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# The 4th International Seminar on Sustainable Urban Development

To cite this article: 2018 IOP Conf. Ser.: Earth Environ. Sci. 106 011001

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# Preface

First of all, praise be to Allah, the Cherisher and Sustainer of the world, for His blessing for all of us. He who has provided us a chance so that we could be here. On behalf of the Organizing Committee, it is our great pleasure to welcome you to the 4<sup>th</sup> International Seminar on Sustainable Urban Development (ISoSUD) 2017, held by Faculty of Landscape Architecture and Environmental Technology, Universitas Trisakti, Jakarta, Indonesia at Hotel Ciputra. To the academicians, our colleagues from overseas universities, guests, participants, students, please accept our gratitude, warm welcome and appreciation.

Let me deeply express a special appreciation to the speakers:

- 1) Assoc. Prof. Dr. Nada Khaleefah M. A. Alrikabi from Environmental & Urban Planner, Center of Urban and Regional Planning for Post Graduate Studies, Baghdad University, Iraq
- 2) Dr. Yusnani Mohd Yusof from Urban Planning, Affordable City, Universiti Brunei Darussalam
- Prof. Dr. Pasi Lehmusluoto, from Department of Limnology and Environment Protection, 3) University of Helsinki, Finlandia
- 4) Prof. Dr. Mohd. Razman Salim, from Department of Environmental Engineering, Faculty of Civil Engineering, Universiti Teknologi Malaysia, Malaysia

Achieving the SDGs requires of governments, private sector, civil society and citizens alie to make sure we leave a better planet for future generations. All 17 Goals interconnect, meaning success in one goal affects success for others. In short word, this is the greatest chance we have to improve life for future generations. Some of the goals that in major issue of 4<sup>th</sup> International Seminar on Sustainable Urban Development are:

- Goals No. 6: Clean Water and Sanitation •
- Goals No. 7: Affordable and Clean Energy •
- Goals No. 11: Sustainable Cities and Communities
- Goals No. 13: Climate Action •
- Goals No. 14: Life Below Water
- Goals No. 15: Life on Land

The seminar gathers eminent speakers and scholars that are engaged in Sustainable Urban Development field. The theme for this event is:

# THE SUSTAINABLE DEVELOPMENT GOALS: IMPROVE LIFE FOR FUTURE **GENERATION**

The sub-themes of the seminar include, but not limited to :

- 1) Ecological Disaster Mitigation and Adaptation
- 2) Urban Environemntal Management
- 3) Environmental Technology

This year, the committee received over than 150 papers both in oral or poster presentation from 15 universities in Indonesia and from UK, Japan, Malaysia, Italy, and Iraq as well. Selected papers will be proposed to be published in IOP Publisher Conferences Series: Earth and Environmental Science, indexing by Scopus and Thomson Reuters. Other selected papers will

be published in National Journal accredited by Ministry of Research and Higher Education of Indonesia, i.e: Aceh International Journal of Science and Technology published by Universitas Syiahkuala, Aceh and Jurnal Reaktor published by Universitas Diponegoro, Semarang.

We are very grateful to Rector of Universitas Trisakti-Jakarta, Dean of Faculty of Landscape Architecture and Environmental Technology, Head of Environmental Engineering Department, Head of Landscape Architecture Department, Head of Urban and Regional Planning Department, the members of Advisory board, the members of Steering committee, Peer Reviewers, and Organizing committee that very supported and helpful within the preparations and conduction this seminar. Our grateful deliver as well to Association of Experts in Sanitary Engineering (TP) and Environmental Engineering (TL), IATPI (Ikatan Ahli Teknik Penyehatan dan Teknik Lingkungan), for their support to the seminar. Our appreciation is also for all the participants who have actively written excellent research papers. It is desired to have a sustainable seminar to be continuously held in the future times, as we are challenged to make a sustainable building and environment for a sophisticated life. Last but not least, we would like to express our gratitude to all the session chairs, reviewers, participants, institutions for their contribution to 4<sup>th</sup> ISoSUD 2017.

