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USE OF PHOTOSYNTHETIC BACTERIA FOR HYDROGEN SULFIDE REMOVAL FROM ANAEROBIC WASTE TREATMENT EFFLUENT

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Abstract—The feasibility of using photosynthetic bacteria to remove H_2S from anaerobic waste treatment effluent was investigated by growing fixed films of photosynthetic bacteria in a packed column or in a submerged tube system ("phototube"). Growth and enrichment for these organisms depended on constant illumination, anaerobic conditions and a substratum for attachment of the bacteria. Both systems were operated as flow-through processes using effluent from anaerobic (upflow) filters.

Results showed that photosynthetic bacteria in fixed films can be effectively used for H_2S removal. Removal efficiencies of 81–95% were obtained on a 24-h retention time. Residual H_2S remained in the process effluent. The submerged "phototube", showed dramatic improvement over the column, yielding a final effluent completely devoid of H_2S, at significantly shorter retention times and higher loading rates than the column. Performance appeared dependent on cell–H_2S contact and adequate illumination. The green photosynthetic sulfide-oxidizing bacterium, *Chlorobium*, was identified as a common organism in this phototube.

This biological sulfide removal process offers the following advantages over currently used physical-chemical techniques: simplicity, no need for aeration or chemical additives and odor-free. Much research in process design is necessary before pilot or full scale application of the technique is possible.

INTRODUCTION

Hydrogen sulfide (H_2S) is a major problem associated with anaerobic stabilization of sulfur containing organic wastes and with a number of manufacturing processes which generate it during operation. Not only does H_2S have an unpleasant rotten-egg-like odor, but it is also a common cause of industrial injury and fatality at accumulated concentrations of about 400 mg m^{-3} and higher (Waldbott, 1973). Property damage attributable to H_2S is considerable (McCarty, 1974; Metcalf & Eddy Inc., 1972; Roy & Trudinger, 1970; Sawyer & McCarty, 1978). Types of damage include direct corrosion of metals, indirect corrosion of metals and concrete by H_2SO_4 formed by its oxidation and discoloration of metal containing surfaces by metallic sulfide formation. Metal and concrete sewerage, pipelines that convey raw biogas and biogas burning engines are especially susceptible to damage.

As the most reduced form of sulfur, H_2S has a high oxygen demand of 2 mol O_2 mol^{-1}. In water it reacts rapidly with dissolved oxygen and may cause depletion of O_2 in effluent receiving waters needed to sustain aquatic life. In gaseous form H_2S only reacts with atmospheric oxygen in the presence of heat or light and possibly a catalyst (Camp & Meserve, 1974; Roy & Trudinger, 1970; Ueno & Williams, 1979); therefore can accumulate to dangerous concentrations in enclosed areas unless removed.

Methods for removal of H_2S in common use today are physicochemical processes which involve either direct air stripping or oxidation. Oxidation requires use of oxidizing agents such as air, oxygen, Cl_2, potassium ferricyanide, quinone, manganese dioxide, manganese sulfide, hypochlorite, chlorine dioxide and nitric oxide (Ueno & Williams, 1979; Camp & Meserve, 1974; Taylor, 1972). These methods are energy intensive, often requiring physical treatment such as aeration, heating and centrifugation. These methods are also costly because they may require chemical additives and energy expenditure for physical treatment. In addition, there are environmental problems associated with the chemical additives. These problems include the possible generation of chlorinated organic compounds from organic compounds present when Cl_2 is used and the possible addition of toxic contaminants along with the chemical additives. Because environmental contamination is a major problem to be dealt with and because the costs of chemicals and energy are rising, there is a need for alternative techniques for sulfide removal.

The purpose of this investigation was to examine the possibility of applying photosynthetic bacteria efficiently for sulfide removal from anaerobic waste treatment effluent. The basis for this application is the fact that in nature photosynthetic bacteria play an

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important role in converting sulfur from one oxidation state to another (Roy & Trudinger, 1970; Stanier *et al.*, 1970); photosynthetic bacteria are known to be responsible for the formation of the large elemental deposits of sulfur found in nature (Ivanov, 1968; Roy & Tridinger, 1970). In addition to metabolizing inorganic sulfur, photosynthetic bacteria are able to degrade mercaptans and volatile fatty acids; compounds also associated with odors in anaerobic digester effluent (Kobayashi M., 1977; Trüper & Pfennig, 1978).

Although numerous forms of photosynthetic bacteria exist, only members of the groups of Chromatiaceae (purple sulfur bacteria) and Chlorobiaceae (green sulfur bacteria) are commonly known to metabolize sulfide (Pfennig, 1978; Trüper & Pfennig, 1978).

