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Production of chelating compounds by yeasts in a microbial copper leaching system and its practical implications

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Abstract. A yeast *Rhodotorula glutinis* repeatedly isolated from microbial copper leaching system increased the iron oxidation rate and the copper extraction efficiency of the process. It is proved that yeast could produce hydroxamate type chelating compounds, the practical implications of which are discussed.

Keywords. Chelating compounds; yeasts; leaching.

In the past, microbiology of ore leaching was studied only with regard to chemolithotrophic bacteria belonging to *Thiobacillus* species and the heterotrophic micro-organisms associated with the leaching systems were neglected. We thought it essential to understand the nature of the members of complex communities of copper leaching microbes and how they interact with each other in a closed ecosystem. The closed copper leaching ecosystem comprised of a 2 litre air-lift percolator filled with 100 g finely ground chalcopyrite ore and 9 K basal medium (Silverman and Lundgren 1959) of pH 2·5 inoculated with *Thiobacillus ferrooxidans* culture. From this ecosystem, various heterotrophic micro-organisms were isolated (Paknikar 1983). A culture of *Rhodotorula glutinis* was repeatedly isolated on Davis' yeast extract agar medium. It was not found to be capable of reducing sulphates as reported earlier (Ehrlich and Fox 1967). However, when grown in combination with *T. ferrooxidans* it increased the iron oxidation rate (IOR) and the copper extraction efficiency of the *T. ferrooxidans* culture. The reason for this phenomenon was not known. Meanwhile, our attention was drawn to reports regarding complexing agents produced by micro-organisms (Akers 1981; Neilands 1974) and their use in solubilization and uptake of iron. The present investigation was carried out to test whether *R. glutinis* produced any of these agents in the presence of *T. ferrooxidans.*

The organisms were grown in 500 ml quantities in Erlenmeyer flasks incubated at 30°C in a constant temperature water-bath shaker for 48 h. The culture media and combinations of organisms used were:

(a) 9 K salts medium with 2·5% ferrous sulphate—inoculated with 10 ml suspension of *T. ferrooxidans* containing 10^8 cells/ml;
(b) Davis' yeast extract medium—inoculated with 10 ml suspension of *R. glutinis* containing 10^8 cells/ml;
(c) 9 K salts medium with 2·5% ferrous sulphate and 1% yeast extract—inoculated with 1 : 1 *T. ferrooxidans*: *R. glutinis* combination (10 ml suspension each containing 10^8 cells/ml);
(d) 9 K basal medium with 50 g chalcopyrite ore—inoculated with *T. ferrooxidans* (10 ml of 10^8 cells/ml);
(e) 9 K basal medium with 50 g chalcopyrite ore and 1% yeast extract—inoculated with 1 : 1 *T. ferrooxidans*: *R. glutinis* combination (10 ml suspension each of 10^8 cells/ml).

The IOR was monitored according to the standard procedure (Paknikar and Agate 1987). Copper extraction efficiency was calculated by estimating the total copper leached by the sodium diethyl dithiocarbamate method.

The cells and the inorganic precipitates formed during the growth were removed by centrifugation at 10,000 rpm for 15 min. Culture supernatant was concentrated by flash evaporation at 40°C to about one-tenth of its volume and this concentrate was used to perform a biological assay using a siderochrome auxotroph, *Arthrobacter* JG9 as described by Estep *et al* (1975).

Only the concentrates from flasks (c) and (e) were found to support the growth of *Arthrobacter* JG9, since zones of exhibition were observed after 48 h of incubation. It was also observed that IOR in the case of combination of cultures was increased by 9% than that of *T. ferrooxidans* culture alone. An increase of 8·5% in the copper extraction efficiency was also observed in the case of combination.

The present results indicate that the growth-promoting activity in the concentrates was due to the presence of hydroxamic acids which were synthesized by *R. glutinis* only in the presence of *T. ferrooxidans* and the ferrous iron. It was noted from the literature that some strains of *Rhodotorula* produced rhodotorulic acid, a hydroxamate compound (Atkins and Neilands 1968). It is possible that the ferric ion specific ligands released the competitive inhibition of iron oxidation reaction by binding with the ferric iron. Another possibility might be that the chelating compounds produced by *R. glutinis* could have effected increased uptake and/or partial resolubilization of precipitated ferric iron. Both these interactions, in turn, increased the IOR and hence the copper extraction efficiency of the combination culture. Although, a lot of reconfirmatory chemical analyses are required to speculate a biochemical role of chelating compounds in metal leaching operations, the above data can be considered as a *prima facie* evidence for the production of hydroxamates in a low-pH biological system. The finding is important from the point of application since it could be used for improving the efficacy of the leaching process which could certainly revolutionize this technology.

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