

Figure 4. BET isotherm for Ni²⁺ and Cd²⁺ biosorption by naturally occurring and laboratory-grown *Microcystis*.

nature of biosorption of that particular metal in defined condition.

The application of mathematical models and constants has demonstrated that (i) *Microcystis* is an excellent biosorbent, (ii) the naturally grown cells are better than the laboratory-grown cells, (iii) biosorption is multilayer, and (iv) this biological biosorbent is quite similar to physical biosorbent, hence, it may replace the physical biosorbents. We are currently evaluating the potential of *Microcystis* as well as optimizing different environmental conditions for the use of this test cyanobacterium in removal of heavy metals from aquatics polluted with different metals.

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Solubilization of phosphorus by *Trichoderma viride*

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Solubilization of insoluble phosphates by *Trichoderma* spp. has been described and their solubilizing efficiency compared with that of certain known phosphate solubilizers. *T. viride* proved to be an efficient solubilizer of tricalcium phosphates.

OF the various macronutrients, phosphorus plays an important role in plant growth and reproduction. Further

availability of phosphorus in soil is conditioned by various factors and is a major limiting factor for the growth of plants. Soil organisms, specifically bacterial¹ and fungi^{2,3}, growing in the root region of plants play an important role in supply of phosphorus. Kundu and Gaur¹ have reviewed the role of fungi in solubilization of rock phosphate in soil. The main problem in application of P as a plant nutrient in P-fixing tropical soils is its conversion to unavailable P in soil up to 85%. Therefore, for making P available to plants, several microorganisms are used as P solubilizers.

The soil-borne fungus *Trichoderma* is a very effective biocontrol organism used against several soil-borne plant pathogens. It is now widely used both in India and abroad under several trade names like BINAB and Trichodes.

Although phosphate solubilization by bacteria has been

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Table 1. Microbial solubilization of phosphates

| Organism | Tricalcium phosphate | | | | | Rock phosphate | | | | |
|-----------------------------------|--|--|---------------------|-----------------|--------------------|--|--|---------------------|-----------------|--------------------|
| | Acid phosphatase activity ^a | Maximum P solubilized as P ₂ O ₅ (mg/100 ml broth) | Mycelial mat wt (g) | Turbidity (NTU) | Final pH of medium | Acid phosphatase activity ^a | Maximum P solubilized as P ₂ O ₅ (mg/100 ml broth) | Mycelial mat wt (g) | Turbidity (NTU) | Final pH of medium |
| <i>Aspergillus awamori</i> | 0.027 | 0.456 | 7.0 | 85 | 4.0 | 0.013 | 0.420 | 4.7 | 45 | 4.5 |
| <i>Bacillus megatherium</i> | 0.036 | 0.638 | 0.0 | 73 | 4.0 | 0.051 | 0.530 | — | 50 | 4.5 |
| <i>Trichoderma harzianum</i> | 0.036 | 0.638 | 4.3 | 95 | 4.5 | 0.009 | 0.638 | 2.9 | 31 | 4.0 |
| <i>Trichoderma pseudokoningii</i> | 0.016 | 0.620 | 4.4 | 62 | 4.5 | 0.013 | 0.530 | 3.4 | 57 | 4.5 |
| <i>Trichoderma viride</i> | 0.027 | 0.639 | 4.7 | 85 | 4.0 | 0.009 | 0.585 | 6.3 | 62 | 4.5 |

^aExpressed in µg-P nitrophenol released/100 mg tissue/h. NTU, Nephelometric turbidity unit.

extensively studied⁴, little attention has been devoted to the part played by biocontrol agent *Trichoderma*. The objective of the present study was to test several species of *Trichoderma*, commonly used as biocontrol agents for their ability to solubilize water insoluble phosphates. With this in view, *in vitro* studies were carried out to test the amount of P solubilized by three species of *Trichoderma* and to compare it with P solubilized by *Bacillus megatherium* and *Aspergillus awamori*. Two forms of P mainly tricalcium phosphate (TCP) and rock phosphate (RP) were used for the present study.

Three selected strains of *Trichoderma*, viz. *T. viride*, *T. harzianum*, *T. pseudokoningii* were procured from the Department of Plant Pathology, Tamil Nadu Agriculture University, Coimbatore. Their efficacy was compared with *A. awamori* and *B. megatherium* with proven efficiency to solubilize different phosphates. Pikovskaya's medium described by Pikovskaya⁵ and modified Pikovskaya medium described by Sunder Rao and Sinha⁶ were used in the experiments.

