Enhancement of microbial silica solubilization from magnesite ore through mutagenic treatment of *Bacillus licheniformis*

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Presence of silica in magnesite lowers its refractory value. The chemical beneficiation of magnesite is both uneconomical and troublesome. *Bacillus licheniformis*, a potential silica remover from magnesite ore, was treated with UV, MNNG and EMS successively, to improve its silica-solubilizing capacity. The mutant strain released 348 $\mu g\ ml^{-1}$ of Si in comparison to the wild which released only 128 $\mu g\ ml^{-1}$ of Si. Later, both the strains were compared in a glass air-lift fermenter for removal of silica from Salem magnesite ore where the mutated strain showed a very encouraging result.

MAGNESITE is generally used for the production of refractory bricks. The presence of silica above 2% renders these unsuitable for withstanding very high temperature. The chemical and physical methods of silica removal from such ores are not only uneconomical but also troublesome. The successful application of bioleaching techniques of various sulphide minerals in the recent times prompted us to exploit the process for enrichment of magnesite ore by removing silica from the ore. The release of silica from different ores by various heterotrophic microorganisms is well documented[1-7]. We have isolated some silicate bacteria[8] which can release significant amounts of silica from magnesite ore. The best strain was identified as *Bacillus licheniformis*. It has been studied intensively for its various physical and chemical parameters that affect the release of silica[9-11]. Here, we report the improvement of the strain through various mutagenic treatments and the release of silica from magnesite ore by the mutant in a glass air-lift fermenter.

The microorganism was grown in the medium having the composition (g/l) $(NH_4)_2SO_4$, 0.25; $MgSO_4 \cdot 7H_2O$, 0.25; $Na_2HPO_4 \cdot 2H_2O$, 0.25; KH_2PO_4, 0.25; yeast extract, 1.0; glycine, 1.0; $MnSO_4 . H_2O$, 0.025; $ZnSO_4 . 7H_2O$, 10 ppm. The pH was adjusted to 6.0 with sterile H_2SO_4 (1 N) after sterilization of the medium. Fructose was sterilized separately and added aseptically to the medium as carbon source to have a final concentration of 1.5%.

The air-lift fermenter used earlier[12] was further modified (Figure 1) to avoid various drawbacks encountered during its earlier operation. The modifications were as follows:

i) The main body of the fermenter was enlarged to 365 mm length and 80 mm diameter to accommodate larger amount of ore (500 g).
ii) The joint between the jet and the lower stop-cock was slightly angled to check the back flow of the air into the medium.
iii) A rubber plug was used in place of cotton at the top of the fermenter to avoid the outer contamination by sprinkling of the medium from the shower-back into the cotton plug.
iv) An additional air out-let was installed through the rubber plug so that the fermenters could be attached in a series.

