

BIOLEACHING OF LODAN QUARTZ USING *Aspergillus ficuum*

BIOLEACHING PASIR KUARSA LODAN MENGGUNAKAN *Aspergillus ficuum*

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ABSTRACT

Depending on its purity, quartz has a wide application in industry. Fungi play an important role in the quartz purification. A bioleaching study of Lodan quartz sample from Rembang, Central Java was conducted to obtain a suitable raw material for industrial applications. The microbial process using selected-indigenous fungus of *Aspergillus ficuum* in terms of removing iron, aluminum, and other unwanted metals within quartz. The result was then compared to the chemical leaching using pure citric and oxalic acids. The bioleaching process removed the iron (Fe_2O_3) from the initial content (0.78%) in the original sand sample to reach a level of 0.013%. Almost 98.3% iron was removed. The bioleaching test also removed the aluminum, manganese, chrome, and titanium to a very low level within the 12-day process. The iron content in this treated quartz met the standards for optical and high-quality glass. On the other hand, the chemical leaching using pure citric and oxalic acids concentrations were equal to those that were produced by *A. ficuum* could only removed 70.5% of iron around 0.23% iron and 0.29 % aluminium were still remained in the sand. This fact suggested that the bioleaching method is more effective than the chemical one using the organic acids. The use of fungi to remove iron from quartz has the potential to be an effective method for upgrading the content and the commercial value of the quartz. The experimental results of this study have provided significant opportunity to use biotechnological approach for producing the quartz as a feed material for the high-quality glass industry.

Keywords: *Aspergillus ficuum*, bioleaching, citric acid, oxalic acid, quartz

ABSTRAK

Tergantung kemurniannya, pasir kuarsa mempunyai penggunaan yang luas di industri. Jamur mempunyai peran yang penting dalam pemurnian pasir kuarsa. Penelitian bioleaching pasir kuarsa Lodan, Rembang, Jawa Tengah dilakukan untuk mendapatkan bahan baku yang memenuhi persyaratan sebagai bahan baku industri. Percobaan bioleaching untuk menghilangkan besi, aluminium dan logam-logam lain yang tidak diinginkan dilakukan menggunakan isolat jamur terpilih berasal dari mineral kuarsanya, yaitu *Aspergillus ficuum*. Hasilnya dibandingkan dengan pelindian menggunakan bahan kimia asam sitrat dan oksalat murni. Proses bioleaching selama 12 hari dapat mengurangi atau menghilangkan kadar besi (Fe_2O_3) dalam kuarsa dari kadar awal sebesar 0,78% menjadi 0,013% (penghilangan sebesar 98,3%) serta mampu menghilangkan aluminium, mangan, krom dan titanium hingga kadar sangat rendah. Pelindian secara kimia menggunakan asam sitrat dan oksalat yang besarnya sebanding dengan jumlah maksimum asam sitrat dan oksalat yang diproduksi oleh *A. ficuum* dalam proses bioleaching, hanya mampu menghilangkan 70,5% besi sekitar 0,23% besi serta 0,29% aluminium masih tertinggal dalam kuarsa. Proses bioleaching lebih efektif dibandingkan dengan pelindian menggunakan asam organik dalam upaya meningkatkan kadar dan nilai komersial pasir kuarsa. Hasil penelitian ini telah membuka kemungkinan yang cukup penting untuk menerapkan pendekatan bioteknologi dalam memproduksi bahan baku pasir kuarsa yang memenuhi syarat untuk bahan baku industri optik dan gelas kualitas tinggi.