Phosphate solubilizing activity was assayed in 100 ml aliquots of Pikovskaya's broth. The medium was modified by replacing TCP with RP equivalent of 0.2% P₂O₅. The method was essentially the same as described by Bardiya and Gaur⁷. The flasks were incubated at 32°C for 15 days. The medium was analysed for soluble P. For this 5 ml growth medium was taken out from the flask, filtered through Whatman filter paper (No. 42) and the filtrate analysed for P₂O₅ content by the chlorostannous reduced molybdophosphoric blue method⁸. The final pH of the growth medium and turbidity were noted. For each batch separate control was kept. Phosphatase activity was estimated by the method described by Tabatabai and Bremner⁹.

Data indicated that among three species of *Trichoderma* tested, *Trichoderma viride* was superior to *B. mega-*

therium in solubilizing P from TCP and RP with significant increase in mat weight over test organisms due to addition of rock phosphate.

More phosphate solubilizing (PS) activity and acid phosphatase was observed with TCP treated with *T. harzianum*. The turbidity of culture media was much reduced with RP treatment compared to control.

Table 1 shows that all the three test fungi were capable of solubilizing RP and TCP. RP was solubilized to a lesser extent than TCP as evidenced by maximum solubilization of RP by *T. harzianum* and maximum solubilization of TCP by *T. viride*.

Between two types of phosphates tested, the highest solubilization of TCP was by *T. viride*. Bardiya and Gaur studied RP solubilization by *Aspergillus* sp. and *Pencillium* sp. *A. awamori* and *B. polymyxa* in the present study showed lesser extent of solubilization while comparing the relative efficiency of PS organisms using different phosphates. It was observed that, solubilization of phosphates depended on the nature of phosphates and the organisms. This is the first comprehensive report on *Trichoderma* species solubilizing different insoluble forms of phosphate compounds. Application of *Trichoderma* has a growth-promoting effect on pulses and oil seeds¹⁰. The present findings establish that release of P for growth of plant is an additional advantage of this biocontrol fungus, which is more effective in solubilizing insoluble P than *B. megatherium*.

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Potential distribution of methane hydrates along the Indian continental margins

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Bathymetry, sea-bottom temperature, geothermal gradient within the sediments and latitude are some of the important parameters that control the thickness of Hydrate Stability Zone (HSZ) in marine environment. This thickness for Indian continental margins is calculated from simultaneous solutions of gas-hydrate phase and pressure-temperature equations using the available data set for the parameters listed above. These results are presented as a contour map of potential thickness of HSZ which helps in searching for bottom simulating reflectors. If gas-hydrates occur along the Indian continental margins, then the potential thickness of HSZ can be inferred from this map.

GAS-HYDRATES are ice-like crystalline compounds of water and gas molecules that are stable at sufficiently low temperatures and high pressures. Generally, these conditions can be met either in deep continental margins or in permafrost regions of the world¹. It is estimated that energy in gas-hydrate reserves is twice the known fossil fuel energy reserves of the world. In marine environment, gas-hydrates can be inferred from an anomalous reflector encountered on marine seismic records. These reflectors are observed to mimic the seafloor topography and hence are called the bottom simulating reflectors (BSRs). BSRs are observed to be in close coincidence with the theoretical base of the methane hydrate stability zone².

Gas hydrates can form and accumulate in marine sediments with sufficient gas molecules in pore water,

if the thermodynamic conditions are favourable. For the production of biogenic methane there must be a sedimentation rate greater than 30 m Ma^{-1} , an organic carbon content exceeding 0.5% and a residual methane content $> 10 \text{ ml l}^{-1}$ (ref. 3). Along the Indian continental margins, sedimentation rate and organic carbon content are in excess of the minimum requirements, the average content of organic carbon being 1.5% (ref. 4).

The gas-hydrate exploration program requires identification of BSRs at the appropriate depth corresponding to the base of hydrate stability zone on the seismic sections. In this paper, theoretical base of hydrate stability for the Indian continental margins is computed and presented as a contour map. This map will be useful to the explorationist in setting a depth window for searching BSRs.

