

Catalyzed Trisodium Citrate as a Medium for Heavy Metals Treatment in Green-Lipped Mussels (*Perna viridis*)

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ABSTRACT

This research was carried out to study the heavy metals removal like arsenic (As), lead (Pb), cadmium (Cd), nickel (Ni) and mercury (Hg) from *Perna viridis*. The trisodium citrate with Ru/Mn (25:75)-Al₂O₃ catalyst were used for the treatment medium with optimum treatment conditions at 500 µg/g of dosage, at temperature of 35°C ± 1°C, at pH 7, for 5 hours of duration and in the presence of compressed air (flow rate 895.50 mL/min). The introduction of Ru/Mn (25:75)-Al₂O₃ catalyst at 100°C calcination temperature on the trisodium citrate medium indicates the reduction of heavy metals which achieved the permissible limit by Malaysian Food Regulations (1985) and Commission Regulation of EU (2006). The original concentrations of As: 1.65 ± 0.89 µg/g, Hg: 12.42 ± 0.53 µg/g, Pb: 6.25 ± 0.69 µg/g, Cd: 1.27 ± 0.25 µg/g and Ni: 2.00 ± 1.05 µg/g were reduced to As: 0.75 ± 0.04 µg/g (54.34%), Hg: 0.4 ± 0.02 µg/g (96.79%), Pb: 1.07 ± 0.02 µg/g (82.89%), Cd: 0.32 ± 0.10 µg/g (75.02%) and Ni: 0.76 ± 0.01 µg/g (61.83%). ICP-MS and MHS-AAS techniques were used for the metals analysis and the catalyst was characterized through X-ray Diffraction (XRD), Field Emission Scanning Electron Microscopy (FESEM) and Energy Dispersive X-Ray (EDX) and Nitrogen Absorption (NA).

Keywords: *Perna viridis*; Heavy Metal; Trisodium Citrate; Catalyst

1. Introduction

The green-lipped mussel, *Perna viridis* (Family: Mytilidae) is a local seafood delicacy. This bivalve is a filter feeder which feeds on phytoplankton, small zooplankton and other organic materials. This natural habitat may accumulate heavy metals over the years which enter the water and food cycles through a variety of chemical and geochemical processes [1-5]. Even low heavy metals content will possess toxic effect for human when intake is excessive [6].

Chelating agent is capable of forming a complex ring-like structure when comes in contact with a metal ion [7]. Studies have reported the application of chelating agents on heavy metals removal from human by deferoxamine (DFO) in chelation therapy, deferoxamine (DFO) and deferiprone (DFP) on fish, potassium butyl dithiophosphate (PBD) on wastewater, 1,3-benzenediamidoethanethiolate (BDET²⁻) on groundwater, citric acid on solid waste, oxalic and acetic acid on soil and sodium citrate

on sediment [8-14]. Upon the catalyst application, studies have shown the enhancement toward the removal of polycyclic hydrocarbons (PAHs) in waste incinerator, the production of hydrogen from the supercritical water gasification (SCWG) and naphthenic acid removal in the acid solution model chemical reaction [15-17].

Biomonitoring the *Perna viridis* species have increased the awareness for health effect toward heavy metals contamination. Even though depuration process had been carried out to naturally purify transition metal contamination from the bivalves, only removed small amount of heavy metals while requires long time for process such as Hg (23.3%) in 20 days and Zn (40%), Pb (18.5%) and Cu (63.3%) in 24 days [18]. Hence, no commercially viable industrial technology was currently developed for the removal of the toxic elements. The purpose of the study is to remove heavy metals (As, Hg, Pb, Cd and Ni) from contaminated *Perna viridis* using trisodium citrate with the addition of Ru/Mn (25:75)-Al₂O₃ catalyst. The result should compliment with the permissible limit set by the Malaysian Food Regulations (1985) and Commission Regulation of EU (2006).