Kata kunci : *Aspergillus ficuum*, bioleaching, asam sitrat, asam oksalat, pasir kuarsa

INTRODUCTION

Quartz is used in a number of industries, such as glass, foundry, refractory and ceramic industries. Quartz is a material that appears in nature combined with iron oxides, hydroxides, and heavy metals. A great part of the quartz beds is contaminated by iron oxides at concentrations of 0.2-3% (Götze and Möckel, 2012). Process to purify the industrial minerals, such as quartz sand contaminated by iron and other metals, are essential to render these raw materials used for traditional industrial applications (ceramics, papermaking glass, etc.), less common uses including glass fibre and refractory insulation bricks (Haus, Prinz and Priess, 2012), and for more advanced application that ensure a higher value-added component, such as optical fibers and the production of pure silicon (Platias, Vatalis and Charalampides, 2014).

There is a number of potential sources for different industrial applications. The most stringent chemical specifications are for the chemical and glass industries. In general, the specifications of the raw materials depend on the glass produced, and the purity level of quartz sands is dominated by the iron content. The British Standard BS2975 includes recommended limits for the composition of quartz and for seven different grades of glass (Table 1). Aluminium, magnesium, and potassium levels affect the melting properties and have to be kept at low levels. Titanium and chromium may melt and will color the glass. In addition, the silicon solar grade destined for the photovoltaic application required a very high purity of silica (>99.999%) with Fe must be less than 1 ppm (Xakalashe and Tangstad, 2011).

Purification of quartz sand is extremely important for many industries. Several techniques are available for upgrading quartz by partial removal of iron such as flotation, heavy-media separation or magnetic

separation. But these seldom reduce the iron and other metals to an acceptable level. Other techniques available are based on the use of sulphuric acid, hydrochloric acid, and phosphoric acid as having been demonstrated by Tuncuk and Akcil (2014); Suratman and Handayani (2014); and Zhang *et al.* (2012). Those techniques are very efficient in removing metal impurities, but they are generally costly and have a considerable impact on the environment. In this scenario, the use of organic acids, especially citric and oxalic acids as a leachant agent in the metal removal process from industrial minerals holds potential interest (Vapur, Top and Demirci, 2017). Organic acids dissolve the iron oxides by direct attack of H^+ ions for the mineral matrix, maintaining them in solution by forming soluble complexes and chelates. Organic acids can be produced by fermentation with filamentous fungi such as *Aspergillus* and *Penicillium*. The exhaust fermentation medium can be the leaching agent for dissolving the quartz iron oxides. This biotechnological route that uses microorganism for removing metal contaminants could prove to be cheaper, environmental benign and less complex because producing effluents, which are easy to purify. This process refers to the removal of undesirable mineral components from a sand through interaction with a microorganism, which brings about their selective dissolution (removal) and thereby enriching the desired mineral constituents in the solid one matrix.

Earlier laboratory investigations have shown that bacteria and fungi could be effectively used to remove iron from silica, kaolin, bauxite, and quartz sand (Štyriaková *et al.*, 2012, 2015; Gurevich *et al.*, 2015; Šuba and Štyriaková, 2015). However, no systematic studies on bio beneficiation of quartz for the removal of iron and other impurities metals using indigenous fungi isolated from local-original mineral have been so far reported.

Table 1. Specified grades of quartz for glass industry (Platias, Vatalis and Charalampides, 2014)

| Grade | Type of raw material sands | SiO ₂ (%) | Fe ₂ O ₃ (%) | Al ₂ O ₃ (%) | Cr ₂ O ₃ (%) |
|-------|----------------------------------|----------------------|------------------------------------|------------------------------------|------------------------------------|
| A | Optical and ophthalmic glass | 99.7 | 0.013 | 0.2 | 0.00015 |
| B | Tableware and lead crystal glass | 99.6 | 0.010 | 0.2 | 0.0002 |
| C | Borosilicate glass | 99.6 | 0.010 | 0.2 | 0.0002 |
| D | Colorless container glass | 98.8 | 0.030 | 0.1 | 0.0005 |
| E | Clear flat glass | 99.0 | 0.100 | 0.5 | - |
| F | Colsandd container glass | 97.0 | 0.250 | 0.1 | - |
| G | Glass for insulating fibers | 94.5 | 0.300 | 3 | - |