We believe that this event will be able to facilitate a good networking among researchers, scientists, engineers and practitioners of with common interest especially in sharing the latest research results, ideas, development and applications in the Sustainable Urban Development. We hope you enjoy the seminar and find this experience inspiring and helpful in your professional field. We look forward to seeing you at our upcoming seminar in the next 3 years on 5<sup>th</sup> ISoSUD 2020.

The Editors Dr. Astri Rinanti Dr. Rositayanti Hadisoebroto Dr. Ade Gafar Abdullah Dr. Eng. Asep Bayu Dani Nandiyanto

doi:10.1088/1755-1315/106/1/011001

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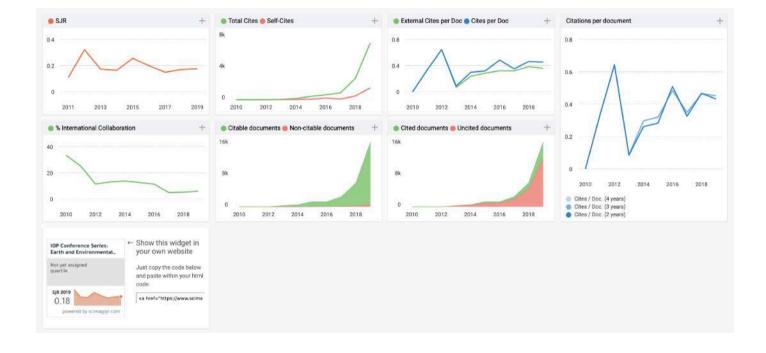




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	View article	n Sustainable Urban Development	
<ul> <li>Open abstract</li> </ul>	View article	20 PDF	
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Papers			
OPEN ACCESS			01
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<ul> <li>Open abstract</li> </ul>	View article	PDF	
• open abstract	(a) view article		
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<ul> <li>Open abstract</li> </ul>	View article	72PDF	
OPEN ACCESS			01
	nic risk mitigatio	n system on structure building	01
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<ul> <li>Open abstract</li> </ul>	View article	™PDF	
OPEN ACCESS	and the second		01
	12: The second s	at southern integrated terminal Bandar Tasik Selatan, Kuala Lumpur	
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<ul> <li>Open abstract</li> </ul>	III View article	POF	
OPEN ACCESS			01
The slope and in	ncision length of	affected local cross abrasion and accretion using ASTER GDEM image analysis	
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<ul> <li>Open abstract</li> </ul>	View article	PDF	
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Table of contents	
Volume 106 2018	
Previous issue     Next issue	
The 4th International Seminar on Sustainable Urban Development 9–10 August 2017 Indonesia	, Jakarta,
Accepted papers received: 18 December 2017 Published online: 31 January 2018	
Open all abstracts	
Preface	
OPEN ACCESS	011001
The 4th International Seminar on Sustainable Urban Development	
+Open abstract 🔄 View article р PDF	
OPEN ACCESS	011002
Peer review statement	
+Open abstract 🔄 View article 🎇 PDF	
Papers	
OPEN ACCESS	012001
Georadar and geoelectricity method to identify the determine zone of sliding landslide	
Y K Dalimunthe and A Hamid	
+Open abstract 🔄 View article 🄁 PDF	
OPEN ACCESS	012090
Biosorption of heavy metal copper (Cu <sup>2+</sup> ) by Saccharomyces cerevisiae	
S A J Ririhena, A D Astuti, M F Fachrul, M D S Silalahi, R Hadisoebroto and A Rinanti	
+Open abstract View article PDF	
OPEN ACCESS	012091
Waste utilization of red snapper (Lutjanus sp.) fish bone to improve phosphorus contents	s in compost
S Ramadhani, B Iswanto and P Purwaningrum	
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#### IOP Conf. Series: Earth and Environmental Science 106 (2018) 012090