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2. Materials and Methods

2.1. Apparatus

As, Pb, Cd and Ni metals were analyzed through Inductive Coupled Plasma Mass Spectrometry, ICP-MS (Perkin Elmer ELAN 6100) while Hg determination was done by Mercury-Hydride Atomic Absorption Spectrometer, MHS-AAS (Perkin Elmer AAnalyst 400). The prepared catalyst was characterized using Nitrogen Absorption (Quantachrome Autosorb-1), Field Emission Scanning Electron Microscopy, X-ray Diffraction (Bruker AXS D8 Automatic Powder Diffractometer), and Energy Dispersive X-Ray (JEOL JSM-6701F). A pH meter (Hanna Instrument) was used to verify the pH of the citrate salt to achieve the desired pH.

2.2. Materials

All reagents used in the study were analytical grade and were used without any purification. All the solutions were prepared using deionized water obtained from the NANO pure water system. Samples were digested using HNO₃ (Sigma Adrich, 65%). All the plastic and glassware were cleaned by soaking in diluted HNO₃ (10%) and rinsed with distilled water prior to use. The element standard solutions used for calibration were produced by diluting a stock solution of 29 elements standard 3 (10 mg/L) supplied by Merck, Germany. The chelating agent used was trisodium citrate dihydrate, C₆H₅Na₃O₇·2H₂O (Sigma Adrich) while for the catalyst, the chemicals were Manganese (II) chloride (MERCK Eurolab), Ruthenium (III) chloride (MERCK Eurolab) and Aluminium Oxide (Sigma Adrich).

2.3. Catalyst Preparation

The catalyst was prepared by dissolved RuCl₃ and MnCl₂·2H₂O with 25:75 ratios in triply distilled water (2 mL). Then Al₂O₃ beds were 3 times impregnated in the catalyst solution for 15 minutes each. The catalyst was then washed with triply distilled water until no color changing of AgNO₃ reagent. Later, it was aged at 80°C for 24 hours before further calcined at 1000°C for another 5 hours. All analysis was done in three series of replicates and the *Perna viridis* was rinsed with deionized water before the metals analysis.

2.4. Sampling

Perna viridis of average sizes of 9.0 cm - 11.5 cm and sea water samples were collected from each 4 sampling site along Pantai Lido-Pendas coastal area (**Figure 1**) from October 2010 to March 2011. These mussel samples were brought back to the laboratory and were stored at -10°C until treatment while the sea water samples were preserved with 65% HNO₃ (5mL/L) for metal analysis.

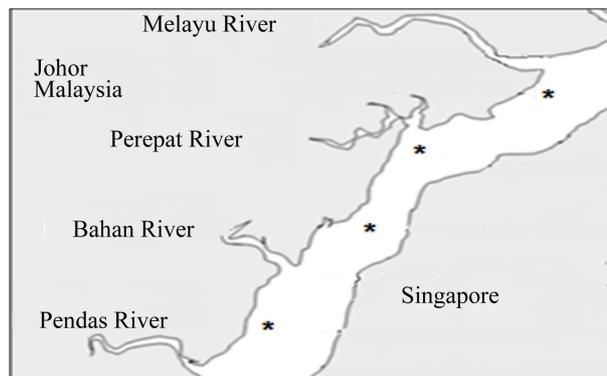


Figure 1. The sampling location from Pantai Lido-Pendas coastal area marked by (*).

2.5. Heavy Metals Removal

Treatment for the heavy metals removal in *Perna viridis* was conducted using trisodium citrate in the presence of Ru/Mn (25:75)-Al₂O₃ catalyst. The samples were put in sack and were soaked in the beaker (250 mL) that contains the chelating agents with stirring for 5 hour under the optimum treatment condition at 500 µg/g of dosage, at temperature of 35°C ± 1°C, at pH 6 - 7, for 5 hours of duration and in the presence of compressed air (flow rate 895.50 mL/min).

2.6. Metal Analysis

All prepared samples were digested using 65% of HNO₃ (APHA 30 30 E). The digestion was done through partial reflux until clear solutions were obtained. After the digestion process, the samples were allowed to cool and filtered through a Whatman Grade 2 filter Paper and then diluted to 50 mL with deionized water. The prepared samples were then analysed for As, Pb, Cd and Ni using ICP-MS while MHS-AAS for Hg. The concentrations are presented in µg/g. To check for contamination and performance of the instrument, procedural blank and standard were analysed once for every ten samples.

