PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

Microbial Production of Energy Sources from Biomass [and Discussion]

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Microbial production of energy sources from biomass

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Anaerobic fermentation of carbohydrates can yield a range of alcohols, fatty acids, esters and the gases hydrogen and methane. In the microbial conversions for ethanol and methane, 93–95% of the calorific value of the carbohydrate is retained in the product. However, the capital and energy costs of operating microbial conversions industrially are generally high. At present, fermentation processes for fuels are generally uneconomic without waste disposal credits, or tax credits. The developments required to improve the economics of bioconversion are (i) higher rates of fermentation, (ii) fermentations at higher concentrations of raw material and products, (iii) product recovery methods which consume little energy.

Ethanol production is unlikely to contribute to liquid fuel supplies in Britain unless cellulose-based processes using crop wastes or urban refuse are developed. Methane is already produced from some wastes and further development of the anaerobic digestion technology can be expected to make a small but significant contribution to energy supplies. Since the scale of use is much smaller and the value higher, products of anaerobic fermentation could make a more significant contribution to supplies of chemicals.

1. Introduction

The net annual production of biomass by photosynthesis has been estimated to be ten times our annual consumption of fossil fuels, and present agricultural production approximately equal to fossil fuel consumption (Klass 1978). About 5% of total biomass is produced by agriculture; of this, less than half is consumed as food or feed, the remainder being discarded as waste. There are thus considerable quantities of biomass produced that could contribute to supplies of fuel and chemicals. However, with the exception of directly burnt wood, biomass requires further processing to produce the fuels and chemicals that are required by present day technologies. The distribution of available biomass and the efficiency of the subsequent processing, in terms of its investment and energy requirements, will determine the extent to which it can contribute to our energy needs.

Biomass is generally harvested as a rather wet, solid material, containing a mixture of chemical species and with a rather low energy density. A variety of processes are available for converting it to useful fuels. Drying and compressing is adequate for some applications. However, liquid and gaseous fuels have a premium value and can be produced from biomass by pyrolysis and by microbial conversion. In this contribution the biochemical options available in microbes will be briefly reviewed and some of the technology available for microbial conversion will be discussed with particular reference to its present limitations and how they might be overcome.

2. Products of anaerobic fermentation

In the presence of oxygen, heterotrophic microbes generally gain energy for the processes of growth and maintenance by the oxidation of organic molecules to carbon dioxide and water; deprived of oxygen, many organisms are capable of using partially oxidized organic molecules, such as carbohydrates, as sources of energy. Biochemically the anaerobic fermentation of carbohydrates is characterized by the oxidation of the sugar to pyruvic acid, by one of several routes. The glycolytic pathway has the overall stoichiometry

$$\text{glucose} + 2P_i + 2ATP + 2ADP + 2NAD \rightarrow 2 \text{ pyruvic acid} + 4ATP + 2NADH_2.$$

There is a net gain of 2ATP, which represents energy available to the cell, and its hydrolysis in the reactions in which it partakes recycles ADP and P_i. For the glycolytic pathway to continue operating it is also necessary to re-oxidize the reduced pyridine nucleotide $NADH_2$, and it is in the diverse mechanisms of re-oxidation that the wide range of fermentation products are formed (table 1).

Table 1. Some products of anaerobic fermentation

acids	alcohols	esters	gases
lactic	ethanol	ethyl acetate	hydrogen
formic	propan-2-ol	ethyl butyrate	methane
acetic	butanol	poly-3-hydroxybutyrate	carbon dioxide
propionic	2,3-butanediol		
butyric	glycerol		

One of the simplest solutions is the single step reduction of pyruvate to lactic acid:

$$CH_3COCOOH + NADH_2 \rightarrow CH_3CHOHCOOH + NAD.$$

Lactic acid is a common fermentation end product which can be produced in almost 100% yield from glucose, conserving 95% of the calorific value of glucose. Lactic acid has some utility as a chemical intermediate and in numerous food applications but is not suitable for fuel use. Its range of applications is restricted by the present production technology, which is costly in terms of energy and chemicals.

