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Effect of surface active agents on bioleaching of Silica and Iron oxide from Indian Chromite Ore by Silica resistant *Aspergillus niger* AB_{200}

Deblina Ball [1*], A.K.Banik [1]

1. Department of Chemical Engineering, University of Calcutta., 92, A.P.C. Road. Kolkata-700009.
[Email: deblina.ball@gmail.com, Tel: +91-9830819998]

Surface active agents were found to have a role in the surface culture fermentations required during bioleaching of silica and iron oxide from chromite ore. All the surface active agents i.e, Sodium Lauryl Sulphate, Tween 20, Tween 40, Tween 80 decreased cell growth of *Aspergillus niger* AB_{200}, silica and iron oxide bioleaching from chromite ore. Sodium Lauryl Sulfate when added in 2% and 3% concentration in the fermentation medium on the zero day of fermentation, totally inhibited the growth of the microorganism as well as silica and iron oxide leaching from Chromite ore. But addition of Sodium Lauryl Sulfate in the fermentation medium on the 3rd and 5th day of fermentation also reduced growth and leaching to some extent. These surface active agents therefore if present in the fermentation medium of chromite bioleaching, reduces silica and iron oxide leaching and therefore should be avoided during the plant set up for bioleaching of chromite ore.

Keyword: Bioleaching, Chromite ore, Surface active agents, *Aspergillus niger*.

1. Introduction

High grade Chromite ore is depleting very fast and so low grade chromite ores would be the choice for obtaining chromium required in metallurgical, refractory and chemical industries. Removal of elements like silica and iron oxide from chromite ore by biological process (bioleaching) would increase the concentration of chromium present in the ore and would convert it into high grade quality. *Aspergillus niger* used for bioleaching of silica and iron oxide from Chromite ore show proper growth and bioleaching in presence of chemical nutrients in the fermentation medium during surface culture fermentation process. Some chemicals were also found to have a negative effect during the surface culture fermentation process.

Surface active agents may be defined as substances which alter the energy relationships at interfaces. Among the manifestations of these altered energy relationships is the lowering of surface or interfacial tension. Compounds displaying surface activity are characterized by an appropriate structural balance between one or more water-attracting groups (hydrophilic or polar groups) and one or more water-repellent groups (hydrophobic, non-polar, or hydrocarbon groups). The electrical charge on the hydrophilic portion of a surface active agent may serve as a convenient basis of classification of these compounds. Dependent upon the nature of this charge, or the absence of ionization, surface active agents have been classified as: Ionic and Non-Ionic [1].

Generally surface active agents are found to have an inhibitory effect on growth of microorganisms. Surface active agents when come in contact with biological cells, frequently causes cytolysis. They generally solubilize lipids and proteins of cell membrane and causes cell lysis [2]. They are also found to precipitate proteins from solutions [3]. It is well recognized that culture media ordinarily have surface tension values lower than that of water, due to their protein content. Reducing the surface tension of the medium may well change the character of growth of microorganisms. In addition to alteration of the character of growth, culture media of low surface tension may depress or prevent growth. But some research has shown that addition of Tween 80 to an optimal level of 0.1%, greatly enhanced the growth of some microorganisms [4]. Behera *et al*. also reported that Tween 20 accelerated the consumption of sucrose by *Aspergillus niger* and thus enhancing the extraction of nickel from chromite overburden [5].

Therefore, considering the different roles of surface active agents on growth of microorganisms, our present study was conducted to find out the effect of surface active agents on growth of *Aspergillus niger* AB_{200} and thereby its bioleaching capacity of silica and iron oxide from chromite ore.

2. Materials and Methods

Chromite ore: Chromite ore used in the study of bioleaching is obtained from the Sukinda Valley, Orissa, India. The ore was crushed to 200 mesh size and the silica and iron oxide content in the ore were estimated [6].

