Identification of organic acids produced during rice straw decomposition and their role in rock phosphate solubilization

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ABSTRACT

The production of organic acids and changes during decomposition of rice straw amended with tricalcium phosphate (TCP) and Udaipur rock phosphate (URP) were studied under laboratory conditions. The organic C content of rice straw decreased and total N increased with time, resulting in a decrease in C:N ratio as the decomposition progressed. The pH decreased to acidic range in all the treatments on day 15, but became alkaline again later on. Soluble P increased at 15 days after incubation, declined later during decomposition, and was highest in the treatments containing TCP and *Aspergillus awamori* inoculation. Citric, oxalic, formic and maleic acids were detected during decomposition of rice straw and maximum amounts were present on day 15. Citric and oxalic acids were responsible mainly for P solubilization from TCP and URP; generally, citric acid was the most effective in P solubilization.

Keywords: organic acids; phosphate solubilization; rice straw decomposition; tricalcium phosphate; Udaipur rock phosphate

Proper disposal of crop residues to conserve plant nutrients is essential to maintain long-term soil productivity. Immobilization of N on direct incorporation of rice straw in soil is caused due to its wide C:N ratio and therefore, composting is a preferred method of straw disposal (Banger et al. 1989, Goyal et al. 2005). In order to hasten decomposition the initial C:N ratio of the straw can be narrowed down by addition of fertilizer nitrogen or nitrogen-rich organic wastes such as poultry manure or sewage sludge low in heavy metals and hazardous organic compounds (Ranalli et al. 2001, Iranzo et al. 2004). Enrichment of compost raw material with rock phosphate before composting to increase the P content is a viable option to meet P requirement of crops (Banger et al. 1989, Manna and Ganguli 1998, Biswas and Narayanasamy 2002). Organic acids and humic substances produced during decomposition are mainly involved in the P solubilization process (Kapoor et al. 1989, Singh and Amberger 1990). However, there is scanty information on types of organic acids produced during decomposition of organic wastes and their role in P solubilization. Therefore, the study was conducted to identify the organic acids produced during decomposition of rice straw and to elucidate the role of organic acids in solubilization of insoluble phosphates.

MATERIAL AND METHODS

Rice straw. Rice straw procured from a farm field near Hisar, India was dried in oven at 80°C and ground to pass through 2 mm sieve. It contained 47.2% organic C, 0.94% total N and 0.076% total P.

Incubation experiment. Five g of rice straw was taken in 150 ml Erlenmeyer flasks and C:N ratio was adjusted to 40:1 by addition of urea. Various treatments with three replicates each were applied as shown in Table 1. Rice straw was amended with tricalcium phosphate (TCP) and Udaipur rock phosphate (URP) at the rate of 10% of rice straw on dry weight basis. The URP, which is of metamorphic-cum-sedimentary origin, was ob-

tained from Rajasthan State Mines and Minerals Corporation, Udaipur, India. It is fluorapatite containing about 69% apatite and 30% total P_2O_5. One ml of compost extract, as a source of inoculum, was inoculated in each flask. Compost extract was prepared according to Pedro et al. (2003) with some modifications. One hundred g of compost was mixed in 500-ml distilled water, shaken for 15 min on a rotary shaker at 140 g and filtered through Whatman No.1 filter paper. Inoculation with a phosphate solubilizing fungus, *Aspergillus awamori,* was carried out. One ml of spore suspension from 96 h grown mother culture containing about 10^8 spore/ml was inoculated in each flask. Moisture was adjusted to 60% of water holding capacity and flasks were incubated at 30 ± 2°C.

