Energy production by microbial photosynthesis

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The large amounts of microalgae produced in waste treatment ponds during sewage purification are a potential source of methane and fertilizer. If techniques can be developed for the selective cultivation of filamentous or colonial microalgae, harvesting of microalgal biomass could be accomplished by low-cost straining or sedimentation methods.

Increasing public and governmental awareness of the serious problems of energy and environment faced by most of the nations of the world is resulting in reappraisals of all potential energy sources. Evaluations of the various energy alternatives must consider, together with technical and economic factors, their environmental and social impact. Here we review the scientific and practical aspects of fuel and fertilizer production from microalgae and photosynthetic bacteria.

Bioconversion

Until less than a century ago, photosynthesis provided over 90% of mankind’s fuels. Even today, on a worldwide basis, the total calorific value of primary photosynthetic production (plant biomass) used by mankind is higher than that of the fossil energy consumed. Only in the industrialised nations with temperate climates are fossil fuels more important than current photosynthetic biomass production in such a comparison. At present, over one-half of the people in the world depend on wood and dung for the majority of their fuels. The diminishing availability of these biofuels to the poorer half of the Earth’s population is a more serious and immediate energy crisis than depletion or monopolistic control of fossil fuels.

Photosynthesis can provide a larger share of mankind’s fuel needs through (1) shifts in relative usage (such as conversion of organic wastes to fuels or reduction in luxury consumption levels of animal protein) or (2) by increasing plant biomass production for direct utilisation or conversion to fuels. The potential of plant biomass production for the exclusive or primary purpose of fuel production is, in practice, limited by a number of constraints—the relatively low efficiency of photosynthesis in solar energy conversion; competition with agriculture or forestry for land, water and fertiliser; marginal economics of biomass production and conversion into usable energy; need for low-energy cultivation technology; and a lack of experience with such processes, and so on. Because most inputs in a bioconversion system would be constant per unit area, the net productivity (expressed as incident solar energy conversion efficiency) is a critical parameter since it must be sufficiently high to justify all costs.

Photosynthesis is limited by its dependence on visible light (about 45% of total solar radiation), reflectance losses (10–20%), a quantum efficiency of only 25%, and metabolic losses, giving a value of 5–6% for the theoretical maximum solar energy conversion efficiency. In normal field conditions, photosynthesis rarely approaches the theoretical limit which applies only in light-limiting conditions. Sustained year-round, solar conversion efficiencies exceeding 1% are unusual in agriculture. In addition, such high yields are often dependent on high fossil fuel subsidies or large human labour inputs, thus reducing the net efficiencies realised. Very high productivities (above 1% efficiency) with favourable net energy ratios are possible only with a few highly productive plants such as sugar cane. The limitations to bioconversion suggest that practical applications will require utilisation either of the most efficient agricultural crops (for example sugar cane) or less efficient but more hardy plants capable of being cultivated in marginal areas with few inputs. The list of plants considered for biomass production is already large; they may be classified as either dry land or aquatic. The aquatic plants may, in turn, be classified as macro (water hyacinths, seaweeds, and so on) and micro (unicellular or small colonial algae and photosynthetic bacteria). We shall consider only the microscopic aquatic plants—microalgae and photosynthetic bacteria—and their potential in bioconversion to fuels, fertilisers, and petrochemical substitutes.

Microalgae in waste treatment

Large scale cultures of microscopic algae have been actively investigated for over twenty-five years. Because of the high protein content of algae and the presumed large potential yields, algal research has emphasised protein production. However, practical applications of algal cultures for food or feed production are limited by high capital costs, moderate yields, poor product quality control, and lack of markets. At present, commercial production is limited to a one-ton-per-day pilot plant in Mexico producing Spirulina (a filamentous blue-green alga) and several smaller operations in the Far East which produce Chlorella.

The development of large-scale algal ponds for domestic and industrial liquid waste treatment has been much more successful. Although originally used only to hold wastes, ponds were observed to substantially reduce pollution indicators—biochemical oxygen demand, odour, and settleable solids. This turned out to be because pond algae photosynthetically generate enough oxygen to allow bacterial oxida-
tion or organic wastes. The released nutrients (carbon dioxide, ammonia, phosphate, etc.) are in turn converted into algal biomass. Design equations have been developed to relate pond area requirements to sewage strength and flows, as well as local solar insolation and climatic conditions. This engineering research has resulted in the widespread adoption of algal ponds (known as ‘oxidation ponds’) in many countries, including the United States, Australia, Israel, South Africa, and India.

