JAQUELINE MARIA DO NASCIMENTO

SOLUBILIZAÇÃO DE FOSFATO DE ROCHA E DESSORÇÃO DE FÓSFORO NO SOLO POR ÁCIDOS ORGÂNICOS E *Aspergillus niger*

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Microbiologia Agrícola, para obtenção do título de *Magister Scientiae.*

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APROVADA: 19 de julho de 2018.

Rafael Vasconcelos Valadares

Christiano da Conceição de Matos

Leonardus Vergütz
(Coorientador)

Gilberto de Oliveira Mendes
(Coorientador)

Maurício Dutra Costa
(Orientador)

Aos meus pais e meu irmão.

Alberto.

Ao Eremitério Nazaré da Serra.

Aos verdadeiros amigos.

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BIOGRAFIA

Jaqueline Maria do Nascimento, filha de Geraldo Lage do Nascimento e de Janete Agostinha Pinto do Nascimento, nasceu no dia 29 de setembro de 1993 na cidade de Nova União, MG. Iniciou a graduação em Agronomia na Universidade Federal de Minas Gerais em março de 2011, graduando-se em julho de 2016. Durante a graduação, realizou intercâmbio acadêmico e cultural na Universidade do Algarve em Portugal. Em agosto de 2016, ingressou no curso de mestrado em Microbiologia Agrícola pela Universidade Federal de Viçosa, concluindo em julho de 2018.

RESUMO

NASCIMENTO, Jaqueline Maria, M.Sc., Universidade Federal de Viçosa, julho de 2018. **Solubilização de fosfato de rocha e dessorção de fósforo no solo por ácidos orgânicos e *Aspergillus niger*.** Orientador: Maurício Dutra Costa. Coorientadores: Gilberto de Oliveira Mendes e Leonardus Vergütz.

O fósforo (P) pode sofrer reações de adsorção, precipitação e imobilização no solo que diminuem a disponibilidade desse nutriente para as plantas. Ao ser fixado a óxidos e hidróxidos de ferro e alumínio, o P passa a compor a fase sólida do solo, sendo de difícil disponibilização para as culturas. Alguns compostos, a exemplo dos ácidos orgânicos, apresentam a capacidade de extrair P de rochas fosfáticas de baixa reatividade. Esses compostos podem ser produzidos por plantas e microrganismos como estratégia para aquisição de P para o crescimento. Pouco se conhece sobre a cinética de solubilização de fosfato de rocha de Araxá por ação de ácidos orgânicos e ainda, sobre a capacidade desses ácidos orgânicos e de microrganismos solubilizadores em disponibilizar o P adsorvido ao solo. Os objetivos desse trabalho foram estudar a cinética de solubilização de fosfato de Araxá por ácidos orgânicos e avaliar a capacidade desses compostos, bem como a de *Aspergillus niger*, de dessorver P a partir de fração de 75-µm de um Oxissol. A cinética de solubilização de fosfato de Araxá foi realizada utilizando-se a técnica de stirred-flow, com soluções de ácido oxálico, cítrico e glicônico 5 ou 10 mmol L^{-1} e suas combinações aos pares na concentração final de 10 mmol L^{-1} (5 mmol L^{-1} para cada componente). As amostras foram coletadas durante 150 min em fluxo contínuo de 1 mL min^{-1} e o P analisado por colorimetria. As partículas de fosfato de Araxá residuais foram analisadas por microscopia eletrônica de varredura (MEV) e a composição mineralógica determinada por difração de raio-X (DRX). A cinética de dessorção de P da fração de 75-µm dos horizontes A e B do LVA foi realizada após incubação por um e 40 dias de contato de P com a fração do solo. A quantidade de P adsorvida ao solo correspondeu a 90 % da capacidade máxima de adsorção de P (CMAP). As soluções extratoras utilizadas foram ácido oxálico, ácido oxálico + cítrico na concentrações 10 mmol L^{-1}, além de água a pH 2,0 e 7,0. Testou-se também a capacidade de *A. niger* em dessorver P das frações do solo em meio de cultura. O ácido oxálico a 10 mmol L^{-1} foi o composto mais eficiente na solubilização de fosfato de Araxá. Ao final do experimento, 43 % do P contido no fosfato de Araxá havia sido solubilizado. A eficiência do ácido oxálico foi aumentada quando combinado com ácido cítrico, a 10 mmol L^{-1}. O processo de solubilização de fosfato apresentou porcentagens de solubilização de P de 71 %. As partículas de fosfato de Araxá sofrem alterações na morfologia e mineralogia quando em contato com ácidos cítrico e oxálico e com a combinação dos dois. O ácido oxálico reduziu fortemente o conteúdo de apatitas do fosfato de Araxá, destacando principalmente a redução no conteúdo de flourapatita. Nos tratamentos com ácido oxálico, observou-se a formação de oxalato de cálcio. O ácido oxálico foi ainda o mais eficiente, dentre os ácidos orgânicos avaliados, em dessorver P do solo. Para o horizonte A, nos tempos de 24 e 960 h de incubação de P com o solo, o ácido oxálico atingiu valores de 35.5 e 32.7 % de P liberado em solução. Para o horizonte B, para os tempos de 24 e 960 h, o P liberado em solução atingiu valores de 22.4 e 18.5 %, respectivamente. Quando na presença de *A. niger*, os valores de dessorção de P foram de 23 e 18 %, no horizonte A, e de 17 e 18 %, no horizonte B, nos tempos de 24 e 960 horas, respectivamente. O ácido oxálico foi o metabólito testado mais eficiente na solubilização de P a partir do fosfato de Araxá. Melhorias na eficiência de solubilização podem ser obtidas com a combinação desse composto com o ácido cítrico. O fungo *A. niger* e os ácidos cítrico e oxálico foram capazes de reverter o processo de adsorção de P ao solo *in vitro*, abrindo perspectivas de aproveitamento do P fixado ao solo na agricultura.

ABSTRACT

NASCIMENTO, Jaqueline Maria, M.Sc., Universidade Federal de Viçosa, July, 2018. **Solubilization of rock phosphate and desorption of phosphorus in the soil by organic acids and *Aspergillus niger*.** Advisor: Maurício Dutra Costa. Co-advisor: Gilberto de Oliveira Mendes e Leonardus Vergütz.

Phosphorus (P) can undergo adsorption, precipitation and soil imobilization reactions that reduce the availability of this nutrient to the plants. When fixed to iron and aluminum oxides and hydroxides, the P starts to form the solid phase of the soil, being difficult to make available to crops. Some compounds, such as organic acids, have the ability to extract P from low reactivity phosphate rocks. These compounds can be produced by plants and microorganisms as a strategy to acquire P for growth. Little is known about the kinetics of solubilization of Araxá rock phosphate (RP) by the action of organic acids and also on the ability of these organic acids and solubilizing microorganisms to make P adsorbed to the soil. The objectives of this work were to study the kinetics of solubilization of Araxá RP by organic acids and to evaluate the ability of these compounds, as well as that of *Aspergillus niger*, to desorb P from the 75-µm fraction of an Oxysol. The kinetics of solubilization of Araxá RP was performed using the stirred-flow technique with solutions of oxalic, citric and gluconic acid 5 or 10 mmol L^{-1} and their combinations in the final concentration of 10 mmol L^{-1} (5 mmol L^{-1} for each component). The samples were collected for 150 min in a continuous flow of 1 mL min^{-1} and the P analyzed by colorimetry. The residual Araxá phosphate particles were analyzed by scanning electron microscopy (SEM) and the mineralogical composition determined by X-ray diffraction (XRD). The P desorption kinetics of the 75-µm fraction of Oxissol horizons A and B were performed after 24 and 960 hours of P contact with the soil fraction. The amount of P adsorbed to the soil corresponds to 90 % of the maximum adsorption capacity of P (MACP). Oxalic acid, oxalic acid + citric acid in 10 mmol L^{-1}, and water at pH 2.0 and 7.0 were found as extraction solutions. An ability of *A. niger* to desorb P from the soil fractions in culture medium was also tested. Oxalic acid at 10 mmol L^{-1} was the most efficient compound in Araxá RP solubilization. At the end of the experiment, 43 % of the P contained in the Araxá RP had been solubilized. The efficiency of oxalic acid was increased when it was combined with citric acid at 10 mmol L^{-1}. The RP solubilization process showed percentages of P solubilization of 71 %. Araxá RP particles undergo changes in morphology and mineralogy when in contact with citric and oxalic acids and the combination of the two. Oxalic acid strongly reduced the apatite content of Araxá phosphate, mainly highlighting the reduction in fluorapatite content. In the oxalic treatments, the formation of calcium oxalate was observed. The oxalic acid was even more efficient, being the results more evaluated, in desorb P of the soil. For the A horizon, in the times of 24 and 960 h incubation of soil P, the oxalic acid got values of 35.5 and 32.7 % of P released in solution. For the B horizon, for the times of 24 and 960 h, the level of 22.4 and 18.5 %, respectively. When in the presence of *A. niger*, the desorption values of P were 23 and 18 % in the A horizon and 17 and 18 % in the B horizon at 24 and 960 hours, respectively. Oxalic acid was the most effective metabolite tested in the solubilization of Araxá RP. Improvements in solubilization efficiency can be obtained by combining that compound with citric acid. The *A. niger* fungus and the citric and oxalic acids were able to reverse the P adsorption process to the soil in vitro, opening prospects for the use of P fixed to soil in agriculture.

SUMÁRIO

INTRODUÇÃO GERAL

A utilização de fontes de fósforo (P) nacionais é limitada pelo fato de o Brasil possuir, em sua maioria, rochas fosfáticas (RFs) de baixa reatividade. Em 2017, o País importou cerca de 5,6 milhões de toneladas de fertilizantes fosfatados solúveis empregados na agricultura brasileira. Apesar da baixa reatividade dos fosfatos nacionais, há interesse crescente em se desenvolver tecnologias baseadas no uso de microrganismos solubilizadores de fosfato que permitam a utilização desses materiais de baixa reatividade para a produção de fertilizantes fosfatados solúveis.

Muitos estudos indicam que alguns microrganismos conseguem solubilizar RFs, melhorando o aproveitamento desse material pelas plantas. Esses microrganismos utilizam mecanismos metabólicos variados, a exemplo da acidificação do meio pela liberação de prótons e ácidos orgânicos, para a solubilização das RFs. O processo de solubilização depende também do tipo de fosfato, sendo os sedimentares mais solúveis que os ígneos e os metamórficos. Em geral, as apatitas são mais solúveis em meios ácidos. Além disso, os ácidos orgânicos, tais como os ácidos oxálico, cítrico, glicônico, itacônico, entre outros, também aumentam a solubilização desse material por meio da formação de complexos com o cálcio. Assim, o uso de ácidos orgânicos, bem como dos próprios microrganismos que os produzem, tem sido o foco de muitas pesquisas na área de biotecnologia visando à produção de adubos fosfatados. No entanto, a cinética de solubilização de apatitas por microrganismos ou metabólitos microbianos permanece desconhecida em sistemas de solubilização contínuos.

Por estar sujeito aos processos de adsorção e fixação na fase sólida do solo, o P torna-se rapidamente indisponível às plantas, principalmente em solos mais eletropositivos, ricos em óxidróxidos de Fe e Al. Após a fertilização com P, o elemento liga-se à fase sólida do solo, por meio de ligações fracas ou de interações eletrostáticas, permanecendo em equilíbrio com a solução. Com o passar do tempo, essas ligações vão se estabelecendo e o P se prende à superfície das argilas por meio de ligações mono e bidentadas. Assim, o P torna-se indisponível, passando a compor fase sólida do solo. Nesse ponto, grande quantidade de energia torna-se necessária para que o elemento volte ao equilíbrio com a solução do solo e torne-se disponível para a absorção pelas raízes.

Por apresentarem capacidade de solubilizar P a partir da estrutura mineral das apatitas de diferentes origens, os microrganismos solubilizadores de fosfato ou metabólitos microbianos podem, possivelmente, influenciar os fenômenos de adsorção e dessorção de P no solo. Assim, os mecanismos utilizados pelos microrganismos do solo para solubilizar RFs poderiam também atuar liberando o P adsorvido ao solo.

Diante do exposto, os objetivos desse trabalho foram estudar a cinética de solubilização de fosfato de Araxá por ácidos orgânicos e avaliar a capacidade desses compostos, bem como a de *Aspergillus niger*, de dessorver P a partir de fração de 75-μm de um Latossolo.

Capítulo 1

REVISÃO DE LITERATURA

Importância e dinâmica do fósforo em solos tropicais

O fósforo (P) é essencial para o crescimento e desenvolvimento das plantas. Esse elemento está associado a moléculas vitais e que fazem parte do metabolismo celular, como ácidos nucleicos, coenzimas, fosfolipídeos e fosfoproteínas (KAPRI e TEWARI 2010). Em solos tropicais, é o nutriente que mais limita o crescimento das plantas, depois do nitrogênio, uma vez que grande parte do P (95-99 %) presente no solo, não é solúvel em água e, por isso, não fica disponível para absorção das raízes (PRADHAN e SUKLA 2005).

Quando absorvido pelas raízes, o P passa a exercer funções importantes, participando de forma direta na fotossíntese, respiração, síntese celular, metabolismo de carboidratos e fixação de nitrogênio, além de participar da regulação enzimática e de vias metabólicas da planta (SCHACHTMAN *et al.*, 1998; VANCE *et al.,* 2003). Por meio de reações químicas, ele é incorporado em diversos compostos, como DNA e RNA, e em moléculas ricas em energia, como o ADP, o ATP e o GTP (BARROSO e NAHAS, 2008).

Apesar de ser nutriente importante na nutrição das plantas, o P é requerido em concentrações baixas, da ordem de 0,1 a 0,5 %, de massa seca produzida (SULTENFUSS e DOYLE, 1999; KAPRI e TEWARI 2010). Por esse motivo, em cálculos de adubação, o P é o macronutriente que é adicionado em quantidades mais baixas em comparação aos outros macronutrientes, como o nitrogênio e o potássio. Por estar relacionado a diversas funções vitais, a limitação de P pode ocasionar efeitos negativos na expansão, área superficial e número de folhas e, ainda, diminuir o crescimento radicular, prejudicando a absorção dos demais nutrientes (SULTENFUSS e DOYLE, 1999; VANCE *et al.,* 2003).

