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Natural Melanin as a Potential Biomaterial for Elimination of Heavy Metals and Bacteria from Aqueous Solution

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Abstract: Development of materials for treatment of heavy metals and bacteria in aqueous solution is still on the way. Although there are many materials developed, there is limit material which could be practical applied to eliminate a wide range of heavy metal ions and bacteria in drinking water. In this study, we investigated the adsorption capacity of melanin extracted from the ink sacs of squids toward heavy metals (chromium and manganese), which normally presented at high concentrations in water sources originated from mining, metal plating and steel making industries, as well as bacteria in aqueous solution. Our results showed that melanin could remove Cr^{6+} and Mn^{2+} effectively (> 97%) with the adsorption capacities to be 5.78 mg/gam and 31.8 mg/gam for Cr^{6+} and Mn^{2+}, respectively. More interestingly, it indicated that melanin could not only eliminate heavy metals but also effectively removed *vibrio parahaemolyticus* bacteria with efficiency more than 90%. The obtained results suggested that melanin, a natural material with high level of biosafety, might be a good adsorbent for removal of heavy metal ions and bacteria in aqueous solution and could be used for advantage treatment of drinking water.

Keywords: Melanin, biomaterial, water treatment, heavy metal, bacteria.

1. Introduction

In Vietnam, heavy metal contaminations in groundwater are very common in several places in the Red and Mekong river deltas [1, 2]. Heavy metal ions removal is a matter of concern because groundwater is the main water source for drinking water in Vietnam, especially in the rural areas. In almost all households in rural areas, groundwater has being treated by sand filtration. However, according to many studies, the levels of heavy metal ions such as chromium and manganese after sand filtration are still higher than the standard values of those in drinking water guided by WHO and/or QCVN 01:2009/BYT of Vietnam [3]. Therefore, finding and development of new materials with high efficiency and economic for treatment of both heavy metals and bacteria in drinking water are necessary. Melanins are polyphenolic amorphous polymers and commonly to be found in animals, plants, bacteria, and fungi [4, 5]. Melanins have many biological functions via their abilities in providing defense against environmental stresses such as ultraviolet light, oxidizing agents and ionizing radiation [6-8]. Previous reports also suggested that melanin-based coating agents could be used for binding

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of lead [9]. Moreover, melanin was also synthesized as nanoparticles and applied as excellent agent for binding to heavy metal ions [10-11], however, there was very few study focusing on investigation the role of melanin as material used for removing of heavy metal ions as well as bacteria from aqueous environment. Therefore, in this study we extracted melanin from ink sacs of the squids and examined the adsorption capacity of melanin toward not only heavy metal ions including chromium and manganese but also bacteria.

2. Materials and methods

2.1. Melanin extraction

Ink sacs of squids were collected from Seafood Company kept in ice and transferred to the laboratory for melanin extraction. Extraction protocol was described in the previous report [12, 13]. Briefly, ink solution from ink sac was extracted and purified in an acid medium. Fifty grams (50 g) of ink solution were added 100 ml of HCl 0.1M. The slurry was ultrasonicated for 15 minutes and stirred for 30 minutes at 30^{o}C and then kept in water bath at 10^{o}C for 24 hours. Next, the solid phase was separated from the supernatant fluid by centrifugation at 10.000 rpm at 5^{o}C for 15 minutes. Pellet was alternatively washed with 0.1M HCl solution, de-ion water, acetone, and de-ion water with three times for each step. Following a 24 hr lyophilization to remove all solvent, melanin pellets were obtained and kept at 4^{o}C. Before using as an adsorbent, melanin pellets were grinded into particles with sizes in the range of < 63 µm by sieving.

