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BIOSOLUBILIZATION OF PHOSPHATE ROCK BY *Penicillium sp*

PELARUTAN BATUAN FOSFAT MENGGUNAKAN Penicillium sp

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ABSTRACT

Phosphorus is an essential element that regulates soil fertility. Its deficiency is replenished by chemical fertilizer made from phosphate rock. An environmentally friendly and economically alternative to chemical processing of phosphate rock is the use of phosphate solubilizing microorganisms. In this research, a potential phosphate solubilizing fungi were successfully isolated from the surface of Cijulang phosphate rock and identified as close relative of *Penicillium sp*. The phosphate biosolubilization capability of the fungus was tested and the influence of leaching parameters such as particle size of mineral, ore concentration (pulp density), and initial pH of medium was investigated using a shake flask study to characterize the solubilization of phosphorus by *Penicillium sp*. The x-ray diffraction data indicated the presence of hydroxyl apatite $Ca_5(PO_4,CO_3)_3OH$ as the main source of phosphorus.The fungal strains of *Penicillium sp* produced oxalic and citric acids during fermentation of glucose which resulted in a drop pH of the growth medium. The results also indicated a potential relationship between the phosphorus biosolubilization and the production of organic acids by the fungus. In addition, particle size, ore concentration and initial pH were also shown to have significant effects on the solubilization of phosphorus from the phosphate rock. The optimal speed of attack was obtained for a surface area of substrate of .-200 mesh. A concentration of 5% solid gave the highest speed of P biosolubilization. The optimum range of initial pH was 6-7 and initial pH began to show an inhibiting effect at 4. The maximum percentage of soluble phosphorus released of 42.8% was attained using -200 mesh particle size, 5% pulp density and initial pH 6 after 16 days of process.

Keywords : phosphate rock, biosolubization, *Penicillium sp*, oxalic acid, citric acid

SARI

Fosfor merupakan unsur esensial yang mengatur kesuburan tanah. Kekurangan fosfor dipenuhi oleh pupuk kimia yang dibuat dari batuan fosfat. Alternatif yang lebih ramah lingkungan dan lebih ekonomis adalah penggunaan mikroorganisme pelarut fosfat. Dalam penelitian ini, jamur yang mempunyai potensi melarutkan fosfat telah berhasil diisolasi dari permukaan batuan fosfat asal Cijulang dan diidentifikasi sebagai Penicillium sp. Kemampuan jamur dalam melarutkan fosfat telah diuji dan pengaruh beberapa parameter seperti ukuran partikel mineral, konsentrasi bijih dan pH awal medium diteliti untuk mengkarakterisasi pelarutan fosfat oleh Penicillium sp. Data x-ray diffraction menunjukkan adanya hidroksil apatit $Ca_5(PO_4,CO_3)_3OH$ sebagai sumber utama fosfor. Strain jamur Penicillium sp memproduksi asam oksalat dan asam sitrat selama fermentasi glukosa yang mengakibatkan penurunan pH medium pertumbuhan jamur. Hasil percobaan juga menunjukkan adanya hubungan antara pelarutan fosfat dengan produksi asam organik oleh jamur. Di samping itu, ukuran partikel mineral, konsentrasi bijih dan pH awal medium mempunyai pengaruh yang signifikan terhadap pelarutan fosfor dari batuan fosfat. Kecepatan pelarutan yang optimal diperoleh pada ukuran partikel -200 mesh dan 5% konsentrasi bijih. Kisaran pH awal optimum adalah 6-7 dan pada pH awal 4 mulai menunjukkan hambatan dalam pelarutan fosfat. Maksimum pelarutan fosfat sebesar 42,8% diperoleh pada kondisi percobaan menggunakan ukuran partikel -200 mesh, 5% padatan dan pH awal 6 setelah proses pelarutan selama 16 hari.

Kata kunci : Batuan fosfat, pelarutan, Penicillium sp, asam oksalat, asam sitrat

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INTRODUCTION

Phosphorus (P) is an essential macronutrient for crop production which largerly exists in phosphate rock (Chen et al., 2008; Elser et al., 2007). However, most of phosphate rock is low grade and not suitable for direct application to soils as phosphate fertilizer because of its low phosphorus content and poor solubility (Ashraf et al., 2007). There are several methods to increase phosphate availability. Although physico-chemical procedures such as magnetic separation, flotation and sulphuric acid could be used for beneficiation of phosphate rock, they are generally expensive (Habashi, 2014). One conventional method is the acidulation of phosphate rock with sulphuric acid (H_2SO_4) or phosphoric acid (H_3PO_4) to produce acidulated phosphate rock (Habashi, 2014; Vassilev et al., 2006).

