The potential of bacterial photosynthesis in recycling of human wastes

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(1) Potential uses of photosynthetic bacteria in the recycling of human wastes have been considered. It is concluded that systems using photosynthetic bacteria may have advantages over purely algal systems under conditions where total recycling is needed and energy is not abundant.

(2) Two systems are proposed in which the metabolic potential of the photosynthetic bacteria might be realized. Both systems include an anaerobic recycling stage in which fermented wastes are converted to biomass by bacterial photosynthesis. In one system gaseous recycling is accomplished by a secondary algal reactor acting under aerobic conditions. In the other, oxygen is regenerated by electrolysis of water, and hydrogen and carbon dioxide are assimilated autotrophically under anaerobic conditions in the same bacterial photosynthesis reactor used for anaerobic recycling.

(3) Advantages and disadvantages arising from the use of photosynthetic bacteria in recycling are discussed, and some potential model systems are proposed.

INTRODUCTION

Interest in photosynthetic recycling has inevitably been focused on systems using green plants, algae, or blue green algae (Burlew 1953; Golueke & Oswald 1959; Myers 1964; Terskov & Gitelzon 1969; Kovrov & Belyanin 1969; Shelef, Oswald & Golueke 1969; Humenik & Hanna 1970; Fogg 1971) since these play by far the most important ecological role in the synthetic reactions of the carbon and nitrogen cycles. Indeed, the major interest in bacterial recycling has centred on the Hydrogenomonas system (Foster & Litchfield 1964; Jenkins 1966) in which the role of green plants in energy conversion and the regeneration of O₂ is replaced by electrolysis of water, and the reduction of CO₂ is coupled to oxidation of hydrogen by the bacteria. This neglect of photosynthetic bacteria seems to me largely justified; certainly, in the recycling of gases and water, the photosynthetic bacteria are of little use. However, the extraordinary metabolic diversity of the photosynthetic bacteria (Kondrat'eva 1963; Pfennig 1967) suggests that they may be of real value in the total recycling of human waste, and it is in this context that I wish to consider their potential.

THE NATURE OF SEWAGE AND ITS TREATMENT

Although I do not want to delve too deeply into this aspect of my discussion, it is necessary to dabble briefly in order to draw attention to a side of this fascinating subject which is of importance in recycling processes—the waste of energy involved in conventional sewage treatments (Jenkins 1963; Casida 1968).
After Jenkins (1963). The carbon cycle involved can be approximately represented as below:

**aerobic oxidation**

\[
(CH_2O)_n + nO_2 \rightarrow nCO_2 + nH_2O
\]

**anaerobic digestion**

- carbohydrates \rightarrow organic acids, alcohols
- fats \rightarrow fatty acids, $H_2$, $CO_2$

**methane bacteria**

- organic acids, alcohols \rightarrow fatty acids, $H_2$, $CO_2$
- fatty acids, $H_2$ \rightarrow methane

**methane combustion**

\[
CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O
\]

**photosynthesis**

\[
nCO_2 + nH_2O \rightarrow (CH_2O)_n + nCO_2
\]
The workings of a sewage treatment plant are shown diagrammatically in figure 1. The raw sewage enters the plant, and after passing through coarse filters, goes into a series of settling tanks. The rate of flow determines the density of solid matter settled out,—rapid flow through the first tank allows only grit to settle—slower flow through subsequent tanks allows a large proportion of the suspended solid material to fall out.

Biological treatments of the precipitated solids and the supernatant fluids are by separate processes. The supernatant fluids are subjected to extensive aerobic oxidation by microorganisms which are either suspended in an activated sludge, or adsorbed onto the surface of an inert filter bed; in either case the result is a complete oxidation of the organic material to the level of CO₂, water, nitrate and sulphate.

