The solubilization of potassium-bearing rock powder by \textit{Aspergillus niger} in small-scale batch fermentations

Maria L. Lopes-Assad, Simoni H. Avansini, Márcia M. Rosa, José R.P. de Carvalho, and Sandra R. Ceccato-Antonini

\textbf{Abstract:} The fungus \textit{Aspergillus niger} was cultivated in culture medium with an alkaline ultramafic rock powder to evaluate the solubilization of potassium for biofertilizer production. The assays were carried out with 2 strains (CCT4355 and CCT911) in small-scale batch fermentations using 125, 500, 1000, and 2000 mL Erlenmeyer flasks, with a nominal volume of 40\%, and rock powder at 0.4\%, shaken at 160 r/min, incubated at 30 °C, and sampled every 7 days for 35 days. The amount of soluble K\textsuperscript{+}, the pH of the culture medium, and the acidity were determined. Both strains solubilized K\textsuperscript{+} from the rock powder to the same extent (approximately 62\%–70\% after 35 days) in the 125 mL flasks; however, the percent solubilization decreased at higher volumetric scales. The results also indicated a difference in strain sensitivity to the increase in volumetric scales in batch fermentation. When filter-sterilized air was injected into the medium, the K\textsuperscript{+} percent solubilization obtained after 4 days of cultivation was similar to that obtained after a 28 day period. The acid production by the fungus may be a mechanism of rock solubilization, in spite of the elevation in pH values probably caused by the increasing hydrolysis of the silicates. Both strains of \textit{A. niger} are recommended for solubilizing potassium from ultramafic rocks, but it is necessary to optimize the oxygen transfer, which seemed to affect the rock solubilization at higher volumetric scales.

\textit{Key words:} rock for crops, biosolubilization, alkaline ultramafic rock, potassium.

\textbf{Résumé :} Le champignon \textit{Aspergillus niger} a été cultivé dans du milieu de culture contenant de la poudre de roche ultrabasique afin d’évaluer la solubilization du potassium en vue de produire des fertiliants biologiques. Les dosages ont été réalisés avec 2 souches (CCT4355 et CCT911) lors d’une fermentation en lot à petite échelle dans des flacons Erlenmeyer de 125, 500, 1000 et 2000 mL, dans un volume nominal de 40 \% et à 0,4 \% de poudre de roche, avec une agitation de 160 r/min et une incubation à 30 °C, échantillonnés à tous les sept jours pendant 35 jours. La quantité de K\textsuperscript{+} soluble, le pH du milieu de culture et l’acidité ont été déterminés. Les deux souches solubilisaient le K\textsuperscript{+} de la poudre de roche à des degrés similaires (approximativement 62 \% – 70 \% après 35 jours) dans les flacons de 125 mL, mais le pourcentage de solubilization diminuait dans des échelles volumétriques plus élevées. Les résultats indiquaient aussi une différence dans la sensibilité des souches à une augmentation de l’échelle volumétrique de la fermentation en lot. Lorsque de l’air stérilisé par filtration était injecté dans le milieu, le pourcentage de solubilization de K\textsuperscript{+} obtenu après quatre jours de culture était similaire à celui obtenu après 28 jours. La production d’acide par le champignon peut constituer un mécanisme de solubilization de la roche, malgré une élévation du pH causée probablement par une augmentation de l’hydrolyse des silicates. Les deux souches d’\textit{A. niger} sont recommandées pour solubiliser le potassium de roche ultrabasique, mais il est nécessaire d’optimiser le transfert d’oxygène qui semble affecter la solubilization de la roche à des échelles volumétriques plus élevées.

\textit{Mots-clés :} « des roches pour les récoltes », solubilization biologique, roche alcaline ultrabasique, potassium.

\textit{[Traduit par la Rédaction]}

\textbf{Introduction}

Countries such as Brazil, China, and India are important food producers and consumers of high amounts of potassium-based fertilizers. In Brazil, around 90\% of the potassium required for agriculture is imported (Barbosa-Filho et al. 2006). Fertilizer production using potassium-bearing minerals (like feldspars and some micas) is not viable because the majority of them are insoluble and their dissolution...
process requires strong chemical and thermal treatments (Nascimento et al. 2008).

Some alternatives have been tried with respect to the incorporation of rocks into soils, with the aim of reducing the use of chemicals and increasing environmental and agricultural sustainability (Martins et al. 2008). Nevertheless, the length of time required to alter the minerals contained in the rocks, and the poor availability of important elements required for plant nutrition, makes this alternative unfeasible.