At present, there is no substitute for phosphate rock as a raw material in the production of phosphate fertilizers in the world. Phosphate rocks are complex raw materials, and are mainly used in the manufacture of phosphate fertilizers. The composition of these rocks varies from one deposit to another. Therefore, phosphate rocks from different sources are expected to behave differently in beneficiation process. Most of the phosphate rocks are of sedimentary origin and are primarily composed of the apatite group in association with a wide assortment of accessory minerals, fluorides, carbonates, clays, quartz, silicates, metal oxides (Ullah et al., 2013).

Phosphate rock is commonly processed chemically with sulphuric acid or phosphoric acid into phosphate fertilizer. This process makes the fertilizer more expensive and also contributes to environmental pollution. Microorganisms may be considered as solubilizing agents for insoluble phosphates rock as an alternative to chemical sulphuric or phosphoric acid. Some microorganisms including bacteria and fungi are known to be involved in the solubilization of phosphate rock (Yasser et al., 2014; Xiao et al., 2009a; Hamdali et al., 2008; Antoun and Babana, 2006). The use of those phosphate-solubilizing microorganisms for the production of phosphate fertilizer has lowered the production cost. Iran has produced a bioleaching phosphate fertilizer for the first time in the world in 2009 (www.iranreview.org). The fertilizer is made of mineral and natural acids which are produced through bioleaching method and constitutes the best substitute for chemical fertilizers. This product is of natural and organic origin and is free from environmental problems, thus being the most suitable fertilizer for agricultural purposes. The new fertilizer also prevents hardening of soil and due to its sponge-like structure, it remains in the earth for two years, thus retaining moisture and preventing dryness of the earth. However, there is no detail information about the process and the microorganisms involved in the making of this biofertilizer.

The type and nature of impurities in low grade phosphate rock varies from deposit to deposit. Sometimes, the type and level of certain impurities may vary even within the same deposit as well. Therefore, each and every phosphate rock may be considered as a special case regarding biobeneficiation technique and technology. The present work aimed to study the potential biosolubilization of phosphate rock reserved located in Cijulang, West Java. The phosphate rock of this area is generally grey to dark grey, hard and compact, and this deposit can not be used directly for phosphate fertilizer.

Recently, efforts are being made in our laboratory for solubilizing phosphate from phosphate rock using *Aspergillus niger* at a laboratory scale (Handayani et.al., 2009). The results showed that 82.2% of phosphate can be extracted using 5% solids of mineral, but the leaching also provided significant high impurities such as aluminium, iron and calcium. Meanwhile, a preliminary screening of phosphate rock samples from Cijulang deposit recently showed the presence of indigenous *Penicillium sp* and has been found to produce organic acids such as citric acid and oxalic acid that can serve as leaching agents for the solubilization of phosphate.

Biosolubilization processes are based on the ability of microorganisms to transform solid compounds resulting in soluble and extractable elements, which can be recovered. Biosolubilization is influenced by a wide range of parameters including physicochemical parameters, microbiological factors of the leaching environment, and the properties of the solids to be leached. These will influence both the growth of the microorganisms as well as their solubilization behaviour (Chandraprabha and Natarajan, 2010; Willscher and Bosecker, 2003). In addition, the metabolic activity of the microorganisms is regulated by several factors that should be controlled to achieve maximum biological action such as pH, percent

solid, particle size of mineral, oxygen, and temperature (Xiao et al., 2009b). Those factors are required for the maintenance of ideal biosolubilizing process conditions; otherwise the process is not enough to solubilize the phosphate content.

The present study aims at characterizing the phosphate rock solubilizing ability of indigenous fungus of *Penicillium sp* that is isolated locally from the Cijulang phosphate rock. The effects of mineral particle size, percent solid, and initial pH are assessed, and the leach liquors are also analyzed at elucidating the possibilities of making phosphate fertilizer in Indonesia by means of biotechnological method.

