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ENHANCEMENT OF PHOSPHATE ROCK SOLUBILITY THROUGH BIOLOGICAL PROCESSES

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Summary—Biological acidulation of a reactive phosphate rock (PR), Sechura, was investigated by exposing ground PR to various combinations of elemental sulphur, soil suspension enriched with Thiobacilli, nutrient solution and lactic casein whey. The incubation was carried out at 30°C for 4 weeks. Weekly measurements were made of the pH of the mixture, phosphorus extractable with water (water-P) and 0.5 M $NaHCO_3$ (bicarb-P) solution, biomass-P, residual elemental sulphur and the Thiobacillus population.

Treatments without elemental S or Thiobacilli culture had little effect on the dissolution of PR. The combination of elemental S and Thiobacilli increased extractable P to nine times that of the untreated PR and with the addition of nutrient solution this was enhanced to 15 times. This was equivalent to 9–10% of stoichiometric acidulation of the PR with sulphuric acid. However, the amount of acid produced, calculated from the sulphur oxidised, was equivalent to that of 14% acidulation.

Use of lactic whey with PR alone or in combination with elemental S showed either an immobilisation of soluble P or very little increase in water-P compared with control values. Amounts of P dissolved during incubation which might have been immobilised were determined by the fumigation–incubation technique for recovering P in the microbial biomass. This was found to be a very small fraction of the total P dissolved during the incubation.

INTRODUCTION

Single superphosphate (SSP) has been the traditional phosphate fertiliser used on New Zealand pastures. Economic pressure on farmers has necessitated the search for more cost-effective P fertilisers. In pastoral soils with a pH of < 6, reactive phosphate rocks (PR) have in some situations been recommended. However, depending on soil and climatic conditions, it could take up to 4 yr of annual application before PR treatments become as effective as superphosphate (Sinclair and Dyson, 1988). One method of reducing this long lag phase is to increase the solubility of PR by treating it with small amounts of sulphuric or phosphoric acids. Such products which are commonly known as partially acidulated phosphate rocks (PAPR), have shown no lag effect and have given high agronomic effectiveness (Rajan, 1986; Hammond *et al.*, 1989; Mackay and Wewala, 1990; Rajan and Watkinson, 1993). Partial acidulation can be effected by industrial processing but this approach is capital intensive. It can also be effected in the field by applying PR after co-granulating with elemental S (Rajan, 1987). When an intimate mixture of PR and elemental S is applied to soil, the inoculated or resident population of chemolithotrophic bacteria viz., Thiobacilli oxidise elemental S to H_2SO_4 which reacts with the PR to form mono and dicalcium phosphates (MCP, DCP) as shown schematically below:

$$2S^\circ + 2H_2O + 3O_2 \xrightarrow{\text{Thiobacilli}} 2H_2SO_4$$

$$H_2SO_4 + PR \longrightarrow MCP + DCP + \text{residual PR.}$$

In this process there is some loss of the acid produced by its reaction with soil, and the cogranulation also adds to the cost of the product. Yet another possibility is *in situ* partial acidulation of PR or PR mixed with elemental S (PR,S) by biological processes, in a soil-free medium. But no such investigation appears to have been conducted.

Our objective was to increase the solubility of PR by treating the PR with different combinations of elemental S, soil culture containing Thiobacilli bacteria and lactic casein whey.

Whey is a by-product of the dairy industry which has a low pH (pH 4.5) and contains many plant nutrients (Bradford *et al.*, 1986). It is speculated that whey, when added with PR may increase the dissolution of PR, but no published evidence exists. In our work, lactic casein whey was mixed with PR,S to investigate if it increased the rate of dissolution of PR because of low pH and also by stimulating microbial activity (Rittenberg, 1969).

MATERIALS AND METHODS

Sechura phosphate rock and elemental sulphur

Ground (< 150 μm) samples of Sechura PR, a reactive PR which is classified as fluorohydroxycarbonate type apatite (Cheney *et al.*, 1979), was used in this study. The samples contained 13.0% total P and 2% sulphate-S. About 43 and 70% of the total P was extractable in 2% citric acid and 2% formic acid solutions respectively. Elemental S used was of commercial grade and the particle size was < 75 μm.

Lactic casein whey

Whey was collected from a nearby dairy company which produces lactose and cheese. The bacterial population in the whey was determined by most probable number technique. The Streptococci were the most dominant group (6.5×10^6 ml^{-1}). The lactic casein whey contained 5.6% total solids including lactose 3.5% and N 0.14%. As reported by Bradford *et al.* (1986) lactic whey generally contains small concentrations of other nutrients such as P 0.64, K 1.50, S 0.14, Ca 1.25 and Na 0.43 g kg^{-1} of whey. It is assumed that whey used in this study would have also contained these nutrients. The pH of the whey was 4.5.

Soil microbial culture

Field moist soil (0–10 cm) was collected from a paddock which had received elemental S for the past 4 yr. Soil samples were treated with 100 μg g^{-1} soil of fine elemental S and kept at 30°C for 2 weeks. Incubated soil was sub-sampled and shaken with distilled water (1:10, soil:water) for 30 min on an end-over-end shaker. The suspension was filtered using Whatman No. 1 filter paper and the filtrate, containing Thiobacilli was used as inoculum. The pH of this microbial culture solution was 6.0 and the Thiobacilli count was about 4.2×10^3 ml^{-1}.

Nutrient solution

A sterilised nutrient solution containing small amounts of N, K, Na and Cl was specifically prepared to sustain higher activity of Thiobacilli once they were introduced to the mixture of PR and S. The solution had a pH of 6.1, and was stored at 4°C in darkness until required. The Thiobacilli also require P, Ca and very small amounts of trace elements which would have been supplied by the PR and thus trace elements were not included in the nutrient solution.

