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Dissolution of Potassium from Silicate Mineral by *Aspergillus* strain

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Abstract

Potassium (K) is the third most important macronutrient for plant growth and development. It is an essential nutrient of life on earth. It plays very important role in physiological and biochemical processes but the concentration of available potassium is very low below 2%. Soil of many areas of India have shown K deficiency .Agricultural practices, water runoff, erosion and leaching reduces the K availability in soil. In India resources of mineral particularly insoluble potassium are present in huge amount so they can be utilized as K fertilizer by application to the agricultural area. As a conventional process such as roasting, smelting etc involve high energy consumption and causes pollution. Biohydrometallurgy: bioleaching is emerging a natural choice for extraction of metals from minerals. The aim of the study was to isolate K solubilizing fungi, for which various rhizospheric soil samples were collected. From these samples total 25 fungal isolates were obtained, which were screened for K solubilization on Aleksandrov agar plates. Out of these, Isolate SDS7, an Aspergillus strain, showed zone of solubilization of 15 mm was selected. Parameter such as particle size of the mineral, pulp density, inoculum size, pH of the medium were optimized in shake flask study. Maximum biodissolution of K was achieved using particle of <44 µm size resulted in 28 ppm solubilization after 21 days of incubation. When pulp density was increased from 0.5% (w/v) to 0.8% (w/v) to 1% (w/v) gave 42 ppm and 53 ppm solubilization in 21 days at pH 6 with 10×10^7 spores / ml inoculum size. Qualitative and quantitative essay of organic acid detection was carried out by standard method showed maximum production of citric and tartaric acid which could be responsible for the K solubilization.

Keywords: Biodissolution, Potash mineral, Solubilization, Optimization.

Introduction

Potassium is the seventh most common element in the soil and third macronutrient but it often receives less attention than N and P. In general, plants are able to gain a very limited amount of K from minerals applied to the soil. It plays very important role in many biological and physiological reactions like enzyme activation, protein synthesis and it also affect the root and stalk development. Plant deficient in K is less resistant to pest and disease[1]. It also replaces the nutrients in the soil which were removed by crop and also confers resistance to biotic and abiotic stresses[2-4].

Indian soil contain approximately 2% soluble K, rest of 98% K present as insoluble form, potassium exist in four form soluble, exchangeable, non exchangeable and mineral rock form[5]. Plants directly take the potassium which is present in soil as soluble form. The most common mineral sources of K in soils are feldspars, biotite and mica[6] consumes 7 Mtpa potash fertilizer but production is 0.5 Mtpa only. Due to less availability and lack of awareness of farmers the use of chemical fertilizers increases in last decades[7], farmer get happy in the beginning for high productivity but slowly it shows ill effect on plants. According to IFA 90% of potash fertilizer produced use in agriculture, from the beginning of 20th centaury.

India ranks 4th in consumption of potassium fertilizers, on an average 1.7 million tonnes of potassium are being imported annually from different country[8]. People started studying the relationship between microbes and mineral[9-10]. Heterotrophic microorganisms have that mechanisms, when growing in presence of minerals, are able to leach metals, leaching of mineral is due to the effect of produced organic acids[11-12], amino acid and other metabolites in broth system.

Friedrich and Ullman have seen that potassium release through production and excretion of organic acids[13-14]. Bioleaching of insoluble mineral is a complex process affected by multifactor (copper). The proper choice of the value of these parameters is necessary if economically feasible process is to be developed. Very few reports were available on bioleaching and solubilization of metals from its insoluble mineral. Sheng and Huang found that silicate solubilizing microorganisms were found to solubilize silicon, Aluminum and potassium release from the mineral[15-16].

