Enhanced solubilization of insoluble silicate from quartz and zeolite minerals by selected *Aspergillus* and *Trichoderma* species

Peningkatan kelarutan silikat sukar larut asal mineral kuarsa dan zeolit oleh spesies *Aspergillus* dan *Trichoderma*

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**Abstract**

Silikon (Si) merupakan komponen utama yang terdapat pada partikel pasir, debu, dan klei tanah. Silika tersedia dalam bentuk asam silika (H₄SiO₄) di dalam larutan tanah tergolong rendah, hanya pada kisaran 3,5-40,0 mg Si L⁻¹. Fungi pelarut silika (FPS) memiliki peran penting dalam meningkatkan ketersediaan silika sukar larut (SiO₂) di dalam tanah untuk tanaman. Tujuan dari penelitian ini untuk menetapkan potensi pelarutan silika dan produksi asam organik tumbuhan isolat FPS di dalam media Bunt dan Rovira dengan menggunakan 0,25% (b/v) magnesium trisilika (Mg₂Si₃O₈), pasir kuarsa, dan zeolit sebagai sumber silika. Pengujian potensi FPS dilakukan dengan menggunakan rancangan acak lengkap dengan tiga perlakuan sumber silika dan tiga ulangan. Hasil penelitian menunjukkan bahwa seluruh FPS yang diuji memiliki kemampuan dalam menghasilkan asam asetat, sitrat, dan oksalat serta dapat meningkatkan kelarutan silika sukar larut. Trichoderma polysporum dan *Aspergillus* niger BCCF194 merupakan isolat terbaik fungi pelarut silika. Lebih lanjut diketahui pula terdapat korelasi positif yang sangat nyata (p<0.05) antara kapasitas pelarutan silika di dalam medium Bunt dan Rovira dengan pasir kuarsa atau zeolit sebagai sumber silika terhadap waktu inkubasi (R²=0,79-0,99) dan produksi asam sitrat (R²=0,97-0,99) dari T. polysporum dan A. niger BCCF194.

**Key words:** *Aspergillus* niger, silicic acid, silicate-solubilizing fungi (SSF), *Trichoderma polysporum*

**Introduction**

Silicon (Si) is a major component of sand, silt and clay particles of soils. Available silica as silicic acid (H₄SiO₄) present in soil solution is considerably low, only in the range of 3.5–40.0 mg Si L⁻¹. To improve plant-available Si in the soil, silicate-solubilizing fungi (SSF) are potentially important in solubilizing insoluble forms of silicate (SiO₂). The objectives of this study were to determine silica solubilizing capacity and organic acid produced by seven SSF isolates on Bunt and Rovira media by using 0.25% (w/v) magnesium trisilicate (Mg₂Si₃O₈), quartz, and zeolite as a silica source. Determination of SSF isolates potential in Si solubilization was carried out in a completely randomized design with three silica sources and three replicates. The results indicated that all SSF were capable of producing acetic, citrate, and oxalic acids and enhancing the solubilization of insoluble silicates. *Trichoderma polysporum* and *Aspergillus niger* BCCF194 were the best isolates of SSF. Furthermore, there was significantly (p<0.05) positive correlation between solubilizing silicon capacity by using quartz or zeolite as a silica source on Bunt and Rovira media with incubation time (R² = 0.79-0.99) and citric acid production (R² = 0.97-0.99) from *T. polysporum* and *A. niger* BCCF194.
converted into water-soluble mono silicic acid, also called ortho silicic acid (H₄SiO₄), absorbed by plants. Various studies have reported weathering of silicates by bacteria for its dissolution to make it available to the plants (Santi & Goenadi, 2017; Chandrakala et al., 2019).