METHODOLOGY

Phosphate Rock

The representative phosphate rock sample was obtained from Cijulang deposit, West Java. The low grade phosphate rock sample was crushed and sieved to collect various size fractions for analysis. The -100 mesh fraction was further ground by using mortar grinder to obtain the desired size fractions. All the sieved samples were dried in an electric oven at 105°C, cooled to room temperature and stored in closed desiccator. These sample fractions were analyzed for mineralogical and chemical analysis.

Microorganism

A strain of *Penicillium sp* was locally isolated from the Cijulang phosphate rock sample using an adopted method of Adeleke et al. (2010). A common fungal growth medium was used for the initial isolation of the fungi, namely potato dextrose agar (PDA) medium. The fungal isolation process was carried out under sterile conditions and involved the addition of 250 mL deionised water to 100 g phosphate rock. The mixture was shaken for 24 h at 60 rpm using a rotary shaker at room temperature. Thereafter, 10 mL of the homogenised mixture was vortexed and 50 µL of the vortexed mixture was inoculated onto pre-prepared plates of PDA agar. The plates were incubated at 37°C for 5 days. To obtain a pure culture of the isolated fungi, mycelia fragments were scraped off the surface of the growth medium and suspended in 1 mL deionised water inside 1.5-mL tubes. The suspension was vortexed to separate the clustered mycelia. A 50 µL aliquot of each suspension was spread onto new plates of PDA with the aid of an autoclaved glass spreader. After 5 days, distinct growing mycelia of the fungi were sub-inoculated onto new plates to obtain pure cultures of the fungi. This method enhanced the purity of the isolates by encouraging growth from individual hyphae. The pure cultures obtained were then transferred onto phosphate-solubilising medium (PSM) by inoculation at the centre of the agar plate. The composition of the PSM was (g/L): $(NH_4)_2SO_4$ 0.10, $MgSO_4 \cdot 7H_2O$ 0.25, $MgCl_2 \cdot 6H_2O$ 5.00, KCl 0.20, $Ca_3(PO_4)_2$ 2.50, $C_6H_{12}O_6$ 10 and agar 20. After 10 days of incubation at 37 ºC, halos forming around the areas of growth of the fungi indicated the phosphate-solubilising ability of these fungi. Fungi that tested positive by this method were then identified as *Penicillium sp* strains.

Solubilization Experiments

The solubilization of phosphate rock was investigated using a shake flask study. The experiments involved the direct use of the fungi and were organized in a completely randomized design with two replications. Biosolubilizations of 10, 20 and 40 g phosphate rock samples with particle size of -200 mesh were conducted in 500 mL Erlenmeyer flasks containing 200 mL of media consisting of (g/L) : glucose 40, $NaNO_3$ 1.5, $MgSO_4.7H_2O$ 0.025, KCl 0.026 and yeast extract 1.6. All the reagents were of analytical grade. The pH of media were adjusted to 6.0 with NaOH. The media were sterilized by autoclaving at 121°C for 15 minutes. As inoculum, 1 ml mycelia suspension was used under aseptic condition. All the flasks were sealed with removable cotton and incubated at room temperature on a rotary shaker at 150 rpm for 16 days of growth period.

In the next experiments, the particles size of phosphate rock samples was varied (-100 +140 ; -140 + 200 and -200 mesh). The same experiments were repeated with varying initial pH (4, 5, 6 and 7). In general, the growth was followed visually from the increase of mycelium. In the time curse, samples were taken out everyday and centrifuged 10,000 rpm for 15 min to remove solid suspension. The supernatants were then analyzed for monitoring pH, the amount of organic acid (oxalic and citric acids) produced and the concentrations of released phosphorus and some metal ions. Autoclaved, uninoculated media containing phosphate rock were serve as abiotic controls.

Analytical Methods

The rock phosphate sample was characterized by X-ray Diffraction (XRD) and X-ray Fluorescence (XRF) analyses. The pH value was recorded with pH meter equipped with glass electrode. The content of phosphorus was determined by using phosphomolybdate method on a UV-Vis 8500 spectrophotometer at 420 nm. The metals concentrations were determined by AAS (atomic absorption spectrophotometry). The concentration of oxalic acid produced by *Penicillium sp* was determined by Bergermann and Elliot method, and the concentration of citric acid was determined by the colorimetric method of Marrier and Boulet.

