INDONESIAN MINING JOURNAL Vol. 19, No. 2, June 2016 : 65 - 77

MINERALOGY CHARACTERS OF CIJULANG PHOSPHATE ROCKS RELATED TO BIOLEACHING PROCESS

KARAKTER MINERALOGI BATUAN FOSFAT CIJULANG PADA PROSES BIOLEACHING

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ABSTRACT

Research on potency test of selected phosphate solubilizing microfungi (PSM) isolates had been conducted. The purpose was to obtain the most potential indigenous microfungi to solubilizing phosphate in bioleaching process. Identification with moist chamber showed that the selected PSM belonged to Penicillium genera. Bioleaching process through measuring process growth and oxalic acid production was effective on the 8th day. Chemical analysis showed that bioleaching process using selected indigenous PSM of phosphate rock was able to increase P_2O_5 content from 38.40 to 49.70% or improve around 11.30%. Experimental condition for such a recovery was -140+200# of sample size an 5% of percent solid. Mineralogy characters of the leached phosphate rocks showed some micro cracks as well as encapsulation by clay minerals. Not all phosphor element was leached by oxalic acid produced by microfungi.

Keywords: phosphate rocks, phosphate solubilizing microfungi (PSM), indigenous, bioleaching, Penicillium

ABSTRAK

Penelitian mengenai uji potensi isolat mikrofungi pelarut fosfat (MPF) terseleksi dari batuan fosfat bertujuan untuk memperoleh isolat mikrofungi asli batuan fosfat yang paling potensial dalam melarutkan fosfat dari batuannya melalui proses bioleaching*. Mikroba terseleksi dengan aktifitas pelarutan tertinggi termasuk ke dalam genus Penicillium. Proses bioleaching melalui pengukuran parameter profil pertumbuhan dan kadar asam oksalat, efektif pada hari ke-8. Analisis kimia basah menunjukkan bahwa proses bioleaching dengan MPF asli batuan fosfat terseleksi dapat meningkatkan kadar P_2O_5 dari 38.40 sampai 49.70% atau bertambah sekitar 11.30% sedangkan kondisi percobaan yang digunakan untuk mencapai perolehan seperti di atas adalah -140+20# ukuran percontoh dan persen padatan 5%. Karakter mineralogi batuan fosfat yang terdeteksi adalah rekahan mikro dan penyelaputan partikel fosfat oleh mineral lempung. Pengamatan secara mikroskopis menunjukkan bahwa tidak semua unsur fosfat terlindi oleh asam oksalat yang dihasilkan mikrofungi.*

Kata kunci: batuan fosfat, mikrofungi pelarut fosfat (MPF), asli, bioleaching, Penicillium

Received: 03 April 2016, first revision: 09 May 2016, second revision: 14 June 2016, accepted: June 2016.

INTRODUCTION

Phosphorus is one of the major nutrients, second only to nitrogen in requirement for plants. Its application is mostly for chemical, fodder, detergent and fertilizer industries. Development of world fertilizer industries in 2009 showed that almost 90% of natural phosphates were used as raw material for superphosphate fertilizer (Sastramiharja et al., 2009). The fact that as an agricultural country, Indonesia requires superphosphate fertilizer around 800.000 tons a year (Budi and Purbasari, 2009) makes the import of superphosphate fertilizer is still required. Normally, Indonesian phosphate deposits are distributed sporadically within limestone caves performing relatively small quantity such as available at Cijulang - West Java (Figure 1). Yet, the newest data regarding Indonesian phosphate reserve are not yet available. In Java, 20 million tons of phosphate resources were already identified. They are included in 48 locations; however, such a figure may bigger than that of identified ones as the guano deposits of Sumatera, East and South Kalimantan as well as Sulawesi have not yet been explored. Moreover, the exploration of marine phosphate deposits has also not yet been conducted. If so, the figure related to Indonesian phosphate resources may increase significantly.

Figure 1. Guano phosphate deposits at Cijulang, West Java

Approximately 95-99% of soil phosphorus is present in the form of insoluble phosphates and cannot be utilized by the plants (Vassileva et al., 1998 *in* Pradhan and Sukla, 2005). The availability of phosphorus for plants can be increased by applying large amounts of fertilizer to soil. However, a large proportion of fertilizer phosphorus after application is quickly transformed to the insoluble form (Omar, 1998 *in* Pradhan and Sukla, 2005). Therefore, very little percentage of the applied phosphorus is available to plants, making continuous application necessary. Phosphorus deficiencies are wide spread on soil throughout the world and phosphorus fertilizers represent major cost for agricultural production.

Most of Indonesia phosphate rock deposits contain low P_2O_5 content, namely less than 20%. Such deposits are suitable only for small scale mining (Wahyudi et al., 2008; Kasno et al., 2009). A good phosphate rock deposit used for superphosphate fertilizer should have minimum P_2O_5 content around 43% (Ridwan, 2011). Improving P_2O_5 content can be conducted both physically and chemically. The former can be accomplished through calcination and magnetic separation while the later is carry out by acidic method using sulphuric, hydrochloric and phosphoric acids. However, the use of such methods has some disadvantages such as high energy and chemical reagent consumptions. The use of chemical reagent also provides hazardous residues that are harmful to the environment. An alternative to change such methods is bioleaching - a biotechnology that beneficiates micro fungi capability to dissolve the phosphate rocks (Saeed et al, 2002).