The well-known gas-hydrate phase diagram illustrates the phase equilibrium among gas-hydrates, free-gas and aqueous solution, and also the physical parameters controlling the formation of gas-hydrate (P, T, and salinity). Phase diagram for continental margins (Figure 1) shows that these conditions can be met in the shallow layers of the sediments at water depths exceeding 500 m to some depth below the seafloor controlled by geothermal gradient. Along the Indian continental margins these conditions can be met at water depths exceeding 750 m (ref. 5). This corresponds to the intersection point of hydrothermal gradient curve for tropical conditions with phase boundary curve. However, introduction of higher molecular weight gases (ethane, propane) to methane allows the gas-hydrates to form at low pressures and

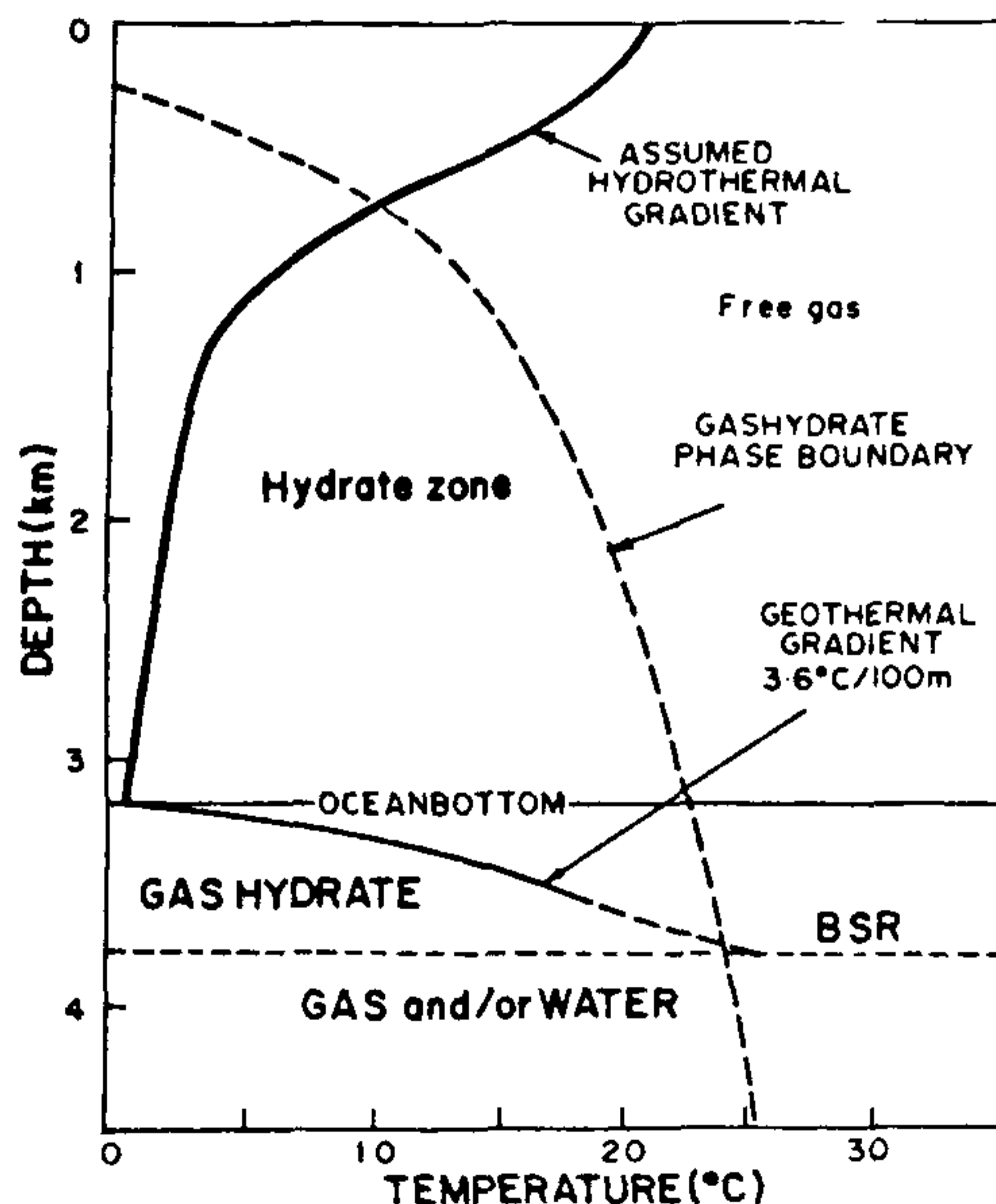


Figure 1. Phase diagram of methane hydrate in marine environment.

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