Experimental procedure

A factorial experiment was set up in which Sechura PR, and PR,S were exposed to different combinations of the following additives: elemental sulphur (S), soil microbial culture (Ct), nutrient solution (Nt) and lactic casein whey (W) (Table 1). Samples (2 g) of Sechura PR were placed into 30 ml glass vials. For treatments with S we mixed the PR samples with 390 mg of elemental S. The PR:S ratio used was similar to that required for making SSP.

The original nutrient solution, whey or microbial culture were diluted to 6-fold, where 5 ml lots of the original solutions either alone or in combination as stated in Table 1, were mixed and the final volume was made up to 30 ml with distilled water. Appropriate amounts of these diluted solutions were added to either PR alone (treatment 1–8) or PR,S (treatment 9–16) to bring the moisture content of the mixture to 21%. All the vials were then kept at 30°C in darkness for 4 weeks. A glass rod was placed in each vial for mixing the PR and additives during the incubation. Glass vials were closed with plastic caps to ensure the retention of moisture during the incubation. These caps were left loose for 30 min at 3–4 day intervals to maintain an adequate supply of O_2. Moisture contents of the incubation mixtures were maintained on a weight basis. Fifty vials were incubated for each treatment. Every week 12 vials were removed from each treatment and samples from lots of two vials were analysed for pH, water-soluble P, bicarbonate P, formic P, elemental S, and microbial biomass-P.

Extraction procedures and analytical techniques

To avoid any sub-sampling errors in measuring the extractable P the glass vials were uncapped and placed into extraction bottles of 250 ml capacity. A

Table 1. Description of the treatment combinations

Treatment No.	Abbreviations	Additives
1	PR	Control (PR + distilled water)
2	PR,Ct	Microbial culture
3	PR,W	Whey
4	PR,W,Ct	Whey and microbial culture
5	PR,Nt,Ct	Nutrient solution and microbial culture
6	PR,Nt,W,Ct	Nutrient solution, whey and microbial culture
7	PR,Nt,W	Nutrient solution and whey
8	PR,Nt	Nutrient solution
9	PR,S	Distilled water
10	PR,S, Ct	Microbial culture
11	PR,S, W	Whey
12	PR,S,W,Ct	Whey and microbial culture
13	PR,S,Nt,Ct	Nutrient solution and microbial culture
14	PR,S,Nt,W,Ct	Nutrient solution, whey and microbial culture
15	PR,S,Nt,W	Nutrient solution and whey
16	PR,S,Nt	Nutrient solution

PR = phosphate rock; S = elemental sulphur; W = whey; Nt = nutrient solution; Ct = microbial culture.

volume of 200 ml of the extractants viz. distilled water or 0.5 M $NaHCO_3$ or 2% formic acid solution were added into the extraction bottles. The contents were shaken on a rotary shaker set at 40 rev min^{-1}, for 30 min at 20°C. After the extraction, the suspension was filtered using Whatman No. 42 filter paper. Both water soluble (water-P) and 0.5 M $NaHCO_3$ extractable P (bicarb-P) were measured by the molybdenum blue method (Murphy and Riley, 1962). Formic acid extractable P (formic-P) was measured by the vanadate method at 420 nm.

The pH of the incubated material was measured after mixing with distilled water (1:2.5, solid:water) and left for 15 min with intermittent stirring before readings were taken.

Elemental S analysis

Amounts of elemental S remaining in the PR,S treatments at different sampling times were determined (Watkinson *et al.*, 1987). Elemental S was extracted with 100 ml of $CHCl_3$ for 4 h on a rotary shaker. Extracts were filtered using glass filter paper and diluted 100-fold with $CHCl_3$ before using for analysis. A 10 μl aliquot was injected into the HPLC system, using a mobile phase of chloroform:methanol (50:50) at 1 ml min^{-1}. The area of the S peak was measured and values were calculated from the standards. The absorbance response was linear against S concentration (0–100 μg S) at 260 nm wavelength.

Microbial biomass-P

Microbial biomass-P in the incubated samples was determined following a modification of the biocidal fumigation–incubation technique (Jenkinson and Powlson, 1976). Chloroform-fumigated and non-fumigated samples were extracted with 0.5 M $NaHCO_3$ (1:100) for 30 min. Values of P obtained for non-fumigated samples were subtracted from the fumigated values and the biomass-P was calculated assuming a k_p of 0.35 (Brookes *et al.*, 1982).

Thiobacillus counts

The Thiobacillus populations in the incubation mixtures were determined by a most probable number procedure (Lee *et al.*, 1988). The incubation mixtures were serially diluted (10-fold), and 1 ml sub-samples from each dilution were added to a set of four tubes containing a thiosulphate medium (Postgate, 1966). Tubes were kept for 6 weeks at ambient temperature.

RESULTS AND DISCUSSION

Effects on pH

Treatments without elemental S (treatments 1–8) showed no significant change in pH during the 4 weeks of incubation [Fig. 1(a), (b)]. The values remained between 6.5–7.2.