O P é adquirido pelas plantas na forma de ortofosfatos divalentes (HPO_4^{2-}) e, ou monovalentes ($H_2PO_4^-$), sendo a última preferencialmente absorvida pelas raízes, a depender do pH. Em geral, o ponto ótimo de absorção do elemento ocorre a valores de pH do solo próximos a 4,5 a 5,0 (RAGHOTHAMA, 1999; NOVAIS *et al.,* 2007). Os ortofosfatos permanecem na solução do solo em concentrações muito baixas, da ordem de 0,1 a 10 μmol L^{-1} (HINSINGER, 2001). A aquisição de P pode se dar pela absorção radicular, a partir da solução do solo, ou, ainda, pela exsudação de diversos compostos orgânicos que melhoram a disponibilidade do elemento na rizosfera, a exemplo das fosfatases e dos exsudatos radiculares (VANCE *et al.*, 2003; DEVAU *et al.*, 2011). Ao secretar enzimas e, ou liberar exsudatos, a planta investe energia na absorção de P, que, de outra forma, poderia ser canalizada para o crescimento. Assim, o P passa a custar mais caro energeticamente, além de haver prejuízo para o crescimento e desenvolvimento vegetal (CURL e TRUELOVE, 1986; MARSCHNER *et al.*, 1986; HARRISON, 1997).

No mundo, o consumo de fertilizantes fosfatados ocupa o segundo lugar em quantidade, perdendo apenas para o consumo de adubos nitrogenados. No Brasil, o consumo de adubos fosfatados atingiu valores de 5,6 milhões de toneladas em 2017, fazendo com que o país seja o terceiro consumidor mundial desses fertilizantes (GLOBALFERT - globalfert.com.br). Estudos indicam que o teor de P em solos de Cerrado dobrou nos últimos 40 anos (GERARD, 2016). Isso ocorre, principalmente, devido às características intrínsecas dos solos, tais como acidez e quantidade de óxidos elevados, sendo essas características altamente favoráveis à ocorrência de reações de P com oxidróxidos de Fe e Al ou com íons secundários, como Fe^{3+} e Al^{3+}, presentes na solução do solo (NOVAIS *et al.,* 2007; SATTARI *et al.,* 2012).

Os fertilizantes fosfatados minerais são provenientes de rochas fosfáticas (RFs) ricas em apatita [$Ca_5(F,Cl,OH)(PO_4)_3$]. O poder de reatividade das RFs varia de acordo com a sua origem e com suas características, sendo as mais relevantes, a substituição isomórfica do íon PO_4^{3-} por CO_3^{2-}, a porosidade e a granulometria. Quanto maior o grau de substituição isomórfica, maior a capacidade de liberar P rapidamente na solução de solo (NOVAIS e SMITH, 1999). As RFs podem ser de origem sedimentar, ígnea e metamórfica. Quando de origem sedimentar, apresentam melhor poder de reação no solo (CORREA *et al.,* 2005). Isso ocorre porque as ligações do P na rocha são mais instáveis, facilitando a troca de íons. As RFs de origem ígnea, por sua vez, apresentam ligações mais estáveis do P com os minerais que as compõem (NOVAIS *et al.,* 1999; VASSILEV *et al.,* 2001).

Para aumentar a solubilidade dessas fontes, alguns processos químicos são utilizados, como o tratamento com ácidos fortes, a exemplo dos ácidos sulfúrico e fosfórico, combinado com temperaturas elevadas. Esses ácidos oferecem condição de acidez inicial ao processo de solubilização, visto que esses minerais são insolúveis em água (NOVAIS *et al.,* 2007), e promovem a dissolução da rocha quase que por completo, o que gera resíduos e subprodutos indesejáveis (GOLDSTEIN *et al.,* 1993).

Em solos tropicais, as concentrações de P são controladas por reações que envolvem a solubilização de minerais que contêm P, a mineralização da matéria orgânica e os processos de adsorção e dessorção do elemento nas superfícies de minerais de argila (MATAR *et al.,* 1992; COMERFORD, 1998; HINSINGER, 2001).

Além disso, outros fatores como pH da solução, força iônica e concentração de metais como Fe, Ca e Al também podem exercer influência nas concentrações do elemento na solução do solo (ACHAT *et al.,* 2016). Esses mesmos fatores vão também influenciar as ligações químicas que ocorrerão entre o P e os coloides do solo (NOVAIS *et al.,* 2007).

As reações que o P pode sofrer, quando presente na solução do solo, variam de simples interações até ligações mais fortes e bem estabelecidas que ocorrem entre o elemento e as partículas do solo. A adsorção ocorre por meio da reação do P com a superfície de oxidróxidos de Fe e Al. Esse processo inicia-se por meio da interação eletrostática do P com a superfície dessas partículas, e em seguida, por meio da troca de ligantes, o elemento é adsorvido (SANYAL e DATTA. 1991; LABOSKI e LAMB, 2003). Enquanto o P permanece interagindo ou ligados fracamente a essas partículas, ele compõe o P-lábil no solo. Nessa condição, o P mantém-se em equilíbrio com aquele presente na solução do solo. A forma de adsorção do P nas argilas é variada (NOVAIS *et al.,* 2007; ACHAT *et al.,* 2016). Dependendo do tipo de argila, essa ligação pode ser mais forte ou mais fraca, empregando maior ou menor energia. Além disso, depois que se inicia, o processo de adsorção não é totalmente revertido, ou seja, parte do fosfato adsorvido às argilas não é devolvido ao solo na sua forma disponível (NOVAIS *et al.,* 2007). A reversão do processo de adsorção é denominada dessorção.

Com o passar do tempo, as interações eletrostáticas e as ligações fracas vão se estabelecendo, formando ligações fortes, mono e bidentadas, que são responsáveis pela perda de equilíbrio com o P da solução (KAFKAFI *et al.*, 1967; PARFITT, 1978). Nesse ponto, o P passa a ser adsorvido com grande energia nas superfícies das argilas e pode difundir-se para o interior das mesmas, formando o P não-lábil. Ao ser fixado ao solo, o P permanece retido, não sendo possível reaver quantidades mínimas desse elemento para a utilização pelas plantas (FONTES e WEED, 1996; NOVAIS e SMYTH, 1999). O P não-lábil não mantém o equilíbrio com o P da solução. A reversão da adsorção de P só é possível a partir do emprego de energia, através do processo de liberação. A adsorção de P é maior em solos que possuem altas quantidades de oxidróxidos de Fe e Al, que são muito ácidos e que apresentam elevado grau de intemperismo (MATAR et al., 1992; COMERFORD, 1998).

O P pode sofrer também reações de precipitação com os íons Fe^{3+} e Al^{3+}, em solos ácidos, e com Ca^{2+}, em solos calcários ou alcalinos, presentes na solução do solo. (SANYAL e DATTA. 1991; VALLADARES *et al.*, 2003; ROLIM NETO *et al.,* 2004).

Solubilização microbiana de rochas fosfáticas

Os microrganismos apresentam capacidade de solubilizar formas insolúveis de fosfatos e esse processo é resultado de mecanismos relacionados ao metabolismo microbiano, a exemplo da excreção de ácidos orgânicos e a acidificação do meio por liberação de prótons (LEITÃO *et al.,* 2010; MAHESWAR e SATHIYAVANI 2012).

Embora exista ampla diversidade de microrganismos no solo, os fungos aparecem com maior frequência envolvidos no processo de solubilização de fosfato (NAHAS 2002). Já foram encontrados muitos gêneros que têm capacidade de promover crescimento vegetal por meio do suprimento de P para as plantas (SILVA FILHO e VIDOR., 2000). Algumas espécies de fungos filamentosos dos gêneros *Aspergillus* e *Penicillium* têm sido estudadas quanto à produção de ácidos orgânicos, sendo evidenciado que apresentam grande potencial para a solubilização de fosfato de cálcio [$Ca_3(PO_4)_2$] (SILVA FILHO e VIDOR., 2000; MENDES *et al.,* 2013). A solubilização por meio dos ácidos orgânicos é o principal mecanismo utilizado por fungos solubilizadores de fosfato (SOUCHIE *et al.,* 2005; BARROSO e NAHAS, 2008; MENDES *et al.,* 2013).

Os ácidos orgânicos são produtos do metabolismo microbiano e, em geral, apresentam maior efetividade no processo de solubilização de RFs de menor reatividade, o que pode ser explicado pelo menor número de carbonatos livres presentes no meio durante o processo. O acúmulo de carbonatos tende a aumentar o pH, reduzindo a acidez e diminuindo a eficiência da solubilização (KOMPBLEKOU-A e TABATABAI, 1994; MASSENSSINI *et al.,* 2015).

Alguns produtos formados durante solubilização de RFs podem prejudicar o processo. Para *Aspergillus niger* por exemplo, a presença de fluoreto no meio pode ser tóxica para seu metabolismo. Estudos demostraram que cepas mutantes têm a capacidade de solubilizar fontes de fosfato que apresentam flúor em sua composição (SILVA *et al.* 2014). Alguns produtos, a exemplo do biocarvão, também podem auxiliar na retirada desses compostos do meio, além de favorecer o processo de solubilização em função do estímulo à produção de ácidos orgânicos (MENDES, *et al.,* 2014). A capacidade e o potencial de um microrganismo em solubilizar RFs estão diretamente ligados às condições a que são submetidos, indicando que alterações no meio, tais como tipo de fonte de carbono e fósforo, podem promover modificações nos mecanismos envolvidos no processo de solubilização (SILVA FILHO e VIDOR, 2000). As condições de cultivo oferecidas aos microrganismos solubilizadores podem ser manipuladas no sentido de favorecer a produção desses ácidos, tornando o microrganismo mais eficiente no processo de solubilização (SILVA FILHO e VIDOR, 2000; MENDES *et al.,* 2015).

Os mecanismos utilizados pelos microrganismos variam de acordo com a fonte de fosfato disponibilizada, podendo ser acidificação do meio, principalmente para fosfato de cálcio [$Ca_3(PO_4)_2$], e produção de ácidos orgânicos, para fosfatos de ferro [$FePO_4$] e de alumínio [$AlPO_4$] (MENDES, *et al*., 2013).

Os ácidos cítrico e oxálico são, dentre os ácidos orgânicos, os mais eficientes para no processo de solubilização (KPOMBLEKOU-A e TABATABAI, 1994; MENDES *et al.*, 2013). O ácido glicônico, apesar de também ser produzido por fungos e bactérias (MENDES *et al.,* 2013), não interfere expressivamente no processo de solubilização em face da baixa capacidade de quelatação desse composto (ILLMER *et al.,* 1995; SCERVINO *et al.,* 2011). A espécie *A. niger* apresenta elevada capacidade de acidificar o meio e produzir ácidos orgânicos, que apresentam atividade de complexação de metais (COUTINHO *et al.,* 2012; MENDES *et al*., 2013; MENDES *et al*., 2013a; MENDES *et al*., 2014). O emprego de resíduos baratos, como os bagaços de cana e de uva, pode ser utilizado como fonte de carbono para a produção de ácidos orgânicos com a finalidade de solubilização de RFs (COUTINHO *et al.,* 2014; MENDES *et al.,* 2014).

Os microrganismos solubilizadores têm sido então apresentados como alternativa economicamente viável e sustentável do ponto de vista biotecnológico, permitindo o uso de RFs de baixa reatividade para a agricultura (BASHAN *et al.,* 2000; MOREIRA e SIQUEIRA, 2006; SHARMA *et al.,* 2013). O manejo de populações de microrganismos no solo ou, ainda, a utilização de microrganismos solubilizadores na forma de inoculantes, pode melhorar o suprimento de fósforo para as plantas após a aplicação de RFs (SILVA FILHO e VIDOR, 2000).

Para otimização do processo de solubilização microbiana de RFs, torna-se necessário o entendimento dos mecanismos que são utilizados pelos microrganismos no processo de solubilização, bem como os fatores do ambiente que interferem no processo. O desenvolvimento de estratégias de manejo que diminuam a dependência dos produtores de fertilizantes fosfatados, muitas vezes onerosos, é de grande interesse para a manutenção da produção agrícola e da sustentabilidade dos ecossistemas.

Atuação de microrganismos nos processos de adsorção e dessorção de P no solo

Nos últimos anos, tem-se observado interesse crescente na promoção de uma agricultura mais sustentável que priorize a diminuição no consumo de insumos. Nesse sentido, o emprego de inoculantes biológicos, principalmente a base de fungos e bactérias, vem se destacando. Alguns desses inoculantes têm a função de promover a mobilização de nutrientes no solo, de uma forma especial o P, buscando melhorar a disponibilidade do elemento para as plantas (OWEN *et al.*, 2015).

A rizosfera e os microrganismos associados têm grande influência na dinâmica do P nos solos, visto que possuem a capacidade de acidificarem o solo rizoférico por meio da exsudação de ácidos orgânicos e da liberação de prótons (COMERFORD, 1998; HINSINGER, 2001), podendo, hipoteticamente, atuar na reversão do P adsorvido. Já se sabe que os ácidos orgânicos produzidos por microrganismos, principalmente fungos, têm maior afinidade com as argilas do solo que o fosfato (SANYAL e DEDATTA, 1991; HINSINGER, 2001; WEI *et al.,* 2010). A capacidade de ácidos orgânicos atuarem na dessorção e liberação do P de latossolos ainda é desconhecida. Alguns trabalhos têm sido realizados buscando entender como o ação de acidos orgânicos pode influenciar as reações do P com o solo. No entanto, as respostas são variadas, haja vista que a mobilização de P pela ação dos ácidos orgânicos varia de acordo com o tipo de solo estudado (JONES e DARRAH, 1994; JONES et al., 2003). Em espodossolos, por exemplo, a eficiência da dessorção de P por ácidos orgânicos foi constatada, sendo o ácido salicílico o mais eficiente no processo de dessorção de P (FOX *et al.,* 1990). A eficácia de ácido cítrico 10 mmol/L^{-1} na mobilização de P de solos ácidos também foi comprovada, dessorvendo de 0,1 a 2 mg kg^{-1}, sendo muito inferior ao $CaCl_2$ (DROUILLON e MERCKX, 2003). Esses autores relataram ainda que, quando o efeito da acidez foi isolado, o ácido cítrico se manteve eficiente na mobilização de P do solo, indicando que, além da acidificação, esse ácido orgânico pode apresentar efeitos de complexação de Fe e Al no solo.

Alguns trabalhos indicam que ácidos orgânicos também atuam impedindo a adsorção de P em argilas, entre eles podem ser citados os ácidos fúlvicos e húmicos, oxálico, cítrico e salicílico (ALMEIDA *et al.,* 2003; ANDRADE *et al.,* 2003; CESSA *et al.,* 2010). A aplicação de ácido cítrico no solo aumentou da concentração de P em plantas de milho após adubação com fosfato monossódico e ácido fosfórico. Valores da ordem de 115 mg dm^{-3} de P foram observadas para cultivos em neossolo quartizarênico e 299 mg dm^{-3} de P para latossolo vermelho (SANTOS *et al.*, 2017). A adsorção de ácidos orgânicos a colóides do solo dá-se com grande energia e, ao ocupar sítios potenciais de ligação do fosfato, o P é mantido em solução, permanecendo, portanto, disponível às plantas (HAYNES, 1984; CESSA *et al.,* 2010).