Only a few species among the very common Rhodospirillaeceae (non-sulfur purple bacteria) are known to be able to tolerate much sulfide or to use it. Sulfide metabolism by photosynthetic bacteria occurs only under anaerobic conditions, and is coupled to CO_2 reduction. Sulfide serves as the sole electron donor and is oxidized to sulfate either directly, or by way of elemental sulfur, as is the case with *Chlorobium* and *Chromatium*, members of Chlorobiaceae and Chromatiaceae, respectively. Some members of Rhodospirillaceae oxidize sulfide directly to sulfate, while there are some that cannot oxidize it further than the elemental sulfur (Hansen & van Gemerden, 1972; Pfennig, 1978). Because there is significant variation in the tolerance to sulfide and the pathways by which these organisms are able to use sulfide, it is important that the proper type of photosynthetic bacteria be present if efficient sulfide removal is required.

The approach used in this work involves a separate unit process for sulfide removal. This approach is necessary because of the need to control environmental conditions, particularly illumination and oxidation-reduction conditions, in order to develop an adequate population of photosynthetic bacteria of the desired type. The units studied were designed as fixed film processes because environmental control in such systems is simple and because large steady-state populations of selected types of organisms can be readily developed and maintained (Kobayashi & Rittmann, 1982).

The use of photosynthetic bacteria for sulfide removal requires neither chemical additives nor aeration; hence it is potentially less costly than currently used techniques. Furthermore, because the photosynthetic bacteria are held in a closed anaerobic system, sulfide removal may occur without volatilization of H_2S to the surrounding atmosphere. Hence, the process is odorless. Because photosynthetic bacteria commonly oxidize H_2S by way of elemental sulfur, it is conceivable that sulfur may be removed in this form instead as sulfate, thereby eliminating the problem of a low pH effluent.

This is the first reported controlled application of photosynthetic bacteria for the purpose of H_2S removal. Although the possible use of photosynthetic bacteria for removal of odor—causing compounds was suggested earlier in the literature (Cooper *et al.*, 1975; Kobayashi M., 1977), no efficiently engineered application was known at the time of this investigation.

MATERIALS AND METHODS

Experimental units

Two separate experimental units were used to examine sulfide removal from anaerobic digestion effluent by photosynthetic bacteria. The first was the raschig ring-filled column shown in Fig. 1 and the second was the submerged tube system shown in Fig. 2.

The column consisted of two concentric acrylic (plexiglass) tubes. The outer tube was 91.4 cm × 15.2 cm (i.d.) × 6.4 mm thick, while the inner tube (light well) was 83.8 cm × 76.2 mm (i.d.). The space between the inner and outer tubes was sealed at the top with a 12.7 mm thick acrylic sheet. The bottom of the unit ended in a cone for influent introduction and sludge collection. Sampling ports were drilled, tapped and $\frac{3}{8}$-in. standard thread (N.P.T.) nylon pipe fittings were inserted. A 25.4 cm long, 40 W tungsten light bulb was inserted into the inner tube as a light source for the photosynthetic bacteria. Two other 40 W bulbs were positioned around the outside column near the mid-point at a distance of about 20.3 cm from the column. The light intensity was controlled through a transformer which kept the light in the inner well at about 105 foot candles and at about 13 ft-candles each at the surface of the column. The inner tube was kept from overheating by introducing air at the bottom. The space between the inner well and the outside tube was packed with $\frac{1}{2}$-in. porcelain raschig rings with a final void volume of approx. 0.25 ft^3 (about 7-l.). The column was operated in the upflow mode and was maintained in connection with two anaerobic (digestion) filters operated in series. The effluent from the anaerobic filters provided the sulfide feed for the experiments. The column was started up by initially filling it up with anaerobic filter effluent, inoculating it at the bottom with photosynthetic bacteria obtained from a previous enrichment culture and maintaining the influent flow at a retention time of about 24 h. The culture of photosynthetic bacteria was allowed to develop for about 3 weeks

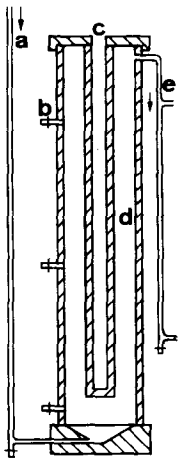

Fig. 1. Photosynthetic column. (a) Influent line; (b) sampling port; (c) light well; (d) raschig ring packed section; (e) effluent line.

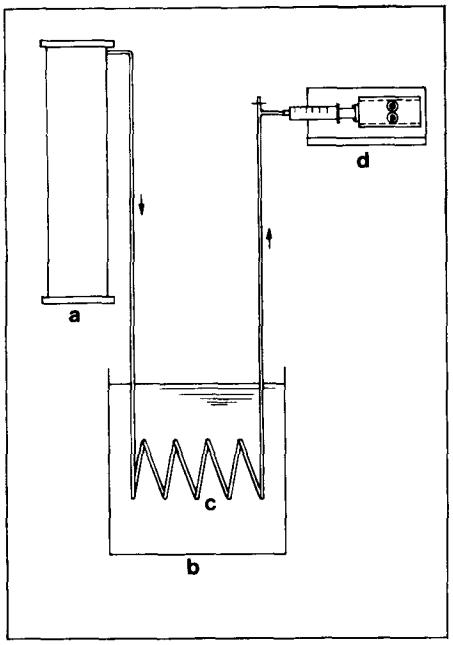

Fig. 2. Photosynthetic tube system for sulfide removal studies. (a) Anaerobic filter; (b) anaerobic water bath; (c) tygon tubing coil containing photosynthetic organisms; (d) syringe pump.