Addition of elemental S alone (PR,S) caused no decrease in pH values [Fig. 1(c)] indicating no oxidation of S to sulphuric acid. This also indicated that there was no contamination by S-oxidising microorganisms in the distilled water added to this treatment. When the microbial culture was added to the mixture of PR and S (PR, S, Ct), the pH of the mixture decreased during the incubation [Fig. 1(c)]. The decrease was slow during the first 2 weeks but in the weeks 3 and 4 it increased, indicating an early lag period during which microbial growth and oxidation of S was slow. These results are comparable with those reported by Rajan and Edge (1980) and Pathiratna *et al.* (1989) who used a North Carolina and a Sri Lankan phosphate rock, respectively. However, addition of nutrient solution to the above mixture (PR, S, Nt, Ct) caused the greatest decrease in pH values [Fig 1(d)]. Within 2 weeks of incubation, pH values in this treatment decreased from 6.7 to 4.9 and after 4 weeks the pH was 4.5. It appears that in the presence of nutrients, microorganisms were able to oxidise the added elemental S much sooner. Addition of whey to this mixture (treatment 14), indicated by a high pH value in this treatment (lowest pH 5.7), apparently retarded the process of S oxidation.

The pH values of the incubation mixtures were determined to investigate the effectiveness of the incubation procedure. A decrease in the pH is a rough measure of the number of non-neutralised H^+ ions in the solution. To measure the effectiveness of biological acidulation of PR we determined the soluble-P in the incubated mixture.

Water extractable P

Water-extractable P measured in control samples during the incubation ranged from 60 to 72 $\mu g\, g^{-1}$ PR [Fig 2(a)]. Inoculation with the microbial culture significantly increased the dissolution of Sechura PR [Fig 2(a)]. Addition of whey alone or with microbial culture (treatments 3 and 4) reduced the water-P in the first week. This was probably caused by some microbial immobilisation of water-P at the initial stages of incubation. This effect was more pronounced where the nutrient solution was also added [Fig. 2(b)]. About 30 $\mu g\, g^{-1}$ PR was immobilised in this treatment during the first week of incubation. Towards the end of the fourth week the amount of water-P in all these treatments increased to 80–86 $\mu g\, g^{-1}$ PR, indicating either the release of the immobilised P or increase in P dissolution caused by microbes.

The amount of water-P in the PR,S mixture remained unchanged throughout the incubation, confirming the absence of acidulation of PR as suggested by a lack of decrease in the pH. Inoculation with microbial culture in the PR,S treatment [Fig. 2(c)] caused a marked increase in PR dissolution. Consistent with the trend in the pH values, the amount of P dissolved in the first 2 weeks was low but increased substantially in the third week to 621 $\mu g\, g^{-1}$ PR

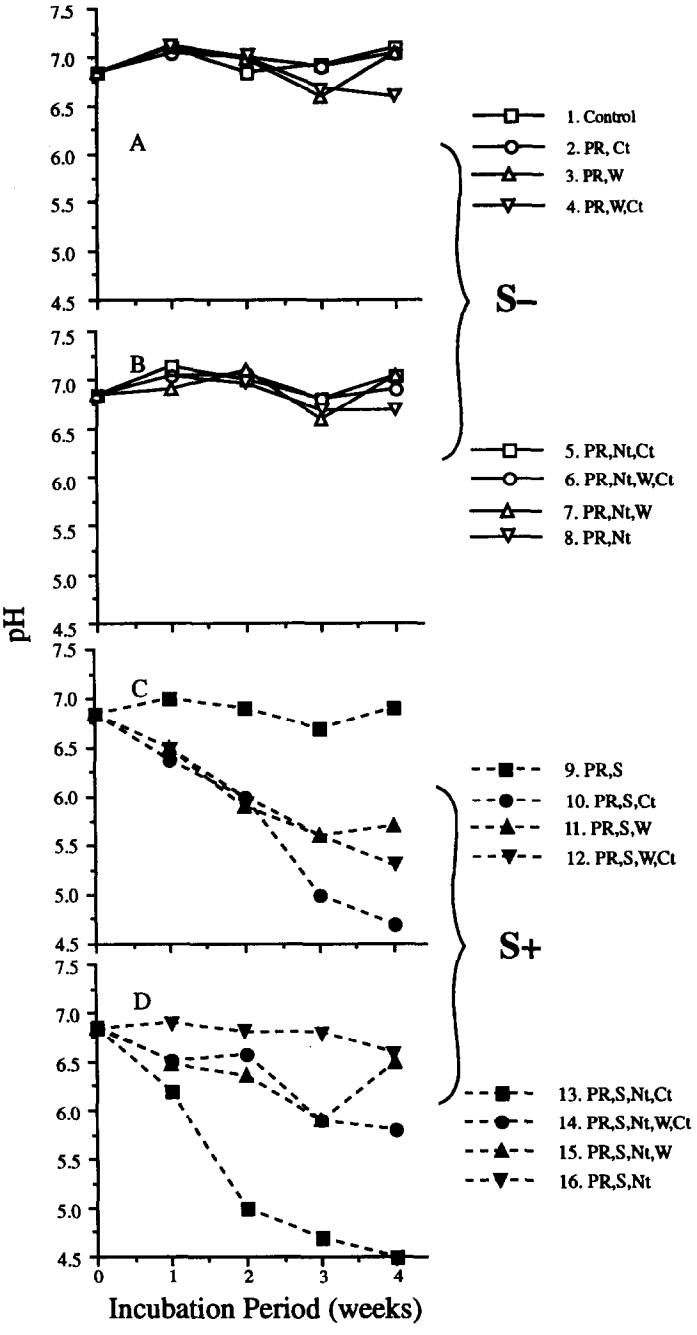

Fig. 1. Changes in the pH values of samples with incubation time. $LSD_{0.05}$ between the treatments = 0.36 (PR = phosphate rock; Ct = microbial culture; S = elemental sulphur; Nt = nutrient solution).

dissolved. The addition of nutrient solution to the PR,S,Ct treatment [Fig. 2(d)] resulted in even higher amounts of P dissolution from the PR. The highest rate of dissolution was again achieved in the third week of incubation suggesting that in this type of incubation there is a lag phase of nearly 2 weeks. The dissolution of P was still continuing at a slower rate when the experiment was stopped and the amount dissolved was 950 $\mu g\, g^{-1}$ PR. The amount of P dissolved was *ca* 0.8% of the total P and was equivalent to that obtained on acidulation of PR with 8% of the stoichiometric amount of sulphuric acid needed.