Phosphate solubilizing microfungi (PSM) play an important role in supplementing phosphorus to the plants, allowing a sustainable use of phosphate fertilizers. The fungi are involved in a range of process that effect the transformation of soil phosphorus (P) and thus are integral component of the soil ‘P’ cycle. Many of them are capable of the soil ‘P’ cycle (Bulut, 2013). PSM application in the field has been reported to increase crop yield. Several mechanisms like lowering pH by acid production, ion chelation and exchange reactions in the growth environment have been reported to play a role in phosphate solubilization by PSM. Among PSM, species of *Aspergillus, Penicillium* and yeast have been widely reported solubilizing various forms of inorganic phosphates (Blackwell, 2011).

Wahyudi et al. (2008) had improved the P_2O_5 content through bioleaching using *Aspergillus niger*. However, the fungi used for bioleaching was not derived indigenously. The leaching was optimized by varying grain size of the sample and its percent solid. Chi et al., (2007) stated that the finer the processed sample, the easier the contact between the mineral and the bioleaching agent. Referring to Chi's statement, Wahyudi et al. (2009) found that the best sample size for bioleaching was -140+200# instead of -200# and the 20% was the minimum condition for percent solid to get economically bioleaching process. Yet, the effectivity of bioleaching decreased when the %solid increased. Based on such an argument, it needs to conduct isolate potential test of phosphate solubilizing microfungi.

Microfungi produce organic acid that can free all phosphatic compounds within the phosphate rock such as $AlPO_4$, $FePO_4$ and $Ca(PO_4)$. The free phosphate is then used by plants. Roni et al. (2013) stated that the best organic acid to solve the phosphor from its clusters was citric acid followed by oxalic acid and the acetic acid while Chi et al. (2007) had been investigated that sulfuric acid produced by *Acidthiobacilus ferooxidans* started effective on the 5^{th} day of bioleaching process and increased the P_2O_5 contents as many a 9%.

Bioleaching study on increasing P_2O_5 content by Wahyudi et al. (2008) showed that the highest oxalic acid production occurred at the 10^{th}-day. Some factors affecting their bioleaching process included type of microfungi, percent solid and particle size. Using PSM of *Aspergillus niger* and particle size of -140+200#, the increasing P_2O_5 was much better than that of particle size of -200#. Nevertheless, Chi et al (2007) who used *Acidithiobacilus ferooxidans* with varying particle size from 400, 270, 140, 70 and 50# showed that particle size of 270# provided the best quantity to increase the P_2O_5 content. Referring to Wahyudi's and Chi's studies, it showed that different microorganism worked better at different particle sizes when solubilizing the phosphor. *Aspergillus niger* was good for coarser particles than 270# while the *Acidthiobacilus ferooxidans* was competent for fine particle.

The objective of this study is to evaluate mineralogy characters related to bioleaching process. The fact that not all phosphate element of Cijulang phosphate rocks dissolved implies that there are some obstacles during the leaching. By studying tailings mineralogy and other bioleaching cases, problem related to bioleaching performance might be solved.

METHODOLOGY

Samples for conducting the study were taken from Cijulang, West Java. The phosphate rocks were then prepared by crushing and sieving the materials to -140+200# and -200# and percent solid of 20%. PSM selection applied methods that were conducted by Widawati et al. (2008), namely rehydration centrifugation (RC) and sodium dodecyl sulfonate (SDS) methods. They were followed by moist chamber method for identifying the selected PSM. PSM selection was determined by potential test of phosphate dissolution within Pikovskaya medium. The selected PSM was represented by the biggest clear zone occurred around the colony. Microfungi growth was analyzed by total plate account method while the oxalic acid profile from bioleaching were evaluated. Previously, the samples were also characterized their mineralogy characters by optical microscope, X-ray diffraction (XRD) and scanning electron microscope-electron dispersive spectrometer (SEM-EDS) methods while its chemical composition employed AAS. Characterization using those instruments was also conducted to the tailings from bioleaching.

RESULTS AND DISCUSSION

Bioleaching test using indigenous PSM improve P_2O_5 content of phosphate rock from Cijulang. Though the leaching provided promised results, some phosphor elements still remained within the tailings. It meant that the indigenous PSM only leached some phosphate minerals due to some mineralogical characters. Mineralogical study on the tailings showed some features regarding the effect of leaching process.

Raw Material Study

Phosphate minerals available within the phosphate rocks consist of various apatites, namely carbonate fluorapatite (francolite) - (Ca, Mg, Sr, $Na)_{10}(PO_4,SO_4,CO_3)_6F_{2-3}$, carbonate hydroxyapatite (dahlite) - $Ca_5(PO_4,CO_3)_3F$ and chlorapatite - $Ca_5(PO_4)_3Cl$. XRD analyses showed that Cijulang phosphate rock contained dahlite, calcite - $CaCO_3$, montmorillonite - Na(Al, $Mg)_2$ Si_4O_{10} $(OH)_2.4H_2O$ and quartz - SiO_2. The fact that dahlite was the phosphate mineral within Cijulang phosphate rocks was confirmed by optical microscope test. The results showed fibrous radiating masses - a special habit of dahlite (Figure 2). In mineral processing point of view, such a feature is an advantage as it will facilitate the leaching reagent to permeate into dahlite body. This will increase the P_2O_5 content. Yet to get the desired content, many factors should be considered in processing the phosphate.