Improved Si management to increase yield and sustain crop productivity appears to be necessary in tropical countries, because the available Si in highly weathered soils such as in Indonesia might be limited. Silicon from natural sources has a potential to mitigate environmental stresses and soil nutrient depletion thereby maintaining sustainable agriculture (Guntzer et al., 2012) and accelerating growth, conferring rigidity to leaves thus maximizing leaf surface area for photosynthesis and mitigating the effects of abiotic stresses like drought, salt and metal toxicity in several plants (Naureen et al., 2015). Furthermore, Pei et al. (2010) indicated that Si might decreases the negative effects of oxidative stress and offers slight resistance to some abiotic and biotic plant stressors. Thus, using Si instead of herbicides and pesticides could reduce harmful environment effects (Vasanthi et al., 2012; Balakhnina & Borkowska, 2013; Karmollahachaab et al., 2013).

Wollastonite (CaSiO₃) is the best-known natural mineral as a Si fertilizer source. However, it is relatively expensive due to limited distribution (Haynes et al., 2013; Tubana et al., 2016). A laboratory incubation study was conducted by Babu et al. (2016) to evaluate the release pattern of H₂SiO₃ from wollastonite and Ca-silicate slag (12% Si) applied to soils varying in clay content and chemical properties. They concluded that the amount of H₂SiO₃ measured in solution (0.1 M NaCl) was influenced by the adsorption capacity of soils, which was highly determined by soil pH, organic matter, and clay content.

In Indonesia, Si sources such as quartz from Bangka-Belitung Province (Santi et al., 2017) and zeolite from Bayah (Kalbuda et al., 2019) are abundant and great interest for Si fertilizer in paddy, sugarcane, corn fields, and oil palm. However, the Si concentration in plants depends primarily on the concentration of silicic acid (H₂SiO₃) in the soil solution and is not correlated to the total Si concentration of the soil. The dissolution and mobilization of silica in the soil by bacteria and fungus are well known. Moreover, solubilization of insoluble Si due to organic acid production by microbes is known to enhance their availability to plants. Silicate solubilizing bacteria have been reported by Santi & Goenadi (2017) and have recently attracted great interest, since solubilization of silicic acid promotes the uptake of both Si and K, thus reducing the need for potash fertilizer (Etesami et al., 2017) and phosphorus (P) (Lee et al., 2019). Daghiino et al. (2010) reported that fungi, while degrading silica-based rocks can also release other mineral nutrients e.g. potassium, iron, and magnesium. The solubilization process by fungi occurs mainly via the production of organic acids and complexing agents and it is faster than that of bacteria. In vitro studies in our laboratory indicated that fungi were more efficient in the solubilization of silica as compared to bacteria (Santi et al., 2018). Thus, a very effective approach for silica solubilization in quartz and zeolite as silica sources is the application of fungi capable of excreting organic acid. The objectives of this study were to determine silicate-solubilizing capacity and organic acid produced especially citric acid by seven silica-solubilizing fungi (SSF) isolates, i.e.: Aspergillus niger strain K0909, BCCF194, A1601, Trichoderma sp., T. polysporum, T. pseudokoningii, and T. viride on Bunt and Rovira solid and liquid media by using magnesium trisilicate (Mg₂O₃Si₃ as control), quartz, and zeolite as a silica source.

**Materials and Methods**

A laboratory study was conducted in Microbiology and Environment Laboratory at Indonesian Research Institute for Biotechnology and Bioindustry (IRIBB), Bogor, in November 2018 - May 2019.

**Quartz mineral**

The mineral used was quartz type collected from Bangka-Belitung province containing 97.1-99.1% SiO₂ (Santi et al., 2017). The quarts analysis was conducted at Chemical Laboratory, IRIBB. Quartz sand samples were air dried and passed through 325 mesh sieve and analyzed for the following: pH, total carbon (spectrophotometer), nitrogen (Kjeldahl), phosphorus (spectrophotometer), potassium (Atomic Absorption Spectrophotometer, AAS), magnesium (AAS), calcium (AAS), total SiO₂ (gravimetry), sulphur, zinc, aluminium trioxide, iron trioxide, manganese dioxide, and cation exchange capacity (CEC) by using SNI 15-0346-1989 standard method (Table 1).

