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The 3rd ASIA FUTURE CONFERENCE

Environment & Coexistence

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AFC
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PROCEEDINGS

The Asia Future Conference aims to provide a platform for those who have studied in Japan and those who are interested in Japan, to meet and discuss about the future of Asia.

アジア未来会議は、日本で学んだ人、日本に関心のある人が集い、
アジアの未来について語る<場>を提供することを目的としています。

The Third Asia Future Conference

Date: Thursday September 29th - Monday October 3rd, 2016

*Including arrival and departure days

Venue: Kitakyushu, Japan

September 30 (Fri): Kitakyushu International Conference Center

October 1 (Sat): University of Kitakyushu (Kitagata Campus)

October 2 (Sun): Study Tour

第3回アジア未来会議

会　期: 2016年9月29日(木)～10月3日(月)

＊到着日と出発日を含む

会議場: 日本・北九州市

9月30日(金): 北九州国際会議場

10月1日(土): 北九州市立大学北方キャンパス

10月2日(日): スタディツアー

Aim of the Conference

The twenty-first century has seen the world thrust into a maelstrom of change and unpredictability. We remain hopeful in the face of rapid technological advancements, but many of us struggle to regain our bearings as longstanding social structures become upended. Internationalization and globalization have long been heralded as the keys for the future, yet a truly global path forward remains elusive, serving only to heighten the sense of uncertainty. As global citizens in this era of change, we are called anew to reexamine our world and our collective future and to seek new multidimensional and inclusive perspectives on myriad global issues.

The achievement of rapid economic development has also led to dramatic changes in Asia. At the same time, a complex set of transnational problems have been brought about by global environmental issues and increased socioeconomic globalization. In the midst of an ever-expanding understanding of "society," the global citizenry—individuals, governments, and the business community—must adopt policies that not only allow for the pursuit of individual interests but also respond to concerns for the peace and happiness of society as a whole. Solving these problems requires the development of multifaceted evaluative and analytical strategies with cooperation across national and disciplinary borders.

The Asia Future Conference is interdisciplinary at its core and encourages diverse approaches to global issues that are both mindful of the advancement of science, technology, and business and also take into consideration issues of the environment, politics, education, the arts, and culture. The Asia Future Conference is organized by the Sekiguchi Global Research Association (SGRA) in partnership with likeminded institutions, in order to provide a venue for the exchange of knowledge, information, ideas, and culture, not only by SGRA members, but also by former foreign students of Japan from various educational institutions throughout the world, their own students and collaborators, and anyone interested in Japan.

The Sekiguchi Global Research Association (SGRA) began operating in Tokyo in July 2000 as a division of the the Atsumi International Foundation, a charitable organization. At its core is a community of non-Japanese researchers who come from all over the world to conduct advanced studies in Japan and obtain doctoral degrees from Japanese graduate institutions. SGRA identifies issues related to globalization and seeks to disseminate research results to a wide audience through forums, reports, and the internet. SGRA's aim is to reach society at large rather than a specific group of specialists through wide-ranging research activities that are inherently interdisciplinary and international. The essential objective of SGRA is to contribute to the realization of responsible global citizens.

We look forward to welcoming a diverse and active group of conference participants.

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* Compilation of Papers is in the attached CD-ROM
* 論文集は添付されたCDの中にあります

1-B-302-E: Innovation (1)

Time: Saturday, 01/Oct/2016: 9:00am - 10:30am Location: B-302
Session Chair: Yutaka Tonooka
Session Chair: Tao Zhang

ID: 245 / 1-B-302-E: 1
Full Paper (5-10 pages)
English
Topics: Natural Environment, Innovation
Keywords: soft clay, rigid pavement, nailed-slab system, pull out, uplift resistance

■Behavior of Uplift Resistance of Single Pile Row Nailed-slab System on Soft Clay

Puri, Anas

Universitas Islam Riau, Indonesia

Nailed-slab Pavement System is a new kind of rigid pavement which proposed by Hardiyatmo (2008). This system consists of a thin reinforced concrete slab, and short piles attached underneath. The installed piles under the slab were functioned as slab stiffeners and anchors. Its performance due to compression loadings were conducted by some researchers. Puri, et.al (2015) reported pull out test on 1-pile row Nailed-slab System. In this paper, the finite element analysis will be done by using the test results from Puri, et.al (2015). According to Puri, et.al (2015), the full scale of 1 pile row Nailed-slab System was conducted on soft clay which consisted of 6.00 m x 1.20 m slab area with 0.15 m in slab thickness, 5 short micro piles (0.20 m in diameter, 1.50 m in length, and 1.20 m in pile spacing) as slab stiffeners which installed under slab. Piles and slab were connected monolithically, then in due with vertical concrete wall barrier on the two ends of slab. The system was loaded by pull out loading on the end of slab. The tested model has sufficiency uplift resistance and linear elastic behavior can be reached the load 13.3 kN (Puri, et.al, 2015). Behavior of Nailed-slab Pavement System can be good modeled in linear elastic zone. Contribution of vertical barrier due to pull out loading should be taken in further research. Anchorage resistance of piles was contributed to the uplift resistance. This uplift capacity is very important to resist the uplift loads around the end of slab.