3. Results

3.1. Heavy Metal Concentrations in *Perna viridis*

The initial concentrations of heavy metals (µg/g) in the *Perna viridis* from 4 sampling points are presented in **Table 1**. 5 batches of sampling were done from October 2010 to March 2011. For this study, the average of initial concentration for 5 samples (represent 5 sampling times) was used to calculate the percentage of heavy metals removed after the treatment. From **Table 1**, the averages of the studied heavy metals were above and exceed the permissible limit by Malaysian Food Regulations (1985) and Commission Regulation of EU (2006).

Table 1. Initial heavy metals concentration in *Perna viridis* with the permissible limit stated by Malaysia and EU.

Sampling time	As ($\mu\text{g/g}$)	Hg ($\mu\text{g/g}$)	Pb ($\mu\text{g/g}$)	Cd ($\mu\text{g/g}$)	Ni ($\mu\text{g/g}$)
October 2010	0.84 ± 0.24	10.20 ± 0.31	0.70 ± 0.27	0.28 ± 0.11	1.03 ± 0.03
November 2010	1.88 ± 0.58	24.42 ± 0.17	9.81 ± 0.55	1.78 ± 0.15	2.00 ± 0.07
December 2010	2.04 ± 0.11	7.48 ± 0.36	5.44 ± 0.23	2.21 ± 0.08	3.59 ± 0.81
January 2011	1.75 ± 0.62	9.00 ± 0.12	8.52 ± 0.18	0.51 ± 0.13	1.44 ± 0.62
February 2011	1.70 ± 0.03	11.02 ± 0.09	6.80 ± 0.12	1.57 ± 0.07	1.97 ± 0.25
Average initial concentration	1.65 ± 0.89	12.42 ± 0.53	6.25 ± 0.69	1.27 ± 0.25	2.00 ± 1.05
Permissible limit,	Malaysia:	As, Cd and Ni (1.00 $\mu\text{g/g}$); Pb (2.00 $\mu\text{g/g}$); Hg (0.50 $\mu\text{g/g}$)			
	EU :	As, Cd and Ni (1.00 $\mu\text{g/g}$); Pb (1.50 $\mu\text{g/g}$); Hg (0.50 $\mu\text{g/g}$)			

3.2. Catalytic Treatment

The preliminary study on the catalytic treatment was done to identify the effect of Ru/Mn (25:75)-Al₂O₃ catalyst towards metals chelation of trisodium citrate. The heavy metals concentration with and without the presence of catalyst were determined. The results are illustrated in **Figure 2**. The result showed that with the presence of Ru/Mn (25:75)-Al₂O₃ catalyst, the percentage removal of the heavy metals increases up to 20% compared to none Ru/Mn (25:75)-Al₂O₃ catalyst in trisodium citrate medium. Hence, the catalyst was optimized to get the optimum catalytic activity.

3.3. Optimization of Catalyst

The calcination temperature of Ru/Mn(25:75)-Al₂O₃ catalyst were varied at 900°C, 1000°C and 1100°C for 5 hours. The catalytic treatment activities are summarized in **Figure 3**. The result shows that calcination temperatures for 1000°C gave higher catalytic activity for the catalyst on As, Pb and Hg when compared with calcination temperature at 900°C and 1100°C. Whereby, Cd and Ni followed the order 900°C > 1000°C > 1100°C and 1100°C > 1000°C > 900°C respectively. The percentage removal achieved at 1000°C was As: 62.66%, Pb: 85.19%, Cd: 77.10%, Ni: 60.20% and Hg: 96.96%.

The optimization for the loading of the catalyst, Ru/Mn (25:75)-Al₂O₃ was varied by 1:1 (2.5 g catalyst in 250 mL sodium citrate) and 1:0.5 (1.25 g catalyst in 250 mL sodium citrate) ratios. The ratio used was based on volume of citrate salt to the weight of the catalyst. The result is illustrated in **Figure 4**.

Figure 4 clearly shows that the presence of catalyst even in a small quantity may enhance the removal rate of the heavy metal in *Perna viridis*. From the result, the dosage of the catalyst at 1.25 g was selected as both dosage of Ru/Mn(25:75)-Al₂O₃ in trisodium citrate did not gave much different in percentage removal of the heavy metals and only differed below 10%.