**Zeolite mineral**

The natural zeolite of the clinoptilolite type (Na₂K₁)(Al₂Si₃O₁₀).24H₂O from Bayah-Banten-Indonesia origin, is one of the world’s most abundantly available and widely used zeolitic minerals. Zeolite samples were air dried and passed through 150 mesh sieves and analyzed for the following: pH, total carbon (spectrophotometer), nitrogen (Kjeldahl), phosphorus (spectrophotometer), potassium (Atomic Absorption Spectrophotometer, AAS), magnesium (AAS), calcium (AAS), total SiO₂ (gravimetry), sulphur, zinc, aluminium trioxide, iron trioxide, manganese dioxide, and cation exchange capacity (CEC) by using SNI 13-3494-1994 standard method (Table 1).
Table 1. Chemical characteristics of quartz and zeolite minerals

| Chemical characteristics | Results Hasil | Chemical characteristics | Results Hasil |
|--------------------------|---------------|--------------------------|---------------|
| Karakteristik kimia       | Quartz Kuarsa | Zeolite Zeolit           | Quartz Kuarsa | Zeolite Zeolit |
| pH in H₂O                | 8.20          | 9.30                     | 99.10         | 81.90         |
| Carbon (%)               | 0.12          | 0.21                     | 0.80          | 3.53          |
| Nitrogen (%)             | 0.03          | 0.03                     | 86.03         | 0.95          |
| P₂O₅ (ppm)               | 30.10         | 0.04                     | 53.20         | 28.90         |
| K₂O (%)                  | 0.01          | 0.74                     | 0.17          | 1.56          |
| MgO (%)                  | <0.01         | 0.18                     | 0.03          | 0.41          |
| CaO (%)                  | 0.05          | 3.43                     | <0.01         | 0.02          |
| Na₂O₃ (%)                | <0.01         | 0.91                     | 1.98          | 167.19        |
| Cr₂O₃ (%)                | <0.01         | <0.01                    | 0.02          | 0.20          |

Silicate-solubilizing fungi

The seven isolates of silicate-solubilizing fungi (SSF) were studied previously regarding their ability to solubilize silicate in Bunt and Rovira solid standard media inoculated with selected strains of *Aspergillus* i.e.: *Aspergillus niger* BCC F194, *A. niger* K0909 and *A. niger* A1601. Whereas, species of *Trichoderma* were *Trichoderma* sp., *T. polysporum*, *T. viride*, and *T. pseudokoningii*, respectively. These fungi were lodged at Microbiology and Environmental Laboratory, IRIBB, Bogor. As a source of inoculum, a piece of inoculant SSF (Ø = 3 mm) was grown on Bunt and Rovira solid medium as described by Vasanthi et al. (2013) consisting of (L⁻¹): 20 g glucose; 1.0 g peptone; 1.0 g yeast extract; 0.5 g (NH₄)₂SO₄; 0.4 g KH₂PO₄; 0.1 g MgCl₂; 0.01 g FeCl₃; 250 mL soil extract; 20 g oxoid agar; 750 mL tap water; pH 6.6-7.0. These media were supplemented with 0.25% magnesium trisilicate (Mg₃O₅Si₃) or modified medium using quartz sand and zeolite as sources of silicate.

Silicate-solubilizing capacity

For assessment in solid Bunt and Rovira medium, petri dishes containing Bunt and Rovira medium at each initial pH condition were inoculated with each culture strain of SSF. The culture dishes were incubated at 28°C for seven days. The diameter of the solubilization halo (translucent area surrounding colonies) was measured after 7 days incubation and the Solubilization Index (SI) expressed as halo diameter (mm)/colony diameter (mm) was calculated (Akintokun et al., 2007). The investigated strains were classified based on their SI as demonstrating low (SI < 2.0), intermediate (2.0 ≤ SI < 4.0) and high (SI ≥ 4.0) solubilization capacities.