In the ethanol fermentation, each molecule of pyruvate is reductively decarboxylated, giving rise to one molecule each of ethanol and carbon dioxide:

$$CH_3COCOOH + NADH_2 \rightarrow CH_3CH_2OH + CO_2 + NAD.$$

Yields from glucose approaching the theoretical 51% (by mass) can be obtained, conserving 93% of the calorific value of the glucose as ethanol. The energy density, 29.6 GJ/t, is nearly twice that of carbohydrates. It can readily be used as a liquid fuel in existing internal combustion engines with minor modifications. It can also be catalytically dehydrated to yield the key chemical intermediate, ethylene.

Many bacteria have pathways that extract further useful energy for the cell in the form of ATP and produce end products that may have more industrial significance in the future. One such group of bacteria is the clostridia, which produce carbon dioxide and the acids formic, acetic and butyric, together with hydrogen from the cleavage of formic acid, and acetone (figure 1). Further reduction of butyric acid and acetone by these bacteria yields butanol and

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

propan-2-ol. The ratios of the end products vary according to the bacterial species and the fermentation conditions. The Propionibacteria produce propionic acid, acetic acid and carbon dioxide from glucose in the approximate molar ratio 4 : 1 : 1, with an overall yield of near 100 %.

Some strictly anaerobic bacteria oxidize hydrogen, by using carbon dioxide as the electron acceptor and producing methane (Wolfe 1971; Zeikus *et al.* 1975). These bacteria are found in mixed cultures and carry out the final step in the production of biogas from carbohydrates. The overall stoichiometry from glucose is

$$C_6H_{12}O_6 \rightarrow 3CH_4 + 3CO_2,$$

with fatty acids, alcohols, hydrogen and carbon dioxide as intermediates. Hydrogen, the other potentially valuable gaseous metabolite, is generally found in low yields from carbohydrates but may be obtained in much higher yields by using photosynthetic microbes (Zajic & Brosseau 1976; Mitsui 1978).

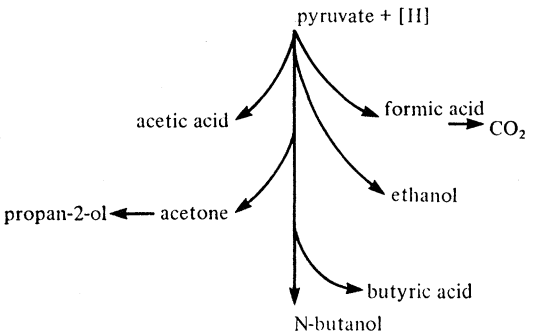

FIGURE 1. Some fermentation products of the clostridia.

In addition to the extracellular products summarized above, there are reduced products of some microbes which accumulate intracellularly to form the greater part of the cell mass. Among them are triglycerides and other lipid products (Woodbine 1959; Whitworth & Ratledge 1974) and the polyester poly-3-hydroxybutyric acid (Stockdale *et al.* 1968).

For those metabolites that are primary products of the pathways of energy generation for the cell, it is often possible to achieve yields approaching the theoretical maxima calculated from the biochemical pathways. This is done by growing the microbes at rates approaching zero. Thus the growth-associated component of substrate assimilation (μ/Y_G) is small compared with the maintenance component (m) of equation (*1*), which has been found to describe substrate assimilation for a range of microbes (Pirt 1965; Mason & Righelato 1976):

$$ds/dt = x\{\mu/Y_G + m\}, \quad (1)$$

where ds/dt represents the rate of assimilation of substrate; x, the microbe concentration; μ the specific growth rate; Y_G, the mass yield of cells from the substrate; and m the non-growth associated rate of dissimilation of substrate. The non-growth associated component (m) is generally thought to represent energy required by the cell for its maintenance (Pirt 1965), and hence substrate consumption described by this component yields little other than the products of energy metabolism (Righelato *et al.* 1968).

At low growth rates, then, the conversion of substrate into cell material is minimized, while conversion to the products of energy metabolism continues. To achieve high overall rates of

substrate conversion, high cell concentrations are required. In practice, high cell concentrations can be maintained at low growth rates by retaining the microbes in the reactor while the substrate processed by the microbes is passed over them. This can be done by immobilizing the microbial cells (Keirstan & Bucke 1977) or by cell recycle (see Pirt 1975 for review).