Sampling and analysis. The organic acids were extracted from decomposing rice straw by distilled water as described by Brinton (1998) with some modifications. The flasks were removed at 0, 15, 30 and 60 days interval, and 25 ml of distilled water was added to each flask, shaken for 30 min on a rotary shaker at 140 g, and filtered through Whatman No.1 filter paper. The pH in the distilled water extract of decomposing rice straw was measured by glass electrode (Anderson and Ingram 1993) and soluble P by the method of John (1970). The residue was dried in an oven at 80°C to constant weight and analyzed for organic C by dry combustion (Nelson and Sommers 1982) and total N by Kjeldhal method (Bremner 1982).

Organic acids in the aqueous extract of decomposing rice straw were measured by HPLC.

Isocratic HPLC (Waters) with software Millennium 32 equipped with a Photo Diode Array detector was used. A column of dimension 4.6 × 250 mm, X-Terra C-18, 5 μ, was operated at ambient temperature. Mobile phase consisted of 2.2 g of KH_2PO_4 per liter in HPLC grade water at pH 4.5 (adjusted with KOH). The samples were filtered through 0.2-micron filter and run for 10 min. The flow rate was 0.9 ml/min with injection volume of 20 μl. Different organic acids were identified by their retention time, corresponding with their standards.

P solubilization by organic acids. Fifty ml of 1 mmol solutions of citric, oxalic, maleic, formic and sulphuric acids were taken in 150 ml Erlenmeyer flasks. One hundred mg of TCP or URP were added in each flask and were shaken for 30 min on rotary shaker at 140 g. The contents were filtered through Whatman No.1 filter paper. The pH and soluble P in the filtrate were determined as described earlier.

RESULTS AND DISCUSSION

Decomposition of rice straw. The initial C:N ratio of straw varied from 39.9 to 41.2 in various treatments; it declined with time as the decomposition progressed (Table 1). The C:N ratio in various treatments varied from 20.0 to 25.5 at 60 days, and was narrower in treatments with TCP or URP.

Changes in pH and soluble P. The addition of TCP or URP increased the initial pH to 8.6 and 8.8 respectively (Table 2). At sampling on day 15,

Table 1. Changes in C:N ratio during decomposition of rice straw at different days of incubation

Treatments	C:N ratio at day			
	0	15	30	60
Control (RS)	40.3	33.0	29.5	25.5
RS + AW	40.2	35.0	28.5	23.6
RS + compost extract	40.2	35.4	28.8	25.2
RS + TCP	39.9	34.1	27.2	21.6
RS + TCP + AW	40.0	34.0	26.0	21.6
RS + TCP + compost extract	40.2	30.4	25.7	22.1
RS + URP	41.2	28.4	23.1	20.0
RS + URP + AW	41.0	30.5	24.1	20.2
RS + URP + compost extract	40.9	29.0	24.5	20.2
LSD *P* = 0.05	NS	1.5	1.3	1.2

RS – rice straw; AW – *Aspergillus awamori*; TCP – tricalcium phosphate; URP – Udaipur rock phosphate

Table 2. Changes in pH during decomposition of rice straw at different days of incubation

Treatments	pH at day			
	0	15	30	60
Control (RS)	7.3	5.9	8.1	8.3
RS + AW	7.2	5.6	7.9	8.1
RS + compost extract	7.2	5.7	8.4	8.6
RS + TCP	8.6	6.4	8.3	8.7
RS + TCP + AW	8.5	5.4	7.8	8.4
RS + TCP + compost extract	8.6	6.2	8.4	8.7
RS + URP	8.8	6.3	8.0	8.5
RS + URP + AW	8.6	6.2	8.1	8.4
RS + URP + compost extract	8.7	6.3	8.3	8.4
LSD P = 0.05	0.3	0.4	0.4	0.3

RS – rice straw; AW – *Aspergillus awamori*; TCP – tricalcium phosphate; URP – Udaipur rock phosphate

the pH was found to decline and was in acidic range (5.4 to 6.3). Inoculation of rice straw with *Aspergillus awamori* along with TCP showed maximum decline in pH. An increase of pH in all the treatments was observed later on. At sampling on day 30, pH increased to 7.8 to 8.4; at 60 days, pH in various treatments ranged from 8.1 to 8.7.