The alteration of rock minerals in natural environments is a well-known process mainly caused by the action of water and organic acids produced by plant roots and by microorganisms that accelerate this alteration. Bacteria, moulds, and actinomycetes are capable of solubilizing elements immobilized in silicates during the decomposition of organic matter, resulting in the production of organic acids (Weed et al. 1969). Satisfactory results for potassium and phosphate solubilization have been obtained using moulds such as Aspergillus, Penicillium, and Fusarium, and bacteria such as Bacillus, Pseudomonas, and Micrococcus (Gaur 1990).

The fungus Aspergillus is known to solubilize phosphate rocks (Illmer and Schinner 1992; Vassilev et al. 1995; Whitelaw 2000; Silva Filho et al. 2002) either by direct dissolution of phosphorus or as a cation chelant, releasing soluble phosphate (Sperber 1958; Cerezine et al. 1988).

The filamentous fungus Aspergillus niger is an exceptionally efficient producer of organic acids, which is one of the reasons for its relevance to industrial processes and its commercial importance (Andersen et al. 2009). The production of organic acids by A. niger is dependent on the pH of the medium, since the greatest quantities of oxalic acid are produced at a pH between 5 and 8, while it is completely absent below pH 3.0 (Ruijter et al. 1999); citric acid production begins at pH 3.0 but is optimal below 2.0 (Karaffa and Kubicek 2003); and gluconic acid is best produced at pH 5.5, although a range of pH 2–8 has been found (Witteveen et al. 1993). Many strains of A. niger are well known for their capacity to produce citric acid under suitable conditions; that is, at a low–medium pH and with aeration, in both submerged and solid-state fermentation (Carvalho et al. 2005). This species is easy to handle and capable of utilizing a great variety of low-cost raw materials with high yields (Grewal and Kalra 1995). However, yields of citric acid were found to depend significantly on the strain of A. niger used. Previous studies have shown that ATCC9142 is the most effective strain, producing the highest quantities of citric acid on a diversity of substrates such as wet corn distillers’ grains (Xie and West 2006), pineapple waste (Tran et al. 1998), autoclaved grape pomace (Hang and Woodams 1985) using solid-state fermentation, and ethanol fermentation coproducts such as corn distillers’ dried grains (Xie and West 2009).

To improve potassic biofertilizer production, aspects that highly influence the biotechnical process must be surveyed. Variables such as pH, temperature, nutrients, oxygen, and agitation influence the process, adding complexity and difficulty to the scale-up. Successful scale-up means a shortened cycle to full-scale production, competitive advantage, and cost savings (Reisman 1993). Because of the difficulty in assessing the factors that affect the scale-up process during cultivation, many large-scale fermentation processes give a lower yield than is expected in the laboratory (Bylund et al. 2000). A critical parameter for judging the suitability of a reactor for cultivating suspension cells is the oxygen transfer rate between the gas and liquid phases (Zhang et al. 2009). In this context, the aim of this research was to evaluate the behavior of 2 strains of A. niger in the solubilization of potassium from an alkaline ultramafic rock powder, in small-scale batch fermentations, at volumetric scales up to 2 L.

**Materials and methods**

### Rock powder

An alkaline ultramafic rock powder (grain size 0.002–0.05 mm) composed of ferromagnesian minerals (olivine, pyroxene), plagioclasmus, and phlogopite, from Lages, Santa Catarina, Brazil, was used in the experiments. The chemical characteristics of the rock were determined at the Laboratório do Centro de Tecnologia Mineral (CETEM) and the Departamento de Geologia, Universidade Federal da Bahia, and indicated 3.44 dag/kg K₂O, 15.09 dag/kg CaO, 16.88 dag/kg MgO, 1.40 dag/kg P₂O₅, and 35.57 dag/kg SiO₂ (Moreira et al. 2006).

### Microorganisms, media, and inoculum preparation

Two strains of A. niger were used, CCT911 (ATCC9142) and CCT4355, the latter isolated from sugar-cane vinasse (CCT is a designation for Coleção de Culturas Tropical in Brazil. Information is available at http://www.fat.org.br/).