Statistically, the optical microscope tests exhibit that the original phosphate rock samples (not fractionated) own dahlite only 5.40% (Figure 3). Of various minerals occur within phosphate rocks, clay minerals that might be montmorillonite seem the dominant one. Its portion within the rocks is 59.05% followed by calcite (20.80%), quartz (6.41%), rock fragments (6.30%) and opaque minerals (2.04%). Those five minerals are categorized impurities and should be noted when the dahlite is processed. SEM-EDS analysis using X-ray method also ascertained that the main phosphate mineral of Cijulang phosphate rock was dahlite. SEM photomicrograph (Figure 4) displayed radiating features of dahlite. The mapping process showed at least eight elements detected within samples. Those are calcium (Ca), carbon (C), phosphor (P), sulfur (S), aluminum (Al), silicon (Si) and iron (Fe). The first three element is dahlite component; the C and Ca are also the elements of calcite while Al, Si and Fe are silicate mineral components. Of the eight-mapped elements; calcium, carbon, phosphor and aluminum, silicon, iron showed strong signal to be well mapped. Sulfur seemed a noise within this sample. Its quantity (0.82%) was supposed to be the cause (Table 1). The material might be came as transported element from somewhere through leaching water. Two detected silicate components, aluminum and silicon, might be came from weathered volcanic rocks where the phosphate rocks associated. Similar to the result of SEM-EDS analysis, XRD analysis of Cijulang phosphate rocks showed that three minerals dominated the material (Figure 5). Those are hydroxyapatite, calcite ($CaCO_3$) and quartz (SiO_2). Hydroxyapatite (HA) was a naturally occurring mineral form of calcium apatite with the formula $Ca_5(PO_4)_3(OH)$, but is usually written $Ca_{10}(PO_4)_6(OH)_2$ to denote that the crystal unit cell comprises two entities. It is one of the phosphate minerals that is known as dahlite and is produced and used by biological micro-environmental systems. Analyzing composition of Cijulang phosphate was also accomplished through chemical analysis using wet method. The analyzed sample consisted of three types, namely head sample (HS) and fraction ones. The original sample (HS) contained P_2O_5 around 17.28% which was then increased to 38.4 and 37.6% when ground into -140+200# and -200# (Table 2).

The grinding process seemed to liberate phosphate mineral from its host. Not only was the P_2O_5 content within Cijulang phosphate rock high but also the CaO substance was also significant. Kusumo et al. (2000) stated that high CaO content had a value added for a phosphate rock to be a natural fertilizer as such a material could increase soil basicity. Though the P_2O_5 content of Cijulang phosphate is relatively high, it is unqualified to be used for super phosphate fertilizer (Ridwan, 2011). It needs improving the content until satisfies the specification for phosphate fertilizer.

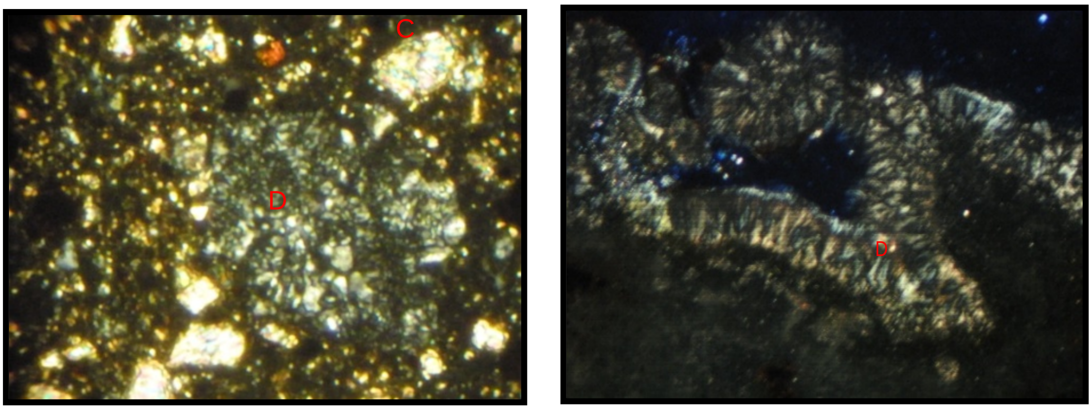


Figure 2. Two dahlite performances showing fibrous radiating masses. D belongs to dahlite and C is calcite.

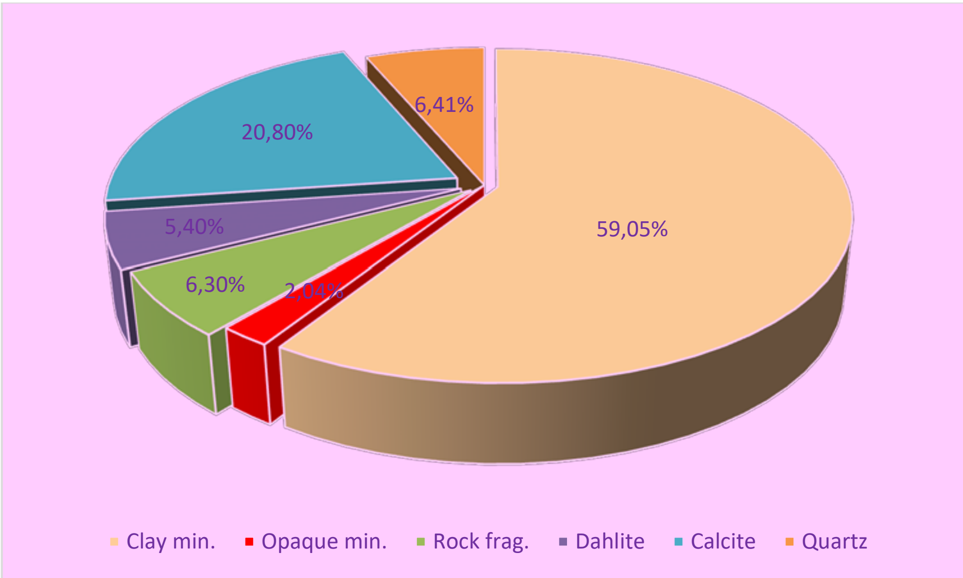


Figure 3. Composition of Cijulang phosphate rocks that consists of dahlite, calcite, quartz, clay minerals, opaque minerals and rock fragments

