolymers synthesized according to the ratio of Asp to Lys of 1:1 allowed the optimal scale inhibition performance. In this paper, we collected nature water as inoculated water, cultivated the mixed bacteria in natural water, studied the biodegradability of PAL (n_{Asp}:n_{Lys} = 1:1) through carbon dioxide evolution tests, discussed the relationship between molecular structure of PAL and biodegradability, and evaluated the environmental performance of PAL.

2. Experimental

2.1. Materials and equipment

Polyacrylic acid sodium salt (PAA), 2-phosphonobutane-1,2,4-tricarboxylic acid (PBTCA), amino trimethylene phosphonic acid (ATMP), and 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP) were industrial products obtained from Changzhou Jingkexiafeng Fine Chemical Co., Ltd. Glucose was of CP grade. Aniline was of AR grade. Other reagents used in the experiment were supplied by Tianjin Fine Chemical Co., Ltd. Testing water was distilled for more than three times until DOC content was lower than 1 mg/L.

Glass instrument was soaked in lotions (50 g $K_2Cr_2O_7$ and 1000 ml H_2SO_4) for 30 min, washed with tap water, soaked in lotions for 60 min, washed with distilled water, and dried at 105 °C to remove all the organic or toxic substances.

2.2. Synthesis and characterization methods of PAL

2.2.1. Synthesis and purification methods of PAL

The synthesis procedure was as follows: A certain amount of aspartic acid and lysine were fully mixed. KH_2PO_4 as catalyst and N-N-dimethyl formamide (DMF) as organic solvent were added into the above mixture to obtain suspension. The suspension reacted several minutes under microwave radiation to gain yellow fluffy product PSID called as intermediate. PSID was fully hydrolyzed by adding an appropriate amount of 6 mol/L NaOH solution. The color of the solution after hydrolyzation turned into orange. The pH was adjusted to 3.84 by using hydrochloric acid, and then excess anhydrous ethanol was added into the solution. The object product (PAL) was obtained after filtering, drying and grinding. When the microwave radiation time and power were changed, PALs with various molecular weights were synthesized.

Purification of PAL: excess anhydrous ethanol was added into 70% PAL solution to obtain the suspension. Then suspension was static settling for 30 min and the deposit was observed. After

filtering, the deposit was added into excess anhydrous ethanol again. The above operation was repeated four times. High-purity PAL was gained after filtering, drying and grinding.

2.2.2. Characterization of PAL

PAL (average molecular weight: 1000−4000) was characterized by Fourier Transform Infrared Spectroscopy (FTIR), ^{1}H NMR and ^{13}C NMR spectroscopy to grasp its structure. For the measurement of FTIR spectrum, the powdered samples of copolymer were mixed with KBr and pressed onto disk. FTIR spectra in the range 400−4000 cm^{-1} of the copolymers were recorded by means of a Perkin Elmer Spectrum 100 spectrometer made in USA.

^{13}C NMR and ^{1}H NMR spectra of PAL were measured with a Bruker ARX400 NMR spectrometer made in Switzerland. 5 mg sample and 1 mL D_2O was used to measurement. The operation parameter for ^{13}CNMR spectra was SI = 32768, SF = 100.61 MHz, WDW = EM, SSB = 0, LB = 1.0 Hz, GB = 0. PC = 1.40. SI = 32768, SF = 400.13 MHz, WDW = EM, SSB = 0, LB = 0.3 Hz, GB = 0 and PC = 1.0 was the operation parameter for ^{1}HNMR spectra.

Molecular weight of PAL was measured on a Perkin Elmer Series 200 LC system (made in USA) equipped with a gel permeation chromatography column. The mobile phase was 0.3 mol/L NaCl−H_3PO_4 solution. And sodium polyacrylate purchased from Sigma Co. was as a standard.

2.3. Methods for biodegradability of PAL

2.3.1. The test device

As shown in Fig. 1, the test device is composed of the gas supply system (1, 2, 3, 9, 10), and the system of detected gas (4), and the CO_2 capture system (5, 6, 7, 8). Among them, the air without CO_2 was supplied by the gas supply system. The CO_2 capture system absorbed CO_2 produced from the detected mixed liquid in three levels. In each level, 100 mL of 0.0125 M $Ba(OH)_2$ was used as the CO_2 absorbent. Moreover, after the adsorption in the third level, water seal was provided to avoid the influence of the ambient air on CO_2 absorption [4].

2.3.2. Preparation of the inoculum solution

Firstly, natural water was taken from the sewage river near Lijinglanwan Residential District in Baoding City in China. The impurities in raw water (containing inoculum) were removed through filtration before the test. After 24 h, filtered water was cultivated on the cultivation plate in a light incubator for 24 h according to the plate counting method. Then the number of inoculation was estimated by streak plate method to determine the appropriate inoculation dilution factor. After dilution, the amount of bacteria in the inoculum solution was 10^4−10^5 CFU/mL (TOC < 4 mg/L).

2.3.3. Preparation of the detected mixed solution

Solution A was prepared with 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4\cdot2H_2O$, and 0.5 g NH_4Cl in a 1000-mL volumetric flask. Solution B was prepared with 22.5 g $MgSO_4\cdot7H_2O$ in a 1000-mL flask. Solution C was prepared with 36.4 g $CaCl_2\cdot2H_2O$ in a 1000-mL flask. Solution D was prepared with 0.25 g $FeCl_3\cdot6H_2O$ and a drop of HCl in a 1000-mL flask.

The detected mixed solution was prepared with 800 mL of H_2O, 10 mL of Solution A, 1 mL of Solutions B, C, and D, and 40 mg tested carbon source substance in a 1000-mL flask, and then diluted to 1000 mL with deionized water. The pH of the mixed solution was adjusted to about 7.4. According to equal carbon content of the tested chemical, the tested carbon source substance corresponded to 95.8 mg PASP (8.3×10^{-4} mol), 80 mg PAA, 100 mg glucose, and 51.7 mg aniline. In addition, in the test of the influence of metal ions on the biodegradation of PASP, the appropriate metal salts were