O potencial de competição de ácidos orgânicos com o fosfato depende de fatores, tais como concentração, persistência no solo e forma da molécula (PAVINATO e ROSOLEM, 2008). Moléculas com maior número de grupos funcionais são mais competitivas pelos sítios de adsorção (HUE, 1991; ANDRADE *et al.,* 2003). A ordem que são aplicados também influencia o processo de adsorção de P, sendo que, quando o nutriente é aplicado posteriormente à aplicação de ácidos orgânicos, a sua adsorção é menor (ALMEIDA *et al.,* 2003; ANDRADE *et al.,* 2003; CESSA *et al.,* 2010).

Em solos ácido, é comum ocorrer a precipitação de P com Fe^{3+} e Al^{3+} presentes na solução do solo. Os ácidos orgânicos podem limitar essa reação por meio da formação de complexos organometálicos com esses íons, impedindo que esses elementos se combinem com o fosfato e tornando-o novamente indisponível no solo (CESSA *et al.,* 2010). Uma parte do P no solo pode combinar-se com Ca^{2+} em solos com pH próximo a 7,0 e superior. No entanto, o composto formado nesse caso é mais susceptível à solubilização por microrganismos rizosféricos por meio da geração de acidez (SOUCHIE *et al.,* 2007).

Novas formas estratégias de disponibilização do P nos solos podem contribuir para a redução no uso de fertilizantes fosfatados e maior economia no setor agrícola, além da preservação do ambiente (RODRIGUES *et al.*, 2016). Nesse contexto, os fatores que influenciam as ligações de P com os solos têm sido foco de muitas pesquisas nos últimos anos. O P adsorvido ou fixado nos solos tem potencial de ser liberado pela atividade da microbiota rizosférica (LYNCH, 2007; RICHARDSON *et al*, 2011, GERARD, 2016).

Ácidos orgânicos têm se mostrado eficientes na solubilização de RFs de baixa reatividade (ILLMER *et al.,* 1995; MOREIRA E SIQUEIRA, 2006; MENDES *et al.*, 2013; MENDES *et al.,* 2014; VASSILEV *et al.,* 2013; VASSILEV *et al.,* 2013a). Assim como na mobilização de P em alguns solos (FOX *et al.,* 1990; DROUILLON e MERCKX, 2003; SANTOS *et al.*, 2017). Portanto, acredita-se que os ácidos orgânicos microbianos possam atuar na reversão do fenômeno de adsorção de P a oxidróxidos de Fe e Al presentes nos solos tropicais.

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Capítulo 2

ROCK PHOSPHATE SOLUBILIZATION BY ORGANIC ACIDS IN STIRRED-FLOW SISTEM

Rock phosphate solubilization by organic acids in stirred-flow sistem

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Abstract

The use of rock phosphates (RPs) in agriculture is usually preceded by pre-treatments aiming at increasing RP solubility. Some microorganisms are able to excrete organic acids that release phosphorus (P) from stable forms, such as P-Ca, present in RP apatites. However, it is not known how the kinetics of solubilization of P by the action of these organic acids occurs. Then, the objectives of this work were to study the kinetics of solubilization of Araxá RP and to observe changes in morphology and mineralogy of this material after the contact with organic acids. The kinetics of solubilization of Araxá RP was carried out using the stirred-flow technique, with solutions of oxalic, citric and gluconic acids at the final concentrations of 5 or 10 mmol L^{-1} and their combinations in pairs at the final concentration of 10 mmol L^{-1} (5 mmol L^{-1} for each component). The samples were collected for 150 minutes in a continuous flow of 1 mL min^{-1} and the P analyzed by colorimetry. The residual Araxá RP particles were analyzed by scanning electron microscopy (SEM) and the mineralogical composition determined by X-ray diffraction (XRD). Oxalic acid at 10 mmol L^{-1} was the most efficient compound in Araxá RP solubilization. At the end of the experiment, 43 % of the P contained in the Araxá RP had been solubilized. The efficiency of oxalic acid was increased when combined with citric acid at 10 mmol L^{-1}. The phosphate solubilization process showed percentages of P solubilization of 71 %. Araxá RP particles undergo changes in morphology and mineralogy when in contact with citric and oxalic acids and the combination of the two. Citric acid led to small reductions in the apatite content of Araxá RP, except for fluorapatite. Oxalic acid, however, greatly reduced the content of these minerals. Oxalic acid is most efficient organic acid tested in P solubilization from Araxá RP in a stirred-flow system and improvements in solubilization efficiency can be obtained by combining that compound with citric acid. The oxalic acid decreasing expressively the peacks of the minerals of fluorapatite during the solubilizations processe.

Key words: oxalic acid; phosphorus; complexation; acidolysis

Introduction

The consumption of soluble phosphates in the world is second only to that of nitrogen-based fertilizers. Brazil alone consumed about 5.6 million metric tons of P_2O_5 in 2017 (GLOBALFERT - globalfert.com.br). The use of soluble phosphate is costly to agricultural production, since the majority of all phosphate fertilizers used in the country are imported (FAO – faostat.fao.org). Thus, the use of national low-reactivity phosphate ores for the production of soluble fertilizers becomes an attractive alternative for a sustainable food production.

RPs present different natural reactivities depending on their origin as igneous, sedimentary, or metamorphic (CORREA *et al.,* 2005). In order to produce soluble phosphates, chemical processes are commomly used, generating toxic wastes (GOLDSTEIN *et al.,* 1993). For example, the use of strong acids, such as sulfuric acid, has been widely used (GOLDSTEIN *et al.,* 1993). It promotes the destabilization of apatite structure, leading to increased available P content, Also, when in the soil, the susceptibility to react with other ions is facilitated (GOLDSTEIN *et al.,* 1993; NOVAIS *et al.,* 2007)

Many RP deposits show poor quality, with low-reactivity rocks (NOVAIS *et al.,* 1999; VASSILEV *et al.,* 2001). The commercial exploitation of Brazilian RPs is still low and, to meet its agricultural demand for P, the country has become the fourth largest importer of RP in the world (GLOBALFERT - globalfert.com.br).

Due to P adsorption and fixation in Brazilian soils, the use of soluble phosphate fertilizers is high and it becomes necessary to increase P doses applied to the soil to compensate for P losses due to adsorption to clays and reactions with cations, such as Al^{3+}, Ca^{2+} and Fe^{3+} (NOVAIS *et al.,* 2007; SATTARI *et al*., 2012). Many microorganisms have the ability to solubilize RPs by the excretion of organic acids and the acidification of the surrounding medium (LEITÃO *et al*., 2010; MAHESWAR and SATHIYAVANI, 2012). Organic acids released by root and microbial cells chelate cations in RPs and in the soil, causing equilibrium to be displaced towards products, with consequent P release (KPOMBLEKOU-A and TABATABAI, 1994; NAUTIYAL *et al.,*2000).

Despite the considerable amount of literature available on microbial RP solubilization in batch systems (ILLMER *et al.,* 1995; MOREIRA E SIQUEIRA, 2006; MENDES *et al*., 2013; VASSILEV *et al.,* 2013; VASSILEV *et al.,* 2013a) little is known about the kinetics of RP solubilization in continuous systems that allow product removal and the determination of solubilization kinetics governed by selected microbial metabolites. Oxalic and citric acids are reputed to be efficient at RP solubilization (KPOMBLEKOU-A e TABATABAI, 1994; MENDES *et al.*, 2013), but the changes in RP mineralogy along the process of solubilization remains unknown. Knowledge on changes in RP mineralogy during microbial solubilization is important for the proposal of new strategies for the optimization of microbial RP solubilization.

The objectives of this work were to study the solubilization kinetics of Araxá RP in a continuous stirred-flow system with solutions of organic acids and to evaluate the structural and mineralogical changes caused by contact with organic acid.

Material and Methods

The experiments were conducted at the Soil Fertility Laboratory and at the Microbial Ecology Laboratory of Universidade Federal de Viçosa (UFV), Viçosa, MG, Brazil. Samples of Araxá RP (13.97 % total P) was used in the experiments.

Kinetics of Araxá RP Solubilization by organic acids

Araxá RP solubilization kinetics was studied following a *stirred-flow* technique (Figure 1) [STRAWN and SPARKS, 2000] with extractant solutions of gluconic, citric, and oxalic acids at 5 and 10 mmol L^{-1}. Additional treatments corresponding to the combination of citric, oxalic and gluconic acids, in pairs, at a final concentration of 10 mmol L^{-1} (5 mmol L^{-1} for each component), and water at pH 2.0 and 7.0, were also included. The extractant solution was maintained in a continuous flow of 1 mL min^{-1} for 150 min and stirred at 600 rpm in the reactor chamber. Ten milligrams of Araxá RP were placed in the reactor chamber. Extractant solution samples were collected every four minutes and solubilized P was determined according to Murphy and Riley (1962). A total of 39 P measurements were done for each replication.

Accumulated solubilized P was determined by the following formula (SPARKS, 1998):

$$q(t_i) = \{[\sum(p_{ni} - p_{si})J\Delta t] + (P_n\ (t_i) - P_S\ (t_i)]\ V\}\ m$$

where, $q(t_i)$ is retention when both efluent fraction and chamber concentrations of sorptive are considered; p is the concentration of phosphorus in the collected fraction; P

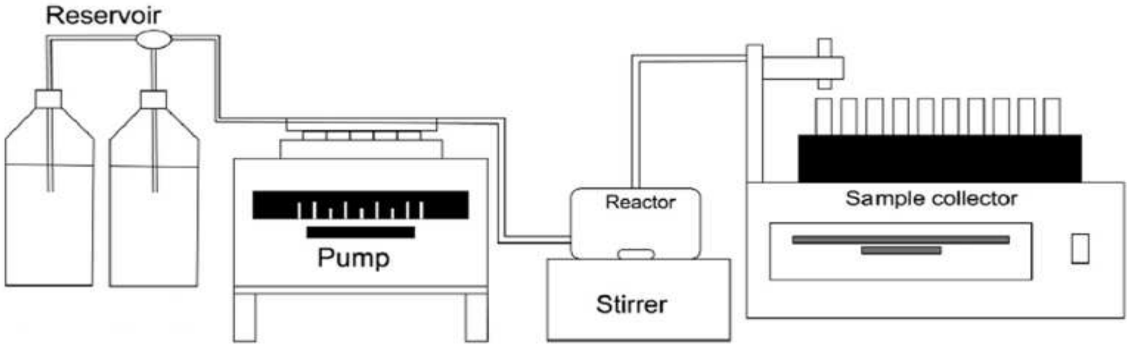

Figure 1. Stirred-flow tecnique: extractant solution in the reservoir is pumped into the reactor chamber where it gets into contact with the particulate material under study (Araxá rock phosphate or the 75-µm fraction of the soil). The suspension formed in the chamber is constantly agitated at 600 rpm. After passing through the reaction chamber at 1 mL min^{-1}, the extractant solution containing solubilized materials is collected in Eppendorff tubes in a sample collector.
Fonte: Guedes *et al.* (2016)

is the phosphorus concentration in the chamber; t_i is time at the end of sample collection; Δt is the length of collection period; J is the flow rate; V is the volume of solution in the chamber; m is mass of RP in the chamber.

The experiments were conducted in a randomized block design with three replications in time. The obtained data were submitted to ANOVA and processed with the R Studio software, using the ExpDes package.

The significant interactions were employed and the averages compared by the F test ($p < 0.01$). When relevant, the data were submitted to regression analyses.

Changes in Araxá RP mineralogy during contact with organic acids

The experiment was done in 250-mL Erlenmeyer flasks by mixing 41.9 mg of Araxá RP with 100 ml of solution containing organic acids or water with adjusted pH as follows: 1) oxalic at 10 mmol L^{-1}; 2) citric acid at 10 mmol L^{-1}; 3) oxalic acid + citric acid at a final combined concentration of 10 mmol L^{-1} (5 mmol L^{-1} for each component) and 4) water with pH 2.0 ajusted with HCl 1%. In the treatment with oxalic acid, contact with Araxá RP corresponded to one and 24 h. For the remaining treatments, solubilization time corresponded to only 24 h. The flasks were incubated at 25 °C and 150 rpm. P solubilization was quantified by taking 1 mL of the supernatant and analyzing solubilized P as described above.

Finally, after incubation, the supernatant was eliminated, RP residues collected, transferred to 50-mL Falcon tubes, and washed three times with deionized water. The residues were then air-dried and submitted to X-ray diffraction (XDR) and scanning electron microscopy (SEM). X-ray diffractometry was performed using a Panalytical X'Pert PRO with Co (CoKα) radiation. The X-ray diffraction patterns were collected in the range of 4 to 50 ° 2θ at a scanning speed of 0.06 ° 2θ per second, with a potential generator of 40kV and a current generator of 40 mA.

For scanning electron microscopy, the procedure described by Haddad et al. (2007) was used. The residue was placed on stubs and left overnight in a dessicator with silica. The residue was metallized with a gold layer of 20 to 30 nm in a Quorum Q150R S metallizer and observed in a Leo 1430VP Scanning Electron Microscope.

Results

Kinetics of Araxá RP Solubilization by organic acids

Oxalic acid was shown to be the most efficient RP solubilizer when compared to citric or gluconic acids (Figure 2; Table 1). The maximum percentages of P solubilization with oxalic acid were 36 and 43% at the concentrations of 5 and 10 mmol L^{-1}, respectively (Figure 2A). Araxá RP treatment with oxalic acid led to higher solubilization P than those obtained with water at pH 2.0 (34 %) and water pH 7.0 (0.16 %). Citric acid was capable of solubilizing 19 and 21 % of the total P in Araxá RP for the concentrations of 5 and 10 mmol L^{-1}, respectively (Figure 2B). For gluconic acid, percentages of solubilized P obtained at the end of the experiment were 1.2 and 2.6 % (Figure 2C). Oxalic + citric acid led solubilization percentages of 71 % (Figure 2D). The treatments with oxalic + gluconic acid and citric + gluconic acid led to solubilization percentages of 41 and 14 %, respectively.

Araxá RP solubilization with the organic acids tested in the stirred-flow system varied with time until stabilization (Table 1). The stabilization of the solubilization rates occurred at different times depending on the acid used and its concentration (Table 1). The lowest solubilization rates recorded corresponded to those obtained after stabilization of P release (Table 1). Oxalic acid at 10 mmol L^{-1} and oxalic acid + citric acid at the final combined concentration of 10 mmol L^{-1} were the most efficient treatment at Araxá RP solubilization (Table 1).