Fungal spores were produced for the inoculum as follows: initially the fungi were inoculated in MYPG slants (3 g/L malt extract, 3 g/L yeast extract, 8 g/L peptone, 10 g/L glucose) and then incubated at 30 °C for 5 days. A volume of 6 mL of Tween 80 (0.1%) was added to each culture tube following scratching for the release of spores. A suspension of 10⁷ spores/mL was obtained after spore counting in a Neubauer chamber.

The medium for the solubilization assays was Crocken and Nyc’s, in accordance with Cerezine et al. (1988), modified by eliminating a potassium source and adding a phosphate source (2.85 g/L sodium citrate, 1 g/L ammonium phosphate, 0.5 g/L magnesium sulphate, 0.132 g/L calcium chloride, 10 g/L glucose). Before autoclaving at 120 °C for 20 min, the rock powder (0.4% m/v) was added and the pH adjusted to 7.0.

### Solubilization assays and sampling

The small-scale batch fermentations were carried out in Erlenmeyer (conical) flasks in volumes of 125, 500, 1000, and 2000 mL, in which proportional volumes of culture media (50, 200, 400, and 800 mL), spore suspension (0.5, 2, 4, and 8 mL), and rock powder (0.2, 0.8, 1.6, and 3.2 g) were added.

Flasks in triplicate were shaken at 160 r/min, incubated at 30 °C, and sampled every 7 days, over a 35-day period. The samples were removed, filtered through Whatman paper, and the supernatant was cooled to 4 °C and kept at this temperature until analysis. For the 125 mL flasks, the sample constituted the total volume of the flask; for the other scales, a volume of 15 mL was taken from the flask.

Two control settings were established: culture medium plus rock powder and culture medium plus fungal spores, with the
same culture and sampling conditions and flask scales, with the exception of the 2000 mL flasks.

The experiments were conducted according to a randomized complete design with factorial treatment, under controlled conditions.

**Analysis**

Soluble potassium (K$^+$) was determined by flame emission photometry; pH, using a digital pH meter; and titratable acidity, by titration of the samples with 0.05 mol/L NaOH solution to pH 7.0.

Initially, the results obtained (at 35 days of cultivation) in the control settings were compared to those in which the fungus was inoculated with the rock powder, to evaluate the influence of the fungus on rock solubilization. Following this, an analysis of variance of the results from each flask scale (125, 500, 1000, and 2000 mL) and fungal strain was conducted, for each parameter (pH, titratable acidity, and K$^+$). The means for each flask scale and fungal strain over the incubation period were compared using Tukey’s test with a $p$ value of 0.05 to evaluate the potential for K$^+$ solubilization. The behavior of each fungal strain in response to the incubation times was evaluated by regression analysis, by considering the unfolding of the triple interaction (strain × flask scale × incubation time) in the analysis of variance and adjusting the second-grade polynomial equations. The statistical analysis was conducted with SAS version 9.1 (SAS Institute Inc. 2002).

**Results**

At the end of 35 days of cultivation, the K$^+$ concentrations in the culture medium inoculated with *A. niger* and rock powder were higher than the concentrations measured in the control settings (Table 1), indicating the accelerating effect of the fungus on the solubilization process, regardless of flask volume and fungal strain. As much as 62%–70% of the K$^+$ was released from the alkaline ultramafic rock in the assays in the 125 mL flasks, for both strains (Table 1).

To evaluate how efficiently the 2 *A. niger* strains solubilized, a comparison was made between the means of the parameters analyzed. This showed that in the 125 mL flasks there were no significant differences between CCT911 and CCT4355 concerning K$^+$ concentration, pH, and titratable acidity (Table 2).

However, with the flask scale-up, the strains differed in their behavior. In the 500 mL flasks, the K$^+$ concentration was still similar in both strains, but significant differences in pH value and titratable acidity were observed. In the 1000 and 2000 mL flask volumes, all the measured values were statistically different between the 2 fungal strains, with the exception of the titratable acidity in the 2000 mL flasks (Table 2).

As the objectives of this work were to evaluate the behavior of 2 strains of *A. niger* and the kinetics of the solubilization process for each strain, the time course was not analyzed.

### Table 1. Mean potassium concentration and percent solubilization$^a$ determined at 35 days of cultivation, in the culture medium, in the assays of varying flask volumes (40% nominal volume) in small-scale batch fermentations using 2 strains of *Aspergillus niger* (CCT911 and CCT4355) and alkaline ultramafic rock powder, in triplicate.