Silica solubilizing capacity was assayed in 50 mL liquid and solid Bunt and Rovira medium with 0.25% (w/v) insoluble magnesium trisilicate, quartz, and zeolite respectively. Inoculation was performed by transferring two pieces of inoculant (Ø = 3 mm; 10⁶ spores) on 250 mL Erlenmeyer flask containing 50 mL of liquid culture Bunt and Rovira medium. The cultures were incubated on a mechanical shaker at 100 rpm (28°C) for 4, 8, and 12 days. Silica solubilizing capacities was determined by using American Standard Testing and Material (ASTM D859-16) method. The dissolved silicate in the form of silicic acid (H₂SiO₃) was measured using a spectronic 21 spectrophotometer at 660 nm wavelength (Rinder & Oelkers, 2014).

Effect of organic acids on silica dissolution

This experiment was carried out to clarify the relative strength of different types of organic acids in solubilizing silica from quartz sand and zeolite by using ASTM D859-16 method with colorimetric measurement. Citrate, acetate and oxalate were added separately in different concentrations (0, 100, 200, 300, 400, and 500 ppm) to 50 mL of Bunt and Rovira medium containing 0.25% (w/v) quartz or zeolite, then incubated for eight days on a mechanical shaker at 100 rpm in 28°C. The dissolved silicate in the form of silicic acid (H₂SiO₃) was measured using a spectronic 21 spectrophotometer at 660 nm wavelength (Rinder & Oelkers, 2014).

Further, the SSF producing of organic acid was determined in liquid Bunt and Rovira with 0.25% (w/v) magnesium trisilicate, quartz sand and zeolite. Seven strains of SSF were grown in liquid Bunt and Rovira medium for 8 days, then incubated at a mechanical shaker at 100 rpm in 28°C. The type and concentration of organic acid produced by *Aspergillus* and *Trichoderma* species were determined on 8 days incubation as described by Jamal et al. (2005).

High-performance liquid chromatography (HPLC) Agilent HP Series 1100 was used to identify and quantify organic acids concentration in the culture with 8 days incubation. Samples
were collected, filtered through a 0.45 μm cellulose membrane, and injected into a Supelco gel C-610H 9 μm chromatographic column sizing 30 cm x 7.8 mm (Marra et al., 2015).

Effect of citric acid producing by SSF isolates on silica dissolution

The SSF producing citric acid was determined in liquid Bunt and Rovira with 0.25% (w/v) quartz and zeolite as sources of silica. Seven strains of SSF were grown in liquid Bunt and Rovira medium for 4, 8, and 12 days, then incubated at a mechanical shaker at 100 rpm in 28 °C. Citric acid assay was done according to improved pyridine-acetic anhydride method suggested by Marier and Boulet (Chetan et al., 2018). About 1 mL of pyridine and 5 mL of acetic anhydride added and incubated for 30 min in ice bath. The standard was prepared by different volumes of citric acid (100 mg of citric acid was dissolved in 100 mL of deionized water (DW), i.e., 0.2–1 mL in 5 different test tubes. The volume was made up to 1 mL with DW. One milliliter of pyridine and 5 mL of acetic anhydride were added. The blank was prepared by taking 1 mL of DW in the place of citric acid. It was then kept for incubation in ice bath for 30 minutes. Concentration of citric acid was found by plotting graph of absorbance versus concentration and measured at 420 nm wavelength with the development of color density.

Results and Discussion

Silica originated from quartz and zeolite minerals

Selected chemical analysis showed that the quartz sand has alkaline reaction (pH H2O=8.2) and mainly composed a high content of SiO2, and less of Fe2O3, Al2O3, TiO2, and K2O (Table 1). Quartz sand obtained has a good quality with a cadre of over 99.1% total SiO2. The result of this analysis showed that impurities usually present in the quartz sand were free and coated iron oxides, aluminum, and smaller amounts of magnesium, potassium and calcium minerals. According to Klein & Philpotts (2013), quartz has a specific crystalline form (hexagonal), thus physically and chemically resistant to weathering. Furthermore, a zeolite sample has alkaline reaction (pH H2O = 9.3) and been characterized by high values of SiO2 and CEC (Table 1). Bai et al. (2019) studied that zeolite form have many different crystalline structures, which have large open pores (sometimes referred to as cavities) in a very regular arrangement and roughly the same size as small molecules.

