Study on the absorption effect of hydrolyzed hoof horn powder on cadmium ions

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Abstract. Animal hoof horn has complex porous structure and rich keratin, crushed and sieved, adding 5% KOH in the quality of the hoof horn powder, and mixing at 1:5 ratio of solid to liquid, adding water at 121°C, Hydrolysis at 2 atmospheric pressure for 5 hours, the horn powder was completely hydrolyzed into the hydrolysis products containing sulfhydryl and disulfide bonds, the hydrolysates of the powder were added into 50mg•L⁻¹, 2.0mg•L⁻¹ cadmium Ion solution, centrifuged for 17h and then centrifuged to detect the residual cadmium ions. The results showed that the best particle size was treated with 100 mesh fine mesh powder, the lowest residual Cd²⁺ concentration in the solution was over 75%, and the high concentration 50mg•L⁻¹ was the best, the residual amount of Cd²⁺ was 14.887mg•L⁻¹, the removal rate was 70.24%. The adsorption effect of the hydrolyzate of Hoof horn powder on cadmium ion solution with concentration of 2.0 mg•L⁻¹. However, the effect was not significant. When the dosage reached 30ml, the residual amount of Cd²⁺ was reduced to 1.238mg•L⁻¹ and the removal rate was 38.10%.

1. Introduction
The situation of heavy metal pollution is aggravating day by day. At present, there are many treatment methods for wastewater containing cadmium. For example, the chemical precipitation method is simple in operation and operation, and the cost is low. However, the secondary pollution can be caused by improper treatment[1]-[3]; The membrane separation method has good selectivity, simple method, easy operation and good effect, and does not cause secondary pollution to the environment. However, the filtration membrane has high cost and is difficult to be recycled and reused[4][5]; the biological adsorption method has the advantages of low cost, High efficiency and low environmental pollution[6],[7]. However, it is very difficult to screen strains in activated sludge, and the cost is very high, so there are some limitations; the electrolysis method does not pollute the environment, and the effect is good, but the cost is high; Ferrite method can simultaneously treat a variety of heavy metal ions, the formation of the precipitate easy to separate, but the high cost, time-consuming and can not be recycled; Ion exchange resin method, high efficiency, low cost, easy recovery, simple operation and so on. Although there are many existing methods, it is particularly difficult to effectively reduce the concentration of heavy metal ions in effluent to the prescribed emission standards[8].
China is a big meat processing country, with abundant horn-keratin protein resources, but most are not fully utilized, and some even causing pollution. Animal hoofs crude protein content as high as 75%-80%, contains a large number of keratin, after hydrolysis under high temperature and pressure peptide breaks produce keratin peptides, disulfide bonds exposed, which can be combined with cadmium ions to produce precipitation complexes. The removal of cadmium ions in wastewater has a good effect, with the potential of adsorption of heavy metals in wastewater and research value, but also provides a "waste treatment" technical ideas and possible[3],[9][10].

2. Materials and methods

2.1. Experimental equipment and materials

2.1.1. Experimental equipment. WYS2200 atomic absorption spectrophotometer (WanYi, Anhui), autoclave YXQ-LS-50SII vertical pressure steam sterilization pot (Boxun, Shanghai), TDZ5-WS/TDZ5WS low-speed multi-Centrifuge (XiangYi, Hunan), ZH-DC type constant temperature shaker.

2.1.2. Experimental Materials. The adsorbents used in the experiment were taken from the abandoned animals' hooves in a nearby slaughterhouse and rich in phosphorus, calcium, various trace elements and other unknown factors, and the crude protein content was as high as 75%-80%. After pulverizing and grinding, a 20-mesh (0.850 mm diameter), 60 mesh (0.25 mm diameter), 100 mesh (0.150 mm diameter) nylon sieve was made into solid powder of different particle sizes and stored in a desiccator for further use.

2.1.3. Experiment required reagents. CdCl$_2$$\cdot$2.5H$_2$O, KOH and other reagents were of analytical grade, concentrated HNO$_3$ is a good grade pure reagent, the experiment used in wastewater containing cadmium CdCl$_2$$\cdot$2.5H$_2$O laboratory preparation.

2.2. Experimental methods

2.2.1. Hydrolysis keratin preparation. The solid-liquid ratio of 1:5 was used to add 100% KOH with a mass of 5% of the hoof horn powder. Hydrolysis was carried out at 121°C and two atmospheric pressures for 5 hours under high pressure and high temperature. The horn powder was completely hydrolyzed to a liquid state.