2.2. Heavy metal ion adsorption experiment

The solutions $K_2Cr_2O_7$ 0,2mg/ml and $MnSO_4$ 0,2mg/ml were prepared for experiments. Batch experiments were carried out in the glass conical flasks (50 mL) with 20 mL of the heavy metal ion solution. Except for the experiment to investigation of the effect of initial concentration of ion, C_o of 5 mg/L was kept constant in all experiments. Melanin with a solid to liquid ratio of 0.5% was applied in all experiments except for the experiment to determine the effect of solid to liquid ratio. Initial pH of 4.0 was kept constant for all experiment except for the experiment to determine the effect of initial pH. This pH of 4.0 was the best choice for heavy metals removal experiments because of the similarity to the pH value in the practical conditions. Moreover, at the alkali pH values, heavy metals could be precipitated as hydroxide forms; and at low pH values, it was not suitable for real applications. The mixture was then strongly mixed using a shaker (Jeiotech BS-31, Korea) at speed of 150 rpm at the temperature of 25 ± 2^{o}C that was reasonable. The supernatant was thereafter filtered through a 0.45 µm filter membrane and used to analyze for ion concentration. The Mn^{2+} ion concentrations were estimated by catalytic oxidization of Mn^{2+} to Mn^{7+} in H_2SO_4 solution using $K_2S_2O_8$ as oxidizing agent and $AgNO_3$ as a catalyst. Mn^{7+} was then analyzed by spectrophotometer (UV-VIS 1201) at λ = 520 nm. The pH of solution was adjusted by HNO_3 or NaOH solution. The Cr^{6+} ion concentrations were determined by spectrophotometric method. Briefly, in pH medium of 1-2 with presence of H_3PO_4 acid, Cr^{+6} reacts with 1,5-diphenylcarbazide to form a colored-complex of Cr^{6+}-1,5-diphenylcarbazide and to be measured at 540 nm. The removal efficiency was calculated using equation (1).

$$\text{Removal efficiency (\%)} = \frac{(C_o - C_t)}{C_o} \times 100 \quad (1)$$

Where, C_o and C_t are the concentrations of ion at initial and time t, respectively.

2.3. Bacterial adsorption experiment

Firstly, *vibrio parahaemolyticus* bacteria was activated and cultured in liquid LB medium. Concentrations of bacteria were checked by measuring the optical density (OD) at the wavelength 620 nm. At the time of the OD of the bacteria medium reached to 0.3, melanin was added and mixed by using a shaker (Jeiotech BS-31) at speed of 150 rpm and at the temperature of 25 ± 2^{o}C for certain times. Then, bacteria concentrations were determined by measuring the OD values and also by culturing in the solid LB dish for 24 hrs to count the number of colonies formed on the LB dish.

2.4. Statistical analysis method

Statistical analysis in this study was performed according to the method previously described [14]. Results from three independent experiments in each group were statistically analyzed by Student's t-test. The SPSS (version 18) software package (SPSS Japan Inc.) was used for these statistical analyses, and the significance level was set at $p < 0.05$.

3. Results and discussion

3.1. Chromium ion (Cr^{6+}) adsorption efficiency of melanin

We firstly examined the adsorption efficiency of Cr^{6+} by melanin with dose dependence. Activated carbon was also used as a control. Initial concentration of Cr^{6+} was 2 mg/ml and tested concontrations of melanin and activated carbon were 0, 1, 2, 4, 10, 15 and 20 mg/ml. Adsorption experiments were set at 25^{o}C, pH:4 and 1 hour of adsorption time.

The standard curve and results were showed in figure 1. In general, adsorption capacity of melanin was much higher than that of activated carbon. Adsorption capacity depended on the concentration of the adsorbent. The higher concentration of the adsorbent the bigger amout of Cr^{6+} to be removed.

