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PHOTOCONVERSION OF ORGANIC SUBSTRATES INTO HYDROGEN USING PHOTOSYNTHETIC BACTERIA

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ABSTRACT

Under nitrogen-limited conditions, photosynthetic bacteria photoconvert a wide variety of organic substrates nearly totally into H_2 and CO_2. More than 98% of the chemical energy of defined organic compounds even from dilute solutions can be recovered as combustible energy of the H_2 produced. Not calculating the chemical energy input, radiant (solar) energy recoveries are approximately 5% over a wide range of incident light intensities. Batch cultures can photoproduce H_2 at rates of 175 ml per gram dry weight of cells per hour (equal to a volume of H_2 per equivalent volume of liquid medium every 4 to 6 hours) when incubated in saturating light. With periodic refeeding, rates remain constant for several weeks. In closed containers H_2 pressures of 735 psig can be generated. In principle, this pressure can be used to decrease storage volume of the gas, to move it through pipelines or to provide required process pressures. Alcohol stillage and food processing wastes are excellent photoconvertible substances. When non-photosynthetic bacteria synthesizing appropriate polysaccharases are included in co-culture with photosynthetic bacteria, cellulose and other polysaccharides can be converted to H_2 and CO_2, albeit at low rates. Prospects for enhancing the photoconversion reactions of photosynthetic bacteria by environmental and genetic manipulations are discussed.

INTRODUCTION

The biological capacity to take up or evolve molecular hydrogen probably occurs only in the microbial realm of the biosphere. These phenomena are especially prevalent among photosynthetic microorganisms, which include the photosynthetic bacteria, cyanobacteria (formerly known as blue-green algae) and eucaryotic algae.

Two general classes of enzyme complexes, hydrogenases and nitrogenases, are closely associated with the final H_2 evolving act. However, the primary functions of the enzymes are quite distinct. Hydrogenases normally catalyze hydrogen uptake or consuming reactions, whereas nitrogenases are normally operative in biological nitrogen fixation, wherein atmospheric N_2 is reduced to the level of ammonia. Nevertheless, under appropriate conditions both can catalyze a different reaction, the photoproduction of H_2. A more complete description of these enzyme complexes and their relationships to photosynthesis is given in Ref. 4.

Hydrogen produced from cyanobacteria and algae is ultimately derived exclusively from water, an obviously plentiful source. However, water-splitting by these organisms simultaneously produces oxygen as well, which inactivates the hydrogenases and nitrogenases responsible for hydrogen production. Oxygen-inactivation severely restricts the rates and especially the duration of hydrogen photoproduction in these systems.

Photosynthetic bacteria, on the other hand, do not split water in light-driven reactions. Instead they utilize reduced carbon (or sulfur) compounds that can be hydrated and dehydrogenated to produce H_2. Half the electrons come from the reduced substrate and half come from water. No oxygen is produced to inhibit H_2 photoproduction as the oxidizing equivalents are removed as CO_2.

Hydrogen evolution in Rhodopseudomonas capsulata is nearly totally mediated by the nitrogenase enzyme complex (2). The presence in the growth medium of atmospheric nitrogen, ammonia, or compounds yielding appreciable levels of ammonia through catabolism (though not necessarily amino acids) inhibit or abolish H_2 production, promoting cell growth instead (1). Applied use of photosynthetic bacteria to photoconvert organic substrates into hydrogen therefore requires limitation and control of the amount of nitrogen added to the cultures.

RESULTS AND DISCUSSION

More than 100 distinct wild-type strains of 13 species of photosynthetic bacteria have been examined for their capacity to photoconvert organic substrates into H_2. One of the highest evolution rates from a wide variety of substrates was achieved using Rps. capsulata SCJ. Unless otherwise noted the data were obtained from this organism.

Stoichiometry and Rates of Hydrogen Production

The photoconversion stoichiometry for several organic substrates is indicated in Table 1. Using N-limited (and therefore non-growing) cultures nearly all of a substrate, such as lactic acid, is converted to H_2 and CO_2. At the cessation of gas production no detectable level of lactic acid remains in the medium, indicating the bacterium's capacity to scavenge low concentrations of substances from dilute sources.