ID: 355 / 1-B-302-E: 2
Full Paper (5-10 pages)
English
Topics: Natural Environment, Sustainability, Innovation
Keywords: brown color of rice straw filtrate, heavy metals, L-☒-arabino-furanosidase enzyme, heavy metal adsorption, water purification.

■Reducing Lead and the Brown Color from the Filtrate of Heavy Metal's Elimination

Kohar, Indrajati

University of Surabaya, Faculty of Pharmacy, Indonesia

Heavy metals contamination is a major concern in the world. Many studies showed that rice straw as agricultural waste could adsorb heavy metals from polluted water, and it is cheap. The drawback of rice straw is that it produced brown water, which is unacceptable for household purposes. We used enzyme L-☒-arabino-furanosidase and it reduced the brown color considerably. In our previous study, we found that the variables with the highest responds in terms of % Pb adsorbed were: enzyme-50, amount of enzyme : straw = 2 : 1, 1 hour incubation time, amount of washing: 5 x 5 ml, place of harvesting low land, and ground straw. Either demineralised water or Pb solution could be used to wash the straw. To invest the feasibility for use in daily life, especially in remote and poor villages, a more thorough and in depth study is conducted to optimize the usage of the enzyme.The addition of enzyme : straw = 1:4, and 1 hour incubation time contributed significantly toward the reduction of the brown color. To obtain bigger surface areas, the straw was shredded, not ground. However, the increase of enzyme and incubation time resulted in a decrease of the elimination of lead. This might be due to the elimination of lignin from the straw, whereas lignin also contributes to the elimination of the heavy metal. Maximum lead reduction was obtained at 0 gram enzyme and 22.3 minutes incubation time and amounts 0.63 mg Pb/g straw.

ID: 612 / 1-B-302-E: 3
Full Paper (5-10 pages)
English
Topics: Natural Environment, Social Environment, Innovation
Keywords: Fuel, plastic waste, pyrolysis reactor design, temperature distribution, CFD

■Performance Test of Pyrolysis Reactor Design for Producing Fuel from Plastic Waste

Arista, Febri Aditya Pratama; Hartulistiyoso, Edy; Yulianto, Muhamad

Bogor Agricultural University, Indonesia

The increasing amounts of plastic wastes generate enormous environmental problems. Plastics pyrolysis process is considered as alternative technology to produce fuels. Almost all of the pyrolysis experiments conducted in laboratory condition, and heat transfer is not investigated. Cylindrical reactor was designed, which has dimension of 0.31 m in diameter and 1 m high. In order to understand the temperature distribution in the reactor, five thermocouples were placed to measure temperature at the bottom to the top of the reactor, with the different position of each thermocouple of 0.19 m respectively. The Computational Fluid Dynamic (CFD) was used to know the contour of temperature inside the reactor.

This paper aims to discuss on the performance and investigates the temperature distribution in the plastic pyrolysis reactor. Pyrolysis process was occurred with 400 of pyrolysis temperature process. From 1,500 g of plastic wastes, 15.72 g of fuel and 691.98 g of solid residues were formed during the process. Temperature distribution pattern along the reactor showed that temperature decreased vertically to the top of reactor. Temperature validation using CFD simulation was approaching the value of temperature measurement on reactor. This fact shows that the pyrolysis process of plastic can produce fuel in the designed reactor.

ID: 947 / 1-B-302-E: 4
Short Paper (2-3 pages)
English
Topics: Natural Environment, Innovation
Keywords: fatty acid salts, anti-amoeba activity, Hartmannella vermiformis

■Application of fatty acid salts as biocontrol agents in the bathroom

Tanaka, Aya[1]; Era, Mariko[1]; Kawahara, Takayoshi[2]; Kanyama, Takahide[2]; Morita, Hiroshi[3]

[1]Graduate School of Environmental Engineering, The University of Kitakyushu; [2]Shabondama Soap Co., Ltd.; [3]Faculty of Environment Engineering, The University of Kitakyushu

Objective

Hartmannella vermiformis is kind of amoeba seen in the plumbing environment. Fatty acid salts are a main component of soap in one surfactant; it has also been made for reporting high antibacterial effect on bacteria. But study of the effects of fatty acid salts against H. vermiformis is few. Then, we investigate the anti-amoeba activities of fatty acid salts against H. vermiformis.

Materials & Methods

Hartmannella vermiformis NBRC 50599 was chosen as tested amoeba. Nine fatty acid salts including potassium butyrate (C4K), caproate (C6K), caprylate (C8K), caprate (C10K), laurate (C12K), myristate (C14K), oleate (C18:1K), linoleate (C18:2K) and linolenate (C18:3K) at 350 mM and pH 10.5 were used as anti-amoeba activity. The cell suspension of amoeba (3.0×104 spores/mL) was mixed with each of the fatty acid salts (final concentration of 175 mM). The anti-amoeba activity was observed at 10, 60, 180 min after mixture.