Silicate-solubilizing capacity

Numerous field studies have shown that supplying crops with adequate plant-available Si (mono-poly-silicic acid) can suppress plant disease, reduce insect attack, improve environmental stress, and increase crop productivity. For the purpose of supplying silica in the form of silicic acid (H2SiO3) for plant, there is a growing interest in the effectiveness of bacteria and fungi as mineral weathering agent (Santi & Goenadi, 2012; Goenadi & Santi, 2013; Kang et al., 2017). Microorganisms are the principal bio-weathering agents and contribute to soil arrangement by providing supplements, such as P, K, and Si, which bolster plant development (Bin et al., 2019). Different groups of researchers have developed the liquid screening media containing different silicon sources i.e.: feldspar, muscovite, biotite, and magnesium trisilicate (Santi & Goenadi, 2017; Vasanthi et al., 2018).

In these research, three strains of Aspergillus and four species of Trichoderma were selected in order to search the highly potential of SSF for solubilizing silicate originated from quarts and zeolite. All the seven SSF were studied previously regarding their ability to solubilize silica in Bunt and Rovira solid medium using magnesium trisilicate (2MgO.3SiO2) as a basic of silica source (Vasanthi et al., 2018). The highest diameter of clear zone in Bunt and Rovira solid medium after 7 days incubation was observed for T. polysporum and Trichoderma sp. These diameters were categorized as a high solubilization index (SI).

Both of Aspergillus and Trichoderma species have the ability to solubilize silica from initial sources of magnesium trisilicate (MgO2Si3) in Bunt and Rovira liquid medium. Silicate solubilizing capacity increased at 12 days incubation time. The amount of Si solubilized from magnesium trisilicate was directly related to the incubation time. The ability to solubilize silica in Bunt and Rovira liquid medium by Trichoderma species was higher than Aspergillus (Table 2). Furthermore, silica solubilizing capacity of T. polysporum in 8- and 12-days incubation on Bunt and Rovira liquid medium was less than T. viride and T. pseudokoningii. Results of this research indicated that no correlation between solubilizing silicate capacity and solubilization zone on solid Bunt and Rovira medium by using magnesium trisilicate as a silica source (Vasanthi et al., 2013).

Aspergillus and Trichoderma species also have ability to solubilize SiO2 from initial sources of quartz sand (Bangka-Belitung province) and zeolite from Bayah, Banten. These isolates grew well with variety of mycelia colors as response on different source of silica on solid Bunt and Rovira media (Figure 1 & 2). Quartz and zeolite have higher SiO2 content than magnesium trisilicate (65% SiO2), i.e.: 99.1 and 81.9%. All species of Aspergillus and Trichoderma have significantly (p < 0.05) positive correlation with incubation time in solubilizing silica originated from quartz, R2=0.79-0.88 (Aspergillus), R2=0.80-0.99 (Trichoderma) and from zeolite, R2=0.94-0.98.
Table 2. The ability of SSF isolates in solubilizing silicate in a liquid and solid Bunt and Rovira medium using 0.25% magnesium trisilicate as a silica source

| Isolat fungi pelarut silika | Solubilizing silicate from 2MgO.3SiO$_2$ (ppm) | Kelarutan silika dari 2MgO.3SiO$_2$ (ppm) | Solubilization Index (SI) |
|-----------------------------|-----------------------------------------------|------------------------------------------|---------------------------|
|                             | Incubation time (day) | Waktu inkubasi (hari) | 7 days incubation time | Masa inkubasi 7 hari |
|-----------------------------|-----------------|-----------------|------------------------|-------------------------|
| A. niger BCCF194            | 45.0 d*)        | 52.3 g          | 1.4 g                  | 2.1 b (intermediate)    |
| A. niger K0909              | 39.7 f          | 67.3 f          | 106.8 f                | 1.9 b (low)             |
| A. niger A1601              | 52.1 b          | 72.8 d          | 108.2 c                | 1.8 c (low)             |
| T. polysporum               | 51.7 c          | 75.0 c          | 110.0 d                | 4.0 a (high)            |
| T. viride                   | 42.7 e          | 85.9 a          | 124.0 c                | 1.0 d (low)             |
| T. pseudokoningii           | 38.2 g          | 86.8 a          | 128.7 b                | 2.0 b (intermediate)    |
| Trichoderma sp.             | 53.9 a          | 68.3 e          | 130.1 a                | 4.0 a (high)            |

