S-oph enzyme for efficient degradation of Polyvinyl alcohol: Soluble expression and Catalytic properties

Xinyu Wang
South China University of Technology

Jiaxuan Li
South China University of Technology

Xiaoshan Lin
South China University of Technology

Yi Zhang (✉ btyzhang@scut.edu.cn)
South China University of Technology  https://orcid.org/0000-0002-1568-6602

Research Article

Keywords: Oxidized PVA hydrolase (oph), Heterologous expression, Molecular chaperone, Lytic protein, Enzymatic properties

Posted Date: February 2nd, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2516649/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Version of Record: A version of this preprint was published at Molecular Biology Reports on August 29th, 2023. See the published version at https://doi.org/10.1007/s11033-023-08712-x.
Abstract

Background

Polyvinyl alcohol (PVA) is one of the most widely used water-soluble polymers with great mechanical properties. However, water-soluble polymers are one of the major organic pollution sources in streams, river, and marine ecosystems. Once dispersed in aqueous systems, they can directly interfere with the life cycle of aquatic organisms due to their direct toxicity. Therefore, it is urgent to develop efficient microorganisms or enzyme to degrade it. The oxidized PVA hydrolase (OPHase) plays an important role in the pathway of PVA biodegradation. It is the key enzyme in the second step of PVA completely degradation.

Methods and Results

The s-oph gene was cloned from laboratory isolated strain Sphingopyxis sp. M19. The s-oph gene was expressed in the E. coli system pET32a/s-oph expression vector in the form of an inclusion body. By binding with the molecular chaperone, pET32a/s-oph/BL21 (DE3)/pGro7 was constructed successfully, which enabled the s-oph gene to achieve soluble expression in E. coli. The s-oph gene expressed protein was purified at the yield of 16.8 mg L$^{-1}$, and its catalytic activity reached 852.71 U mg$^{-1}$. In the s-oph enzyme reaction system, the degradation efficiency of PVA can be increased to 233.5% compared to the controls.

Conclusion

The s-oph enzyme had PVA degradation characteristics, high efficiency, specificity, and stability. The s-oph enzyme has good practical application potential in alleviating plastic pollution and protecting the environment.

Introduction

Polyvinyl alcohol is a polymeric compound with excellent physical properties that are widely used in paper, textile, and adhesive industries [1]. Globally, the annual consumption of polyvinyl alcohol is to be measured in hundreds of thousands of tons [2]. The production of plastic products far exceeds that of other material products [3]. A large amount of consumed polyvinyl alcohol is discharged into the environment, PVA is difficult to degrade and improper disposal methods cause serious pollution of soil and water quality [4, 5]. The widespread use of PVA has led to various environmental problems [6], for example, the difficult degradation of PVA will result in poor reoxygenation performance of water, high COD of wastewater, and destruction of the soil environment. The discharge of PVA-containing wastewater into water will promote the release and migration of heavy metals in rivers, lakes, and Marine sediments, resulting in more serious environmental problems. We have degraded PVA by various physical and
chemical methods, but found that traditional treatment methods do not alleviate the threat of PVA to the ecosystem after consuming valuable resources [7]. Biodegradation is considered a highly promising treatment method for effective PVA removal [8]. PVA has received increasing attention in recent years because of its biodegradable properties, but the number of microorganisms capable of degrading PVA is limited and not very effective, so the search for new microorganisms or enzymes with greater degradation capacity is imminent. The discovery of new microorganisms or enzymes that can degrade PVA faster or more easily would be beneficial to improve PVA pollution in wastewater or the environment [9].

Polyvinyl alcohol hydrolase (OPH) is a type of polyvinyl alcohol degrading enzyme, and there are three main PVA degrading enzymes reported in the literature, namely PVA oxidase (SAO), PVA dehydrogenase (PVADH), and oxidative PVA hydrolase (OPH) [10]. Today the recognized PVA hydrolysis reaction has two main steps: 1) oxidation of two adjacent hydroxyl groups of PVA to form a diketone structure, that is, oxidized PVA was generated. (SAO or PVADH + PQQ); 2) hydrolysis of oxidative PVA, thus breaking the long chain of PVA (aldolase or OPH). In the second step of the PVA degradation reaction, the OPH enzyme can completely hydrolyze PVA into small molecules such as monoketones and carboxylic acids. The OPH enzyme that controls the second step of the PVA degradation has been heterologously expressed in *E. coli* in addition to having a high degradation rate, which is suitable for large-scale production [11, 12].