Again using the example of lactic acid, chemical energy (free energy of formation) is 97% conserved in the form of evolved H_2 (free energy of combustion). The reaction is driven, of course, by the additional energy input of absorbed light. Assuming the biomass substrate to be free or a credit and therefore ignoring its chemical energy input, 4.9% of the incident radiant energy up to saturation is recoverable as combustible energy of H_2.

Rates of hydrogen production are dependent upon light intensity and bacterial density. With saturating light a constant rate of 175 ml H_2 per gram dry weight per hour can easily be maintained. This is equivalent to a volume of H_2 per equivalent volume of medium every 4-6 hours at normal cell densities.

Duration of Hydrogen Production

With constant light intensity on batch cultures, H_2 is photoproduced at constant rates until the substrate is exhausted (Fig. 1). The initial rate is resumed with the refeeding of additional substrate. After 4-5 refeedings salt buildup from the neutralized organic acids approximates 2%, which is inhibitory to active H_2 photoproduction in this strain. Preliminary tests with continuous-flow (rather than batch) cultures produce H_2 at slightly higher rates and for more prolonged periods of time.

Substrates Utilized

A wide variety of organic substrates can be utilized by photosynthetic bacteria. Table 2 indicates a number of substrates capable of supporting growth and hydrogen production (though not all have been tested for the latter function). No single isolate is capable of using all of the listed substrates, but Rps. capsulata SCJ can catabolize nearly all.

Whereas sugars and sugar alcohols can be photoconverted directly into hydrogen, indigenous nonphotosynthetic microbes rapidly ferment these substrates to alcoholic or acidic products with a resultant, inhibitory drop in pH. Prior sterilization of the substrate probably would not be economically feasible. Organic acids and alcohols on the other hand, are essentially non-fermentable by most bacteria, yet these are the preferred substrates for photosynthetic bacteria. Contamination levels in cultures maintained on unsterilized organic acid media have not risen above about 2% during two years of subculturing.

Preliminary experiments with the neutralized soluble fraction from alcohol stillage indicate that 60-80% of the residues (calculated as

TABLE 1 Stoichiometry of Photosynthetic Bacterial Conversions

1. $C_3H_6O_3$ (Lactic Acid) + 3 H_2O $\xrightarrow{h\nu}$ 3 CO_2 + 6 H_2

2. $C_2H_{12}O_2$ (Glucose) + 6 H_2O $\xrightarrow{h\nu}$ 6 CO_2 + 12 H_2

3. $C_2H_4O_2$ (Acetic Acid) + 2 H_2O $\xrightarrow{h\nu}$ 2 CO_2 + 4 H_2

4. $C_4H_6O_5$ (Malic Acid) + 3 H_2O $\xrightarrow{h\nu}$ 4 CO_2 + 5 H_2

5. $(CH_2O)_n$ (Biomass) + H_2O $\xrightarrow{h\nu}$ CO_2 + 2 H_2

Reaction Extent:	>98% complete for example 1 (>1350 liters H_2/kg substrate).
Energy Conversion:	4.9% of radiant energy (or 97% of chemical energy) in example 1 is conserved as H_2.
Evolution Rates:	1 vol H_2 · equal vol. cells^{-1} · min.$^{-1}$ or 175 ml H_2 · g dry wt^{-1} hr^{-1}.