# Biosorption of heavy metal copper (Cu<sup>2+)</sup> by Saccharomyces cerevisiae

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Abstract. This research aims to study the optimum effect of contact time and pH adsorption of copper  $(Cu^{2+})$  from electroplating industry waste by dried beer waste *S.cerevisiae*. This research conducted using batch culture with pH variation 2,3,4,5, and 6, contact time variation 60, 90, 120, 150, 180 minutes, 150 rpm at room temperature ( $\pm$  28°C), initial Cu<sup>2+</sup> concentration 33,746 mg/l, and biosorbent mass 200 mg & 500 mg. The adsorption of heavy metal ions  $Cu^{2+}$  occurs in all variations of pH and contact time at optimum pH. The optimum adsorption occurs at pH 4 with contact time 120 minutes for both 200 mg (41.60%) and 500 mg (61.04%) beer waste biosorbent. Cell morphology seen with Scanning Electron Microscope (SEM) analysis shows the change of cell wall that gets damaged from Cu2+ adsorption. It also proved by the decreased concentration of initial high concentration carboxyl groups. The adsorption process of this research complies to Freundlich Isotherm with R<sup>2</sup> value closest to 1 and followed first order kinetic.

Keywords: contact time, copper, pH adsorption, S.cerevisiae

#### 1. Introduction

Various research regarding metal ion reduction treatment has been done, such as oxidation-reduction, precipitation, adsorption, compaction, electrolytic recovery, and ion exchange. These processes are expensive in which some technological problems exist especially when applied to diluted metal solutions. Therefore, the search for clean and competitive technologies is strongly recommended. Biological treatment is usually considered as an effective method and can significantly reduce the quantity of heavy metals in aqueous solutions [1]. Study of biosorption as an alternative treatment considered effective in cost and easy to apply. Biosorption can adsorb more heavy metals than chemical precipitation on bigger pH range [2]. Biosorption capacity is mainly influenced by three kinds of influential factors: metal ionic characteristics (e.g., atomic weight, ion radius, valence) the nature of the biosorbents (e.g., cell age), and biosorption conditions (e.g., pH, temperature, contact time) [3].

This research aims to study the effect of contact time and pH to adsorption of Cu<sup>2+</sup> ion by biosorbent S. cerevisiae, determine the adsorption efficiency of  $Cu^{2+}$  ion contain in electroplating wastewater by biosorbent S. cerevisiae, and determine functional groups involved in  $Cu^{2+}$  adsorption process.

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# 2. Research Method

### 2.1. Biosorbent Preparation

*S. cerevisiae* that found in beer waste can be used as biosorbent. Beer waste centrifuged at 3600 rpm for 60 minutes. Biomass that formed in the bottom of centrifuged tube collected into a petri dish and then put it in the oven with temperature 80°C for 28 hours to let it dry. Dried biomass then grind with mortar and passed through screener (30 mesh) until it becomes powder.

2.2. Industrial Electroplating Wastewater Characterization. Characterization utilized with Atomic Absorption Spectrophotometry (AAS).

# 2.3. $Cu^{2+}$ adsorption on pH variation

Dried biosorbent of 200 mg and 500 mg were put into 70 mL electroplating wastewater contained  $Cu^{2+}$  then solution pH was adjusted to variation 2, 3, 4, 5, and 6. After that the solution was put on shaker 150 rpm for 90 minutes. Then the solution was centrifuged with 4000 rpm for 30 minutes to separate biomass with the solution. The concentration of  $Cu^{2+}$  remainder in solution determined by *Atomic Absorption Spectroscopy* (AAS) analysis.

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#### 3. Results and Discussion

The result of electroplating wastewater characterization indicates that it contained 33,764 mg/L  $Cu^{2+}$  without any other metals detected.

#### 3.1. Determining Optimum pH

The potential of hydrogen (pH) is one of the main factors that affects biosorption process. Metal ions adsorptions depends on solution pH, which influences electrostatic binding of ions to corresponding functional groups [4].

