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Anaerobic Fermentation: Alcohol Production [and Discussion]

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Anaerobic fermentation: alcohol production

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Anaerobic fermentation of carbohydrates by yeasts and bacteria leads to the production of a range of alcohols, acids and esters. Three alcohols, ethanol, isopropanol and butanol, are currently made industrially by fermentation, though, in most places, production from petroleum is cheaper than the biological conversion. The conversion of glucose to ethanol can be achieved at approaching the theoretical maximum efficiency of 51 % (by mass) based on the biochemical route, retaining 93 % of the energy content of the carbohydrate. However, the cost of carbohydrate feedstock and the processing cost are generally high compared with the value of the alcohol. The costs and energy balances for alcohol production from a range of feedstocks are discussed. Technological improvements, particularly in reduction of capital and energy requirements, could substantially reduce the processing costs.

Developments can be expected in (i) the application of continuous fermentation and immobilized cells; (ii) the development of organisms with increased tolerance to alcohols, wider substrate ranges and higher temperature optima; and (iii) lower energy requirements for recovery of alcohols.

Given reasonable technical progress, the wider adoption of bioconversion of carbohydrates to alcohols and similar bulk chemicals will depend on the relative costs of carbohydrate to fossil hydrocarbons. In some regions of high agricultural productivity, and without petroleum, bioconversion processes are already in use and it seems likely that this trend will become more widespread over the next few decades.

INTRODUCTION

Recently, there has been renewed interest in finding alternative liquid fuels to petrol and alternative sources of chemicals to petroleum fractionation; hence, the old fermentation processes for alcohols and other products are under review. In this contribution the types of chemical and fuel products that can be produced by fermentation will be discussed and the conditions required for them to become economically useful will be examined by looking at the supply of carbohydrate raw material and at the present production technology and likely developments.

Fermentation, the anaerobic catabolism of carbohydrates, proceeds by the oxidation of sugars to pyruvic acid, which process yields the cell energy and produces reduced nucleotides and a number of products that are potentially useful to man (table 1). Ethanol is the most widely known of these, as an industrial product, made by the reductive decarboxylation of pyruvate. From each mole of hexose, 2 mol of ethanol are generated conserving over 90 % of the calorific value of the sugar in the product. The energy density, 29.6 GJ/t, is nearly twice that of carbohydrates. It is a non-corrosive liquid at normal temperatures and can readily be used as a fuel in existing internal combustion engines, alone or in mixtures with petrol. Dehydration yields ethylene, which is generally obtained from naphtha and is a pivotal compound in petrochemical processes. Ethanol is made industrially by means of a number of yeasts, usually of the genera *Saccharomyces* and *Schizosaccharomyces*.

In addition to ethanol, butanol, isopropanol, acetone, butanediol, butyric acid and propionic acid have been made industrially, though few of these are still manufactured by fermentation.

As well as the extracellular products, there are reduced, water immiscible products of some microbes that accumulate intracellularly to form the greater part of the cell mass. Among them are triglycerides (Woodbine 1959; Whitworth & Ratledge 1974) and the polyester, poly-3-hydroxybutyrate (Stockdale *et al.* 1968). Most of the compounds listed in table 1 are, or could be, important as fuels or chemical feedstocks. These would be low margin, high volume products, giving rise to a type of business with which the fermentation industry has not traditionally been involved. The logistics of supply of raw materials, the potential economies of scale, and the sensitivity to process energy demand raise new questions. Perhaps the most critical of these is the supply of carbohydrates from agriculture.

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		
acrylic			

Table 2. Agricultural production of fermentable carbohydrates

crop	latitudes N/S	starch or sugar / t ha^{-1} per year	energy in farming / GJ/t	references
sugar cane	0–30	8–15	2.3–3.1	Austin *et al.* (1978) Da Silva *et al.* (1976)
cassava	0–30	5–14	3.7	Da Silva *et al.* (1976)
sorghum	0–40	7	4.6	Da Silva *et al.* (1976)
maize	20–50	4–6	9	Scheller (1977)
sugar beet	40–60	4–8	4.1–5.1	Austin *et al.* (1978)

Carbohydrates for fermentation

World use of fossil carbon as fuels is approximately equal to the total carbon produced agriculturally. Chemical consumption is an order of magnitude lower in volume, but it represents, nonetheless, about 10^8 t a^{-1}. So, for fermentation to make a significant worldwide contribution to either fuel or chemical feedstocks, substantial quantities of carbohydrate must be provided. The use of agricultural surpluses and by-products, which are, or can be, relatively cheap will not make a significant impact on supplies. So we must look at primary production of carbohydrates for fermentation. The crops considered as suitable substrates for fermentation are the major starch-and-sugar-yielding plants (table 2). The most efficient crops in terms of land area required are the tropical plants, sugar cane and cassava. The costs of production, measured in terms of the energy equivalent of fertilizers, pesticides, fuel and machinery, are lower per tonne of fermentable carbohydrates from cane and cassava than for carbohydrate from crops in temperate regions. The by-product, lignocellulosic fibre, is an important part of the crop for sugar cane, as it provides the fuel for sugar processing or alcohol production. This could, perhaps, be true also of cassava, sorghum and maize, as they generate sufficient fibre residue, most of which is, at present, not collected. The total dry biomass is two- to fourfold higher than the sugar or starch yield, and the costs would be proportionately lower for the total carbohydrate. There would, therefore, be a substantial economy attached to technologies able to convert the whole plant biomass into fuels or chemicals.