before experiments were started. The inoculum was developed by maintaining a 4-l. anaerobic filter on domestic waste water for several months in subdued sunlight. Samples from the photosynthetic column were collected from the influent and effluent lines and at the vertical ports of the column. To avoid losing H_2S, samples were collected with a pasteur pipet connected to the sampling outlet on one end and submerged under a measured amount of zinc acetate solution (10 ml 100 ml^{-1} sample) in order to fix immediately the sulfide as zinc sulfide. Samples were analyzed within 2–3 days.

The photosynthetic tube system ("phototube") consisted of a 12.8 m long, 3.2 mm i.d. tygon tubing connected to an effluent line from the anaerobic (digestion) filters. A sampling port in the influent line of the photosynthetic tube was used for removing liquid. Because tygon tubing is permeable to oxygen, the tubing was submerged 20.3 cm in a water bath containing a reducing agent (sodium sulfite, Na_2SO_3, 30 mg l^{-1}) and a catalyst cobalt chloride ($CoCl_2$, 0.5 mg l^{-1}), to promote anaerobic conditions. The reducing agent was replenished at 1–2-day intervals. There was no measurable oxygen in the water bath. The tygon tubing was connected to a syringe pump fitted with a 100-ml hypodermic syringe.

A timer was used to regulate the pump at the desired rate. A 25.4 cm long, 40 W tungsten bulb was positioned about 25.4 cm above the water bath to provide illumination. The intensity of this light source was 350 ft-candles at the surface of the water bath. Sampling septa consisting of latex rubber tubing, 2–3 cm long, were spliced into the tygon tubing at 6-in. intervals in the "phototube". The septa were changed after each experiment in order to avoid leakage. The "phototube" was filled with anaerobic filter effluent (see Experimental feed), innoculated with liquid from the bottom of the photosynthetic column, then maintained on flow rates of 0.28 and 0.48 ml min^{-1}. Samples were not taken until a thick growth of pigmented cells appeared. Samples were drawn with tuberculin syringes (1-ml) and 23 gauge needles were used to withdraw 1-ml samples. All samples were analyzed for sulfide and elemental sulfur; however, only influent and effluent samples were analyzed for sulfate because of the large sample volume required in the analysis.

Experimental feed

The sulfide feed supplied to the photosynthetic column and tube systems was derived from the liquified fraction of water hyacinths ground down in a commercial food blender. The water hyacinth treatment scheme was part of a project to improve digestion efficiency of water hyacinths (Kobayashi H., 1981). The liquified hyacinth waste was fed to anaerobic (digester) filters to which the photosynthetic systems were connected to polish effluent from the filters. Bacterial activity in the anaerobic filters released sulfur found in proteins and other biochemical components of hyacinth tissue. In addition, inorganic sulfate found in the water used in the grinding process was reduced to sulfide by bacterial activity.

Concentration of the sulfide feed was controlled by dilution of the feed to the anaerobic (digestion) filters.

Analytical methods

Sulfides were determined by the colorimetric method of Pachymar (Brock *et al.*, 1971), but modified for immediate analysis of small samples drawn from the "phototube". Such samples were transferred immediately to tubes containing zinc acetate, to fix sulfide as zinc sulfide, plus reagents for the colorimetric test.

Elemental sulfur (S^o) was determined by the colorimetric method of Bartlett & Skoog (1954).

Sulfate was determined by the turbidimetric method described in *Standard Methods* (APHA, 1975).

Media for culture of photosynthetic bacteria

The Hungate roll tube technique for anaerobic culture of bacteria (Hungate, 1968) was applied in the preparation of media, originally described by van Niel, for photosynthetic bacteria (1971). The resulting media are described below:

(1) *Medium for sulfide-tolerant bacteria.* The ingredients of media for high sulfide tolerant bacteria were (per liter of solution): KH_2PO_4, 0.33 g; NH_4Cl, 0.33 g; $MgCl_2 \cdot 6H_2O$, 0.33 g; KCl, 0.33 g; 10 ml Pfennig's heavy metals solution; 1 ml B_{12}, 0.002% w/v; 1 ml resazurin, 0.1% w/v; 0.02% w/v $CaCl_2 \cdot H_2O$; Na_2CO_3, 0.1% w/v; and $Na_2S \cdot 9H_2O$, 0.06% w/v. A mixed salt solution was prepared with KH_2PO_4, B_{12}, NH_4Cl, $MgCl_2 \cdot 6H_2O$, KCl, heavy metals, resazurin and deionized water. An excess of 200 ml of water was added to each liter of solution which was then boiled under 100% N_2 until the excess water was evaporated; dissolved O_2 was removed by this process. Flushing with 100% N_2 was continued while the medium cooled to room temperature. The pH of the cooled solution was adjusted so that addition of Na_2S, $CaCl_2$ and Na_2CO_3 solutions (after autoclaving and just before use) would yield a medium with a final pH of 6.7–7.2. Roll tube media were prepared by adding 2% agar to this medium.

