



## PRODUCTION OF BIOGAS FROM CELLULOSE AND BENZOATE USING ANAEROBIC BACTERIAL CONSORTIA

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### Abstract:

*Among the energy sources, the depletion of non-renewable energy is of great concern in the present days. The non-renewable energy deposits are continuously depleted by human withdrawals. An alarming situation of continuous depletion in the natural resources by an increased consumption of energy, the urge to look out for alternate ways for energy production in on the high stand. Anaerobic bacteria are known to be efficient energy producers. In the present study, the anaerobic bacterial hydrogen production from different consortia isolated from different sources was studied. Anaerobic consortia were isolated from different sources by inoculating into specific media, Beibl and Pfennigs medium anaerobically in the light (2000 lux). Bergey's Manual of Systematic Bacteriology (1994) was adopted for identification of phototrophic bacteria. Growth was determined using UV-Vis spectrophotometer at 660nm. The effect of two major substrates i.e. Benzoate and Cellulose has been investigated. The amount of biogas produced varied with benzoate and cellulose as substrates. Among the 10(ten) consortia, for most of the consortia cellulose was confirmed to be utilized as a major substrate for the production of biogas.*

**Keywords:** Photosynthetic Bacteria; Hydrogen; Cellulose; Benzoate.

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### 1. Introduction

Contemporary human society is based on non-renewable fossil fuels (oil, coal, natural gas), which represents more than 80% of our energy consumption. The rapid growth of the human population, improvement of living standards and the parallel increase of industrial demands all call for more energy. The inevitable exhaustion of economically and energetically exploitable fossil fuels will not meet the energy needs of consumers. Because the increasing price of their recovery, fossil fuels gradually lose their competitive edge. This tendency is accompanied with

the alarming phenomenon of climate change, which is the result of burning fossil fuels and releasing excess amounts of greenhouse gases to the atmosphere threatening the living conditions in our planet. Therefore, worldwide attention is paid to the replacement of the current energy carriers with alternative, renewable ones with rapid technology development. These alternatives have a common character, i.e. the energy comes from renewable sources and the raw materials for energy generation are reformed at the rate of utilization. One of the most promising alternatives is biogas. The simplest and most common use of biogas is to burn it producing simultaneously heat and electricity. The fermentation residue can substitute artificial synthetic fertilizers; therefore it is an excellent soil nutrient replacement. The main component of the biogas is methane, depending on the fermentation substrate it makes up to 50-75% of the gas, in addition it contains carbon dioxide (28-48%) and other gases (~1-2%) for example hydrogen sulphide and nitrogen compounds. The fossil “natural gas” comprises usually more than 90% of methane. This value can be achieved in the case of biogas, if carbon dioxide is removed from the raw gas. After gas cleaning the heat value of biogas may be equivalent to natural gas, thus the gas can be fed to the gas network. It has long been known that biogas production also occurs spontaneously in marshes, landfill sites, where anaerobic environment can develop. The biogas formation process takes place under anaerobic conditions, but facultative anaerobes are also involved in the breakdown of organic materials. The anaerobic conversion of various materials is a complex chain of biochemical process which is carried out by a diverse microbial community the composition of this community depends on various environmental factors. The events leading to biomass decomposition follow each other in precise order, each step is carried out by a specific microbial group. The different types of microbes are interdependent and operate in harmony. The conversion of complex organic molecules into methane can only be successful if the bacteria develop specific community in which each species can make a living, and leaves behind a product for the following group of microbes as food. Biogas production is therefore a complex microbiological process; the microorganisms constitute a complex food chain where biogas is the end product. In the present study, the anaerobic bacteria hydrogen production from different consortia isolated from ten different sources was studied. The effect of two major substrates i.e. Benzoate and Cellulose has been investigated.

## 2. Materials and Methods

The phototrophic bacteria were isolated from the effluent samples by enrichment techniques by inoculating into the medium and incubated anaerobically in the light (2000 lux). Bergey's Manual of Systematic Bacteriology (1994) [6] was adopted for identification. Growth was determined by measuring optical density at 660 nm using UV-Vis spectrophotometer. Thirty ml of bacterial culture was centrifuged at 10,000xg for 10 min. The harvested cells were washed with 0.3% saline and the cells were suspended in the basal medium. Ten day old cultures of photosynthetic bacteria isolated from ten different sources were inoculated (1% v/v) into basal medium containing two different carbon sources i.e. Benzoate and Cellulose with ammonium chloride (0.5%) was used as nitrogen source and B12 as the growth factor. The incubation period was 196 hrs after inoculation of the consortium. The technique used for hydrogen measurement was water displacement technique.