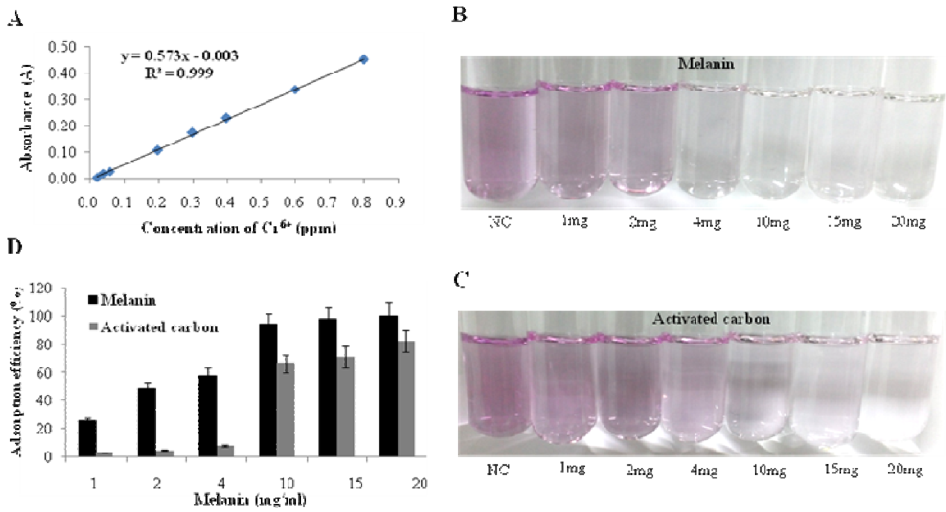

Figure 1. Removal of chromium ion by melanin. (A) Standard curve for chromium analysis; Removal of chromium by melanin (B) and activated carbon (C) with dose dependence; (D) Adsorption efficiency of chromium by melanin and activated carbon.

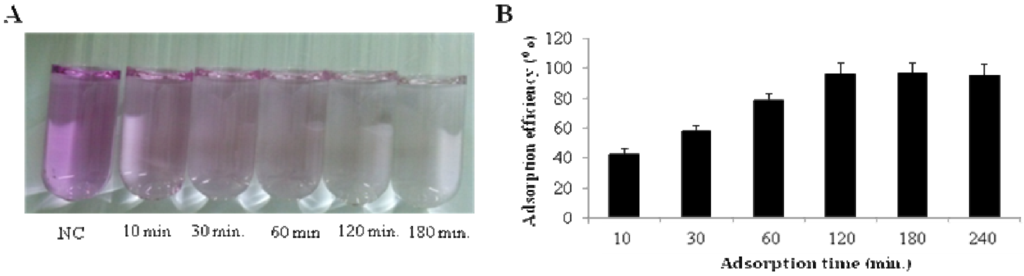

Figure 2. Chromium adsorption efficiency by melanin with time dependence was showed in a picture (A) and in a graph (B).

At the concentration of 4 mg/ml, melanin could adsorb about 60% amount of Cr^{6+} in the aqueous solution and at the concentration of 10, 15 and 20 mg/ml, melanin could remove Cr^{6+} with adsorption efficiency more than 96, 97, 98%, respectively. While, at the same conditions, activated carbon at concentration of 4 and 10, 15 and 20 mg/ml had adsorption efficiencies to be 8 and 62, 67, 78%, respectively. We then investigated the adsorption efficiency of Cr^{6+} by melanin with time-dependence (figure 2).

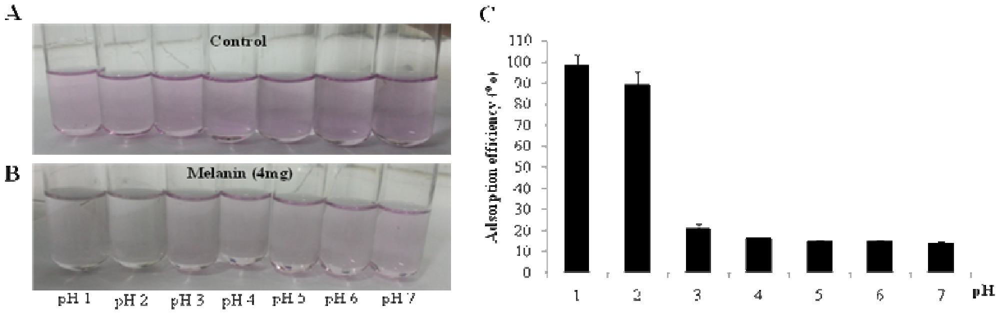

Figure 3. Effect of pH on chromium adsorption efficiency by melanin was showed in pictures (A and B) and in a graph (C).

