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SOLUBILISATION OF ROCK PHOSPHATE BY *THIOBACILLUS FERROOXIDANS*

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MICROBIAL solubilisation of rock phosphate, especially of the low grade variety, is receiving greater attention in recent years. This process can compensate for the higher cost of preparing phosphate fertilizers in the industries. India possesses substantial deposits of rock phosphate which till now remains unutilized due to the presence of several impurities and a low phosphorus content[1]. Laboratory studies have been carried out to utilize rock phosphates, rendering them soluble by microbial means in presence of a suitable carbon source[2-4]. We report here, for the first time, the involvement of iron-oxidizing chemoautotroph *Thiobacillus ferrooxidans* for such a solubilisation process.

The cells of *T. ferrooxidans* were grown in the modified 9K medium and harvested accordingly[5]. Rock phosphate samples were collected from Indian origin. For our experiment the samples were ground to < 63 microns particle size and analysed[6]. The slurry of rock phosphate, mixed with or without pyrite *viz* Pyriteferous shales of Amjhore (India), was made in acidified water (pH 2.4 adjusted with 0.01N H_2SO_4) containing 0.1% ammonium sulphate. The suspensions in 250 ml Erlenmeyer flasks were inoculated with 5 ml cell suspension (2×10^9 cells/ml) and incubated at 28°C on a rotary shaker (150 rev/min). The amount of phosphate released from rock phosphate was estimated[7] periodically. The uninoculated samples were treated in the same manner served as controls.

The results (figures. 1a & b) show the efficiency of the bacterial strain *T. ferrooxidans* in solubilizing phosphate from a sterilized pyrite mixed low grade rock phosphate (P_3-MRP). It could be concluded that the maximum phosphate leach rate was achieved at 6% (w/v) rock phosphate concentration (16.4 mg phosphate/l/hr), whereas the maximum solubilisation (98%) was obtained at 2% (w/v). Considering the release of phosphate with time (figure 1a) it was evident that the extent of solubilisation increased steadily up to 15 days, although it continued upto 25 days. The increase in solubility of phosphate between 20 and 25 days was very small (figure 1b).

The effective role of pyrite during this process is reflected from another low grade rock phosphate sample P_1-MRP (compare B with C, figure 2). The deleterious effect of the presence of calcite to restrict phosphate release was observed. This can be due to the secondary reactions in which Ca^{2+} ions released from calcite at pH 2.4 may effect the solubility product of calcium phosphate. The sample in association with pyrite showed 38.0% phosphate solubilisation, whereas 56% solubilisation was possible when calcite free sample, obtained by the treatment with triammonium citrate solution[8] was used (compare C with D, figure 2). Maximum solubilisation (72.5%) was achieved when the medium with calcite free sample was supplemented with essential nutrients (0.05% magnesium sulphate and 0.01% potassium chloride), other than nitrogen source (compare D with E, figure 2). The calcite free unsterilized, uninoculated sample also showed significant phosphate solubilisation (compare A with F, figure 2). However, the bacterium showed nearly similar phosphate release from calcite free sterilized and unsterilized samples (compare D with H, figure 2). The strain was found equally effective in solubilizing phosphate from high grade rock phosphates (unpublished data).

It seems evident from these results that *T. ferrooxidans* together with acidiphilic microbes present in the samples is efficient in solubilizing phosphate from rock phosphate, irrespective of their total phosphate content and other chemical constituents (table 1). The