Addition of whey to the PR,S alone or in combination with microbial culture or nutrient solution [Fig. 2(c) and (d)] showed only a small increase in P dissolution [Fig. 2(c), (d)]. These values are in accord with those for pH [Fig. 1(c), (d)].

Sodium bicarbonate extractable P

The bicarbonate-extractable P in control samples ranged between 74–82 $\mu g\, g^{-1}$ PR which is slightly

higher than the water-P [Fig 3(a)]. The PR, Ct treatment showed a small increase in bicarb-P in the first week but values decreased as the incubation continued. Similar to the results of water-P, treatments receiving whey showed a decrease in the amounts of bicarb-P, which was probably due to microbial immobilisation. This was followed by an immediate increase in P in the case of PR,W,Ct but a delayed increase in the PR,W treatment.

Treatments 9–16 showed similar amounts of P dissolved in terms of bicarb-P as was achieved for water-P [Fig. 3(c),(d)]. There was a linear relationship between water-P and bicarb-P (Fig. 4). Thus either one of these could be used as an extractant for measuring the soluble P in the fertiliser mixture.

Formic acid-P

Phosphorus extracted from PR with a 2% formic acid solution has been found to correlate closely with the agronomic performance of PRs (Rajan *et al.*,

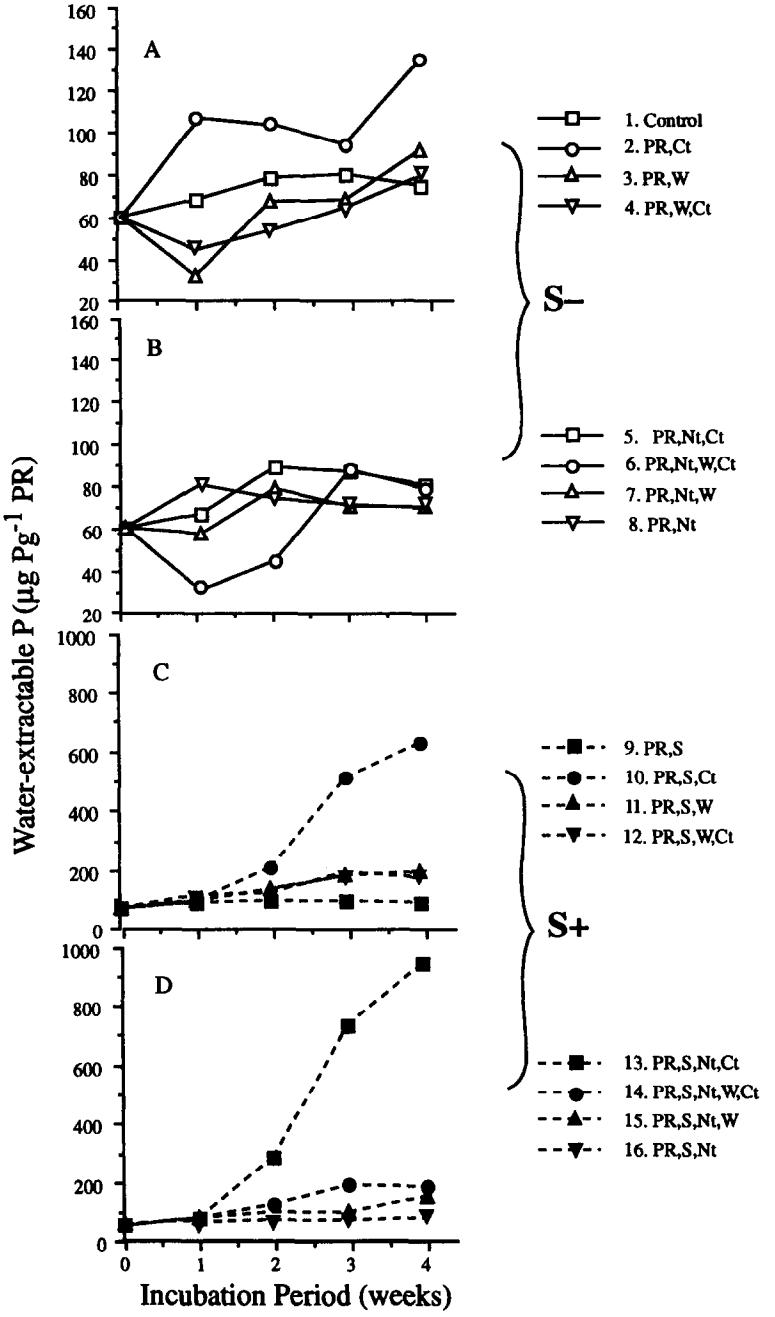

Fig. 2. Changes in the water extractable-P of samples with incubation time. $LSD_{0.05}$ between the treatments = 1.28 (PR = phosphate rock; Ct = microbial culture; S = elemental sulphur; Nt = nutrient solution).