In our previous work, we have screened some strains with degradation ability such as *Bacillus* sp. DG01, *Bacillus* sp. DG02, *Paenibacillus* sp. DG03, and *Sphingopyxis* sp. M19 strains. The gene encoding the degradation enzyme of PVA (NO. OP934066) was from *Sphingopyxis* sp. M19. We found that the gene sequence of *Sphingopyxis* sp. M19 degrading PVA had high homology with the soph gene of *Sphingopyxis* sp. 113P3 (NO. BAD95542.3), and the amino acid sequence was 100% coincidence. Therefore, we will name the OPH enzyme in *Sphingopyxis* sp. M19, also S-oph. Our laboratory used the existing *Sphingopyxis* sp. M19 strain as the experimental strain cloned the s-oph gene and expressed it in *E. coli*. The heterologous soluble expression of the s-oph gene in conventional host cells is of great significance for the research, production, and application of polyvinyl alcohol degrading enzymes.

We chose the prokaryotic expression vector plasmid pET32a, which contains a tag encoding thioredoxin and a pro-soluble tag trxA, which can help the disulfide bond to fold correctly and thus achieve soluble expression of the protein, in addition to the properties of pET32a such as high expression commonly. If the s-oph gene is expressed as an inclusion body, the trxA tag can also bind well to the molecular chaperone and achieve soluble expression more efficiently [13]. The software simulation of the protein expression results of the analysis of the s-oph gene is shown in Fig. 1.

**Materials And Methods**

**Materials and biology**
Common chemical materials were purchased from Sangon Biotech (Shanghai) and Macklin (Shanghai), with p-nitrophenyl acetate (PNPA) purchased from Energy Chemical (Anhui, China). The rEK enterokinase was purchased from Vazyme Biotech (Nanjing), SacI and NotI restriction endonucleases and seamless cloning ligase were purchased from Sangon Biotech (Shanghai, China). BCA protein quantification kit was purchased from Thermo Scientific (China). Genome extraction, plasmid extraction, and reaction solution recovery kits were purchased from Tiangen Biotech (Beijing, China). Luria-bertani (LB) (10 g Tryptone L^{-1}, 5 g Yeast Extract L^{-1}, 10 g NaCl L^{-1}) and Terric Broth (TB) (12 g Tryptone L^{-1}, 24 g Yeast extract L^{-1}, 0.4% Glycerin, 9.4 g K_{2}HPO_{4} L^{-1}, 2.2 g KH_{2}PO_{4} L^{-1}, pH 7.2–7.4) media used in this study are among the most common media for growth and protein expression of Escherichia coli. Antibiotics, such as 20 µg Chloramphenicol (Cm^{+}) mL^{-1} and 100 µg Ampicillin (Amp^{+}) mL^{-1}, were also used. Strains are shown in Supplementary Table S1. The primers used in this experiment are shown in Supplementary Table S3.

S-oph gene clone from Sphingopyxis sp. M19

The preserved glycerol bacteria (25% glycerin, -80℃) were inoculated in an LB liquid medium overnight for activation. The bacterial broth was collected and total DNA was extracted using the Tiangen Genome Extraction Kit, which was operated according to the kit manual.

The s-oph gene was obtained by PCR amplification with cDNA (The primers F/R in Supplementary Table S3) as a template, and the corresponding signal peptide was removed. The cDNA was synthesized from DNA extracted from Sphingopyxis sp. M19. The PCR product should be a single band of approximately 1095 bp in size. The cloned s-oph gene was sequenced by Sangon Biotech (Shanghai) Co., Ltd. The sequencing results showed that the s-oph gene was 1095 bp in length and encoded 365 amino acids.

Recombinant plasmid construction

The insertion of the s-oph gene into plasmid pET32a was achieved by a method, in which NotI and SacI double digested pET32a and s-oph PCR recovered products were ligated by a seamless cloning kit [14]. The ligated recombinant plasmids were transformed into DH5α receptor state (Tsingke Biotechnology, Beijing, China). Colony PCR and double digestion of recombinant plasmid pET32a/s-oph were used for preliminary verification. The system is shown in Supplementary Table S2.