Results

C12K, C18:1K, C18:3K and LAS showed high anti-amoeba effect for 10 min against H. vermiformis. As a result of the MIC measurement test for the fatty acid salt, C12K=1.36 mM, C18:1K=0.17 mM, C18:3K=0.34 mM.

These results suggest utility that fatty acid salts use as bathroom cleaner.

`Reducing Lead and Brown Color from Heavy Metal's Elimination's Filtrate

Indrajati Kohar[1], Ratih[2], Cynthia Lynawati[3], M. Arbi Hadiyat[4]
Ni Nyoman Tri Puspaningsih[5], Leon Janssen[6], Kestrilia Rega[7]

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ABSTRACT
Heavy metals contamination is a major concern in the world. Many studies showed that rice straw as agricultural waste could adsorb heavy metals from polluted water, and it is cheap. The drawback is that it produced brown water, which is unacceptable for household purposes. Enzyme L-α-arabino-furanosidase reduced the brown color considerably. In the previous study, enzyme-50: straw = 2 : 1, 1 hour incubation time, amount of washing: 5 x 5 ml, place of harvesting: low land, and ground straw showed the highest responds in terms of % Pb adsorbed. Either demineralised water or Pb solution could be used to wash the straw. To invest the feasibility for use in daily life, especially in remote and poor villages, a more thorough and in depth study was conducted to optimize the usage of the enzyme. The addition of enzyme : straw =4:1, and 1 hour incubation time contributed significantly toward the reduction of the brown color. However, the increase of enzyme and incubation time resulted in a decrease of the elimination of lead. This might be due to the elimination of lignin from the straw, whereas lignin also contributes to the elimination of the heavy metal. Maximum lead reduction (0.63 mg Pb(II)/g straw) was obtained at 0 gram enzyme and 22.3 minutes incubation time.

Key words: brown color of rice straw filtrate, heavy metals, L-α-arabino-furanosidase enzyme,adsorption

Introduction

Heavy metals contamination of ground and surface water is of growing concern in many parts of the world, particularly in developing countries in which large populations have to use these sources for drinking and cooking water.

Indonesia is facing substantial heavy metal pollution of ground- and surface water in industrialized areas**.** A study by Sutomo[1] found that there was Pb in drinking water in an area in Yogyakarta and its impact on children. Yuliandari et al. [2] reported that the blood samples of pregnant women, breast feeding mothers and children under five years old in Kenjeran area (in Surabaya) contained heavy metals such as Cd, Hg, and Pb. Kohar et al. [3] in their study on the hair of autistic and non autistic children and adults also found an interesting results, that the hair of the autistic children contained twice as much lead as the hair of non autistic adults, and the adults hair also contained twice as much lead as in the hair of non autistic children.

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Many studies have been conducted to eliminate heavy metals from water resource such as flocculation, titration, using activated charcoal, and ion exchange, precipitation by chemicals, etc. However, because these methods are costly, the development of a more cost-effective and environmentally friendly remediation system is necessary. In order to find more cost-effective and environmentally friendly methods, several studies have been carried out since many years ago, by using living and dried plants, and agricultural wastes, such as soybean hulls, sugarcane bagasse, rice hulls, rice straw, barley straw, rice milling by product, etc., treated or untreated straw, studies on single or mixed metals solutions, or on industrial effluents [4], [5], [6], [7], [8], [9], [10], [11], [12], [13], [14].

On the other hand, Indonesia also has vast number of padi fields, and the production of rice is over 50 million tons per year, and the resulting rice straw is considered agricultural waste that is usually burnt in the fields, thus yielding a lot of smog. Yet, rice straw has not received much attention as a potential remover of heavy metals so far, and the results appear to be inconclusive. Although straw has been a good adsorbent for heavy metals in solution there is a drawback, i.e. the brown color which is produced when straw is soaked in water,

the filtrate has brown color, and of course it cannot be used in everyday needs, not to say as drinking water. Some means and materials have been used to clean the filtrate from the brown color, such as bentonite, zeolite, sand, charcoal, and carbo adsorbent[15]. However, the results were inconclusive.

