Isolation and growth kinetic studies of novel isolates from Indian Ocean nodules

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Marine isolates from sea nodules can be worthy catalysts to explore the possibilities of faster bioleaching of low index ocean nodules mined from Indian Ocean. The native microbes were isolated from Indian Ocean Nodules in Artificial Sea water Nutrient Broth at pH 7.0. Organisms have been found to tolerate up to 7% salt concentration. These species were found to be typically elongated gram positive bacilli and having a good generation time (0.7-1.3 h). The faster growth kinetics was found to useful in sequential adaptation on various metal concentrations. Both species exhibited good resistance towards 1000 ppm Ni (II) concentration; whereas resistance towards Cu(II) and Co(II) could only been seen at a metal concentration of 100 ppm thereby proving their efficacy in metallurgical applications.

[Keywords: sea nodules, metals, bioleaching, native isolate, adaptation, metal tolerance]

Introduction
Ocean Nodules serves as the richest and reliable secondary resource of various metals for want of exploitation1-4. For decades, mining companies eyed with interest the fist-sized lumps of mineral littering the floor of the world's deep oceans5. Made largely of manganese dioxide and other oxides, these mysterious nodules harbours valuable metals like copper, nickel, iron, manganese, zinc and cobalt. Available estimates show that Ni and Co availability in the nodules is about 5-40 times higher than in land based resources. The current on-going practices for processing of sea nodules are applicable for resources having up to 2% w/w copper. The huge tonnage of sea nodules are also mined out daily and dumped containing less than 0.5% copper and other metals6-9. The cost of mining on the seabed at least 4 kilometres down makes the prospect unattractive to most companies, but a bacterium that inhabits the nodules could improve the economics of deep-sea mining by making extraction cheaper by bio-leaching10-13. The current practices of leaching involve ammonial leaching and chemical leaching by acids. While the former is done under high pressure and involves the risks of explosions, the latter uses highly corrosive acids like HCl, HNO3, H2SO4 which are very corrosive and dangerous to handle. Also, the recovery of Co and Fe is lesser when compared to bioleaching methods14. Therefore bioleaching technique is not only novel but also extremely safe. The researchers isolated a marine Bacillus from the nodules and fed nodules with the initial concentrations of Co, Cu and Ni being 17, 114 and 115 ppm, respectively, for 1% pulp density. Around 45% Co and 30% of Cu and Ni were dissolved at pH of 8.2 in 10 h14. It was noted from the above work that pH is one factor which, if brought down to 6-7, can be quite economical for process applications.

Hence, the paper illustrates isolation of native marine isolates from the sea nodules and characterize them using various biochemical techniques and to study their growth kinetics. Later these, bacterial species can be used for further bioleaching of these nodules itself and to recover metals from them.

Materials and Methods

Microbial Culturing
Artificial Sea Water Media (ASWNB) was used to cultivate the microorganisms from the sea nodules, whose composition14 in (g/L) is as follows: Sodium Chloride-28.13, Potassium Chloride-0.77, Calcium Chloride-1.6, Magnesium Chloride-4.8, Sodium Bicarbonate-0.11, Magnesium Sulphate- 3.5, Peptone-5, Beef Extract-3.5. The above components were mixed and the pH was adjusted to 7.0. 20 g/L bacteriological agar was added per 100 mL of ASWNB for making media plates. The isolation of bacteria was carried out by initially boiling sea
nODULES in water for about 30 minutes in order to remove the surface contaminants and make use of the in-situ microorganisms. These sea nodules were then allowed to cool and ground for a particle size of -300μm size. Above powdered fractions were then added to flasks containing 100 mL of autoclaved ASWN Agar media. In order to optimize the conditions for growth of bacteria, the pH was adjusted to 7, 4.5 and 2.0; unless stated otherwise. Flasks were incubated in an orbital shaker with temperatures set at 35°C and 100 rpm. The pH was adjusted using 10N H₂SO₄ and 2N NaOH on daily basis using a pH meter [Model-Toshniwal; CL 54]. Redox potential was measured against a saturated calomel electrode. Growth of the bacteria was ensured by carrying out cell counting in Petroff-Hauser chamber and OD estimation at regular intervals. An initial culture of bacteria was prepared by boiling sea nodules and performing a spread plate technique. Then, at regular intervals different colonies were streaked onto ASWNA Agar using quadrant streaking method. The tail colonies from these plates were viewed under the microscope for their purity and were again subcultured. Thus, different bacterial colonies were obtained by regular sub-culturing activity. Later two different pure strains of bacteria were obtained which were named 7-BIOSN and α-BIOSN.