Citric and oxalic acid is a common metabolite excreted by several fungi under specific conditions. To seek the best fungi, it is needed to isolate more fungi with better properties to enhance metal removal from the mineral. Preliminary screening of mineral samples from quartz sand deposits in Lodan, Rembang, Central Java showed the presence of a number of fungi. Among other species such as *Penicillium oxalicum*, *Trichoderma viride*, *Aspergillus oryzae*, and *Aspergillus ficuum*, the fungus of *Aspergillus ficuum* is favoured as the best citric and oxalic acid production species from this deposit (the paper of this matter is in progress). This microorganism can accelerate the reactions of metal solubilization by producing organic and inorganic acids, creating metal-complexing ligands, changing redox condition or mediating formation of secondary mineral phases.

The purpose of the present study is the removal of iron and other metals from quartz sand originated from Lodan by means of bioleaching using *Aspergillus ficuum*, and to evaluate this fungus performance. The results were then compared with chemical leaching using pure citric and oxalic acid solutions. Very low levels of iron and other impurities must be reached in the sand after bioleaching to permit its use for high-quality glass industry (maximum 0.013% of Fe_2O_3). Further target values are <0.01% Fe_2O_3 for tableware and crystal glass application and <1 ppm for pure silicon production (99.9999% silica sands).

METHODOLOGY

Mineral samples

The sample of quartz sand was taken from the quartz deposit in Lodan, Rembang, Central Java. The sample was oven dried, and based on previous experiments with quartz, for experimental testing, the quartz sample was ground to 241 μm to improve metal leaching by increasing the surface area. The sample was analysed using X-ray diffraction for characterization and X-ray fluorescence for chemical composition of the mineral.

Microorganism

The *Aspergillus ficuum* used in this study was isolated from the quartz sand samples

of the Lodan. The sand was heating at 80°C for 15 min to kill the non-sporo forming species. Several fungi were obtained and purified by colonies reisolation on the PDA (Potato Dextrose Agar) plates to obtain pure strain cultures. Four fungi strains were successfully isolated and screened. By a series of morphological, biochemical dan physiological tests, the four fungi were identified. The results indicated that they were *Penicillium oxalicum*, *Trichoderma viride*, *Aspergillus oryzae*, and *Aspergillus ficuum*. The *A. ficuum* strains were—then chosen for this bioleaching study since the fungus was predominant and found to be the best citric and oxalic acids production strains compared to other three strains.

Leaching Experiment with Fungi

Bioleaching of the 30 g quartz samples was carried out in 500 ml Erlenmeyer flask containing 300 ml of medium consisting of (g/l): glucose 90, NaNO_3 2.5, KH_2PO_4 1.5, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5, yeast extract 2.0. The pH value of the solution was adjusted with 2 M NaOH (pH 6.0), buffered with Tris 1 M at the beginning of incubation, then sterilized at 121°C for 30 min. The submerged culture media were inoculated with a spore suspension of *A. ficuum* concentration of 5×10^6 spores/ml under aseptic condition. The number of spores was determined using a Neubauer counting chamber. The flasks were incubated at room temperature on a rotary shaker at 250 rpm for 14 days. The amount of citric and oxalic acid produced, the concentration of released metals ions and the rate of metals removal were measured and determined every day. The abiotic control in the absence of *A. ficuum* was incubated under the same condition. After being incubated, the culture solutions were separated from the biomass by means of filtration.