Colony PCR was performed on the recombinant plasmid using universal primers T7 and T7T, and the product should be a single band of approximately 1834 bp. To further confirm the pET32a/s-oph vector, the plasmid was extracted from the corresponding colony shake and verified by double digestion with NotI and SacI restriction endonucleases. The agarose gel of the digested product should show two bands, one of about 5800 bp and the other of about 1095 bp, for the plasmid pET32a and s-oph gene, respectively. The initially verified bacteria solution was sent to the company for sequencing (Sangon Biotech, Shanghai, China).

Expression of recombinant plasmid encoding S-oph protein
The recombinant plasmid pET32a/s-oph containing the s-oph gene was transferred into E. coil BL21 (DE3) chemosensory cells (Tsingke Biotechnology, Beijing, China). The recombinant bacteria pET32a/s-oph/BL21 (DE3) were picked and inoculated in an LB medium containing 100µg mL⁻¹ Amp+ and cultured overnight. The seed solution was incubated in resistant LB at 1% transfer volume until the OD₆₀₀ of the bacterial solution reached 0.6–0.8, and expression was induced by adding isopropyl-β-d-thiopyranoside (IPTG). Since most of the exogenous genes are expressed in inclusion bodies, temperature gradient and inducer concentration gradient were established to induce the culture. The temperature gradients were 37°C, 32°C, 30°C, 28°C, 25°C, 16°C, and the inducer concentration gradients were 0.1, 0.3, 0.5, 0.7, and 1 mM. Bacteria were collected at 12000 rpm for 1 min, resuspended with Buffer A (20 mM Tris/HCl, 500 mM NaCl, 20 mM imidazole, pH 7.8), and subjected to sonication (100 W, 3 s on 3 s off, 5 min) in an ice-cold water bath. The cells were broken in a water bath, and the whole solution, supernatant, and precipitate were collected and treated with SDS-PAGE loading buffer, respectively, and boiled at 100°C for 5 min. They were then detected by 12% SDS-PAGE(80 V 30 min, 120 V 60 min).

**Purification of recombinant S-oph protein**

In order to successfully purify the target protein, we also needed to perform western blot experiments [15, 16] due to the purification of the target protein using nickel columns and the need to ensure the presence of His-tag. The specific method was as follows: after SDS-PAGE, the target band was transferred to the PVDF membrane (80 V for 90 min), and the membrane was removed after the transfer. The residual transfer solution was washed off with TBST (Tris/NaCl/Tween-20, pH 7.4–7.6) (5 min), closed with 3% skim milk for 1.5 h, washed with TBST for 15 min, incubated with His-antibody for 2 h. The membrane was washed with TBST three times, 15 min/time. Exposure to observe the strip results.

After confirming the soluble expression, the optimal expression conditions were confirmed. The cell pellets were collected at 4°C and 8000 rpm for 7 min. Resuspend the cell cultures and ultrasonically crush it. Cell cultures were separated by centrifugation at 8000 × g for 20 min and the cell pellets were resuspended in binding buffer (20 mM Tris/HCl, 500 mM NaCl, 20 mM imidazole, pH 7.8), and cell disruption was performed by sonication (Ultrasonication conditions: variable amplitude rod 6, power 75%, 2 s on 3 s off, 40 min ~ 60 min) in an ice-water bath. The suspension was centrifuged (12,000 × g at 4°C for 50 min), and the supernatants were filtered through a 0.22-µm filter and purified with a Ni–NTA 6 FF (Pre-Packed Gravity Column) (Sangon, Shanghai, China). All proteins were purified using the standard nickel affinity chromatography procedure with elution buffer (20 mM Tris/HCl, 500 mM NaCl, 500 mM imidazole, pH 7.8). The target proteins were collected after validation by SDS-PAGE, dialyzed at 4°C in preservation buffer (25 mM Tris/HCl, 150 mM NaCl, pH 8.0), concentrated by ultrafiltration, and the concentrations were measured by the BCA kit.

