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An Increase of Silicon Recovery from *Oryza sativa* L. Husk by Cow Rumen Fluid Treatment

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Abstract

Si is one of the beneficial nutrients and has various essential roles to cope with stresses situation in the graminaceous plant. The availability of Si on earth is abundant however the form that can be readily uptake by the plant is limited, and without external addition, the silica content in the cultivated land would be depleted and decreasing plant growth, recycle Si from the decaying plant is a better alternative to protect from that situation. Rice husk is a leftover paddy by-product having significant Si content that can be reused. This study offered a biological way to treat the rice husk using rumen fluid as a better alternative to the high energy consuming thermal procedure. Rice husk was macerated in rumen fluid, the kinetic and model of lignocellulose degradation were evaluated. The study in Si release in liquid media i.e. 0.05 M hydrochloric acid, 0.1 citric acid and aquades, and Si extraction in alkaline solution 1 M KOH using the rumen-treated husk showed that this method could be a better alternative to develop high Si content of solid and liquid fertilizer, respectively.

Keywords: biodegradation, lignocellulose, rice husk, rumen fluid, silica

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1. Introduction

Silicon (Si) has various essential roles in graminaceous plants i.e. wheat, paddy, corn, etc. Its existence is found to relieve several forms of biotic and abiotic stresses. Studies reported that Si provides a mechanical barrier through the deposition in non-soft tissue as protection from insects and pathogens, and as a chemical inducer i.e. alters the presence level of herbivore-induced plant volatiles compound [1]. Moreover, Si application is found to alleviate the stresses caused by temperatures, salinity, and elements toxicity [2]. It increases the tolerance of salt concentration, protects from the oxidative stress damage, and reduce the inhibitory effect of Na^+ [3]. The silicon soil amendment succeeds to increase the yield and supresses the diseases in paddy due to the thickening in the epidermal layer to reduce palatability and digestibility in the pests and stimulation of insects-resistance biochemical pathway [4].

The availability of Si is abundant in the world. It is the second most abundant element in the earth's crust in crystalline form, quartz, or silicon oxide (SiO_4, SiO_2). However, not all of the available Si can be taken directly by plants, the crystal phase is un-dissociated due to having lower solubility which is 10^{-4} M than the amorphous one, $10^{-2.74}$ M that is normally present in soil [5]. The absorbed Si is distributed and accumulated in the aboveground plant biomass, particularly in straw and grain tissue and having strong correlation with the presence of other elements i.e. C, N and P [6]. The Si-derived from plants is very essential as the accumulated Si, in the form of phytolith, will be recycled in the soil solution and can be reabsorbed by the new plants once the decay of plants occurred [7].

Rice (*Oryza sativa* L.) is commonly known as Si accumulator, more than 50% of Si uptake is deposited in straw, which is 59% of the total biomass [8]. While the rice husk contains Si as much as 91.42% of its ash content, and it could achieve up to 20% of rice husk in dry weight, and the rest comprise cellulose (35-40%), hemicellulose (15-20%), and lignin (20-24%) [9]–[11]. The common method in Si recovery from rice husk is through chemical and combustion process, the product i.e. husk charcoal or biochar is subjected to alkaline reaction and then apply acid solution to precipitate Si in a form of H_2SiO_3 [12]. These robust methods have disadvantages such as high-energy usage due to high-temperature operation, and requires neutralization step due to strong alkaline and acid consumption in extraction processes [13].

A biological approach in rice husk treatment is a potential alternative in Si recovery, as such, it can be celebrated in mild temperature and consuming less toxic chemicals. The process proposed was conducted using rumen cow fluid due to the composition consists of lignocellulose-degrading bacteria and enzyme mixture [14]. Studies reported that rumen fluid has the activity of each complex enzyme cellulase, hemicellulase, and lignolytic enzyme with the highest value at 80, 250, and 35 U/L respectively [15]. Therefore, the lignocellulose structure of rice husks can be loosened enabling the access of Si-leaching chemicals. The investigation of rumen fluid application for this purpose was rarely studied, commonly it applies to lignocellulose substrate for methane formation and nutritional uptake for animal feed. Using cattle rumen fluid for riverbank grass waste treatment at 38.6°C resulted in 50% decrease of chemical oxygen demand which was representing the amount of organic compound in the mixture [16]. Pretreatment of rice straw, to produce biomethane, using rumen fluid succeeded to reduce the total solid up to 65% after 5 incubation days [17], and when applied to the grass clipping to enhance hydrolysis performance, the content of soluble chemical oxygen demand increased up to 6.5 g/L after 3 days of incubation period, indicating the lignocellulose hydrolysis occurred [18].