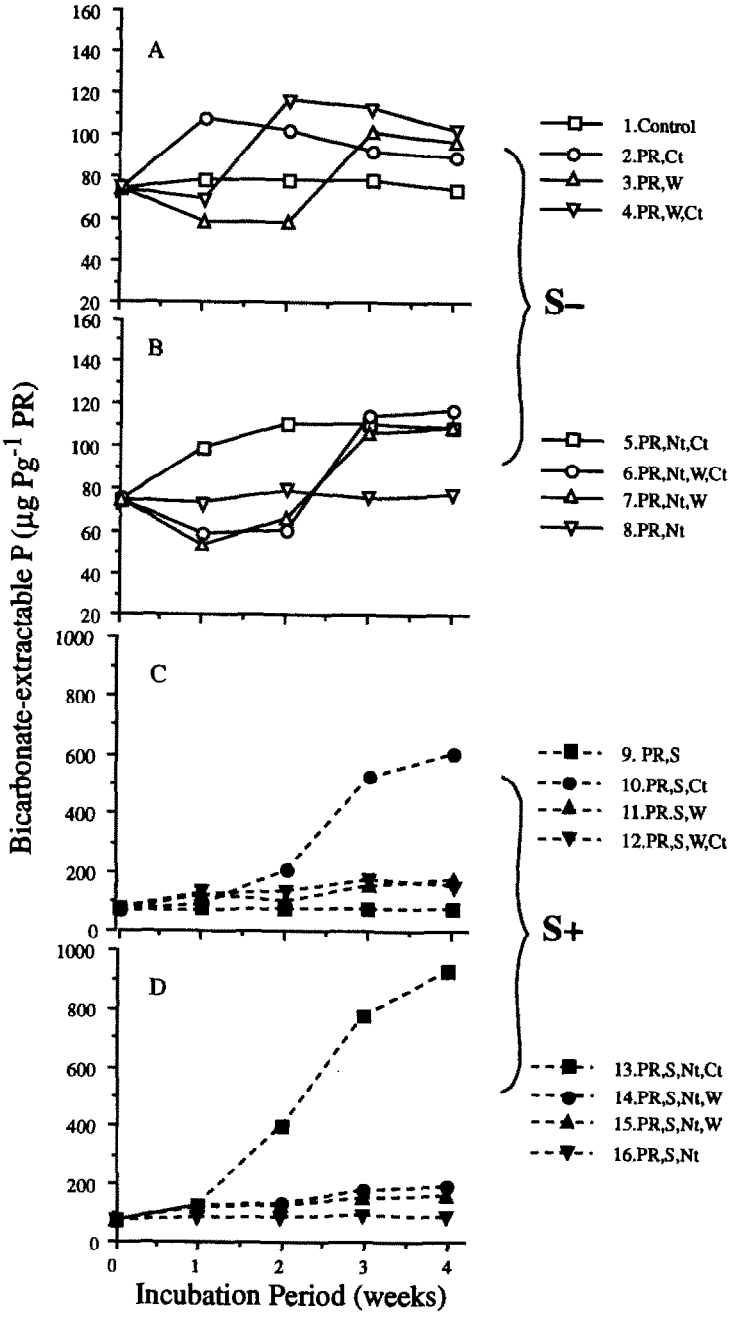

Fig. 3. Changes in the bicarbonate extractable-P of samples with incubation time. $LSD_{0.05}$ between the treatments = 1.23 (PR = phosphate rock; Ct = microbial culture; S = elemental sulphur; Nt = nutrient solution).

1992). We also extracted the incubation mixture with formic acid to measure not only the monocalcium phosphate (MCP) form of dissolved P but also the secondary product if any, i.e. dicalcium phosphate (DCP). However, due to a large background associated with formic acid extracts (extracting up to 70–73% of the total P in Sechura PR), this method was not sensitive enough to measure the effect of treatments on the dissolution of PR. Hence we have not presented results for the formic acid extractable-P.

Thiobacilli counts

Counts of Thiobacilli were made only in the treatments which showed significantly high levels of PR dissolution, i.e. treatments PR,S,Ct and PR,S,Nt,Ct. The initial number of S-oxidising *Thiobacilli* were low, (~100 in every vial: Table 2) but once these microbes were exposed to the mixture of PR and elemental S, they proliferated quickly. Within 24 h, the number of S-oxidising microbes increased to 10 times

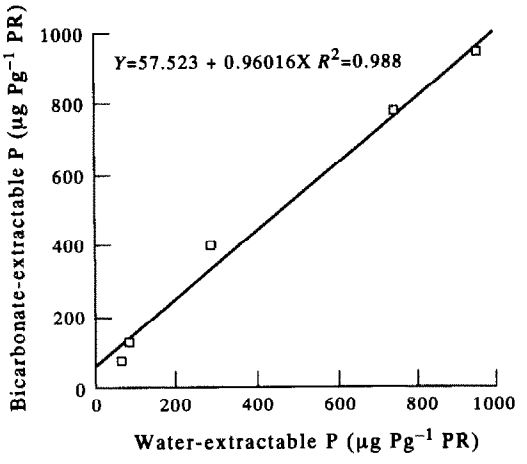

Fig. 4. Relationship between water extractable and bicarbonate extractable P in treatment 13 (PR,S, nutrient solution and microbial culture).

in treatment PR,S,Ct and 50 times in PR,S,Nt,Ct. It was evident that addition of nutrients encouraged faster growth of microorganisms. During the second and third week of incubation, the S-oxidising microbial populations in both treatments were the same. However, towards the end of the incubation, the number of Thiobacilli in treatment PR,S,Nt,Ct was approximately twice that in PR,S,Ct. Yet P dissolution values in these two treatments were significantly different from each other. Treatment PR,S,Nt,Ct showed significantly higher amounts of P dissolution after 2–4 weeks compared with PR,S,Ct. When the numbers of Thiobacilli are the same in both treatments one would expect a similar rate of S oxidation and hence the same efficiency in dissolving PR. The absence of a positive relationship between Thiobacili numbers and the rate of S oxidation agrees with the findings of Lee *et al.* (1988). However, it is evident that the addition of nutrients significantly increased the oxidising ability of Thiobacilli. The effects of different combinations of nutrients and their optimisation is currently being examined.