3. Process technology

Many of the products discussed in § 2 have been made industrially in the past, though, except in a few special cases, fermentation processes have been superseded by routes using fossil reserves. The reasons are twofold: (i) the high cost of fixed carbon from agricultural sources, i.e. carbohydrates, relative to the cost of fixed carbon as oil, and (ii) the high capital and energy cost of fermentation processes. The price gap between petrochemicals and agricultural products appears to be narrowing and might be expected to bring the basic feedstocks to comparable costs over the next few decades. Hence, given the high biochemical efficiency of the bioconversion routes measured in energy and mass terms, it is the efficiency of the process technology that will determine whether and when fermentation methods can be used.

Table 2. Operating costs for ethanol fermentation

(Basis: 100000 t/a of 95 % ethanol.)

	£/t	
fixed costs	25	
labour	20	
materials (excluding carbohydrate)	15	
water	8	
electricity	4	
steam	27	£99/t

Most fermentations are operated as rather dilute aqueous processes. The substrate is provided in solution or finely dispersed in water and the fermentation is carried out in large volume reactors. For fuel and chemical use the product is usually required more or less pure, and so it then has to be removed from the aqueous phase. As product concentrations are generally only 1–2 %, many tonnes of water have to be separated for each tonne of product. Thus the costs arise in (i) the large mass of capital equipment for fermentation and recovery; (ii) the energy for preparation of the raw material and (iii) the energy for separation of the product from the reaction mixtures.

(*a*) *Ethanol production*

The relative significance of the capital investment and the energy can be seen in a cost breakdown of the ethanol fermentation (table 2). The major items of the operating costs for ethanol production, excluding the cost of the carbohydrate, are energy costs (32 %), most of which is used in distillation, and capital-related costs (26 %). Some ways in which these two components may be decreased are discussed below.

(i) *Capital costs* of ethanol distilleries are markedly influenced by the productivity of the fermentation step (figure 2). In the present operating range for conventional batch fermentations the productivity is 1–4 kg ethanol per m^3 of fermentor per hour, and the investment is about £100 per tonne-year on large scales of operation. The costs shown in figure 2 refer to a plant taking in its carbohydrate as a prepared syrup; the investment would increase to at least

£200 per tonne-year if the biomass preparation costs were included (Anderson 1978; Carvalho *et al.* 1978). Continuous fermentation processes are being developed to increase the fermentor productivity (Coombs *et al.* 1978; Cysewski & Wilke 1978; Rosen 1978). The basis for the methods are retention of yeast cells in the fermentor by separation and recycle from the product stream or by continuous evaporation of the fermentation broth. Yeast concentrations of up to 100 kg m^{-3} can be obtained and productivities of up to 82 kg m^{-3} h^{-1} have been reported in laboratory experiments (Cysewski & Wilke 1978). The concentration of ethanol, which also affects the capital cost of a distillery, has a much greater effect on the energy consumption and is discussed in § 3 (*a*) (ii).

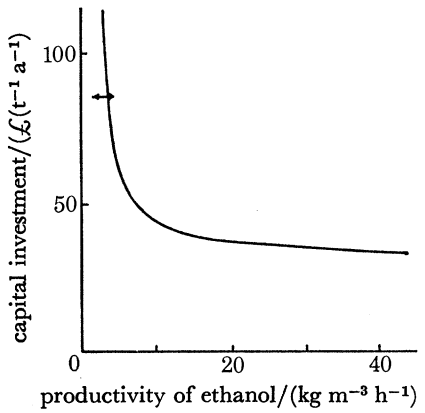

FIGURE 2. Effect of fermentation productivity on capital investment in a distillery. Basis: 100 000 t/a of 95 % ethanol from molasses. Arrow indicates present operating range.

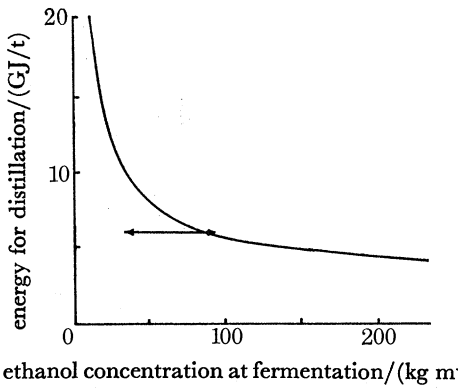

FIGURE 3. Effect of ethanol concentration on energy used in distillation to 95 % (Bungay & Walsh 1978). Arrow indicates present operating range.