The purpose of the study was to examine the influence of incubation time in lignocellulose degradation using rumen fluid on the amount of Si extracted using maceration method and to evaluate the kinetic model in rice husk lignocellulose biodegradation. The objective was to increase the Si release in several liquid media with the pretreatment using rumen fluid based on incubation time and to compare the extraction results between biodegradation and thermos-degradation treatment, and to determine each kinetic coefficient in cellulose, hemicellulose and lignin degradation. The information given in this investigation can be used to develop Si-containing solid and liquid fertilizer with more efficient in energy and chemicals consumption.

2. Materials and Methods

2.1. Materials

The rice husk was collected from Bunihayi Village, in Subang, West Java, Indonesia. It was air-dried at room temperature and ground to the size of 40-mesh using a kitchen grinder (Philips, HR2116). The rumen fluid was obtained from a local slaughterhouse in Cikuda village, in Jatinangor, West Java. The fluid was kept in the temperature range of 38-40°C close to the body temperature of the ruminants [17]. The chemicals used such as H_2SO_4, KOH, 1-amino-2-naphthol-4-sulphonic acid, molybdic acid, hydrochloride acid, oxalic acid, and acetic acid were analytical grades and obtained from chemicals warehouse of School of Life Sciences and Technology Institut Teknologi Bandung.

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2.2. Methods

The experiments focused on husk lignocellulose degradation in variation of time, and the rumen-treated result was compared to the husk char with a certain amount of carbon content. The result from optimum time would be subjected to Si release assay in liquid media, and the comparison of Si extraction using alkaline solution for husk charcoal, rumen-treated husk and untreated husk as a control. This experiment was conducted in duplicate.

2.2.1. Husk char synthesis

The rice husk of 10 g in a lid covered chamber was placed in the furnace. It was maintained at various temperature 350ºC for 90 minutes [19]. The ash content of the char resulted will be determined at the temperature of 950ºC for 60 min [20].

2.2.2. Husk degradation using rumen fluid

The rice husk powder of 10 g with 15% moisture content was added into 200 ml rumen fluid in a 300 ml Erlenmeyer flask. After that, it was incubated at 38ºC, for 24, 48, 72, 96, and 120 hr in an incubator shaker. The mixture then was filtered through Whatman No.1 filter paper and dried at 105ºC for 24 h.

2.2.3. Lignocellulose content measurement

Lignocellulose content in each sample was measured using Chesson-Datta method [21]. The sample of 1 g was added into 150 ml distilled water and refluxed for 2 hr at 100ºC. After that, it was filtered, and dried under 105ºC for 24 hr to determine the hot water soluble (HWS) content. The previous treatment was subjected to hemicellulose determination, 0.5 M H_2SO_4 of 150 ml was added, and refluxed at 100ºC for 2 hr. Then it was filtered and dried as the previous one. The hemicellulose-lack residue was added into 10 ml of 72% (v/v) H_2SO_4 and incubated in room temperature for 4 hr, after that it was diluted to 0.5 M, and refluxed for 2 hr at 100ºC. After filtered and dried, the cellulose content was measured. The residue left was then pyrolised at 575ºC for 2 hr. After that, the sample was cooled, and the ash and lignin content were determined. The entire component was determined using the equation as follow:

$$\text{Hot water soluble (HWS)}(\%) = \frac{a-b}{a} \times 100\% \quad [1]$$

$$\text{Hemicellulose } (\%) = \frac{b-c}{a} \times 100\% \quad [2]$$

$$\text{Cellulose}(\%) = \frac{c-d}{a} \times 100\% \quad [3]$$

$$\text{Lignin } (\%) = \frac{d-e}{a} \times 100\% \quad [4]$$

$$\text{Ash } (\%) = \frac{e}{a} \times 100\% \quad [5]$$

Where a, b, c, d, and e were the initial sample mass, mass without HWS, mass without HWS and hemicellulose, mass without HWS-hemicellulose-cellulose, and ash, respectively.

