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DESULFURIZATION OF PYRITIC COAL BY PHOTOSYNTHETIC SULFUR BACTERIA

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ABSTRACT

Two strains of photosyntheitic sulfur bacteria were isolated after enrichment on pyrite - containing medium from certain localities in Egypt. . Isolates were able to grow autotrophically on FeS_2 as an electron donor . They were tentatively identified as Chromatium MNII and Chlorobium NY2 . Both were able to desulfurize pyritic coal anaerobically . Sulfur leaching efficency reached 83% in case of Chromatium MN11 and 88% in case of Chlorobium NY2 . A precipitate of ferrous compound like illite , H_2S gas , and elemental sulfur were detected after desulfurization .

Key words : Chromatium sp. , Chlorobium sp. Desulfurization , Coal , pyrite leaching .

INTRODUCTION

The contribution of microorganisms other than the acidophilic becterium Thiobacillus ferrooxidans to the solubilization of minerals has been demonstrated (for review see Kelly et al . , 1979; Lundgren silver , 1980 ; Brierley , 1986 ; and Liselotte et al. , 1990) Interestingly , the use of photosyntheitic anoxygenic bacteria for leaching purposes has not been throughly investigated .

The idea of the present work depends on the fact that most strictly anaerobic phototrophic bacteria are capable of fixing CO_2 trough anoxygenic photosynthetic process in which a sulfur compound more reduced than sulfate acts as the electron donor among this group of bactria , and its oxidation product is used to fix CO_2 and to produce elemental sulfur . Elements sulfur is deposited as sulfur

produce elemental sulfur. Elemental sulfur is deposited as sulfur globules within the cell or outside the cell according to the following equation :

$$2\ H_2s + Co_2 \text{ ----------} 2\ S + H_2O + (CH_2O) \qquad (1)$$

The oxidation of H_2s to elemental sulfur takes place rapidly in the cell and sulfur is slowly oxidized to sulfate as follows :

$$2\ S + 3\ CO_2 + 5\ H_2O \text{ ------- } 2\ H_2SO_4 + 3\ (CH_2O) \qquad (2)$$

In the present investigation H_2S was practically replaced by pyrite then by coal containing pyrite. The isolated phototrophic bacteria attack pyrite in coal instead of HS converting it to HS according to the following suggested equation :

$$FeS_2 + 2\ H_2O \xrightarrow[\text{Sulfur bacteria}]{\text{Photosynthetic}} H_2S + Fe(OH)_2 + S \qquad (3)$$

Deposited sulfur is either precipitated intracellularly or extracellularly according to the isolate and the formed ferrous hydroxide is possibly precipitated as ferrous compound like illite. The formed H_2S acts in turn as an electron donor and the reaction proceeds as in equation (1). Extra sulfur precipitation increased the conversion to sulfates according to equation (2).

MATERIALES AND METHODS

Sample localities : Soil samples (mud) collected from madinet naser, a suburb of Cairo, were used for the isolation of photosynthetic sulfur bacteria. Coal samples colected from Maghara area in Sinai Peninsula, Egypt, were kindly provided by Prof. N. A. Eissa Head of

physics Dept. Fac. of Sci. AL-Azhar Univ.

Pyrite constitutes 3% (w/w) of Maghara coal (Eissa et al., 1989).

Enrichment and Isolation : The following ferrous sulfide medium was used for isolation of green and purple sulfur bacteria; (a) besal medium : NH_4Cl, 1g; KH_2PO_4, 1g; $MgCl_2$, .5g; NaCl, 5g; tap water 700mL. Distribute as 70mL aliquots in 100mL wide-mouth ground-glass stoppered bottles, (b) NaHCO, 5g; tap water 100mL, (c) FeCl. 6HO, 0.005g; tap water 100mL, (d) FeS (fine powder), 20g; presterilized tap water 100mL. Sterilize solutions a, b, and c at 121 C for 15 min, cool and aseptically add, to each basal midium bottle, 10mL of c, and 4.75mL of d, pH of the midium is 6.7 + 0.2. inoculate each bottle with 5g mud and incubate at room temprature (about 25oC) and in the dark for about 12 h then in the normal day light (300 - 500 Lux in the early morning, 3000 to 5000 Lux during noun and afternoon and dark at night) for two weeks.