Microbial biomass-P

It has been suggested that the amounts of P held in microbial cells in soils represent a readily-available source of P for plant uptake (Kapoor and Haider, 1982; Brookes *et al.*, 1984). In our study, attempts were made to determine the amounts of P which was dissolved from the PR during the incubation but had been assimilated by the microbial biomass and which could not be measured by the water or bicarbonate extraction methods. Results showed that treatments receiving whey had significantly higher amounts of microbial biomass-P than the other treatments (Fig. 5). The amounts of microbial P recovered in whey treatments corresponded with the decrease in P measured by water-P or bicarb-P (Figs 2 and 3) at the respective sampling periods. The highest amount of microbial P recovered from the PR,S,Nt,W,Ct treatment was 16 $\mu g\ P\ g^{-1}$ PR. It is possible that the immobilisation of soluble P was caused by *Streptococcus* spp which were present in large numbers ($6.5 \times 10^6\ ml^{-1}$) in the added whey. However, the amounts of P recovered from microbial cells was a very small fraction of the amounts of P dissolved particularly from PR,S,Ct and PR,S,Nt,Ct treatments (Fig. 2). Hence the microbial biomass P in this particular system of incubation appears to be of little significance. It may also be possible that the fumigation method used was ineffective in recovering the proportion of P held in the microbial biomass.

Table 2. Changes in the number of Thiobacillus per vial with time

Incubation Period	Treatment 10 (PR,S,Ct)	Treatment 13 (PR,S,Ct,Nt)
Initial	~100	~100
24 h	9.5×10^2	4.9×10^3
1 week	2.4×10^7	2.4×10^7
2 weeks	2.4×10^7	2.4×10^7
3 weeks	2.3×10^8	2.3×10^8
4 weeks	2.3×10^6	6.2×10^6

Oxidation of elemental S

Elemental sulphur remaining in the mixture was measured at intervals to estimate the amount of sulphur oxidised. This was done only in the control and treatments having high P dissolution, viz. PR,S,Ct and PR,S,Nt,Ct. In control samples recovery of the added S ranged from 97 to 104% throughout the incubation period, indicating no oxidation of S in this treatment. In the PR,S,Ct treatment, during the first week of incubation only 2.1% of the added S was oxidised (Table 3) whereas at the end of the 4 weeks 9.0% was oxidised. Treatment PR,S,Nt,Ct showed a much faster rate of S oxidation, where 3.4% S was oxidised in the first week and 14.5% of S at the end. The amounts of acid produced after 4 weeks is equivalent to about 14% of the stoichiometric amount of H_2SO_4 needed to fully acidulate the PR. The values obtained for water-P and bicarb-P indicate only 9–10% acidulation.

Assuming that all of the acid produced from the oxidation of S reacted with the PR, we can calculate the amount of water-P in the fertiliser mixture. A comparison of observed and calculated water-P enabled the determination of the reaction dynamics between acid and PR. The difference between the calculated and the observed water-P values varied during the incubation. In the first 2 weeks of incubation, the difference between these two values was high (Fig. 6). But with time this difference narrowed, and towards the end of incubation, the observed values were 70–80% of the calculated values. Relatively large differences between the calculated and observed values in the first couple of weeks, indicates the time lag between acid production and its reaction with PR under the experimental conditions.

Our study showed that acidulation of PR can be effected in a soil-free medium by biological means, by incubating the PR with elemental S and a Thiobacillus inoculum. Addition of nutrient solution,