(ii) *Energy consumption* in distilleries at present is approximately equal to the fuel value of the ethanol produced (Anderson 1978). Studies on fuel ethanol production from a number of agricultural crops show that energy consumption in the fermentation and associated plants is at least two-thirds of the energy value of the ethanol produced (table 3). Most of the energy is required for distillation of the fermentation broth and for concentrating the residual liquor from the stills. Low pressure steam is required for this and can be generated by burning low-grade fuels such as bagasse or straw. All of the projects for generating fuel alcohol from biomass therefore depend on the use of crop by-products as fuel in order to obtain a substantial, favourable net energy ratio.

TABLE 3. ENERGY USED IN ALCOHOL PRODUCTION FROM BIOMASS

(Units are gigajoules per tonne of ethanol.)

crop ...	sugar cane	cassava	sorghum	maize
reference ...	(1) (2)	(1)	(1)	(3)
farming	6.2 8.9	8.2	10.2	18.2
industrial	16.3 18.3	18.9	15.9	41.7
crop waste use	15.9 16.7	11.7	*ca.* 16	*ca.* 40
net energy out	22.6 18.7	13.8	*ca.* 19	*ca.* 9

(1) Da Silva *et al.* (1976).
(2) Rawlings, C.S.I.R.O. (personal communication).
(3) Scheller (1977).

Collection and fuel use of the crop by-products is a technology well established for sugarcane bagasse which is the main fuel used in sugar production; for wheat straw, corn trash, etc. the technology remains to be established. At the same time potentially competitive technologies for the use of the crop by-products are being developed, e.g. building materials, paper, pyrolysis, anaerobic digestion, and hydrolysis to yield sugars for food or fermentation. Hence these by-products may have more valuable uses in the future than simply burning as a low-grade fuel. There is, therefore, a need to develop methods for recovering ethanol that are less energy-demanding than conventional distillation of dilute solutions, and methods that are less energy-demanding than evaporation of the dilute residues, of separating water from the still bottoms.

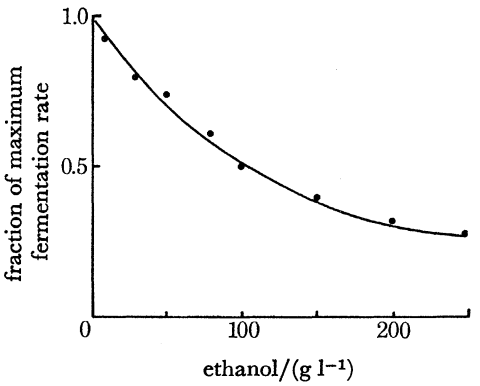

FIGURE 4. Effect of ethanol concentration on fermentation rate (S. Brown, D. E. F. Harrison, S. Oliver & R. C. Righelato, unpublished data). Fermentation rate measured as carbon dioxide evolution by *Saccharomyces cerevisiae* over 40 min at 10 g l^{-1} glucose and specified ethanol concentrations.

Some approaches to reduction in energy costs include vacuum distillation (Ramalingam & Finn 1977) and ternary azeotrope distillation (Jackman & Cook 1978) of the fermenting broth and membrane filtration for concentrating alcohol and the residues. Our approach is to increase the concentration of ethanol produced by the organisms. Present technology is mostly in the range 40–90 g l^{-1} in the fermentation broth (figure 3). Ethanol acts as an inhibitor of fermentation and yeast growth, so that the rate of alcohol production falls with increasing alcohol concentration. The inhibition of fermentation approximately obeys non-competitive inhibition kinetics and we have found that some yeasts are capable of fermentation at ethanol concentrations up to 250 g l^{-1}, albeit for short periods of time (figure 4). Further studies of the kinetics of ethanol inhibition and the selection of resistant yeast strains may enable us to develop the bioconversion process to operate at concentrations of ethanol well above the present operating levels. The energy required for distillation and residue evaporation for a broth containing 200 g l^{-1} would be a little over half that required for 80 g l^{-1}. However, such processes would present a new set of limitations: the preparation of the carbohydrate raw material at concentrations above the 200–300 g l^{-1}, that can be made at present from starch, without evaporation or other energy-demanding techniques.