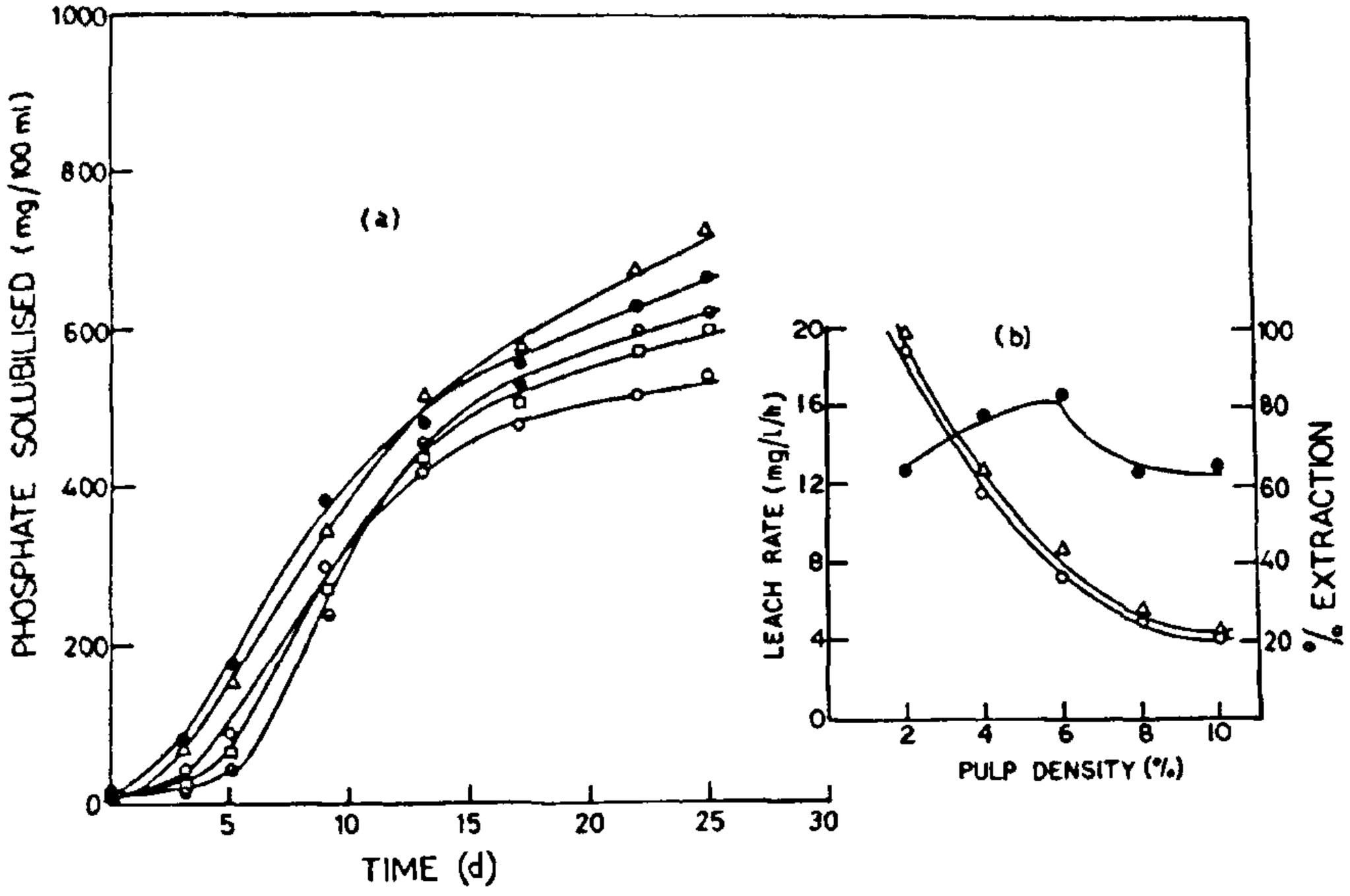

Figure 1. Influence of *Thiobacillus ferrooxidans* on solubilisation of phosphate from the sterilized rock phosphate sample, P_3-MRP. **(a)** Solubilisation of phosphate at various pulp densities of rock phosphate (%): 2 (O); 4 (△); 6 (●); 8 (□); 10 (◒). **(b)** Phosphate leach rate (●), and percent extraction at 20 days (O) and 25 days (△).

