Arsenic Removal from *Pinctada martensii* Enzymatic Hydrolysate by Using Zr(IV)-Loaded Chelating Resin

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**Abstract** The present study investigated the removal of inorganic arsenic from *Pinctada martensii* enzymatic hydrolysate through unmodified resin (D296) and Zr(IV)-loaded chelating resin (Zr-D401). By loading Zr to macroporous chelating resin D401, the as exchange adsorption active sites are generated. This transforms D401 from a material that does not have the arsenic adsorption capacity into a material that has excellent arsenic exchange adsorption capacity. The static adsorption experiments were conducted to investigate the optimal removal condition for D296 and Zr-D401. The experimental results show that: the optimum condition for D296 is that T=25 ℃, pH=5, resin additive amount = 1 g (50 mL)-1, and contact time = 10 h, the corresponding arsenic removal rate being 65.7%, and protein loss being 2.33%; the optimum condition for Zr-D401 is that T=25 ℃, pH=8, resin additive amount = 1 g (50 mL)-1, and contact time=10 h, the corresponding arsenic removal rate being 70.3%, and protein loss being 4.65%. These results show that both of the two resins are effective in arsenic removal for preserving useful substance. Our research provides scientific evidence and advances in the processing technology for heavy metal removal in shellfish.

**Key words** *Pinctada martensii*; enzymolysis; arsenic removal; chelating resin

1 Introduction

The pearl oyster *Pinctada martensii* is a typical kind of shellfish in the South China Sea. It is widely distributed near the coast of Guangdong and Guangxi provinces. Following the development of the pearl industry, *Pinctada martensii* is now cultured at very large scales. The *Pinctada martensii* oyster meat contains proteins, bioactive peptides, amino acids and taurine, and other minerals that are valuable sources for human nutrition (Zhang et al., 2000). However, due to the limitation of the processing technology, only a small part of oyster meat was utilized. The remainder of the meat has been discarded as aquatic product processing wastes, which is not only wasteful but also causes environmental pollution (Zheng et al., 2009; Zheng et al., 2012). As a processing technology for shellfish, protein enzymatic hydrolysis converts shellfish’s under-utilized by-products into acceptable forms without losing nutritional value, which obviously has high economic profit (Clemente, 2000). In addition, enzymatic hydrolysis can improve the functional properties of protein. These functional proteins then display various biological activity, such as antioxidant (Barkia et al., 2010), antihypertensive (Je et al., 2009), ACE inhibitory (Dai et al., 2012), antimicrobial (Liu et al., 2008), etc.

However, along with the industrial development, tons of wastewater are dumped into the ocean. These hazardous substances such as heavy metals are not subject to degradation processes but tend to accumulate in sediments. *Pinctada martensii* are filter feeders straining particulate food from the surrounding water. This special feeding mode of shellfish causes the accumulation of pollutants in the shellfish, which consequently causes the percentages of heavy metals and other toxic substances to exceed the safety levels (Bourgoin, 1990; Li et al., 2003; Katano et al., 2003). There have been several reports related to the technology for removing hazardous substance from shellfish. The most common method is shellfish depuration technique (Qiao, 2007). However, such methods are mainly focused on the removal of bacteria (Martinez et al., 2009), shellfish poison (Xie et al., 2013) and microorganism (Ho and Tam, 2000) from shellfish, and they do not work well with heavy metal and arsenic removal. Moreover, there are other techniques, such as the use of chitosan (Liu et al., 2010; Sun et al., 2010) and cation exchange resin, which are employed for the removal of heavy metals in enzymatic hydrolysate. But these studies are mainly focused on Cd and Lead ions...
purification, and there have not been many useful techniques that deal with arsenic removal (Yang et al., 2012). Nevertheless some methods used in the arsenic removal from industrial wastewater can provide guidance for our removal experiment (Mohan and Pittman, 2007; Biswas et al., 2008; Pan et al., 2007; Ratna et al., 2004).