Almost all presently used oxidation ponds are the ‘facultative’ type—one to three metres in depth and arranged in cells of up to 50 hectares, and mixed only by wind action and perhaps recirculation of effluent. The bottom of these ponds are thus anoxic, resulting in fermentation of the settled sludge and algal, while the surface is kept aerobic by the algae, preventing odours from developing (a prime consideration in sewage treatment). The liquid detention time (days required to displace one pond volume) is long, from weeks to months. This design is optimal for waste treatment but not for algal growth. If algal biomass production is to be maximised, the ponds must be shallow (30–50 cm), have short detention times (2–4 days in summer), and be mechanically mixed (to minimise algal settling and prevent thermal stratification). These types of ponds termed ‘high-rate’, are much more efficient solar energy converters and can be very effective in waste treatment, provided that the algae are removed before discharge of the pond effluent.

The high algal concentrations in pond effluents represent a load of organic matter and nutrients which are regarded by regulating agencies as environmentally undesirable. High-rate ponds produce well above 100 mg l\(^{-1}\) of algae (dry weight) and even facultative ponds normally have effluents of above 50 mg l\(^{-1}\). These levels of suspended solids are higher than those allowed by present US waste treatment plant discharge regulations; thus, algae must now be removed from oxidation pond effluents. This step is, of course, also required for conversion of algal biomass into usable fuels.

**Microalgal harvesting**

Concentrating (at least 100-fold) a dilute suspension of microscopic algae is a considerable engineering problem. Most oxidation pond algae are below 20 \(\mu m\) in diameter, too small for low-cost straining, filtration, or sedimentation. Only the more expensive centrifugation and chemical flocculation reliably remove algae from pond effluents. Centrifugation requires high capital investments in large centrifuges as well as power to run them. Chemical flocculation is somewhat more cost-effective and is being installed in a few large scale oxidation pond systems in the US, but the weight of chemicals (alum, lime or polyelectrolyte) required often surpasses that of the algae harvested. The flocculated algae are collected either by sedimentation or flotation and expensive extraction steps are then needed before the algae can be used for bioconversion. Furthermore, the chemical–algal sludge is difficult to dewater and dispose of. Thus, high capital and operational costs make chemical flocculation unsuitable for bioconversion systems. Less expensive methods of removing algae from effluents are to settle them in the pond by chemical flocculation, or by prolonged (2–3 week) isolation of ponds from further sewage flows. These methods, although economical, are not suitable for algal biomass production since the algae settle on the pond bottom as a thin layer of sludge which slowly decomposes.

Water reservoirs often exhibit blooms of algae which impart foul tastes and odours to the water and which are removed by large scale ‘micro-strainers’. Almost all such algae occur as long filaments or large colonies, which accounts for the effectiveness of microstrainers—rotating straining devices (Fig. 1) in the purification of reservoir waters. Oxidation pond algae, on the other hand, tend to occur as unicellular or small colonial types, generally under 20 \(\mu m\) in any one dimension, which are too small to be effectively strained. Only the sixth and seventh most common algae in oxidation ponds—the filamentous blue-green alga *Oscillatoria* and the colonial spined green alga *Microactinium*, can easily be harvested by microstrainers. If such types of algae were normally prevalent in high-rate oxidation ponds, microalgal harvesting would no longer be a limiting economic factor in algal biomass production from liquid wastes.