The enterokinase rEK cleaves the lysogenic tag trxA. The rEK specifically acts on the AspAspAspAspLys site of the polypeptide chain. 2 U rEK was added for every 1 mg trxA/s-oph protein and reacted at 4°C for 64 h. After cleavage, it was dialyzed in rEK buffer (20 mM Tris/HCl, 100 mM NaCl, pH 8.0) with 20% polyethylene glycol for 8 h. The trxA and rEK were removed, and the s-oph enzyme solution was in the
dialysis bag. After being concentrated by ultrafiltration with 20% glycerol and filtered by 0.22 µm membrane on an ultraclean table for sterilization, it was stored in the refrigerator at -80°C.

**S-oph enzyme activity assay**

Enzyme activity assay conditions: 2 mmol L\(^{-1}\) of PNPA (pre-dissolved in acetonitrile) was added in potassium phosphate buffer (50 mmol L\(^{-1}\), pH 6.0), preheated at 37°C for 1 min, and the rate of product (p-nitrophenol) production was measured at 405 nm after addition of the appropriate amount of enzyme [17].

Definition of s-oph enzyme activity: the amount of enzyme required to catalyze the hydrolysis of 1 µmol of p-nitrophenol (PNP) per minute at 37°C is defined as one unit of enzyme activity.

Assay system: In a 50 mmol L\(^{-1}\) pH 6.0 potassium phosphate buffer system, 1 µL of pre-prepared 2 mol PNPA L\(^{-1}\), 20 µL of enzyme solution (set up three groups: blank, trxA/s-oph protein, s-oph protein), and the buffer was made up to 1 mL. After mixing the system thoroughly, three samples of each group were taken in a 96-well plate and measured with an enzyme standard at dual wavelengths (primary wavelength 405 nm, secondary wavelength 700 nm).

**Temperature stability assay**

The enzyme activity was measured at different temperatures in phosphate buffer solution with pH 6.0. The relative enzyme activity was calculated by taking the enzyme activity without disposal as 100%.

**pH stability assay**

The enzyme solution was placed at room temperature in different pH buffers, held for different times, and the enzyme activity was measured. The relative enzyme activity was calculated by taking the original enzyme activity as 100%.

The system was 600 µL: 20 µL s-oph enzyme solution, 1 µL PNPA, and buffer make-up. Three parallel measurements were performed.

**Metal ions catalysis assay**

In the enzyme activity assay system, different concentrations of different metal ions and chelating agents (pre-matched concentration of 1 mol L\(^{-1}\), 1:1000 addition) were added to determine the enzyme activity. The original enzyme activity without the addition of new ions was used as 100% to calculate the respective relative enzyme activity. The system was 600 µL: 20 µL s-oph enzyme solution, 1 µL PNPA, 1 µL ion or chelator (extract pre-matched), and buffer make-up. Three parallel measurements.

**S-oph enzyme degradation characterization**

The reaction system: 100 µL PVADH enzyme solution, 4 mg PVA 1799, 0.2 mM DCIP, 1 mM CaCl\(_2\), 6 µM PQQ, and 50 mM pH 7.2 phosphate buffer were added to a total reaction volume of 1 mL. The PVADH
enzyme solution, PQQ, and CaCl$_2$ were warmed at 37°C for 10 min before the reaction [18]. The enzymatic reaction is carried out at room temperature.

Four group reactions were set up for the degradation experiments in such system with or without the s-oph enzyme, both in the presence or absence of the PVADH enzyme. The rate of the PVADH degradation in the absence of the s-oph enzyme was taken as 100%, and the change in the overall reaction rate with the addition of the s-oph enzyme was calculated.

**Results**

**Cloning and sequence analysis of s-oph gene from Sphingopyxis sp. M19**

The s-oph gene was cloned from the total DNA of *Sphingopyxis sp. M19* by PCR. The PCR product was recovered by agarose gel electrophoresis assay. The gel results were shown in Fig. 2(a) that the s-oph gene was successfully cloned. The DNA was sequenced by Sangon Biotech (Shanghai) Co., Ltd. and the sequence results were correct for the next step.

**Construction of E. coli pET32a/s-oph/BL21 (DE3) recombinant heterologous expression systems**

The agarose gel used for colony cloning PCR is shown in Fig. 2(b). The results show that the s-oph gene was successfully ligated to pET32a. Figure 2(c) shows the agarose gel used for *N*otI and *S*acI double digestion products. The gel shows that pET32a and s-oph gene were successfully ligated. The verified successful bacteria solution was sent to Bioengineering (Shanghai) Co., Ltd. for sequencing, and the sequencing was correct, which proved that the recombinant plasmid pET32a/s-oph was successfully constructed.