Leaching Experiments with Organic Acids

For comparison with the bioleaching processes above, the chemical leaching test was also performed using pure organic citric and oxalic acid. Another objective of the work was to investigate the use of organic acids as a cheaper and more environmentally acceptable alternative compared to sulphuric or hydrochloric acids. Reagents from Sigma Aldrich and distilled water were used in the experiments. A 30 g of quartz sample was

put into a 500 ml Erlenmeyer flask, then added by 300 ml of pure citric and oxalic acid solution at concentration of 0.21 M and 0.43 M respectively, which corresponded to the concentration of citric and oxalic acids produced by *A. ficuum* in the bioleaching process. The flask was put on the rotary shaker at 250 rpm at room temperature for 10 hours. A 3 ml of sample solution was taken periodically every hour to monitor the dissolved metal extracted during the course of the process.

Analytical Methods

The mineral and chemical composition of quartz sample were characterized by X-ray diffraction (XRD) and X-ray fluorescence (XRF) analyses. The amount of citric acid produced by *Aspergillus ficuum* was determined by the titration method of Marrier and Boulet, and oxalic acid was determined using a spectrophotometer at 525 nm (Milton Roy Spectronic 401) based on the method developed by Bergemann and Elliot. An Atomic Absorption Spectrophotometer instrument (Shimadzu AA 680) was used to determine the concentrations of metal ions (iron, aluminium, manganese, chrome and

titanium ions) in the liquid samples taken during the leaching process and in the mineral characterization studies.

RESULTS AND DISCUSSION

Characterization of the Lodan Quartz Mineral

The results of mineralogical analyses of the Lodan quartz sample using XRD is presented in Table 2 and Figure 1. It can be seen that quartz is the main component (93%) containing 4% of orthoclase, 3% of calcite. The XRF result shown in Table 3 indicates that the quartz having a high content of Fe (0.78%) and Al (0.77%) as the main impurities.

Table 2. Mineral composition of Lodan quartz sample

| Mineral | Chemical Formula | % |
|------------|--|----|
| Quartz | SiO ₂ | 93 |
| Orthoclase | K(Al,Fe)Si ₃ O ₈ | 4 |
| Calcite | Ca(CO ₃) | 3 |

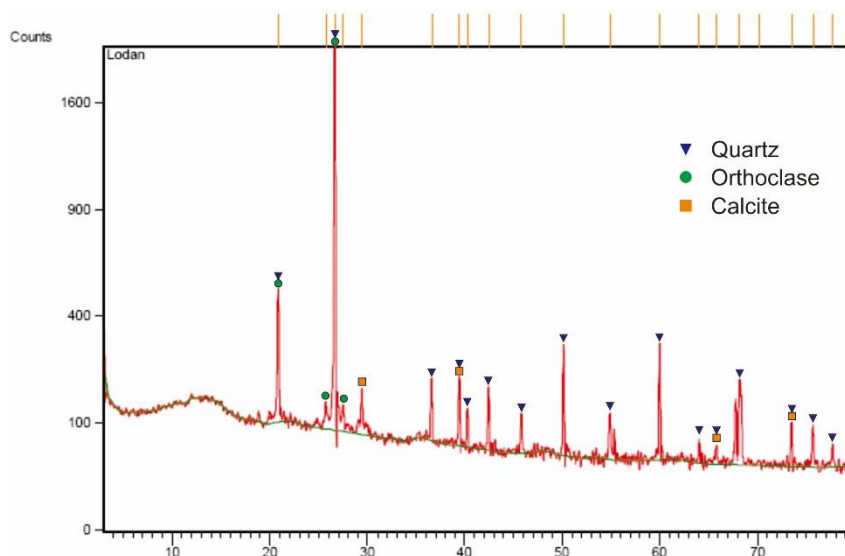


Figure 1. Characterization of Lodan quartz sample by XRD analyses

Tabel 3. Chemical composition of Lodan quartz sample and impurities

| Location | Concentration of SiO ₂ and impurities (%) | | | | | | | | | | |
|----------------|--|------|------|------|----|-------|-------|-------|----|----|----|
| | SiO ₂ | Al | Fe | Ca | Mg | Mn | Cr | Ti | V | Zr | Cu |
| Lodan, Rembang | 97.41 | 0.77 | 0.78 | 0.66 | ud | 0.031 | 0.014 | 0.072 | ud | ud | ud |

Note : ud = undetectable

Bioleaching with Fungi

The release/removal of metal ions from quartz was used as an indicator of mineral dissolution and beneficiation of quartz quality in this study. The main purpose of the bioleaching process was to obtain a quartz with much lower metal content than in the original sample. In particular, the target values were Fe_2O_3 concentration at least 0.013% for the high-quality glass industry. Minimum extraction yields or iron removal of 98% was thus required from the bioleaching process.