(*b*) *Methane production*

Anaerobic digestion to yield biogas, i.e. methane and carbon dioxide, avoids the energy-expensive recovery problems of ethanol in that the product, being gaseous, disengages itself from the fermentation broth. The anaerobic digestors used on municipal sewage works and

increasingly for farm and industrial effluents are mixed cultures of bacteria that hydrolyse complex organic matter, including cellulose, into simple monomers and convert these to fatty acids, hydrogen and carbon dioxide which are further metabolized to methane. As an effluent treatment process it can be very efficient since most of the organic matter is converted to methane and carbon dioxide and only a small proportion to microbial biomass, the sludge content of the effluent from the digestor. The gases can be burnt directly or refined to pipeline grade substitute natural gas which could be used for methanol production.

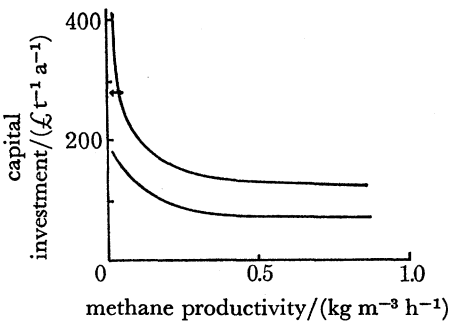

FIGURE 5. Effect of digestor productivity on capital investment in methane production. Upper curve, 10000 t/a of methane; lower curve, 100000 t/a of methane. Arrow indicates present operating range.

(i) *Capital costs*. The main drawback of conventional anaerobic digestion is the slow rate of the microbial process. Generally a single tank is used as a homogeneous continuous reactor, with residence times of 20 days or more. Hence very large digestor volumes are required and this forms a large part of the capital cost (Beneman 1978). Decreases in residence time and hence digestor capacity would therefore have a significant effect on the investment (figure 5). The data presented by Donelly (1977) show a methane productivity of *ca.* 0.05 kg m^{-3} h^{-1} with a microbial biomass concentration of *ca* 5 kg m^{-3}. It is possible that improved methods of retention of microbes in the reactor could lead to concentrations up to ten times this and proportionate increases in the rate of methanogenesis. Two-stage digestion systems have also been suggested as a method of increasing productivity (Gosh & Klass 1978). Even so, the capital investment per tonne of methane is similar to or higher than that for ethanol.

Simpler forms of anaerobic digestors are being developed which may substantially reduce the capital cost and make it possible to apply digestion on small scales and in situations without waste disposal credits (Lovelidge 1976; Patel 1977). An alternative approach is the use of landfill sites designed for gas collection. In such systems the biomass generated as either urban refuse, crop waste or crops from marginal land is buried, in some cases mixed with sewage sludge. The methane produced by anaerobic decay of the organic matter is collected via pipes sunk into the landfill. Gas recovery from urban refuse landfill sites is done at present at a number of sites in the U.S.A. and is reported to be economically viable, though variable, owing to inconsistencies in landfill design and composition (Anon. 1978). Design and operation of landfill sites with gas production as a primary objective may overcome some of these problems.

(ii) *Energy efficiency*. Despite the high capital cost, the operating costs for anaerobic digestion of £10–20/t methane excluding capital, are low relative to ethanol production because of the low cost of recovery of the product. Donelly (1977) reports an energy use for a digestor of 0.14 GJ h^{-1} for an output of 4.3 GJ h^{-1}. Although the biological process is exergonic the rates

are usually insufficient to maintain the temperature of the digestor at 30–40 °C and it is necessary to couple the digestion process to a supply of low-grade heat, often available on industrial sites. Failing such a supply, part of the gas may be used, with a concomitant lose of efficiency.

The energy yield from the input biomass should be high relative to ethanol since the whole of the crop or waste may be digested at an efficiency of at least 50 % (Beneman 1978). However, the overall energy economics and costs will depend on waste disposal credits and on the cost and credits involved in converting the effluent sludge from the digestor into feed or fertilizer.

(*c*) *Other processes*

A limitation of the ethanol fermentation is that the yeasts used are capable of metabolizing a limited range of carbohydrates; starch and cellulose, the major carbohydrate in biomass cannot be used without prior hydrolysis. Work currently in progress in a number of laboratories involves the use of an ethanol-producing thermophilic organism *Clostridium thermocellum*. It can be calculated from the results of Wiemer & Zeikus (1977) that, during the linear growth period on cellulose, ethanol, hydrogen, acetic acid and carbon dioxide were synthesized in the approximate molar ratio 1 : 1 : 0.5 : 1. The potential advantages of a process based on this organism are that the majority of the biomass may be consumed, as in anaerobic digestion for methane, that the products are more valuable than methane, and that it can be operated at a temperature of at least 60 °C, giving a lower consumption of energy in distillation of ethanol than the conventional ethanol fermentation.