(2) *Medium for sulfide sensitive photosynthetic bacteria.* The low sulfide medium was prepared by adding 10 g yeast extract, 2 g malic acid and 1 ml (0.1% w/v) resazurin to 1-l. of water. The solution was boiled, cooled and the pH was adjusted to give a final pH 7.0 with or without Na_2S and Na_2CO_3. The medium was subsequently modified to include 30% (v/v) anaerobic filter effluent. Roll tube media were again prepared by adding 2% agar to this medium.

(3) *Media for characterization of sulfide sensitive photosynthetic bacteria.* Media used for characterizing low sulfide-tolerant photosynthetic bacteria were modifications of selective media described by van Niel (1971) for *Rhodopseudomonas palustris* and *Rp. acidophilia.* These media were selected on the basis of preliminary visual identification of the isolates by color, shape, motility and budding. Two

types of basal salts media were prepared: one developed for *Rp. palustris* contained 0.00002% (w/v) *p*-amino benzoic acid (growth factor), 0.2% (w/v) thiosulfate, and 0.25% (w/v) benzoate; the other for *Rp. acidophila* contained 0.25% (w/v) citrate. The basal salts solution consisted of 0.2% (w/v) $NaHCO_3$, 0.1% (w/v) NH_4Cl, 0.05% (w/v) KH_2PO_4, 0.05% (w/v) $MgCl_2$, Pfennig's trace metal solution (1 ml $100\ ml^{-1}$) and deionized water. The basal salts solution was boiled under O_2-free 100% N_2 and cooled. The extra components for the two specific media were then added, the pH adjusted to 7 and the liquid media dispensed into tubes and autoclaved.

Isolation of photosynthetic bacteria

The inoculum was collected from different sampling ports of the photosynthetic column by a syringe and needle technique and injected into a sterile oxygen-free serum bottle outgassed with 100% N_2. The bottle was thoroughly shaken before 0.5 ml aliquots were withdrawn and inoculated into agar tubes containing high or low sulfide media. Serial dilutions (to 10^{-8}) were made and the tubes were incubated under an incandescent lamp at room temperature.

Purification of photosynthetic bacteria

The low sulfide organisms were purified by successive "picks" and dilutions of individual colonies into fresh roll tube media. This procedure was continued until only one colony type was observed. The same isolation procedure was not effective for the high sulfide-tolerant organisms due to unknown factors and possibly oxygen sensitivity. Colonies of green photosynthetic bacteria were sufficiently well separated for isolation and microscopic examination.

Characterization of photosynthetic bacteria

Liquid medium for low sulfide-tolerant photosynthetic bacteria (described previously) was introduced by sterile hypodermic syringe and needle into roll tubes containing pure cultures. The tubes were agitated and incubated under an incandescent lamp (25 W) until a liquid culture developed. Serial decimal dilutions of the cultures were made in tubes containing the media specific for *Rp. palustris* and *Rp. acidophila*. The tubes were incubated for 3 weeks, then examined for cell growth.

The high sulfide tolerant organisms were not purified.

RESULTS

The photosynthetic column

Figures 3, 4 and 5 show the effect of light variation on the distribution of various forms of sulfur in the photosynthetic column and effluent. The column was maintained on a 24-h retention time during these experiments. Elemental sulfur (S°) was not detected by chemical or visual methods in the effluent. However, S° globules were seen by phase contrast microscopy in the column samples. All calculated data were based on the weight of sulfur present as H_2S or sulfate (SO_4^{2-}). Figure 6 shows the reduction of sulfide by the photosynthetic column during a 5-month period. Theoretical hydraulic retention times in the column were estimated from the void space ($0.008\ m^3$) before cellular growth. Sulfide loading and removal rates were calculated from the sulfide reduction data and are presented in Table 1.

The photosynthetic tube

Initial concentrations of sulfide were measured in the influent of the phototube and the rates of removal were followed by sampling at various points in the phototube at corresponding intervals of contact time. Samples were analyzed for sulfide, sulfate and S° by turbidometric or colorimetric techniques (see Materials and Methods). In addition, S° was also observed visually. Microscopic (low power) examination of scrapings collected at the inlet and effluent from the system contained small, amorphous bodies of S°. These sulfur globules were verified by microscopic comparison with pure S° under the × 100 oil immersion objective of a Zeiss phase-contrast microscope.