**Expression of the s-oph gene in E. coli**

The s-oph protein was concluded that the expression product of s-oph gene was about 39.4 kDa. However, SDS-PAGE showed that the expression of s-oph in pET32a/s-oph/BL21 (DE3) was approximately 57.6 kDa, which was due to the fusion of s-oph with trxA and S-tag within pET32a, this being the fusion protein trxA/s-oph. SDS-PAGE results showed an expressed protein of approximately 57.6 kDa size in pET32a/s-oph/BL21 (DE3), which is consistent with the size of the target protein. However, the expression of s-oph gene induced by temperature and IPTG concentration gradient was expressed in inclusion bodies (Supplementary Figure S1). The expression of eukaryotic proteins in *E. coli* usually results in the formation of insoluble material. The expression of soluble proteins in *E. coli* is always a challenge [19].

Protein misfolding and aggregation are the main causes of bacterial inclusion body formation, and solving the problem of proteolysis usually considers the addition of molecular chaperones to induce co-expression [20]. For example, Raziyeh Malekian et al used molecular chaperones to achieve soluble expression of GM-CSF protein in *E. coli* [21]; Safar Farajnia et al found that molecular chaperones have
significant advantages in improving soluble expression of Fab antibody in *E. coli* [22]; Shuaiying Peng et al studied found that co-expression of PFA with molecular chaperone proteins significantly increased the soluble expression of PFA [23]. So we continued to combine molecular chaperones to alter the soluble expression of s-oph proteins [24].

To the heterologously soluble expression of *s-oph*, the recombinant plasmid pET32a/s-oph and the molecular chaperone pTF16/pKJE7/pGro7 were cotransformed into BL21 (DE3) by sensory transformation. The pET32a/s-oph/BL21 (DE3)/pTF16, pET32a/s-oph/BL21 (DE3)/pKJE7, and pET32a/s-oph/BL21 (DE3)/pGro7 were screened for Amp$^+$ and Cm$^+$ double antibodies, three recombinant bacteria were obtained.

The *s-oph* gene of pET32a/s-oph/BL21 (DE3)/pTF16, pET32a/s-oph/BL21 (DE3)/pKJE7, pET32a/s-oph/BL21 (DE3)/pGro7 was expressed with IPTG in LB liquid medium containing 0.5 mg arabinose mL$^{-1}$, 100µg Amp$^+$ mL$^{-1}$ and 20µg Cm$^+$ mL$^{-1}$ resistance. The results were shown in Fig. 3, which achieved soluble expression of the target protein.

**Exposure of target protein His-tag**

After co-expression of the pET32a/s-oph recombinant plasmid with the molecular chaperone, the target protein could not hang on the column during purification. The results of Western Blot showed that the target protein had no His-tag exposure. The analysis showed that the upstream solubilizing tag trxA and S-tag of the target fragment wrapped His-tag when they acted with molecular chaperone to change the protein folding mode again. It could not be exposed by using 8 M urea and 6 M guanidine hydrochloride denaturants [25, 26]. Recombinant proteins are commonly used for heterologous expression enzyme production, but recombinant proteins may interfere with protein spatial structure because of tag exposure. So we also need to choose tags carefully when constructing recombinant protein expression systems. The His-tags are generally considered to have the least impact on the properties of fusion proteins because they do not tend to form ordered elements. And it is small enough to not significantly affect solubility or size [27]. Therefore, we still purified the target protein from the pathway of processing exposed His-tag. One 6 × His-tag and three 6 × His-tags were added downstream of the target fragment. And then the three molecular chaperones pTF16/pGro7/pKJE7 interacted with them, respectively, and were verified by Western Blot that three 6 × His-tags were over-polarized and wrapped again, and one 6 × His-tag was successfully exposed (Fig. 4(a)). Combined with the expression level and tag exposure (Fig. 4(b)), pET32a/s-oph/BL21 (DE3)/pGro7 was selected for bulk expression of the purified target protein.