A preliminary study using -α-arabino-furanosidase enzyme has been conducted in eliminating the brown color (which is due to lignin) of the straw's extract, and it showed a satisfactory result, which is extracting lignin from the straw and the end result is clear water with low concentration of heavy metal (Pb) [16]. To provide clear drinking and cooking water for the villagers in the villages with contaminated wells, the process is supposed to be simple; if possible: a) using whole straw; b) use as little as possible enzyme; c) preferably without washing. If not possible: then wash the straw with contaminated water.
So, it is imperative to continue the research using the enzyme. In this research the optimization of the process was carried out by varying on the amount of enzyme (ml), and time of incubation (h). The screening experiments showed that the variables which gave the highest respond in terms of mg Pb(II) adsorbed/g straw, were enzyme-50 : straw = 4 : 1 (4 g of enzyme for each 1 g of straw), 1 hour incubation time, amount of washing : 5 x 5 ml, place of plant: low land, and size of straw: ground. As for the type of washing liquid, both either demineralised water or Pb(II) solution were the same[17]

Materials and Method:

1. Materials:

Rice straw from lowland (Mojokerto area. East Jawa).Demineralised water (Faculty of Pharmacy Laboratory). α-L-arabinofuranosidase enzyme (University of Airlangga). Lead acetate.$3H_2O$ p.a., and Standard stock Lead solution (1000 ppm) (Merck).

2. Instrumentation:

Spectrophotometer (Cintra, Hewlett Packard, USA). Gram Balance. Analytical Balance (Sartorius. type BJ 210S), Inductively Coupled Plasma Spectrometeter - **ICPS** (Fisons, model 3410 ARL, USA), with argon as reactant gas.

3. Work Protocol

3.1. Preparation of the rice straw.

Rice straw from lowland was dried under the sun for 1 day, then was shredded/punched to break the skin. The length of the straw was around 30 cm. To simplify the preparation of the straw, it was shredded or punch, not ground.

3.2. Preparation of the enzyme

The ratio of the straw and the enzyme were 1:0 = did not use enzyme, just sprayed with demineralised water; 1:2 = 4 grams of straw : 8 g of enzyme, and 1:4 = 4 grams of straw : 16 grams of enzyme.

The enzyme which was used in this step was produced in "application" medium, which contains defined salts. The enzyme which was used in the precious step was produced in "Luria Bertani" medium which was more expensive. The changing of the type of the enzyme was carried out to make the process cheaper, so that it can be applied in the community with minimum /less cost. The "applied" enzyme was produced using *Bacillus subtilistermophile*, which was inoculated into a solid medium, and then was grown about 16 – 18 hours. Then the bacteria were moved to a liquid medium. 1% of the liquid medium was taken to be able to produce a bigger scale. Then it was harvested, and centrifuged, and then the supernatant liquid and pellets were obtained. Because the enzyme was an extracellular enzyme, the supernatant was taken.

3.3. Preparation of washing liquid (5 ppm Pb(II) solution).

Washing liquid was prepared by dissolving $(CH_3COO)_2Pb.3H_2O$ (9.15 mg) in demineralised water to make 1000 ml solution (5 ppm of Pb(II)). Pb(II) solution was used to wash the straw, to make it easier to apply this method in the village, i.e. to wash the straw with contaminated well water. In the previous step it was proven that Pb solution as washing liquid did not affect the adsorbtion of Pb(II) by enzyme-pretreated straw.

3.4. Experiments on the Adsorption of Pb(II) by the filter Paper.

A working solution of 5 ppm Pb(II) was prepared from a standard solution of Pb(II) 1000 ppm. Six experiments were conducted. The first one was measuring the intensity of the Pb(II) solution without filtration by the ICP, then the second experiment up to the sixth were measuring the intensities of Pb(II) after filtration by filter paper Whatman 41. All measurements in this step were carried out at λ 283.306 nm.

Adsorption of Pb(II) by the filter paper =

[(Concentration of 5 ppm Pb(II) solution without filtration)-(Average concentration of 5 ppm Pb(II) after filtration)] / concentration of 5 ppm Pb(II) solution without filtration) x 100% → (% adsorbed by filter paper).

3.5. Preparation of Pb(II) Working Standard Solution and of the Calibration Curve

Pb(II) solutions (0.5; 1; 2; 5 and 10 ppm) were prepared from 1000 ppm standard solution, each one of them were filtered by Whatman 41 filter paper, their intensities were measured by ICP at λ 283.306 nm. and a calibration curve was made by plotted the concentrations vs the intensities of Pb(II) solution.

3.6. Checking the Reliability of the Calibration Curve.

To make sure that the result of the ICP measurements were correct, the calibration curve need to be checked as follows:

5,0 ml Standard solution (1000 ppm Pb(II)) was pippeted to a 100,0 ml volumetric flask, then demineralised water was added to the mark (100,0 ml). From this solution was pippeted 2,0 and 5,0 ml into 50,0 ml volumetric flasks, demineralised water was added to the marks, then the solutions were filtered to make 2 ppm and 5 ppm Pb(II) solutions.

1. 2 ppm and 5 ppm solutions were measured and determined their concentrations using the present calibration curve.
2. Conclusion was made by :

a. If the results of ICP reading show a different not more than 5% of the 5 ppm standard Pb(II) solution, the calibration curve was still good and can be used in the experiment.

b. If the results of ICP reading show a different more than 5% of the 5 ppm standard Pb(II) the calibration curve cannot be used, and a new one must be made.