Purification and maintenance : Green and purple sulfur bacteria were recorded after 10 - 13 days respectively. Eriched cell populations were removed using pasteur pipettes and directly used as inocula for agar shake dilution tubes containing 3.3(w/v) washed agar according to van Niel (1932), Larsen (1952), and Pfennig (1965). The agar solution (supplemented with the above mentioned medium) was first dispensed in 3 mL amounts into test tubes stoppered with cotton plugs. The agar tubes were kept molten in a water bath at 55 C and inoculated as above. Ten serial dilution tubes were prepared for each sample. All tubes were

then hardened in cold water and immediately sealed with 3mL of sterile overlay consisting of 1 part parafin wax and 3 parts parafin oil. Tubes were incubated in the dark for 12 h then as mentioned above. Well-separated purple-red and greenish colonies were removed with sterile Pasteur pipetts and suspended in anaerobic medium for further purification in agar shake dilutions. Purification was repeated and purity was checked using a phase contrast microscope (type II Carl Zeiss) and by the use of A-C medium (Difco). Pure caltures were maintained in 10 mL screw capped tubes containing 1% pyrite mineral medium.

Identification of pure cultures : Identification of isolates was based on the following criteria : cell morphology, sulfur deposition (microscopic investigation), color of cell suspension, and absorption spectra of cell suspensions as described by Cohen-Bazire et al., (1957) to identify the predominant bacteriochlorophyll and type of carotenoid pigments.

Assessment of pyrite oxidation : Bottles inoculatted with bacterial strains unde investigation were tightly closed and stoppers were sealed by a strong adhesive tape. Bottles were incubatted horizontally. All bottles were rotated four times/day to insre proper mixing of cells with pyrite particles. Seven day liquid cultures containing 1% Fes_2 preinculated with test organisms wre filtered through whatman No.1 ashless filter paper.

The filtrate was centerifuged at 1000 g for 15 min. The precipitate on the filter paper (pyrite residues), cell pellet and the supernatant were

oven dried at 100 C. Dried fractions were measured spectroscopically by a beam of gamma rays using the Mossbauer spectroscope type MR - 260 / MR - 360 for the detection of any iron containing compound such as Fes_2 according to the method mentioned by Montano (1981). Killed bacterial suspensions were also inoculated to a set of three control bottlescontaining pyrite medium and treated as above.

Coal desulfurization : i- Cultural conditions : The isolation medium was used to monitor desulfurization efficiency. Crushed (particle size 60 mesh) was added as 5% (w/v) instead Maghara coal of pyrite. Cultures were inoculated by test organisms and incubated as stated before for 10 days. One set of control bottles was prepared and inoculated with dead bacterial suspensions. Another set of control bottles were prepared by lowering the pH of the medium to 4.0, those bottles were not inoculated. ii- Analyses : Sets of 100 mL cultures (the test was repeated ten times) of each isolate were collected after 3, 4, 5, 6, 7. 8, 9, & 10 days respectively. Final pH of each set was measured using a glass electrode. A total of 3 x 100 mL cultures were used for total protein determination by treating cells with 0.1 M NaOH at 95 C for 30 min and measuring color reaction with folin reagent according to Lowry et al.,(1951). In order to get rid of the black coal particles prior to treating cells with Naoh, cultures were filtered through Whatman No.1 filter paper. A standard curve was made for this purpose using bovine serum albumin. Rest of cultures were filtered through Whatman No.1 ashless filter paper. coal particles were oven dried and kept for

further spectroscopic analysis. The filtrate of each culture was centrifuged at 1000 g for 15 min. The supernatant and the cell pellets were oven dried and kept for further analysis. Control bottles were also treated as above. All dried fractions were examined with Mossbauer Spectroscope type MR-260/MR-360 provided with soft-ware and a Calculation of desulfurization efficiency : Efficiency of desulfurization is calculated using the working parameters of each treatment as follows

Area of pyrite = Amplitude x line width

$$\text{Desulfurization efficiency} = \frac{\text{Area of pyrite (head sample)} - \text{Area of pyrite (treated sample)}}{\text{Area of pyrite (head sample)}} \times 100$$

Results

Tow bacterial isolates were obtained out of 50 cultures, that were able to grow autotrophically on pyrite. they were tentatively identified as Chlorobium NY2 and Chromatium MN11. Characteristics leading to such identification are presented in Table (1). The ability of both isolates to desulfurize pyrite was confirmed by mossbauer spectroscopic analysis. Typical absorption peaks of pyrite are presented in Fig (1 A & D).Mossbauer spectroscopic analysis of Chlorobium NY2 and Chromatium MN11 cultures containing 1% FeS_2 showed that : i- Typical pyrite peaks were recorded in the filter paper precipitate of the control and inoculated cultures. the area of pyrite is smaller for inoculated samples, then in control uninoculated samples this may be

due to the ability of both isolates to desulfurize (Fig. 1 A, B, & C).ii-No absorption spectra indicative of pyrite were recorded in the supernatant of both isolates although it is detected in the supernatant of the control medium. iii-A pyrite peak was recorded in the precipitate of the control bottles whereas ferrous compound like illite spectra were recorded in the cell pellet of both isolates (Fig. 1 D, E,& F). .Desulfurization of Maghara coal : A spectroscopic analysis of a head sample of Maghara coal showed tow absorption peaks one is characteristic of pyrite and the other is characteristic of szomolnokite FeSO4 .H2O (Fig. 2 a).