**Purification of target protein**

The recombinant protein s-oph was purified using the principle of nickel column purification. The s-oph protein was collected from the gradient eluate. The optimal expression conditions (Fig. 5(a), (b)) were 25°C 220 rpm, 0.7 mmol IPTG L$^{-1}$ incubation for 16 h, and then the recombinant protein was purified by
crushing the sample preparation with a nickel column. Figure 5(c) shows the SDS-PAGE results of the purified products of recombinant proteins. The target band was about a single band of 57.6 kDa, and the trxA/s-oph protein purification result was pure and free of spurious bands.

The purified protein was dialyzed in a preservation buffer. The protein was concentrated by ultrafiltration as in Fig. 5(d). The s-oph gene was heterologously expressed and its soluble protein was purified at the yield of 16.8 mg L\(^{-1}\). The s-oph protein was stored at -80°C after cleavage of the trxA tag with rEK enterokinase and removal of impurities by filtration and sterilization.

**S-oph enzymatic properties**

The C-OD\(_{405}\) standard curve corresponding to the concentration and absorbance of p-nitrophenol (PNP) was measured (Supplementary Figure S2), and the amount of PNP produced in the degradation reaction was calculated to convert the s-oph enzyme activity. The trxA/s-oph enzyme activity was 566.94 U mg\(^{-1}\), and the s-oph enzyme activity was 852.71 U mg\(^{-1}\). According to the experimental data, the s-oph enzyme obtained by heterologous expression and purification has high activity.

**Temperature stability of s-oph enzyme**

At 25°C and 37°C, the enzyme activity did not decrease significantly, and the relative enzyme activity remained above 60% at 8 h. As can be seen from the Fig. 6(a), above 50°C is not suitable for the reaction. At 50°C, the relative enzyme activity of s-oph in 1h reaction decreased to less than 60%, while at 60°C and 70°C, the relative enzyme activity was only 50% after 1h reaction. This indicates that the optimal operating temperature of s-oph enzyme is 25–37°C, that is, the direct reaction at room temperature can exert the high activity of s-oph enzyme for PVA degradation reaction. This is consistent with the characteristic that s-oph enzyme is convenient to use. It can be reacted at room temperature without special temperature disposal, which also lays the operational basis for large-scale use of s-oph enzyme for PVA degradation.

**pH stability of s-oph enzyme**

The enzyme activity was measured in the buffer system pH 4.0–11.0. As the results can be seen from the figure that the acid environment was not suitable for the reaction, and the enzyme activity would not decrease significantly in the alkaline environment. In general, at the same reaction time, the relative enzymatic activity of s-oph was positively correlated with the pH of the reaction system. As can be seen from the Fig. 6(b), the lowest relative enzyme activity was 78.79% (reaction system pH 4.0) and the highest was 95.84% (reaction system pH 11.0) after one hour of reaction. After three hours of reaction, the relative enzyme activity decreased to 56.01% (reaction system pH 4), 61.88% (reaction system pH 6.0), 67.11% (reaction system pH 7.0), 76.50% (reaction system pH 9.0) and 84.58% (reaction system pH 11.0) in turn. In the subsequent reaction time, the decrease of relative enzyme activity was relatively gentle. When the reaction lasted for 8 hours, only the relative enzyme activity of s-oph in alkaline pH environment was more than 50%. The pH change of the degradation reaction system also affects the
activity of s-oph. According to the experimental results, the relative activity of s-oph in alkaline environment is higher. These indicate that the s-oph enzyme is adapted to an alkaline environment and an acidic environment is not suitable for the s-oph enzyme to maintain its enzymatic activity. So the alkaline soil environment can be preferred when putting the enzyme for biochemical degradation later.

**Tolerance of s-oph enzyme to metal ions**

Some conclusions can be obtained from the 20 min s-oph enzyme activity test from Fig. 7. Among them (Ions that may be included in practical applications), Mn$^{2+}$, NH$_4^+$, and EDTA have a positive catalytic reaction for the s-oph enzyme, Ni$^+$, Co$^{2+}$, and Na$^+$ have an obvious inhibitory effect on enzyme activity. Ni$^+$ and Co$^{2+}$ have a rapid decrease in relative enzyme activity at 2 mM, and enzyme activity completely lost at 16 mM; Li$^+$/Cu$^{2+}$ have relative enzyme activity below 60% at 16 mM, Cu$^{2+}$ concentration up to 120 mM, Na$^+$ and Cu$^{2+}$ at 50 mM, Ca$^{2+}$ at 90 mM and NH$_4^+$ at 160 mM, all of them have a positive catalytic effect, and the highest catalytic efficiency is at the concentration node of 20 mM. Combined with the natural environmental ion presence concentration, the s-oph enzyme reaction in the environment is almost always favorable to the positive state of the enzyme reaction.