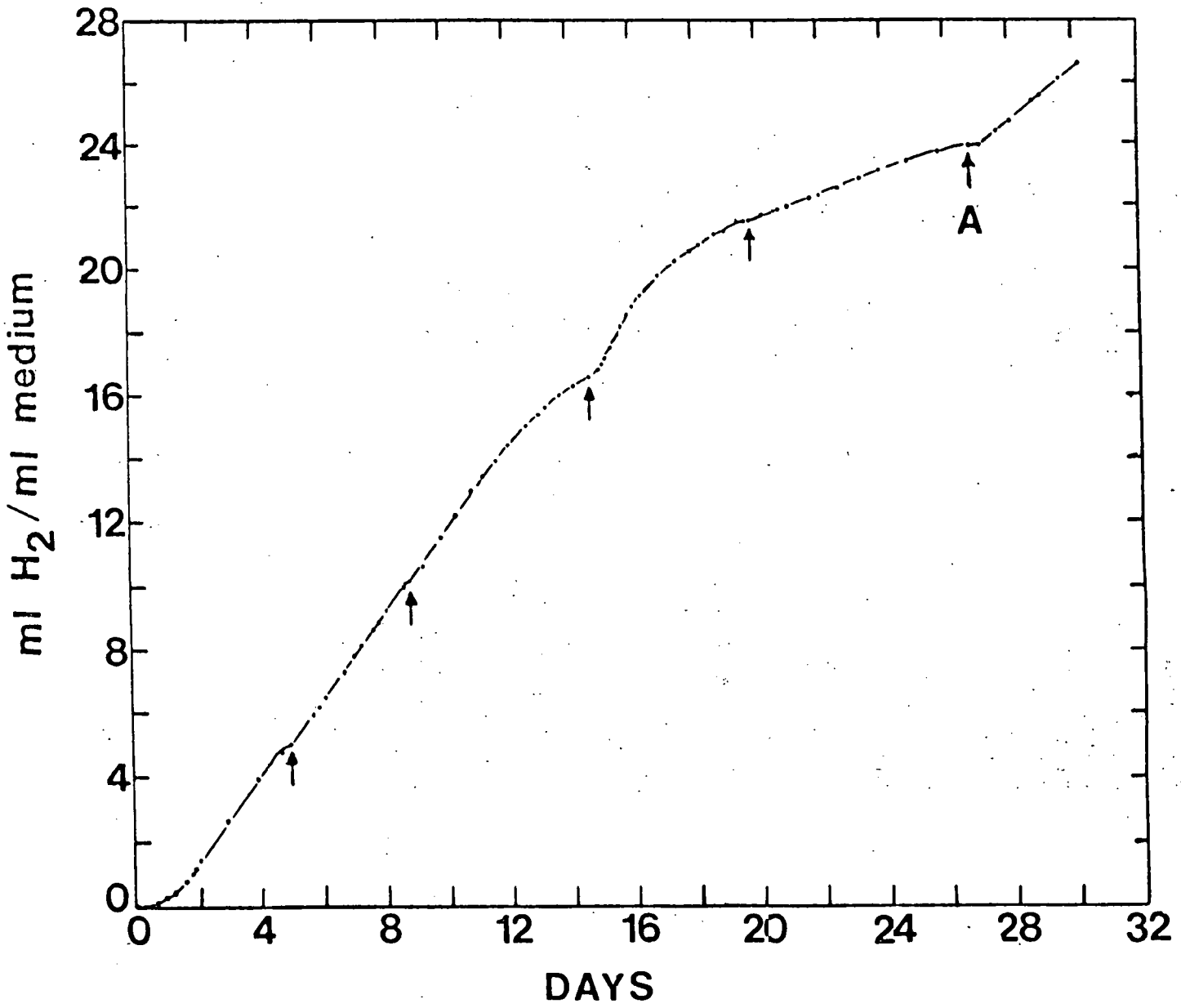

Fig. 1. Duration of hydrogen production from sodium lactate mediated by Rps. capsulata SCJ. Additional volumes of sodium lactate (40 mM) were added at points indicated by the arrows. The culture was centrifuged at time A and resuspended in fresh medium without a nitrogen source.