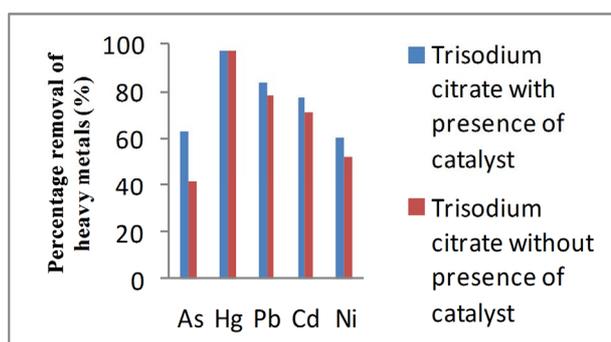


Figure 2. Percentage removal of heavy metals in *Perna viridis* in the presence of Ru/Mn (25:75)-Al₂O₃ catalyst at 900°C calcination temperature in trisodium citrate (500 $\mu\text{L/L}$) medium with pH 7, at 35°C \pm 1°C and for 5 hours.

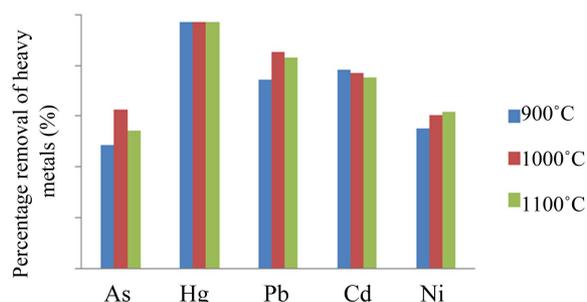


Figure 3. Percentage removal of heavy metals removal for catalyzed trisodium citrate with Ru/Mn (25:75)-Al₂O₃ catalyst (1.25 g) at various calcination temperatures for 5 hours.

4. Characterization of Potential Catalyst

In this study, the characterization techniques of potential catalysts used were carried out by Nitrogen Absorption (NA), Electron Microscopy (FESEM), X-Ray Diffraction Analysis (XRD) and Field Emission Scanning Energy Dispersion X-Ray (EDX).

4.1. NA

The surface area and pore diameter of Ru/Mn(25:75)-

Al₂O₃ catalyst were presented in **Table 2**. The results followed the order of 1000°C > 1100°C > 900°C of calcination temperature.

At 1000°C, the catalyst achieved the highest surface area (9.576 m²·g⁻¹) and highest average pore diameter (84.887 nm) compared to other calcination temperature. In general, bigger pore size creates bigger surface area of the catalyst, hence giving more active sites. However, higher calcination temperature leads to sintering effect and cause migration of particles size to grain in which the particles size increases and surface area decreases while low calcination temperature was unable to create high pore size on the surface of the catalyst which lead to low surface area. Even though the N₂ adsorption-desorption isotherms analysis recognized that the catalyst to be Type IV and mesopores at all temperature, but the hysteresis loop as shown in **Figure 5** indicates the narrower pore size of the catalyst at 1000°C calcinations temperature.

It was proven as it gave higher uptake of nitrogen at high relative pressure and formation of the hysteresis loop at high relative pressure compared to calcination temperature at 900°C and 1100°C. Thus, high catalytic activity achieved at 1000°C calcination temperature was due to the higher surface area with bigger and narrower pore size.

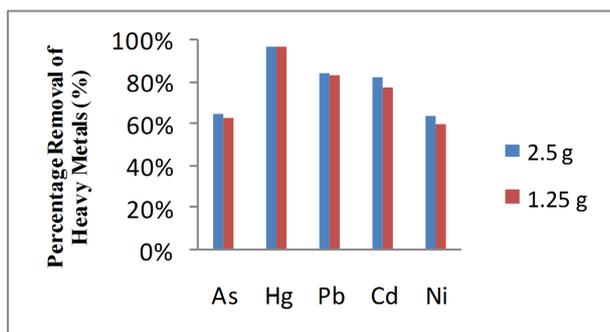


Figure 4. The percentage removal of heavy metals for different dosage of Ru/Mn (25:75)-Al₂O₃ catalyst calcined at 1000°C in the trisodium citrate (500 µL/L) at neutral pH (pH 6 - 7), temperature of 30°C - 35°C with stirring for 5 hours.