2.2.2. Adsorption particle size screening experiments. The animal hoof crushed, respectively, 20 mesh, 60 mesh, 100 mesh nylon mesh, each take 10g were added 100ml Cd$^{2+}$ concentration of 1mg•L$^{-1}$, 2mg•L$^{-1}$, 3mg•L$^{-1}$, 4mg•L$^{-1}$, 5mg•L$^{-1}$, 6mg•L$^{-1}$, 7mg•L$^{-1}$, 8mg•L$^{-1}$, 9mg•L$^{-1}$ and 10mg•L$^{-1}$ were placed in a shaker for 17 hours and then centrifuged Filtration, the residual concentration of Cd$^{2+}$ in the supernatant was measured by atomic absorption spectrometry, and the optimal particle size of Cd$^{2+}$ solution was determined by comparing the residue of Cd$^{2+}$.

2.2.3. Equilibrium adsorption experiment. The animal hoof hydrolyzate were taken 5ml, 10ml, 20ml in a 50ml volumetric flask to 50ml, and then the diluted solution was added to 50ml concentration of high concentration of 100mg•L$^{-1}$ Cd$^{2+}$ solution samples At this point, the concentration of Cd$^{2+}$ in the solution was 50 mg•L$^{-1}$. The sample was shaken and adsorbed on a thermostatic shaker at 25°C for 17h with a shaking rate of 180 r•min$^{-1}$. After the adsorption was completed, the sample was centrifuged (4000r•min$^{-1}$, 5min) and filtered. The supernatant was added with concentrated nitric acid Decontamination to remove the organic matter, the supernatant to be clarified and the color lighter, the atomic absorption spectrometry measured Cd$^{2+}$ residue. Each sample to do three parallel samples, so as the blank control groups.

According to the above method, the animal hoof hydrolyzate were taken 5ml, 10ml, 20ml, 30ml in 50ml volumetric flask to 50ml, and then the diluted solution was added to 50ml concentration of high
concentration of 4.0 mg•L⁻¹ Cd²⁺ solution, the concentration of Cd²⁺ in the solution was 2.0 mg•L⁻¹. The sample was shaken and adsorbed on a thermostatic shaker at 25°C for 17h with a shaking rate of 180r•min⁻¹. After the adsorption was completed, the sample was centrifuged (4000r•min⁻¹, 5min) and filtered. The supernatant was added with concentrated nitric acid Decontamination to remove the organic matter, until the supernatant clear and lighter color, atomic absorption spectrophotometry Cd²⁺ residual concentration. Each sample to do three parallel samples, and a blank control.

3. Results and analysis

3.1. Hoof horn powder on the adsorption of Cd²⁺ effect

Shown in Figure 1, trends of 20 mesh, 60 mesh, 100 mesh animal hoof horn powder adsorption of different Cd²⁺ solutions. The removal of Cd²⁺ was significantly better than that of blank control group(CK). The residue of cadmium ions in the Cd²⁺ solution with the diameter of 100 mesh was the smallest, the adsorption rate was above 75% and the particle size For the 60 mesh hoof horn powder adsorption rate of 50% or more, and 20 mesh fineness of the hoof horn powder adsorption rate of about 30%, indicating that the smaller the diameter of the hoof horn powder, the better the adsorption effect. As can be seen from the trend of the three curves in the figure, as the solution concentration increases, the adsorption capacity of the hoof horn powder also increases, but the adsorption capacity of the powder balance adsorption does not reach that of the hoof horn powder, indicating that the porous structure has a good adsorption of Cd²⁺. Overall, 100 mesh animal hoof horn powder had the lowest concentration of Cd²⁺ with the best Cd²⁺ adsorption capacity, with an average adsorption rate of 78.47% and an adsorption capacity of 0.0985 mg•g⁻¹.

![Figure 1. Adsorption of Different Kinds of Powder with Different Size Containing Cd²⁺ in Solution](image)

3.2. Hoof horn powder hydrolyzate adsorption of high concentrations of Cd²⁺

As shown in Table 1. and Figure 2., as the amount of hydrolyzate added to the hoof horn powder increased from 5ml to 10ml and 20ml gradually, the apparent adsorption of Cd²⁺ solution with high concentration of 100mg•L⁻¹ And the residual Cd²⁺ concentration decreased with the increase of hydrolysates. When the dosage reached 20ml, the sample was shaken and adsorbed on a 25°C temperature-controlled shaker for 17h with a shaking rate of 180r • min⁻¹, After centrifugation (4000r • min⁻¹, 5min), the Cd²⁺ concentration was reduced from 50mg•L⁻¹ to 14.887mg•L⁻¹ by centrifugation (4000r•min⁻¹ and 5min), the removal rate was 70.24%, which was 2.35 times of the blank sample And 10ml dosage of the removal rate slightly higher. From the overall removal rate curve, we can see that the amount of Cd²⁺ adsorbed
and bound by hydrolysates gradually increases with the increase of the dosage of hydrolysates of the hoof horn powder, and the adsorption-saturation trend gradually appears.