Changes in Araxá RP mineralogy and morphology during contact with organic acids

Treatment with oxalic and citric acids, singly or in combination, led to changes in the morphology of Araxá RP particles (Figure 3). Formation of putative calcium oxalate crystal was also observed for all the treatments with oxalic acid and this was later confirmed by XRD analyses (Figure 4). Calcium oxalate appeared as druses and tetragonal crystals (Figure 3G). RP contact with oxalic acid at 10 mmol L^{-1} for one or 24 h led to more notable changes in RP morphology (Figure 3C and 3D). Water at pH 2.0 did not cause any major changes in the morphology of the mineral particles (Figure 3B). The oxalic + citric acid at 10 mmol L^{-1} altered mineral morphology, but led to the formation of less calcium oxalate. This treatment apparently caused greater changes in morphology than either acid alone (Figure 3F).

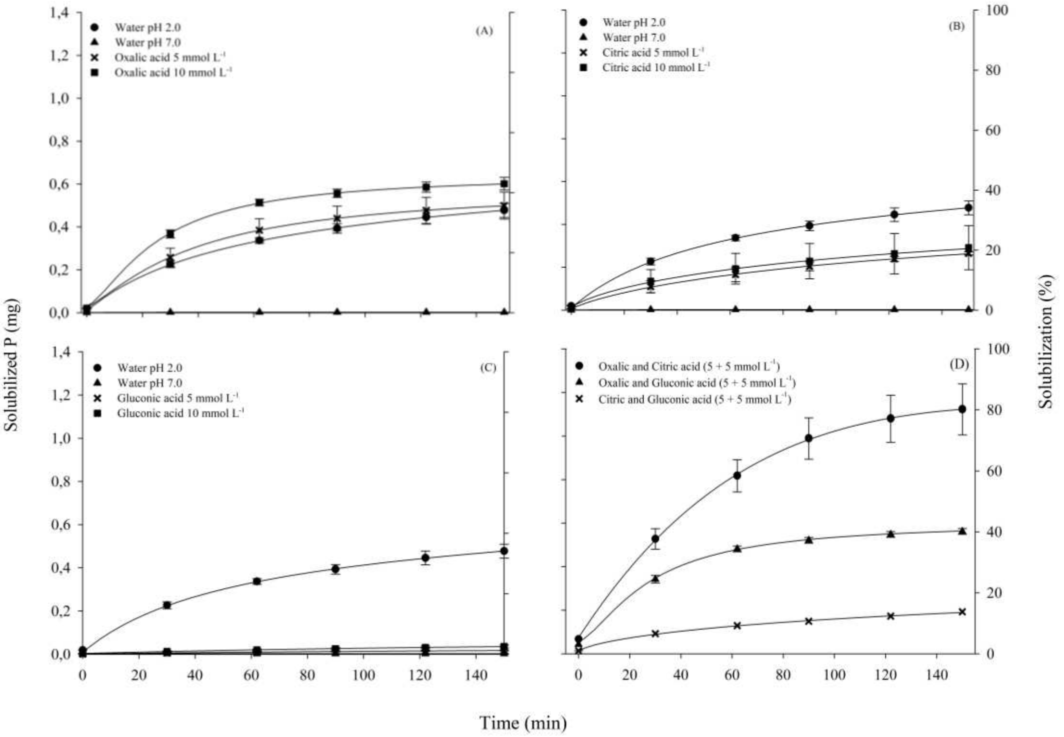

Figure 2. Solubilized P and percentage of solubilization of Araxá rock phosphate treated with oxalic (A), citric (B), and gluconic (C) at the final concentrations of 5 and 10 mmol L^{-1} and their combination in pairs (D) at the concentrations of 5 + 5 mmol L^{-1} and water at pH 2.0 and 7.0, in a stirred-flow system with a continous flow of 1 mL min^{-1} and 600 rpm, at room temperature. Regression equations see supplementary table 1.

Table 1. P solubilized, solubilization percentage, minimun (MISR) and maximum (MASR) solubilization rate, desorption rate stabilization time (SRET) and stabilization rate (SR) for Araxá rock phosphate treated with by oxalic acid, oxalic and citric acid together, water pH 2.0, and water pH 7.0 in a stirred-flow system with a continous flow of 1 mL min^{-1} and 600 rpm, at room temperature.

EXTRACTANT SOLUTION	**TOTAL P SOLUBILIZED (µg)**	**SOLUBILIZATION (%)**	**MISR (µg min^{-1})**	**MASR (µg min^{-1})**	**SRET (min)**	**SR (µg min^{-1})**
Oxalic acid 5 mmol L^{-1}	500 c	36 c	1	11	90	1
Oxalic acid 10 mmol L^{-1}	602 b	43 b	1	18	82	1
Citric acid 5 mmol L^{-1}	264 d	19 d	1	6	74	1
Citric acid 10 mmol L^{-1}	291 d	21 d	1	7	62	1
Gluconic acid 5 mmol L^{-1}	17 e	1.2 e	0.1	0.4	14	0.1
Gluconic acid 10 mmol L^{-1}	36 e	2.6 e	0.2	0.6	38	0.2
Oxalic and Citric acid 5 + 5 mmol L^{-1}	992 a	71 a	2	18	106	2
Oxalic and Gluconic acid 5 + 5 mmol L^{-1}	576 b	41 b	0.5	12	146	0.5
Citric and Gluconic acid 5 + 5 mmol L^{-1}	190 d	14 d	0.9	4	142	0.9
Water pH 2.0	476 c	34 c	1	8	118	1
Water pH 7.0	2.3 e	0.16 e	0.01	0.25	34	0.01

Lines with the same small letter are not statistically different by the Scott-knott test (p<0,05).

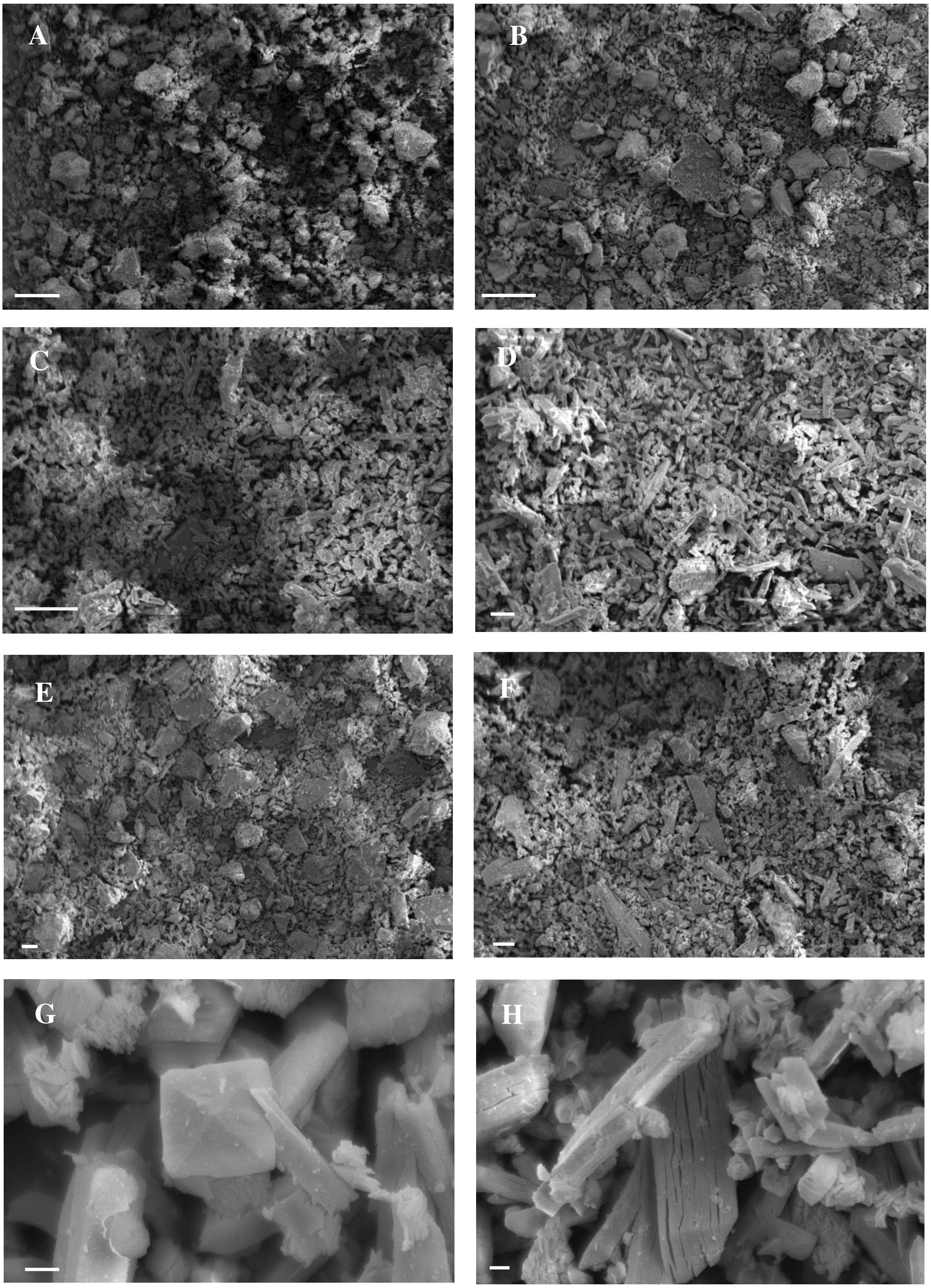

Figure 3. Changes in Araxá RP particles by organic acids. (A) Araxá RP particles without treatment (control). (B) (C) Oxalic acid 10 mmol L^{-1} for 1h treatment. (D) Oxalic acid 10 mmol L^{-1} for 24h treatment. (E) Citric acid 10 mmol L^{-1} treatment. (F) Oxalic acid + Citric acid 10 mmol L^{-1} treatment. (G) Tetragonal calcium oxalate crystal. (H) Calcium oxalate druses and multi-layer minerals undergoing disaggregation. Notice spaces between layers. Marker bars = (A) 100 µm (B) 20 µm (C), (D); (E) and (F) 10 µm and (G) and (H) 2 µm.

XRD analyses showed general decreases in apatite peaks in the treatments with oxalic and citric acids, singly or combined (Figure 4). A fluorapatite peak, detected in the control treatment, was eliminated by the treatment with oxalic acid for 24 h (Figure 4). In all treatments with oxalic acid, a remarkable formation of calcium oxalate crystals was detected (Figure 4).

Discussion

The use of organic acids in the stirred-flow system promoted increases in P solubilization. The organic acids tested, besides promoting the acidification of medium, have been reported to act as chelating agents for the products of RP solubilization, *i.e.* Ca^{2+}, shifting the equilibrium of the reaction towards the release of P and the accompanying cation (KPOMBLEKOU-A and TABATABAI, 1994). Calcium phosphates, such as Araxá RP, are sensitive to decreases in the pH of the medium and are partly solubilized by acidification (NAUTIYAL *et al*, 2000; MENDES *et al.*, 2014). Since water at pH 2.0 was not as effective as citric and oxalic acids at Araxá RP solubilization, acidification alone has a limited contribution to apatite solubilization. Our results put in evidence that the additional removal of the solubilization products through chelation of cations by organic acids is essential for enhancing apatite solubilization. Additonally, the stirred-flow technique used in our work allows the removal of all the products of the reaction, avoiding the accumulation of compounds that can inhibit the continuity of solubilization (SPARKS, 1998).

Among the organic acids tested in our work, oxalic acid at 10 mmol L^{-1} presented the highest solubilization of Araxá RP, releasing 43 % of the total P present in this apatite. In fact, oxalic acid has been repeatedly shown to be one of the most efficient metabolites at RP solubilization (MENDES *et al.,* 2013; DUARTE, 2017; MURTA, 2017) due to its strucutural caracteristics, such as the number of carboxyl groups. When combined with citric acid, the efficiency at Araxá RP solubilization increased to 71 %. This effect cannot be attributed to an increased drain for Ca^{2+} in the mixture, since the combined final concentration of the two acids was kept at 10 mmol L^{-1}. Hypothetically, the combination of organic acids can corrode RP particles in a way to increase the accessibility of the solubilizing agents to susceptible minerals within the RP particle, improving RP solubilization.

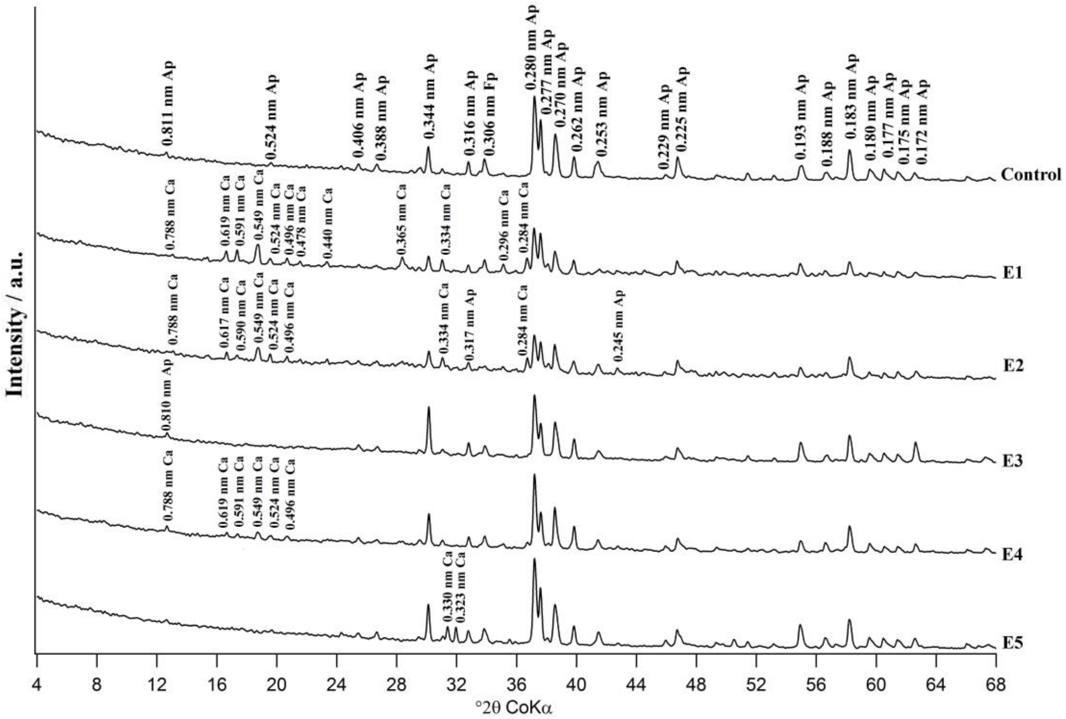

Figure 4. X-ray diffraction pattern of Araxá RP treated with different organic acids. Control (without treatment). E1 – Oxalic acid 10 mmol L^{-1} - 1 h. E2 – Oxalic acid 10 mmol L^{-1} - 24 h. E3 - Citric acid 10 mmol L^{-1}. E4 – Oxalic acid + Citric acid 10 mmol L^{-1}. E5 – Water pH 7.0. Ap – apatite. Fp – Fluoroapatite. Ca – calcium oxalate.