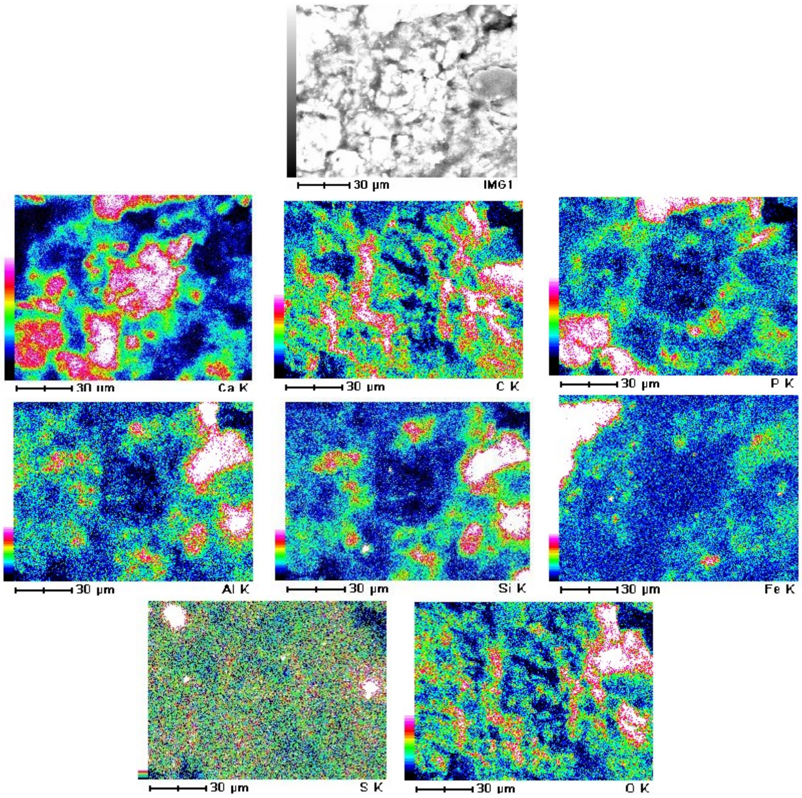
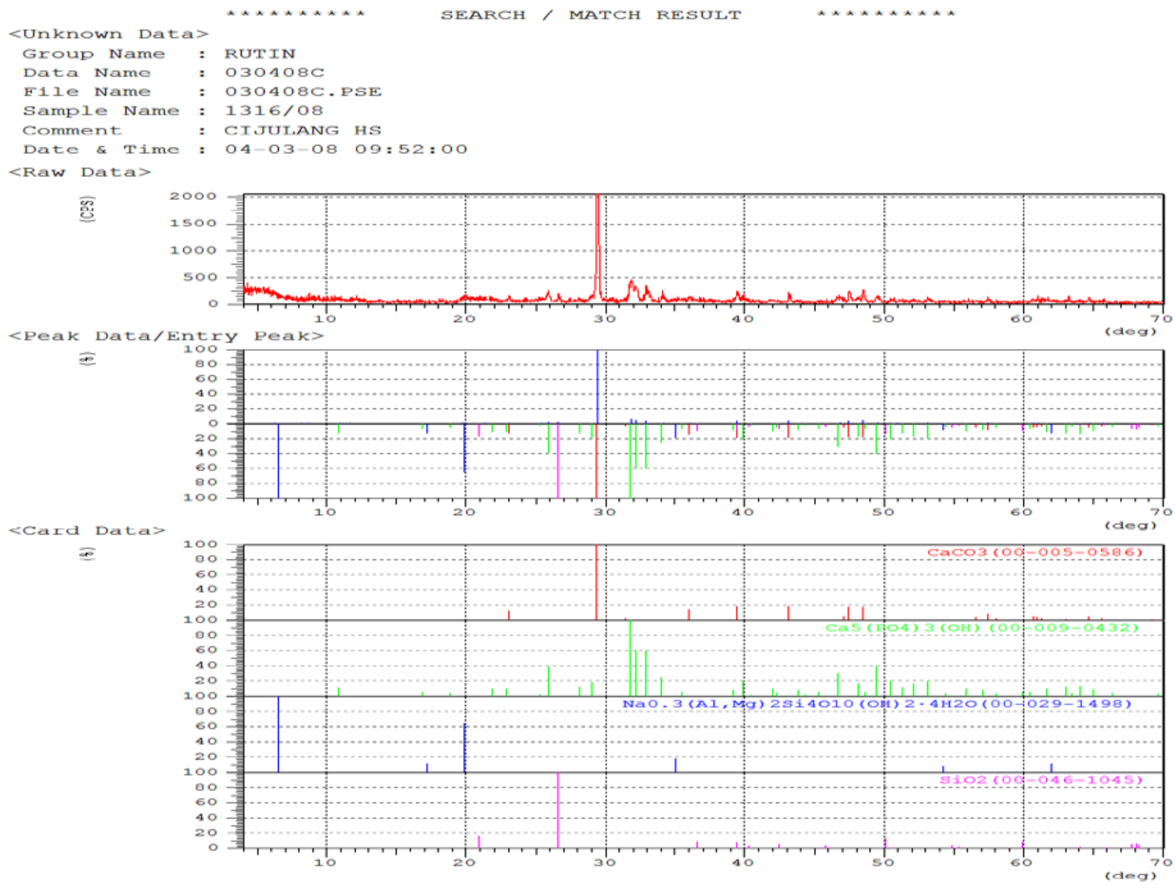