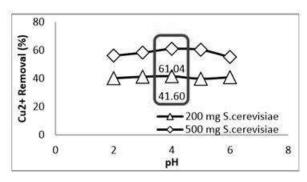


Figure 1. Effects of pH variation towards  $Cu^{2+}$ removal (%) at contact time 90 minutes, room temperature (± 28°C), and  $Cu^2$  initial<sup>+</sup> concentration 33,746 mg/L.

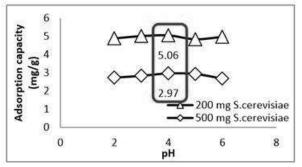


Figure 2. Effects of pH variation towards adsorption capacity (mg/g) at contact time 90 minutes, room temperature ( $\pm$  28°C), and Cu<sup>2+</sup>, initial concentration 33,746 mg/L.

Based on Figure 1  $Cu^{2+}$  adsorption efficiency increased from pH 2 until pH 4 which has the highest adsorption efficiency for all biosorbent samples whereas at pH 5 until pH 6  $Cu^{2+}$  removal efficiency decreased and has the lowest removal efficiency. It correspond with previous study that shows the optimum adsorption for  $Cu^{2+}$  ion happens at pH 4 until 5 [5]. The adsorption of heavy metal  $Cu^{2+}$  occurs

because of ions exchange mechanisms between heavy metal ions and carboxyl groups, phosphate, and amino which are the main components of *S.cerevisiae* cell wall, capable of acting as ion exchangers and complex formers with metal ions. At the low pH, concentration of protons is high and the ion exchange sites become solidly protonated, therefore metal adsorption will occur better at low pH [6].

As seen in Figure 2, the highest adsorption capacity also happen at pH 4, which compatible with previous study said the removal efficiency of  $Cu^{2+}$  occurs at a maximum of 3 g/L or 3 mg/mL biosorbent concentration but the removal efficiency will decrease if there is additional biosorbent concentration used [7]. This may occur due to the presence of biosorbent bundles that cause a decrease in surface area for adsorption, resulting in decreasing adsorption capacity. Previous study report at pH 4.5 200 mg *S. cerevisiae* adsorb 2,042 mg/g Cu<sup>2</sup> [8]. Based on these things it can be concluded that pH 4 is the optimum pH for biosorbent *S. cerevisiae* to adsorb Cu<sup>2+</sup>.

### 3.2. Determining Optimum Contact Time

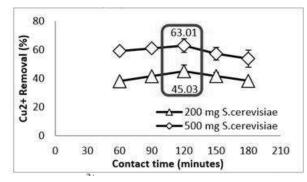


Figure 3.  $Cu^{2+}$  removal (%) towards contact time variation (minutes) at pH 4 (optimum), room temperature (± 28°C), and initial  $Cu^{2+}$  concentration 33,746 mg/L.

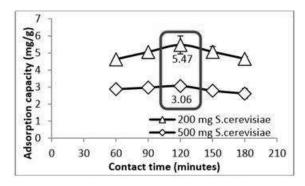


Figure 4. Adsorption capacity (mg/g) towards contact time variation (minutes) at pH 4 (optimum), room temperature ( $\pm$  28°C), and initial Cu<sup>2+</sup> concentration 33,746 mg/L.

Based on Figure 3  $Cu^{2+}$  removal efficiency keeps increasing until optimum at 120 minutes and decreasing at contact time 150 minutes. Previous study shows that optimum contact time for  $Cu^{2+}$  removal happened at 90 minutes and stay stable until 120 minutes [8]. 500 mg biosorbent *S. cerevisiae* has larger surface area than 200 mg *S. cerevisiae*, therefore 500 mg *S. cerevisiae* has higher  $Cu^{2+}$  removal efficiency than 200 mg *S. cerevisiae*.

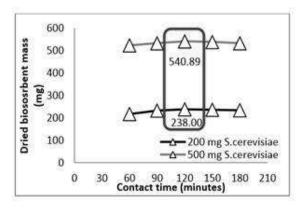


Figure 5. Dried biosorbent mass (mg) towards contact time at pH 4 (optimum), room temperature ( $\pm$  28°C), and initial Cu<sup>2+</sup> concentration 33,746 mg/L.