Table 2. Surface area (S_{BET}) and BJH desorption average pore diameter, d (nm) of Ru/Mn (25:75)-Al₂O catalysts calcined at 900°C, 1000°C and 1100°C for 5 hours.

Calcination Temperature (°C)	S_{BET} (m ² ·g ⁻¹)	D (nm)
900	7.369	64.160
1000	9.576	84.887
1100	8.454	67.993

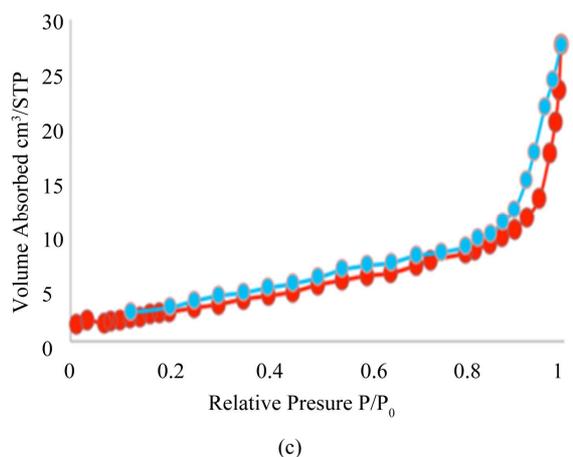
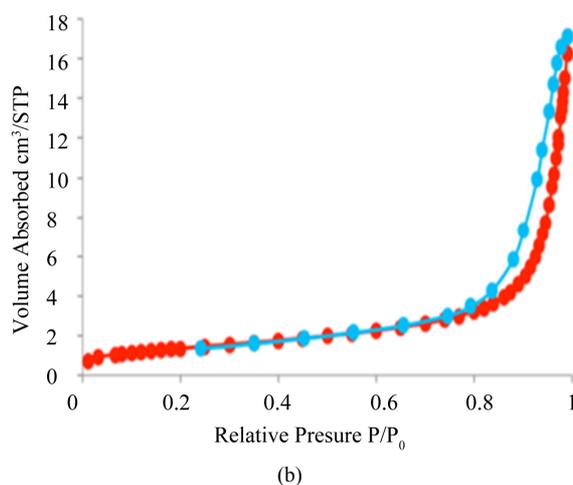
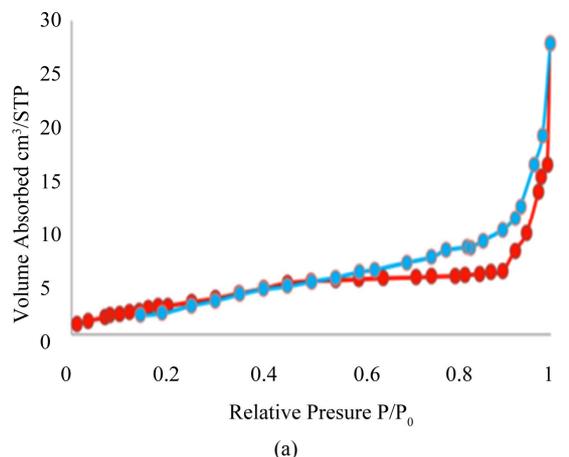


Figure 5. Isotherm plots of Ru/Mn (25:75)-Al₂O₃ catalyst calcined at (a) 900°C (b) 1000°C and (c) 1100°C for 5 hours.

4.2. XRD

Figure 6 shows the XRD pattern of Ru/Mn (25:75)-Al₂O₃ catalyst. All calcination temperature showed the presence of Al₂O₃ support in rhombohedral phase with RuO₂ tetragonal phase and Mn₂O₃ hexagonal phase as the active site species. At 900°C, Al₂O₃ (rhombohedral)