Figure 4. SEM-EDS analysis of Cijulang phosphate rocks detected fibrous radiating performance and 8 phosphate-forming elements

Table 1. Result of SEM-EDS analysis of Cijulang phosphate rocks.

Element	(keV)	mass%	Error%	At%	Compound		mass%	Cation	K
C K	0.277	36.56	0.47	76.91	C	36.56	0.00	11.6433	
O		24.41							
Al K	1.486	2.82	0.62	1.32	Al2O3	5.33	1.65	2.5885	
Si K	1.739	3.27	0.56	2.95	SiO2	7.01	1.83	3.6325	
P K	2.013	5.25	0.63	2.14	P2O5	12.04	2.67	8.7698	
S K	2.307	0.82	0.62	0.65	SO3	2.05	0.40	1.2755	
Ca K	3.690	21.84	0.55	13.76	CaO	30.55	8.57	37.3861	
Fe K	6.398	5.02	1.05	2.27	FeO	6.46	1.41	6.7918	
Total		100.00		100.00		100.00	16.53		

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          **********        SEARCH / MATCH RESULT        **********
Group Name  : RUTIN
Data Name   : 030408C
File Name   : 030408C.PSE
Sample Name : 1316/08
Comment     : CIJULANG HS
<Entry Card>
No.   Card          Chemical Formula                          S        L            d      I      R
                    Chemical Name (Mineral Name)                       Dx     WT%     S.G.
 1  00-005-0586 CaCO3                                       0.562 0.684(13/45) 0.778 0.487 0.259
               Calcium Carbonate ( Calcite, syn )                 2.71                 R-3c
 2  00-009-0432 Ca5(PO4)3(OH)                               0.094 0.378(17/56) 0.623 0.608 0.143
               Calcium Phosphate Hydroxide ( Hydroxylapat         3.15                 P63/m
 3  00-029-1498 Na0.3(Al,Mg)2Si4O10(OH)2·4H2O               1.000 1.000( */ 6) 1.000 1.000 1.000
               Sodium Magnesium Aluminum Silicate Hydroxi         -----                P
 4  00-046-1045 SiO2                                        1.000 1.000( */58) 1.000 1.000 1.000
               Silicon Oxide ( Quartz, syn )                      2.65                 P3221
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Figure 5. Result of XRD analysis showing four detected minerals, namely calcite, hydroxyl apatite, (Na, Mg) silicate and quartz.

Table 2. Composition Cijulang phosphate rocks, HS = head sample, -140+200# and -200# samples = fractioned phosphate rocks

Sample code	SiO_2	Al_2O_3	Fe_2O_3 %	CaO	P_2O_5
HS	14,16	7,71	5,35	26,9	17,28
-140+200#	0.75	2.68	0.33	46.62	38.4
-200#	0.65	3.08	0.32	46.61	37.6

Bioleaching Tests

Most of soil microbes have potency as bio-fertilizer, mainly those that lives within rhizosphere. One of them relates phosphatic-solvent microbes. Such microbes have a capability to increase the growth and production of the plants (Widawati et al., 2008). Yet its mechanism has not been known but it is supposed to involve complex process including dissolution of polypeptide compounds, oxidation and reduction (Ruangsanka, 2014). Samples for isolating microbes were phosphate rocks. To remove the water content that might be still available within samples, the samples used wind-drying method as the method was relatively secured from composition destruction (Sterflinger, 2000). Isolated PSM was accomplished by testing the potency of phosphate dissolution in Pikovskaya medium (Malviya et al., 2011). Clear zone formation within the medium showed that the microorganism were available. The clear zone occurred due to the dissolution of tricalcium phosphate or $Ca_3(PO_4)_2$. The selected PSM was determined by the largest dimension of the clear zone surrounding the colony (Ginting et al., 2006; Maryanti, 2006). Figure 6 shows the selected PSM available in Cijulang phosphate rocks and successfully isolated for bioleaching Identification of selected PSM isolates showed that the isolated PSM belonged to *Penicillium sp*. genus. Such the microfungi had grown-fast character and spread fast in various environments in either soil, air, or rotten plants (Blackwell, 2011; Kiziewicz et al., 2013).

When conducting bioleaching; two items should be observed, namely the growth of Penicillium sp. and produced oxalic acid during the leaching (Yasser et al. 2014). A series of bioleaching tests had been conducted using 6 combinations of particle size and percent solid (Table 3) and found that during 10-day leaching, the microfungi had shown the growing capability since Day-1 and continued through Day-8 but then declined from Day-9 through 10. Of 6 variables, sample with label P = -140+200+5% retained higher cell development compared to other samples and showed the colony number around 68 $x10^6$ CFU/ml. Yet the figure showed that the *Penicillium* sp. resulted from selection test did not undergo adaptation phase as the fungi came indigenously from the phosphate rocks. The adaptation phase is not always necessary if the growing medium and environment of the fungi was similar to the previous ones.