In our work, combination of two organic acids led to a solubilization percentage that was higher than that obtained with H_2SO_4 when acting on sedimentary phosphate rocks (KPOMBLEKOU-A and TABATABAI, 1994), a strong acid commonly used in the manucture of soluble phosphate fertilizers. Araxá RP solubilization rates varied according to the type of acid, used singly or in combination, and water pH. Generally, solubilization rates decreased with time until reaching a stability stage. At this time, P release from Araxá RP could still be observed, but at solubilization rates very close to zero. From this stage on, remaining P-bearing minerals would be exhausted in rather long times. The higher RP solubilization rates at the beginning of the experiments in the stirred-flow system can be possibly explained by the immediate dissolution of more reactive phosphate minerals and the removal of soluble P.

Citric acid at 10 mmol L^{-1} solubilized lower amounts of P when compared to oxalic acid. However, at stabilization, solubilization rates for this acid was higher than that observed for oxalic acid at the same stage (Table 1). This is an indication that different organic acids may act on the solubilization of distinct components of the Araxá RP and reinforces that mixtures of different microbial metabolites may be more efficient at RP solubilization and be closer to the process of P disponibilization in the soil and plant rhizosphere.

Gluconic acid was the least efficient of all the acids and acid combinations tested. When combined with other organic acids, Araxá solubility improved, but remained low. In fact this compound is monocarboxylic, which gives it lower chelating power when compared to the other acids with more carboxyl groups (KPOMBLEKOU-A and TABATABAI, 2003; SCERVINO *et al.*, 2011 MENDES *et al.*, 2014). Additionally, gluconic acid has a low ability to acidify the medium. The gluconic acid solutions at 5 and 10 mmol L^{-1} used in our experiments had pH values of 6.6 and 5.6, respectively. Thus, based on our results, for a phosphate solubilizing microorganism that produces high amounts of gluconic acid, microbial RP solubilization can only be effective if the microorganism possesses other complementary acidification mechanism, such as H^+ extrusion, NH_4^+ and S_2^- chemolithotrophic oxidation, among others. This hypothesis must yet be tested. As the final concentration of the organic acids tested, singly or in combination, was maintained at 10 mmol L^{-1}, it is possible that the oxalic and citric acid concentrations were not enough to counterbalance the decrease in acidification and chelating ability brought about their combination with gluconic acid.

The best performance of combined acids may also be related to the distinct material that they attack when in contact with Araxá RP. This synergic action may reflect complementary solubilization mechanism acting upon RPs that are very complex, both in chemical and mineralogical composition, as exemplified in the work of Mendes *et al.* (2014) Since in our work we have studied the solubilization of a single RP, its possible that other chemical properties of the organic acids tested may have had an impact on the solubilization efficiency of each compound, such as the number of carboxyl groups, spatial shape of the molecule, molecular mass, and other physico-chemical property affecting organic acid interaction with the surface of RP particles. To our knowledge, this is the first work that describes the kinetics of RP solubilization by different organic acids.

When in contact with the organic acids tested, Araxá RP particles undergo morphological changes that evidence distinct modes of action of the organic acids tested on the minerals present in Araxá RP. The formation of calcium oxalate crystals was remarkable in the treatments with oxalic acid and indicate that the calcium oxalate precipitates may be the main mechanism for the removal of solubilization products, namely calcium, from the solubilization medium. This may also explain the higher efficiency of oxalic acid as RP solubilizing agent. Calcium oxalate can have different forms (needles, disks, dumbbells, and druses) and exist as whewellite, the monohydrate form, and weddellite, the dehydrate one. The monohydrate form generally monocyclic crystals and the dehydrate, tetragonal ones (ARNOTT, 1995). In our experiments, both crystal shapes were observed. The formation of oxalate crystal was decreased in the treatments with oxalic and citric acids combined. This probably occurred because the presence of citric acid could inhibit the formation of monohydrate calcium oxalate crystals (RYALL *et al.*, 1981).

XRD analyses confirmed the identity of calcium oxalate crystals and allowed to follow changes in Araxá RP mineralogy during organic acid treatment. Oxalic acid caused decreases in the apatite peaks and eliminated fluorapatite from Araxá RP. On the other hand, fluorapatite remained in the samples treated with citric acid. Thus, since fluoride released from RP during solubilization is toxic to microorganisms (MENDES *et al.,* 2014a), microbial RP solubilization based on oxalic acid use must take into account strategies for fluoride elimination microorganisms (MENDES *et al.,* 2014a) to prevent microbial inhibition. Also, citric acid may be less efficient to solubilize fluoride-rich RPs.

Conclusions

Oxalic acid is most efficient organic acid tested in P solubilization from Araxá RP in a stirred-flow system and improvements in solubilization efficiency can be obtained by combining that compound with citric acid. The oxalic acid decreasing expressively the peacks of the minerals of fluorapatite during the solubilizations processe.

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Capítulo 3

PHOSPHORUS DESORPTION IN THE SOIL BY ORGANIC ACIDS AND *Aspergillus niger*

Phosphorus dessorption in the soil by organic acids and *Aspergillus niger*

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Abstract

Phosphorus (P) can undergo adsorption, precipitation and soil fixation reactions that reduce the availability of this nutrient to the plants. When fixed to iron and aluminum oxides and hydroxides, the P starts to form the solid phase of the soil, being difficult to make available to crops. Some compounds, such as organic acids, have the ability to extract P from low reactivity phosphate rocks. These compounds can be produced by plants and microorganisms as a strategy to acquire P for growth. Little is known about the ability of organic acids and microorganisms to make P adsorbed to soil. The objectives of this work were to study the kinetics of desorption of P by organic acids and to evaluate the ability of *Aspergillus niger* in desorb P of 75-µm fraction of a Oxissol. The desorption kinetics of P of the 75-µm fraction of Oxissol horizons A and B were performed after one and 40 days of P contact with the soil fraction. The amount of P adsorbed to the soil corresponded to 90 % of the maximum adsorption capacity of P (MACP). The extractive solutions used were oxalic acid, oxalic acid + citric at concentrations 10 mmol L^{-1}, (5 mmol L^{-1} for each component) in addition to water at pH 2.0 and 7.0. The ability of *A. niger* to desorb P from the soil fractions in culture medium. The oxalic acid was the most efficient in desorbing soil P. The P released in solution reached values of 35.5 and 32.7 % of the adsorbed P at the times of one and 40 days, respectively, for A horizon and the P released in solution reached values of 22.4 and 18.5 % of the adsorbed P in the times of one and 40 days, respectively. When in the presence of *A. niger*, the desorption of P reached values of 23 and 18 % in the A horizon and 17 and 18 % in the B horizon, at times of one and 40 days, respectively. Citric and oxalic acids and *A. niger* fungus were able to reverse the P adsorption process to the soil *in vitro*, opening prospects for the use of P fixed to soil in agriculture.

Key words: Oxisol, adsorption, phosphate solubilizing microorganisms

Introduction

The consumption of animal protein driven by population growth causes the global demand for food to grow (TILMAN *et al*., 2011, ALEXANDRATOS and BRUINSMA, 2012). Although there is much debate about where intensification of agriculture must take place to support this increase (KUYPER and STRUIK, 2007), there is an agree that tropical regions play a key role in global agriculture (ALEXANDRATOS and BRUINSMA, 2012). However, these regions depend on external inputs to maintain crop productivity, since soils are highly weathered and poor in terms of nutrient supply, mainly phosphorus (P) (PALM *et al.,* 2007).

Soils from tropical regions exhibit high P adsorption capacity, so the phosphate fertilizers that are added react with Fe and Al oxides, becoming unavailable over time for crop adsorption. Brazil has approximately 25% of cultivable soils, which adsorb large amounts of P, which produce soybean, maize, sugarcane, rice and wheat (ROY *et al*., 2016). The intensification of agriculture in these regions implies a large input of inputs, mainly phosphates. A large proportion of these phosphate fertilizers are adsorbed to the soil, resulting in a surplus of 20 Tg of P in the last 40 years in Brazilian soils (ROY *et al*., 2016). The accumulation of P in the soil can cause problems in the environment. In places like North America and Europe, P accumulated in the soil is lost by artificial runoff and is carried to bodies of water causing great damage to these ecosystems (SHARPLEY *et al*., 2014).

In general, access to residual P in the soil could contribute to a decrease in demand for phosphate fertilizers, helping farmers to cope better with input price changes (SATTARI *et al*., 2012; MEW, 2016). Strategies to reduce the adsorption of P in soils that naturally have this characteristic, it is necessary to use the finite stocks of phosphate rocks (ROY *et al*., 2016) in a more aware and sustainable way.

A considerable amount of inorganic P from soluble sources adsorbed to soils, making this compartment an important P reservoir that can hypothetically be accessed by plants through rhizosphere and microbial processes (LYNCH, 2007; RICHARDSON *et al*., 2011).

Generally, the organic acids have higher affinity for clays than phosphate (WEI *et al*., 2010) and compete with the latter for adsorption sites in the soil (HAYNES, 1984; CESSA *et al.,* 2010). The potential for competition with phosphate depends on acid concentration, number of carboxyl groups, molecule shape, and persistence in the soils (PAVINATO and ROSOLEM, 2008). Also, organic acids of plant or microbial origin can also form organometallic complexes with Fe^{3+} and Al^{3+}, increasing P availability by the dissolution of phosphate precipitates with these ions (PAVINATO and ROSOLEM, 2008).

In addition, doubts remain on the susceptibility of reversing strong P adsorption to weathered tropical soils. Since P adsorption may involve strong covalent bond formation with ion oxides and hydroxydes along time, this process is assumed to be irreversible (KAFKAFI *et al*., 1967; PARFITT., 1978). The action of microorganism, such as phosphate solubilizing fungi, and their metabolites, including citric and oxalic acids may be effective for releasing strongly adsorbed P from the soil. To our knowledge, this hypothesis has not been tested so far.

The objectives of this work were to study the kinetics of desorption of P by citric and oxalic acids and to evaluate the ability of *Aspergillus niger* in desorb P of 75-µm fraction of a Oxissol.

Material and Methods

The experiments were conducted at the Soil Fertility Laboratory and at the Microbial Ecology Laboratory of Universidade Federal de Viçosa (UFV), Viçosa, MG, Brazil. Samples from a Oxissol (Table 1) were used in the experiments. The soil was previously ground in a mortar and passed through a sieve to generate the 75-µm fraction.

The fungus *A. niger* FS1 belongs to the collection of Phosphate Solubilizing Fungi of the Microbial Ecology Laboratory. Unless otherwise described, the fungus was maintained in potato-dextrose-agar (PDA) at 30 °C and transferred to fresh PDA every seven days.

Table 1. Physical and chemical characteristics of the A and B horizons of Oxissol collected in Viçosa, MG, Brazil

Soil	**pH H_2O**	**P**	**K**	**Ca^{2+}**	**Mg^{2+}**	**Al^{3+}**	**H + Al**	**SB**	**CTC (t)**	**CTC (T)**	**V**	**m**	**MO**	**P-rem**	**Fe**	**Clay**	**Silt**	**Sand**
		mg dm^{-3}		$cmol_c$ dm^{-3}							%		dag kg^{-1}	mg L^{-1}	mg dm^{-3}	%		
A horizon	5.2	1,1	115	0,6	0,2	0,7	7,59	1,09	1,79	8,68	13	39	3,22	10,7	96.0	44	10	46
B horizon	4.9	0,8	6	0,1	0,0	0,3	3,47	0,12	0,42	3,59	3	71	1,55	3,8	24.3	57	9	34

pH in H_2O – Relation 1:2.; P – K – Fe – Extractant Mehlich 1; Ca – Mg – Al – Extratant: KCl – 1mol/L; SB – Exchangeable Bases Sum; CTC (t) = Effetive cation exchange capacity; CTC (T) = Cation exchange capacity at pH 7.0; V = Saturation by Bases Index; m = Aluminium Saturation Index; MO – Organic Matter – Oxidation $Na_2Cr_2O_7$ 4N + H_2SO_4 10N; P-rem = Remaining phosphorus.

Desorption of P from Oxissol by organic acids

The maximum phosphorus absorption capacity (MPAC) of the soil was determined following the method described by ALVAREZ and FONSECA (1990) and the data obtained were submitted to ANOVA (f test at $p < 0.05$) and averages were used to adjust the Langmuir model (Figure 1). Soil field capacity was determined using the Moisture Equivalent method according to Ruiz *et al.* (2003). Forty grams of the 75-µm fractions of the A and B horizons of the Oxissol were mixed with 12.8 mL of a KH_2PO_4 solution containing 50.76 mg of P. This corresponded to a P quantity equivalent to 90 % of MPAC, *i.e.,* 1.27 mg P g^{-1} soil. The soil samples were maintained at 80 % field capacity for one or for 40 days. P desorption was done by the stirred-flow technique (Figure 2) (STRAWN and SPARKS, 2000) with extractant solutions tested corresponded to 1) oxalic acid at 10 mmol L^{-1}; 2) oxalic + citric acid at a final combined concentration of 10 mmol L^{-1} (5 mmol L^{-1} for each component; 3) water at pH 2.0 and 4) water pH 7.0. The extractant solution was maintained in a continuous flow of 1 mL min^{-1} for 150 min and stirred at 600 rpm in the reactor chamber. One grama of Oxissol were placed in the reactor chamber. Extractant solution samples were collected every four minutes and solubilized P was determined according to Murphy and Riley (1962). A total of 39 P measurements were done for each replication.