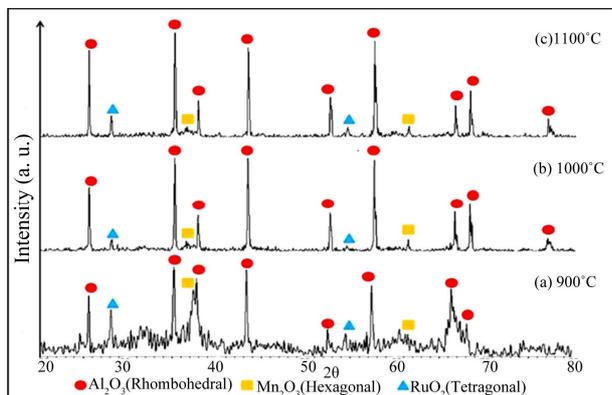


Figure 6. XRD Diffractograms of Ru/Mn (25:75)-Al₂O₃ catalysts calcined at (a) 900°C (b) 1000°C (b) and (c) 1100°C for 5 hours.

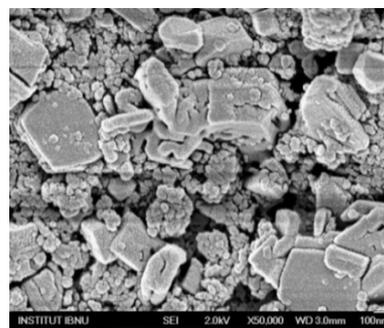
was revealed at $2\theta = 35.131^\circ$ (I_{100}), 43.329° (I_{96}), 57.410° (I_{91}), 25.550° (I_{68}), 68.089° (I_{52}), 52.475° (I_{47}), 37.323° (I_{46}) and 66.398° (I_{35}); RuO₂ (tetragonal) at $2\theta = 28.076^\circ$ (I_{100}) and 54.965° (I_{39}) while Mn₂O₃ (hexagonal) at $2\theta = 36.466^\circ$ (I_{100}) and 61.872° (I_{39}). For 1000°C, Al₂O₃ (rhombohedral) was found at 2θ values of 35.126° (I_{100}), 42.323° (I_{96}), 57.462° (I_{91}), 25.548° (I_{68}), 68.217° (I_{52}), 52.519° (I_{47}), 37.776° (I_{46}), 66.483° (I_{35}) and 76.869° (I_{15}); RuO₂ (tetragonal) at $2\theta = 28.076^\circ$ (I_{100}) and 54.454° (I_{39}) while Mn₂O₃ (hexagonal) at $2\theta = 36.316^\circ$ (I_{100}) and 61.211° (I_{50}). Whereby, at 1100°C, Al₂O₃ (rhombohedral) was determined at $2\theta = 35.162^\circ$ (I_{100}), 43.350° (I_{96}), 57.514° (I_{91}), 25.574° (I_{68}), 68.187° (I_{52}), 52.5459° (I_{47}), 37.780° (I_{46}), 66.493° (I_{35}) and 76.901° (I_{15}); RuO₂ (tetragonal) at $2\theta = 28.041^\circ$ (I_{100}) and 54.966° (I_{39}) while Mn₂O₃ (hexagonal) at $2\theta = 36.451^\circ$ (I_{100}) and 61.921° (I_{50}).

The result revealed the amorphous state for the catalyst at 900°C compared to crystalline state at 1000°C and 1100°C. The high peak intensity of RuO₂ tetragonal at 1100°C compared to 1000°C calcination temperature will increase the crystallinity and may also decrease the surface area as indicated by NA analysis. Therefore, high catalytic activity at 1000°C calcination temperature was mainly due to the presence of crystalline phase at high surface area.

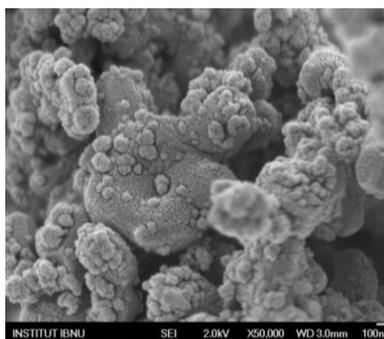
4.3. FESEM

The morphology of Ru/Mn(25:75)-Al₂O₃ catalysts were shown by FESEM micrographs in **Figure 7**. All the images show the formation of aggregation and agglomeration. Through FESEM mapping pattern in **Figure 8**, both Ru and Al species were identified to be in cluster pattern at 900°C calcinations temperature while only Ru species was clustered for 1000°C and 1100°C calcinations temperature.