RESULTS AND DISCUSSION

Characterization of Phosphate Rock

Mineralogical analysis of low grade phosphate rock sample reflected the presence of hydroxyl apatite as the main mineral with other accessory minerals such as calcite, montmorillonite and quartz. The XRD analysis result of the Cijulang phosphate rock sample is presented in Table 1. The chemical composition of the mineral as the result of XRF analyses is presented in Table 2. It is shown that the phosphate rock sample with -200 mesh of particle size contained major component of P_2O_5 (19.56%), CaO (23.37%), SiO_2 (16.71%), Al_2O_3 (7.71%) and Fe_2O_3 (5.7%). Other oxides such as K_2O, Na_2O, MgO and TiO_2 were in low concentrations.

Table 1. Mineralogical analyses of Cijulang phosphate rock

Mineral identified	Mineral formula
Hydroxyl apatite	$Ca_5(PO_4,CO_3)_3OH$
Calcite	$CaCO_3$
Montmorillonite	$Na.3(Al,Mg)_2Si_4O_{10}(OH)_2.4H_2O$,
Quartz	SiO_2

Effect of Mineral Particle Size

The amount of energetic substrate available for fungal growth depends on the surface provided by phosphate rock. This area is determined by particle size and solid concentration. The effects of those two parameters upon biosolubilization rate were studied.

The experiments were carried out in Erlenmeyer flasks with 5% of pulp density mineral suspensions agitated at 150 rpm at room temperature. The mineral suspensions were composed of different subsieve size fractions of phosphate rock (-100+140; -140 + 200 and -200 mesh). The solubilization rates are presented on Figure 1. It can be seen that particle size had a significant effect on phosphorus solubilization. The phosphorus solubilization rate increased with decreasing particle size. The highest phosphorus extraction was 42.8% and it was obtained with the size fraction of the largest spesific surface area of -200 mesh. In all treatments, phosphorus concentration curves were typically sigmoid.

The influence of particle size in the use of fungal treatment can be explained by the fact that there is a greater surface area of minerals exposed to microbial activity when smaller particle size are used (Modak et al., 2001). The efficiency of the solubilizing process is naturally expected to increase with a decrease in the particle size as this may tend to liberate more calcareous material from the apatite matrix. Fine grinding of the phosphate feed can increase the efficiency of the biosolubilizing process, but the problems related to handling and filtration of fine phosphates, may not allow grinding to exceed a certain limit. Also, higher energy for grinding would cause an extra cost to the process (Ashraf et al., 2007), and therefore this should be taken into consideration.

Table 2. Chemical composition of Cijulang phosphate rock

Chemical Composition (%)	P_2O_5	Al_2O_3	Fe_2O_3	K_2O	Na_2O	CaO	MgO	TiO_2	SiO_2
Head sample	19.56	7.71	5.70	0.212	0.069	23.37	0.535	0.437	16.71
-100 mesh	18.26	0.15	6.00	0.209	0.091	22.19	0.550	0.467	17.82
-140 mesh	19.54	9.52	5.83	0.208	0.068	23.61	0.534	0.457	16.39
-200 mesh	19.56	7.71	5.70	0.212	0.069	23.37	0.535	0.437	16.71

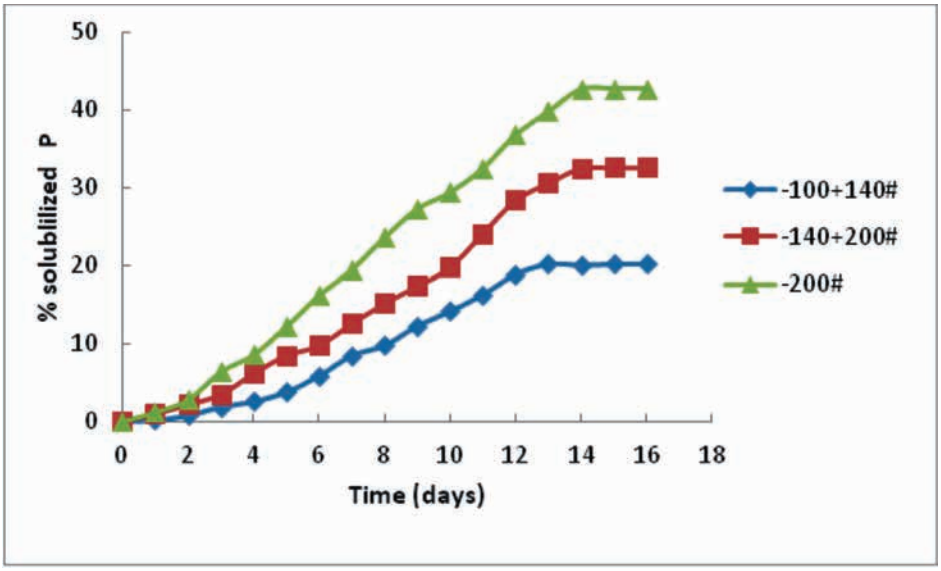

Figure 1. Effect of particle size on the solubilization of phosphorus from phosphate rock