TABLE 2 Substrates Utilized by Photosynthetic Bacteria

SUGARS
Cellobiose
Sucrose
Glucose
Fructose
Mannose
Galactose
Ribose
Xylose

SUGAR ALCOHOLS
Mannitol
Sorbitol

SUGAR ACIDS
Gluconic Acid
Glucuronic Acid

ORGANIC ALCOHOLS
Ethanol
Glycerol
Propanol
Butanol
Isopropanol

INORGANIC DONORS
Sodium Thiosulfate
Hydrogen Sulfide

ORGANIC GASES
Methane
Carbon Monoxide

ORGANIC ACIDS
Acetic Acid
Lactic Acid
Pyruvic Acid
Formic Acid
Succinic Acid
Propionic Acid
Butyric Acid
Isobutyric Acid
Citric Acid
Fumaric Acid
Malic Acid
Tartaric Acid
Valeric Acid
Caproic Acid
Caprylic Acid
Pelargonic Acid
Malonic Acid
Glycolic Acid

AROMATIC ACIDS
Benzoic Acid

NATURAL SUBSTRATES
Alcohol stillage
Fermented Corn
Fermented Milk
Fermented Orange Juice
Fermented Grapefruit
Fermented Peach

$(CH_2O)_n$ after evaporation) can be photoconverted into hydrogen. More extensive applied attempts to utilize this substrate have recently been undertaken. Fruit and vegetable wastes are also excellent substrates once they have been anaerobically fermented to organic acid and alcohol products with subsequent neutralization.

Certain biomass substrates cannot currently be used to photoproduce H_2 and should be noted. An obvious example is sewage, which is an excellent medium for growth of photosynthetic bacteria, but the high fixed nitrogen content represses nitrogenase synthesis and inhibits H_2 production activity. Wild-type photosynthetic bacteria can aid in clarifying sewage and other high-nitrogeneous wastewater streams, but cannot produce H_2 from them. Cellulose has been tested as a substrate for many photosynthetic bacterial strains and only one, Rps. palustris E_c, exhibited slight growth. No strain has been found capable of attacking lignin, although free phenylpropanes can be catabolized by some.

Gas Pressures Generated

When photosynthetic bacteria produce gas in enclosed pressure vessels they can generate pressures at least as high as 735 psig (Fig. 2). This pressure in all likelihood does not indicate the true equilibrium of the reaction, but rather reflects exhaustion of the substrate as well as inhibition by decreasing pH due to the bicarbonate produced. The highly exergonic nature of H_2 production in these bacteria is directly attributable to the unique nature of the nitrogenase complex and its being driven by the energy of light conserved as ATP.

While transparent containers capable of withstanding high pressures may not be feasible on a commercial scale, it is reasonable to consider producing moderate pressure hydrogen (under 5 atm) for storage, transmission, and process use.

Improving Solar Biogasification

Through proper strain selection and environmental or genetic manipulations, the usefulness and efficiencies of photosynthetic bacteria as photoconversion agents can certainly be further improved.

A simple expedient to enhance H_2 production rates would be to use mixed cultures of different strains of photosynthetic bacteria that absorb in different portions of the solar spectrum. They co-exist very successfully since there is very little competition for the same wavelengths of light, epecially those of the infrared. A mixture of three strains of photosynthetic bacteria and a cyanobacterium will produce a "biological blackbody" which readily absorbs all wavelengths of light from 350 nm to 1050 nm. Unfortunately, of the pure strains examined to date which exhibit prolonged and high rates of H_2 production, none contain chlorophyllous pigments other than bacteriochlorophyll a.

Acid-tolerant strains of photosynthetic bacteria would also be of value in applied photoconversion schemes since fermented, nitrogen-limited biomass wastes are usually rather acidic. Presently, these fermented wastes must be neutralized before being biologically photoconverted. Acid-tolerant strains would obviate this