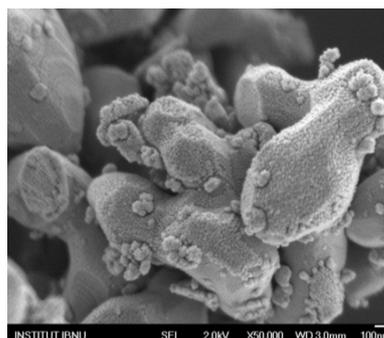
High catalytic activity for the catalyst at 1000°C calcinations temperature was determined to be inhomogeneous nano particle sizes which vary from 41.4 nm to



(a)



(b)



(c)

Figure 7. FESEM micrographs of Ru/Mn (25:75)-Al₂O₃ catalyst calcined at (a) 900°C, (b) 1000°C and (c) 1100°C for 5 hours (Magnification: 50,000×, Scale bar: 1 cm = 100 nm).

85.1 nm. Besides, the particles were shown to project out from the catalyst surface with high aggregation and was parallel with Ru mapping profile which shown to be more clustered at 1000°C compared to 1100°C. This morphology will increase the surface area of the catalyst and it was in agreement with the result obtained through NA analysis. Whereby, the surface area was lower at 900°C as the particles with large number of square shape and uniform small spherical at diameter varies from 74.5 nm to 122.0 nm were more flatten on the catalyst surface. Meanwhile at 1100°C, a capsule like shape and inhomogeneous small spherical shape with diameter sizes varied from 34.4 nm to 103.6 nm were presented. The particles were projected out from the catalyst surface but tend to more agglomerated which also lower the surface area.

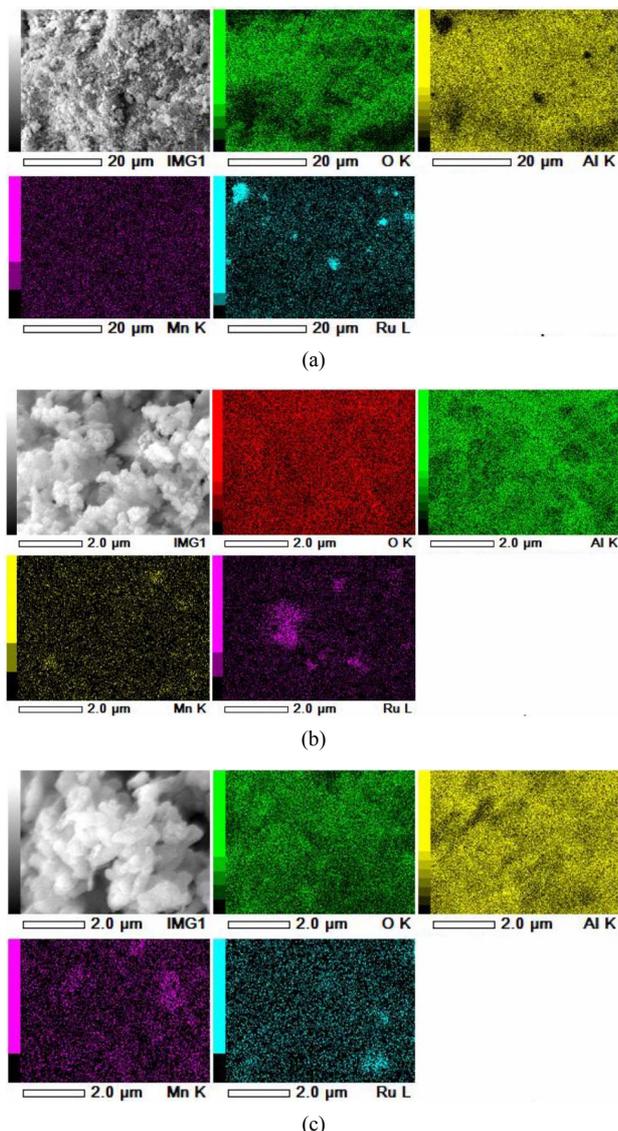


Figure 8. FESEM Mapping pattern for Ru/Mn (25:75)-Al₂O₃ catalyst calcined at (a) 900°C (b) 1000°C and (c) 1100°C for 5 hours. (Magnification: 20,000x, Scale bar: 1 cm = 2.0 µm).