Results and Discussion

Microbiological techniques were dedicated to isolate the pure strains from sea nodules in pre-defined media and efforts made are described for obtaining two pure isolates from a complex system of gram positive and negative cells. Later these two strains were inoculated in ASWNB and left for culturing. Samples at regular intervals were collected from broth for their OD measurement and cell counting activity to study their growth kinetics. The pictures depicted in Fig. 1(a,b) and Fig. 2(a,b) are respectively for final pure isolates of 7-BIOSN and α-BIOSN.

Growth Kinetics of isolates: The final isolates were grown in ASWNB broth at pH 7.0 and 35°C. Samples for assessment of growth kinetics and cell count were taken at regular intervals of time.

A) Growth Kinetics of 7-BIOSN

Over the growth period of 7-BIOSN, the pH initially increased from 7 to 7.15 and later decreased to 6.89 after 16 hours of growth. Conversely the Eh
increased from 166 mV to 522 mV. This isolate had an exponential rise in cell count as depicted in Fig. 3, where it reached a maximum cell count of $3 \times 10^9$/mL in 16 h. The optical density were measured which also showed an exponential rise. In (Cell count (t)/initial cell count) vs. time, also called a first order plot was made and a straight line was fit in to obtain specific growth rate $\mu$. $R^2$ value of 0.9892 indicates a very decent fit. $\mu$ was calculated from the slope of the straight line (Fig. 4) and was found to be 0.5222 h$^{-1}$. From this, generation time was calculated to be 1.327 h.

B) Growth Kinetics of $\alpha$-BIOSN

Over the growth period of $\alpha$-BIOSN the pH initially increased from 7.02 to 6.84 after 16 hours of growth. Conversely, the Eh decreased from 176 mV to 458 mV. This isolate also had high rise in cell count reaching the exponential phase in cell count as depicted in Fig. 5, where it reached a maximum cell count of $5 \times 10^9$/mL in 16 h. The optical density were measured which also showed an exponential rise. In (Cell count (t)/initial cell count) vs. time, also called a first order plot was made and a straight line was fit in to obtain specific growth rate $\mu$. $R^2$ value of 0.9884 indicates a very decent fit. $\mu$ was calculated from the slope of the straight line (Fig. 4) and was found to be 0.9401 h$^{-1}$. From this, generation time was calculated to be 0.7371 h.

The relatively high growth rate of bacterial species (deduced from its generation time) clearly inferred on effective metal tolerance of these species omnipresent in sea nodules. The comparative studies of growth characteristics for 7-BIOSN and $\alpha$-BIOSN in the presence of NaCl revealed different growth pattern for both the isolates. 7-BIOSN was found to have a stationary growth feature upto 5% Concentration of NaCl, whereas, $\alpha$-BIOSN was observed to highly halophilic and able to multiply even at 7% NaCl concentration. However in presence of NaCl concentration more than 7%, longer exponential phase was observed.

![Fig 3 — Change in cell count of 7BIOSN with respect to optical density (OD) at 600nm with time.](image1)

![Fig 4 — Variation in cell count with time for estimation of generation time for 7BIOSN and $\alpha$-BIOSN](image2)

![Fig 5 — Change in cell count of $\alpha$BIOSN with respect to Optical Densinity (OD) at 600nm with time.](image3)
Biochemical Tests

Biochemical characteristics are one of the important methods for classification of organisms. This is mainly based on the various biochemical reactions taking place in their metabolic and normal pathways. There microbiological and biochemical features as characterized are summarized in Table 1. It clearly depicts the efficiency of bacterial species to survive in a high salt concentration, by means of monosaccharide metabolism and eliciting a good cellular multiplication.

Metal Tolerance

The purely isolated species from sea nodules were tested for metal toxicity evaluation by adaptation on various concentrations of synthetic salts of copper, nickel and cobalt. It was found that both species exhibited good resistance towards 1000 ppm Ni (II) concentration; whereas resistance towards Cu(II) and Co(II) could only been seen at a metal concentration of 100 ppm (Table 2). These values can be improvised on subsequent sub-culturing for definite applications in bioleaching of low-index sea nodules and pose as a definite alternate technology for future.

Conclusions

The following major conclusions can be drawn based on the above study: Two marine species isolated from the polymetallic Indian Ocean nodules were found to grow well in artificial seawater nutrient broth at neutral pH. The species were tolerant to NaCl concentration of nearly 5-7%, suiting to their application environment. Bacterial species possessed a relatively higher growth rate and a good resistance to metal concentrations. Both species exhibited good bacterial resistance towards 1000 ppm Ni (II) concentration; whereas resistance towards Cu(II) and Co(II) could only been seen at a metal concentration of 100 ppm, proving their amenable utilisation in bioleaching processes.

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References