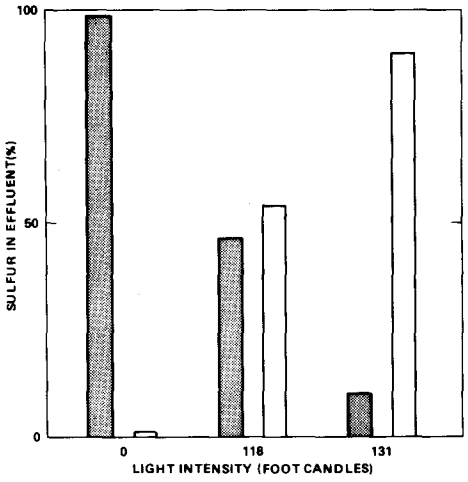

Fig. 3. Sulfur balance in effluent from the photosynthetic column under varying conditions of light availability. □ Sulfate; ■ sulfide.

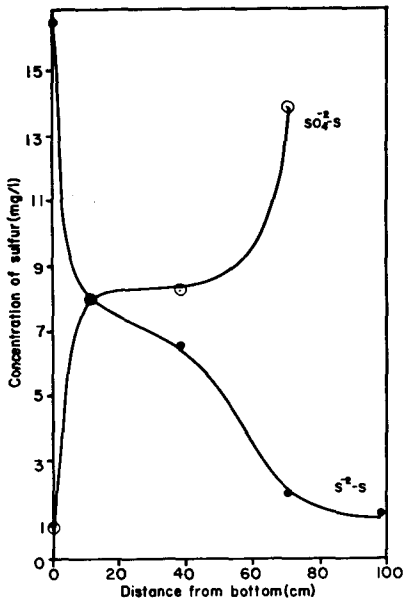

Fig. 4. Sulfur balance within the photosynthetic column when all lamps were turned on.

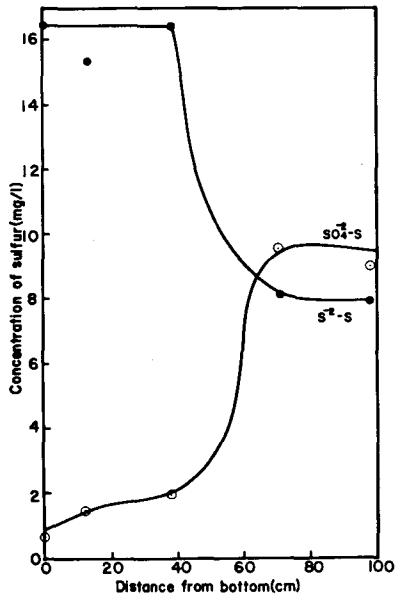

Fig. 5. Sulfur balance in the photosynthetic column after one of the outside lamps was turned off.

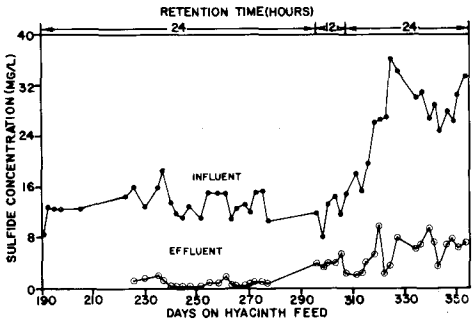

Fig. 6. Sulfide removal by the photosynthetic column.

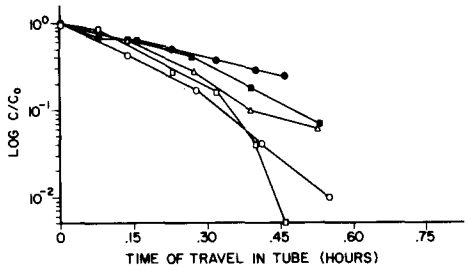

Fig. 7. Semilog plot of sulfide removal. □, 18.9; ●, 22.9; ○, 14.3; ■, 23.9; △, 21.3. C_0, initial concentration (mg l^{-1}); C, concentration at time of measurement (mg l^{-1}).

Figure 7 shows a semi-logarithmic graph of the sulfide removal rates from several experiments on the phototube. Table 2 gives the actual initial and final sulfide concentrations and the two flow rates used in this study. The kinetic data calculated from Fig. 7 for sulfide removal are also given in Table 2. Simple, first order kinetics were used in making these calculations as follows:

1. The simple first order reaction:

$$-t = (\ln C/C_0)/k.$$

Table 1. Sulfide removal by the photosynthetic column

OLR* (kg m^{-3} day^{-1})	Retention time in column (h)	S^{2-} loading rate (mg day^{-1})	S^{2-} in effluent (mg day^{-1})	Average % removed
0.48	24	114	9.6	92
0.96	12	194	57.6	70
0.96	24	107	5.6	95
1.44	24	244	47.2	81

*Organic loading rate of feed to the anaerobic filter system.

Table 2. Sulfide removal by the photosynthetic tube

Average pump flow rate (ml min^{-1})	Velocity (m min^{-1})	C_0 (mg l^{-1})	k* (h^{-1})	S^{2-} load (g l^{-1} day^{-1})	Tube length (m) required to reduce C_0 to 1 mg l^{-1}			Time (h) required to reduce C_0 to 1 mg l^{-1}		
					Actual	Expected	%	Actual	Expected	%
0.29	0.037	14.3	7.6	0.8	0.88	1.11	79	0.40	0.50	80
		21.3	5	1.1	1.20	1.29	93	0.54	0.58	93
		23.9	4.6	1.3	1.32	1.33	99	0.60	0.60	100
0.48	0.061	18.9	2.9	1.6	1.51	2.05	74	0.41	0.56	73
		22.9	6.3	2.0	3.05	2.16	141	0.83	0.59	141

*Average k used in calculations $k = 5.3\ h^{-1}$.
†% = (actual/theoretical) × 100.
‡C_0 = initial S^{2-} concentration.
§Load based upon volume in 1 m of tubing.