In our experiment, we transform D401 from a material that does not have the arsenic adsorption capacity into a material that has arsenic exchange adsorption capacity by loading Zr to macroporous chelating resin D401. The relevant research indicates that Zr-loaded resin showed strong selectivity and retention of As (V) and As (III) (Suzuki, et al., 2000). In the present paper, we used unmodified resin (D296) and Zr (IV)-loaded chelating resin (Zr-D401) as purification material, and used Pinctada martensii enzymolysis hydrolyzate as samples to study the best technology and conditions to remove arsenic. Our research provides scientific evidence and advances in the processing technology for heavy metal removal in shellfish.

2 Materials and Methods

2.1 Material and Equipment

Pinctada martensii oyster meat was purchased from Nansha fish market, Guangzhou (China); macroporous strong-based anion exchange resin D296 and macroporous chelating resin D401 were from the chemical factory of Nankai University (China); trypsin and flavourzyme were from Guangxi Pang Bo Biological Technology, LLP (China). Zirconium reserving liquid (adding 48.34 g ZrOCl₂·8H₂O and certain amount of buffer solution of pH 6.5) was from Guangxi Pang Bo Biological Technology, LLP (China). The static adsorption experiments were conducted to investigate the removal behavior of arsenic by using macroporous strong-based anion exchange resin D296 and Zr (IV)-loaded chelating resin D401 (Zr-D401). The static adsorption experiments were conducted to investigate the removal behavior of arsenic by using macroporous strong-based anion exchange resin D296 and Zr (IV)-loaded chelating resin D401 (Zr-D401).

2.2 Experimental Method

2.2.1 Preparation of enzymatic hydrolysate

Dispersions of Pinctada martensii oyster meat (20% w/w) were prepared in deionized water. The hydrolysis process was carried out in a shaking water bath incubator. Trypsase (at 50℃ for 1 h) and flavor enzyme (at 50℃ for 3 h) were applied in turn to obtain the enzymatic hydrolysate. After hydrolysis process, the mixtures were heated at 100℃ for 5 min to inactivate the enzymes.

2.2.2 Resin pretreatment

After soaking in dehydrated alcohol for 24 h, the resin was soaked in 3% HCl and 3% NaOH solution, respectively, with stirring every half hour for a total of 6 h. Then resin was fully washed with deionized water until being neutral, followed by drying at 40℃ until constant weight.

2.2.3 Preparation of the Zr (IV)-loaded chelating resin D401 (Zr-D401)

Resin was modified according to the method proposed by Biswas (Biswas et al., 2008). In order to load zirconium, 40 g dried macroporous chelating resin D401 was equilibrated with 1 L zirconium reserving liquid at 30℃ for 24 h. The resin was then washed with deionized water until neutral pH, followed by drying at 40℃ until constant weight. The amount of Zr (IV) ion loaded onto the resin was calculated from the difference of the metal concentrations in the solution before and after loading.

2.2.4 Determination of total nitrogen loss rate

Micro-Kjeldahl method (Chang, 2010) was developed to determine total nitrogen (TN) content in enzymatic hydrolysate.

$$\text{Nitrogen loss rate} = \frac{\text{TNb} - \text{TNa}}{\text{TNb}} \times 100\%$$

where TNb is the content of total nitrogen before removal, and TNa is that after removal.

2.2.5 Determination of inorganic arsenic

The inorganic arsenic content in enzymatic hydrolysate was determined by hydride generation-atomic fluorescence spectrometry (Feng and Fu, 1998). Given the inorganic arsenic content in enzymatic hydrolysate before and after removal, the arsenic removal rate is calculated as follows:

$$\text{Arsenic removal rate} = \frac{C1 - C2}{C1} \times 100\%$$

where C1 and C2 are the contents of inorganic arsenic in enzymatic hydrolysate before and after removal, respectively.

3 Results and Discussion

3.1 Effect of pH on the Removal of Arsenic by D296 and Zr-D401 Resin

The static adsorption experiments were conducted to investigate the removal behavior of arsenic by using macroporous strong-based anion exchange resin D296 and Zr (IV)-loaded chelating resin D401 (Zr-D401).