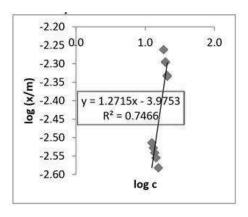


Figure 6. Isotherm Freundlich.

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The same tendency can be seen in Figure 4, adsorption capacity for 200 mg biosorbent *S. cerevisiae* is in the range 5-6 mg/g with highest capacity at 5.47 mg/g at and for 500 mg biosorben *S. cerevisiae* is in the range 3 mg/g with the highest capacity at 3.06 mg/g at optimum pH and contact time.

Figure 5 shows a correlation between dried bisorbent mass with contact time. Initial 200 mg dried biosorbent mass increased up until 238.00 mg while initial 500 mg increased up to 540.89 mg. The increasing dried biosorbent weight occurs due to heavy metal ion  $Cu^{2+}$  presence from solution that adsorb into biosorbent *S. cerevisiae* in accordance with the quantity  $Cu^{2+}$  adsorb from solution into biosorbent *S. cerevisiae* cell wall, other than that there's a chance of impurities in solution that goes through the filter and weighted together with dried *S. cerevisiae* biosorbent. As contact time increase, the weight of dried biosorbent will increase until the saturation point which is 120 minutes and begin to decrease at 150 minutes.

#### 3.3. Determining Optimum Contact Time

Adsorption isotherm used to determine the occurrence of  $Cu^{2+}$  adsorption characteristic, and a curve will be generated to shows the maximum adsorption capacity of *S.cerevisiae* biosorbent based on the characteristic. Calculation of adsorption isotherm value of this study using Langmuir, Freundlich, and BET isotherm. The use of adsorption isotherms is to determine the most representative description of this study biosorption process.

Langmuir isotherm assumes that adsorbent surface has a certain number of adsorption points which is proportional to the surface area of the adsorbent and adsorption only occurs on the monolayer thus the adsorption process can only occur in the adsorbent that has not been filled by any adsorbate. Whereas for Freundlich Isotherm used to explain non-ideal adsorption process on the heterogeneous surface because of functional groups difference in adsorbent (Figure 6). BET isotherm assumed that adsorbent has homogenous surface and adsorbat molecule can form more than one layer on the surface [9].

From calculation results shown in Table 1, adsorption process of this study follows Freundlich Isotherm with the highest  $R^2$  value between Langmuir and BET Isotherm.  $R^2$  value of Langmuir Isotherm is not so different than Freundlich value, according to Freundlich the adsorbent surface is heterogeneous, composed of different classes of the adsorption region, but each class adsorption follows Langmuir isotherm, therefore the adsorption tends to be similar.

#### 3.4. Adsorption Kinetics

Adsorption kinetics is a study to determined reaction order and speed rate of adsorption process that happened. The reaction of the biosorption rate provides important information to create a batch scale biosorption system. The approach used in calculating the adsorption kinetics in this study is first order and second order.

$\mathbb{R}^2$			R	2
Isotherm Langmuir	Isotherm	Isotherm BET	First Order	Second
	Freundlich			Order
0.71	0.7466	0.4731	0.0448	0.0351

Table 1. R<sup>2</sup> Value calculation of adsorption isotherm and kinetics.

Based on  $\mathbb{R}^2$  value seen in Table 1, the value of first order kinetic adsorption is closer to 1 than second order thus it can be said that the adsorption kinetics that occurs tend to follow the first order. The first-order reaction is a reaction whose velocity depends only on one of the reactive substances which or is proportional to one of the reactant forces [10].