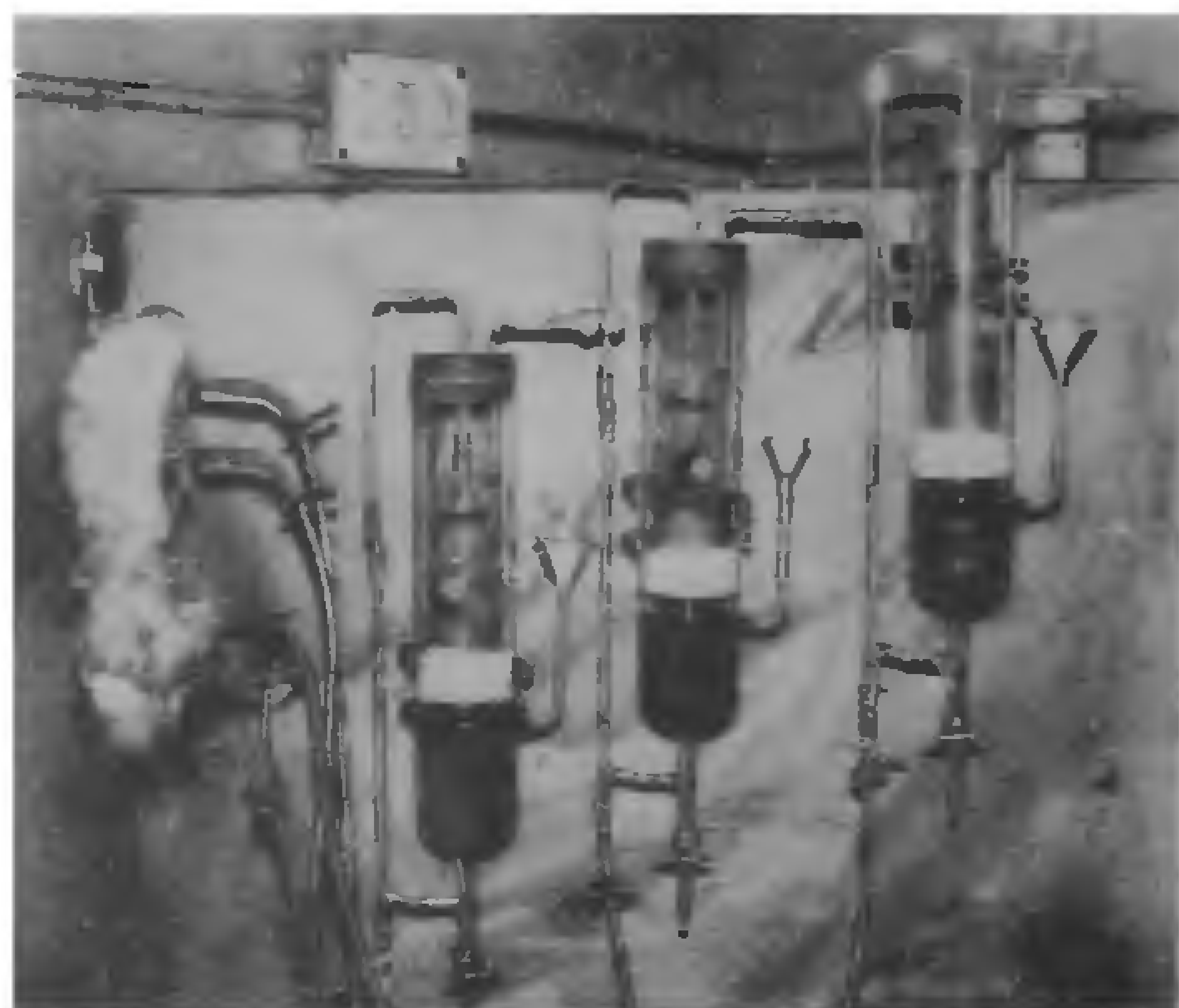

Figure 1. Modified air-lift fermenter.

The fermenter charged with 500 g of magnesite ore[12] was sterilized in an autoclave at 1.1 kg/cm^2 for 20 minutes. Sterilized medium (980 ml) along with the carbon source was introduced into the fermenter through the side arm funnel maintaining an aseptic condition followed by the addition of 20 ml of 24 h grown culture (10^6 cells ml^{-1}) as inoculum. In the control, no inoculum was added. The medium was continuously circulated through the ore by passing the sterilized air from a compressor at 37°C. At various incubation periods the ore samples were taken out aseptically from the fermenters, washed repeatedly in running tap-water and then in distilled water. Silica and magnesium content (%) were estimated following the methods of Washington[13] and Indian Standards[14] respectively.

A typical mutation selection programme involved several mutational steps, so that better and better strains were successfully selected for the desired property. In fermentation process, it is considered better to follow mild mutagenic treatment by strong ones, while a strong mutation should be followed by weaker treatment capable of inducing productive changes[15].