The conducted experiment was focused on the optimization various factors that could induce higher level of K solubilization, which will be helpful for the transformation of insoluble potash to soluble form[17] and to improve farmer profit by using biofertlizer instead of chemical fertilizer. (Mtpa; million tones per acre)

Materials and Methods

Sample collection, enrichment and isolation of potassium solubilizers: Different soil samples like rhizospheric soil of seasonal and herbal plants, ceramic industry soil from various regions of Gujarat and India were collected. Individual soil samples and all soil samples were mix together to make a composite from which 2.5gm of soil was added to 25ml of medium and shaken for 48 h at 150 rpm at 28±2ºC. Serial dilution was carried out using individual sample as well as enriched samples, plated on modified Aleksandrov agar medium containing (g% w/v) (glucose 4.0, $MgSO_4$ 0.05, $CaCO_3$ 0.01, NaH_2PO_4 0.2, NH_4SO_4 0.2, $FeCl_3$ 0.0006, Feldspar 0.5) in 100 ml of deionized water pH adjusted to 7.0. Feldspar sample was collected from mine chemical constituents of mineral sample used in this study was mentioned in Table-1.

Screening of K solubilizers: Potassium solubilizing microorganisms were obtained on modified Aleksandrov medium by spot test method[18]. Plates were incubated at 28±2 ºC for 10 days. The diameter of the solubilization zone was measured and best solubilizers were further screened for quantitative analysis[19]. For quantitative analysis potassium solubilizing microorganisms inoculated in liquid broth and kept at 28±2 ºC for 10 days. After each 24 h sample was withdrawn and solubilized k was measured by flame photometer using standard solution.

Optimization of K solubilization by *Aspergillus* SDS7: Solubilization of potassium from the insoluble feldspar powder was carried out in modified Aleksandrov medium broth in Erlenmeyer flasks in incubator shaker at neutral pH and 28±2 ºC. All the experiments were conducted in triplicate. *Aspergillus* SDS7 was inoculated in broth and incubated for several days at 150rpm. A known amount of sample was withdrawn at every 24 h for analysis of K, pH and organic acids released. Sample was centrifuged at 8000g for 15 min. Quantitative analysis of organic acid was done by pyridine method[20]. Effect of pH on solubilization of K was studied by adjusting different initial pH 4, 5, 6, 7, 8 using 0.01N HCl and 0.01N NaOH and all others parameters were kept constant. To check the optimum inoculums size spore count was carried out using Neubauer Chamber, spore count was set as $2x10^7$, $4x10^7$, $6x10^7$, $8x10^7$, $10x10^7$. Whereas pulp density was standardize from 0.1 to 1 in terms of g%. The particle size of the mineral was also optimizing from 44µm to 320 µm.

Results and Discussion

Various physicochemical properties like; pH, EC, organic carbon, nitrogen, K_2O, zinc, iron, copper and manganese content has been presented in Table-2. Total 25 fungal isolates were obtained from different soil samples. Potasssium solubilizing efficiencies of these isolates were determined to screen out prominent strains having high solubilization capacity on the basis of both qualitative and quantitative. *Aspergillus* SDS7 showing 15mm zone, used for further studies for optimization.

The effect of pH range from 4- 8 at 0.5% (w/v) pulp density, 28±2 ºC temperature, $4x10^7$ inoculum size with <44µm size particles on potassium solubilzation was examined and results are presented as solubilzation after 21 days of incubation Figure-1. As the pH of the medium plays a very important role in growth, metabolism and various activities of microorganisms, most of the fungi prefer acidic pH for their growth and development. An appreciable increase in K solubilization (25.16 ppm) at pH 6 with increase in citric acid concentration 45.5 ppm in 21 days was observed as compared to the 12, 7, 22, 8 ppm at pH 4, 5, 7, 8 with corresponding change in citric acid amount 9, 15, 40, 9 ppm respectively. Potassium solubilization was very low at acidic and basic pH due to decreased activity of fungi. At pH 4, K solubilzation was decreased. Release of K may be due to the production of organic acids by *Aspergillus* SDS7[1] this causes reduction in pH[21-23]. Organic acid produced by *Aspergillus* SDS7 may cause destruction of mineral structure[24].