Experiments were perfomed under conditions of temperature 25^{o}C; pH: 4; with initial concentration of melanin was 4 mg/ml and initial concentration of Cr^{6+} was 2 mg/ml. Adsorption times were set for 10, 30, 60, 120, 180 ans 240 minutes. The obtained results indicated that adsorption efficiency of melanin was time-dependence and reached to saturated adsorption capacity at the time of 120 minutes with removal efficiency more than 97%. After that, we conducted experiments to examine the effect of pH on adsorption capacity of melanin (figure 3). Tested pH were set in the range from 1.0 to 7.0. Initial concentrations of melanin and Cr^{6+} were 4 mg/ml and 2 mg/ml, respectively. Experiments were carried out at the temperature of 25^{o}C for 10 minutes. It was found that the lower pH of the solution the stronger adsorption ability of the melanin. After 10 minutes, melanin could remove 98%, 91% and about 20% amount of Cr^{6+} out of solution at pH 1, pH 2 and other pH values (pH:3-7), respectively.

3.2. Manganese ion (Mn^{2+}) adsorption efficiency of melanin

We next investigated the adsorption efficiency of melanin toward Mn^{2+} in aqueous solution. Similarly as the case of chromium

(Cr^{6+}), we first established standard curve and then conducted experiments to examine the effect of melanin with dose dependence on Mn^{2+} removal efficiency (figure 4). The tested concentrations of melanin and activated carbon (as a control) were set of 1, 4, 10, 20, and 30 mg/ml. Experiments were carried out under conditions of temperature: 25^{o}C, pH: 4, adsorption time: 1 hour. The obtained results showed that melanin was much more better than activated carbon in removal of Mn^{2+} out of soulution (figure 4). Although adsorption efficiency of Mn^{2+} by melanin also depended on the using dose of melanin, the removal efficiency of Mn^{2+} was very high even at the very low concentrations of melanin. Particularly, at the concenttration of 1 mg/ml, melanin could remove about 50% amount of Mn^{2+} in the solution. And the saturated adsorption efficiency was reached at the concentration of 20 mg/ml with removal capacity of 93%.

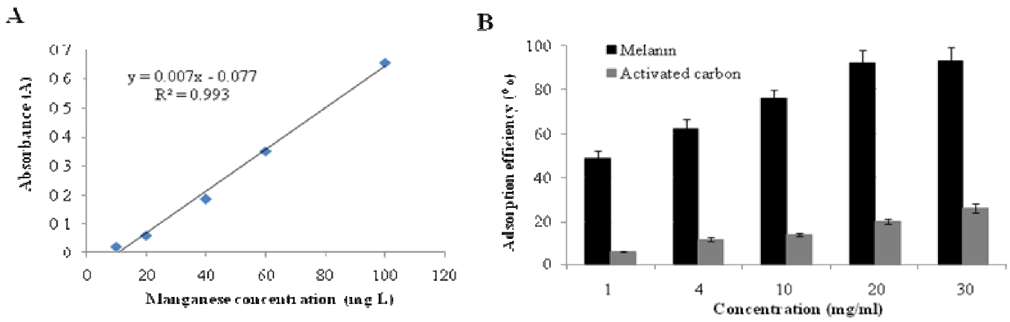

Figure 4. Removal of manganese ion by melanin. Standard curve for manganese analysis (A); Efficiency of manganese removals by melanin with dose (activated carbon was used as a control) (B).

The effects of adsorption time and pH on Mn^{2+} adsortion efficiency were also examined and the results were showed in the figure 5. The time-dependent experiments were carried out at temperature: 25^{o}C, pH:4, initial concentration of melanin and Mn^{2+} were 4 mg/ml and 2 mg/ml, respectively. The obtained results showed that adsorption efficiency reached to the saturation after 60 minutes with removal capacity of 86% amount of Mn^{2+}.