#### 3.5. Analisis Fourier Transfer Infra-Red Spectrophotometer (FTIR)

Biosorbent *S.cerevisiae* has three main functional groups which are carboxyl groups, amino groups, and hydroxyl groups. *S. cerevisiae* cell wall composed of a-mannan and b-glucan, protein, lipid, and inorganic substances particularly phosphate, chitin, and chitosan. Proteins that construct *S.cerevisiae* cell wall are rich in glutamic acid residues, aspartate, serine, threonine, and asparagine. Based on FTIR

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analysis it is known that biosorbent *S. cerevisiae* has high carboxylic acid, carboxyl group, and amine. In this FTIR results chitin included in acid carboxyl functional groups which in the 3277/cm area, which indicates the peak of O-H.

The carboxyl group in the 1625/cm region indicates the peak presence of C = O, and amine at 1234/cm indicates the peak presence of N-H. The content of carboxylic acid decreased from 92.27% to 92.04%, carboxyl group decreased from 98.04% to 89.77%, Amine from 94.17% to 92.33%, and alkane from 95.93% to 94.37%. This indicates the presence of functional groups involved in the adsorption of  $Cu^{2+}$  ions which cause changes in functional groups in the biosorbent of *S. cerevisiae* either in the presence or the content of functional group present in the biosorbent.

#### 3.6. Scanning Electron Microscope (SEM) Analysis

*Scanning Electron Microscope* (SEM) is a type of electron microscope that uses a focused beam of highenergy electrons to produce images of a sample by creating a variety of signals at the surface of the sample [11]. SEM produces high-resolution images of a sample surface, capturing a complete size of approximately 1 - 5 nm and can produce 3-dimensional shape characteristics so as to provide easier data about the analysed samples [12].

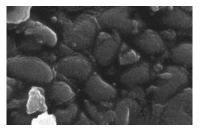
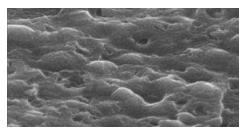


Figure 7. Control *S. cerevisiae* seen with SEM (500x)



**Figure 8.** *S. cerevisiae* after contact with  $Cu^{2+}$  in optimum condition seen with SEM (500x)

Based on Figure 7 and Figure 8 we can see the difference in cell morphology before and after contact with  $Cu^{2+}$ . As seen on Figure 8 *S.cerevisiae* cell wall looks shrivelled and have more holes than before contact (Figure 7) which look more hydrated and plump. This morphological change occurs due to  $Cu^{2+}$  ion adsorption process that happened in the cell wall during contact with electroplating wastewater. The change that happened is not too significant because the biosorbent is used in a state of death & dried so no metabolism progress occurs and disturbed by the absorption of  $Cu^{2+}$  ions.

# 4. Conclusion

From this research, we can conclude that contact time and pH affect the adsorption of  $Cu^{2+}$  by biosorbent *S.cerevisiae*, with optimum contact time 120 minutes and optimum pH 4. Optimum  $Cu^{2+}$  adsorption efficiency happens at pH 4, contact time 120 minutes, 500 mg *S.cerevisiae*, initial  $Cu^{2+}$  concentration 33.764 mg/l, at room temperature ( $\pm$  28°C), shaker speed 150 rpm is 63,01%. This research adsorption process follows Freundlich Isotherm with the highest R<sup>2</sup> value between Langmuir and BET isotherm which is 0.7466. Reaction kinetic of this research follows first order kinetics with R<sup>2</sup> value higher than second order value. Several functional groups involved in adsorption of heavy metal  $Cu^{2+}$  are carboxyl acid, carboxyl groups, and amine which proved by the decreased concentration of these functional groups.

# Acknowledgment

The authors would like to thank Directorate for Research and Community Services as well as Directorate General for Strengthening Research and Development at the Ministry of Research, Technology, and Higher Education Indonesia for funding this study through Universities Prime Research Grant Program (Penelitian Unggulan Perguruan Tinggi-PUPT) 2016.