Accumulated solubilized P was determined by the following formula (SPARKS, 1998):

$$q(t_i) = \{[\sum(p_{ni} - p_{si})J\Delta t] + (P_n\ (t_i) - P_S\ (t_i)]\ V\}\ m$$

where, $q(t_i)$ is retention when both efluent fraction and chamber concentrations of sorptive are considered; p is the concentration of phosphorus in the collected fraction; P is the phosphorus concentration in the chamber; t_i is time at the end of sample collection; Δt is the length of collection period; J is the flow rate; V is the volume of solution in the chamber; m is mass of RP in the chamber

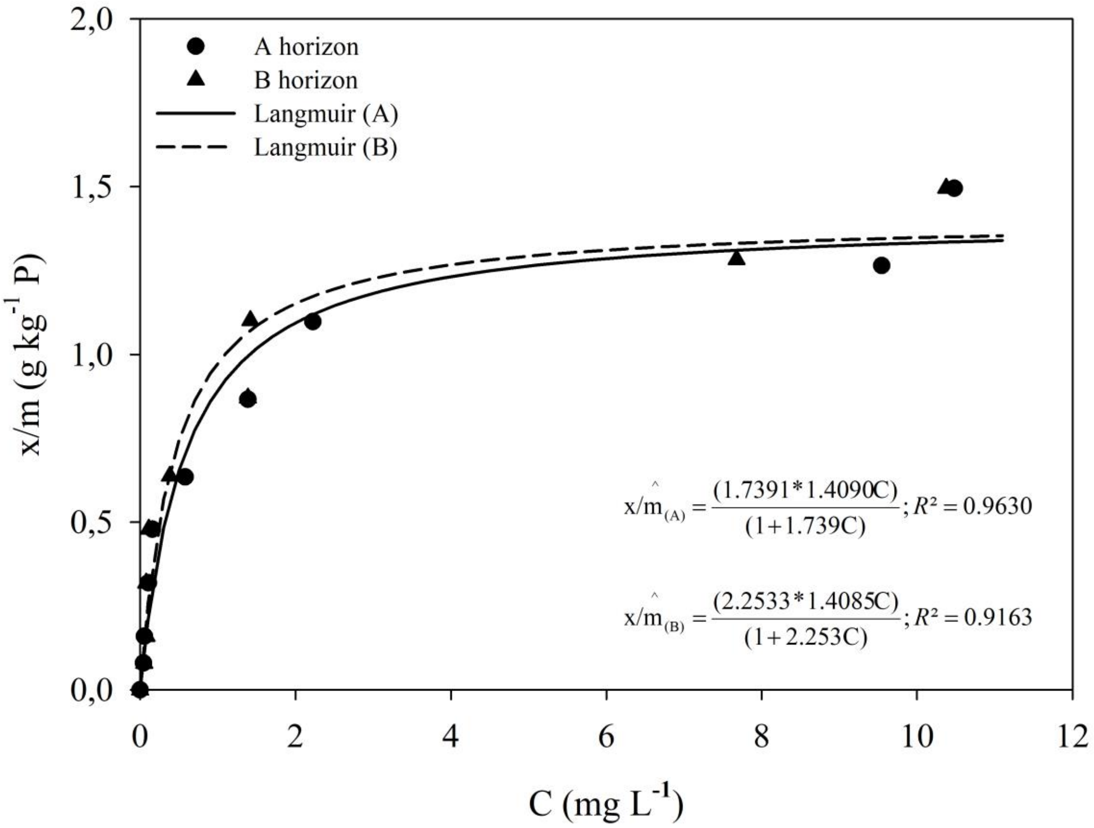

Figure 1. Maximum adsorption capacity of P for soil from A and B horizons of Oxissol from Viçosa, MG, Brazil

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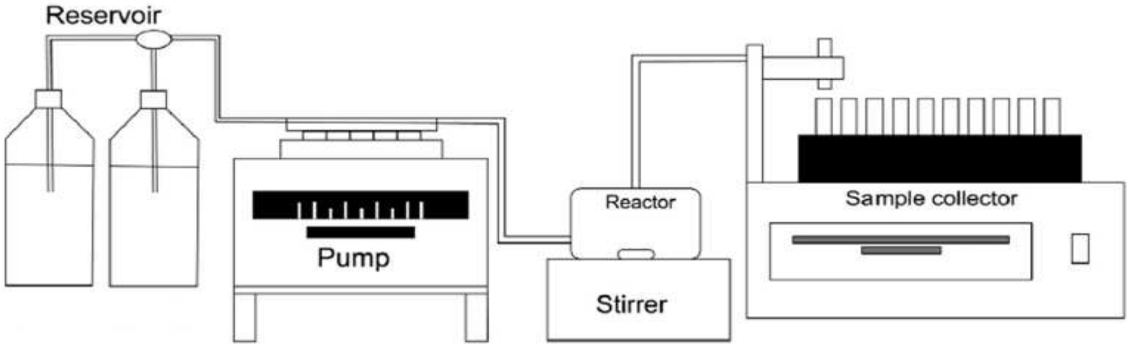

Figure 2. Stirred-flow tecnique: extractant solution in the reservoir is pumped into the reactor chamber where it gets into contact with the particulate material under study (Araxá rock phosphate or the 75-µm fraction of the soil). The suspension formed in the chamber is constantly agitated at 600 rpm. After passing through the reaction chamber at 1 mL min^{-1}, the extractant solution containing solubilized materials is collected in Eppendorff tubes in a sample collector.
Fonte: Guedes *et al.* (2016)

Filter membranes from the reactor chamber containing residual soil particles were collected and air-dried. The retained soil particles were then removed from the filter membrane with a spatula and broken in an agatha mortar. The residual soil particles were collected, air-dried, and submitted to X-ray diffraction analyses as described above.

The experiments were conducted in a randomized block design with three replications in time. The obtained data were submitted to ANOVA and processed with the R Studio software, using the ExpDes package.

The significant interactions were employed and the averages compared by the F test ($p<0.01$). When relevant, the data were submitted to regression analyses.

P desorption by A. niger *FS1*

125-mL Erlenmeyer flasks containing 50 mL of NBRIP medium (NAUTIYAL, 1999) and supplemented with 20 g L^{-1} of A and B horizons soil fractions, previously treated to achieve 90 % of CMAP, were inoculated with 1 mL of spore suspension prepared in 0.1 % Tween 80 containing 10^6 spores of *A. niger* grown in PDA for 7 days at 30 °C. The flasks were incubated in a horizontal shaker for 7 days at 30 °C and 150 rpm. At the end of the incubation, culture supernatants were filtered through quantitative pore 22 µm filter paper JP42 (Quanty®), and used to determine soluble P, pH, and titratable acidity. The material retained in the filter paper was collected, dried at 65 °C until constant weight and then incubated in a muffle at 500 °C for for 5 hours.

Fungal biomass was calculated by subtracting the residue mass obtained fater incineration from that obtained after drying at 65 °C. Soil without fungal growth was submitted to the same procedure to allow corrections of weight loss due to soil organic matter oxidation.

The experiment was conducted in a completely randomized design, with three replicates. The data obtained was submitted to ANOVA and means were compared by the Tukey test ($p < 0.01$).

Results

Desorption of P from Oxissol by organic acids

For the desorption experiments, the maximum adsorption capacities of P in the Oxissol, A and B horizons, were previously determined and corresponded to 1.409 and

1.408 g kg^{-1} P, respectively (Figure 1). For the A and B horizon, P desorption for the treatments without P addition was low (Figure 3A). For the adsorption time of one and 40 days, P desorption decreased in the following order: oxalic acid > oxalic acid + citric acid > water pH 2.0 > water pH 7.0 (Figure 3C and E) Generally, P desorption was higher for the adsorption time of one day (Table 2). Desorption percentage varied from 0.17 to 36 %, with the highest values obtained in the treatments with oxalic acid (Table 2). Desorption rates varied from 0.007 to 13 µg min^{-1} (Table 2). Stabilization time were shorter for the treatments with P adsorption for one day (Table 2). For the B horizon, P desorption for the treatments without P addition was also low (Figure 3B), similarly to that found for the A horizon. For the adsorption time of one and 40 days, P desorption decreased in the following order: oxalic acid > oxalic acid + citric acid > water pH 2.0 > water pH 7.0 (Figure 3D and F) Generally, P desorption was higher for the adsorption time of one day (Table 3). Desorption percentage varied from 0.18 to 22.4 %, with the highest values obtained in the treatments with oxalic acid (Table 3). Desorption rates varied from 0.001 to 7 µg min^{-1} (Table 3). As for the A horizon, stabilization times were shorter for the treatments with P adsorption for one day (Table 3).

The organic acid tested did not lead to changes in soil mineralogy, with no decreases XDR peaks of hematite, gibbsite, goethite e kaolinite for both A and B horizons (Figure 4). On the other hand, oxalic acid led to decreases in the quartz content in the soil fractions used (Figure 4).

P desorption by A. niger FS1

A niger FS1 was capable of desorbing P from the 75-µm fractions of the A and B horizons of the Oxissol (Figure 5A, B, C, D). P desorption was higher for the treatments with one day of P adsorption for the A horizon. The fungus led a porcentage of P desorption that vaired from 17 to 23 % depending on the soil horizon and adsorption time. In the control treatments, without P adosrption, P release was low.

Growth of *A. niger* in the medium NBRIP suplemented with the soil fractions caused significant decreases in pH (Figure 6A and B). Titratable acidity was higher for the treatments with no P adsorption and decreased for the treatments with P adsorption 24 and 960 h, with slight difference between soil horizons (Figure 6C and D).

Fungal biomass did not differ among soil horizons and P adsorption times.

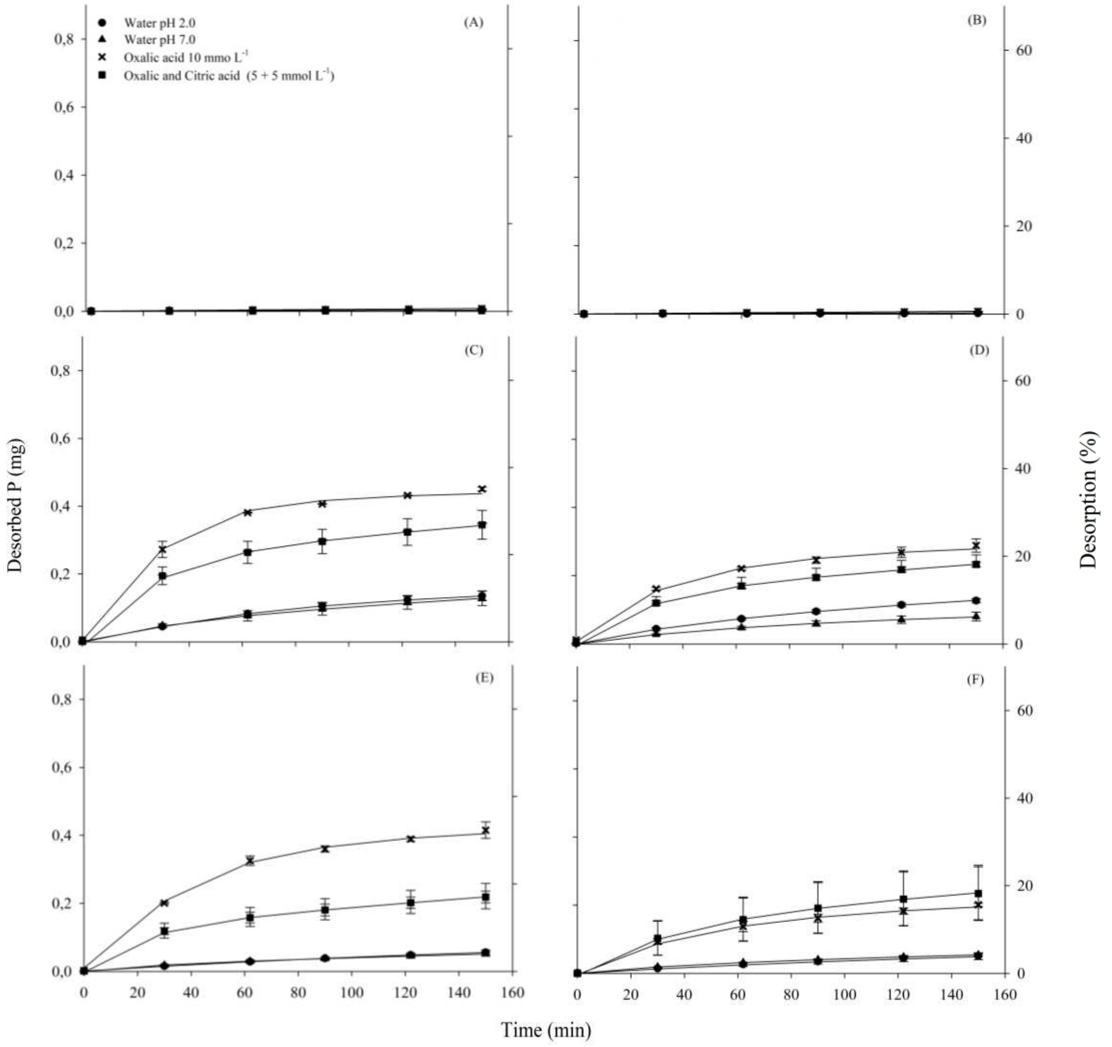

Figure 3. Desorption percentage from the A and B horizon of a Red-Yellow Latasol, from Viçosa, Minas Gerais, Brazil, in a stirred-flow system using organic acids (oxalic acid and oxalic + citric acid at 5 + 5 mmol L^{-1}) and water at pH 2.0 and 7.0. (A) and (B) Treatments with soil without P addition. (C) and (D) Treatments with P adsorption for one day. (E) and (F) Treatments with P adsorption for 40 days. Regression equations see supplementary tables 2 and 3.

Table 2. P desorbed, desorption percentage, minimun (MIDR) and maximum (MADR) desorption rate, desorption rate stabilization time (DRET) and stabilization rate (SR) for 75-µm fraction from the A horizon of a Red-Yellow Latosol treated with by oxalic acid, oxalic acid + citric acid, water pH 2.0, and water pH 7.0 in a stirred-flow system with a continous flow of 1 mL min^{-1} and 600 rpm, at room temperature.

TOTAL P ADSORPTION TIME	**EXTRACTANT SOLUTIONS**			
	Oxalic acid 10 mmol L^{-1}	**Oxalic and Citric acid 5 + 5 mmol L^{-1}**	**Water pH 2.0**	**Water pH 7.0**
DESORBED (µg)				
Control	6.92 Ac	8.37 Ac	3.99 Ac	5.99 Ac
One day	451.09 Aa	345.23 Ba	135.57 Ca	128.07 Ca
40 days	415.36 Ab	218.57 Bb	5.95 Cb	50.71 Cb
DESORPTION (%)				
Control	0.35 Ac	0.42 Ac	0.20 Ac	0.30 Ac
One day	35.55 Aa	27.85 Ba	10.68 Ca	10.09 Ca
40 days	32.73 Ab	17.22 Bb	4.39 Cb	4.13 Cb
MIDR (µg min^{-1})				
Control	0.04	0.04	0,007	0.2
One day	1	1	0.5	0.3
40 days	1	0.5	0.2	0.5
MADR (µg min^{-1})				
Control	0.09	0.09	0.06	0.5
One day	13	10	2	2
40 days	13	5	1	1
DRET (min)				
Control	130	138	-	-
One day	50	66	130	102
40 days	66	134	38	30
SR (µg min^{-1})				
Control	0.04	0.04	0,007	0.2
One day	1	1	0.5	0.3
40 days	1	0.5	0.2	0.5

Columns with same small letter are not statistically different by Tukey test. Lines with the same capital letter are not statistically different by the Tukey test ($p<0.01$).