<table>
<thead>
<tr>
<th>Flask volume (mL)</th>
<th>A.niger + medium</th>
<th>Rock powder + medium</th>
<th>A.niger + rock powder + medium</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CCT911</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>125 mL</td>
<td>0.60</td>
<td>0.85 (29.1%)</td>
<td>1.81 (62.0%)</td>
</tr>
<tr>
<td>500 mL</td>
<td>0.26</td>
<td>0.36 (12.3%)</td>
<td>1.05 (35.9%)</td>
</tr>
<tr>
<td>1000 mL</td>
<td>0.48</td>
<td>0.62 (21.2%)</td>
<td>1.45 (49.6%)</td>
</tr>
<tr>
<td><strong>CCT4355</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>125 mL</td>
<td>0.39</td>
<td>0.66 (22.6%)</td>
<td>2.04 (69.8%)</td>
</tr>
<tr>
<td>500 mL</td>
<td>0.28</td>
<td>0.40 (13.7%)</td>
<td>1.16 (39.7%)</td>
</tr>
<tr>
<td>1000 mL</td>
<td>0.24</td>
<td>0.42 (14.4%)</td>
<td>1.06 (36.3%)</td>
</tr>
</tbody>
</table>

$^a$The percent solubilization was calculated as follows: mean of soluble K$^+$ concentration (mEq/L) / total potassium concentration in the rock powder (2.921 mEq/L).

### Table 2. Potassium concentration, pH, and titratable acidity obtained in the assays of varying flask volumes (40% nominal volume) in small-scale batch fermentations using 2 strains of *Aspergillus niger* (CCT911 and CCT4355) and alkaline ultramafic rock powder.

<table>
<thead>
<tr>
<th>Flask volume (mL)</th>
<th>CCT911</th>
<th>CCT4355</th>
<th>CCT911</th>
<th>CCT4355</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Potassium (mEq/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>125 mL</td>
<td>1.48 aA</td>
<td>1.51 aA</td>
<td>0.86 aB</td>
<td>0.85 aB</td>
</tr>
<tr>
<td>500 mL</td>
<td>6.06 aA</td>
<td>6.41 aA</td>
<td>6.99 aB</td>
<td>7.93 bB</td>
</tr>
<tr>
<td>1000 mL</td>
<td>8.06 aA</td>
<td>6.27 aA</td>
<td>7.94 aC</td>
<td>7.10 bC</td>
</tr>
<tr>
<td>2000 mL</td>
<td>8.06 aA</td>
<td>6.27 aA</td>
<td>7.94 aC</td>
<td>7.10 bC</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>125 mL</td>
<td>6.06 aA</td>
<td>6.06 aA</td>
<td>7.94 aC</td>
<td>7.94 aC</td>
</tr>
<tr>
<td>500 mL</td>
<td>7.94 aC</td>
<td>7.94 aC</td>
<td>7.47 aB</td>
<td>7.47 aB</td>
</tr>
<tr>
<td>1000 mL</td>
<td>7.10 bC</td>
<td>7.10 bC</td>
<td>8.18 bD</td>
<td>8.18 bD</td>
</tr>
<tr>
<td>2000 mL</td>
<td>8.18 bD</td>
<td>8.18 bD</td>
<td>8.18 bD</td>
<td>8.18 bD</td>
</tr>
<tr>
<td><strong>Titratable acidity (mEq/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>125 mL</td>
<td>0.39 aB</td>
<td>0.39 aB</td>
<td>0 bB</td>
<td>0 bB</td>
</tr>
<tr>
<td>500 mL</td>
<td>0.39 aB</td>
<td>0.39 aB</td>
<td>0 bB</td>
<td>0 bB</td>
</tr>
<tr>
<td>1000 mL</td>
<td>0.39 aB</td>
<td>0.39 aB</td>
<td>0 bB</td>
<td>0 bB</td>
</tr>
<tr>
<td>2000 mL</td>
<td>0.39 aB</td>
<td>0.39 aB</td>
<td>0 bB</td>
<td>0 bB</td>
</tr>
</tbody>
</table>

Note: Means followed by different noncapitalized letters in the columns and by different capital letters in the lines differ significantly ($p < 0.05$) by Tukey’s test. Number of replicates to calculate each mean = 18.
reached. Both strains showed similar behavior in the 500 and 1000 mL flasks, with maximum solubilization occurring at around 28 days, as seen in the regression curve. However, in the highest volume flask, 14–21 days of cultivation was the optimum period for K⁺ solubilization for both strains, but this occurred at significantly lower levels (Fig. 1).