Upon desulfurization by phototrophic isolates one peak only is recorded showing a small area of pyrite due to bacterial leaching (FIG 2 b & c). Ferrous compound like illite spectra were also recorded in the precipitate of both isolates (Fig. 2 d & e). Spectroscopic analysis of cell debris of both isolates proved to be free from pyrite. phase contrast microscopic observations of cell pellet after desulfurization showed the deposition of intracellular sulfur granules in case of Chromatium MN11. Mossbauer spectroscopic analysis of Maghara coal particles precipitated after incubation of both control and experimental bottles showed that 83% leaching of pyrite was achieved after 7-8 days of incubation in case of Chromatium MN11 and 88% leaching by Chlorobium NY2 (Fig 3). All measurements and calculations of efficiency were made in relation to control uninoculated bottles containing 5% w/v coal. interestingly, increasing incubation period to

Table (1) : Characterstics of the isolated bacteria.

Identification criteria	Isolate NY2	Isolate MN11
Cell shape	Vibroid	ROD
Sizes	0.9 - 1.3 um wide	3.0 - 6.5 um wide
	1.2 - 1.8 Long	9.1 - 17.4 um long
Motility	non-motile	motile
Color of cell suspension	green - brown	purple red
Intracellular sulfur deposition	-	+
Type of main bacteriochlorophyll	c[1]	a[2]
Type of main carotenoid pigment	Chlorobactene[9]	Okenone[4]
Proposed identification	Chlorobium[9]	Chromatium[1]

(1) Bacteriochlorophyll c (758 nm)
(2) Bacteriochlorophyll a (840 nm)
(3) Chlorobactene (458)
(4) Okenone (522 nm)

10 days did not affect the leaching efficiency. final pH measurements of both cultures showed a drop towards the acidic range represented as 4.1 for Chromatium MN11 and 3.9 for Chlorobium NY2 after the seventh day of incubation (Fig.4)

Lowering the pH to 4.0 in the second set of control bottles has no effect on pyrite and typical absorption peaks were recorded (notrepresented). On the other hand, growth measurements represented as total protein content (mg/100 mL) showed decreased values after the