Table 3. Sulfur balance in the photosynthetic tube

Sulfur form	0.29 ml min^{-1} (0.44 h)*		0.48 ml min^{-1} (0.66 h)*	
	Influent (S-mg l^{-1})	Effluent (S-mg l^{-1})	Influent (S-mg l^{-1})	Effluent (S-mg l^{-1})
Sulfide-S	18.9	0	23.9	0
Sulfate-S	0	14.0	0	20.3
Elemental sulfur	0	1.5	0	2.9
Total	18.9	15.5	23.9	23.2

*Retention time at which 0.5 mg l^{-1} S^{2-} remained.

2. Theoretical (expected) length of tube required:

$$\text{length(m)} = t \times 60 \times v.$$

3. Actual length of tube obtained from sulfide reduction vs tube length at different sampling sites.

4. Reaction constant (k) obtained from slope of plot of $\log C/C_0$ vs tube length; this was verified by calculation.

5. Actual time = actual tube length/($v \times 60$).

6. Sulfide loading rate (g day = $s \times f \times 60 \times 24$.

The symbols used were:

t = theoretical (expected) time of travel (h)
v = velocity (m min^{-1})
C_0 = initial sulfide concentration
k = reaction constant (h^{-1})
l = length (m)
f = flowrate (ml min^{-1})
s = sulfide concentration ml^{-1}.

The distribution of three different forms of sulfur in the influent and effluent of the phototube is given in Table 3. Two different rates were evaluated.

Photosynthetic bacteria

The photosynthetic column contained green and brown zones; the brown zones were closer to the light source than the green. Samples from different parts of the column mainly contained non-motile rods and extracellular S°. The latter was presumptively identified by its characteristic birefringence (Trüper & Pfennig, 1978). Reddish-purple to brown samples contained many motile, flagellated rods, undergoing division by budding. A few spirillum-shaped and other morphological types of bacteria were also observed. In these latter samples, S° was not observed.

Green photosynthetic bacteria (*Chlorobium* type) predominated at the inlet of the "phototube". They were followed by a mixture of green and purple (mainly *Rhodopseudomonas* and some *Rhodospirillum* types) photosynthetic bacteria for about one meter of the tube length. The rest of the tube was populated by purple photosynthetic bacteria.

Reddish-purple colonies developed in low sulfur media inoculated with a sample from the photosynthetic column. Microscopic examination revealed reddish, motile, rod-shaped bacteria similar to those described earlier. The reddish colonies were eventually purified by "picking" and diluting colonies in repeated subsequent transfers. The isolates resembled the predominant organisms in the photosynthetic column and the "phototube". These bacteria (Fig. 8b) were reddish-purple rods ranging from 1–1.5/3–5 μm (width/length), motile by flagellar type locomotion and dividing by budding in the manner described for *Rp. palustris*, *Rp. acidophila* and *Rp. viridis* (Trüper &

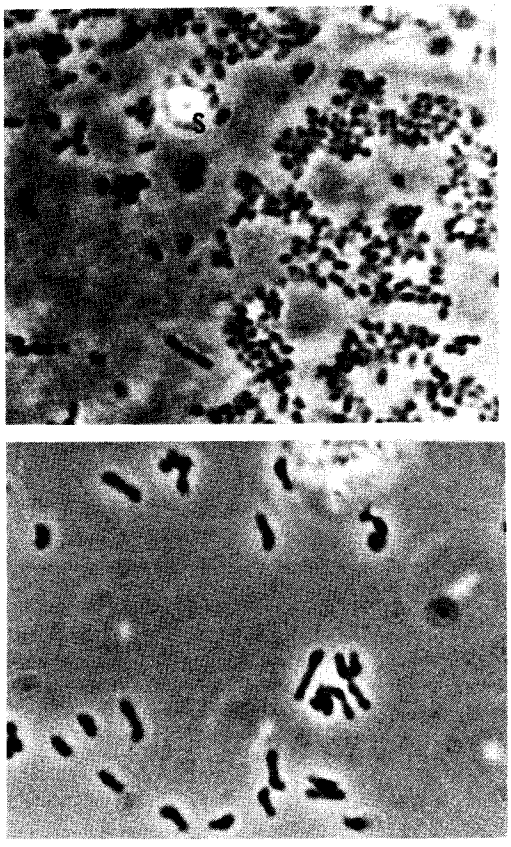

Fig. 8. Phase contrast micrograph (70 × oil objective) of photosynthetic bacteria. (A) Green photosynthetic bacteria and extracellular sulfur deposit (S). (B) Reddish-purple photosynthetic bacteria.