Referring to the oxalic contents that were produced during the process, it was found that the acid increased exponentially since Day 1 through Day 10; however, there were no significant differences of the content among six varying samples. Overall, the quantity of produced oxalic acid when leached by the fungi varied from 75 to 85%. Sample with labelled P = -140+200+5%, P = -140+200+12.5% and P = -140+200+20% yielded similar produced oxalic acid quantity, namely 85%. Comparing cell development and oxalic acid production, the acid production was in accordance with microfungi growth. Start on Day 1 through 8, the fungi grew exponentially (Figure 6) and were followed by stationary phase on the next day; later, the cells died on Day 10. The oxalic acid was optimally produced on Day 8. High concentration of oxalic acid detected after cell mortality indicated that the acid was not used as a nutrition for growing phase but accumulated as intermediate compound within fungi metabolism.

Leaching experiment showed that particle size of -140+200# and percent solid of 5% provided the highest oxalic acid (Figure 7). It seemed that such parameters were the optimum condition for the experiment. Employing various sample feeds and indigenous microfungi, it showed that the increase of P_2O_5 content varied from 37.6 - 38.4% to 47.4 - 49.7% (Table 3). The highest recovery was achieved by experiment that used sample with size of -140+200# and percent solid of 5%. The increase of P_2O_5 and CaO occurred due to the phosphate mineral was leached and releasing P element to be P_2O_5 while the Ca developed into CaO (Wahyudi et al., 2008).

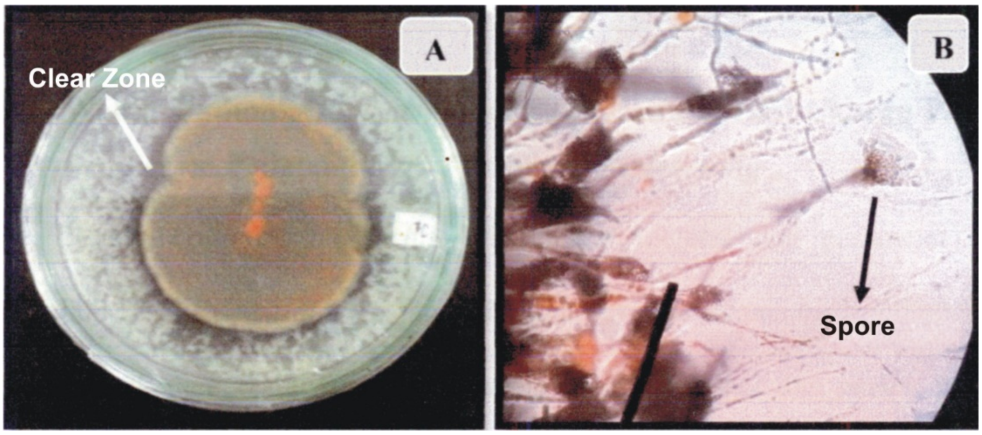

Figure 6. The selected PSM for dissolving phosphate element showing the biggest clear zone (A) and identified PSM (*Penicillium* sp)

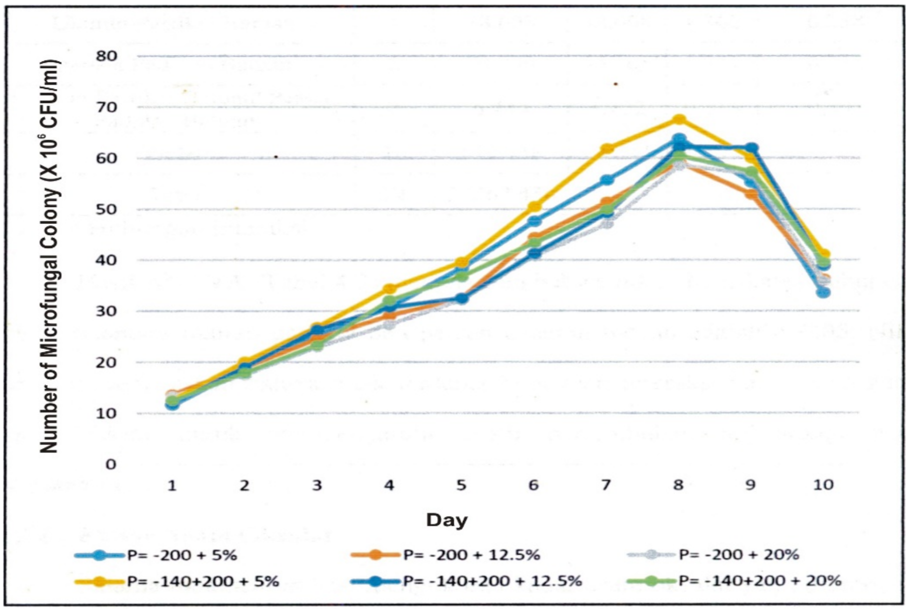

Figure 6. The growth of indigenous phosphate solubilizing microfungi during bioleaching proses