**Algal population dynamics**

Control of algal species in ponds (whether for biomass harvesting, aquaculture, or protein production), must be based on an understanding of the specific factors affecting algal population dynamics. The following are a few generalisations about the importance of various factors. When high strength wastes (for example, food processing wastes) are serially diluted, inoculated, and incubated in the light, different biotypes will appear in the various dilutions. The most concentrated wastes will contain only anaerobic fermentative bacteria. Photosynthetic bacteria appear in the first dilution followed by a graduation of flagellated euglenoids, blue-green algae, green algae, and finally diatoms and nitrogen-fixing blue-green algae. Such successions can also be seen in outdoor ponds going from heavily loaded anaerobic ponds to the final ‘polishing’ or ‘tertiary’ ponds. Often, only one or two algal species comprise over 95% of the algal biomass in sewage ponds that are not allowed to fully stabilise (through fast dilution and heavy organic loading). A greater number of algal species is found in the more lightly loaded tertiary ponds, indicating greater stabilisation of the wastes and algal populations. The filamentous blue-green alga *Oscillatoria* is usually found at higher temperatures (being the predominant algae reported from Indian oxidation ponds) and in ponds receiving wastes low in nitrogen (such as canner wastes). The many environmental, biological, operational and even historical factors which affect algal population dynamics in ponds interact in many ways, complicating the task of understanding algal population dynamics. For example, is is known how seeding of ponds with algae from operating oxidation ponds will affect the establishment and subsequent history of algae in the ponds. In outdoor systems, it is not possible to achieve a steady state due to constantly changing environmental conditions. Temperature, for example, is an important factor in algal successions. The greatest problem is the lack of detailed ecological or limnological data from operating oxidation ponds. The low species diversities and rapid succession of the predominant algal species in ponds indicate a versatile system which can be affected by many environmental conditions.
Algal species control

How, then, can algal populations be controlled so as to maintain selected algal species (or algal types) for prolonged periods? Sunlight and temperature cannot be significantly controlled, although they are critical to the succession of algal species. Pond loadings, depth, and mixing can be controlled, however; their particular effects on algal species are not yet known and would be influenced by additional environmental conditions. Thus, the selective species control methods must be adjustable in the face of changing conditions. Two such methods are presently being studied at our laboratory—size-selective recycle, and nutrient limitations.

The following is an example of size-selective recycle. In a pond operated at a five-day detention time, the predominant alga Chlorella is accompanied by a small percentage of Microactinium. Chlorella is the preferred food of many zooplankton such as Daphnia, whereas Microactinium is seldom grazed because its spines, which protrude in all directions (Fig. 2), obstruct ingestion by rotifers and similar zooplankton. The zooplankton cannot flourish because their population growth cannot keep up with the short detention time. If a relatively coarse screen were used to remove the Daphnia from the pond effluent and recycle it into the pond, a population explosion of Daphnia would be predicted to cause a diminished Chlorella population and thus lead to predominance of the undigestable Microactinium. Such biological control of algal species has yet to be demonstrated in ponds.

A more direct method of size-selective recycle has been used in outdoor ponds to achieve algal species control. It involved recycling to the pond a certain fraction of the algae harvested by a microstrainer. If a pond is heavily inoculated with harvestable species and all the algae stranded from the pond are re-introduced into the pond, these algae, because they are not washed out of the pond, will have an advantage over faster-growing but non-harvestable algae. Eventually, most of the algae in the pond will be of the desired, harvestable type and the amount recycled to the ponds can be decreased. To prevent non-harvestable algae from returning, the fraction of harvested algae that must be continuously recycled is proportional to the ratio of the relative growth rates. In practice, this recycling fraction must be larger since harvesting is neither completely selective nor totally effective. Thus, the growth rate of the desirable harvestable filamentous or colonial algal species must not be too far below that of the unwanted single-cell alga. The faster dilution rates or higher algal concentrations required by cell recycling also set limits to the allowable degree of recycling. It is important to note that such recycling is a size-specific, not a species-specific control method. Other strains of harvestable algae which spontaneously appear in the ponds will be recycled and become dominant if they grow faster than the inoculated strain. Inoculations and recycling might be alternated so as to allow true species control. The principles of selective biomass recycling are also applicable to any other harvesting method (such as settling) and to other large scale microbial processes including activated sludge procedures, and single-cell protein production. Indeed, in the activated sludge process, the practice of returning settled bacterial biomass to the reactor results in selection for settleable bacterial flocs.

The other method now under investigation for establishing harvestable species in ponds is based on nutritional limitation. The major nutrients of algae are water, sunlight, carbon, nitrogen, and phosphorus. The degree to which any one of these nutrients is limited will affect algal populations. To maximise biomass production, sunlight should be the only limiting nutrient, since productivity is proportional to solar energy conversion efficiency. However, the final yield of algae will be determined by the limiting nutrient in the wastes, which for domestic sewage is carbon. Treatment of sewage and other liquid wastes cannot be considered complete until nutrients causing eutrophication in receiving waters (N or P) are removed from sewage treatment plant effluents, and that means adding an additional source of carbon to algal ponds. Carbon dioxide produced by a power plant or any other source could easily be brought to ponds for this purpose and would allow growth of the harvestable algae (maintained through selective recycle) up to the nitrogen algal growth potential of the sewage. An additional crop of algae could be obtained from the residual phosphate in the effluents of these ponds through further carbonation.