IOP Conf. Series: Earth and Environmental Science **106** (2018) 012090 doi:10.1088/1755-1315/106/1/012090

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by Perpustakaan Faltl

Submission date: 12-Apr-2023 11:06AM (UTC+0700) Submission ID: 2062238837 File name: File\_8.pdf (278.27K) Word count: 2838 Character count: 15504

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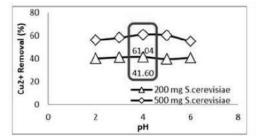


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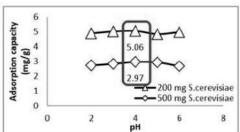


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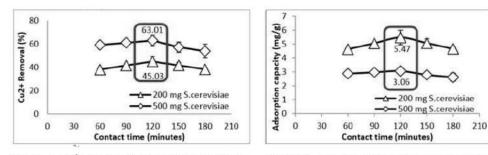


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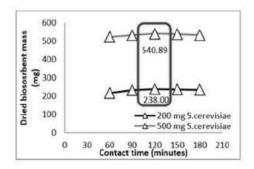


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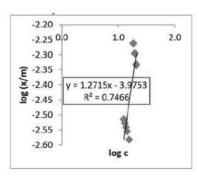


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Figure 5 shows a correlation between dried bisorbent mass with contact time. Initial 200 mg dried biosorbent mass increased up until 238.00 mg while initial 500 mg increased up to 540.89 mg. The increasing dried biosorbent weight occurs due to heavy metal ion  $Cu^{2+}$  presence from solution that adsorb into biosorbent *S. cerevisiae* in accordance with the quantity  $Cu^{2+}$  adsorb from solution into biosorbent *S. cerevisiae* cell wall, other than that there's a chance of impurities in solution that goes through the filter and weighted together with dried *S. cerevisiae* biosorbent. As contact time increase, the weight of dried biosorbent will increase until the saturation point which is 120 minutes and begin to decrease at 150 minutes.

#### 3.3. Determining Optimum Contact Time

Adsorption isotherm used to determine the occurrence of  $Cu^{2+}$  adsorption characteristic, and a curve will be generated to shows the maximum adsorption capacity of *S.cerevisiae* biosorbent based on the characteristic. Calculation of adsorption isotherm value of this study using Langmuir, Freundlich, and BET isotherm. The use of adsorption isotherms is to determine the most representative description of this study biosorption process.

Langmuir isotherm assumes that adsorbent surface has a certain number of adsorption points which is proportional to the surface area of the adsorbent and adsorption only occurs on the monolayer thus the adsorption process can only occur in the adsorbent that has not been filled by any adsorbate. Whereas for Freundlich Isotherm used to explain non-ideal adsorption process on the heterogeneous surface because of functional groups difference in adsorbent (Figure 6). BET isotherm assumed that adsorbent has homogenous surface and adsorbat molecule can form more than one layer on the surface [9].

From calculation results shown in Table 1, adsorption process of this study follows Freundlich Isotherm with the highest  $R^2$  value between Langmuir and BET Isotherm.  $R^2$  value of Langmuir Isotherm is not so different than Freundlich value, according to Freundlich the adsorbent surface is heterogeneous, composed of different classes of the adsorption region, but each class adsorption follows Langmuir isotherm, therefore the adsorption tends to be similar.

#### 3.4. Adsorption Kinetics

Adsorption kinetics is a study to determined reaction order and speed rate of adsorption process that happened. The reaction of the biosorption rate provides important information to create a batch scale biosorption system. The approach used in calculating the adsorption kinetics in this study is first order and second order.

	$\mathbb{R}^2$		R	2
Isotherm Langmuir	Isotherm Freundlich	Isotherm BET	First Order	Second Order
0.71	0.7466	0.4731	0.0448	0.0351

Table 1. R<sup>2</sup> Value calculation of adsorption isotherm and kinetics.

Based on  $\mathbb{R}^2$  value seen in Table 1, the value of first order kinetic adsorption is closer to 1 than second order thus it can be said that the adsorption kinetics that occurs tend to follow the first order. The first-order reaction is a reaction whose velocity depends only on one of the reactive substances which or is proportional to one of the reactant forces [10].