3.7. Blank experiments

To demineralised water was added 1 gram of pretreated straw, then water was added until 200 grams, then it was left for 1 hour, then was filtered by Whatman ≠ 41 filter paper. The straw was dried under the sun, and referred as enzyme-pretreated-straw, the filtrate was weighed and the intensity was measured by ICP, and the absorbance was measured by Spectrophotometer. The intensity of the water itself was also measured. These data are used to calculate the amount of Pb(II) adsorbed.

3.8. Sample preparation

Rice straw (punched, 30 cm length) was weighed (4 grams), then water or enzyme, α-L-arabinofuranosidase 50ºC (8 or 16 grams) was sprayed to the straw and incubated at ambient temperature at different incubation times. The incubation time was counted after the entire water or enzyme was sprayed to the straw, then after the certain incubation time, the straw was washed with Pb solution (1 x 60 ml), and then was filtered with filter paper. The filtrate was discarded and the straw was dried under the sun. The enzyme pretreated-straw was soaked in 5 ppm Pb(II) solution, left for 1 hour, then was filtered. The color of the filtrate was measured by spectrophotometer at 473 nm and the Pb(II) content by ICPS. The incubation time was not too long, only up to 60 minutes, because in the previous experiment, the incubation time more than 60 mins did not show significant different in terms of the absorbance of the filtrate.

Calculation of Pb(II) adsorbed/g straw: [(Initial concentration (ppm)/1000) x the weight of solution (gram)]-[(final concentration (ppm)/1000) x weight of filtrate (gram)] / weight of straw (gram)

3.9.*Response Surface Methodology*

In this study,*Response Surface Methodology* was applied to find optimum independent variables setting which will obtain maximum response. This methode is used to develop, to enhance and to optimise the process[18].

Result and Discussion

1. Calibration curve of Pb(II) solution

Table 1. Concentrations and Intensities of Pb(II) solution

Concentration	Intensities
0.0	0.046
0.5	0.061
1.0	0.077
2.0	0.100
5.0	0.192
10.0	0.333

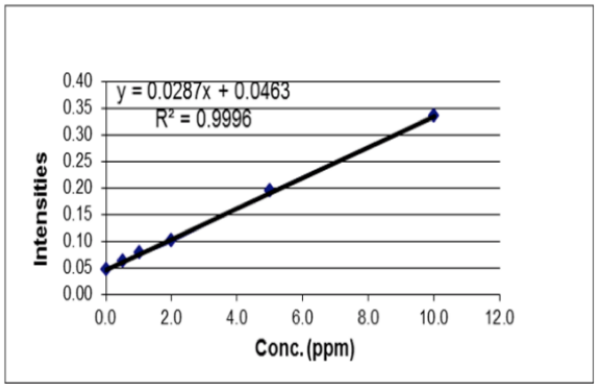

Fig 1. Calibration curve of Concentration (ppm) vs Intensities of Pb(II) solutions

2. Adsorption of Pb(II) by the Filter Paper

It was found that the average of Pb adsorbed by the filter paper is around 0.66%. Because the filter paper can influence the adsorption of Pb, all the

solutions in the experiments are filtered prior measuring by the ICP.

3. Statistical Analyses of the Optimization Condition of The Amount of Enzyme and of The Incubation Time vs mg Pb adsorbed/g straw

Screening experiment
From the previous experiment, it was known that there were 2 variables that has an effect towards the adsorption of Pb, i.e. the ratio of straw:enzyme and the incubation time.

Determination of starting (initial) point
1) The Results around the starting point.

Table 2. Design Faktorial 2^2

Natural variables		*Coded variables*		Response (y)	Absorbance	
ξ_1 (gram)	ξ_2 (mins)	X_1	X_2	(mgPb adsorbed/g straw)	Value	Symbol
0	0	-1	-1	0.44	0.053	+++
0	60	-1	1	0.36	0.022	+
16	0	1	-1	0.43	0.025	++
16	60	1	1	0.43	0.011	+
8	30	0	0	0.41	0.028	++
8	30	0	0	0.45	0.034	++
8	30	0	0	0.42	0.038	++
8	30	0	0	0.42	0.017	+
8	30	0	0	0.39	0.022	+

Remarks:
ξ_1 : Natural variable for ratio of straw:enzyme
ξ_2 : Natural variable for incubation time
X_1 : Coded variable for ratio of straw:enzyme
X_2 : Coded variable for incubation time

Remarks of the value of absorbancewe:
+ = absorbance 0.000-0.022: clear solution
++ = absorbance 0.023-0.045: pale yellow
+++ = absorbance 0.046-0.068: turbid yellow
++++ = absorbance 0.069-0.091: brownish yellow

Checking linearity around starting point
H_0 = *linear* model is not significant
H_1 = *linear*model is significant
Conclusion = H_0 is accepted because the test statistic value: P>0.05

❖ Statistical Analyes using *software* Minitab 14 with *Response Surface* Method (*Linear-Interaction* Model) :