*) Means in the same column followed by the same letter are not significantly different according to Duncan’s multiple range test at $\alpha = 0.05$

*) Angka dalam kolom yang sama yang diikuti oleh huruf yang sama berarti tidak berbeda nyata menurut uji jarak berganda Duncan pada $\alpha = 0.05$

Figure 1. The growth of *Aspergillus niger* (from left to right) BCCF194, Korbe 0909, and A1601 strains on solid Bunt and Rovira media containing quartz sand (above) and zeolite (below)

Gambar 1. Pertumbuhan *Aspergillus niger* strain (dari kiri ke kanan) BCCF194, Korbe 0909, dan A1601 di dalam media padat Bunt Rovira yang mengandung pasir kuarsa (atas) dan zeolit (bawah)

Figure 2. The growth of (from left to right) *Trichoderma polysporum*, *T. Pseudokoningii*, *T. viride*, and *Trichoderma* sp. on solid Bunt and Rovira media containing quartz sand (above) and zeolite (below)

Gambar 2. Pertumbuhan (dari kiri ke kanan) *Trichoderma polysporum*, *T. Pseudokoningii*, *T. viride*, dan *Trichoderma* sp. di dalam media padat Bunt Rovira yang mengandung pasir kuarsa (atas) dan zeolit (bawah)
(Aspergillus) and $R^2=0.93-0.97$ (Trichoderma), respectively. Among the SSF in this research, T. polysporum has the highest ability in solubilizing silicate from quartz and zeolite. The significantly positive correlation at the level of $p<0.05$ between incubation time and the amounts of silica released from quartz and zeolite by T. polysporum were $R^2=0.80$ and $R^2=0.95$. All Aspergillus niger strains and Trichoderma species have potential capacity in solubilizing silica from magnesium trisilicate (MgO-Si) i.e.: 91.4-130.1 ppm, but showed poor in solubilizing silica from zeolite (17.6-19.4 ppm) and quartz (7.7-9.0 ppm). These phenomena suggested that silica solubilizing capacity depends on the type of mineral as source of silica. As reported by Klein & Philpotts (2013) and Bai et al., (2019), quartz having a specific crystalline form, whereas zeolite have open pores in many different crystalline structures (Grismer & Collison, 2017), thus physically and biologically resistant to weathering. Further, the lowest solubilizing silica in quartz and zeolite compared to magnesium trisilicate because crystalline quartz and zeolite will dissolve only very slowly in hot watery alkaline solutions. Hydroxyl ions primarily attack and subsequently break the stronger siloxane bonds (Si-O-Si) located on the surface of the silicone component (Ehrlich et al., 2010), while amorphous SiO$_2$ in magnesium trisilicate will be readily dissolved at room temperatures.

**Effect organic acid on silica dissolution**

Production of organic acids is an important mechanism for solubilizing silicate from insoluble silicate from quartz sand and zeolite. According to Cuadros (2017), interaction of various microorganisms with the siliceous rocks takes place in three major ways: (i) bacteria and fungus solubilize silicate minerals by the secreted organic acids, (ii) microorganisms utilize silica by incorporating it into their cells, keeping it there for a period of time in an altered or unaltered condition, and then, releasing it into fermentation medium in the form of free silicic acid (H$_2$SiO$_3$), and (iii) they also assimilate silica by taking it in dissolved form, polymerizing the same within the cells and thus silica becomes an integral part of the organism itself and is not released prior to death.