An increase in soluble P with time up to 60 days was observed in treatments without TCP and URP (Table 3). Addition of TCP or URP increased soluble P at 15 days, however, it declined subsequently. The RS + TCP + AW treatment showed a maximum content of soluble P. The soluble P

was significantly higher in TCP treatments compared to URP.

Organic acids. The HPLC analysis of water extract of the decomposing rice straw at different treatments in various intervals revealed the production of organic acids (Table 4). None of the organic acids were detected initially in the rice straw. The organic acids detected during decomposition of rice straw at various sampling times were citric, oxalic, formic and maleic acid. Results of all the treatments indicate that citric and oxalic acids were two major acids produced. Concentration of organic acids detected at 15 days of decomposition

Table 3. Changes in soluble P during decomposition of rice straw at different days of incubation

Treatments	Soluble P (µg/g straw) at day			
	0	15	30	60
Control (RS)	7.7	12.4	16.1	17.6
RS + AW	7.9	14.1	17.1	18.2
RS + compost extract	7.8	14.3	14.8	17.2
RS + TCP	8.1	53.6	48.2	45.3
RS + TCP + AW	8.1	56.2	51.7	46.8
RS + TCP + compost extract	8.4	55.2	44.8	41.2
RS + URP	8.8	16.8	15.8	14.6
RS + URP + AW	9.0	19.8	15.8	14.8
RS + URP + compost extract	9.1	18.6	15.2	14.2
LSD P = 0.05	0.5	0.3	0.2	0.4

RS – rice straw; AW – *Aspergillus awamori*; TCP – tricalcium phosphate; URP – Udaipur rock phosphate

Table 4. Organic acids produced during decomposition of rice straw at different days of incubation

Treatments		Organic acids (μmol/g straw) at days		
		15	30	60
Control (RS)	Citric	0.03	0.00	0.00
	Oxalic	0.01	0.00	0.00
RS + AW	Oxalic	0.13	0.06	0.04
	Citric	0.16	0.09	0.06
RS + compost extract	Citric	0.09	0.03	0.00
	Oxalic	0.02	0.01	0.00
RS + TCP	Maleic	0.16	0.09	0.03
RS + TCP + AW	Oxalic	0.46	0.16	0.09
	Citric	0.84	0.48	0.14
RS + TCP + compost extract	Oxalic	0.09	0.07	0.01
	Citric	0.11	0.08	0.06
	Formic	0.24	0.10	0.00
	Maleic	0.01	0.01	0.00
RS + URP	Citric	0.12	0.09	0.03
RS + URP + AW	Oxalic	0.26	0.14	0.06
	Citric	0.24	0.19	0.09
RS + URP + compost extract	Oxalic	0.08	0.01	0.00
	Citric	0.06	0.00	0.00

RS – rice straw; AW – *Aspergillus awamori*; TCP – tricalcium phosphate; URP – Udaipur rock phosphate

was higher compared to other time intervals. The maximum amount of citric (0.84 μmol/g straw) and oxalic acid (0.46 μmol/g straw) were produced in the RS + TCP + AW treatment (Table 4).

P solubilization by organic acids. The P solubilizing efficiency from TCP and URP by organic acids (citric, oxalic, formic and maleic acid) detected during decomposition of rice straw was compared with H_2SO_4 (Table 5). The initial pH of different acids varied from 2.8 to 3.1, which increased with addition of TCP and URP. After reaction of TCP and URP with 1 mmol H_2SO_4 pH increased to 5.4 and 6.5, respectively. The mineral acid (sulphuric acid) released 10.8 μg P/ml from TCP and 1.5 μg P/ml from URP. Citric acid showed maximum P solubilizing efficiency of 54.1 and 6.5 μg P/ml with TCP and URP, respectively, followed by oxalic, maleic and formic acids. The P solubilizing efficiency of different organic acids was higher with TCP as compared to URP.