rock phosphate solubilisation by this process has a correlation with pyrite oxidizing ability of this bacterium[9]. The availability of pyrite crystal surfaces for such oxidation will gradually reduce with the increase in rock phosphate addition. As such, maximum solubilisation at lower pulp densities of rock phosphate was thus favoured. Considering both phosphate leach rate and percent extraction, its effective leaching concentration was found between 2 to 4% (w/v). In another experiment it was found that the maximum amount of pyrite that could be used was 8% (w/v) but then total particle concentration of pyrite *vs* rock phosphate, should be between 10 to 12% (w/v) to give effective solubilisation and bacterial activity. Comparing the results of uninoculated sterilized and unsterilized samples, it can be stated that, pyrite oxidizers are always present in the samples. These may be utilized to solubilize phosphate from rock phosphate without any additional cost of bacterial inoculum. As such, the enhancement of process could be possible by the cumulative effect of iron oxidizers already present. Furthermore, no sterilization is necessary for this process which is beneficial for field scale studies. Addition of essential bacterial nutrients, helps in increasing the rate of phosphate solubilisation indicating that their relative amounts are not ubiquitious in the studied samples. The presence of calcite to restrict bacterial activity as observed earlier[10] is also evident here.

It could be concluded from these results that the soil treatment with rock phosphate in combination with pyrite and *T. ferrooxidans* might show a way in the near future for better soil management.

Table 1. *Chemical composition of rock phosphate samples Location: Mussoorie, India*

Constituents (%)	Sample No.	
	P_1-MRP	P_3-MRP
P_2O_5	17.5	26.2
LOI	14.1	10.4
CaO	48.9	40.8
MgO	2.3	1.6
SiO_2	13.6	2.5
RO	3.6	18.5