Fungi are known to play major role in dissolution of minerals like silicates (Lee et al., 2019). Several beneficial fungi have been reported for their positive impacts on plant under different stress conditions through better uptake of these minerals. Solubilization of insoluble silica due to organic acid production by fungi is known to enhance their availability to plants (Ameen et al., 2019). Various studies have reported weathering of silicates by bacteria for its dissolution to make it available to the plants (Chandrakala et al., 2019). From this study showed that the increased citrate, oxalate and acetate concentration in the modified Bunt and Rovira medium, apparently related to the significantly increased ($p < 0.05$) in available silica concentration in solution (Figure 3). It indicated that citrate has the strongest influence on the solubilization silica from quartz sand ($R^2=0.89$) and zeolite ($R^2=0.85$). The result of the study suggested that the major mechanism of silica solubilization is due to the action of organic acids synthesized by SSF as reported by Bist et al. (2020) that the major mechanisms for the solubilization of silica by fungi is acidolysis. These phenomena lead to the assumption that citric acid is responsible for lowering the pH medium providing proton (H$^+$) to increase the silica solubilization. There was relationship between the pKa values of the acids and the amounts of silica dissolution. Citric acid has a higher dissociation constant (pKa = 3.14-6.40) than oxalic acid (pKa = 1.25) and acetic acid (pKa = 4.75).

![Graph 1](image1.png)

![Graph 2](image2.png)

**Figure 3.** Correlation between concentration of selected organic acid (citrate, oxalate, and acetate) and solubilization of silica from quartz sand (left) and zeolite (right) in modified Bunt and Rovira liquid medium

*Gambar 3. Hubungan antara konsentrasi asam organik (sitrat, oksalat, dan asetat) terhadap kelarutan silika dari sumber pasir kuarsa (kiri) dan zeolit (kanan) di dalam modifikasi medium cair Bunt dan Rovira*
Regarding the effect organic acid on silica dissolution, our study showed that *Aspergillus* and *Trichoderma* isolates produced acetic, citric, and oxalic acids in the presence of quartz and zeolite as silica sources in Bunt and Rovira liquid medium. Acetic acid was produced in higher amounts by *Aspergillus* and *Trichoderma* species than citric and oxalic acids (Table 3). However, further study is necessary to confirm the results particularly under different incubation time on organic acid production and its correlation between silica solubilizing capacity and organic acid production of the SSF.

**Effect of citric acid producing by SSF isolates on silica dissolution**

During our laboratory study, both *Aspergillus* and *Trichoderma* species produced high amount of citric acid, which is considered responsible for solubilization of silica originated from quartz and zeolite. There was significantly (p < 0.05) positive correlation between citric acid production and incubation time by all of *Aspergillus* strain (R²=0.85-0.99) and *Trichoderma* species (R²=0.96-0.99), except isolates *T. pseudokoningii* (R²=0.56) and *T. viride* (R²=0.44) in quartz as a source of silica (Figure 4-5). The highest citric acid production in Bunt and Rovira liquid medium by using quartz and zeolite as a silica sources in 12 days incubation was observed for *A. niger* BCCF194 i.e.: 889 ppm (quartz), 741.5 ppm (zeolite), and *A. niger* K0909 i.e.: 839 ppm (quartz) and 686.5 ppm (zeolite). Whereas, *T. polysporum* 684 ppm (quartz) and 509 ppm (zeolite), *Trichoderma* sp. 751.5 ppm (quartz) and 589.0 ppm (zeolite). Similar result was reported by Makut & Ekeleme (2018) that *A. niger* has higher potential in producing citric acid than *Trichoderma* isolate. Both silica dissolution and citric acid production have positive correlation with incubation time. The highest silica solubilizing capacity and citric acid production by *Aspergillus* and *Trichoderma* species were achieved at 12 days incubation.