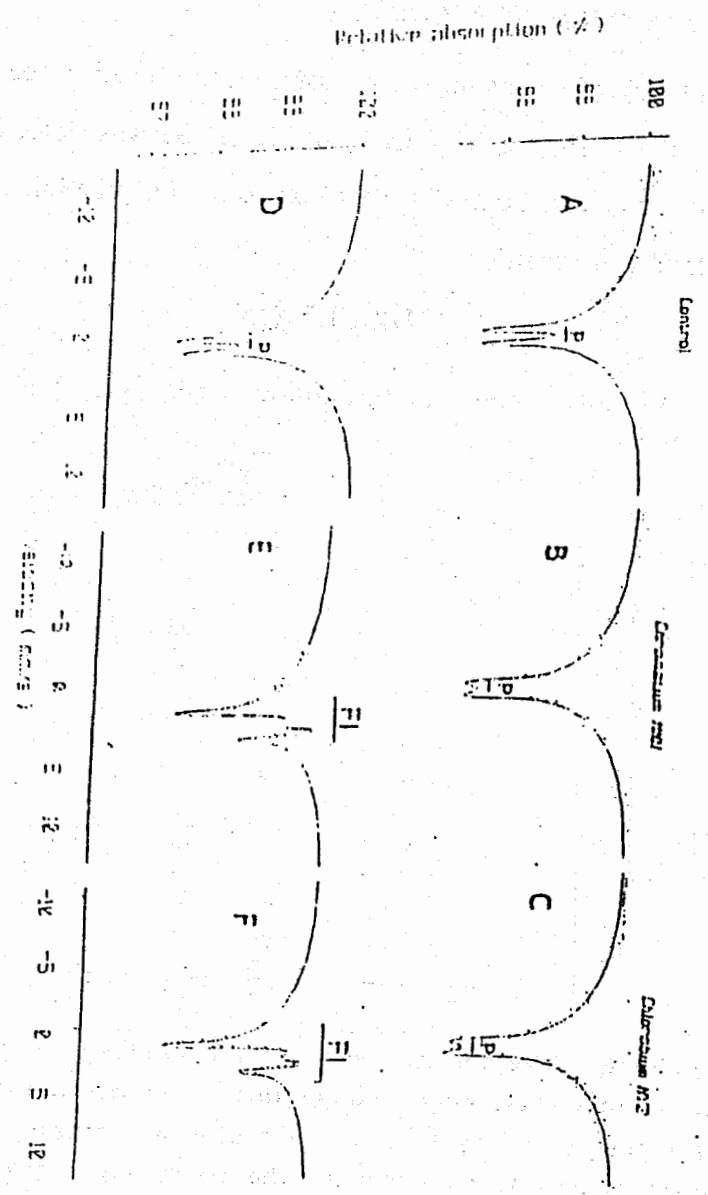

Figure (1) : Mossbauer spectroscopic analysis of inoculted and control pyrite containing media. A, b, & C: Filter paper precipitate of control and isolates. P : Pyrite, FI : Szomolnokite.

seventh day of incubation which could be attributed to the recorded drop in pH (fig. 4). Hydogen sulfide (H_2S) was detected in all inoculated culture bottles in the second day of incubation using lead acetate filter paper strips.

DISCUTION

The results indicate that these strains are able to grow

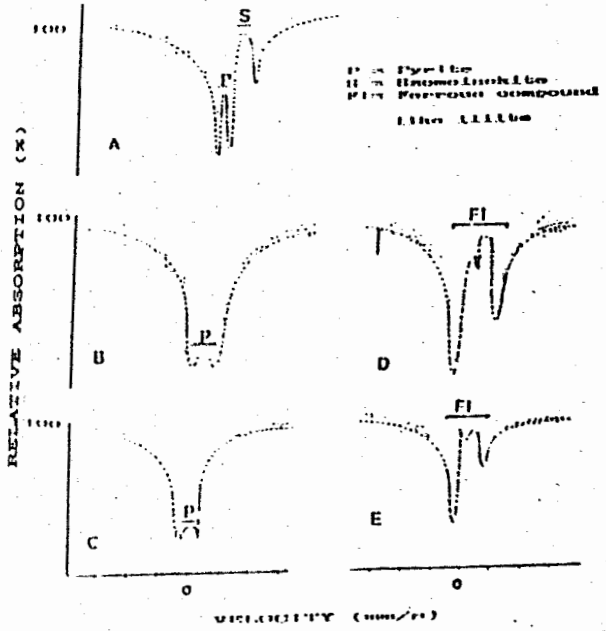

Figure (2): Mossbauer spectroscopic analysis of reference and treated Maghara coal samples. A : Spectrum of the filter paper precipitate of control coal sample after 10 days; B and C : spectra of the filter paper precipitates after 10 days incubation with Chromatium MN11 and Chlorobium NY2; D and E : Spectra of cell debris of both strains collected after 10 days.

autotrophically on pyrite containing medium. Complete identification of the two strains is beyond the scope of the present study, therefore they

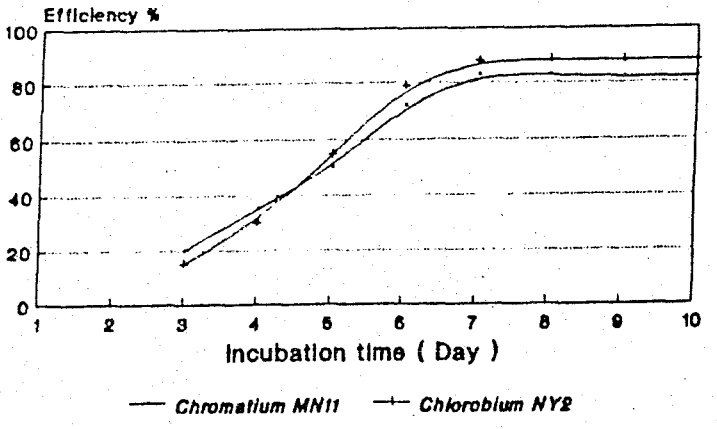

Figure (3): Desulfurization efficiency of Chromatium MN11 and Chlorobium NY2.