Given the method of enzyme production, processing steps and the structure of the enzyme, the stability of the enzyme is difficult to control. If the stability is higher, it will be extremely beneficial to the production and use of the enzyme [28]. These tests of temperature, pH, and metal ions indicate that the s-oph enzyme reaction is highly efficient, convenient, and suitable for mass production use, with its simple operation and wide reaction conditions, that can be easily accomplished.

**S-oph enzyme degradation characteristics**

Figure 8(a) shows that the s-oph enzyme does not act significantly in the reaction under the conditions without PVADH enzyme pre-degradation, which indicates that the s-oph enzyme is specific in its action. In the oxidative PVA degradation reaction, the s-oph enzyme can greatly increase the reaction rate and achieve the complete degradation of PVA. Figure 8(b) shows that the reaction rate increases significantly after adding the s-oph enzyme to the PVA degradation system, and the increase rate reaches a maximum of 233.5%. The maximum rate reached at 35 min, and then leveled off after 65 min, that maintained at around 180%. The lowest improvement ratio in the whole system can reach 168.4%. This indicates that the s-oph enzyme can significantly improve the efficiency in the PVA degradation reaction. It can make the whole degradation process faster and better, in addition to play the key role of that degrade the large molecules to generate small molecules. Therefore, it is more suitable to put the s-oph enzyme in a naturally alkaline environment, it is more efficiently to reduce PVA pollution in soil and wastewater [29].

**Discussion**

Environmental protection is always the theme of the times. Plastic pollution has attracted hot discussion because of its difficulty in solving, high cost and wide spread area [30]. Under the current trend, many materials are replaced with biodegradable PVA [31].
degradation methods of PVA degradation on the environment shows that biodegradation is the most environmentally friendly degradation method with great research potential [32], which has aroused extensive attention to the modification of biodegradable plastics and the occurrence process of biodegradation [33, 34]. Microbial strains with the potential to degrade plastic materials can be further transformed into usable products, such as enzymes, which will be very conducive to ecosystem stability [35].

Firstly, Escherichia coli heterologous expressed proteins are characterized by short periodicity and high yield. Secondly, the specificity and efficiency of s-oph enzyme degradation play a key role in the complete degradation of PVA. S-oph enzyme degradation helps to reduce collateral damage to environmentally healthy microorganisms and enhances the efficiency of PVA degradation. Therefore, the s-oph enzyme in the second step of the PVA biodegradation reaction has great potential for industrial production and large-scale applications. However, there are several undesirable disadvantages related to the activity, expression, production and degradation characteristics of the PVA degrading enzymes found so far. For example, the SAO from different sources varies greatly in nature [36]; PVADH needs to bind the coenzyme PQQ to have catalytic activity [37].

Anyway, we've got the s-oph enzyme. Consistent with previous studies, exogenous genes expressed in large intestine are generally inclusion bodies, formed by insoluble substances. Adding molecular chaperones will change the folding mode of target proteins and increase protein solubility. The inconsistency is that the target protein becomes soluble and the tag is embedded and cannot be purified. The reason for the inconsistency is that after the folding mode of the target protein is changed, the pro-soluble tag and the molecular chaperone interact to produce structural changes that make His-tag wrapped and cannot be exposed. His-tag is wrapped and cannot be exposed, and neither treatment with denaturant can expose it. The problem that can be further studied is to add flexible arms when constructing recombinant plasmids [38] to change the solubility of the target protein, change the tag [39, 40] or change the arrangement of amino acid hydrophobic groups and the successful pairing of soluble and functional enzymes that can be screened by algorithms [41]. The study of the spatial structure of proteins is also important for their expression purification [42]. In this study, His-tag was added downstream of the target fragment, the existence of His-tag was confirmed by Western Blot, and the label exposure was enhanced by urea. The purification of s-oph gene soluble expression was successfully achieved.