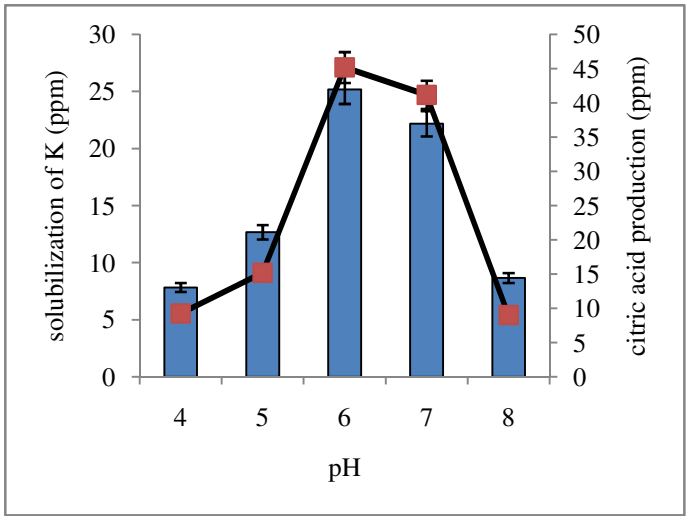

Figure-1
Effect of pH on K solubilization

Pulp density (PD) for the solubilzation of potassium was varied in the range of 0.1 – 1% (w/v) using mineral particles <44µm size using $4x10^7$ spore concentration, while shaking at 150 rpm at 7.0 pH and 28±2ºC. Data presented in Figure-2 shows increase in solubilization of K with time. At 0.6% pulp density, potassium solubilization of 29 ppm and citric acid was 50.66 ppm was obtained in 21 days. Increase in pulp density to 0.8% and 1.0%, potassium solubilzation was found to be nearly same 41.26 ppm and 52.16 ppm respectively. The rise in citric acid was observed 62.83 ppm and 69.5 ppm for 0.8% and 1.0% PD in 21 days.

Inoculum size (Is) for the maximum solbilization of potassium was studied from $2x10^7$–$10x10^7$ spores/ml with 0.5% (w/v) PD, <44µm size particle, neutral pH at 28±2ºC and 150 rpm.

Influence of inoculum size can be seen from the results shown in Figure-3. As the spore concentration increases solubilization of potassium and citric acid concentration was increased. At $2x10^7$ inoculum size, potassium solubilization of 9.5 ppm and 12.66 ppm of citric acid produced. As inoculums size increases from $4x10^7$–$10x10^7$, solubilzation increased 19.83 – 30.83 ppm and36.5 – 54.86 ppm of citric acid.