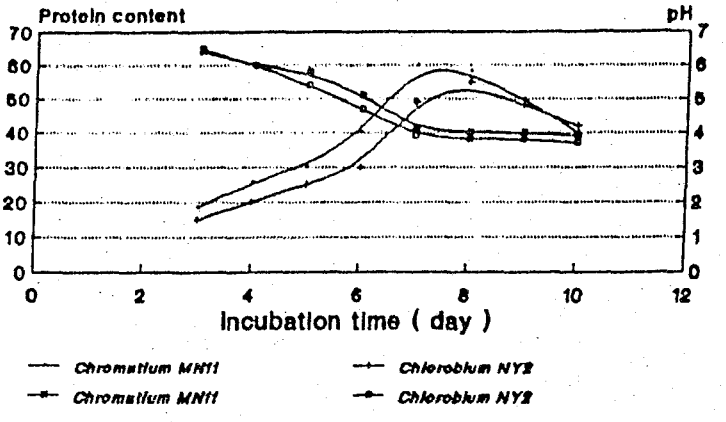

Figure (4): Total protein and pH changes during desulfurization.

were tentatively identified as Chromatium sp MN11 and Chlorobium sp NY2 according to pfennig and Truper (1989). Criteria applied for their identification were based on cell shape, motility, sulfur depositon and type of bacteriochlorophyll and carotenoid pigments.

Chemical analysis of maghara coal samples showed the following composition (% w/w) : Ash, 12.28; C, 66.4; H, 5.11; N, 1.05; S, 4.20; & 10.75 (Eissa et al., 1989). pyrite constitutes about 3.0% of Maghara coal.Sulfur leaching efficiency of other methods ranged from 50 to 70%. Treatment of Maghara coal with 5% HCL at 150 followed by concentrated nitric acid treatment resulted in complete leaching of inorganic sulfur, but this process caused damage to coal matrix (Eissa, et al., 1989, Troma, and Bosecker, 1982 and wheelock, 1977). The first step in the present investigation was to adapt some anoxygenic phototrophic bacteria to utilize pyrite instead of hydrogen sulfide. Pyrite is a partially soluble material, therefore, it is separated from other constituents of the medium by filtration through Whatman No.1 ashless filter paper. The filterate was in turn centrifuged to separate growing cells for the purpose of deticting any pyrite or intermediate iron product that may form inside bacterial cells under investigation. Resultes confirmed that both strains were able to desulfurize pyrite (Fig.1). Upon using crushed Maghara coal samples instead of pyrite, similar results of pyrite leaching were recorded (Fig.2). One problem that face this method is the precipation of coal particles. Rotation of bottles as well as the used grain size of coal particles helped to great extent to overcome this problem.Moreover the concentration of 5% Maghara coal (60 mesh) is found good enouhgh to insure proper dispersion in the mdium upon rotation. Coal is precipitated within 30 minas a very thin layer on the side of the incubation bottles (bottles were incubated

horizontaly), hence did not affect the illumination process. Samples were randomly collected using pasteur pipettes from the coal slurry and investigated microscopically. Heavy populations were investigated near the coal precipitate rather than near the surface of incubation bottles.

interestingly, both photosynthetic strains were recorded to resist low pH ranges. this was confirmed by total protein determination which showed maximum total protein content in the 6-8 day of incubation where pH was about 4 (Fig.4). Afinding that some purple and green sulfur photobacteria can resist or grow at low pH is rather vague, however this is beyond the scoupe of the present investigation.

A conclusion that the isolated photosynthetic sulfur bacteria Chromatium MN11 and Chlorobium NY2 can be used in coal desulfurization and pyrite leaching from Maghara coal is now clear. they showed higher efficiency in pyrite leaching in addition to the ease of manipulation of cultures. We did not know much about the exact mechanism in leaching of organic sulfur but we recommend thier used as efficient pyrite leaching organisms since they were proved to remove 83 an 88% of pyrite from Maghara coal samples where other methods such as thermal treatment, and floatation removed 60 to 70% pyrite (Eissa et al.1989).

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استخدام البكتريا للتخلص من الكبريت الموجود فى الفحم

د . عصام غانم

قسم النبات كلية العلوم -

جامعة الأزهر - القاهرة

تم عزل وتعريف نوعين من البكتريا الكبريتية ، والتى تنمو وتتغذى ذاتيا فى وجود Fe_2S والذى يعتبر مانحا للالكترونات تنمو البكتريا وتتغذى على الكبريت وبذلك فمن الممكن أن تستغل للتخلص من الكبريت فى المركبات ذات الأهمية الاقتصادية المحتوية على الكبريت وخصوصا الفحم البيريتى .