The s-oph enzyme yield is high and the degradation effect is good, which is of great significance for large-scale industrial applications to alleviate environmental plastic pollution. However, to make the s-oph enzyme more beneficial, some later treatment of the enzyme is needed for better results, such as the immobilization of the enzyme. Immobilized enzymes will show significantly improved pH, thermal and storage stability compared to free enzymes. This will bring great convenience to enzyme application, such as thermal stability, which can exert the influence of temperature on enzyme catalysis to a greater extent [43]. Therefore, enzyme immobilization is often considered by biomaterials as a way to improve enzyme stability and reusability [44]. The combination of immobilized enzymes and heterologously
expressed enzyme-producing enzymes is expected to lead to significant advances in advanced biological enzyme applications in various fields [45].

**Declarations**

**CONFLICT OF INTEREST**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**AUTHOR CONTRIBUTIONS**

YZ conceived and designed the research. XW conducted the experiments. JL contributed to the analysis of data. XL prepared the materials. XW wrote the manuscript. YZ modified the manuscript. All the authors read and approved the submitted version.

**ETHICS DECLARATIONS**

This article does not contain any studies with human participants or animals performed by any of the authors.

**FUNDING**

The Joint Funds of the National Natural Science Foundation of China, Grant number: U1301231.

**SUPPLEMENTARY MATERIAL**

The supplementary materials for this article are submitted together.

**AVAILABILITY of DATA and MATERIALS**

The datasets supporting the conclusions of this article are included within the article and its additional files.

**References**


Figures

(a) Simulation diagram of s-oph enzyme analysis. (a) Signal peptide prediction diagram (b)/(c) The s-oph protein 3D structure (d) The s-oph protein pull-down diagram

Figure 1

Simulation diagram of s-oph enzyme analysis. (a) Signal peptide prediction diagram (b)/(c) The s-oph protein 3D structure (d) The s-oph protein pull-down diagram
Figure 2

Construction of recombinant plasmids. M: Marker. (a) Amplification of the target gene s-oph (b) Colony PCR identification results (c) Recombinant plasmid double digestion
Figure 3

Co-expression of recombinant plasmids combined with different molecular chaperones. M: Marker; S: The supernatant fractions of cells after sonication; P: The insoluble pellets of cells after sonication; 0: no molecular chaperone. pGro7/pKJE7/pTF16: BL21 series molecular chaperone; +: The cells were induced with IPTG; -: The cells were not induced with IPTG.
Figure 4

Species-specific binding and binding activity of proteins were detected by western blot. Species-specific assays were detected by western blot for s-oph enzyme and His-tag with different strains that bind different molecular chaperones. M: Marker. (a) a: pET32a/s-oph untreated inclusion bodies; 0: pET32a/s-oph without His-tag; 1: pET32a/s-oph with 1-6 × His-tag; 3: pET32a/s-oph with 3-6 × His-tags. (b) a: Buffer A; a + 6: Buffer A + 6 M guanidine hydrochloride; a + 8: Buffer A + 8 M urea; p: PBS; p + 6: PBS + 6 M guanidine hydrochloride; p + 8: PBS + 8 M urea.
To explore the optimal expression conditions and purify the target protein. M: Marker; P: The insoluble pellets of cells after sonication; S: The supernatant fractions of cells after sonication; LB/TB: Culture medium; a: flow-through solution; b: equilibration solution; (a) Protein expression under different temperature conditions (b) Protein expression in different media (c) 1-3: elution collection tubes; 10%-100%: elution gradient; (d) 1-2: fusion protein after ultrafiltration concentration.
Figure 6

Temperature and pH stability test of s-oph enzyme. (a) Temperature stability (b) pH stability (c) Stability of s-oph enzymatic degradation in different pH systems.
Figure 7

The catalytic effect of different ions on s-oph activity and ion tolerance test. (a) Effects of common ions on s-oph enzyme (b) Tolerance to trace ions (c) Tolerance to macroions
Figure 8

Activity of s-oph enzyme in degradation of PVA. (a) p: addition of PVADH enzyme; p(0): no PVADH enzyme; s: addition of s-oph enzyme; s(0): no s-oph enzyme. (b) Fold increase in reaction rate after addition of s-oph enzyme

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Graphicalabstract.tif
- SupplementaryMaterial20230126.pdf