Exploitation of the above knowledge made it possible to proceed through the following mutation selection programme with a view to isolating mutants of remarkable potency for better silica solubilization.

$$\text{Wild sp.} \xrightarrow{\text{UV}} M_1 \xrightarrow{\text{MNNG}} M_2 \xrightarrow{\text{EMS}} M_3,$$

where M_1, M_2 and M_3 represent the superior silica solubilizing mutants selected from the survivors following the mutagenic treatments. The methods for all mutagenic treatment were as described earlier[16]. After each mutagenic treatment, properly diluted cell suspensions were spread on silica agar plates[8] and incubated at 37°C for 4 days. Colonies showing improved solubilization zone[8] in comparison to control were primarily selected as improved strains. The five superior mutants showing higher solubilization index[8] in comparison to the wild strain and other mutant isolates, were later on inoculated into the fresh liquid medium to verify the actual quantity of Si released by each strain as reported earlier[8–10]. The best mutant at each step of mutation was selected on the basis of its efficiency to release maximum silica and minimum magnesium in the liquid broth in comparison to the wild type and other mutants.

The wild strain of the *B. licheniformis* was highly sensitive to ultra violet (UV) irradiation. Ninety-nine per cent of the cells were killed at 30 sec exposure to UV (100 ergs mm^{-2} sec^{-1}). A number of strains isolated after UV irradiation (40–50 sec of UV exposure) showed improved solubilization zone. The release of Si and Mg by 5 superior mutant strains is shown in Table 1. U_{68} released more Si than the wild strain (U_0), which was considered to be the best mutant at this mutational step.

When U_{68} was further subjected to *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG) treatment for 20 minutes, at 100 μg ml^{-1}, more than 99% of the cells were killed. However, unlike UV irradiated cells, maximum improved solubilization zone by the cells was observed in the range of 85–95% killing (25–30 μg ml^{-1} of MNNG treatment). The 5 superior mutants showing the quantity of Si and Mg released, are given in Table 2. A high increase in the release of Si was

Table 1. Release of Si and Mg by the improved mutant strains obtained through UV irradiation

Strain no.	Si released* (μg ml^{-1})	Mg released* (μg ml^{-1})
U_0	128	26
U_{66}	134	34
U_{68}	148	26
U_{74}	130	36
U_{82}	136	24
U_{91}	132	28

*After 96 h.
U_0 = (*Bacillus licheniformis*, wild strain).

Table 2. Release of Si and Mg by the MNNG-treated improved strains

Strain no.	Si released* (μg ml^{-1})	Mg released* (μg ml^{-1})
$U_{68}N_0$	146	24
$U_{68}N_{33}$	242	26
$U_{68}N_{39}$	235	32
$U_{68}N_{43}$	239	39
$U_{68}N_{53}$	227	27
$U_{68}N_{66}$	267	29

*After 96 h.
$U_{68}N_0 = U_{68}$ (UV mutant strain).

Table 3. Release of Si and Mg by EMS-treated improved strains

Strain no.	Si released* (μg ml^{-1})	Mg released* (μg ml^{-1})
$U_{68}N_{66}E_0$	264	28
$U_{68}N_{66}E_{61}$	319	27
$U_{68}N_{66}E_{68}$	321	29
$U_{68}N_{66}E_{73}$	261	24
$U_{68}N_{66}E_{77}$	348	22
$U_{68}N_{66}E_{89}$	251	26

*After 96 h.
$U_{68}N_{66}E_0 = U_{68}N_{66}$ (MNNG mutant strain).

achieved by the mutant $U_{68}N_{66}$ obtained through MNNG treatment in comparison to U_{68} obtained through UV treatment. $U_{68}N_{66}$ was considered to be the best mutant at this step.

Being a relatively low lethal mutagen, 1% EMS brought down 99% cell killing of $U_{68}N_{66}$ upon an exposure of 1 h. However, a number of colonies showed improved solubilization zone in the killing range of 97–98% (i.e. 45–60 min of treatment). The superior mutants showing the quantity of Si and Mg released are shown in Table 3. $U_{68}N_{66}E_{77}$ was considered to be the best mutant at this step.