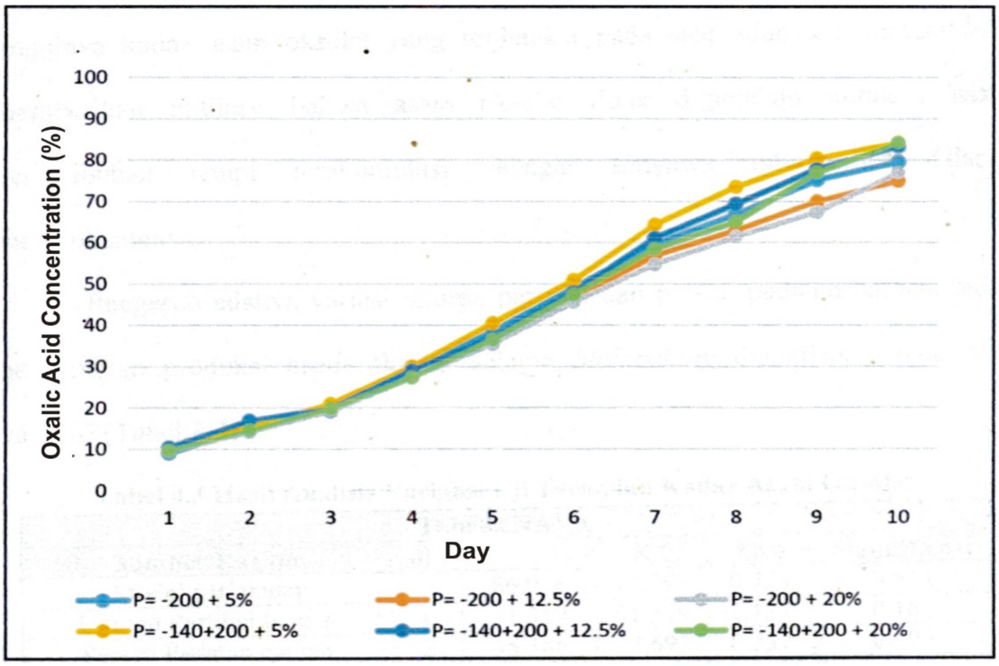

Figure 7. Oxalic acid concentration from day-1 through day-10 of bioleaching proses

Table 3. Result of bioleaching test for Cijulang phosphate rocks using indigenous *Penicillium* sp.

Feed Condition		P_2O_5 Content	CaO Content	P_2O_5 Content	CaO Content	P_2O_5 Increase (%)	CaO Increase (%)
Particle Size	% Solid	Prior Bioleaching		After Bioleaching		After Bioleaching	
	5%			49.7	48.2	11.3	2.0
-140+200#	12.5%	38.4	46.2	47.4	47.3	9.0	1.1
	20%			48.3	47.5	9.9	1.3
	5%			48.1	47.8	10.5	1.7
-200#	12.5%	37.6	46.1	49.2	48.4	9.6	2.3
	20%			48.5	49.3	9.9	3.2

In its activity, the phosphate solubilizing microfungi releases several organic acids such as citric, glutamic, succinic, lactic, oxalic, glyoxal, malic, fumaric, tartaric and aceto butyrate acids. The increase of the acids results in decreasing the pH sharply and dissolving Ca-phosphate. A stable complex compound of Ca^{2+}, Mg^{2+}, Fe^{3+} and Al^{3+}, known as chelate, is also developed and releases the phosphor (Raharjo et al., 2007). The phosphate solubilizing microfungi (PSM) of Cijulang phosphate rocks, *Penicillium* sp. produced the oxalic acid and leached the phosphate rock. Referring to the 49%-P_2O_5 content (Table 3), it seems that such a microfungi is prospective to leach the rocks.

Tailings Characterization After Bioleaching

Bioleaching by *Penicilium* sp. could increase the P_2O_5 to 49% and exceeded the minimum specification for triple phosphate fertilizer. Therefore, it is necessary to do a characterization for tailings after bioleaching Moreover, if referring to the leaching result that was only 49%, not at least 80%. Such a study can help improving leaching implementation. SEM-EDS analyses using X-ray mapping method of the after-leaching materials showed the feature of non-leached dahlite mineral as shown in Figure 8. The illustration showed that after bioleaching some dahlite minerals were completely leached by the fungi (D1), another was partly leached (D2). The phosphorus pentoxide was still available within both minerals, noted by the green color, however, such concentrations were not as many as that of Dahlite D3. The red color of the D3 implied that the P_2O_5 were not completely leached. Mineralogical characters of the D3, such as less particle porosity or unliberated particles, were supposed to be the cause; the oxalic acid produced by the fungi could not optimally reach such particles. Mineralogical condition of the tailings from bioleaching also found the unleached calcite due to encapsulation by clay mineral (Figure 9). Such condition results in ineffective contact between calcite and the lixiviants.

CONCLUSIONS

Carbonate hydroxyapatite known as dahlite - $Ca_5(PO_4,CO_3)_3F$ are the main phosphate mineral found within Cijulang phosphate rock. The rocks also contain calcite, quartz, clay mineral, opaque mineral and rock fragments. The clay mineral is supposed to be montmorillonite. Encapsulation performance found within the sample is supposed to be the cause imperfect leaching as the contact between the lixiviants and desired particles turns out to be ineffective.

The potential indigenous PSM found in Cijulang phosphate rocks is *Penicillium sp*. The fungi is effective enough to dissolve phosphor element within the rocks. Bioleaching using such a microfungi, -140+200# of feed size and 5% of percent weight provided the highest P_2O_5 solubilization, namely 49%. The figure exceeds the requirement of P_2O_5 for superphosphate fertilizer (43%). Prior bioleaching, the content of P_2O_5 within the rocks is only 38.4% but then increases 11.3%.