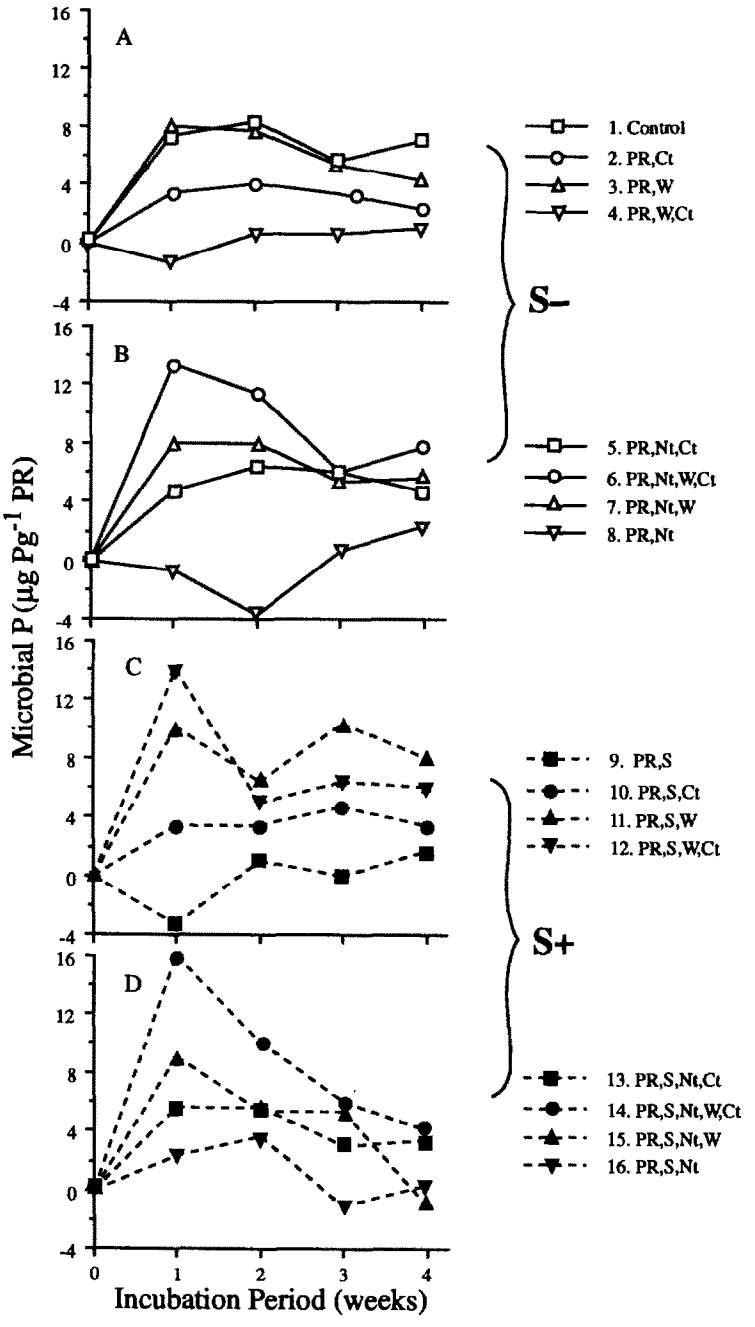

Fig. 5. Changes in the microbial biomass-P of samples with incubation time. $LSD_{0.05}$ between the treatments = 1.49 (PR = phosphate rock; Ct = microbial culture; S = elemental sulphur; Nt = nutrient solution).

Table 3 The percent of added elemental sulphur oxidised during incubation (elemental S added = 390 mg vial^{-1})

Incubation Period	Treatment 10 (PR,S,Ct)*	Treatment 13 (PR,S,Ct,Nt)*
Time 0	0.0	0.0
1 week	2.1 ± 0.4	3.4 ± 0.7
2 weeks	3.6 ± 0.6	7.2 ± 0.8
3 weeks	6.7 ± 1.2	12.3 ± 0.6
4 weeks	9.0 ± 0.8	14.5 ± 0.8

*Values are mean of two replicates ± SE.

particularly N, seems to have encouraged microbes to utilise S as a substrate and therefore oxidise elemental S more efficiently. In a separate study we found less dissolution of PR when NH_4NO_3 and KNO_3 were excluded from the nutrient solution (unpubl. data). Selective microbial strains will have specific nutrient requirements. As a first phase in our study, we used a microbial culture extracted from the local soil which had been fertilised with elemental S for the past 4 yr,

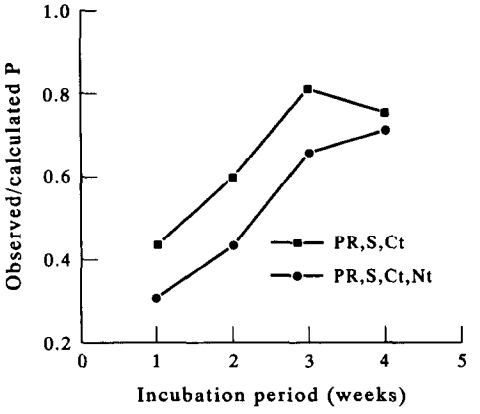

Fig. 6. Changes in the ratio between calculated and observed acidulation of PR with sulphuric acid during the incubation (PR = phosphate rock; Ct = microbial culture, S = elemental sulphur, Nt = nutrient solution).

rather than using an imported pure culture. This avoids the possible problem of a new culture adapting to the local soil environment when such fertilisers are applied.

The evidence for the effectiveness of the incubation for oxidising added S can be indirectly assessed by the pH values of the PR,S mixture (Swaby, 1975; Rajan and Edge, 1982). In our study where P dissolution was the highest, pH of the mixture decreased from 6.7 to 4.5 within 3 weeks of incubation. Swaby (1975) concluded that pH measurements can also be related to the amounts of elemental S oxidised during the incubation. However, our results do not entirely support this conclusion. Measurements of elemental S remaining at specific times in treatments where microbial culture and nutrients were added to the PR,S mixture showed that the oxidation of elemental S continued (Table 3) while pH values in these treatments remained relatively unchanged [Fig. 1(c),(d)]. This is because some of the H^+ produced during the dissociation of sulphuric acid would have been neutralised by the PR and therefore free H^+ as measured by pH would not give true values of the amounts of S oxidised as found in our study.

The use of acidic lactic casein whey (pH 4.5), was found to have very little effect on the dissolution of P in PR only treatments. There are three possible reasons for this which may be largely responsible for underestimating the effect of whey in dissolving P from PR [Fig. 2(a)]. Firstly, the acidic effect of whey could have been substantially reduced due to dilution by distilled water or with the other additives used in our study. Secondly, whey contained large numbers of Streptococci bacteria which appeared to have immobilised small amounts of soluble P, as evident from the higher amounts of microbial-P found in these treatments [Fig. 4(c), (d)]. Thirdly, whey contains a significant concentration of Ca^{2+} (Bradford *et al.*, 1986) which is known to deter PR dissolution (Robinson and Syers, 1990).