Effect of Pulp Density (Solid Concentration)

A series of experiments were carried out with different pulp density suspensions from 5 to 20% phosphate rock in sterile and inoculated runs and the data are summarized in Figure 2. Phosphorus solubilization increases as a function of time and decreasing pulp density. The highest phosphorus solubilization was found in the lowest pulp density of 5% and the lowest phosphorus solubilization was observed in the highest pulp density of 20%. The phosphorus solubilization gradually increased with increasing in incubation period up to 14th day and after that the leaching activity was stopped and further phosphorus solubilization was not observed because of the deficiency of nutrient in the culture media. It was found that the solubilized phosphate concentration in the sterile controls were 80% lower than those obtained during inoculated runs.

The increase of pulp density led to a lower solubilization efficiency. A pulp density of 5% was likely to be the upper limit for phosphate biosolubiliza-

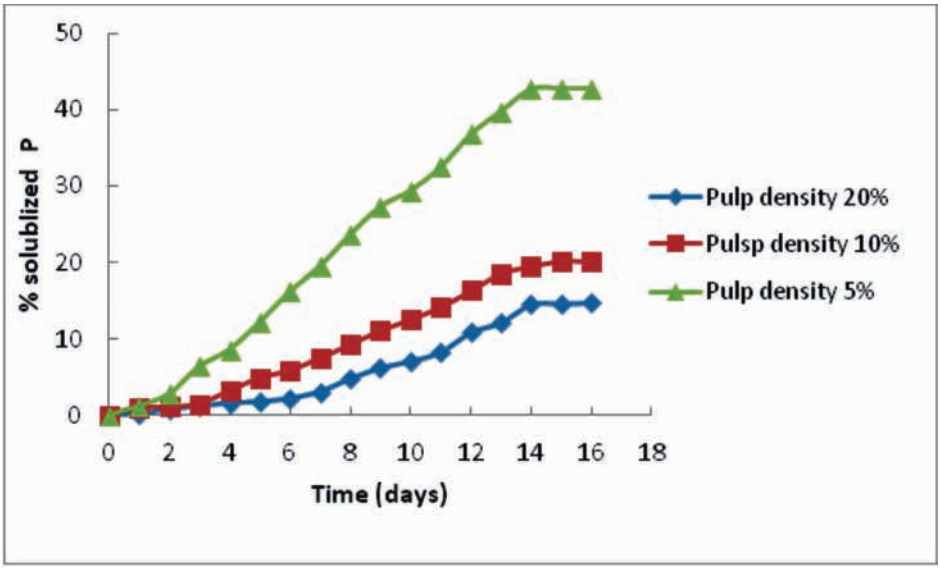

Figure 2. Effect of pulp density on the solubilization of phosphorus

tion. This phenomenon can be explained by Jain and Sharma (2008) who stated that a high solid to liquid ratio in the biological system may result harmful for the fungal growth and development.

Phosphorus Solubilization by *Penicillium sp* and the Change of pH

After 24 hours of incubation in shaking flask, appeared tiny and light yellow coloured beads. The size and number of beads increased, and then small hypae appeared on the surface of beads. The hypae were branched, scale like with knobby ends, and the growth of mycelia was prominent. The filamentous mass was formed and became condensed later.

The effect of culture time and pH on the solubilization of phosphorus is shown in Figure 3. It can be seen that the concentration of phosphorus increased with the increasing culture time. In the initial 12 h of inoculation, the concentration of phosphorus hardly changed. The reason is that there was an adaptive process when the *Penicillium sp* was tranferred to a novel condition, and the growth of it was in inducement phase. Then the *Penicillium sp* gradually adapted to the novel condition, and began to enter the growth phase slowly. The fraction of phosphorus leached was different at different culture time. With increasing culture time, the fraction of phosphorus leached increased rapidly, but when being culture over 14 days, the fraction of phosphorus leached hardly increased. Figure 3 also showed that the pH gradually decreased during the culture process, and it decreased to 3.2 when the growth of *Penicillium sp* was stable. Along with the process of biosolubilization and *Penicillium sp* growing, the concentration of H^+ increased continuously thus causing the decreasing pH.