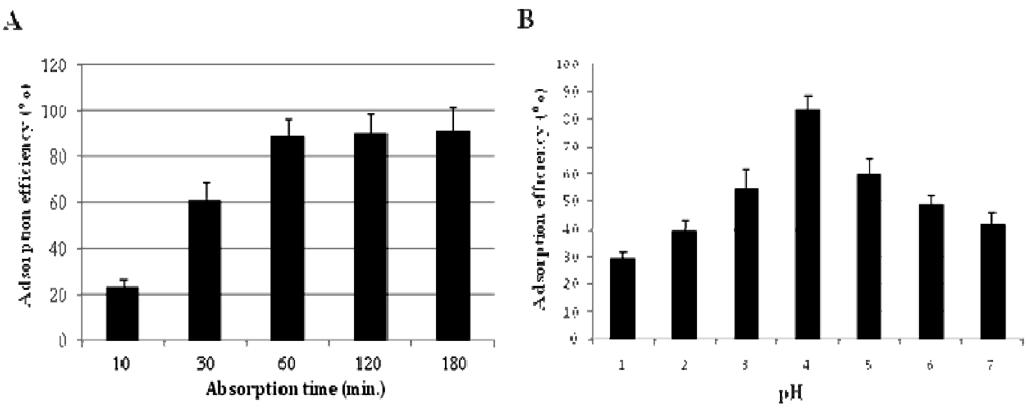

Figure 5. Removal of manganese ion by melanin. Efficiency of manganese removals by melanin with time (A) and pH (B) dependences.

We also investigated the effect of pH on Mn^{2+} adsorption efficiency by melanin. Experiments were conducted with conditions of temperature: 25°C, adsorption time: 60 minutes, initial concentration of Mn^{2+} : 2 mg/l and concentration of melanin: 4 mg/ml. The results showed in the figure 5 indicated that the pH:4 was the best for the Mn^{2+} removal with adsorption efficiency reached to 87%.

3.3. Efficiency of bacterial adsorption by melanin

Vibrio parahaemolyticus bacteria usually presents in water as harmful agent and causes death of fishes and shrimps. In this study, *vibrio parahaemolyticus* bacteria was batch cultured in the LB medium until the optical density (OD) reached to 0.1, 0.2, 0.4, 0.6, and 0.8. After that, cultured solutions were shaked with melanin at concentration of 1.0 mg/ml for 1 hour and let stand for 5 minutes before the optical density of the supernatants were measured by spectrophotometer at 620 nm (figure 6-A), and the pigment pellets were analysed by Image J software (figure 6-B, C). The obtained results showed melanin with 1.0 mg/ml could reduce the initial OD of 0.1, 0.2, 0.4, 0.6 and 0.8 bacterial solutions to 0.015, 0.02, 0.049, 0.16 and 0.35, respectively (figure 6-A).

After standing for 5 minutes, although the initial melanin were added with the same amount (1.0 mg/ml) for all tubes, we observed the gradually increased volume of pigment pellets in the bottom of the tubes.This fact was because of the adsorption of bacteria on the melanin and led to increasing the volume of the pellets. The pigment pellets were taken as photos (figure 6-B) and measured by Image J software (figure 6-C). The obtained results suggested that melanin was able to adsorb bateria in the solution.

We then investigated adsorption efficiency of bacteria by melanin with dose dependence. *Vibrio parahaemolyticus* bacteria cultured until the OD reached to 0.4, 5ml bacteria medium were treated with melanin at various concentrations of 0, 5, 10, 15, 20 and 25 mg/5ml (i.e. 1, 2, 3, 4, 5 mg/ml) for 1 hour before culturing in the solid LB dishes and kept in the incubator at 30°C for 24 hours. After that, colonies were counted and presented in the figure 7. The results showed that treatment with melanin at concentrations of 1, 2, 3, 4, 5 mg/ml decreased 2.3, 2.8, 5.4, 5.7 and 5.1 folds of the number of colonies formed in the dishes compared with that of control dish, respectively.