In such a final algal production and phosphate stripping pond, only nitrogen-fixing algae could grow (Fig. 2). Nitrogen-fixing algae are blue-green and all planktonic forms are filamentous. Thus, they are easily harvested and, since growth is selective, no recycling would be required.
Wherever phosphate detergents are used, nitrogen-fixing blue-green algal biomass can be produced in large quantities (exceeding the production of the initial ponds), thereby increasing the potential N fertilizer output from the ponds to a level above that present in the wastes.

**Algal Bioconversion**

In the multi-pond system of waste treatment and algal biomass production as exposed above and indicated in Fig. 2, two ponds in series (in practice several more would be required) are used to grow successive crops of different algal types harvestable by microstrainers or similar inexpensive methods. In such ponds, energy and plant nutrients are recovered from wastes and augmented by the processes of carbon dioxide enrichment and nitrogen fixation. The most direct and practical method for conversion of the algal biomass would be methane fermentation. Direct burning is not feasible since it would require dewatering of algae, a process requiring more energy than the algae could yield.

More than one-half of the heat of combustion of the algae (10,000 BTUs per lb algae) can be converted into methane gas with digester loadings, temperatures, and detention times similar to those of sewage sludge. Many problems arise in including the possible resistance to degradation by part of the algal biomass, ammonia build-up in fermenters, and dewatering properties of the residual sludge. Of particular concern is the cost of conventional sludge digesters; covered anaerobic ponds might prove to be the most cost-effective large scale digestion systems. The production costs of methane would be part of the overall sewage treatment costs, making it a valuable by-product of algae-based waste treatment systems. The residues from algal digestion would contain virtually all the nitrogen and phosphorus of the algal biomass. These could be disposed of as fertilizer on agricultural land. In principle, all necessary agricultural fertilizer could be produced in this way—one acre of algal ponds providing the fertilizer required by 10 to 50 acres of modern agriculture.

The total amount of methane that can be obtained from the algal biomass produced during sewage treatment is limited to only a few percent of local natural gas needs. It has been demonstrated in laboratory studies that algae can be cultivated on the residues from algae methane fermentations. Therefore, by recycling the fermentation residues to the algal growth ponds algal biomass production can be expanded to the limits of available water and land. However, in such cases, no waste treatment or fertilizer production credits would help underwrite the economics of methane production. In principle, capital investments (into pond construction) and operational costs (including energy inputs) could be low enough to allow large scale algal biomass production and conversion systems. The feasibility of such large scale 'nutrient integrated' systems will depend on the solution of many technical and economic problems.

**Algal Productivity**

So far we have emphasized the problem of algal harvesting, species control, and bioconversion because they must be solved before large scale microalgal biomass production can become feasible. However, it is the harvestable yields of algal biomass (expressed as g m⁻² day⁻¹ or MT per hectare per year) that determines the potential of algal systems for energy and fuel production. There is no strong theoretical basis for expecting higher yields with algae than with higher plants. Vertical shade adaptation of leaves in a crop plant is inherently a more efficient arrangement than the uniform pigmentation of algae in a mixed water column. The reflectance of sunlight from the water surface is another disadvantage of algal ponds. However, algal ponds have several compensating features, primarily the capacity for continuously supplying all nutrients so that only light is limiting. Thus continuous culture makes possible a quick, graded response to environmental conditions and complete light absorption. The hydraulic nature of a pond system allows for simple and inexpensive operations. However, continuous cultivation also means harvesting of a dilute suspension of the microalgae. This is inherently more expensive than the periodic harvesting possible with microalgae or land plants.