The data showed that solubilization of phosphorus started immediately after the production of organic acids and solubilized phosphorus contents were clearly detected in leach solution of *Penicillium sp* on 2nd day of incubation. The amount of soluble phosphorus increased linearly from 2nd to 14th day of incubation in all treatments except in control where only 20% phosphorus was recorded during the whole incubation period. *Penicillium sp* solubilized maximum phosphorus quantity of 42.8 % after 14 days.

With the passage of time, pH of growth media dropped due to sufficient amount of biologically generated organic acids and high concentration of soluble phosphorus was recorded. Fast release of phosphorus from phosphate rock was noted at low pH values. Phosphorus solubilization data revealed that extend of phosphorus solubilization had a positive significant correlation with the amount of fungal generated organic acids in the biosolubilization process. Greater concentration of organic acids resulted in high phosphorus solu-

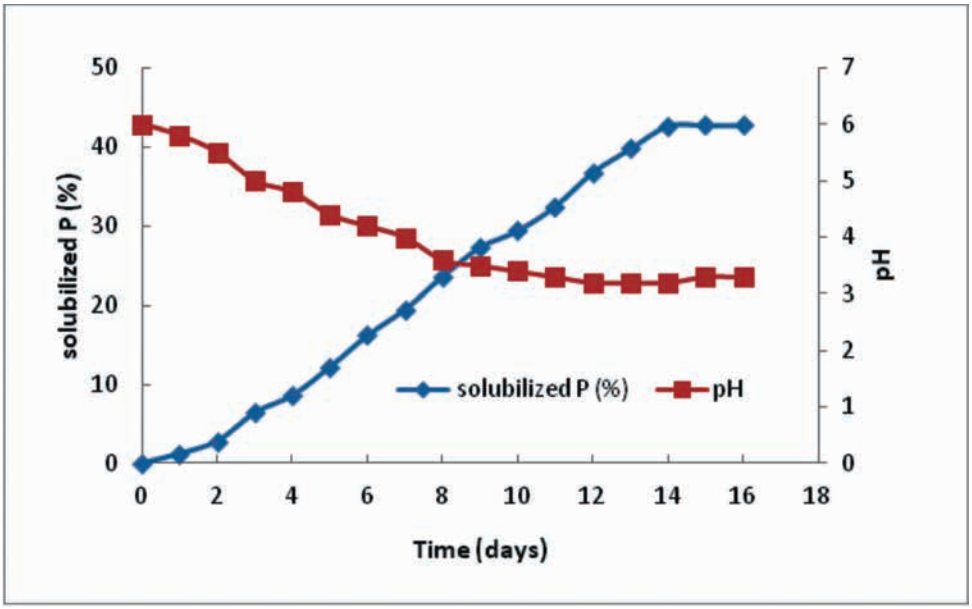

Figure 3. pH profil during solubilization of phosphorus by *Penicillium sp*

bilization rate and low quantity of organic acids caused low phosphorus solubilization in the leach solution. Therefore, the importance of organic acid in phosphorus solubilization from phosphate rock was well established. In this way it was clear that high organic acid producing microorganism strains were more efficient in phosphorus solubilization than low organic acid producing fungal strains. The strain of *Aspergillus niger* in previous study done by Handayani et al. (2009) was more efficient and exhibited high phosphorus solubilization capacity when compared to *Penicillium sp* in this present study because the quantity of organic acid produced by *Aspergillus niger* was almost 2-fold greater that those of *Penicillium sp*. *Aspergillus niger* solubilized 82% of P during 10 days of incubation while P solubilization from phosphate rock by *Penicillium sp* in the current study was in the range of 14,8 to 42,8 % for longer incubation of 14 days. This finding is in accordance with Bhattacharya et al. (2013) who reported that the particular acids released and their concentrations differed between the fungi as did the rate of phosphorus solubilization.