The sludge formed by precipitation in the primary settling tanks, together with sludge from the final settling tank is subjected to an anaerobic digestion process. Digestion occurs in two stages. In the first (acid) phase, complex organic molecules are degraded by anaerobic bacterial fermentations. In the second (neutral) stage these simpler molecules are partially converted to gaseous products (mainly methane, H₂ and CO₂) by anaerobic dismutation catalysed by the methane bacteria. The combustible gases are vented off, or in more refined plants, burnt to provide useful energy (Golueke & Oswald 1959) and the solubilized organic material is returned to the sewage input to be oxidatively degraded to CO₂, water, etc. Intractable sludge is removed and treated by drying and spreading as manure. The object of sewage treatment is the complete oxidative degradation of the organic material to its simple stable forms. The chemical energy of the raw sewage is almost entirely dissipated as heat.

**ALGAL RECYCLING AND SEWAGE TREATMENT**

In a comprehensive series of papers Golueke, Oswald and their collaborators (Golueke & Oswald 1959; Shelef, Oswald & Golueke 1969) have considered the use of algae in the treatment of sewage and in total recycling, and in addition several groups of workers associated with the Soviet space effort have made great progress in the design of working algal recycling plants (Terskov & Gitelzon 1969; Kovrov & Belyanin 1969; Doucha et al. 1969). Humenik & Hanna (1970) have recently studied a symbiotic algal–bacterial culture as a working system for sewage treatment. These systems have several common features. They involve the oxidative degradation of organic matter to the CO₂, H₂O level by aerobic bacterial processes, and the reductive assimilation of these products by photosynthesis to produce algal biomass, which is cropped, and O₂. The two complementary processes involved (oxidative degradation and photosynthetic assimilation) may occur either in series, or in parallel—the systems are essentially aerobic and represent model ecological imitations of the biosphere.
The chemical nature of sewage is shown in table 1. The major organic constituents are at the reduction level of carbohydrates, proteins and fatty acids, that is at a similar energetic level to the constituents of living cells. Other constituents are at the level of fermentation products. The waste of energy involved in sewage treatment coupled to algal recycling is apparent from a consideration of the carbon cycle involved (figure 2).

**Table 1. Chemical composition of sewage**
(Data from Painter, Viney & Bywaters 1961, Shelef, Oswald & Golueke 1969.)

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Concentration</th>
<th>mg l(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty acid (% C)</td>
<td>22.8</td>
<td>301</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>17.1</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Fatty acid esters</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Soluble acids</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>Amino acids</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>Creatinine</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>Amino sugars</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Amides</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Detergent</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>Unaccounted</td>
<td>25.3</td>
<td></td>
</tr>
<tr>
<td>HCO(_3)</td>
<td>323</td>
<td></td>
</tr>
<tr>
<td>Cl(^{-})</td>
<td>302</td>
<td></td>
</tr>
<tr>
<td>SO(_4)(^{2-})</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>37.5</td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>PO(_4)(^{3-})</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Mg</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Al, Fe, Ti, Zn, Cu, B</td>
<td>&lt; 10 mg l(^{-1})</td>
<td></td>
</tr>
<tr>
<td>Sr, Mn, Ba, Cr, Ni, Pb</td>
<td>&lt; 1 mg l(^{-1})</td>
<td></td>
</tr>
<tr>
<td>Co, Mo, Sn, V, Ag, Cd, Zr, Bi</td>
<td>&lt; 0.1 mg l(^{-1})</td>
<td></td>
</tr>
</tbody>
</table>

Under circumstances in which abundant energy is available—sunlight on Earth or on a Moon station for instance—this waste of energy is tolerable, and the advantages of algal or green plant photosynthesis far outweigh energetic considerations. However, there are obviously hypothetical situations in which recycling with a minimal energy loss might be advantageous. Clearly a major gain in energy conservation would arise if recycling of waste could be achieved by an anaerobic process.