The results of the fungal leaching process were shown in Table 4 and Figure 2. It can be seen that 98.3% of iron can be removed from the quartz to reach a final concentration of 0.013% in the sand. Moreover, high removal rates of aluminium, calcium, titanium can be achieved, and manganese and chrome can be removed completely (100% removal). This maximum removal was obtained after 12 days of the process (Figure 2). The iron and aluminium removal started 2 days after incubation. The removal rate was very high between day 4 and day 11 of the process, then reached the maximum removal after 12 days. This result has a significant improvement in terms of process time compared to work done by Štyriaková *et al.* (2012) who performed bioleaching of iron oxide coatings from quartz sand using heterotrophic bacteria and *Bacillus spp* in a much longer period of a process for 63 days at 24°C. Moreover, in another related study, Arslan and Bayat (2009) have reported that the bioleaching of iron oxide by *Aspergillus niger* resulted in the removal of 47.7% of total iron in the quartz sand, and the iron content decreased from 0.31 to 0.164% in the bioleached quartz sand. Therefore, *Aspergillus ficuum* used in this present study was being more efficient with higher efficiency leaching rate of iron. The role of microbes actions and the reactions behaviour might be different from one mineral to another because each mineral has its specific chemical composition and mineral

characteristics. However, the ferro-enzymatic reduction must be involved in the reactions, so it is important to choose the best microbe strains.

Indigenous microbes that were obtained from the original ore can adapt naturally to the certain circumstance in the ore slurry during bioleaching process such as high heavy metal concentrations so that they can better overcome the disadvantage and propagate than non-indigenous microbes. As the propagation of microbes dramatically increases, the concentration of microbes increases quickly, so the leaching rate is accelerated, which is much faster than non-indigenous microbes.

In this bioleaching process, *A. ficuum* produced citric and oxalic acid starting after 2 days of incubation and reached maximum concentration of 41 and 39 g/l after 10 days of the process as shown in Figure 2. Compared to research done by Handayani and Suratman (2016) that produced 23 g/l citric acid and 21 g/l oxalic acid in a bioleaching process for 20 days using *Aspergillus sp* isolated from nickel sand, this work yielded almost two times of citric and oxalic acids production.

Iron and aluminium ions were generated in the solution that was obviously seen, started from the second day of the process. It can also be seen that dissolution (removal) rate gradually increased as the citric and oxalic acids concentration in solution increased. After the first 12 days the organic acid concentration remained stable and the removal rate also stable. The sigmoid form of the curves and the strong relationship between organic acid concentration and rate of iron and aluminium removal indicates that the reaction of iron and aluminium removal is catalyzed by the organic acid generated in the solution. It is suggested that the highest removal of those metal ions corresponded with the maximum in organic acid production by the fungi.