Response Surface Regression: mg Pb adsorbed vs Code amount , Code time i

The analysis was done using coded units.
Estimated Regression Coefficients for mg Pbterjerap/g jerami

Term	Coef	SE Coef	T	P
Constant	0.41667	0.006498	64.124	0.000
Code amount of enzyme	0.01500	0.009747	1.539	0.184
Code inc time -	0.02000	0.009747	-2.052	0.095
Code amount of enzyme*	0.02000	0.009747	2.052	0.095
Code inc time				

S = 0.01949 R-Sq = 68.3% R-Sq(adj) = 49.3%Analysis of Variance for mg Pb adsorbed/g straw

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	3	0.004100	0.004100	0.001367	3.60	0.101
Linear	2	0.002500	0.002500	0.001250	3.29	0.123**
Interaction	1	0.001600	0.001600	0.001600	4.21	0.095
Residual Error	5	0.001900	0.001900	0.000380		
Lack-of-Fit	1	0.000020	0.000020	0.000020	0.04	0.847
Pure Error	4	0.001880	0.001880	0.000470		
Total	8	0.006000				

Path of Steepest Accent was conducted after Factorial 2^2 step was carried out. However, in the previous step i.e. factorial 2^2 step, it was found that the linearity was not conformed, which was shown by the linear value = 0.123** (linearity was conformed if the value P < 0.05), for that, the *steepest accent* step (the adding of Δ is continuously carried out until maximum/minimum point of the response is obtained) can be neglected and the experiment can be done straight to the next step, i.e.

1. Checking the curvature around the initial point by using *CCD (Central Composite Design).*
2. PSA *(Path of Steepest Accent*) was not conducted
3. Checking the initial point was not carried out, but checking the curvature around the initial point will be conducted straight away.
4. Experimental Design by CCD (*Central Composite Design*)

Table 3. Central Composite Design (CCD)

Natural variables		***Coded variables***		**Response (y) (mgPb adsorbed/g straw)**	**Absorbance**	
ξ_1 **gram**	ξ_2 **mins**	X_1	X_2		**Value**	**Symbol**
0	0	-1	-1	0.44	0.053	+++
0	60	-1	1	0.36	0.022	+
16	0	1	-1	0.25	0.037	++
16	60	1	1	0.12	0.020	+
8	30	0	0	0.41	0.028	++
8	30	0	0	0.45	0.034	++
8	30	0	0	0.42	0.038	++
8	30	0	0	0.42	0.017	+
8	30	0	0	0.39	0.022	+
19.5	30	1.414	0	0.18	0.036	++
0	30	-1.414	0	0.48	0.041	++
8	73	0	1.414	0.13	0.039	++
8	0	0	-1.414	0.34	0.051	++

Remarks on absorbance values :
+ = absorbance 0.000-0.022 clear solution
++ = absorbance 0.023-0.045 pale yellow
+++ = absorbance 0.046-0.068 turbid yellow
++++ = absorbance 0.069-0.091 brownish yellow

Data analyses of CCD (*Central Composite Design*)
H_0 = regression model *Full Quadratic* is not significant
H_1 = regression model *Full Quadratic* is significant
Conclusion = H_0 was rejected because test statistic value P<0.05

❖ Statistical Analyses by *software* Minitab 14 with *Response Surface Method* (*Full Quadratic* Model) :

Response Surface Regression: mg Pb adsorbed versus Code amount, Code time i
The analysis was done using coded units.
Estimated Regression Coefficients for mg Pb adsorbed/g straw

Term	Coef	SE Coef	T	P
Constant	0.41800	0.009308	44.908	0.000
Code amount of enzyme	-0.10679	0.007359	-14.512	0.000
Code incubation time	-0.06338	0.007359	-8.612	0.000
Code amount enzym*Code amount enzyme	-0.04150	0.007893	-5.258	0.001
Code incubation time*Code incubation time	-0.08902	0.007893	-11.278	0.000
Code amount of enzyme*Code incubation time	-0.01250	0.010406	-1.201	0.269

S = 0.02081 R-Sq = 98.4% R-Sq(adj) = 97.2%

Analysis of Variance for mg Pb adsorbed/g straw

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	5	0.185399	0.185399	0.037080	85.60	0.000
Linear	2	0.123349	0.123349	0.061675	142.38	0.000**
Square	2	0.061424	0.061424	0.030712	70.90	0.000**
Interaction	1	0.000625	0.000625	0.000625	1.44	0.269
Residual Error	7	0.003032	0.003032	0.000433		
Lack-of-Fit	3	0.001152	0.001152	0.000384	0.82	0.548**
Pure Error	4	0.001880	0.001880	0.000470		
Total	12	0.188431				