Whey contains a range of nutrients which are commonly utilised by microbes. Hence it was expected that the combination of whey treatments with soil microbial culture would increase the activity of S-oxidising microbes without the addition of nutrient solution. The results shown in Figs 3 and 4 reflect an adverse effect of adding whey to the microbial culture. One reason for this could be microbial competition (Kuenen *et al.*, 1985), where a large population of Streptococci would have utilised available nutrients more effectively and indirectly starved the Thiobacillus population, at least until the easily metabolisable source of carbon (lactose) was available for Streptoccoci. The decrease in pH values in some of these treatments towards the third and fourth week of incubation supports this explanation.

The highest dissolution of PR measured (treatment 13, PR,S,Nt,Ct) was not able to match the amount of water-soluble P generally found in commercially-available PAPR products. However, our results are encouraging from the viewpoint of producing PAPR in a soil-free medium and by biological processing. Further work is currently under way to determine the limiting factors in achieving higher dissolution of PR with the aim of devising a simple, cost-effective method of producing biological PAPR.

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REFERENCES

Bradford J. B., Galpin D. B. and Parkin M. F. (1986) Utilization of whey as a fertilizer replacement for dairy pasture. *New Zealand Journal of Dairy Science and Technology* **21**, 65–72.

Brookes P. C., Powlson D. S. and Jenkinson D. S. (1982) Measurement of microbial biomass phosphorus in soil. *Soil Biology & Biochemistry* **14**, 319–329.

Brookes P. C., Powlson D. S. and Jenkinson D. S (1984) Phosphorus in the soil microbial biomass. *Soil Biology & Biochemistry* **16**, 169–175.

Cheney T. M., McClellan G. H. and Montgomery E. S. (1979) Sechura phosphate deposits, their stratigraphy, origin and composition. *Journal of Economic Geology* **74**, 232–259.

Hammond L. L., Chien S. H., Roy A. H. and Mokwunge A. U. (1989) Solubility and agronomic effectiveness of partially acidulated phosphate rocks as influenced by their iron and aluminium oxide content. *Fertilizer Research* **19**, 93–98.

Jenkinson D. S. and Powlson D. S. (1976) The effects of biocidal treatments on metabolism in soil. I. Fumigation with chloroform. *Soil Biology & Biochemistry* **8**, 351–359.

Kapoor K. K. and Haider K. (1982) Mineralisation and plant availability of phosphorus from biomass of hyaline and melanic fungi. *Soil Science Society of America Journal* **46**, 953–957.

Kuenen J. G., Robertson L. A., and Gemerden H. Van (1985) Microbial interaction among aerobic and anaerobic sulfur-oxidizing bacteria. *Advances in Microbial Ecology* **8**, 1–60.

Lee A., Watkinson J. H., and Lauren D. R. (1988) Factors affecting oxidation rate of elemental sulphur in a soil under ryegrass dominant sward. *Soil Biology & Biochemistry* **20,** 809–816.

Mackay A. D. and Wewala G. S. (1990) Evaluation of partially acidulated phosphate fertilizers and reactive phosphate rock for hill pastures. *Fertilizer Research* **21,** 149–156.

Murphy J. and Riley J. P. (1962) A modified single extraction solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* **27,** 31–36.

Pathiratna L. S. S., Waidyanathan U. P. De S. and Peries O. S. (1989) The effect of apatite and elemental sulphur mixture on growth and P content of *Centrocema pubescens*. *Fertilizer Research* **21,** 37–43.

Postgate J. R. (1966) Media for sulphur bacteria. *Laboratory Practice* **15,** 1239–1244.

Rajan S. S. S. (1986) Partial acidulation of an unground phosphate rock, II. Plant availability of phosphate. *Fertilizer Research* **4,** 287–296.

Rajan S. S. S. (1987) Phosphate rock and phosphate rock/sulphur granules as phosphate fertilizers and their dissolution in soil. *Fertilizer Research* **11,** 43–60.

Rajan S. S. S. and Edge E. A. (1980) Dissolution of granulated low grade phosphate rock, phosphate/sulphur (Biosuper) and super phosphate in soil. *New Zealand Journal of Agriculture Research* **23,** 451–456.

Rajan S.S.S. and Watkinson J. H. (1993) Unacidulated and partially acidulated phosphate rock, Agronomic effectiveness and the rate of dissolution of phosphate rock. *Fertilizer Research.* **33,** 267–277.

Rajan S. S. S., Brown M. W., Watkinson M. K. B. and Upsdell M. (1992) Extractable phosphorus to predict agronomic effectiveness of ground and unground phosphate rocks. *Fertilizer Research* **32,** 291–302

Rittenberg S. C. (1969) The role of oxogenous organic matter in the physiology of chemolithotrophic bacteria. *Advances in Microbial Physiology* **3,** 159–196.

Robinson J. S. and Syers J. K. (1990) A critical evaluation of the factors influencing the dissolution of gafsa phosphate rock. *Journal of Soil Science* **41,** 597–605.

Sinclair A. G. and Dyson C. B. (1988) An interim report on the MAF 'National Series' forms of phosphate fertilizer trials, herbage dry matter production for growing seasons 1982/83 to 1986/87 inclusive. MAFTech, Wellington.

Swaby R. J. (1975) Biosuper-Biological super phosphate. In *Sulphur in Australian Agriculture* (K. D. Mclachalan, Ed.), pp. 213–220. Sydney University Press, Sydney.

Watkinson J. H., Lee A., and Lauren D. R. (1987) Measurements of elemental sulfur in soil and sediments, field sampling, samples storage, pretreatment, extraction and analysis by high-performance liquid chromatography. *Australian Journal of Soil Research* **25,** 167–178.