

## PHOTOSYNTHETIC BACTERIA: ARE THEY PRESENT IN BIOGAS DIGESTERS ?

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### ABSTRACT

Attempts were made to isolate photosynthetic bacteria in translucent biogas digesters made of fibreglass reinforced plastic which allow light to pass through. The results revealed the presence of purple non sulphur bacteria possibly of the genus *Rhodospseudomonas* in the digester.

Anaerobic digester is a microbial system in which several groups of microorganisms must interact effectively if organic matter is to be converted to methane. Pfenning (1967) established that under anaerobic conditions, organic matter is fermented and release end products such as hydrogen, acetic, propionic, lactic and butyric acids and ethonolamines. Such compounds would accumulate under anaerobic conditions if they are not oxidised by bacteria which respire with nitrate (nitrate reducers) sulphate (sulphate reducers). Besides these two groups of bacteria, there exist two more groups which can utilise these compounds. They are the methane and photosynthetic bacteria can thrive in anaerobic niches whether it is natural or man made which have access to light.

The outstanding physiological property of photosynthetic bacteria is the ability to grow rapidly, strictly under anaerobic conditions, using light as the source of energy. (Gest, 1972) In the present investigation, an attempt was made to isolate photosynthetic anaerobes especially the purple non sulphur bacteria in a biogas digester made of fibreglass reinforced plastic which permits light to pass through.

### MATERIALS AND METHODS

#### Collection of samples

Slurry samples were collected from the digester from the sample outlet in presterilised bottles. The samples were then diluted in dilution medium (0.5% bicarbonate; 0.1% carbonate; 0.0001% resazurin).

#### Enumeration of purple non sulphur bacteria

The population of purple non sulphur bacteria was enumerated using RCVBN medium (Weave *et al.*, 1975) adapting roll tube technique. The roll tubes were then incubated under incandescent light atmosphere (25 W tungston lamps) for about 3-4 weeks. The individual colonies showing pink colour were counted.

#### Spectral analysis of photosynthetic anaerobes

The total cell suspension of purple non sulphur bacteria was scanned in DU-64 spectrophotometer in UV and visible range and their absorption maxima recorded.

#### Spectral analysis of bacteriochlorophyll

The pigment analysis of phototrophically grown cells of the purple bacteria were measured after culturing the cells in a large container (2 l) for about 30 days. The cells were harvested after centrifugation at 15000 g for 15 minutes. The pellets were extracted at 15° C with methanol - diethyl ether - petroleum ether (5:2:1v/v) for 5 min. Insoluble materials were removed by centrifugation at 15000 g for 10 min. The supernatant was taken for bacteriochlorophyll spectral analysis. The absorption maxima was measured in DU-64 Beckmann spectrophotometer (Redlinger and Fuller, 1985).

### RESULTS AND DISCUSSION

The population of non sulphur purple bacteria was found to be  $26 \times 10^6$  /ml; and  $18.3 \times 10^6$  /ml respectively during the three samplings. The population showed reduction over time (Table 1). The growth of purple non sulphur bacteria in the biogas digester slurry was well evidenced.

**Table 1. Population of purple non sulphur bacteria**

Incubation time (days)	Population (10/ml)
10	26.00
20	22.67
30	18.30

**Table 2. Absorbtion peaks of total cell suspension of *Rhodopseudomonas* sp.**

Peak No.	Absorbance	Wavelength (nm)
1	0.080	808
2	0.120	858

**Table 3. Absorbtion peaks of bacteriochlorophyll extract of *Rhodopseudomonas* sp.**

Peak No.	Absorbance	Wavelength (nm)
1	2.600	772
2	1.200	647
3	1.000	600
4	3.400	413

Pfenning (1967) stated that under anaerobic conditions, organic substances are fermented and end product released such as hydrogen, acetic, propionic, lactic, butyric acids and ethonolamines are oxidised by bacteria like sulphate and nitrate respirers, methanogens and phototrophs. Schlegel (1985) reported the presence of phototrophs in anaerobic niches whether natural or man made. In the present investigation, the environment from which the purple sulphur bacteria isolated had the anaerobic condition and had access for light, which are the essential features for the growth of phototrophs.

The total cell suspension was scanned for absorbtion maxima under UV and visible range which showed peaks at 865nm (Table 2). Pfenning (1967) reported that the absorbtion maxima of BChl "a" of purple bacteria occurs in the spectral forms of viz., B 800, B 850 and B 870 - 890. Schlegel (1985) suggested BChl "a" has two distinct spectral forms at 808nm and 868nm. The present study revealed the presence of BChl "a" in

the cultures tested which could present only in the purple bacteria. Weaver *et al.*, (1975) reported that the *Rhodopseudomonas capsulata* grown anaerobically in light showed two peaks at 858nm and 802nm. Hence the culture in the present study is temporarily assigned to the genus *Rhodopseudomonas*.

### Spectral analysis of bacteriochlorophyll

The bacteriochlorophyll extracted in the methanol diethylether and petroleum ether, when scanned under UV and visible range of light shwoed peaks at 772nm, (Table 2). Weaver *et al.*, (1975) evidenced the BChl "a" of *Rhodopseudomonascapsulata* at 772nm and a secondary absorbtion peak at 600nm. The other peaks caused may be due to other purple bacteria in the culture.

The results of the present study revealed the presence of purple non sulphur bacteria possibly belonging to the genus *Rhodopseudomonas* in the translucent biogas digester which would play a role in the generation of hydrogen and thereby facilitating hydrogen utilising methanogens in the production of methane.

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