**Anaerobic recycling with photosynthetic bacteria**

The ecology and physiology of the photosynthetic bacteria have been admirably reviewed by Kondrati'eva (1963) and by Pfennig (1967). Both authors have emphasized the predominantly anaerobic ecology of the photosynthetic bacteria—
only a few members of the Athiorhodaceae are able to grow aerobically, while members of the Thiorhodoceae and Chlorobacteriaceae suffer lethal oxidation on illumination under aerobic conditions.

Under anaerobic conditions a recycling process occurs in nature in which organic matter is fermented by various microbes to the level of lactate, ethanol, etc. In the absence of O₂, these fermentation products are in part oxidized by bacteria which respire with nitrate and sulphate, but in the main they are metabolized either by the methane bacteria, or by the photosynthetic bacteria. The photosynthetic bacteria are able to use as substrates for cell growth all the products of fermentative metabolism, and in addition the reduced sulphur and nitrogen, and the methane produced by the methane bacteria. The anaerobic ecology of soils and muds therefore constitutes a complete recycling system—in fact an anaerobic culture in a closed bottle containing mud and water kept in dim light will live for years (Pfennig 1967). The driving force for such a cycle is of course the light absorbed by the photosynthetic bacteria. The viability of such a cycle and the role of the photosynthetic bacteria, arise mainly from three characteristics of the bacteria:

(1) Their ability to exist photoautotrophically on inorganic substrates like H₂, N₂, CO₂, H₂S and NH₃.

**Figure 2. Energetics of recycling.** The ordinate shows the energy level of metabolites, the vertical arrows show changes in energy (per mole of glucose) which occur during the process:

\[
\Delta G^\circ (\text{pH 7}) \quad \text{kJ mol}^{-1} \quad \text{kcal mol}^{-1}
\]

- glucose \(\longrightarrow\) 2 lactate \(\Delta G^\circ = 198 \quad 47.4\)
- glucose \(\longrightarrow\) 6 CO₂ + 6H₂O \(\Delta G^\circ = 2870 \quad 686\)
- 2 pyruvate (metabolism) \(\longrightarrow\) glucose \(\Delta G^\circ = 29 \quad 7\)
(2) The very wide range of organic substrates on which they exist photo-heterotrophically.

(3) The ability of the photosynthetic bacteria to transform organic compounds entirely into cell substance.

Some of the substrates which are used by photosynthetic bacteria, together with their roles in metabolism, are shown in table 2. Perhaps it should be emphasized that this wide range of substrates reflects a fairly wide range of species and strains. Nevertheless, it is clear from ecological considerations that the photosynthetic bacteria, as an ecological group, possess the widest of capabilities for the utilization of substrates at the level of the products of anaerobic fermentation.

**Table 2. Substrates of the Photosynthetic Bacteria**

(Data from Kondrat’eva 1963; Pfennig 1967; Fuller 1969.)

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Bacteria</th>
<th>Metabolic role</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{H}_2 )</td>
<td>S, NS</td>
<td>H donor</td>
</tr>
<tr>
<td>( \text{H}_2\text{S} )</td>
<td>S</td>
<td>H donor</td>
</tr>
<tr>
<td>( \text{Na}_2\text{S}_2\text{O}_3 )</td>
<td>S</td>
<td>H donor</td>
</tr>
<tr>
<td>S</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>( \text{CH}_4 )</td>
<td>NS</td>
<td>C source and H donor</td>
</tr>
<tr>
<td>( \text{CO}_2 )</td>
<td>S, NS</td>
<td>C source</td>
</tr>
<tr>
<td>( \text{N}_2 )</td>
<td>S, NS</td>
<td>N source</td>
</tr>
<tr>
<td>( \text{NH}_3 )</td>
<td>S, NS</td>
<td>N source</td>
</tr>
<tr>
<td>Simple organic acids</td>
<td>S, NS</td>
<td></td>
</tr>
<tr>
<td>Amino acids</td>
<td>S, NS</td>
<td></td>
</tr>
<tr>
<td>Peptones</td>
<td>S, NS</td>
<td></td>
</tr>
<tr>
<td>Fats, oils</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Sugars</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Alcohols</td>
<td>NS</td>
<td>growth substrates with</td>
</tr>
<tr>
<td>Ketones</td>
<td>NS</td>
<td>mixed roles</td>
</tr>
<tr>
<td>Aromatics</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Fatty acids</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Hydroxyacids</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

S, sulphur bacteria (Thiorhodaceae, Chlorobacteriaceae)
NS, non-sulphur bacteria (Athiorhodaceae).