**Table 3.** The organic acids produced by SSF in modified liquid Bunt and Rovira medium using Mg₃O₇Si₃, quartz, and zeolite as a silica sources after 8 days incubation time

| Silicate solubilizing fungi (SSF) isolates/Isolat fungi pelarut silika | Mg₃O₇Si₃ | Quartz | Zeolite |
|---------------------------------------------------------------|---------|--------|--------|
| Sumber silika                                                 |         |        |        |
|                                                               | Acetate | Citrate | Oxalate | Acetate | Citrate | Oxalate | Acetate | Citrate | Oxalate | Acetate | Citrate | Oxalate |
| *A. niger* BCCF194                                            | 1.03 a C | 0.80 a A | 0.22 a A | 0.93 d B | 0.81 a A | 0.14 ab B | 0.79 d C | 0.63 a B | 0.07 c C |         |         |         |
| *A. niger* K0909                                              | 1.38 a A | 0.79 b A | 0.10 d B | 1.14 a B | 0.77 a A | 0.10 ab B | 1.15 b B | 0.61 a B | 0.12 b A |         |         |         |
| *A. niger* A1601                                              | 0.59 g B | 0.54 e A | 0.14 b A | 0.51 e C | 0.44 d C | 0.09 b C | 0.76 e A | 0.49 b B | 0.12 b B |         |         |         |
| *T. polysporum*                                               | 1.24 b A | 0.55 e A | 0.15 b A | 0.94 d C | 0.61 b A | 0.15 ab B | 1.17 a B | 0.37 c C | 0.08 c A |         |         |         |
| *T. viride*                                                   | 0.95 d B | 0.59 d A | 0.13 c A | 1.11 b A | 0.54 c B | 0.10 ab B | 0.43 g C | 0.38 c C | 0.11 b AB|         |         |         |
| *T. pseudokoningii*                                           | 0.91 e A | 0.53 c A | 0.09 d C | 0.93 d A | 0.48 d B | 0.19 a B | 0.57 f B | 0.36 c C | 0.16 a B |         |         |         |
| *Trichoderma* sp.                                             | 0.75 f B | 0.74 c A | 0.22 a A | 1.04 c A | 0.57 bc B | 0.10 ab B | 1.04 c A | 0.50 b C | 0.16 a B |         |         |         |

Coefficient variable (%) = 1.5 2.8 3.6 0.7 3.5 1.8 0.6 6.7 4.2

*) Means in the same column and row followed by the same letters at the same organic acid are not significantly different according to Duncan’s multiple range test at α = 0.05

*1) Angka dalam kolom dan baris yang sama yang diikuti oleh huruf yang sama pada jenis asam organik yang sama berarti tidak berbeda nyata menurut uji jarak berganda Duncan pada α = 0.05
Enhanced solubilization of insoluble silicate from quartz and zeolite

Figure 4. Correlation between citric acid production by *A. niger* strain and incubation time in Bunt and Rovira liquid medium containing quartz (left) and zeolite (right) as a silica source

Gambar 4. Hubungan antara produksi asam sitrat oleh strain *A. niger* terhadap waktu inkubasi di dalam medium cair Bunt dan Rovira yang mengandung pasir kuarsa (kiri) dan zeolit (kanan) sebagai sumber silika

Figure 5. Correlation between citric acid production by *Trichoderma* isolates and incubation time in Bunt and Rovira liquid medium containing quartz (left) and zeolite (right) as a silica source

Gambar 5. Hubungan antara produksi asam sitrat oleh isolat *Trichoderma* terhadap waktu inkubasi di dalam medium cair Bunt dan Rovira yang mengandung pasir kuarsa (kiri) dan zeolit (kanan) sebagai sumber silika
Conclusion

Trichoderma polysporum and A. niger BCCF194 isolates performed high abilities in solubilizing silica from quartz sand and zeolite. Both fungi were able to produce considerable amounts of organic acids (citrate, oxalate, and acetate) in the culture medium in the presence of quartz and zeolite as a silica source and these organic acids were important components responsible for silica solubilization by Aspergillus and Trichoderma species. Acetic acid was the main organic acid produced by these species, but citric acid was more effective than oxalic and acetic acids in solubilizing silica from quartz and zeolite. The amount of Si solubilized and citric acid produced by SSF were directly related to the incubation time.

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