Table 4. Metal removal from quartz by bioleaching for 12 days.

| Treatment | Concentration of SiO_2 and impurities (%) | | | | | | | | | | |
|--------------|--|------|-------|------|----|-------|-------|-------|----|----|----|
| | SiO_2 | Al | Fe | Ca | Mg | Mn | Cr | Ti | V | Zr | Cu |
| Raw Material | 97.41 | 0.77 | 0.78 | 0.66 | Ud | 0.031 | 0.014 | 0.072 | ud | ud | ud |
| Bioleaching | 99.70 | 0.02 | 0.013 | 0.01 | Ud | Ud | ud | 0.01 | ud | ud | ud |

Note : ud = undetectable

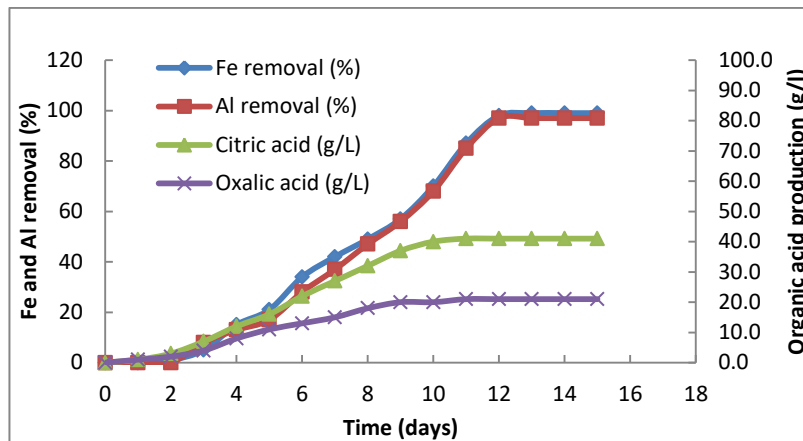


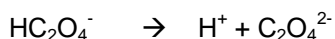
Figure 2. Production of citric, oxalic acids (g/l), and iron and aluminium removal (%) during bioleaching process of quartz by *Aspergillus ficuum*

A. ficuum produced enzymes and organic acid specifically to degrade and solubilize minerals. They break down mineral structures and extract elements required for metabolism or structural purposes (mineralised). This is especially important for ions such as Fe and Al. The chemical reactions of citric and oxalic acid with the oxides of iron, aluminium and calcium can be drawn as follows (Salmimies *et al.*, 2012; Hernández *et al.*, 2013):

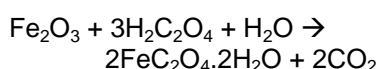
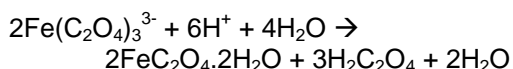
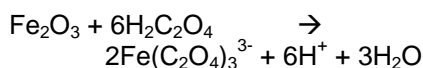
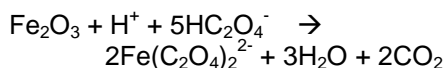
In solution, oxalic acid ($\text{H}_2\text{C}_2\text{O}_4$) dissociates releasing bi-oxalate ion (HC_2O_4^-):



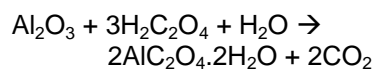
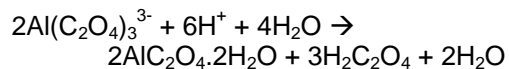
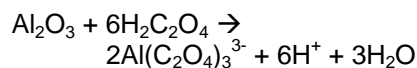
The bi-oxalate ion formed dissociates releasing the oxalate ion ($\text{C}_2\text{O}_4^{2-}$):



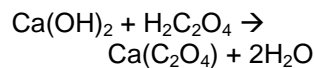
Of these species, it is considered that the bi-oxalate is responsible for the dissolution of the iron:



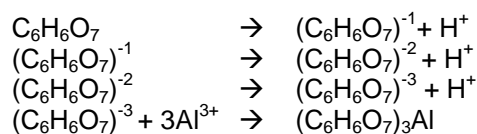
Reactions with aluminium oxide:



Reactions with calcium oxide :



Chemical reaction citric acid with aluminium can be drawn as follows :



Fungi interact with minerals and synthesize an array of organic compounds that have been shown to affect the mobility of metal ions. Their biological activity was high and dependent on the production of organic acids, such as citric and oxalic acids. The samples from Lodan quartz deposit were characterized by a high number of fungi. This fact suggests that they were the representatives of the microbial community, which was active in this environment. They play an important role in the iron and the metal removal from quartz.