On the basis of even such a qualitative analysis, it may be concluded that rates of algal biomass production would not be necessarily higher than those observed in intensive agriculture, algal production, however, being potentially less demanding on inputs and efforts. It is not possible at present to make comparisons—many reported peak agricultural productivities are suspect on a number of accounts (methodology, border effects, and so on), and algal productivity is normally based on extrapolations from short term, small scale experiments. A figure of 50 MT per ha per yr (12 gm m⁻² d⁻¹) of dry-weight, ash-free algal production has been presented as a reasonable figure of potential productivities. This would represent a total solar energy conversion efficiency of about 2% at 30° latitude. It might be possible to perfect algal pond systems with higher efficiencies. To achieve this requirement, careful pond design, water treatments and genetic selection of the algal strains to be cultivated. For example, the addition of carbon dioxide to ponds could be used (in addition to the reasons listed above) to maintain an adequate pH in the ponds (it may go above 10 in the afternoons, resulting in a sharp decline of photosynthesis). This would not only extend the daily growing period, but also reduce photorespiration and the potential for photo-oxidative death. Another factor which could increase photosynthetic efficiencies and which may account for some of the very high yields reported is the phenomenon of photoheterotrophy. Many algae assimilate preformed organic compounds, particularly under low light intensities (as may be expected in the bottom of ponds). Since little light energy is required in the photoheterotrophic production of biomass, calculated efficiencies are high because the energy content of the liquid wastes are ignored. Controlled photoheterotrophic growth would increase the performance (load rates, organic waste destruction, and biomass production) of the primary ponds receiving settled sewage. Photosynthetic bacteria are, of course, photoheterotrophic by nature and are potentially useful in waste treatment systems. Another suggestion for improving photosynthetic efficiencies would be through decreasing the accessory pigment concentration of the algae to decrease self-shading and photoinhibition.

An important aspect to be considered is the reduction in net photosynthetic efficiencies resulting from energy inputs into pond operations such as dilution, mixing, fertilization, harvesting and concentrating, species control, and so on. If the large scale production of algae for energy production is to be feasible, these inputs must be minimal. A slight net energy gain has been calculated from waste-grown algae using available production methods. In principle, net algal productivity should not be limited by the energy requirements of cultivation which are low; mixing at slow speeds (about 5 cm s⁻¹) is sufficient for thermal destratification and requires little energy. Harvesting by microstrainners is likewise not energy intensive. Water usage in pond systems is mainly through evaporation; the ability of microalgae to grow in brackish or saline waters would make it possible to use unrecyclable water exhaustively through use of a terminal evaporative pond system. Fertilisation obviously would be too expensive if mineral or synthetic fertilizers are used; nutrient recycling, whether from wastes or internally, is required. Nitrogen fixation must make up extra nitrogen requirements at a probable reduction in photosynthetic efficiencies estimated at 10%. Including energy
inputs and conversion losses, a net production of 200 M BTUs per acre per year of methane seems to be a feasible goal\textsuperscript{19,27}.

**Future developments**

The use of microbial photosynthesis for energy production is not limited to methane and fertilisers. Biophotolysis—the decomposition of water to yield hydrogen gas—has been demonstrated with algae in the laboratory\textsuperscript{19,29}. Heterocystous blue-green algae can be used to produce hydrogen and oxygen simultaneously in a system of horizontal glass tubes\textsuperscript{30}. Other algae might be capable of alternating daytime oxygen with night-time hydrogen production. Photosynthetic bacteria can produce hydrogen from organic substrates\textsuperscript{19}, presenting a potential alternative to methane fermentation\textsuperscript{19}. The production of specialised chemicals is possible. The large scale culture, of the salt-loving alga, *Dunaliella*, consisting of 80% glycerol, has been proposed (M. Aaron personal communication). Algal species containing high concentrations of fats and lipids may be used for liquid fuel production. Petrochemical feedstocks of various types may theoretically be derived from algae. However, such technologies are probably going to take longer to perfect.

Microalgal bioconversion will have a beneficial impact on environment and society as long as it does not conflict with food production or develop into gigantic centralised conversion systems. The prospects of very large scale sites (hundreds or thousands of square miles) converted to pond systems is not imminent; technological development will certainly at first favour smaller locally integrated waste treatment-algal bioconversion-nutrient recycling systems. These would be of particular value where adequate insolation is combined with a need for sanitary sewage disposal and fertiliser supplies. Such pond systems may expand, as practicable, to dispose of salt-laden agricultural drainage water by evaporation and to provide a significant fraction of local fuel needs. Energy production from microalgae might have little immediate overall impact on energy consumption of advanced industrial countries, except where favourable local situations exist. Algal bioconversion is specifically important to underdeveloped countries since such technology would require little or no investments in scarce raw materials or complex ‘hardware’. The application of sophisticated cultivation technology should be within the reach of even the poorest countries. The environmental and social impacts of algal pond systems or other bioconversion technology are expected to be minor compared with present or alternative energy sources. In the future, the developing technology of bioconversion can present mankind an alternative to depleting fossil fuels and dangerous reliance on nuclear energy.

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