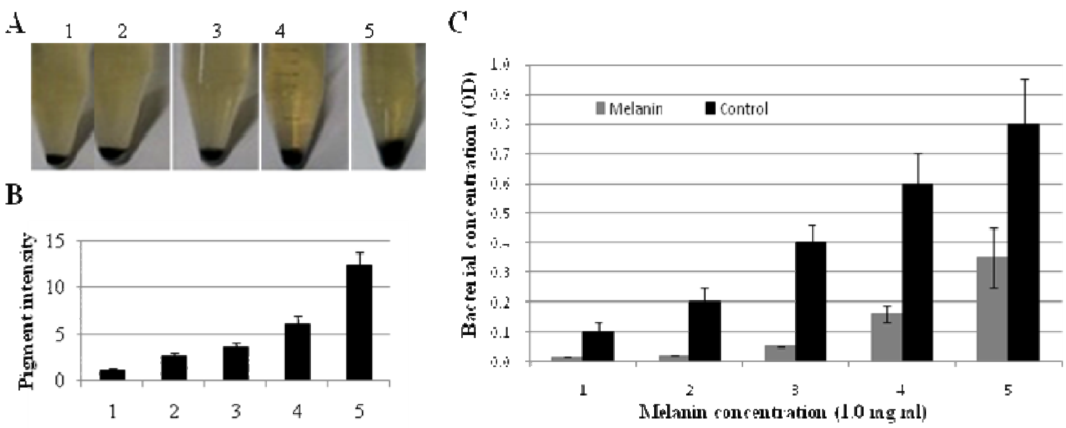

Figure 6. Efficiency of bacterial removal by melanin with dose dependence was showed in pictures (A and B) and in a graph (C).

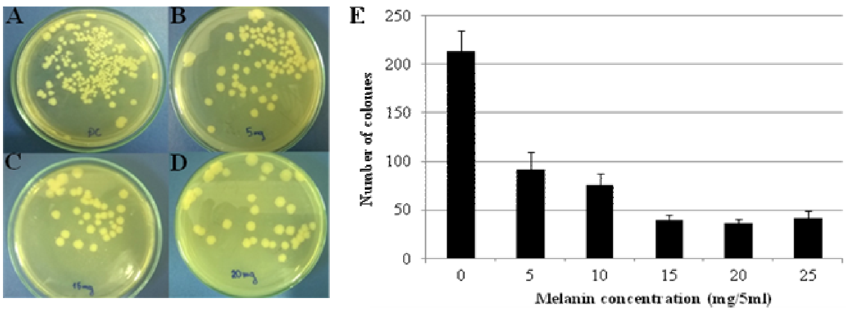

Figure 7. Number of colonies formed o LB dishes with or without melanin treatment at different concentrations were showed in pictures (A-D) and in a graph (E).

4. Conclusions

In this study, we investigated the ability of melanin extracted from ink sacs of squids in eliminating of heavy metal ions including Cr^{6+} and Mn^{2+} as well as *vibrio parahaemolyticus* bacteria in aqueous solution with high removal efficiencies. Ink sacs of the squid are considered as useless material and normally to be discarded by seafood companies. Melanin content in ink sacs of squid is account for about 10% [12, 13]. Vietnam is a seafood exporting country, in which squid is one of the main products. That means amount of melanin discarded by seafood companies every year is very big. Extracted melanin from ink sacs of squid is a natural black pigment, which could be applicable in many types of industries including cosmetic, medicine, addictive food, and environment. In this research we succeded in extracting melanin and used it for elimination of heavy metal ions and bacteria in aqueous solution. Our results indicated that melanin had strong ability in removing of Cr^{6+} and Mn^{2+} with high adsorption efficiencies. The adsorption capacities of melanin were 5.78 mg/gam and 31.8 mg/gam for Cr^{6+} and Mn^{2+}, respectively. More interestingly, our results showed that melanin could also eliminate *vibrio parahaemolyticus* bacteria effectively. The obtained results of this study suggested that melamin could be used as a biomaterial for advange treatment of water polluted with heavy metal ions especially Cr^{6+} and Mn^{2+} as well as bacteria.

Acknowlegments

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References

[1] Winkel Lenny H.E, Trang PTK, Lan VM, Stengel K., et al , Arsenic pollution of groundwater in Vietnam exacerbated by deep aquifer exploitation for more than a century, PNAS 108 (2011)1246.

[2] Johanna B, Berg M, Stengel C, et al, Contamination of drinking water resources in Mekong delta floodplains: Arsenic and other trace metals pose serious health risks to population, Environment International 34 (2008) 756.

[3] Do AT, Kuroda K, Hayashi T, et al, Household survey of installation and treatment efficiency of point-of-use water treatment systems in Hanoi, Vietnam, Journal of Water Supply: Research and Technology-AQUA, 63 (2014) 154.

[4] Tarangini K and Mishra S, Production, characterization and analysis of melanin from isolated marine Pseudomonas sp. using vegetable waste, Research Journal of Engineering Sciences 2 (2013) 40.