### 3. Results and Discussion

Conventional methods of hydrogen production are found to be causing environmental pollution and are energy expensive (Nath & Das, 2003; Logan, 2004; Nath & Das, 2004). Due to rapid industrialization there is a greater demand for energy requirement. Fossil fuels should be replaced with renewable energy sources which are sustainable. (Kim et al., 2004, Nandi et al., 1998). Hydrogen represents a promising clean alternative energy to fossil fuels, as its combustion produces only energy and water. However, actual production of hydrogen comes from catalytic reforming based on the use of fossil fuel. Numerous microorganisms can produce hydrogen during dark fermentation, an anaerobic process of biomass degradation. Biogas is a promising candidate as the technology of its production may combine the treatment of various organic wastes with the generation of an energy carrier for the most versatile applications [1]. Biogas can be converted to heat and/or electricity, and its purified derivative, biomethane, is suitable for every function for which fossil natural gas is used today. The decomposition of organic materials by a microbial community is carried out under anaerobic conditions [2]. Photosynthetic bacteria are also proven to be one of the major sources for biogas production. Major reports also indicate that the use of Photosynthetic bacteria for biogas production. This study emphasises on the substrate specificity of Photosynthetic bacteria for Biogas production.

In continuation of our earlier work, on exploring the biotechnological applications of Photosynthetic bacteria [7-28]. Different photosynthetic bacterial consortia were isolated using enrichment techniques from different places in Hyderabad, Telangana and Rourkela, Orissa. The log phase cultures which are ten day grown cultures was used to assess their potential of producing biogas. The results are tabulated as below (Table 1,2,&3). Consortium 10 isolated from a soil sample of Rourkela produced the highest amount of hydrogen in benzoate as carbon source compared to other photosynthetic bacteria followed by consortium 8 and consortium 2.

Table 1: Consortium isolated from different sources

Consortium 1	Soil sample, OU Campus, Hyderabad
Consortium 2	Water sample, OU Campus, Hyderabad
Consortium 3	Sewage water, Tarnaka, Hyderabad.
Consortium 4	Sewage water, Tarnaka, Hyderabad.
Consortium 5	Stagnant water, Begumpet, Hyderabad
Consortium 6	Stagnant water, Begumpet, Hyderabad
Consortium 7	Pond water, Safilguda, Hyderabad
Consortium 8	Pond water, Safilguda, Hyderabad
Consortium 9	Soil sample, Rourkela.
Consortium 10	Soil sample, Rourkela.

Table 2: Effect of benzoate on the growth of different consortia

Photosynthetic consortia	O.D at 660 nm	Biogas produced (ml/30ml vessel)
Consortium 1	1.178	6.5±0.3
Consortium 2	1.6561	7.0±0.2
Consortium 3	1.2186	6.0±0.2
Consortium 4	1.4069	6.3±0.1

Consortium 5	1.0421	6.0±0.2
Consortium 6	1.5847	6.9±0.4
Consortium 7	1.5642	6.8±0.3
Consortium 8	1.6532	7.1±0.3
Consortium 9	1.2354	6.2±0.4
Consortium 10	1.8791	7.3±0.2

Table 3: Effect of cellulose on the growth of different consortia

Photosynthetic consortia	O.D at 660 nm	Biogas produced (ml/30ml vessel)
Consortium 1	1.9052	7.2±0.3
Consortium 2	1.7892	6.8±0.2
Consortium 3	1.3545	6.3±0.4
Consortium 4	1.9845	7.2±0.3
Consortium 5	1.2568	6.2±0.2
Consortium 6	1.9825	7.3±0.4
Consortium 7	1.0250	6.1±0.2
Consortium 8	1.9875	7.4±0.3
Consortium 9	1.4562	6.3±0.5
Consortium 10	2.0652	7.5±0.4

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