by Bernard Dixon

CHEMICALS FROM ALGAE AND PLANT TISSUE

Single cell protein and polyhydroxybutyrate are not the end of the story." That forecast was made two years ago by Eric Howells from ICI's Corporate Research and Technology Department. The real "end of the story", he suggested, was the use of stable, safe, versatile microorganisms like the ICI Pruteen bacillus as industrial workhorses for fabricating a whole range of organic chemicals.

The very month when Howells's words appeared in *Chemistry and Industry* (7 August, 1982), the French Atomic Energy Commission and Solar Energy Commission were consorting with industrial partners (Elf Aquitaine, Total, and Rhone-Poulenc) to establish a body dedicated in part to the concept of chemical manufacture via novel biotechnology. Despite its title, suggesting singleminded devotion to familiar tactics of biomass production, the resulting Association for Research into Solar Bioenergy (ARBS) has focussed its eager gaze on algae and plant tissue—not as sources of energy but as producers of chemicals.

Speaking at Biotech 84, held recently in London, Claude Gudin gave one of the first glimpses of what the ARBS has achieved thus far. The plan, Gudin explained, was to screen plant cells and microalgae for their skill and versatility in generating substances with commercial potential—those, saleable in the medium price range, that account for at least 30 percent of total biomass.

The plant studied most intensively has been *Euphorbia characias,* a source of *cis*-polyisoprene. Until recently, botanists believed that undifferentiated plant tissues could not synthesize secondary metabolites (although presumably they carried the genetic information to do so). On the other hand, dependence on differentiated structures posed obvious problems for bioreactor design. Such an appraisal is now being revised. With *Euphorbia,* achievement of photosynthetic growth seems to be the key. Claude Gudin and his colleagues first established callus cultures containing sucrose as the organic carbon source. Then they transferred tissue to suspension culture, and used a chemostat to grow these cells, with declining sugar concentration plus illumination and a rising level of carbon dioxide. The outcome was an autotrophic strain of *Euphorbia* which, like the intact plant, does synthesize *cis*-polyisoprene.

As well as developing this and related systems (including pectin production by *Myrtillocactus*), ARBS workers have been assessing the biochemical productivity of microalgae. With solar energy conversions of around three percent (double those of even the most efficient higher plants), the various genera yield a considerable variety of marketable products. They include long chain (C27-C31) hydrocarbons from *Botryococcus* species, polysaccharide gelling agents released by *Chlamydomonas,* acrylic acid and polyacrylates made by *Phaecocystis,* sorbitol from *Dunaliella,* and triglycerides from *Neochloris.* In addition to these compounds, readily available as intra- or extracellular products from naturally occurring strains, the terrestrial microflora must contain many more.

The reactor built by Gudin's team for liquid cultures comprises an array of glass tubes presenting an area of six square meters to the incident sunlight. Between the outlet from this assembly and the inlet to a circulating pump, a carbonation column introduces carbon dioxide while extracting oxygen. When *Botryococcus braunii* was introduced into the apparatus, it grew prolifically for several weeks, with an energy conversion yield of 3.5 percent even in winter, and despite the emergence of *Chlorella* and other contaminants. Hydrocarbons accumulated in pericellular sheaths around the *Botryococcus,* but in much lower quantities than expected because of competition in the heterogeneous population. Gudin and his co-workers now intend to optimize cultural conditions for their producer organism and to optimize productivity by genetics and strain selection.

In a concurrent series of experiments, *Chlamydomonas mexicana* (acquired from Ralph Lewin at Scripps Institution of Oceanography) was the key performer. The ARBS group plans to engineer a process harnessing the capacity of this alga to generate polysaccharides for gelling and thickening agents. Preliminary trials have established that continuous cultures can run for up to ten weeks, and efforts are now under way to maximize polysaccharide output.

A third approach being pioneered by the French workers is to immobilize microalgae by suspending them in a prepolymer, which is then polymerized to become polyurethane foam containing entrapped algal colonies. One organism manipulated in this way is *Porphyridium cruentum,* another potentially prolific source of polysaccharide thickening agents. When foam carrying immobilized algae is placed in a glass column reactor, with carbon dioxide bubbling upwards, a cell-free solution carrying polysaccharide can be tapped off at the bottom. The system goes through three phases: an initial period of cell destruction and no detectable photosynthesis; a growth period when surviving algae divide and begin to photosynthesize; and a steady state, with stable photosynthesis and rare cell division. By restricting nitrogen between the latter two phases, Gudin and his team have found they can enhance polysaccaride production over several weeks of the steady state.

Again, productivity remains to be maximized. In other cases, harvesting is the problem awaiting solution. *Botryococcus braunii,* for example, secretes hydrocarbons when immobilized, as it does when free-living. The ARBS researchers now need to perfect means of collecting such insoluble materials, which do not pass into the reactor fluid. And an added dimension to their work with plant cells is the possibility that so-called "redundant" DNA may specify substances not found in mature plants. What price novel structures, generated by bringing these sleeping genes into action?

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