The C:N ratio of rice straw decreased with incubation time in all the treatments. The decline in C:N ratio with time is one of the reliable indices of decomposition of organic wastes (Ranalli et al. 2001, Goyal et al. 2005). Decline in pH at 15 days was caused by the production of organic acids during rice straw decomposition, which increased later due to utilization of organic acids by other microorganisms. Moreover, production of NH_3 during transformation of organic nitrogenous compounds also tends to neutralize organic acids (Atkinson et al. 1996). However, the changes in the quantity of organic acids were not commensurate with the changes in pH. This therefore suggests that there might be other factors involved in the regulation of pH, such as NH_4^+, NO_3^-, fulvic and humic acids, which are also known to influence the pH (Simandi et al. 2005).

The TCP- and URP-amended treatments showed significantly higher soluble P at 15 days compared to control, which may be attributed to greater mobilization of P from added P sources. The TCP resulted in significantly higher soluble P compared to URP in all the treatments. The TCP being amorphous in nature with simple structure is solubilized easily, whereas URP is a crystalline mineral con-

Table 5. pH changes and water soluble P during solubilization of insoluble phosphates by different organic acids

Treatments	Initial pH	Final pH		Soluble P (µg/ml)	
		TCP	URP	TCP	URP
Sulphuric acid	2.8	5.4	6.5	10.8	1.5
Citric acid	3.0	4.7	5.8	54.1	6.5
Oxalic acid	2.9	5.1	6.4	33.2	4.0
Formic acid	3.1	5.0	6.8	11.3	3.1
Maleic acid	2.9	5.0	6.0	9.0	2.8
LSD $P = 0.05$	0.1	0.2	0.2	1.2	0.4

TCP – tricalcium phosphate; URP – Udaipur rock phosphate

taining apatite and is solubilized slowly. Soluble P declined subsequently after 15 days due to reaction of soluble P with other components of TCP and URP such as soluble Ca and Fe. Inoculation with *A. awamori* resulted in a higher content of soluble P, as this fungus is known to solubilize P by production of organic acids (Biswas and Narayanasamy 2002). The present study revealed the production of citric, oxalic, formic and maleic acids during decomposition of rice straw. Rashid et al. (2004) also reported production of citric and oxalic acids as major organic acids during P solubilization in the pure culture of *Aspergillus flavus, Aspergillus niger* and *Penicillium canescens.*

The solubilization efficiency of different organic acids was compared taking inorganic acid sulphuric acid as control. The pH of the acid solution increased with addition of P sources (TCP or URP). A higher increase in pH was observed at reaction with URP compared to TCP. It was due to the presence of free carbonates in rock phosphate that reduced the extent of solubilization; these results confirm the previously observed phenomenon that a large part of organic acids is diverted towards neutralization of free carbonates (Narayanasamy and Biswas 1998). Moreover, the URP has a crystalline structure containing apatite with low reactivity with acids. The nature of organic acids produced has a considerable effect on the solubilization of insoluble phosphates. Citric acid showed maximum P solubilizing efficiency followed by oxalic, maleic and formic acids. The position and type of functional group within each acid seems to be a dominant factor that influences the amount of released P (Kpomblekou and Tabatabai 1994). Less P was released by sulphuric acid compared to organic acids. Kim et al. (1997) reported that HCl was able to solubilize less P

from hydyoxyapatite than citric and oxalic acids at same pH. The ability of citric and oxalic acids to release P was greater than that of mineral acids, due to chelating of calcium ions associated with P in rock phosphate.

The availability of P from TCP and URP increased during decomposition of rice straw and treatments with *A. awamori* resulted in a higher level of soluble P as compared to the control without *A. awamori,* which increased the mobilization of P present in rock phosphate. Thus, it can be concluded that organic acids produced during rice straw decomposition by microorganisms play an important role in rock phosphate solubilization.

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