Microorganism: The parent of *Aspergillus niger* was isolated from the soil of North Bengal and silica tolerant *Aspergillus niger* AB_{200} was developed from the parent culture which was used for the present study [6].

i. Growth medium and growth conditions:

The cultures were maintained on agar slants having composition: glucose – 5%, $NaNO_3$ – 0.2%, KH_2PO_4 – 0.1%, KCl – 0.05%, $MgSO_4.7H_2O$ – 0.05%, Na_2SiO_2 – 0.18%, Agar – 4%. pH was adjusted to 4.8-5.0 using 0.1(N) HCl. The slants were incubated for 7 days at 30 ^{0}C.

ii. Preparation of Inoculum:

Full grown slant cultures were scrapped off and suspended in 100 ml double distilled water. The suspension contained 1.4×10^6 spores/ml and was used as inoculum [6].

Composition of the synthetic medium and conditions for bioleaching:

The following synthetic media was used for bioleaching studies:- glucose – 10%, $NaNO_3$ – 0.2%, KH_2PO_4 – 0.1%, KCl – 0.05%, $MgSO_4.7H_2O$ – 0.06%. pH – 4.5.

Surface culture fermentation was carried out using 100 ml Polypropylene flask which contained 0.3 gm chromite ore and 30 ml fermentation media. 6 ml inoculum was added to each flask and incubated at 33 ^{0}C for 7 days.

iii. Addition of Surface Active agents in the fermentation media:

The effects of varying concentrations 1%, 2% and 3% of different surface active agents, namely Sodium Lauryl Sulphate (Ionic), Tween 20 (Non – Ionic), Tween 40 (Non – Ionic), Tween 80 (Non – Ionic) added at different time intervals(0 day, 3rd day, 5th day of fermentation) on the growth of *Aspergillus niger* AB_{200}, silica and iron oxide bioleaching were studied. Each experiment was carried out with one set of control which did not contain the particular surface active agent in the fermentation media, which was under study.

iv. Estimation of silica and iron oxide in the fermentation media:

The organism leach out silica and iron oxide from the ore into the fermentation media, which is then estimated colorimetrically by silicomolybdate method [7] and thiocyanate method [8] respectively.

v. Estimation of Dry cell weight:

The cells were separated from the fermentation media by filtration with Whatman no.1 filter paper, washed with distilled water and filtered once again. The mass was dried in an oven at 100 ^{0}C for 24 hrs.it was then weighed to obtain the dry cell mass [6].

vi. Statistical Analysis

All data were expressed as Mean ± SEM, where n=6. Statistical analysis were performed according to student's t distribution. The level of significance for two tail test was determined from the table with critical values of " t ".

3. Result and Discussion

Table 1: Effect of Surface Active agents on silica and iron oxide leaching from chromite ore.

Surface Active agents	Day of Addition	Concentration (%)	Cellular growth Dry weight (mg/ml)	Silica leaching (%)	Iron oxide leaching (%)
Control	-	0.0	15.20 ± 0.054	70.76 ± 0.065	75.30 ± 0.086
Sodium Lauryl Sulphate	0	1	4.95 ± 0.056	20.32 ± 0.075	22.45 ± 0.068
		2	-	-	-
		3	-	-	-
	3	1	11.42 ± 0.045	53.26 ± 0.075	66.00 ± 0.085
		2	10.86 ± 0.056	47.86 ± 0.067	57.55 ± 0.076
		3	8.32 ± 0.055	37.25 ± 0.074	42.20 ± 0.079
	5	1	15.15 ± 0.052	70.76 ± 0.057	75.30 ± 0.068
		2	14.83 ± 0.075	67.76 ± 0.066	71.24 ± 0.085
		3	13.86 ± 0.057	64.20 ± 0.069	70.24 ± 0.075
Tween 20	0	1	6.82 ± 0.048	29.33 ± 0.075	39.24 ± 0.068
		2	4.46 ± 0.058	18.26 ± 0.063	20.20 ± 0.096
		3	2.56 ± 0.037	7.60 ± 0.068	18.00 ± 0.059
	3	1	12.80 ± 0.065	58.36 ± 0.075	70.80 ± 0.069
		2	11.86 ± 0.075	54.32 ± 0.064	65.42 ± 0.076
		3	10.26 ± 0.065	47.18 ± 0.085	55.20 ± 0.075
	5	1	15.10 ± 0.045	70.76 ± 0.076	75.30 ± 0.055
		2	15.02 ± 0.046	70.76 ± 0.087	75.30 ± 0.068
		3	14.60 ± 0.043	69.22 ± 0.095	74.78 ± 0.128
Tween 40	0	1	8.36 ± 0.057	37.34 ± 0.075	40.24 ± 0.065
		2	5.24 ± 0.066	22.36 ± 0.065	32.65 ± 0.086
		3	2.36 ± 0.038	8.74 ± 0.067	15.76 ± 0.062
	3	1	12.95 ± 0.054	60.63 ± 0.046	70.20 ± 0.075
		2	11.64 ± 0.057	57.24 ± 0.068	66.25 ± 0.077
		3	10.38 ± 0.047	48.64 ± 0.068	55.70 ± 0.055
	5	1	15.15 ± 0.027	70.76 ± 0.054	75.30 ± 0.046
		2	15.00 ± 0.046	70.76 ± 0.065	75.30 ± 0.045
		3	14.70 ± 0.045	68.26 ± 0.075	73.24 ± 0.057
Tween 80	0	1	7.36 ± 0.057	32.24 ± 0.068	40.65 ± 0.085
		2	4.20 ± 0.054	17.24 ± 0.065	20.20 ± 0.086
		3	2.15 ± 0.068	6.30 ± 0.076	10.55 ± 0.072
	3	1	12.40 ± 0.034	57.36 ± 0.065	70.00 ± 0.075
		2	11.38 ± 0.054	53.80 ± 0.044	65.24 ± 0.075
		3	9.36 ± 0.054	42.20 ± 0.068	54.00 ± 0.078
	5	1	15.02 ± 0.075	70.76 ± 0.064	75.30 ± 0.075
		2	14.80 ± 0.065	68.26 ± 0.056	74.00 ± 0.088
		3	14.55 ± 0.085	67.76 ± 0.055	72.22 ± 0.067