Table 3. P desorbed, desorption percentage, minimun (MIDR) and maximum (MADR) desorption rate, desorption rate stabilization time (DRET) and stabilization rate (SR) for 75-µm fraction from the B horizon of a Red-Yellow Latosol treated with by oxalic acid, oxalic acid + citric acid, water pH 2.0, and water pH 7.0 in a stirred-flow system with a continous flow of 1 mL min^{-1} and 600 rpm, at room temperature.

TOTAL P ADSORPTION TIME	**EXTRACTANT SOLUTIONS**			
	Oxalic acid 10 mmol L^{-1}	**Oxalic and Citric acid 5 + 5 mmol L^{-1}**	**Water pH 2.0**	**Water pH 7.0**
	DESORBED (µg)			
Control	7.11 Ac	7.99 Ab	3.58 Ac	5.14 Ab
One day	288.77 Aa	233.38 Ba	127.14 Ca	80.47 Ca
40 days	234.33 Ab	201.23 Aa	54.47 Bb	50.67 Bab
	DESORPTION (%)			
Control	0.35 Ac	0.40 Ab	0.18 Ac	0.26 Ab
One day	22.40 Aa	18.97 Aa	10.02 Ba	4.29 Ca
40 days	18.45 Ab	15.80 Aa	4.29 Bb	3.97 Bab
	MIDR (µg min^{-1})			
Control	0.04	0.04	0.001	0.01
One day	1	1	0.2	0.4
40 days	1	1	0.5	0.5
	MADR (µg min^{-1})			
Control	0.09	0.09	0.01	0.02
One day	7	7	1	1
40 days	7	5	1	1
	DRET (min)			
Control	130	138	-	-
One day	118	50	102	130
40 days	106	46	30	30
	SR (µg min^{-1})			
Control	0.04	0.04	0.001	0.01
One day	1	1	0.2	0.4
40 days	1	1	0.5	0.5

Columns with same small letter are not statistically different by Tukey test (p<0.05). Lines with the same capital letter are not statistically different by the Tukey test (p<0.05).

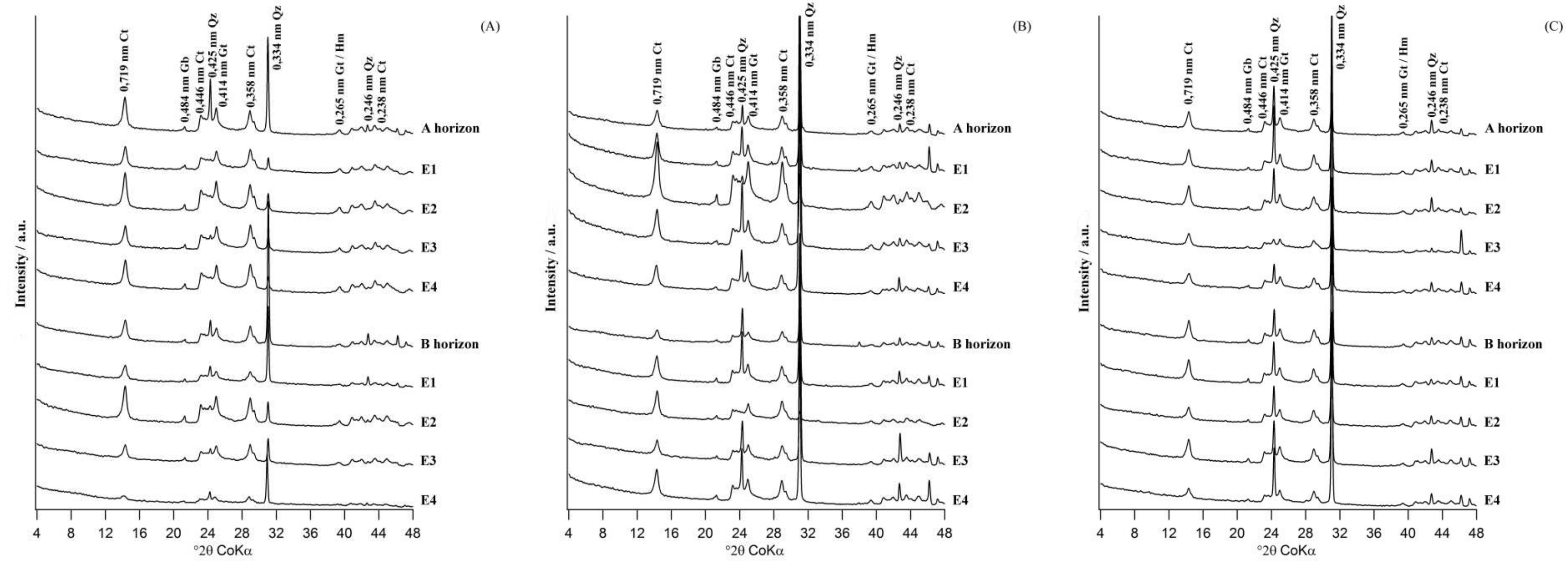

Figure 4. X-ray diffraction pattern of 75-µm fraction from the A and B horizon of a Red-Yellow Latosol treated with different organic acids. (A) 75-µm fraction of A and B horizon without P addition. (B) 75-µm fraction of adsorption P for 24 h. (C) 75-µm fraction of adsorption P for 960 h. (A horizon and B horizon in the graph represent the control). E1 – Oxalic acid 10 mmol L^{-1}. E2 – Oxalic acid + Citric acid 10 mmol L^{-1}. E3 – Water pH 2.0. E4 – Water pH 7.0. Ct – Kaolinite. Gb – Gibbiste. Gt - Goethite. Hm – hematite. Qz – Quartz.

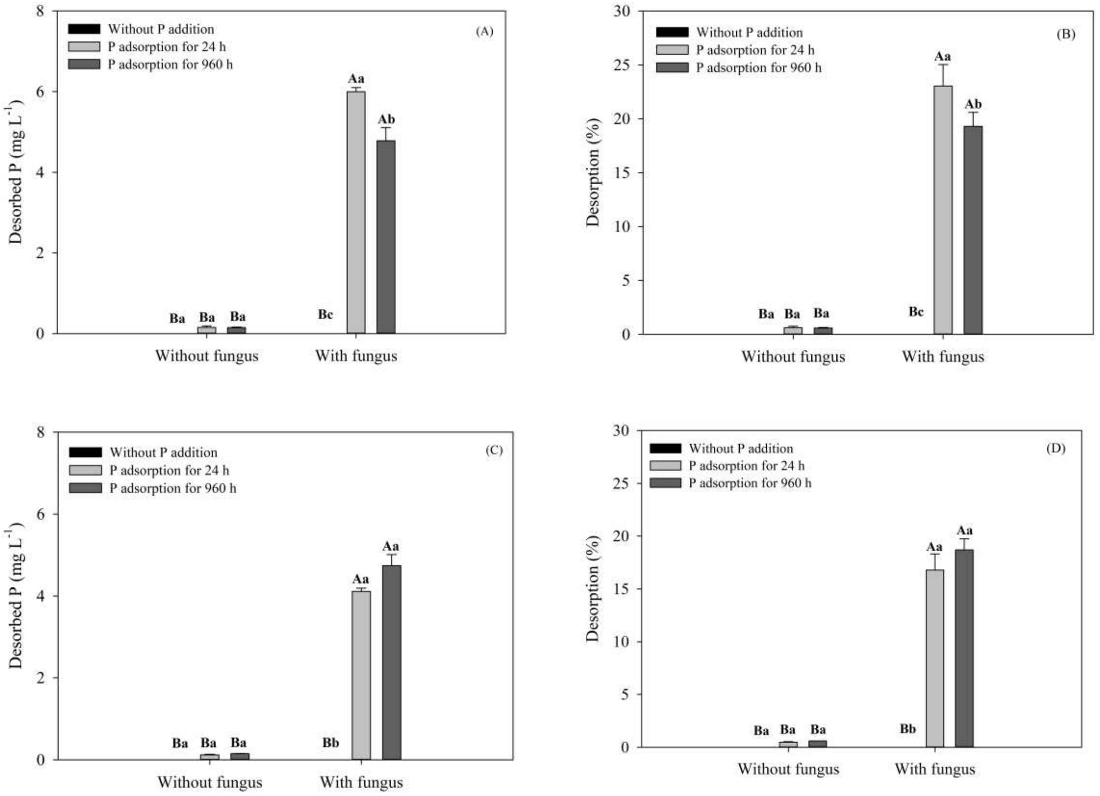

Figure 5. Desorbed P and percentage desorption by *Aspergillus niger* in 75-µm fractions of the A (A) and (B) and B (C) and (D) horizons of a Red-Yellow latosol with different P adsorption times, after seven days of incubation in NBRIP medium at 30 °C and 150 rpm. Within each fungal inoculation treatment, columns with same small latter are not statistically different by Tukey test ($p<0,01$).

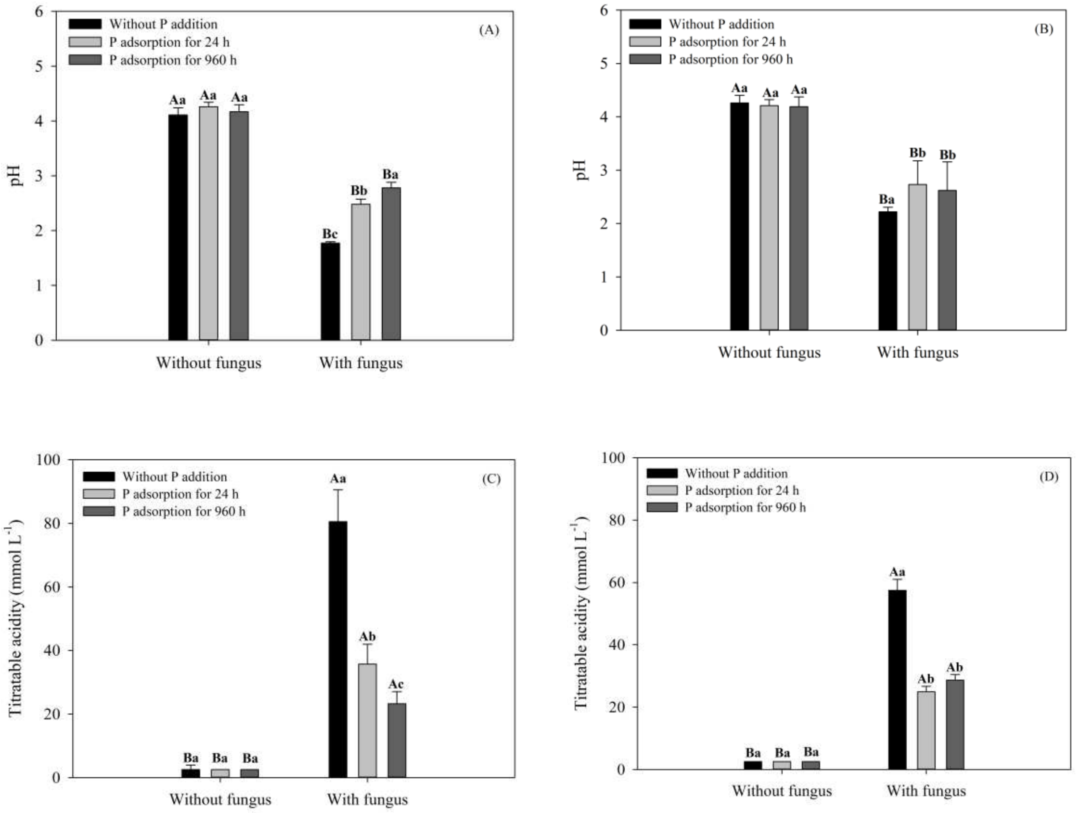

Figure 6. pH and titratable acidity in NBRIP medium supplemented with 75-µm fractions of the A (A) and (B) and B (C) and (D) horizons of a Red-Yellow latosol with different P adsorption times, after seven days of incubation of *Aspergilus niger*, at 30 °C and 150 rpm. For the same P adsorption treatment, columns with same capital letter are not statistically different by Tukey test (p<0,01). Within each fungal inoculation treatment, columns with same small latter are not statistically different by Tukey test (p<0,01).

However, in the treatments with no P adsoption, fungal biomass was very small and could not be measured (Figure 7A). The yield of P per biomass unit did not vary in relation to the treatments tested (Figure 7B).

Discussion

Oxalic acid was also efficient at desorbing P from the 75-µm fraction of both A and B horizons of the soil tested. P desorption was higher in the treatments with 24 h of contact of P with the soil fractions, indicating that, with this adsorption time, P was held with less energy in the soil clays. Desorption values were higher for the A horizon. Coincidently, this soil lay presented higher P-rem values (Table 1), suggesting a higher content of ion and aluminum oxydes and hydroxydes to which P is bound with higher energy.

P binding energy was not determined in our experiments, but it was assumed that, along time, adsorption to soil particles would retain P with greater strength. This, in fact, was reflected in the lower values of P desorption in the treatments with the soil fractions that were kept in contact with P for longer periods, namely 40 days. P desorption in the soil through the action of organic acids must include mechanisms such as binder exchange and the extraction of P bound to Al and Fe via the formation of carboxylated complexes (FOX *et al.,* 1990, GERKE, 1992).

Gerke (1994), studying P desorption in Luvisols and Podzols, obtained values of 0.5 and 0.12 mmol g^{-1} soil, respectively. The author attributed his results to the continuous removal of P in the continuous flow system used in comparison to those obtained in batch systems. This is in agreement with our experiments, in which the the continuous flow allowed higher P desorption than in the batch system with *A. niger.*

The XRD analyzes did not indicate changes in the Oxissol mineralogy treated with the organic acids tested. This indicates that P desorption did not involve the dissolution and elimination of clay minerals such as goethite, hematite, and kaolinite, rather, P desorption must have affected surface-interacting P or P precipitated with Fe^{3+} and Al^{3+} (CESSA *et al.,* 2010). Fernández *et al.* (2008) and Fernández *et al.* (2008a) investigated the effects of redox potential decreases by microbial activity on the reversibility of non-labile P forms, after 30 days of adsorption. No P release was observed and this was attributed to the longtime of contact of P with the soil and the greater stability of goethite and gibbsite under the experimental conditions tested.