**Artificial Anaerobic Recycling Using Photosynthetic Bacteria**

I have attempted in the discussion above to emphasize two points.

(a) Conventional recycling processes using aerobic degradation, followed by, or together with, algal photosynthesis, are energetically wasteful.

(b) The ecology and physiology of the photosynthetic bacteria suggests that they may be able to catalyse an anaerobic recycling which avoids much of the energetic waste of the conventional systems.

I propose in the rest of my discussion to consider two possible recycling systems in which this potential of the photosynthetic bacteria might be realized, and to point out some of the advantages and disadvantages of such systems.
**Anaerobic Recycling Coupled to Algal Photosynthesis**

The major drawback of a recycling system using photosynthetic bacteria is its complete inadequacy in gaseous recycling—the bacteria do not produce oxygen. Anaerobic recycling therefore has to be coupled to an oxygen producing system. In figure 3, I have represented a possible recycling system using separate anaerobic and aerobic stages. Solid and liquid wastes would be fed to an anaerobic digester for fermentation. The effluent from this would pass by way of a settling tank to an anaerobic photosynthetic reactor. The sludge from the settling tank would be recycled to the digester. In the anaerobic photosynthetic reactor, the fermentation products from the digester would be stoichiometrically converted to bacterial biomass. The bacteria would be cropped from the effluent of the reactor, and the supernatant fluid passed to the algal photosynthetic reactor. This second reactor would in addition be fed directly by waste gases. If the anaerobic stage proved to be efficient, the algal reactor would be receiving only those waste products which had already reached a stable aerobic oxidation state. These would be converted stoichiometrically to algal biomass and O₂, while the reactor simultaneously 'purified' the carrier waters. If the anaerobic reactor was less than absolutely efficient, a small proportion of organic waste spillover would be handled by the algae. If any large scale organic spillover occurred, aerobic bacteria would be needed in the algal stage to cope with its aerobic digestion (Humanik & Hanna 1970). The total biomass (which would in either case be predominantly algae) would be cropped from the effluent of the aerobic reactor.
This two stage photosynthetic recycling system may have a number of advantages over conventional recycling systems.

1. It would be energetically less wasteful in its chemistry.
2. Because of the differences in spectral absorption between the algae and the photosynthetic bacteria, a much greater proportion of light from a broad spectrum source could be used. The system would then be less wasteful of radiant energy.
3. The system could provide total recycling, and in this sense would be superior to a solely algal system.

A possible and attractive alternative to the algal system discussed above is an anaerobic recycling reactor coupled to electrolysis of water, the latter acting as an oxygen generating system. A schematic diagram of such a system is shown in figure 4. Solid and liquid wastes would be processed, essentially as in the algae-linked system above, by anaerobic recycling. Exhaled gases would pass through a separator in which water vapour and CO\(_2\) would be condensed from the other gases. The water would be electrolysed, and the oxygen regenerated. Additional water could be fed in to regulate the oxygen supply. The CO\(_2\) and H\(_2\) would be fed to the anaerobic photosynthesis reactor and assimilated to carbohydrate. The overall stoichiometry of the system should be self-adjusting, as is apparent from the equations for the carbon cycle shown below.