2.2.4. Degradation efficiency and Kinetics

The degradation efficiency of the lignocellulose content of the husk was calculated according to Eq. 6 [17] as follows:

$$\eta_j = \frac{m_{i,ini} - m_{i,fin}}{m_{i,ini}} \quad [6]$$

Whereas the kinetics mechanism was evaluated using the Michaelis-Menten equation as a form below:

$$\frac{d\eta_i}{dt} = v_{max,i} \frac{(1-\eta_1)}{K_{m,i} + c_{o,i}(1-\eta)} \quad [7]$$

Where η_i is degradation efficiency [dimensionless] of composition i such as cellulose, hemicellulose, or lignin of the rice husk, v_{max} is the maximum rate of degradation [g/L-h], Km is half-saturation constant for each component concentration [g/L], and $c_{o,i}$ is the initial component concentration [g/L].

2.2.5. Si release assessment from rumen-treated husk and husk char in liquid medium

The liquid media used were distilled water, 0.1 M citric acid solution [22], 0.05 M hydrochloric acid [23], and at this step the focus was emphasized to performance of each type sample in Si release. A total of gram from each sample was added into 200 ml of each medium and incubated at room temperature in an orbital shaker at 130 rpm. The observation was carried out at a specified time for 36 hr. The silica content in the liquid media was analysed using the ASTM Method: Standard Test Method of Silica in Water (2000) [24].

2.2.6. Si extraction from rumen-treated husk and husk char

A total of 25-gram amount from each sample was added to 100 ml of 1 M KOH solution. It was incubated at 38ºC, 225 rpm, and the observation was taken in 2 hr interval time. The mixture was filtered through a Whatman 1 filter paper and the silica content in the filtrate was measured according to the previous method.

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3. Results and discussion

3.1. Husk Char and Lignocellulose degradation of rumen-treated husk

Thermal process resulted in husk char with carbon content (volatile matter and fixed carbon) as much as 72.38 %, this value was about the same with several reports conducting the procedure at the same temperature which was 72.8% on dry basis [25], [26]. Whereas the initial content of cellulose, hemicellulose, and lignin in rice husk for this investigation were 43, 17, and 26% by weight, respectively, the value obtained in this investigation were not the same to other literatures with the average such as 33.43, 20.99, and 18.25% respectively [27], [28]. The treatment of husk by using rumen resulted in an increase of the degradation with the time courses as shown in Fig. 1. The highest efficiency achieved for cellulose, hemicellulose, and lignin were 89, 17, and 21% respectively after a 120-hour period of incubation.