From the statistical analyses above, it was seen that *Full Quadratic* Model did fit for mg Pb adsorbed/g straw in step *CCD (Central Composite Design)*, by *linear* value = 0.000** (P<0.05), *square* = 0.000** (P<0.05) and did not fit for 0.548** (P>0.05). This means that the above modeling did fit, so that it can be concluded the highest (top) point of the three dimensional model of ratio of straw:enzyme, incubation time and mg Pb(II) adsorbed/g straw has been found. The three dimensional mathematical equation is:

$$y = 0.418 - 0.107\, x_1 - 0.063\, x_2 - 0.042\, x_1^2 - 0.089\, x_2^2 - 0.013\, x_1 x_2$$

Remarks :

- x_1 = optimum amount of enzyme
- x_2 = optimum incubation time

5. Determining optimum value

From the model above, the x_1 and x_2 value can be obtained, which will produce maximum y and it can be calculated with the following equations:

$$x_x = -\frac{1}{2} B^{-1} b$$

$$x_x = -\frac{1}{2}\begin{bmatrix} \beta_1^{\,2} & \frac{\beta_1\beta_2}{2} \\ \frac{\beta_1\beta_2}{2} & \beta_2^{\,2} \end{bmatrix}^{-1} \begin{bmatrix} \beta_1 \\ \beta_2 \end{bmatrix}$$

$$-\frac{1}{2}\begin{bmatrix} -0{,}107 \\ -0{,}063 \end{bmatrix}\begin{bmatrix} -0{,}042 & -0{,}007 \\ -0{,}007 & -0{,}089 \end{bmatrix}^{-1} = \begin{bmatrix} -1{,}23096 \\ -0{,}25712 \end{bmatrix}$$

Code of amount of enzyme to get optimum *response*:

$$x_1 = -1.23096$$

Code of incubation time to get optimum *response*:

$$x_2 = -0.25712$$

Calculation for optimum value of the real amount of enzyme and incubation time:

- For optimum amount of enzyme: -1.23096 = 0 gram, because the value is below – 1, whereas the code – 1 referred to 0 gram amount of enzyme
- For optimum incubation time

$$\frac{x - 30}{0 - 30} = \frac{-0{,}25712 - 0}{-1 - 0}$$

$$-x + 30 = 7{,}7136$$

$$x = 22{,}2864 \text{ min} = 22{,}3 \text{ min}$$

By statistical analyses using *response surface* method, it was found that 0 gram enzyme (code -1.23096) and 22.3 minutes incubation time (code -0.25712) yield 0.63 mg Pb adsorbed/g straw as maximum. To be able to see the condition of the filtrate in the position of maximum mg Pb adsorbed/g straw, vertical and horizontal line can be drawn in the *countour plot* (**Fig 2**) below. The values of the filtrate's absorbance vs amount of enzyme and incubation time can be observed as follows:

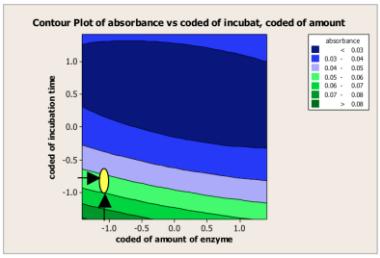

Fig 2. *Countour plot* of Optimum absorbance vs Amount of Enzyme and Incubation time

From the yellow dot in Fig 2, it can be seen that even though the mg Pb adsorbed/g straw is maximum, the absorbance is not satisfactorily (absorbance around 0.05-0.06). The color was still yellowish (pale yellow - turbid yellow, see **Table 3**).

To confirm the results, another experiment was conducted:

Experimental Results of Anova Factorial Design

Table 4. Experimental Results

Natural variables		***Coded variables***		**Response (y) (mgPb adsorbed/g straw)**	**Absorbansi**	
ξ_1 **gram**	ξ_2 **mins**	X_1	X_2		**Value**	**Symbol**
0	0	-1	-1	0.16	0.027	++
0	0	-1	-1	0.18	0.046	+++
0	0	-1	-1	0.44	0.053	+++
0	30	-1.414	0	0.47	0.035	++
0	30	-1.414	0	0.48	0.041	++
0	30	-1.414	0	0.45	0.042	++
0	60	-1	1	0.52	0.057	+++
0	60	-1	1	0.36	0.022	+
0	60	-1	1	0.51	0.057	+++
8	0	0	-1.414	0.34	0.051	+++
8	0	0	-1.414	0.47	0.048	+++
8	0	0	-1.414	0.50	0.051	+++
8	30	0	0	0.42	0.017	+
8	30	0	0	0.42	0.038	++
8	30	0	0	0.41	0.028	++
8	60	0	1	0.42	0.021	+
8	60	0	1	0.44	0.035	++
8	60	0	1	0.42	0.035	++
16	0	1	-1	0.25	0.037	++
16	0	1	-1	0.35	0.054	+++
16	0	1	-1	0.43	0.025	++
16	30	1	0	0.43	0.030	++
16	30	1	0	0.46	0.044	++
16	30	1	0	0.46	0.027	++
16	60	1	1	0.09	0.019	+
16	60	1	1	0.12	0.020	+
16	60	1	1	0.43	0.011	+

Remarks of **table 4**:
+= absorbance 0.000-0.022: clear solution
++= absorbance 0.023-0.045: pale yellow
+++= absorbance 0.046-0.068: turbid yellow
++++= absorbance 0.069-0.091: brownish yellow

1) Anova

Anova factorial statistical analyses:

H_0 = there is no significant different between the results of different treatments

H_1 = at least there is one significant different between different treatments.