(a) anaerobic fermentation

$$\text{(CH}_2\text{O})_n \rightarrow \frac{1}{2}n' \text{C}_3\text{H}_6\text{O}_3,$$

(b) anaerobic heterotrophic photosynthesis

$$\frac{1}{2}n' \text{C}_3\text{H}_6\text{O}_3 \rightarrow \text{(CH}_2\text{O})_n,$$
Bacterial photosynthesis in recycling

\[(\text{CH}_2\text{O})_n + n\text{O}_2 \rightarrow n\text{CO}_2 + n\text{H}_2\text{O},\]

**c)** respiration

\[2n\text{H}_2 + n\text{CO}_2 \rightarrow (\text{CH}_2\text{O})_n + n\text{H}_2\text{O},\]

**d)** anaerobic CO\(_2\) fixation (autotrophic photosynthesis)

\[2n\text{H}_2\text{O} \rightarrow 2n\text{H}_2 + n\text{O}_2.\]

**e)** electrolysis

The system proposed above has a number of features in common with the *Hydrogenomonas* recycling system (Foster & Litchfield 1964; Jenkins 1966; Bongers 1970) but offers a number of additional advantages.

1. It provides a complete recycling system.
2. The chemistry is less wasteful of energy.
3. The metabolic adaptability of the photosynthetic bacteria would be reflected in the adaptability of the system as a whole.
4. The photosynthetic bacteria are able to use light as an energy source.

The last of these points is a somewhat dubious advantage. When natural light is available, algal systems are probably the most convenient recycling process; when energy has to be derived from other sources, the electrolysis of water may well be more efficient as an energy conversion process than conversion of electrical energy to light, and conversion of the light back to chemical energy with the low efficiency found in practice (Burlew 1953; Göbel 1969). However, use of the relatively efficient sodium lamp, with its major emission (at 589 nm) at the yellow bacteriochlorophyll peak, may minimize this energetic disadvantage.

**Model systems using photosynthetic bacteria**

Photosynthetic bacteria have been cultured under continuous chemostatic conditions in a number of laboratories (Cohen-Bazire & Sistrom 1966; Lippert & Pfennig 1969; Göbel 1969; Munson & Burris 1969; Jones & Phipps 1971). This work together with the extensive work on algal systems, on *Hydrogenomonas* recycling (Foster & Litchfield 1964; Jenkins 1966; Bongers 1970) and on sewage treatment (Jenkins 1963; Pipes 1966; Bungay & Bungay 1968; Casida 1968) provides a firm basis for the design and technology of a recycling system using photosynthetic bacteria. An immediate possibility is the design of model recycling
systems using pure cultures in serial chemostatic cultivation, or simple mixed cultures. Thus an anaerobic carbon cycle, using a fermentative microbe to produce lactate from glucose and a photosynthetic bacterium to reverse the process, might provide a simple mixed culture system for the study of competitive and synergistic effects. Another possibility is an anaerobic coupled carbon and sulphur cycle using Desulphovibrio and Chlorobium in serial or mixed culture. Such systems would provide valuable models for more complex ecological situations.

Conclusions

Under certain limited circumstances the photosynthetic bacteria may prove of use in the operation of recycling systems. Experience may show that this utility has a wider application but it seems more likely that in the near future, photosynthetic recycling systems, especially in situations in which natural light energy is available, will be of the type using mixed cultures of bacteria and algae under aerobic conditions. Under conditions where the availability of energy is limited, and there is no natural light, the greater chemical efficiency of an anaerobic recycling process might be advantageous. In these circumstances, the use of photosynthetic bacteria in systems like those described would be very much more practicable.

One major disadvantage of bacterial recycling is the unpalatable nature of the crop (Waslien, Calloway & Margen 1969; Whatley 1971). It seems unlikely that unprocessed photosynthetic bacteria will ever be fit to eat—anyone who has cropped them will be aware of the odours of putrification they produce. Clearly a major effort must be made to find methods of extracting the useful food from the crop. Unless this can be done, the most elegant recycling processes will be useless.

I am grateful to my colleagues and especially to Dr J. B. Jackson, Department of Biochemistry, Bristol, for interesting comments and discussions.

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