High capital investment and energy consumption, and the problems related to the inhibition of organisms by their products, discussed above in § 3 (*a*) with respect to the ethanol fermentation, are more apparent with the fermentations for other alcohols, acetone and fatty acids. It is possible that some of the solutions developed for ethanol production may also be applicable to these products. An alternative approach to the search for useful fermentation chemicals, to which little attention has yet been paid with the exception of methane, is the production of water-immiscible products (table 1).

Conclusions

Anaerobic fermentation of carbohydrates can yield a wide variety of end products of value as chemicals or as energy sources. The factors mediating against its use for the production of alcohols and fatty acids are the high capital cost and the cost of recovery of the products from the fermentation broth. Current developments are bringing both capital and operating costs down although there is a need for lower energy-demanding technology in recovery of the water-soluble products.

For ethanol it appears that energetically economic processes can be operated from sugar cane and corn provided the crop residues can be used as a low value fuel. For the present, in most situations the cost is greater than that of gasoline or of petroleum-derived ethanol. However, there already exist several situations in which ethanol produced by fermentation is used as a liquid fuel and as a chemical intermediate.

Anaerobic digestion of biomass-containing effluents is an economic proposition in many situations where some form of effluent treatment is required. As a waste treatment process it has a low energy consumption and provides, as a by-product, methane, which can make a significant contribution to the energy needs of the producer. Technological improvements at

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

present in development can be expected to reduce the capital cost and hence make it a more attractive method of waste treatment, and perhaps extend it to the generation of methane as a primary product from waste crops or urban refuse.

The overall contribution that fermentation could make to the energy economy of a densely populated, highly developed country such as Britain is small. For instance, liquid fuel for transport, which accounts for about 15% of our total energy consumption, is about $1.3 - 10^9$ GJ/a (U.K. I.S.E.S. 1976). To produce this as ethanol from carbohydrates would require most of our present arable land. While we cannot satisfy our food needs from home production in the U.K., it is unlikely that substantial amounts of energy will be produced in this way. However, some crop by-products, urban refuse and industrial effluents could be used, at least for methane production. While these could not fully support our energy needs by any means, they could make significant contributions to many individual locations. If technology for the production of ethanol from cellulose wastes becomes available it too might make a significant contribution to energy supplies. The potential consumption of carbohydrate for chemicals is of an order of magnitude lower than that required for energy and food supplies, and it is possible that biomass will make a significant contribution (Coombs *et al.* 1978).

In countries with high photosynthetic productivities and proportionally low consumption, the contribution from biomass could be much greater, and these countries could become primary producers of chemical and energy feedstocks based on carbohydrates and their conversion products.

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

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Discussion

D. O. Hall. What do you think of the role of genetic engineering in improving strains of microbes for these purposes?

R. C. Righelato. There is clearly scope for strain improvement in a number of areas: increased tolerance to products such as fatty acids and alcohols, operation at higher rates and temperatures, and direction of mixed product fermentations to a single valuable product. Genetic engineering is one of many techniques available for strain improvement and may well be useful, perhaps most particularly in extending the range of substrates that an organism can use.

D. O. Hall. There are agricultural surpluses within the E.E.C. that could be used as feedstocks for alcohol production.

R. C. Righelato. The surpluses are not nearly as big as the press would sometimes have us believe. For instance, the present 'sugar mountain' only represents two months supply for the European Sugar market. This would have a tiny impact on energy supplies. Moreover, the mountains or molehills occur irregularly and it would not be economically feasible to have large fermentation plants built for only occasional use, or at E.E.C. crop prices.

Sir William Hawthorne, F.R.S. Perhaps the best thing to do with biomass is to eat it.

R. C. Righelato. Yes. The first thing to do with biomass is to eat it. However, a lot of the biomass we produce is inedible, at least for humans. So large quantities of agricultural waste and by-products could be made available for production of energy sources such as alcohol and methane.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES