lectins and these interactions are characterized by association constants in the range of 1.0×10^3 to 1.0×10^5 M^{-1} (refs 6, 7). It is interesting to note that K_a values for the porphyrin–lectin interaction reported here are in the same range as those for lectin-hydrophobic ligand and porphyrin–serum protein interaction[21]. However, the interaction of porphyrins with lectins has more potential for their targeted delivery because of the preferential interaction of the lectins with tumour cells.

Con A could be completely freed of the porphyrin from a Con A–H_2TPPS mixture by gel permeation chromatography on a column of Sephadex G-50, clearly demonstrating that the association of porphyrin with the lectin is reversible. Therefore, in a given solution containing lectin and porphyrin, the porphyrin will exist in two forms: associated with the lectin, and free in solution. Though only partial association with the lectin is seen in the present study, the magnitude of K_a values is such that considerable improvement in the preferential localization of the porphyrin in tumour tissues may be expected when lectin–porphyrin mixtures are used as compared to using porphyrin alone in photodynamic therapy. In this regard, it can be noted that, with an association constant of *ca.* 5×10^4 M^{-1}, under typical *in vitro* PDT conditions, use of about 10^{-5} M of the porphyrin and a ten-fold molar excess of lectin would provide more than 80% of the lectin-bound porphyrin molecules that are capable of localizing in the tumour. Nonetheless, studies with cultured cells and animal models are required to evaluate the tumour selectivity that can be achieved by using lectin–porphyrin complexes in PDT. Additionally, further increase in the tumour localization of the porphyrins can be envisaged by covalently linking them to the lectins. Future studies should be aimed in this direction.

In summary, this communication reports the first demonstration of the interaction of porphyrins with lectins. In view of the current interest in porphyrins as photosensitizers in photodynamic therapy for the treatment of cancer, this suggests that lectins may be useful in tumour-specific targeted delivery of porphyrins.

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Bioleaching of copper from ferromanganese sea nodule of Indian ocean

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Ferromanganese sea nodule is an abundant low-grade metal reserve of Indian ocean. However, it can be a potential substrate for bioextraction of metals like copper. It has been found that 85% of copper could be leached in seven days in batch fermentation using *Aspergillus* spp. at 30°C. Bioleaching of copper was found to be organic-acid mediated. Scanning electron micrographs revealed eroded areas indicating biosolubilization of copper from the nodule.

BIOLEACHING of valuable metals like copper, gold, uranium has become a prime concern of biometallurgists due to depletion of high-grade metal reserves. The potential and ability of microorganisms in mineral biotechnology have opened new avenues for the studies on microbial leaching[1,2]. Leaching of metals using microbes from low-grade ores has many advantages over chemical leaching because of its eco-friendly and cost-effective nature. Chemolithotrophs, viz. *Thiobacillus*, *Sulfolobus*, *Bacillus*, are amongst the potential microbes used for bioleaching[3–5]. However, since they are all acidophiles, alternatives to these extremophiles are being searched for substrates which shift the pH of the leach liquor above 5.0 (ref. 6) like ferromanganese sea nodule of Indian ocean.

Ferromanganese sea nodule of Indian ocean is a complex polymetallic sea deposition[7,8]. They are hydrogenous sea depositions containing about 0.60% of copper in association with other metals (Table 1).

Bioleaching of copper from sea nodule was studied and optimized using two isolates of *Aspergillus*, viz. *Aspergillus foetidus* and *Aspergillus japonicus*. These have been isolated from Indian rock phosphate and are good phosphate solubilizers, in alkaline conditions[9]. In addition, these are good organic acid producers[10].

Bioleaching of copper from ferromanganese sea nodule was carried out in modified Czapek Dox's minimal medium[11] without adjusting the pH, with a composition of glucose 1.0% w/v, $NaNO_3$ 0.6% w/v, KH_2PO_4 0.15% w/v, and KCl 0.05% w/v in distilled water. Five-day-old cultures of each species were used for preparing spore suspension in normal saline. Spores (5×10^7) of the respective test organisms were inoculated in 50 ml of the leaching medium contained in 250 ml Erlenmeyer flasks supplemented with 1% w/v of ground sea nodule (100 mesh size). The pH of the medium checked after autoclaving was 6.8 ± 0.2. The flasks were inoculated and the cultures were incubated at 30°C at 200 rpm for five days, initially. The effect of different parameters on bioleaching, viz. growth conditions, inoculum size, incubation period, phosphate, nodule, nitrogen, glucose concentration and temperature was investigated.

The amount of copper leached or solubilized was determined by atomic absorption specrtrophotometer (Shimadzu AA-649-13). Uninoculated control and leached nodule were also examined through scanning electron microscope (Japan Electron Optics Limited; Model JSM-840) to study the possible changes on the surface of the nodule during bioleaching.

Table 1. Metal composition of the ferromanganese sea nodule of Indian ocean

Metal	Composition (%)
Iron	14.00
Manganese	6.50
Nickel	0.74
Copper	0.60
Cobalt	0.14

Sea nodule composition provided by National Metallurgical Laboratories, Jamshedpur.

These ferromanganese sea nodules were also subjected to different concentrations of chemical leaching using different types of organic acids in relation to time.

In presence of sea nodule *A. foetidus* could grow and leach copper both as static and shake cultures. However, the leaching efficiency was more in shake cultures attributable to enhanced interaction between sea nodule and the growth medium (Figure 1 *a*).

We are presenting here the results on only *A. foetidus*, as *A. japonicus* behaved in the same fashion and hence, it could be extrapolated that the mechanism of bioleaching is the same in both the fungi.

It is clear from Figure 1 *b* that maximum leaching is achieved after 7 days of incubation. However, pH of the culture filtrate decreased to as low as 2.6 on the 4th day and thereafter, it showed a gradual increase and pH reached up to 6.7 ± 0.2. Initial decrease in the pH during growth is due to the production of organic acids in the medium and the subsequent rise in pH is most probably due to the consumption of these acids by some metabolic or chemical processes. The culture filtrate revealed the presence of oxalic, citric, and fumaric acid[10].

It is well known that the presence of inorganic phosphate affects both growth of microorganisms and organic acid production[12]. In the present investigation also a significant effect on bioleaching of copper by *A. foetidus* was observed at varying concentration of phosphate (Figure 1 *c*). Maximum leaching was observed at 0.20% of KH_2PO_4 (w/v). A decrease at higher concentration could be due to inhibitory effect of higher phosphate concentrations on solubilization of metals[13]. Figure 1 *d* reveals that nodule density had a profound effect on the leaching efficiency. Maximum leaching was achieved at 1% (w/v) nodule concentration. Low metal extraction at higher nodule density may be because of unidentified substances contained within the nodule, which most probably have a strong inhibitory effect on the growth of the organism[14]. Slight variation in nitrogen ($NaNO_3$) concentration did not affect bioleaching efficiency of *A. foetidus*.

Glucose at a concentration of 2% (w/v) supported 85% leaching (Figure 1 *e*). Though bioleaching occurred in the temperature range of 25°C–37°C, the maximum was at 30°C (Figure 1 *f*). There was a sharp decline in bioleaching at and above 40°C as there was no growth at this temperature.

The present results indicated that *A. foetidus* is a potential bioleaching organism under conditions where acidophiles fail. A six-fold increase in the bioleaching efficiency by *A. foetidus* could be achieved by optimization of various parameters and the process was

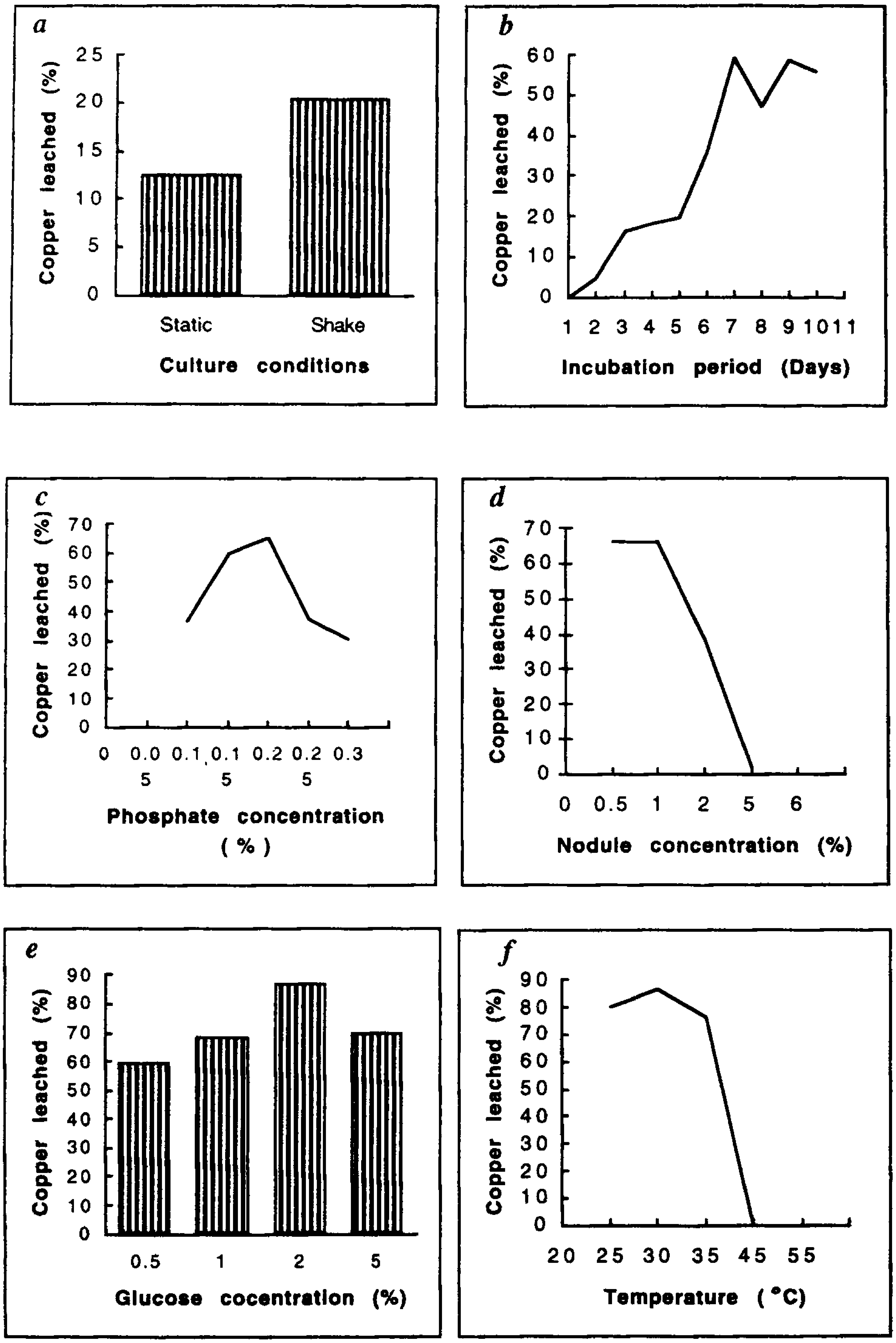

Figure 1. Bioleaching of copper (%) from ferromanganese sea nodule by *Aspergillus foetidus* in modified Czapek Dox's minimal medium at 30°C in shake cultures. Effect of (*a*), static and shake cultures; (*b*), Incubation period; (*c*), Phosphate concentration; (*d*), Nodule concentration; (*e*), Glucose concentration; (*f*), Temperature.

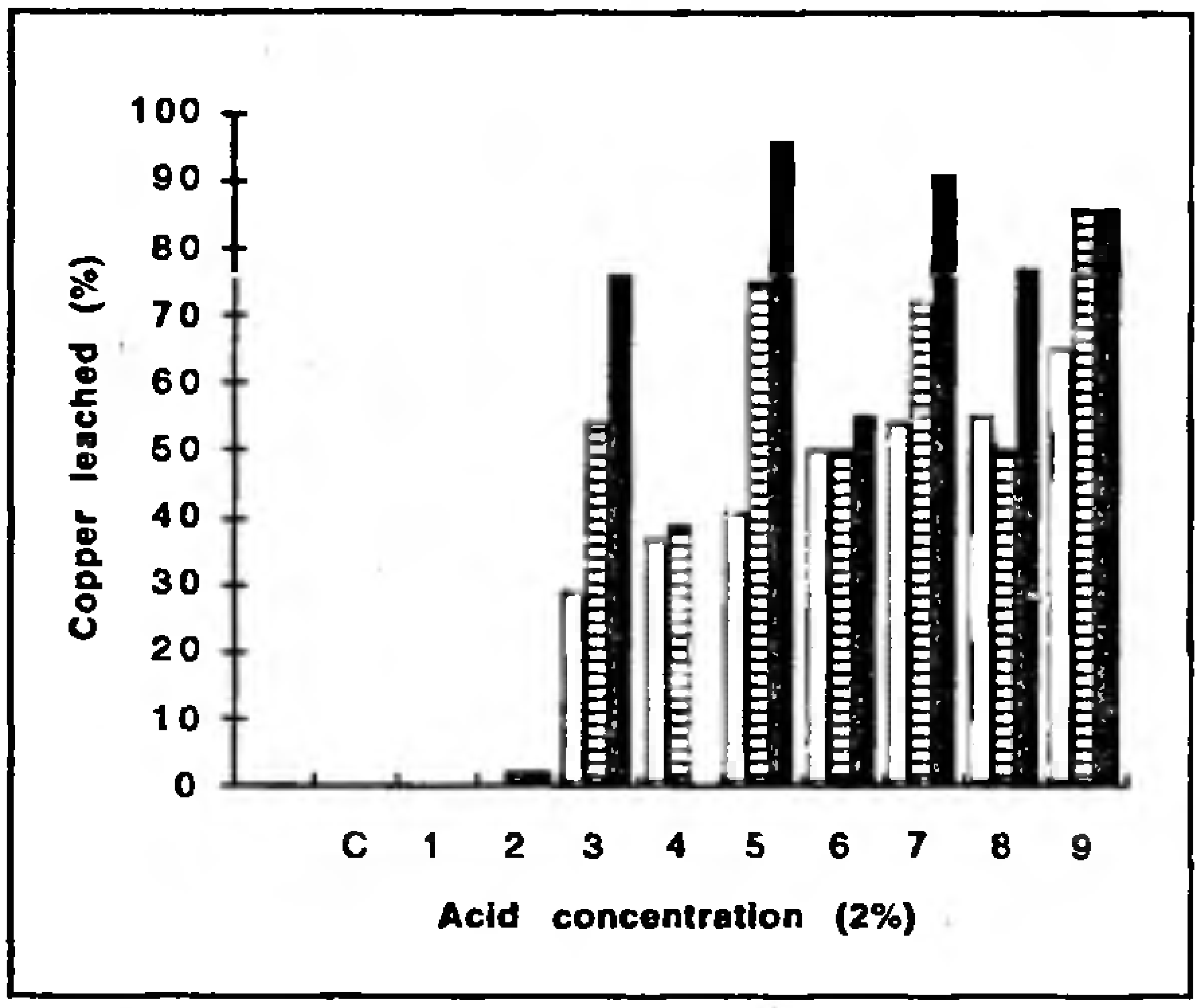

Figure 2. Effect of organic acids on bioleaching of copper from ferromanganese sea nodules. C, Control; 1, Acetic; 2, Succinic; 3, α-Ketoglutaric; 4, Oxalic; 5, Lactic; 6, Malonic; 7, Citric; 8, Malic; 9, Tartaric.

Figure 3. Scanning electron micrograph of ferromanganese sea nodule. *a*, Control; *b*, Perforations (→) on the nodule surface after seven days of leaching.

found to be organic-acid mediated. This was further confirmed by chemical leaching. Metal leaching with pure organic acids revealed that copper leaching from ferromanganese sea nodule is organic-acid mediated (Figure 2). Ninety-one per cent leaching could be achieved with 2% (w/v) oxalic acid. Increase in the solubility of metal ions at acidic pH is because of complexation and chelation[6]. This checks precipitation of metal hydroxides which is important in case of high metal concentrations.

Based on the results it is possible to develop a two-step strategy for bioleaching, the first comprising of production of culture filtrate of the organism and in the second step, subsequent use of this for leaching. Thirty-one per cent leaching could be achieved in 48 h at 30°C using 4-day-old culture filtrate (data not shown).

Scanning electron micrographs of the uninoculated control (Figure 3 *a*) and leached nodules (Figure 3 *b*) showed eroded surface area with small holes at places from where metal has been leached out from the nodules. *A. foetidus* is a versatile fungus that grows in a wide range of pH conditions and produces organic acids during growth, which help in bioleaching. Hence, it is possible to design one-step and two-step bioreactors for the exploitation of the abundant low-grade metal reserves lying unattended and unclaimed within the sea for metal(s) recovery.

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