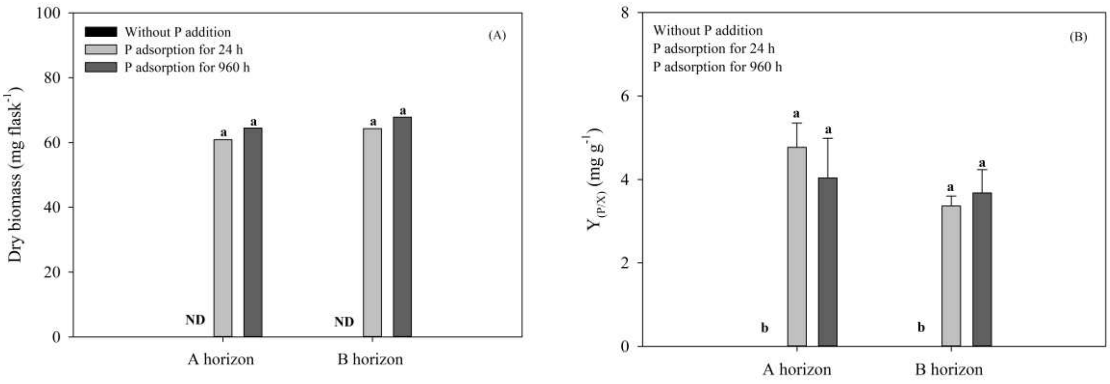

Figure 7. Biomass (A) and yield (B) in NBRIP medium supplemented with 75-µm fractions of the A (A) and B (B) horizons of a Red-Yellow latosol with different P adsorption times, after seven days of incubation of *Aspergilus niger*, at 30 °C and 150 rpm. For the same soil horizon, columns with same small letter are not statistically different by Tukey test (p<0,01).

Santos *et al.* (2017) evaluated the efficiency of citric acid application of disponibilizing P to maize grown in the greenhouse. The authors concluded that among the combinations of citric acid and P doses applied, the best combination was 115 of P mg kg^{-1} and 299 of P mg kg^{-1}, for a Red Neosol and Red Latosol, respectively.

In the literature, some studies point out that *A. niger* is a promising microorganism in the solubilization of low reactivity RPs (ZEROUAL *et al.,* 2012; MENDES *et al*., 2015; MENDES *et al.,* 2014; VASSILEV *et al.,* 2013; VASSILEV *et al.,* 2013a), without immobilizing significant amounts of P. In our work, *A. niger* was able of reversing P adsorption in all treatments tested. This microorganism proved efficient in the removal of P from the soil and was able to grow and develop. As it has been reported in the literature, the main mechanisms of RP solubilization by this fungus correspond to medium acidification and organic acid production, especially oxalic acid (MENDES *et al.,* 2014) The same mechanisms must have worked for reversing P adsorption by this fungus. In our experiment, P desorption by *A. niger* showed negative correlations with medium pH and titratable acidity.

For the soil with no P adsorption, almost no P was detected in the medium and the fungus grew poorly. It was not possible to identify the organic acids produced by *A. niger*. The fungus reduced the levels of titratable acidity in the treatments with P addition to the soil, suggesting, as reported in the literature, that organic acid release by fungi must be a strategy for nutrient acquisition, among other functions (SOUCHIE *et al.,* 2005; BARROSO e NAHAS, 2008; MENDES *et al.,* 2014).

Noteworthy is the fact that both *A. niger* FS1 and organics acids were capable of reverting P adsorption to the soil fractions tested. Our results unveil the potential of soil microorganisms and their metabolites for the development of new strategies for improving P use efficiency in agriculture, especially in the tropics where P fixation in the soil is one of the major constraints of productivity.

Conclusions

Both *A. niger* and oxalic acid are able to reverse P adsorption to soil particles. This opens up new perpsectives for use RF solubilizing fungi and their metabolites to optimize phosphate use efficiency in agriculture.

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SUPPLEMENTARY MATERIAL

Table S1. Regression equations of the solubilized P and percentage of solubilization of Araxá rock phosphate treated with oxalic, citric, and gluconic and their combinations, at the final concentrations of 5 and 10 mmol L^{-1}, and water at pH 2.0 and 7.0, in a stirred-flow system with a continous flow of 1 mL min-1 and 600 rpm, at room temperature.

EXTRACTANT SOLUTION	SOLUBILIZED P (mg)	R²	SOLUBILIZATION (%)	R²
Oxalic acid 5 mmol L^{-1}	$\hat{y} = \frac{(260.56 * 102.49 + 58.51x^{1.26})}{(102.49 + x^{1.26})}$	0.9999	$\hat{y} = \frac{(1.86*102.49+ 41.88x^{1.26})}{(102.49 + x^{1.26})}$	0.9999
Oxalic acid 10 mmol L^{-1}	$\hat{y} = \frac{(311.34 * 110.11 + 64.48x^{1.45})}{(110.11 + x^{1.45})}$	0.9994	$\hat{y} = \frac{(2.21*110.21 + 46.14x^{1.45})}{(110.21 + x^{1.45})}$	0.9994
Citric acid 5 mmol L^{-1}	$\hat{y} = \frac{(4080.22*75.77 + 55.76x^{83.78})}{(75.77 + x^{83.78})}$	0.9999	$\hat{y} = \frac{(29.16*75.77 + 39.91x^{83.78})}{(75.77 + x^{83.78})}$	0.9999
Citric acid 10 mmol L^{-1}	$\hat{y} = \frac{(101.45*53.16 + 57.81^{78.27})}{(53.16 + x^{78.27})}$	0.9986	$\hat{y} = \frac{(61.38*53.16 + 41.27x^{78.27})}{(53.16 + x^{78.27})}$	0.9986
Gluconic acid 5 mmol L^{-1}	$\hat{y} = \frac{(1381.39 + 16288.23x)}{(1 + 3456.15x+ 229090668.02x^{2})}$	0.9997	$\hat{y} = \frac{(872.60*284.41 + 4.52x^{91.49})}{(284.41 + x^{91.49})}$	0.9998
Gluconic acid 10 mmol L^{-1}	$\hat{y} = \frac{(3373.78 + 34711.06x)}{(1 + 3762.78x + 547226964.37x^{2})}$	0.9996	$\hat{y} = \frac{(19.93*278.10 + 11.7x^{85.28})}{(278.10 + x^{85.28})}$	0.9999
Oxalic acid + Citric acid 10 mmol L^{-1}	$\hat{y} = \frac{(743.72 + 184.80x)}{(1 + 5947.60x + 285494.6x^{2})}$	0.9998	$\hat{y} = \frac{(6.33*155.45 + 100.66x^{1.28})}{(155.45 + x^{1.28})}$	0.9990
Oxalic acid + Gluconic acid 10 mmol L^{-1}	$\hat{y} = \frac{(578.12*143.82 + 60.40x^{1.50})}{(143.82 + x^{1.50})}$	0.9983	$\hat{y} = \frac{(4.24*160.33 + 42.8x^{1.54})}{(160.33 + x^{1.54})}$	0.9987
Citric acid + Gluconic acid 10 mmol L^{-1}	$\hat{y} = \frac{(103.15*52.32 + 49.49x^{68.38})}{(52.32 + x^{68.38})}$	0.9972	$\hat{y} = \frac{(87.24 + 37.9x)}{(1 + 307.65x + -644302.22x^{2})}$	0.9994
Water pH 2.0	$\hat{y} = \frac{(8638.87 + 112.66x)}{(1 + 185.01x + 9672163.16x^{2})}$	0.9986	$\hat{y} = \frac{(99.37*66.75-+ 47.71x^{1.01})}{(66.75-+ x^{1.01})}$	0.9992
Water pH 7.0	$\hat{y} = \frac{(48297.17 + 32400.43x)}{(1 + 13.22x + 1716218.55x^{2})}$	0.9836	$\hat{y} = \frac{(346.64 + 232.59x)}{(1 + 13.04x + 469513.55x^{2})}$	0.9927

Table S2. Regression equations desorbed P and desorption percentage from the A horizon of a Red-Yellow Latasol, from Viçosa, Minas Gerais, Brazil, in a stirred-flow system using organic acids (oxalic acid and oxalic acid + citric acid) at 10 mmol L-1 and water at pH 2.0 and 7.0.

EXTRACTANT SOLUTION	P DESORBED (mg)	R²	DESORPTION (%)	R²
Without P adition				
Water pH 2.0	$\hat{y} = \dfrac{(0.0044)}{(1 + e^{1.66-0.024*x})^{1/1.017}}$	0.9924	$\hat{y} = \dfrac{(0.195)}{(1 + e*(-3.85 - 0.0186x)^{1/0.0063}}$	0.9922
Water pH 7.0	$\hat{y} = (0.000035*1.0055x)x0.41$	0.9978	$\hat{y} = 0.018 *1.006x * x0.398$	0.9977
Oxalic acid 10 mmol L^{-1}	$\hat{y} = \dfrac{(0.00060 + .000072x)}{(1 + 0.0046x - 0.000015x^2)}$	0.9999	$\hat{y} = \dfrac{(0.0301 + 0.0036x)}{(1 + 0.0046x -0.000015x^2)}$	0.9999
Oxalic acid + Citric acid 10 mmol L^{-1}	$\hat{y} = \dfrac{(0.0086 - 4.185*x)}{(1 + 0.017x + 0.0048x^2)}$	0.9988	$\hat{y} = \dfrac{(0.428)}{(1 + e*(-4.185 - 0.0174x)^{1/0.0048}}$	0.9988
Adsorption P for 24 h				
Water pH 2.0	$\hat{y} = \dfrac{(0.0012*258.40 +0.194x^{1.27})}{(258.40 + x^{1.27})}$	0.9999	$\hat{y} = \dfrac{(0.0373 + 0.0017x)}{(1 + 0.0090x - 0.00003x^2)}$	0.9990
Water pH 7.0	$\hat{y} = \dfrac{(-0.00069 + 0.0022x)}{(1 + 0.0128x + -0.000018x^2)}$	0.9990	$\hat{y} = 0.0436 - 0.0337*0.798x + 0.0014x$	0.9996
Oxalic acid 10 mmol L^{-1}	$\hat{y} = (0.245 - 0.259*0.96x +0.00065x)$	0.9972	$\hat{y} = \dfrac{(0.0030x + 0.000165)}{(0.0022x + 0.0769)}$	0.9922
Oxalic acid + Citric acid 10 mmol L^{-1}	$\hat{y} = \dfrac{(0.0048*386.42 + 0.45x^{1.87})}{(386.42 + x^{1.87})}$	0.9934	$\hat{y} = \dfrac{(0.0098 + 4.38x^{0.97})}{1886.46^{0.97}}$	0.9999
Adsorption P for 960 h				
Water pH 2.0	$\hat{y} = (0.107 -0.106*e(-0.0051x^{0.99})$	0.9999	$\hat{y} = \dfrac{(0.0951*258.40 + 15.32x^{1.27})}{(258.40 + x^{1.27})}$	0.9999
Water pH 7.0	$\hat{y} = \dfrac{(x)}{(6.02 + 0.07x + -0.000098x^2)}$	0.9998	$\hat{y} = \dfrac{x}{(6.020 + 0.073x -0.000098x^2)}$	0.9990
Oxali acid 10 mmol L^{-1}	$\hat{y} = (0.128 - 0.130*0.954x + 0.00061x)$	0.9977	$\hat{y} = 19.80 - 20.48*0.959x + 0.053x$	0.9980
Oxalic acid + Citric acid 10 mmol L^{-1}	$\hat{y} = \dfrac{(0.0100*206.76 + 0.448x^{1.51})}{(206.76 + x^{1.51})}$	0.9980	$\hat{y} = \dfrac{(0.360*386.63\ 35.57*x^{1.87})}{(386.625 + x^{1.87})}$	0.9940

Table S3. Regression equations desorbed P and desorption percentage from the B horizon of a Red-Yellow Latasol, from Viçosa, Minas Gerais, Brazil, in a stirred-flow system using organic acids (oxalic acid and oxalic acid + citric acid) at 10 mmol L-1 and water at pH 2.0 and 7.0.

EXTRACTANT SOLUTION	P DESORBED (mg)	R²	DESORPTION (%)	R²
Without P adition				
Water pH 2.0	$\hat{y} = \frac{(0.00075 -+ 0.000035x)}{(1 + 0.0090* - 0.00003*^{2})}$	0.9990	$\hat{y} = \frac{(11.74x)}{(131.15 + x)}$	0.9993
Water pH 7.0	$\hat{y} = (0.00087 - 0.000675*0.798x + 0.000029x)$	0.9996	$\hat{y} = x(4.29 + 0.0785x(-0.775))(-1/0.77)$	0.9996
Oxalic acid 10 mmol L^{-1}	$\hat{y} = \frac{(0.000263 + 0.0113x^{1.59})}{(96.27^{1.59} + x^{1.59})}$	0.9915	$\hat{y} = (12.12 - 12.18*0.960x + 0.0466x)$	0.9990
Oxalic acid + Citric acid 10 mmol L^{-1}	$\hat{y} = \frac{(0.00020 + 0.0876^{0.97})}{(1886.46^{0.97} + x^{0.97})}$	0.9999	$\hat{y} = \frac{(0.196*70.52 + 24.94x^{1.22})}{(70.52 + x^{1.22})}$	0.9970
Adsorption P for 24 h				
Water pH 2.0	$\hat{y} = \frac{(0.149x)}{(131.15 + x)}$	0.9993	$\hat{y} = (8.26 - 8.20*e(-0.0050x^{0.999})$	0.9999
Water pH 7.0	$\hat{y} = \frac{(x)}{(526.93 + 9.276x^{0.85})}$	0.9996	$\hat{y} = \frac{(0.0095 + 0.073x)}{(1 + 0.0141x - 0.00002x^{2})}$	0.9999
Oxalic acid 10 mmol L^{-1}	$\hat{y} = (0.149 - 0.154*0.962x\ 0.00057x)$	0.9982	$\hat{y} = (10.084 - 10.26*0.954x + 0.0479x)$	0.9977
Oxalic acid + Citric acid 10 mmol L^{-1}	$\hat{y} = \frac{(0.0069*70.52 + 0.321x^{1.22})}{(70.52 + x^{1.22})}$	0.9970	$\hat{y} = \frac{(0.790*206.76 + 35.32x^{1.51})}{(206.76 + x^{1.51})}$	0.9980
Adsorption P for 960 h				
Water pH 2.0	$\hat{y} = \frac{(0.0015 + 0,14x^{1,03})}{(273.45^{1,03} + x^{1,03})}$	0.9999	$\hat{y} = (7.03 - 6.93*e(-0.0050x^{1.02})$	0.9999
Water pH 7.0	$\hat{y} = \frac{(x)}{(1160.66 + 27.74x^{0.81})}$	0.9997	$\hat{y} = \frac{(x)}{(14.71 + 0.35x^{0.81})}$	0.9997
Oxali acid 10 mmol L^{-1}	$\hat{y} = \frac{(-0.0033*90.0096 + 0.253x^{1.14})}{(90.0096 + x^{1.14})}$	0.9958	$\hat{y} = \frac{(-0.318*90.01 + 19.88*x^{1.14})}{(90.01 + x^{1.14})}$	0.9958
Oxalic aci + Citric acid 10 mmol L^{-1}	$\hat{y} = (0.153 - 0.159*0.973x + 0.00057x)$	0.9990	$\hat{y} = 12.05 - 12.52*0.97x + 0.045x$	0.9990