The pH and titratable acidity values are depicted in Figs. 2 and 3. Within 7 days, the pH decrease in the 125 mL flasks was more remarkable with CCT911 than with CCT4355. However, at higher volumetric scales, the pH values did not vary substantially, or even increased, with time (Fig. 2). The titratable acidity reached its highest levels with the smallest volumetric scale (the 125 mL flasks), and this was similar for both fungal strains. Very low or null acidity values were found in the solubilization assays of the higher volume flasks (Fig. 3).

The morphology of A. niger pellets is seen in Fig. 4, showing longer hyphae arising from the pellet in the control setting without the rock powder. Shorter hyphae were seen in the fungal pellets growing in the medium to which alkaline ultramafic rock powder had been added, in the 125 mL flasks with CCT4355. In the 2000 mL flask, after 14 days the typical pellet morphology was not found anymore. Instead, there was a white mass at the bottom of the flask, which suggests cell lysis (data not shown).

Discussion

The present high cost of conventional potassium fertilizers justifies further investigation of potassium silicate minerals and their host rocks as alternative sources of K⁺ (Manning 2010). A biotechnological process to accelerate K⁺ release from potassium-bearing rocks may be an interesting approach compared to the slow release of potassium that occurs when rocks are incorporated into the soil. In this context, the present work has strongly proven the benefits of inoculating A. niger into the solubilization process of alkaline ultramafic rock, as well as highlighting some areas for further research, which may lead to biofertilizer production on a larger scale.

Lopes-Assad et al. (2006) have verified in assays that as much as 53.7% of the total K⁺ in a rock can be solubilized with the strain CCT4355, using alkaline ultramafic rock after 21 days of incubation. Cerezine et al. (1988) have solubilized as much as 87% of the phosphorus from fluorapatite within 11 days with A. niger. Using agroindustrial wastes from olive production in solid-state fermentation, Vassileva...
et al. (1998) observed that 42% of phosphorus was solubilized with *A. niger* and phosphate rock. In this paper, the highest percent solubilization that took place (62%–70%) was observed after 35 days of assays for both strains; however, the maximum degree of solubilization possible may not have been reached.

The ability of *A. niger* to produce organic acid is well known, and this could be the mechanism involved in the rock solubilization, consequently releasing basic ions.

Lian et al. (2007) observed that the potassium solubilization rate showed a positive dependence upon pH when the fungus *Aspergillus fumigatus* and minerals were mixed directly and exhibited no correlations with solution acidity if cell–rock contact was restrained. The authors suggested that the fungus promoted K⁺ release by means of at least 3 routes: complexation of soluble organic ligands; immobile biopolymers such as the insoluble components of secretion; and mechanical forces in association with the direct physical contact between cells and mineral particles. In this paper, K⁺ solubilization may also have been enhanced by the direct contact between the fungus and rock powder, although an effect on the morphology of pellets was observed in the culture medium with the rock powder (shorter hyphae arising from the pellets).

However, Vyas and Gulati (2009) verified that no relationship could be ascertained between the amount of organic acids and the solubilization of rock phosphates by *Pseudomonas* strains. Some of the phosphate substrates utilized had a fluoroapatite structure, with a high rate of substitution of phosphate with carbonate. The higher rate of solubilization and lower quantities of organic acids detected in the presence of a phosphate substrate (North Carolina rock phosphate) could be due to the higher reactivity and greater diversion of organic acids in the neutralization of free carbonates.

The oscillation in pH values between the assays may indicate that as organic acids were produced by the strains of *A. niger*, the hydrolysis of silicates contained in the rock powder was increasing, releasing alkalinizing cations. This observation can explain the fact that even at high pH values, the solubilization was expressive, with a significant release of K⁺ from the rock. In submerged fermentation, oxalic acid is produced preferably when pH values increase (Andersen et al. 2009). Another explanation is the production of CO₂ by fungal respiration. When this gas is dissolved in water it forms carbonic acid, and this may lead to cation solubilization, which in turn liberates alkalinizing cations responsible for the increase in pH values.