#### 3.5. Analisis Fourier Transfer Infra-Red Spectrophotometer (FTIR)

Biosorbent *S.cerevisiae* has three main functional groups which are carboxyl groups, amino groups, and hydroxyl groups. *S. cerevisiae* cell wall composed of a-mannan and b-glucan, protein, lipid, and inorganic substances particularly phosphate, chitin, and chitosan. Proteins that construct *S.cerevisiae* cell wall are rich in glutamic acid residues, aspartate, serine, threonine, and asparagine. Based on FTIR

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analysis it is known that biosorbent *S. cerevisiae* has high carboxylic acid, carboxyl group, and amine. In this FTIR results chitin included in acid carboxyl functional groups which in the 3277/cm area, which indicates the peak of O-H.

The carboxyl group in the 1625/cm region indicates the peak presence of C = O, and amine at 1234/cm indicates the peak presence of N-H. The content of carboxylic acid decreased from 92.27% to 92.04%, carboxyl group decreased from 98.04% to 89.77%, Amine from 94.17% to 92.33%, and alkane from 95.93% to 94.37%. This indicates the presence of functional groups involved in the adsorption of  $Cu^{2+}$  ions which cause changes in functional groups in the biosorbent of *S. cerevisiae* either in the presence or the content of functional group present in the biosorbent.

#### 66. Scanning Electron Microscope (SEM) Analysis

Scanning Electron Microscope (SEM) is a type of electron microscope that uses a focused beam of highenergy electrons to produce images of a sample by creating a variety of signals at the surface of the sample [11]. SEM produces high-resolution images of a sample surface, capturing a complete size of approximately 1 - 5 nm and can produce 3-dimensional shape characteristics so as to provide easier data about the analysed samples [12].

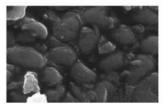


Figure 7. Control S. cerevisiae seen with SEM (500x)

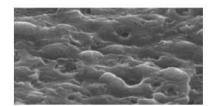


Figure 8. S. cerevisiae after contact with Cu<sup>2+</sup> in optimum condition seen with SEM (500x)

Based on Figure 7 and Figure 8 we can see the difference in cell morphology before and after contact with  $Cu^{2+}$ . As seen on Figure 8 *S.cerevisiae* cell wall looks shrivelled and have more holes than before contact (Figure 7) which look more hydrated and plump. This morphological change occurs due to  $Cu^{2+}$  ion adsorption process that happened in the cell wall during contact with electroplating wastewater. The change that happened is not too significant because the biosorbent is used in a state of death & dried so no metabolism progress occurs and disturbed by the absorption of  $Cu^{2+}$  ions.

#### 4. Conclusion

From this research, we can conclude that contact time and pH affect the adsorption of  $Cu^{2+}$  by biosorbent *S.cerevisiae*, with optimum contact time 120 minutes and optimum pH 4. Optimum  $Cu^{2+}$  adsorption efficiency happens at pH 4, contact time 120 minutes, 500 mg *S.cerevisiae*, initial  $Cu^{2+}$  concentration 33.764 mg/l, at room temperature ( $\pm$  28°C), shaker speed 150 rpm is 63,01%. This research adsorption process follows Freundlich Isotherm with the highest R<sup>2</sup> value between Langmuir and BET isotherm which is 0.7466. Reaction kinetic of this research follows first order kinetics with R<sup>2</sup> value higher than second order value. Several functional groups involved in adsorption of heavy metal  $Cu^{2+}$  are carboxyl acid, carboxyl groups, and amine which proved by the decreased concentration of these functional groups.

#### 3 cknowledgment

The authors would like to thank Directorate for Research and Community Services as well as Directorate General for Strengthening Research and Development at the Ministry of Research, Technology, and Higher Education Indonesia for funding this study through Universities Prime Research Grant Program (Penelitian Unggulan Perguruan Tinggi-PUPT) 2016.

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doi:10.1088/1755-1315/106/1/012090

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