Leaching with Organic Acids

The primary advantage of using citric and oxalic acids is in the formation of soluble complexes during leaching that is both photochemically and microbiologically degradable. The chemical leaching operation, performed with citric and oxalic acids as the leaching agents was carried out in an analogous way with bioleaching experiments, except that *A. ficuum* was not involved in the process. The citric and oxalic acids used (0.21 M and 0.43 M respectively) corresponded to the amount of citric and oxalic acids produced by *A. ficuum*, and therefore used for the chemical leaching process. The results presented in Table 5 show that chemical leaching gave values lower than those with bioleaching. The maximum removal of iron and aluminium was only 70.5% and 62.3%, respectively; and 0.23% of iron and 0.29% of aluminium still remained in the treated sand. Those results did not meet high-quality glass specifications. This leaching capacity of organic acids is intrinsic and not due to the influences of *A. ficuum*'s activity. This observation clearly indicates that the activities of *A. ficuum* gave positive effects in enhancing metal removal from the sand. Abiotic leaching with laboratory prepared solutions of these organic acids was not as effective as bioleaching indicating that compounds other than the identified organic acids may contribute to leaching performance. Similar results are reported by Hassanien, Desouky and Hussein (2014) also Brisson, Zhuang and Alvarez-Cohen (2016) where the bioleaching method is more effective than the chemical one using organic acids.

CONCLUSIONS

The ferrous quartz sand from Lodan, Central Java has been subjected to the biotechnological method in order to reduce the iron and other metals content, so as to

attain a high value-added product. The comparison of metal removals from quartz by bioleaching and chemical leaching shows that the bioleaching results had a better performance. Bioleaching process could remove 98.3% of iron (Fe_2O_3) and reduced all other metals from the quartz sand significantly to a very low level within 12 days of the process. Iron removal by chemical leaching gave a lower value of 70.5%.

It is clear that microbial leaching mechanism had a role to play in the bio-beneficiation process of quartz by *A. ficuum*. This fungus strain has a good ability to remove iron and other metals impurities from quartz sand in comparison with chemical leaching. Since significant amounts of citric and oxalic acids were present in the liquid phase, iron and other metals can be solubilized. Probable reasons behind incomplete iron removal lie in the oxidation state at which the iron is present in sand matrix. Hematite (Fe_2O_3) is a refractory oxide while lower valence oxides such as goethite and magnetite (Fe_3O_4) are more readily soluble.

The reported results, however, establish the significant potential of biobeneficiation for the removal of iron and other metal ions from quartz adequate for industrial application, especially for optic and high-quality glass industry. Through appropriate control of biological process and optimization of influencing parameters such as type of medium, biomass content, pulp density and particle size of the mineral, possibilities of enhancing iron removal definitely exist to reach further target values of <0.01% Fe_2O_3 for tableware and crystal glass application and <1 ppm for pure silicon-photovoltaic production. Future experimentation is planned by utilising parameters that will be further optimised to improve the process rate. After that stage, a flow sheet of the process will be designed to evaluate the cost of the process.

Table 5. Metal removal from quartz by leaching with organic acids for 8 hours

| Treatment | Concentration of SiO_2 and impurities (%) | | | | | | | | | | |
|-------------------|--|------|------|------|----|-------|-------|-------|----|----|----|
| | SiO_2 | Al | Fe | Ca | Mg | Mn | Cr | Ti | V | Zr | Cu |
| Raw Material | 97.41 | 0.77 | 0.78 | 0.66 | ud | 0.031 | 0.014 | 0.072 | ud | ud | ud |
| Chemical Leaching | 98.03 | 0.29 | 0.23 | 0.10 | ud | 0.01 | ud | 0.01 | ud | ud | ud |

Note : ud = undetectable

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