Conclusion = H_0 is accepted

General Linear Model: Mg Pb adsorbed vs Amount of Enzyme, Incubation time

Factor	Type	Levels	Values
Amount of enzyme	fixed	3	0, 8, 16
Incubation time	fixed	3	0, 30, 60

Analysis of Variance for mg Pb adsorbed/g straw, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Amount of Enzyme	2	0.038807	0.038807	0.019404	2.08	0.154
Incubation time	2	0.047652	0.047652	0.023826	2.55	0.106
Amount of Enzyme*Inc time	4	0.121304	0.121304	0.030326	3.25	0.036*
Error	18	0.167867	0.167867	0.009326		
Total	26	0.375630				

S = 0.0965708 R-Sq = 55.31% R-Sq(adj) = 35.45%

From the results of the statistical analyses above, it was found that there was an interaction between the amount of enzyme and incubation time, which can be seen by the valueof P= 0.036* where there is an interaction when the value of P<0.05.

***InteractionPlot* (*data means*) of the Amount of Enzyme and Incubation Time vs Absorbance in the Release of Lignin Experiments and of mg Pb(II) adsorbed/g straw**

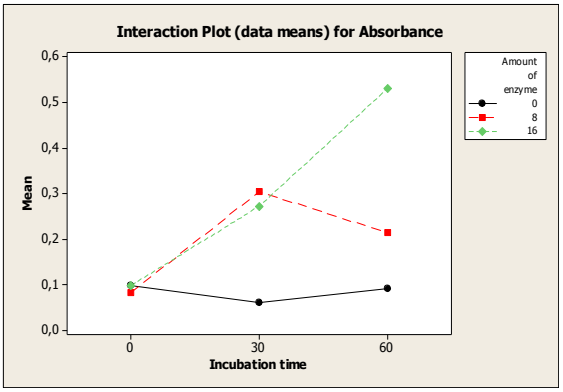

Fig 3. *InteractionPlot* of the Amount of Enzyme and Incubation Time vs Absorbance in the Release of Lignin Experiments

Fig 3 showed that the adding of enzyme contribute significantly toward the absorbance of the filtrate. And also the incubation time as well, the longer the incubation time, the higher the absorbance of the filtrate.

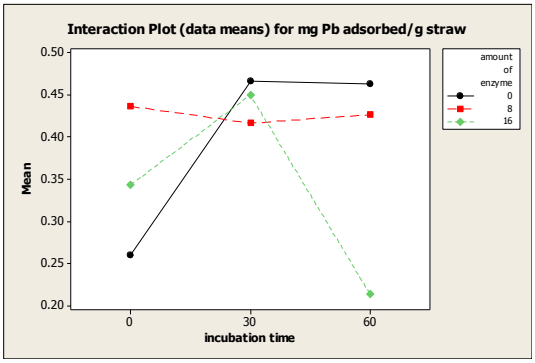

Fig. 4. *Interaction Plot* of the Amount of Enzyme and Incubation Time vs mg Pb adsorbed /g straw

Fig 4 showed the relations between the release of lignin (the brown color)and the adsorbing of Pb by straw. In the 0 minutes incubation time, there were a variation in the adding of enzyme (0, 8, and 16 grams for each gram of straw) on the results of mg of Pb(II) adsorbed/g straw, and this might be due to the in-homogenity of the straw in the first place, so that it cannot be sure whether the content of cellulose, and lignin in all the straw was the same. While when 16 grams of enzyme was added (straw:enzyme = 1:4), at longer incubation time (60 mins), the adsorption of Pb(II) was dropped significantly. Because the added enzyme and the incubation time was increase, the more lignin was also released, and because lignin also has the activity to adsorb heavy metals, there was less active site for Pb(II) adsorption[19], which make the adsorption of Pb(II) considerably decreased.

Conclusion

It can be concluded that at zero enzyme and 1 hour incubation time, the mg Pb(II) adsorbed is optimum i.e. 0,63 mg/g straw, but the color of the filtrate is still pale yellow to turbid yellow.

Because of the limitation of the statistical analyses, the result is also cannot be 100% reliable. In this study, the mg of Pb adsorbed/g straw obtained in the repetition/replication were fluctuated, this might due to the possible in-homogenous of the straw, so that it could not be determined whether the lignin and cellulose content in all the straw sample was homogenous. It is advisable that in the future study to use more samples, and then to choose a lower coefficient of variance (CV), or to apply another program of statistical analyses which can compare the interaction of less homogenous inter variable. The activity and mechanism of lignin on the adsorption of heavy metals is also important to explore.

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