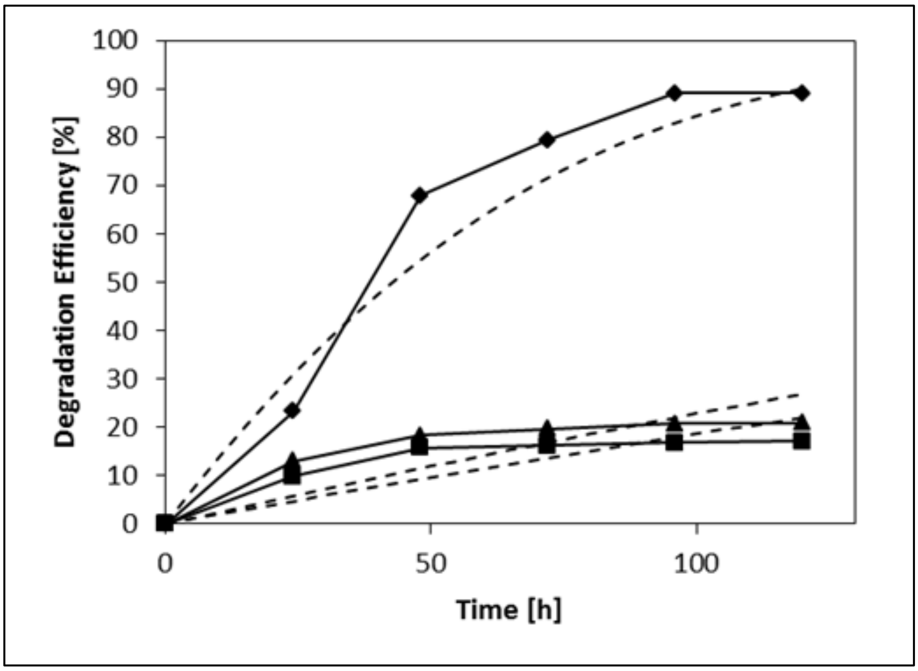

Figure 1 Lignocellulose profile of rumen-treated husk for 120 hours (♦) cellulose, (■) hemicellulose, and (▲) lignin content, the dashed lines are the predicted model kinetics.

The cellulose degradation was the highest, and the significant increase took place from 24 to 96 h time courses, after that it tended to be stationer, while the hemicellulose and lignin showed the similar pattern. Treatment using rumen fluid on rice biomass found to be effective in reducing lignocellulose as investigated in rice straw treatment that the stationer level was achieved at 96 h of incubation time with the efficiency for cellulose, hemicellulose, and lignin was 46, 59, and 21 % respectively [17], comparing with this results the current investigation had higher activity in cellulose degradation and lower for hemicellulose one.

This results also implied that the rumen fluid had specialized enzymes secreted by the indigenous microorganisms for lignocellulose degradation, the presence of this microbial community have been detected through several modern molecular techniques [29]. Several investigations in the potential microorganism of rumen fluid were carried out, a significant reduction in content was achieved on cellulose in 72 h treatment as much as 23%, and it was found that the dominated microorganism was *Firmicutes*, and this particular group of bacteria are the potential cellulase producer [30], [31]. Another investigation also showed that the particular microorganism has the ability to degrade lignin due to extracellular peroxidase [32]. The lignin degradation in this investigation was higher than the hemicellulose. Its value was almost close to other investigations when using rumen to degrade wheat straw and corncob, with efficiency at 25.5 and 30% respectively [33].

3.2. Kinetic model of husk lignocellulose degradation

The calculated kinetics parameter v_{max} for cellulose, hemicellulose, and lignin can be shown in the Table 1.

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Table 1 Kinetic parameters value i.e. maximum rate (v_{max}), and saturation constant (K_m) for lignocellulolytic activity in the rumen fluid.

Component	Kinetic parameters	
	v_{max} (g/L-h)	K_m (g/L)
Cellulose	0.77	33.11
Hemicellulose	0.04	13.25
Lignin	0.08	19.87

The kinetics for lignocellulose degradation using rice husk as a substrate particularly by a rumen fluid is not yet reported, the kinetic coefficients calculated were the apparent values not the intrinsic one due to the biocatalyst consists of many microbes. However, as a comparison the values could be compared to the intrinsic values of kinetic for cellulase produced by *A.fumigatus* Z5 at 37.8 g/L for K_m, and 437.3 µmol/min-mg for v_{max} [34], and for lignolytic enzyme from *Pleuorotus* sp. was calculated to be 250 mM and 0.33 µM/min for K_m and v_{max} respectively [35].

3.3. *Silica release from char and rumen-treated husk*

At this step, the study was trying to mimic the natural environment of soil for Si leaching from organic material using both weak and strong acids, these acid concentrations were selected due to commonly applied in inorganic material leaching in the soil [22], [23]. Based on the solvent, all type of sample had the highest solubility in 0.05 M hydrochloric acid solution, whereas the lowest for each sample category was obtained in distilled water solvent (Figure 2.). For the across type sample rumen-treated husk in HCl solution had the most soluble Si, after achieved the equilibrium condition at 24 hr, after that it was followed by husk char in HCl, then rumen-treated in 0.1 citric acid solution, then husk char in citric acid and control in the HCl. The less soluble Si came from control and husk char in distillate water.