The experimental results indicated that *Penicillium sp* decreased pH of growth media significantly as compared to control during 16 days of leaching period. In all the test, the final pH was considerably lower. The pH decreased from 6-7 to 3.6 - 3.2 for 16 days of process. A similar observations had been recorded by Sharma (2011). It was further observed that pH reduction in leach media closely linked with the concentration of organic acids produced by *Penicillium sp*. Citric and oxalic acid were produced by *Penicillium sp* using glucose as energy source. Decrease in pH was observed due to those organic acid production via incomplete oxidation of glucose as follows (Saeed et al., 2002):

$C_6H_{12}O_6 + 4.5O_2 \rightarrow 3C_2H_2O_4 + 3H_{20}$ (oxalic acid)

$C_6H_{12}O_6 + 1.5O_2 \rightarrow C_6H_6O_7 + 2H_{20}$ (citric acid)

The maximum concentrations of oxalic and citric acids produced by *Penicillium sp* in this experiment were found to be 12.67 and 8.14 g/L, respectively.

Penicillium is able to leach metal by acetolyses and complexation reactions. Citric acid contains three carboxylic groups and one hydroxyl group at 25°C as possible donor of proton (H^+). When citric acid is dissociated in aqueous solution and aluminium ion (Al^{3+}) present in the system, a complexation reaction may take place. The overall chemical reactions is as follows (Ambreen et al., 2002):

$C_6H_6O_7 \rightarrow (C_6H_6O_7)^{-1} + H^+$

$(C_6H_6O_7)^{-1} \rightarrow (C_6H_6O_7)^{-2} + H^+$

$(C_6H_6O_7)^{-2} \rightarrow (C_6H_6O_7)^{-3} + H^+$

$(C_6H_6O_7)^{-3} + 3Al^{3+} \rightarrow (C_6H_6O_7)_3Al$

Oxalic acid contains two carboxylic groups at 25°C as possible donor of proton (H^+). The chemical reaction which covers the dissolution of aluminium from ore in leaching system is (Ambreen et al., 2002):

$C_2H_2O_4 \rightarrow (C_2HO_4)^- + H^+$

$(C_2HO_4)^- \rightarrow (C_2O_4)^{2-} + H^+$

$3(C_2O_4)^{2-} + Al^{3-} \rightarrow Al_2(C_2O_4)_3$ Aluminium oxalate

According to Saeed et al. (2001), the attack of organic acids on the minerals present in the ore-matrix involved both the release of phosphorus (PO_4^{3-}) and some other associated metal ions (Al^{3+}, Ca^{2+}, Mg^{2+}, Fe^{3+}, Na^+, K^+) solution and simultaneously, the complexation/chelation of dissolved metals. The anions and the protons of an organic acids are able to leach metals by acidolysis and complexolysis phenomena. During leaching studies, some other metal ions were also dissolved from the rock sample, namely Fe 22,5%, Ca 19,1% and Al 18,4%.

Effect of Initial pH

The experiment of this parameter was performed to identify the optimum pH region for solubilization of phosphate. The effect of initial pH on the fungal growth and the rate of phosphate solubilization was studied in the initial pH range of 4-7, and the results were presented in Figure 4.

Figure 4 shows that the phosphorus biosolubilization rate is dissimilar under different initial pH values in the culture media, and with the culture time increasing, the pH decreased gradually. The highest phosphorus solubilization rate was 42.8% when the initial pH value and culture time are 6-7 and 16 days, respectively. Therefore, the optimal range of initial pH value of the phosphorus solubilization rate was 6-7, and the biosolubilization of phosphorus was reduced when the initial pH value is lower than 5. Considering that organic acid