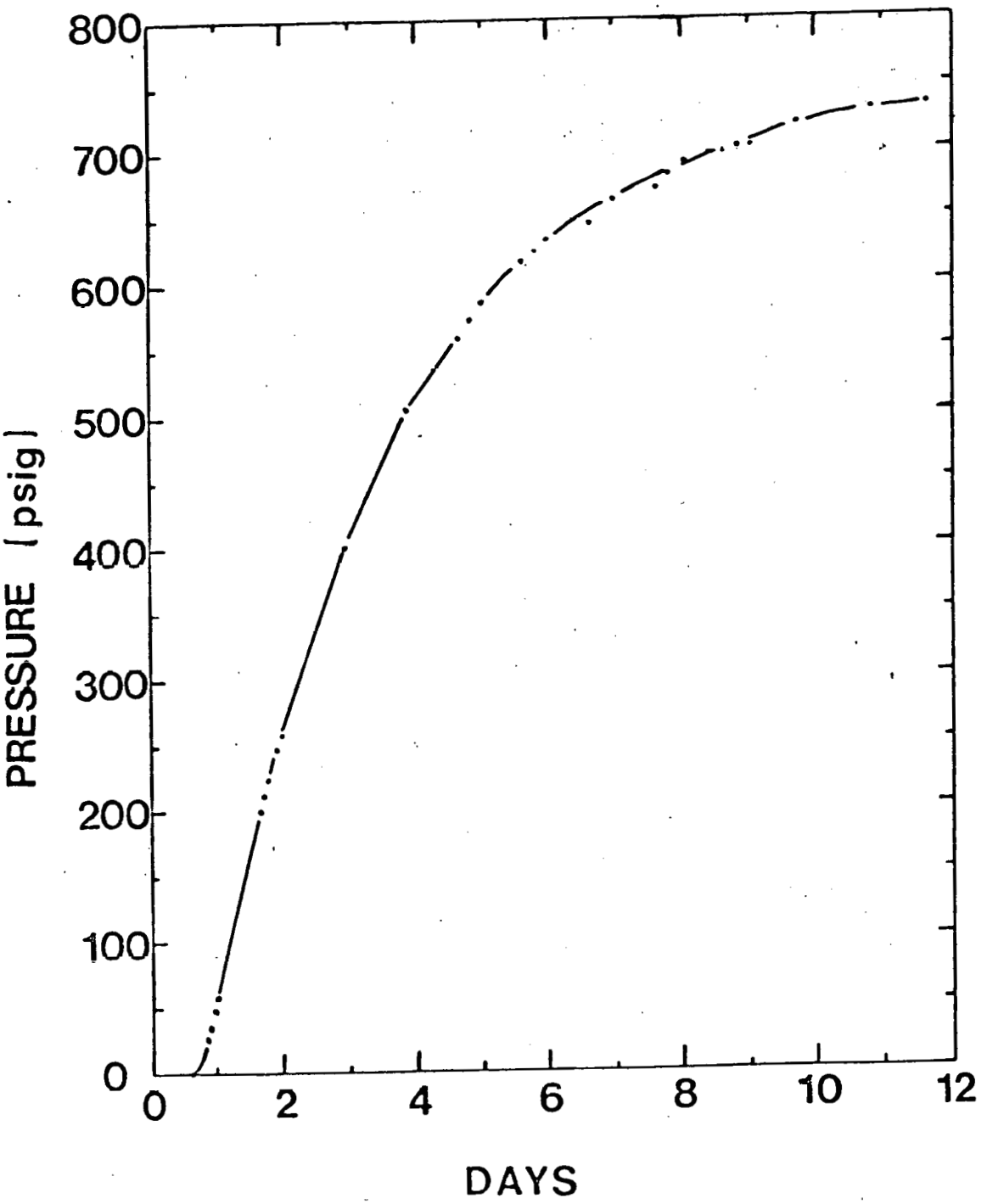

Fig. 2. Pressure generations from evolved H_2 and CO_2 by photoconversion of sodium lactate (40 mM) using Rps. capsulata SCJ. The vessel was a Fischer-Porter hydrogenation vessel (87 ml) fitted with a 1000 psi dial pressure gauge.

requirement. A few such strains of Rps. acidophila and Rhodomicrobium vanniellii have been tested for H_2 production at lowered pH, but none as yet has exhibited high activity.

Thermophillic photosynthetic bacteria may have merit as well, due to their enhanced metabolic rates and the greater stability of their enzymes to physical and chemical influences. At least one genus of thermophiles has been described, but has not been surveyed for H_2 production.