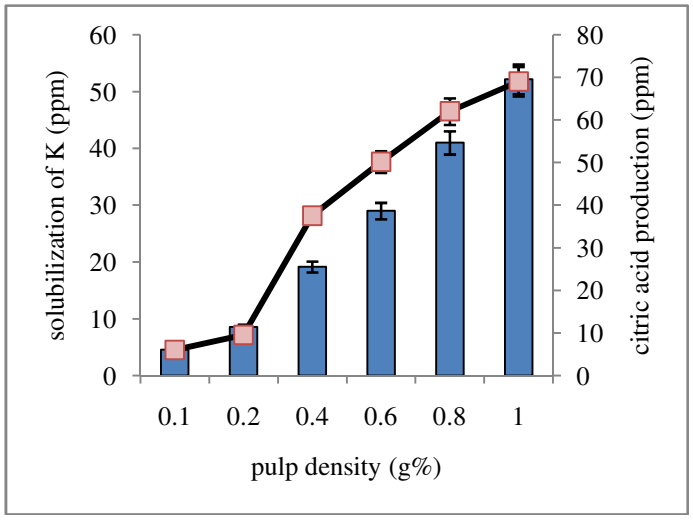

Figure-2
Effect of pulp density on K solubilization

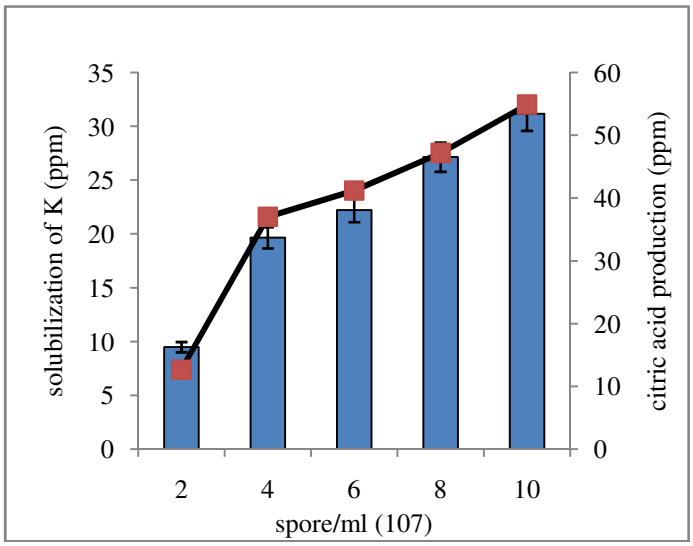

Figure-3
Effect of inoculum size on K solubilization

Effect of particle size on solubilization of potassium is shown in Figure-4. This result indicated that fine particles have better leaching ability but after certain condition, the permeability of the mineral retarded with drastic fall in solubilization after 21 days. Solubilization was found to be 26.83 ppm in 21 days at neutral pH and 0.5% (w/v) PD with mineral particle 35µm size. Whereas 18.83 ppm of K was solubilzed with particle 44 µm size and 34.83 ppm of citric acid produced, as the particle size decrease the solubilization of K was increased due to large surface area for microbial activity. Particle size had a significant role in solubilization.

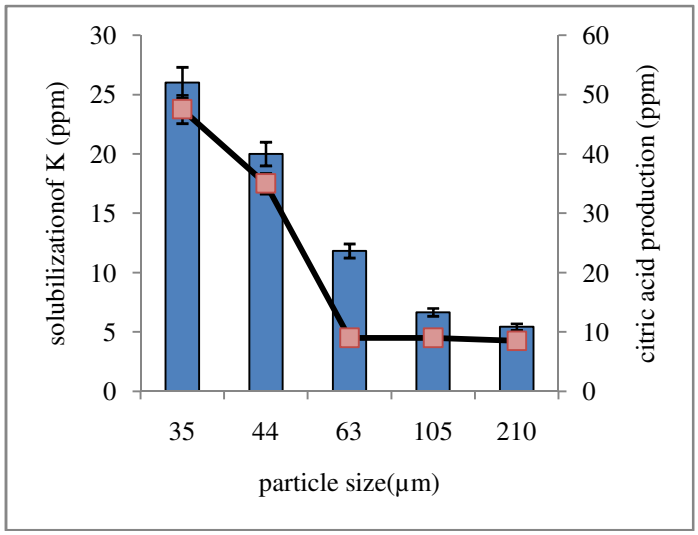

Figure-4
Effect of particle size on K solubilization

Conclusion

Very few reports are available on optimization of potassium solubilizatiion by heterotrophic microorganisms. The results obtained from the current study concerning[25] the optimization of feldspar solubilzation, 25 isolates were obtained from the rhizospheric soil of seasonal plants and ceramic industrial soil. Among 25 isolates, *Aspergillus* SDS7 studied for optimum conditions for maximum solubilization of K. The amount of K released by isolates ranged from 4.5-52.16 ppm. It was seen that pH 6.0 was the best for K solubilization and citric acid production, 25.16 ppm and 45.55 ppm respectively. $10x10^7$ inoculum size was optimum at 0.5% pulp density at neutral pH, as the pulp density increases solubilization of K was increased. 4.5 ppm with 0.1% PD and 52.16 ppm with 1% PD at neutral pH.

26.83 ppm, 47.83 ppm of K and citric acid produced with Particle size 35µm. It was seen that the isolate was growing best with pH 6.0 and produced organic acids like citric, tartaric in broth, which cause the weathering of mineral which enhances the dissolution of mineral. It was concluded that isolate can grow and solubilize K at acidic pH. They can be applied on acidic soil as biofertilizers. However, the actual mechanism of dissolution is some extent unclear and need further investigation.

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