Pfennig, 1978). The isolate used citrate but not benzoate as a carbon source, was sulfide sensitive and unable to grow in medium containing thiosulfate, benzoate and *p*-amino benzoic acid.

Roll tube media containing high sulfide concentrations inoculated with samples from the photosynthetic column developed greenish colonies of various shades. Microscopically, the colonies contained cells which were predominantly short, green, non-motile rods characteristic of *Chlorobium* (Trüper, 1978). Extracellular S° deposits often occurred alongside the cells. Attempts to pure culture the green bacteria were not successful; however, the wide colony separation made it possible to "pick" individual colonies for examination. Figure 8(a) is a phase contrast photomicrograph of the predominant green photosynthetic bacteria. As the culture aged, algae and *Rhodopseudomonas* type cells appeared in the roll tube.

DISCUSSION

Though photosynthetic bacteria are generally present in aerobic and anaerobic wastewater treatment facilities (Croft, 1971; Siefert *et al.*, 1978; Torien, 1976) they seldom play a perceptible role because usual conditions are not favorable for their proliferation. The basis for the sulfide removal process examined in this investigation was the optimization of environmental conditions necessary for selective proliferation of photosynthetic bacteria. This was accomplished by providing anaerobic conditions and an adequate, constant light source.

The predominant organism in the photosynthetic column and in the influent section of the "phototube" (Fig. 8a) was tentatively identified as *Chlorobium limicola*. This identification was based on the description of Trüper & Pfennig (1978). *C. limicola* is a green, rod-shaped organism often occurring in chains, capable of anoxygenic photosynthesis requiring H_2S as its electron source, has a high tolerance for sulfides, lacks gas vacuoles, may require B_{12} and deposits S° extracellularly. Isolation of these organisms is difficult because of requirement for syntrophic growth factors such as B_{12} and also because of the fastidiously anaerobic nature of these organisms. The presence of other types of pigmented organisms in the photosynthetic column suggest that organisms other than *C. limicola* may also be active in sulfide removal. Their role, however, may be secondary since they usually succeed *Chlorobium* in aged roll-tubes of mixed cultures, and appear to be relatively intolerant of sulfide.

Reddish-purple phototrophic bacteria (Fig. 8b) were present in the effluent lines from the photosynthetic column and in the phototube. A pure culture of these organisms was obtained after several transfers of colonies from low sulfide media. The isolate was identified as a species of *Rhodopseudomonas* by its bud formation for vegetative reproduction and its reddish-purple color in anaerobic culture; its ability to use citrate but not benzoate narrowed the choice tentatively to *Rp. acidophila*. Identification based on moles percent guanine + cytosine of the DNA was not done. The rhodopseudomonads appeared to be sensitive to sulfide in pure culture but were insensitive when grown with other organisms. Their sulfide sensitivity suggested that their function in the "phototube" was not sulfide removal but utilization of organic compounds by photoorganotrophic metabolism. In pure culture these photoorganotrophic organisms may use various simple organic and some ring compounds (Dutton, 1969; Sojka, 1978). They are, however, unable to use macromolecules such as proteins, starch and chitin (Pfennig, 1978). In nature they are frequently found just above the zone of green photosynthetic bacteria in a stratified layer where less sulfide is present (Pfennig, 1978). In this niche, they presumably metabolize organic compounds produced by sulfide-oxidizing green photosynthetic and other bacteria capable of breaking down complex macromolecular compounds.

Microbial ecologists have often classified photosynthetic bacteria by their preference for specific sulfide concentrations (Pfennig, 1978; Zinder & Brock, 1978). However, the present results show that such classifications based on observations of stratified aquatic systems, may not be pertinent to the phototube/column system. For example, the green sulfur bacteria supposedly grow selectively in a concentration of 4–8 mM sulfide, while the so-called non-sulfur purple bacteria supposedly grow at sulfide concentrations from 0.4 to 2 mM. The sulfide concentration in the present column ranged from 0.26 to 1.46 mM. Yet the full spectrum of green to reddish-purple organisms indicated that they grew at sulfide concentrations much lower than traditionally predicted.

Results of experiments testing the effect of variation in light availability (Figs 3, 4 and 5) showed that sulfide oxidation in the photosynthetic column was dependent upon the presence of light. The predominance of green photosynthetic cells in the column and influent section of the phototube and the presence of S° deposists in the column indicated that the primary pathway was via oxidation of sulfide to S° to sulfate. The fact that S° was not found in the effluent even though it was seen in samples taken from within the column implied that it was converted to sulfate during the 24-h retention time.