As previously mentioned, we have not found any strains of photosynthetic bacteria that would appreciably catabolize polysaccharides by themselves. However, in concert with non-photosynthetic bacteria which make appropriate polysaccharases, the released sugars and organic acids from polysaccharides can be assimilated by photosynthetic bacteria and converted into H_2. As yet the rates remain very low and of short duration, both of which can be attributed to the rate-limiting steps of the non-photosynthetic member. Using a Bacteroides strain in co-culture with Rps. capsulata, cellulose (Whatman No. 1 filter paper) can be photoconverted into H_2 at a rate of about one volume of medium per two weeks. Alternatively, agar conversion to H_2 can be mediated by a co-culture of Rps. sulfidophila BSW8 and an untyped marine isolate at rates four-fold higher, but the duration is limited to less than one week. Perhaps a more promising approach may be to utilize a major algal product, such as the glycerol produced in high amounts by Dunaliella, either in continuous or two-stage mixed cultures.

Genetic techniques also can be employed to amplify photoconversion processes. Rps. capsulata mutant W12 is defective in its capacity to fix nitrogen and only produces small amounts of H_2. Since hydrogenase is present in the organism, the nitrogenase enzyme complex is responsible for at least the majority of the H_2 evolved in the wild-type (2). Strain W52, however, is defective in the uptake of H_2, and the rate of H_2 production is increased relative to that of the wild-type. This indicates that H_2 evolved by nitrogenase is incapable of being reoxidized in this type of mutant, and therefore, the H_2 yield is higher.

Rates of H_2 production also might be improved by creating mutants genetically derepressed for nitrogenase. Such a strain would permit the photoconversion of high-nitrogeneous biomass wastes such as sewage. A partially derepressed mutant of R. rubrum has been generated by Weare (3). The mutant exhibited a 75% increase in H_2 production over the wild-type parent. It is important to remember, however, that a mutant bacterium operating in an applied system would have to compete favorably with revertants and the indigenous, wild-type photosynthetic bacteria present in the unsterilized biomass wastes. Periodic reinoculation of mutants may keep their titers sufficiently high, but at the present time they are still laboratory phenomenona.

Another means to circumvent the inhibition of H_2 production by ammonia would be to isolate or create a bacterial strain that uses hydrogenase rather than nitrogenase as the terminal, evolving enzyme. Also, H_2 production utilizing hydrogenase is less energy intensive as it is not driven by ATP, and, as a result, the rates of production may be much

higher. Along this line, Rps. sulfidiphila BSW8 is a natural isolate that can derepress its hydrogenase activity to a level 20-50 times higher than most other photosynthetic bacteria. Although the conditions are less than optimal as yet, about 5% of the H_2 evolving activity of this strain is impervious to the presence of ammonia and therefore presumably mediated by the hydrogenase enzyme.

At present we do not know what rate-limiting steps are involved in the photoproduction of H_2, but in vivo genetic engineering may well be used to remove them as they are discovered.

CONCLUSION

Photosynthetic bacteria offer promise as a low technology method for converting dilute biomass substrates into combustible, gaseous H_2. Although environmental conditions and strains have not been optimized as yet, the process is considered feasible today from a scientific standpoint given an appropriate biomass source. From an engineering standpoint the system remains untested, but a small scale process design unit will be designed and constructed in the summer of 1981. "Thin" stillage waste from an alcohol plant will be the initial substrate utilized.

REFERENCES CITED

1. Hilmer, P. and Gest, H., "H_2 Metabolism in the Photosynthetic Bacterium, Rhodopseudomonas capsulata", J. Bacteriol. 129, 724-39 (1977).

2. Wall, J.D., Weaver, P.F. and Gest, H., "Genetic Transfer of Nitrogenase - Hydrogenase Activity in Rhodopseudomonas capsulata", Nature 258, 630-1 (1975).

3. Weare, N.M., "The Photoproduction of H_2 and NH_4^+ by a Derepressed Mutant of Rhodospirillum rubrum", Biochim. Biophys. Acta 502 486-94 (1978).

4. Weaver, P.F., Lien, S. and Seibert, M., "Photobiological Production of Hydrogen", Solar Energy 24, 3-45 (1980).