**Table 1. Hoof horn powder hydrolyzate adsorption of high concentrations of Cd\(^{2+}\).**

| Hydrolyzate Dosage/ml | Cd\(^{2+}\) remaining Mean concentration /mg•L\(^{-1}\) | N | Standard deviation/S | Removal rate/% |
|-----------------------|------------------------------------------------------|---|----------------------|----------------|
| CK                    | 35.042                                               | 3 | 0.287                | 29.92          |
| 5                     | 17.084                                               | 3 | 0.767                | 65.84          |
| 10                    | 15.155                                               | 3 | 0.725                | 69.69          |
| 20                    | 14.887                                               | 3 | 0.455                | 70.24          |

**Figure 2. Adsorption of Cd\(^{2+}\) by high-concentration of hydrolysate**

3.3. *Hoof horn powder hydrolyzate adsorption of low concentrations of Cd\(^{2+}\)*

As shown in Table 2 and Figure 3, as the amount of hydrolysate added to the hoof horn of animals increased from 5ml to 10ml, 20ml and 30ml from 0ml, there was a certain value for Cd\(^{2+}\) solution with a low concentration of 4mg•L\(^{-1}\). However, the residual Cd\(^{2+}\) concentration decreased with the increase of hydrolysate was not obvious, even fluctuated. When the dosage was up to 30ml, the sample was shaken for 17h on a 25°C temperature-controlled oscillator, The concentration of Cd\(^{2+}\) was reduced from 4mg•L\(^{-1}\) to 1.238mg•L\(^{-1}\) after removal of the filtrate by centrifugation (4000r•min\(^{-1}\), 5min) 38.10%, slightly higher than that of the blank sample and other dosing samples, and the concentration of Cd\(^{2+}\) in the solution decreased obviously. However, the trend of Cd\(^{2+}\) residual concentration with different dosing amount was not obvious. According to the overall removal rate curve, with the increase of hydrolysate dosage, the amount of Cd\(^{2+}\) adsorbed and bound by hydrolysates also gradually increased, and the residual concentration gradually approached the lower limit of adsorption, gradually showing the trend of adsorption saturation.

**Table 2. Hoof horn powder hydrolyzate adsorption of low concentrations of Cd\(^{2+}\)**

| Hydrolyzate Dosage/ml | Cd\(^{2+}\) remaining Mean concentration /mg•L\(^{-1}\) | N         | Standard deviation/S | Removal rate/% |
|-----------------------|------------------------------------------------------|-----------|----------------------|----------------|
| CK                    | 1.395                                                | 3         | 0.0372               | 30.25          |
| 5                     | 1.247                                                | 3         | 0.0579               | 37.65          |
| 10                    | 1.293                                                | 3         | 0.0408               | 35.35          |
| 20                    | 1.257                                                | 3         | 0.0246               | 37.15          |
| 30                    | 1.238                                                | 3         | 0.0686               | 38.10          |
4. Discussion

Animal hoof is rich in phosphorus, calcium and trace elements and other unknown factors, rich in keratin, contents of crude protein up to 75%-80%, keratin is a special resistance to hard protein, which Its structure is extremely complicated by intermolecular disulfide bonds, hydrogen bonds, salt bonds and other cross-linking interactions. Its disulfide bond is most critical during hydrolysis. After keratin hydrolysis Can produce peptides, oligopeptides and free amino acids[11], on the free Cd\(^{2+}\) also has strong adsorption complex properties, its protein thiol, carboxyl has strong ion binding capacity, especially the mercapto binding Extremely strong, can be combined with very low concentrations of inorganic ions[12].

In this study, the adsorption of heavy metal Cd\(^{2+}\) was studied based on this principle. The results showed that the adsorption effect of the powder was very obvious. After the animal's hoof crushed, the protein shrank and became porous fine particles, which increased the specific surface area. The adsorption also plays a supporting role, but not a major one, since the prepared blood meal is 60 mesh and belongs to relatively large particles. The particle size of 100 mesh foot powder after treatment of cadmium ion solution in the smallest amount of cadmium ions, the adsorption rate of 75% or more, the particle size of 60 mesh horn of the powder is more than 50% adsorption rate, and 20 mesh fine. The degree of hoof horn powder adsorption rate of about 30%, indicating that the smaller the diameter of the hoof horn powder, the better the adsorption effect. As can be seen from the trend of the three curves in the figure, as the solution concentration increases, the adsorption capacity of the hoof horn powder also increases, but the adsorption capacity of the powder balance adsorption does not reach that of the horn powder, indicating that the porous structure has a good adsorption of Cd\(^{2+}\) Effect, the adsorption equilibrium point still need to continue to explore.