Environment changes, especially pH changes, affect not only the dissociation of Zr and the exchange-adsorption behavior of inorganic arsenic on the anion exchange resin but also the speciation of arsenic, which is considered to be the most significant factor for the removal of arsenic.

With adding 1 g Zr-D401 or D296 resin per 50 mL hydrolyzate, keeping constant temperature 25℃ and stirring for 12 h, the arsenic adsorption efficiency under different pH conditions was observed as shown in Fig.1.
Though adding 1g Zr-D401 or D296 resin per 50 mL hydrolysate, keeping constant temperature 25°C for 12h, and adjusting pH to 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, the content of inorganic arsenic in enzymatic hydrolysate after removal was determined.

When other conditions remain unchanged, pH has great influence on arsenic removal effect. During the removal process, Macroporous strong-based anion exchange resin D296 has 2 maximum points: at pH 5 with arsenic removal rate up to 63.4%, and at pH 8 with arsenic removal rate up to 52.1%; for Zr-D401 enzymatic hydrolyzate, the arsenic removal rate is up to 67.7% at pH 8, and at this pH the arsenic removal rate of Zr-D401 is higher than any other pH condition. Similar results have been reported: optimum pH range was found between 4−6 by An et al. (2011) and between 4.5−6.5 by Pakzadeh and Batista (2011) by using the method of ion-exchange. Biswas et al. (2008) reported that arsenate was strongly adsorbed in the pH range from 2 to 6, while arsenite was strongly adsorbed between pH 9 and 10 by Zr (IV)-loaded orange waste gel through the removal of As (V) and As (III).

According to Vaclavikova et al. (2008), the distribution of As (III) and As (V) in the species is shown in Figs.2 and 3. These figures show that at pH values of 5 to 8, the neutral H₃AsO₃ species predominate in As (III), while at pH 8 the H₂AsO₄⁻ species predominate and at pH 5 the HAsO₄²⁻ species predominate in As (V). Since the electrostatic forces between negatively charged As (V) and resin anion [exchange capacity sites] are stronger than neutral As species, it can be interpreted that As (V) is more easily to be removed than As (III). Similar results have been reported in previous studies (Manna et al., 1999). Furthermore, we speculate that enzymatic hydrolyzate of As (V) content was higher than As (III) content due to enzymatic oxidation process. In the aerobic environment of 50°C under the constant temperature heating, according to the Eh-pH diagram for arsenic speciation in the neutral pH oxidizing environments, As (III) is likely to become As (V) due to oxidation (Smedley and Kinniburgh, 2002). Therefore, in enzymatic solution, the As (V) content was higher than the As (III) content. It also explains why inorganic arsenic was more easily to be removed at pH 5 to 8 in enzymatic solution.

3.2 Effect of Contact Time on the Arsenic Removal

Based on the study of the effect of pH on arsenic removal using D296 and Zr-D401, the optimal pH conditions were determined: pH 5 for D296, pH 8 for Zr-D401. At 25°C, by adding 1g Zr-D401 or D296 resin per 50mL hydrolyzate, the arsenic adsorption efficiency under different contact time conditions was observed as shown in Fig.4.

At 25°C, by adding 1g D296 or Zr-D401 resin per 50 mL hydrolyzate, keeping constant temperature at the optimum pH, and adjusting contact time to 0, 15, 30, 60, 120, 360, 600, 900 and 1440 min, respectively, the content of inorganic arsenic in enzymatic hydrolysate after removal can then be determined.

Because of the particularity of the enzymatic hydrolysate, when the contact time increases, microbial growth and organic matter degradation occur. In this case, organic arsenic is transformed into inorganic arsenic and inorganic arsenic content increases. The transformed inorganic arsenic is toxic. Therefore, the long contact time does not provide any benefit on arsenic removal in enzymatic hydrolyzate. Moreover, it will result in loss of

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**Fig.1** Effect of pH on the arsenic removal.

**Fig.2** Arsenite speciation as a function of pH for total As (III) concentration of 50 mg L⁻¹ (constructed by Mineql Plus).