4.4. EDX

The elements composition of Ru/Mn (25:75)-Al₂O₃ catalyst was shown in Table 3. The result showed the presence of Al, O, Mn and Ru elements on the catalyst at all studied calcination temperature. Al and O elements were attributed to the presence of Al₂O₃ as the catalyst support while both Mn and Ru elements exhibited as the active species of the catalyst which appeared as individual phases as Mn₂O₃ and RuO₂ respectively as determined in XRD analysis. The percentage weight of Mn in the catalyst shows inversely proportional to the calcination temperature while the percentage weight of Ru in the catalyst followed the order of 1000°C > 900°C > 1100°C

of calcination temperature. Thus, indicates the high Ru aggregation formation as shown in the Ru mapping profile in FESEM analysis. High percentage of Mn at 900°C on the catalyst not affected the catalytic activity as it act only as a base for Ru active species on the Al₂O₃ support.

5. Proposed Mechanisms of the Catalytic Activity

The catalytic activity can be explained with the postulated reaction mechanism shown in Figure 9.

The reaction begins when trisodium citrate solution was adsorbed on the surface of Ru/Mn (25:75)-Al₂O₃ catalyst (1). Then, the bonding between sodium, hydrogen and oxygen in the compound will be dissociated to form anion and cation (2). At the transition state, citrate ion will bond to the metal ion from the mussel while the sodium ion will bond with OH⁻ from water molecule (3). Later, the production of metal citrate compound and NaOH will be released into the solution leaving the catalyst alone (4). The catalyst will then proceed their catalytic activity with adsorbing new trisodium citrate compound on their surface. Thus, a cyclic catalytic chelation proceeds again.

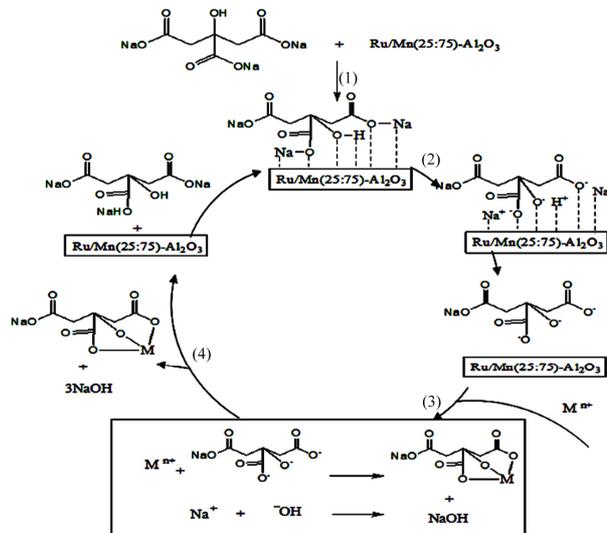


Figure 9. Proposed catalytic chelation cycle for the removal of metal ion from *Perna viridis*.

Table 3. EDX analysis of Ru/Mn (25:75)-Al₂O₃ catalyst calcined at 900°C, 1000°C and 1100°C for 5 hours.

Calcination Temperature (°C)	Weight Ratio (%)			
	Al	O	Mn	Ru
900	53.42	31.94	11.06	3.58
1000	48.31	38.45	9.09	4.15
1100	53.15	37.74	7.60	1.51

6. Conclusion

The obtained results proved that the catalytic treatment gave higher percentage removal of heavy metals in *Perna viridis* compared to treatment using only chelating agent. The Ru/Mn(25:75)-Al₂O₃ catalyst calcined at 1000°C, was found to be the best catalyst to enhance the removal of heavy metals in *Perna viridis*. This catalyst possesses crystalline phase, surface area of 9.576 m²·g⁻¹ with average pore diameter of 84.887 nm, the isotherm of Type IV with hysteresis loop indicating the presence of mesopores, nano particle sizes which vary from 41.4 nm to 85.1 nm and high percentage composition of Ru and Mn on the catalyst with 4.15% and 9.09% respectively. Furthermore, the presence of RuO₂ aggregation species on the catalyst surface suggested as active sites of the catalyst.

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