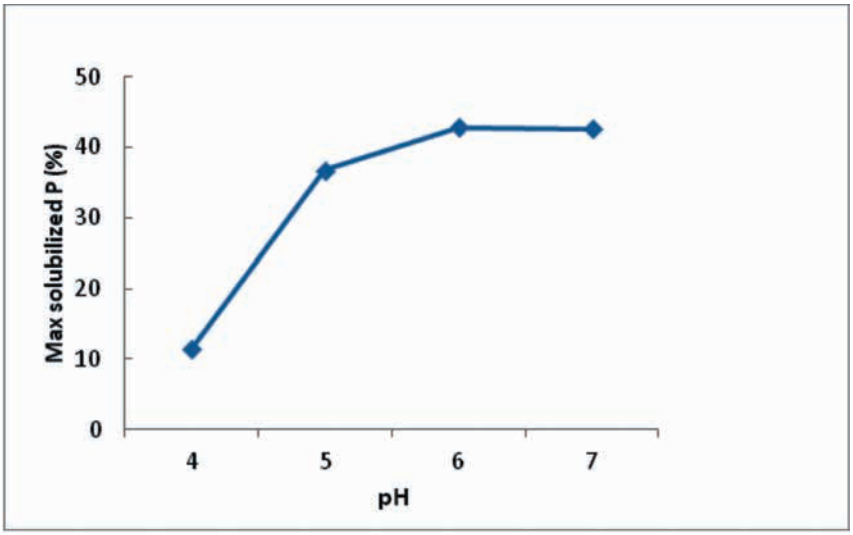

Figure 4. Effect of initial pH on the solubilization of phosphorus

would be generated during the growth of *Penicillium sp*, the pH value in the culture medium should be adequate, thus the initial pH value would not retard the growth of *Penicillium sp*.

The solubilization of phosphate was sensitive to initial pH, especially in the pH region of 6-7 where the rate of solubilization increased sharply as pH decrease. It can be seen that maximum phosphorus solubilization was observed in the medium of initial pH 6-7 with the final pH of the medium was 3.6-3.2. The pH of the medium in all experiments decreased from their initial pH. Organic acids were produced which decreased the pH of the medium to 3.2. Therefore, there was a direct relationship between phosphorus solubilization and decrease in pH of medium due to organic acid production.

The growth of fungi increased sharply at initial pH 6-7, and the difference was seen below pH 5. The quantity of harvested cells produced by fermentation was constantly high in initial pH 6-7 region, but the rate of solubilization by the fungi declined with initial pH 4. Therefore, one of the most important reason would be that pH 6-7 increased the fungal growth, which will in turn lead to an increased production of biogenic substances. These results are in accordance with previous findings by Castro et al. (2000) who stated that oxalic acid could be produced by fungi in growth medium with pH 5-8.

The subsequent solubilization rate is related to the well-known lag, exponential, and stationary phases of fungal growth. The pH has a well-defined effect on the lag phase. At pH 4, the fungi remained in the lag phase over the 3 days of incubation time. At initial pH values ranging from 6 to 7, the lag phase was much shorter. During the exponential growth phase that followed lag phase, the exponential increased in the number of cells directly correlated with the exponential increase in the rate of phosphate solubilization. Exponential phase end abruptly, and the slopes of lines corresponding to this growth phase were similar in the pH range 3.2 – 3.6 at the stationary phase (the end) of fermentation, indicating that the fungal growth depens very little on pH in this range. In the last phase, stationary growth, pH had no effect.

CONCLUSIONS AND SUGGESTION

The present study demonstrated the effective role and the potential efficiency of *Penicillium sp* in the biosolubilization of phosphate from the Cijulang phosphate rock. As shown by the results given in this paper, the optimization of biosolubilization of rock phosphate can be approached by exploring the operating conditions according to the state of knowledge for this method. The three physical-chemical-biological factors of concern have been considered: mineral particle size, solid concentration, and initial pH. The fungal culture used in this study comes from a mixed population which has reached a high degree of adaptation to the rock phosphate substrate. The optimal particle size

and solid concentration have been defined. They are in the same range as usually found and they correspond to a specific area above which the fungal activity cannot increase. The acidity is an inhibiting factor of the growth at a certain level. When all the optimized conditions are applied, 42.8% of phosphorus was dissolved at 5% of pulp density, particle size of -200 mesh and initial pH 6-7 for 16 days of process. The fungal solubilization activity has been shown to be directly carried out through the production of organic oxalic and citric acids during the oxidation of carbohydrates. The significant positive correlation between the solved phosphorus and organic acids produced by *Penicillium sp* suggesting that organic acids may play an important role, although it might not the only possible mechanism for phosphorus solubilization. With *Penicillium sp*, biosolubilization yield was somewhat low and longterm treatment (16 days) was needed to attain a biosolubilization degree of 42.8%. However, it is still possible to solubilize phosphate almost completeley from the mineral when low pulp concentrations are used, but at industrial scale, it is necessary to work with higher pulp concentrations with the aim of obtaining an economic process. These results indicate the challenges and therefore, new efforts must be realized to adapt the fungi to a higher pulp densities, and further research is also needed to increase the fungal capacity for phosphorus solubilization

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