**Fig.3** Arsenate speciation as a function of pH for total As (V) concentration of 50 mg L⁻¹ (constructed by Mineql Plus).
utility of the materials. The experimental results show that at 25°C and with resin additive amount = 1 g (50 mL)^{-1}, pH 5 for D296, pH 8 for Zr-D401, the optimum contact time was 10 h for both D296 and Zr-D401. The corresponding arsenic removal rates were 65.7% and 70.3%, respectively. Based on the comparison of the arsenic removal for the two resins, the removal rate of Zr-D401 increased rapidly within three hours. At contact time of 3 h, the maximum removal rate of 90% was reached. In addition, Zr-D401 had shorter adsorption equilibrium time and higher adsorption capacity.

3.3 Effect of Resin Quantity on Arsenic Removal

The content of inorganic arsenic removed in enzymatic hydrolysate was determined after adding 0.1, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5 and 3 g of Zr-D401 or D296 resin per 50 mL hydrolysate, respectively, and adjusting pH and contact time to the optimum conditions, with temperature oscillating around 25°C. The efficiency of arsenic adsorption under different resin quantities is shown in Fig.5. When the amount of resin is increased from 0.1 g to 1 g, the D296 and Zr-D401 arsenic removal rate is increased markedly from 15.5% to 63.2% and from 18.6% to 66.1%, respectively; whereas when the amount of resin is increased from 1 g to 3 g, the arsenic removal rate is only increased by 7.7% and 13.7%, respectively. These results indicate that 1 g of resin corresponds to a significant turning point. During the experiment, the limiting factors for arsenic removal changed from the amount of resin added and contact area (when the resin is less than 1 g) to the diffusion rate of ions and the existence of competitive ions (when the resin is increased to more than 1 g). Based on economic considerations, the use of 1 g of resin is considered as optimal.

Using the same amount of resin, Zr-D401 has a higher arsenic removal rate than D296 in enzymatic hydrolysate. This can be explained by the two following points. First, the particle size of Zr-D401 is smaller than D296 and has a larger specific surface area than D296 for the same volume. Therefore, Zr-D401 has a larger effective area for adsorption and ion exchange. Second, the active group of Zr-D401 is hydrolysis Zr ion which can be exchanged with OH^- and H_2O ligands. It also has an exchange capacity with anionic form of As and neutral H_3AsO_3.

3.4 Protein Loss Rate Under Optimal Removal Condition

The protein loss rate during the inorganic arsenic removal process was calculated to evaluate the effectiveness for preserving useful substance. Table 1 shows the optimum conditions for D296 and Zr-D401 resin to remove inorganic arsenic from enzymatic hydrolysate and the corresponding protein loss rates. Under the optimal removal condition, the protein loss rates are 2.33% and 4.65% for D296 and Zr-D401 resin, respectively. These results indicate that both of the two resins are effective in arsenic removal for preserving useful substance.

| Resin    | Additive amount | pH | Temperature | Contact time | Protein loss rate |
|----------|-----------------|----|-------------|--------------|------------------|
| D296     | 1 g (50 mL)^{-1}| 5  | 25°C        | 10 h         | 2.33%            |
| Zr-D401  | 1 g (50 mL)^{-1}| 8  | 25°C        | 10 h         | 4.65%            |

4 Conclusions

1) Our study shows that both D296 and Zr-D401 are effective materials for inorganic arsenic removal from enzymatic hydrolysate. By loading Zr to macroporous chelating resin D401, the As exchange adsorption active sites are generated. This transforms D401 from a material that does not have the arsenic adsorption capacity into a material that has excellent arsenic exchange adsorption capacity.

2) Within the scope of the experiment, the optimum conditions for D296 are 25°C, pH = 5, resin additive amount = 1 g (50 mL)^{-1}, and contact time = 10 h, the corre-
sponding arsenic removal rate being 65.7%, and protein loss being 2.33%; the optimum conditions for Zr-D401 are 25°C, pH = 8, resin additive amount = 1 g (50 mL)^{-1}, and contact time = 10 h, the corresponding arsenic removal rate being 70.3%, and protein loss being 4.65%.

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