Observations on sulfide removal by the column fed effluent from an anaerobic filter system (Kobayashi H., 1981) showed that even though sulfide removal was effective (81–95% removed at a 24-h column retention time) sulfide removal was incomplete (Table 1, Fig. 6). These results were attributed to poor illumination and poor contact between cells and sulfide in the effluent. The shading properties of the packing and the uneven distribution of the light sources are the likely causes of the illumination problem. Poor contact was the result of uneven cell distribution resulting from uneven illumination. Back mixing of the influent "front" because of the slow flow

rate may also contribute to the poor effluent quality (presence or residual sulfide) from the column. Figures 4 and 5 especially show the uneven pattern of sulfide removal within the column. These studies prompted the development of the alternate method using the phototube.

The performance of the "phototube" showed marked improvement over the column. The final effluent was completely devoid of sulfide in contrast to the effluent from the photosynthetic column. At approximately the same starting concentration of sulfide in both the column and "phototube", the column required about 18 h to achieve 95% sulfide removal while operating at a loading rate of 0.019 g S^{2-} l^{-1} day^{-1}. The tube was able to accomplish the same efficiency in about 24.6 min while operated at a loading rate of 1.6 g S^{2-} l^{-1} day^{-1}.

The results from experiments at differring pumping rates and initial substate concentrations are shown in Fig. 7. These data showed that removal followed first-order reaction kinetics, at least until 1 mg l^{-1} sulfide was reached. Rates at concentrations higher than this were difficult to measure because of the difficulty of sampling low concentrations of sulfide without loss. Assuming a first-order reaction, kinetic constants were determined by graph and by calculation. The average "k" was used in calculations with collected data to examine data "fit" to the simple first-order decay model. The data are summarized and presented in terms of time required for sulfide removal, and the length of tubing required for removal of sulfide to a concentration of 1 mg l^{-1}. Table 2 shows a reasonable "fit" of data in most cases.

The improved performance in the phototube may be attributed to:

(1) improved illumination because of less shading; (2) better contact between cells and sulfide; and (3) less back-mixing of the influent front because of faster flow rates. Although 12 m of tubing were used for the phototube, no more than 3 m were involved in sulfide removal.

Elemental sulfur was present in the effluent of the phototube but was not seen in the column effluent. Its presence in the phototube effluent may be due to a number of factors: (1) faster flow rates may have swept S° into the effluent; (2) the shorter retention times led to incomplete oxidation. The presence of S° in this effluent raises the possibility of sulfur removal by S° production. Sulfur in this form is relatively inert and less problematical than sulfate; hence its removal in this form may be advantageous.

The functional biomass involved is difficult to estimate because the alternative treatment schemes investigated are both biological fixed film processes. In these fixed film processes total quantities of biomass that can be collected are of little meaning because primary transfer of feed-substrate between the liquid phase and the layered cellular mass occurs at the surface interface. The best estimate of functional biomass is probably that which can be obtained on the basis of surface area available, however even this is a problem in the two systems tested. In the photosynthetic column, the raschig rings caused shading in portions of the column, resulting in uneven illumination and uneven cell growth; the population of cells was a mixture of sulfide metabolizing and non-sulfide metabolizing organisms. In the tube system, although the total population growth appeared to be uniform throughout because of even illumination and a high loading rate, the composition of the biomass changed with the distance from the inlet. The green sulfide metabolizing bacteria predominated at the inlet, but as the distance from the sulfide source increased the population gradually became predominantly composed of the red, non-sulfide metabolizing organisms. The length of the tube over which the population of sulfide using bacteria predominated appeared to be related to the initial sulfide concentration in the feed. As shown in Table 2, at a loading rate of 1.1 g S^{2-} l^{-1} day^{-1} the length of the tube required was about 1.2 m or roughly 0.01 m^2 of surface area, while at 2.0 g S^{2-} l^{-1} day^{-1} the tube length in which the green cells predominated was about 3.1 m or about 0.03 m^2. This estimate, as earlier discussed, does not take into consideration the change in the composition of the population.

A number of aspects of this sulfide removing process using photosynthetic bacteria must be investigated before an actual evaluation or a pilot scale operation can be considered. Among these considerations are the need for an appropriate design which would incorporate the need for a large surface area, good flow characteristics to ensure optimal substrate-surface contact, even illumination, possibly combining use of sunlight with artificial light and simplicity in design for simple servicing. In addition, the illumination requirements of the photosynthetic bacteria should be investigated so as to determine the appropriate illumination intensities so as to minimize energy requirements. Photosynthetic bacteria are known to selectively absorb light mainly in the red section of the light spectrum hence it may be possible to minimize energy requirements by using low intensity light sources in the red, instead of bright white lights.

CONCLUSION

The results of this investigation showed that sulfide removal by photosynthetic bacteria is feasible and may be efficiently accomplished under proper illumination, good cell-sulfide contact and an anaerobic environment.

The organisms primarily involved in sulfide removal in this system appear to be *Chlorobium limicola*. Sulfide is oxidized by these organisms to sulfate via elemental sulfur.

The advantages of sulfide removal by this method are simplicity, little or no loss of H_2S into the atmos-

phere during treatment, lower energy requirements (unless artificial illumination is needed) and no chemical additives.

Much research is needed in the areas of illumination requirement and reactor design before this method can be used on a pilot scale, or even analyzed for process energy requirements.

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