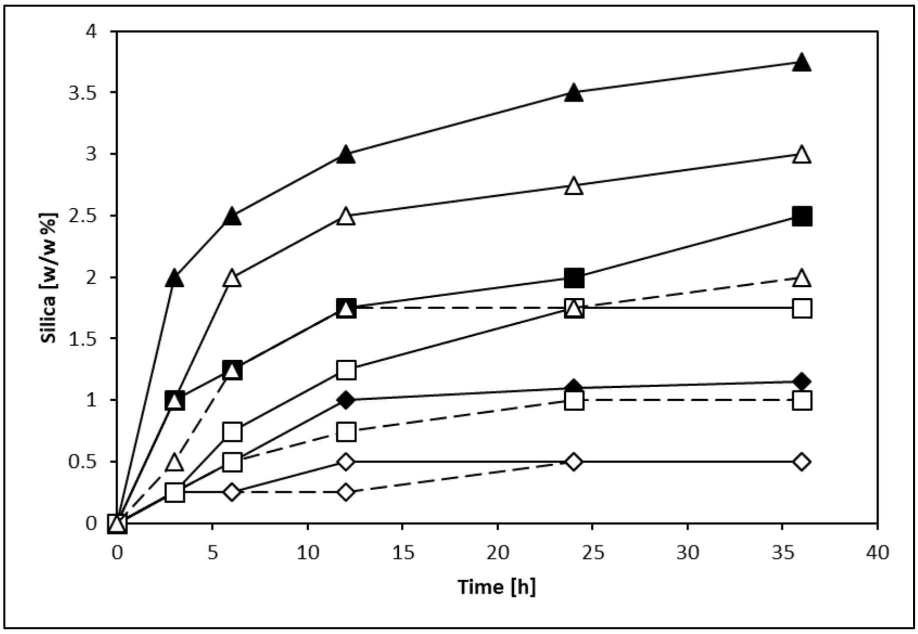

Figure 2 The dissolved silica in sample weight percentage of each treatment i.e. rumen treated husk (full mark), husk char (empty mark) and control (empty mark + dashed line) in several media i.e. distilled water (diamond), 0.1 M citric acid solution (square), and 0.05 M HCl solution (triangle)

The maximum values achieved were 1.15, 2.5, and 3.75% weight of the treated husk in the distilled water, 0.1 M citric acid, and 0.05 M HCl solution respectively. The solubility from all conditions were still far from the total content of silica in the husk at 17%. Whereas for the husk char, the highest yield percentages of silica were 3.0, 1.75, and 0.5%

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for hydrochloric acid, citric acid, and distilled water, respectively. For the control (untreated husk), the highest value was 2.0, 1.0, and 0.5% by sample weight for the corresponding medium, hydrochloric acid, citric acid, and distilled water respectively.

Comparing to the distillate water as the control solvent, the significant increase of dissolve silica occurred in acid solutions, and it was obvious that the type of acids had more influence than the amount of acids concentration as the molarity applied for strong acid, was less than weak acid. The strong acid leads to deconstruct the organic matter due to high affinity of nucleophile attack on the biomass material hence the fibrous structure was loosened, it leads to it wide application in biomass acid hydrolysis [36], as organic material, the decomposed rice husk will allow more inorganic material, which is dominated by Si, to escape. An investigation strong acids including hydrochloric higher than 2.0 M in the treatment to extract silica from biomaterial including rice husk found that it increased organic matter removal impurities [37] and also increased the specific surface area of the particles [38]. Because of classified as dangerous chemicals, strong acid was not applicative, the organic acid as known as weak acids would be highly recommended particularly the citric acid, one of the majority acids in the soil [39].

The extracted silica from the husk char was lower than the yield obtained in the rumen-treated husk. This occurred because the content of extractable organic carbon represented by the lower content of carbon content, and an investigation was shown that the presence of charcoal in the soil lowered the content of extractable organic carbon[40]. The less dissolved char led to less silica released. It preferred to act as an adsorbent rather than soluble compound in the liquid medium [41].