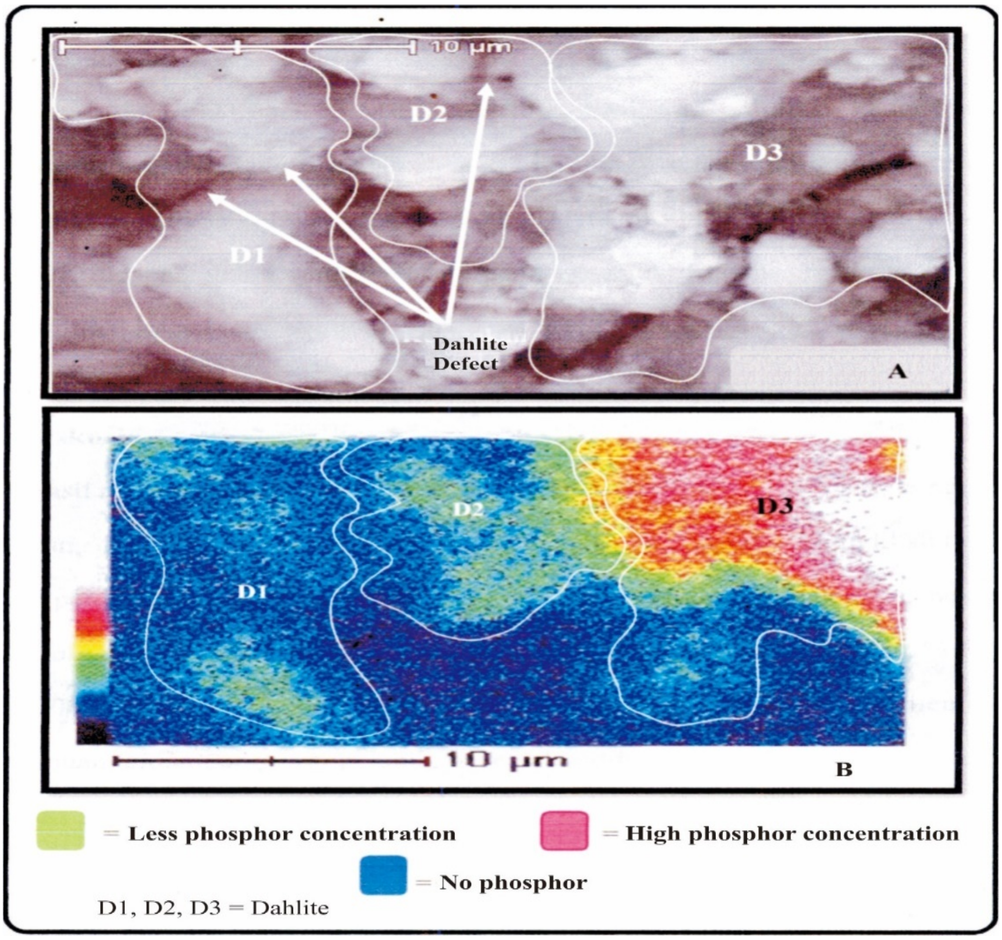

Figure 8. SEM-EDS analysis of the phosphate tailings came from bioleaching process

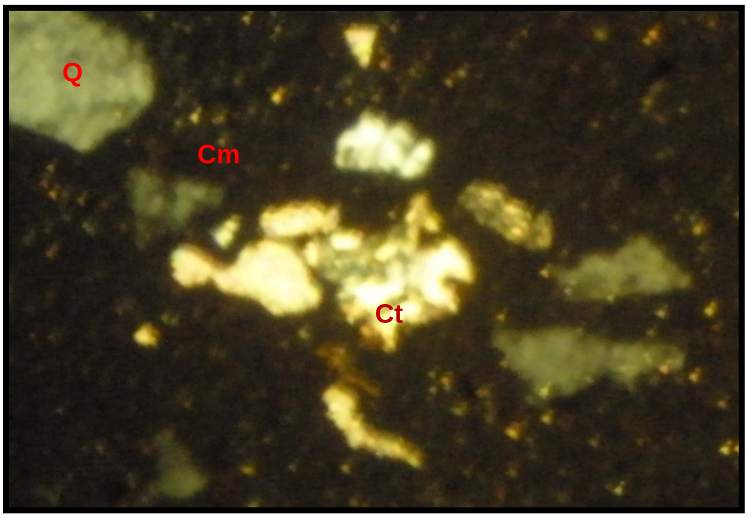

Figure 9. Calcite (Ct) along with quartz (Q) encapsulated by clay minerals (Cm) results in imperfect leaching

The study of adaptive characters for the microfungi and its capability in improving the P_2O_5 within phosphate rocks can be conducted to evaluate its potency when the fungi is directly inoculated on the superphosphate fertilizer. Changing the stirring method from rotary shaking to agitation when conducting bioleaching will result effective infiltration of microfungi mycelium.

ACKNOWLEDGEMENTS

The authors would like to thank Head of Microbiology Laboratory, Padjadjaran University for his permission to use laboratory facilities during the research, Pak Rachmat of Cijulang for providing phosphate rock samples and Staff of Preparation Laboratory, Research Development Centre for Mineral and Coal Technology for their assistance to prepare the samples.

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