Hoof horn powder hydrolyzate contains a large number of peptides, oligopeptides, etc., to the solution of Cd\(^{2+}\) adsorption and complexation, especially for high concentrations of cadmium ions adsorption effect. As the amount of animal hydrolysates added from 0ml blank, gradually increased from 5ml to 10ml, 20ml, the adsorption and removal of Cd\(^{2+}\) solution with high concentration of 100mg·L\(^{-1}\) showed obvious adsorption and removal efficiency, and the residual concentration of Cd\(^{2+}\) The amount of hydrolyzate increased and decreased, when the dosage reached 20ml, the sample was shaken on a 25\(^{\circ}\) temperature-controlled shaker for 17h with a shaking speed of 180r·min\(^{-1}\), after which the sample was centrifuged (4000 r·min\(^{-1}\), 1.5min), the Cd\(^{2+}\) concentration was reduced from 50mg·L\(^{-1}\) to 14.887mg·L\(^{-1}\), and the removal rate was 70.24%, which was 2.35 times higher than that of the blank sample. Compared with the same 5ml and 10ml dosage, Slightly higher As can be seen from the overall
removal rate curve, as the dosage of hydrolyzate of the hoof horn powder increases, the amount of Cd\(^{2+}\) adsorbed and bound by the hydrolyzate also gradually increases, indicating that increasing the amount of the hydrolyzate dosing effectively increases the adsorption capacity of the Cd\(^{2+}\). Increased the effect of adsorption and removal, but the unit dosage increases the adsorption efficiency does not help\[13\].

When the dosage of 20ml was reached, the concentration of Cd\(^{2+}\) in the sample decreased from 4mg•L\(^{-1}\) to 1.257mg•L\(^{-1}\) and the removal rate was 37.15% The removal rate of Cd\(^{2+}\) was 70.24% when the concentration of Cd\(^{2+}\) was high. When the dosage of Cd\(^{2+}\) was 30ml, the concentration of Cd\(^{2+}\) decreased from 4mg•L\(^{-1}\) to 1.238 mg•L\(^{-1}\) and the removal rate was 38.10% The dosage of sample increased slightly, and the concentration of Cd\(^{2+}\) in solution decreased obviously. However, the trend of Cd\(^{2+}\) concentration with different dosage was not obvious, and the residual concentration gradually approached the lower limit of concentration.

5. Conclusion

The adsorption effect of the horn powder is very obvious. After crushing the animal's hooves, the protein shrinks and becomes porous fine particles, which increases the specific surface area and plays an auxiliary role in the adsorption of cadmium ions, but it is not the main effect. The smaller the diameter of the hoof horn powder is, the better the adsorption effect is. The 20 mesh fineness and the 60 mesh hoof horn powder have the adsorption rates of Cd\(^{2+}\) of 30% and 50% respectively, while the particle size of Hoof horn powder of 100 mesh The content of cadmium ions in the cadmium ion solution is the smallest, and the adsorption rate is above 75%, but it does not reach the adsorption capacity of the equilibrium adsorption of the horn powder, indicating that the porous structure has a good effect of adsorbing Cd\(^{2+}\).

Animal hoof horn powder hydrolyzate had obvious adsorption and removal effects on Cd\(^{2+}\) solution with high concentration of 100 mg•L\(^{-1}\), and the residual concentration of Cd\(^{2+}\) decreased with the increase of hydrolyzate. When the dosage reached 20ml, After shaking for 17h at a temperature of 25°C on a thermostatic shaker, the shaking speed was 180r•min\(^{-1}\). After centrifugation (4000r•min\(^{-1}\), 5min) after shaking adsorption, the concentration of Cd\(^{2+}\) down to 14.887 mg•L\(^{-1}\), removal rate of 70.24%. From the overall removal rate curve, we can see that the amount of Cd\(^{2+}\) adsorbed and bound by hydrolysates gradually increases with the increase of the dosage of hydrolysates of the hoof horn powder, and the adsorption-saturation trend gradually appears.

Addition of hydrolysates from the hoof horn had certain adsorption and removal effects on the Cd\(^{2+}\) solution with a low concentration of 4mg•L\(^{-1}\), but the residual Cd\(^{2+}\) concentration decreased with the increase of hydrolyzate. When the dosage reached 30ml, the sample was shaken and adsorbed on a 25°C temperature-controlled oscillator for 17h with a shaking speed of 180r•min\(^{-1}\). The sample was centrifuged (4000r•min\(^{-1}\), 5min) After filtration and digestion, the concentration of Cd\(^{2+}\) was reduced from 4mg•L\(^{-1}\) to 1.238mg•L\(^{-1}\), and the removal rate was 38.10%, which was only slightly higher than that of the blank samples and other dosing samples. However, the Cd\(^{2+}\) concentration in the solution decreased significantly, The amount of Cd\(^{2+}\) adsorbed by the hydrolyzate also gradually increased, and the residual concentration gradually approached the lower limit of adsorption.

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