[5] Magarelli M, Passamonti P, and Renieri C, Purification, characterization and analysis of sepia melanin from commercial sepia ink (Sepia Officinalis), CES Medicina Veterinaria y Zootecnia 5 (2010) 18.

[6] Larsson BS, Interaction between chemicals and melanin, Pigment Cell Res 6 (1993) 127.

[7] Chen S, Xu J, Xue C, Dong P, Sheng W, Yu G, Chai W, Sequence determination of a non-sulfated glycosaminoglycan-like polysaccharide from melanin-free ink of the squid Ommastrephes bartrami by negative-ion electrospray tandem mass spec-trometry and NMR spectroscopy, Glycoconj J 25 (2008) 481.

[8] Brenner M and Hearing VJ, The protective role of melanin against UV damage in human skin Photochem Photobiol 84 (2008) 539.

[9] Kim DJ, Ju KY, Lee JK, The synthetic melanin nanoparticles having an excellent binding capacity of heavy metal ions, Bul. of the Korean Chemical Society 33 (2012) 3788.

[10] Sono K, Lye D, Christine A, et al, Melanin-based coatings as lead-binding agents, Bioinorganic Chemistry and Applications (2012) ArticleID 361803.

[11] Lydén A, Larsson BS, Lindquist NG, Melanin affinity of manganese, Acta Pharmacol Toxicol (Copenh) 55 (1984) 133.

[12] Derby CD, Cephalopod Ink: Production, Chemistry, Functions and Applications, Mar Drugs. 12 (2014) 2700.

[13] Naraoka T, Uchisawa H, Mori H, Matsue H, Chiba S, Kimura A, Purification, characterization and molecular cloning of tyrosinase from the cephalopod mollusk, Illex Argentinus, Eur J Biochem 270 (2003) 4026.

[14] Thang ND, Yajima I, Kumasaka YM, and Kato M, Bidirectional Functions of Arsenic as a Carcinogen and an Anti-Cancer Agent in Human Squamous Cell Carcinoma, PLoS One 9 (2014) 5e96945.

Nghiên cứu khả năng ứng dụng vật liệu sinh học melanin nguồn gốc tự nhiên trong việc loại bỏ ion kim loại nặng và vi sinh vật trong nước

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Tóm tắt: Phát triển vật liệu nhằm loại bỏ ion kim loại nặng và vi sinh vật trong nước đã và đang được nghiên cứu. Mặc dù nhiều vật liệu đã được phát triển, tuy nhiên khả năng ứng dụng trong việc loại bỏ đồng thời nhiều loại ion kim loại nặng cũng như vi sinh vật vẫn có nhiều hạn chế. Cùng với sự phát triển của ngành công nghiệp nặng như khai khoáng, luyện kim, xi mạ,.. crom, mangan là hai kim loại nặng thường có mặt trong các nguồn nước. Trong nghiên cứu này, chúng tôi đã khảo sát khả năng hấp phụ và loại bỏ các ion Cr6+ và Mn2+ cũng như vi sinh vật trong nước bằng vật liệu sinh học melanin tách chiết từ túi mực. Kết quả nghiên cứu cho thấy rằng, melanin có khả năng loại bỏ ion Cr^{6+} và Mn^{2+} hiệu quả lên đến trên 97%, với dung lượng hấp phụ Cr^{6+} và Mn^{2+} lần lượt là 5.78 mg/gam và 31.8 mg/gam. Thú vị hơn, các kết quả nghiên cứu cũng cho thấy rằng melanin có khả năng hấp phụ và loại bỏ vi sinh vật *vibrio parahaemolyticus* với hiệu quả trên 90%. Với những kết quả thu được, chúng tôi cho rằng melanin có khả năng được sử dụng như là một loại vật liệu sinh học an toàn và hiệu quả để loại bỏ đồng thời ion kim loại nặng cũng như vi sinh vật trong quá trình xử lí nước sinh hoạt ở giai đoạn nâng cao.

Từ khóa: Melanin, vật liệu sinh học, xử lí nước, kim loại nặng, vi khuẩn *vibrio parahaemolyticus*.