LOI – Loss due to ignition, RO – All other oxides

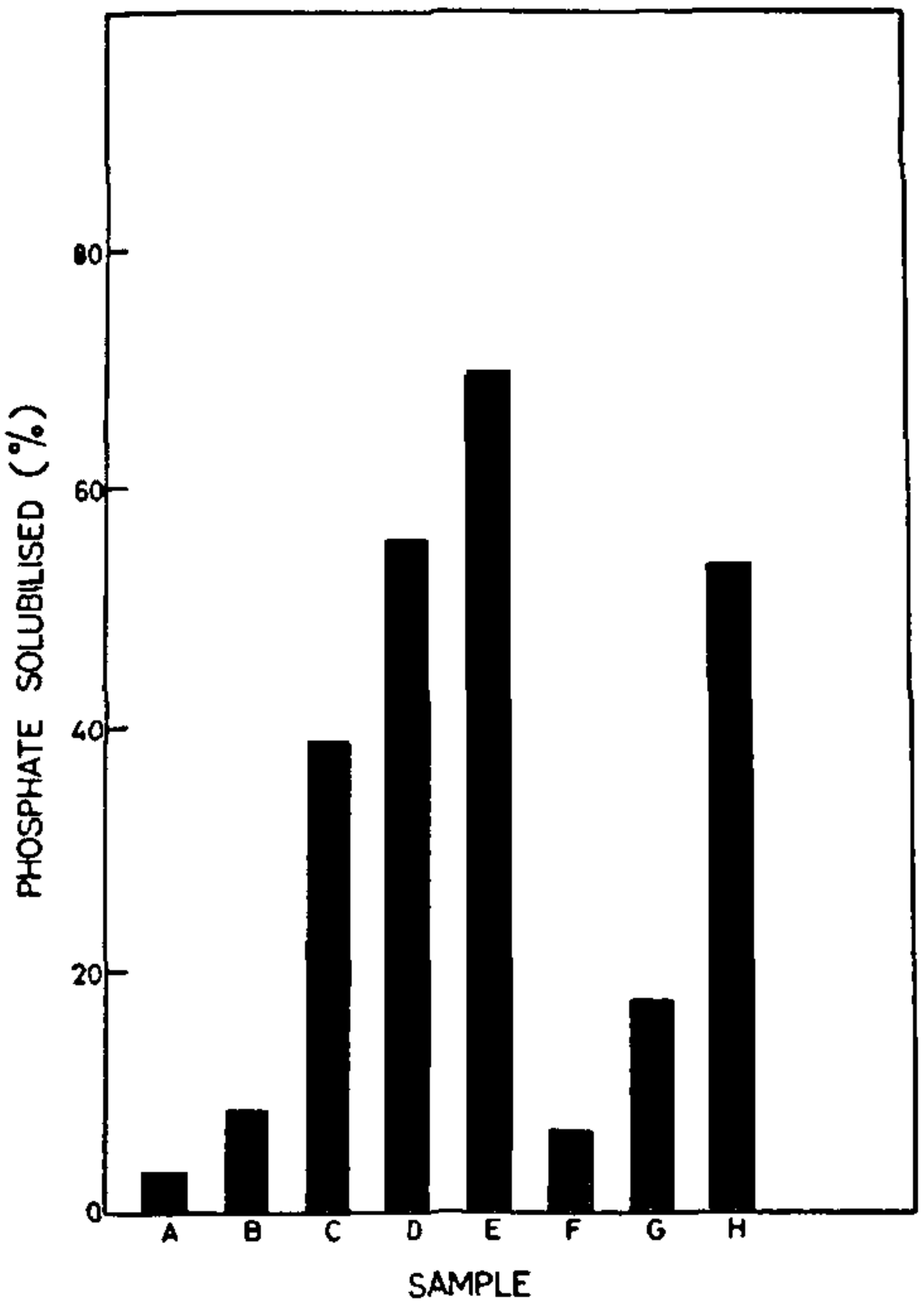

Figure 2. Solubilisation of phosphate from the rock phosphate sample, P_1-MRP, after 25 days of incubation under varying conditions with *Thiobacillus ferrooxidans*. A, sterilized, uninoculated; B, sterilized, inoculatated without pyrite; C, sterilized, inoculated with pyrite; D, calcite free sample, sterilized, inoculated; E, calcite free sample sterilized, inoculated with nutrients; F, unsterilized, uninoculated; G, calcite free sample, unsterilized, uninoculated; H, calcite free sample, unsterilized, inoculated.

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A NEW SPECIES OF *UNCINULA* (ERYSIPHACEAE) FROM KASHMIR

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DURING the surveys conducted in Jammu and Kashmir to collect the members of Erysiphaceae (powdery mildews), a new species of *Uncinula* was collected from *Populus nigra* L. The description of the new fungus is described in this note.

Uncinula populi sp. nov.

Mycelium album, epiphyllum; conidia ellipsoidea, elongata vel oblonga, 28.13–45.00 × 11.25–18.75 μm. Perithecia epiphylla, fusca ad atra, globosa ad subglobosa, hemispherica, 101.25–176.25 μm diam.; cellulae parietes, parvae, angulars 11.25–26.25 × 9.38–16.75 μm, appendiculi 17–40 in quoque perithecium, flexibilis, tenelli, cercinati apice, aseptati, hyalini, 5.63–7.50 μm lati. Asci 5–15, lati, ovati, clavati, oblongi, pyriformes, 52.50–86.25 × 41.25–60.00 μm. Ascosporae interdum 3–8 autem plerumque 5–8, oblongae, elongatae, ovalis, raro globosae ad 16.88–30.00 × 11.25–18.75 μm.

Typus lectus in *Populus nigra* L. a Tulamula (Kashmir), et depositus ad Indian Type Culture Collection I.A.R.I., New Delhi, sub Acc. no. 33326.

Mycelium white, epiphyllous (sometimes amphigenous also), forming a thick mat on the adaxial laminar surface. Conidia ellipsoid, elongate, oblong, 28.13–45.00 × 11.25–18.75 μ, average relative length