Values are expressed as Mean ± SEM, where n=6

From Table 1, it is evident that all the surface active agents i.e. Sodium Lauryl Sulphate, Tween 20, Tween 40, Tween 80 decreased cell growth, silica and iron oxide bioleaching from chromite ore. The inhibitory effect of these surface active agents were to a large extent higher, when they were added in the fermentation medium on the zero day of incubation. Total inhibition of growth and leaching was observed, when Sodium lauryl sulphate was added in the fermentation medium on the zero day of fermentation ($p<0.001$). The surface active agents disrupts the cell membrane of the fungal cells and causes cell lysis, which may be the cause of reduction in growth and leaching capacity of the organism.

4. Conclusion

From the present study, it can be concluded that presence of Surface Active Agents in the fermentation medium during the bioleaching process disrupts growth as well as bioleaching capacity of the microorganism and thereby inhibits the upgradation process of chromite ore by the microbial process. So Surface Active agents or any chemical having such properties should be avoided from the fermentation medium during bioleaching experiments.

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6. References

1. Price D. Certain aspects of the chemistry of surface active agents. Ann N Y Acad Sci 1946; 46:407-426.
2. Van BRM, Lambrecht RS, Collins MT. Effect of polyoxyethylene sorbate compounds (Tweens) on colonial morphology, growth and ultrastructure of Mycobacterium paratuberculosis. APMIS 1990; 98(7-12):901-908.
3. Baker Z, Harrison RW, Miller BF. Inhibition by phospholipids of the action of synthetic detergents on bacteria. J Exptl Med 1941; 74:621-637.
4. Dubos RJ. The effect of lipids and serum albumin on bacterial growth. J Exptl Med 1947; 85:9-22.
5. Behera SK, Sukla LB. Microbial Extraction of nickel from Chromite overburdens in presence of surfactant. Transactions of Nonferrous Metals Society of China 2012; 22(11):2840-2845.
6. Ball D, Banik AK. Studies on bioleaching of silica from Indian chromite ore by a silica tolerant Aspergillus *niger* AB_{200}: Characterisation of some physical parameters and scanning electron microscopic studies. Journal of Indian Chemical Society 2010; 87: 857-862.
7. Jeffery GH, Basset J, Mendham J, Denney RC. Vogel's Text book of Quantitative Chemical Analysis, Edn 5, Longman Scientific and Technical, UK, 1989, 703-704.
8. Jeffery GH, Basset J, Mendham J, Denney RC. Vogel's Text book of Quantitative Chemical Analysis, Edn 5, Longman Scientific and Technical, UK, 1989, 690-691.