Our main aim of the study was to use the bacterial strain for removal of silica from Salem magnesite ore. Experiments conducted in flasks on laboratory shakers provided fundamental information on the strain behaviour, the composition of the medium, suitable type of inoculum, etc., but they provided meagre data on crucial technological factors, which may be assessed only in laboratory fermenters. Thus the glass air-lift fermenter as described earlier was used which gave a highly satisfactory result from the technical point of view. The wild strain could release only 47–45% of silica from the magnesite ore[12] after 24 days of treatment. Table 4, however, shows that the mutant strain, $U_{68}N_{66}E_{77}$, not only increased the silica removal from magnesite ore, but also cut down the time period from 24 days to 8 days in comparison to the wild strain.

Thus, the potency of the mutant strains creates new

Table 4. Removal of silica from magnesite ore in the fermenter after treatment with the mutant $U_{68}N_{66}E_{77}$

No. of days	Silica content* in ore (%)		Total silica removal from ore (%)	
	Control	Treated with bacteria	Control	Treated
0	5.5	5.5	–	–
4	5.2	3.6	5.45	34.5
8	5.2	2.17	5.45	60.5

Control: No bacterial inoculum was added to the fermenter.
* = average of three replicates.

hope for refractory industry, considering the rapid depletion of high grade magnesite in India.

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Taxonomic status of rust on mulberry in India

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The morphology of the rust fungus on mulberry (*Morus alba* L.) reported by earlier workers is not in conformity with generic concepts of any of the genera to which the fungus has been assigned, viz. *Caeoma, Aecidium, Uredo* and *Cerotelium*. The fungus shows characteristic features of the form genus *Peridiopsora* Kamat & Sathe apud Sathe, based on peridial characters and urediniospores. Detailed histopathological studies revealed that the rust fungus occurring on mulberry in India is *Peridiopsora*. Hence the new combination *Peridiopsora mori* (Barclay) Prasad *et al.*, comb. nov. has been suggested for the fungus.

Caeoma mori Barclay[1], *Aecidium mori* Barclay[2], *Uredo mori* Barclay sensu Saccardo[3], *Aecidium mori* (Barclay) Sydow et Butler[4], and *Cerotelium fici* (Cast) Arthur[5,6] have been reported to be the causal organisms of rust disease in *Morus alba* L. and *M. indica* L.

The review of literature reveals that this rust is known to the Indian workers in the field either as *Aecidium mori* Barclay[7] or as *Cerotelium fici* (Cast) Arthur[5,6]. It is noteworthy in this connection that Barclay himself had described this rust under *Caeoma mori* and as such the transfer effected as *Aecidium mori* (Barclay) Syd. & Butler is a valid transfer. As far as its placement under *Cerotelium fici*, it was made out of the confusion arising due to the fact that Barclay while describing the rust under *Aecidium mori* also included the rust on *Ficus* because the uredinial sori of *Uredo fici* Cast. are in fact peridiate and ostiolate[8]. Subsequently, however, the two rusts namely *Aecidium mori* and *Cerotelium fici* were separately treated by Sydow & Butler[4]. *Cerotelium fici*, therefore, is a misnomer for the rust on mulberry in India. The telial stage for the rust on mulberry has not been reported so far, and the rust is believed to be prevailing in its anamorphic state only.

During the course of studies on rust fungus on mulberry in Karnataka, it was noticed that the rust fungus prevailing in the state is not in conformity with generic concepts of either *Caeoma, Aecidium* or *Uredo* and therefore, it was decided to undertake the detailed studies of the same.

Leaves infected with the rust fungus were collected from the mulberry farm of the Institute (KSSDI). The rust was found to manifest itself on the lower (adaxial) surface of leaves in the form of yellowish to reddish brown blisters grouped together (Figure 1).