As the previous explanation, the higher acid concentration led to organic matter leaching [37]. However, in this experiment the acid concentration applied was lower than 0.5 M. As such, it released less silica compare to the treated husk. The untreated rice husk is an analogue to its utilisation directly without any preliminary procedure, and it would be ineffective because of the longer time required in degradation. It had been studied that mushroom, *Pleurotus ostreatus*, required 35 days to increase rice husk digestibility up to 79.4% [42].

3.4. *Si extraction using alkaline solution*

This step was trying to figure out the potential of biological-treated husk as a substitute process to the thermal treatment in a set of Si extraction. Commonly, the Si extraction directly from the char using alkaline solution was performed to obtain Si solution and highly porous bio-char [43]. The extraction effectiveness in alkaline solution was observed for each husk treatment. The husk char content released the highest silica at 25% of the ash weight. And then followed by rumen-treated husk, and untreated (control) husk as much as 17, and 7%, respectively as can be shown in Figure 3.

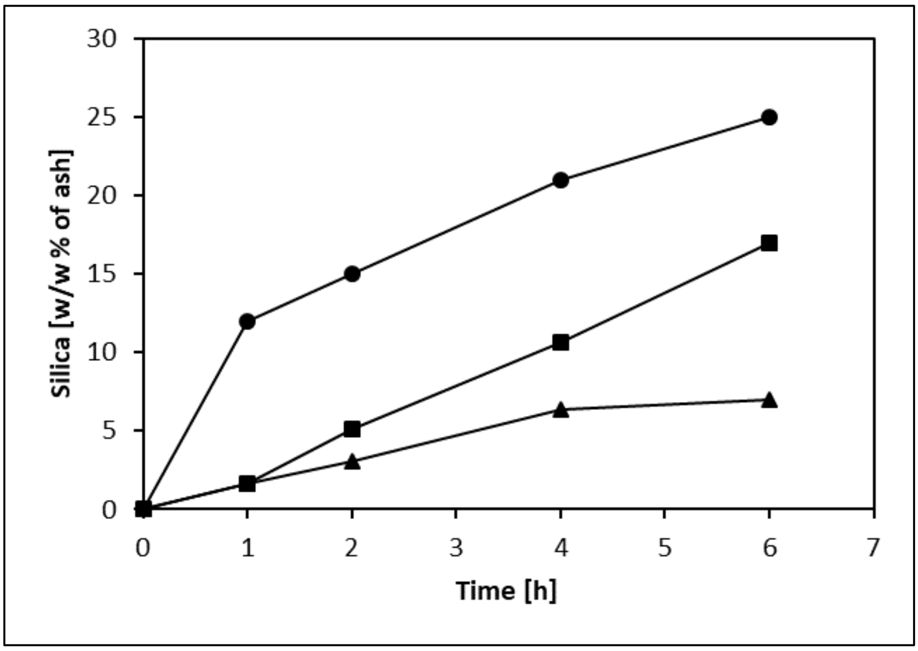

Figure 3 The dissolved silica in weight percentage of the ash from the each husk; (▲) the untreated husk or control, (■) the rumen-treated husk, (●) the char husk, in 1 M KOH solution

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Extraction using the alkaline solution resulted in silica driven off from the char and ash. char with 1.0 M NaOH solution treatment had a more porous structure than untreated based on SEM results [43]. Moreover, 85% silica from ash was recovered after 1.0 M NaOH treatment for 1 hr followed by the addition of 1.0 M hydrochloric acid to attain pH at 7 [44]. França et al. investigated silica extraction from rice husk ash using KOH solution with various concentration and showed that 32.3% recovery from ash was achieved using 1.0 M solution for 6 hr. [45]. The difference between biological treatment and thermal treatment was not too far or less than 50%, it means that biological process could be an alternative to the thermal one in a series of extraction procedures.

4. Conclusion

The rumen treatment on rice husk highlighted the kinetic of lignocellulose degradation including the coefficient values. The assessment of Si release in liquid media was showing the potential of rumen-treated husk as high Si content solid fertilizer due to the highest release result. The result in Si extraction in alkaline solution suggested that the biological treatment can be applied as substitute for the thermal pretreatment in Si extraction with the common procedure. A study to observe the optimum concentration of organic acid in Si release, and the optimum of alkaline solution in Si extraction is highly recommended for the next investigation.

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