**Fig. 2.** pH of the culture medium plus alkaline ultramafic rock powder cultivated with 2 strains of *Aspergillus niger* (CCT911 and CCT4355), during 35 days, at 30 °C and 160 r/min, in small-scale batch fermentations. Each point represents the mean of 3 replicates. ns, not significant.
The numbers obtained for K+ release and solubilization in the 125 mL flasks were not obtained at higher volumetric scales (500, 1000, and 2000 mL), and the substrate oxygen may be the primary reason. Oxygen limitation has drastic effects on fermentation kinetics (Gupta and Rao 2003), acting similarly to substrate limitation (Clark et al. 1995) and influencing the production of secondary metabolites (Katzer et al. 2001). Soccol et al. (2006) stated that dissolved oxygen concentration influences the citric acid formation directly and that the maintenance of oxygen concentration above 25% saturation is crucial (Vandenbergh et al. 1999). Critical dissolved oxygen tension is 9%–12% of air saturation during the growth phase and 12%–13% of air saturation during the production phase (Grewal and Kalra 1995).

Although this work has shown the beneficial influence of the fungus *A. niger* in ultramafic rock solubilization, it has also indicated a difference in strain sensitivity to the increase in volumetric scales in batch fermentation. Fermentation conditions such as pH, temperature, and shaking speed were kept constant during the small-scale fermentations, while other factors were proportionally distributed among the flasks (medium and inoculum volumes, rock powder mass). A working volume of 40% of the nominal volume was used in the experiments. If the shaking speed (160 r/min) did not vary with the increased volumetric scales (from 125 to 2000 mL flasks), it is expected that oxygen transfer ($k_{La}$, volumetric mass transfer coefficient) was decreased, affecting the balance between oxygen transfer and working volume. Zhang et al. (2009) observed this fact using cylindrical containers for mammalian cell cultures, where filling volume was varied as the shaking speed was kept constant. The importance of aeration in the biosolubilization proc-

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**Fig. 3.** Titratable acidity of the culture medium plus alkaline ultramafic rock powder cultivated with 2 strains of *Aspergillus niger* (CCT911 and CCT4355), during 35 days, at 30 °C and 160 r/min, in small-scale batch fermentations. Each point represents the mean of 3 replicates.  

- (**) TAc125 : $R^2 = 0.8439$ ($p > 0.05$)  
- (o) TAc500 : $R^2 = 0.4939$ ($p > 0.05$)  
- (o) TAc1000 : $R^2 = 0.9627$ ($p > 0.05$)

**Fig. 4.** Morphology of *Aspergillus niger* pellets (strain CCT4355) in the culture medium with (A) or without (B) alkaline ultramafic rock powder, in 125 mL flasks, after 21 days of cultivation, at 30 °C and 160 r/min. Scale bar = 25 mm.
less by strains of A. niger for K⁺ release could be confirmed when filter-sterilized air was injected into the medium (around 1500 cm³/min) in the volumetric scale of 2000 mL with the CCT4355 strain, the most sensitive to the scale-up. In this situation, a potassium solubilization of 62% (1.81mEq/L) was obtained after 4 days of cultivation (data not shown), abbreviating substantially the time demanded for solubilization (28 days) in the 125 mL flask volume.

The concentration of trace elements is another relevant factor in the context of rock solubilization. Vandenbergh et al. (1999) reported the influence of trace elements on citric acid fermentation by A. niger. Mirminachi et al. (2002) indicated that citric acid production is impaired in concentration of manganese above 1 ppm with A. niger strain ATCC11414. Ultramafic rocks are also rich in iron, magnesium, and manganese (Oliveira et al. 2006), which are released into the medium by fungal action along with the potassium. Values of 50 ppm of iron and 0.8 ppm of manganese were detected after 14 and 21 days of cultivation, respectively, in 125 mL flasks with strain CCT4355 (data not shown). This can affect, at least partially, the acid production, resulting in diminished solubilization rates. Further investigation is required to survey the effects of these elements on the growth and metabolism of these fungal strains.

The strains of A. niger studied here (CCT911 and CCT4355) are recommended for potassium solubilization from ultramafic rocks. However, from the point of view of establishing a biotechnological process for biofertilizer production, it is necessary to optimize the oxygen transfer, which seemed to affect the rock solubilization at higher volumetric scales. The well-known A. niger strain, CCT911 (ATCC9142), was less sensitive to this parameter; however, CCT4355 has greater potential once environmental conditions are optimized.

Acknowledgements

The authors wish to thank the National Council for Scientific and Technological Development (CNPq), Research and Projects Financing (FINEP), and the Brazilian Agricultural Research Corporation (EMBRAPA) for their financial support.

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