Higher yields per unit of land area and lower energy inputs make growth of carbohydrate in the tropics cheaper than in temperate regions. So it is not surprising that the countries currently exploiting the production of ethanol as a liquid fuel, and of other low price organic chemicals by fermentation, are within the latitudes 40° N–40° S. Within these countries, the substrate is almost always cane sugar. In the temperate countries, price support systems for farmers generally prevent access to lower cost carbohydrate from the warmer parts of the world. In Britain, the cost of starch or sugar has been generally higher than the price of the alcohol or other bulk chemicals to which it can be converted. However, there are worldwide trends and local variations that lead one to suppose that carbohydrates will form a greater proportion of chemical and energy feedstocks in the future:

(*a*) Inflation rates for petroleum and petroleum-derived products have been higher than for agricultural products over the last fifteen years, and the differential rates are expected to continue. Although increases in the oil price are transmitted to agricultural products, the increase is not *pro rata*; it has been estimated that a 10% increase in crude oil prices eventually results in a 3% increase in crop prices.

(*b*) The general trend with commodity crops is for them to decrease in price relative to other goods as agriculture develops.

(*c*) There is an oversupply in the international markets for sugar and some grains, due largely to N. America and E.E.C. farming policies. This results in (i) surpluses and (ii) depressed prices, so that many developing countries are losing sources of foreign exchange.

(*d*) Oil-importing countries are presented with increasingly serious balance of payments problems, which have to be met by exports or by import substitution.

(*e*) There is a need for rural development in many emerging industrial nations, to take the pressure off the overcrowded cities and to improve living standards in the countryside.

The effect of various of these factors on the fermentation industry can be seen in several countries today. In Pakistan, fermentation ethanol is dehydrated to ethylene. In Brazil, an ambitious programme aimed at giving a 17–20% substitution of ethanol for gasoline is well under way. Over 200 distilleries, producing 4 Mt of ethanol per year, are planned. Most of the distilleries use cane juice or molasses, though an experimental distillery based on cassava has been commissioned, and another using babassu, the fruit of a palm, is planned. The chemical industry in South Africa makes ethanol, butanol and acetone by fermentation of molasses. In addition to these existing operations, at least 40 governments are studying the prospects of fuel ethanol programmes in their countries.

Ethanol production technology

The prime determinant of a more general shift to the biomass-based processes for bulk chemicals will be the cost of carbohydrate relative to the cost of fossil sources on a carbon and energy basis. As these costs come together it will be the efficiency of the process technology that will determine whether and, if so, when fermentation will feature as a major contributor.

Fermentations, in general, are operated as rather dilute, aqueous processes: the substrate is provided in solution or finely dispersed in water, and the fermentation is done in batches in large volume reactors. For most uses, the product has then to be separated from the aqueous phase, and, as the product concentrations achieved are generally only a few percent, many tonnes of water have to be removed for each tonne of product. Thus, as well as the substrate, fermentation processes require a large amount of capital equipment and energy for preparing the raw material and separating the product.

Table 3. Cost (£/t) of ethanol production

(Basis: 96% ethanol; 80000 t/a from molasses; 8000 h/a in United Kingdom.)

fixed costs including labour	40
materials	15
water	8
electricity	4
steam, at £5 per tonne	27
molasses, at £50 per tonne	200
total	294

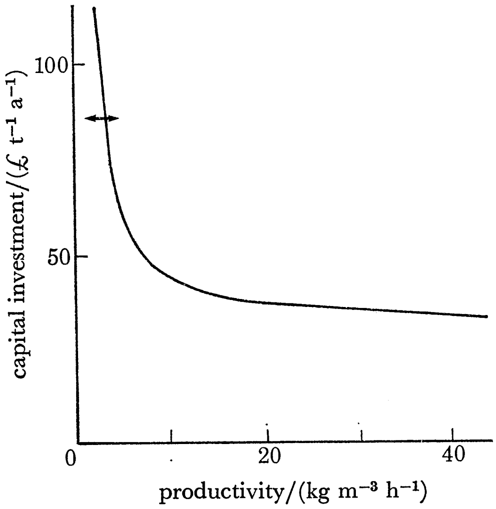

Figure 1. Effect of fermentation productivity on capital investment in a distillery. Arrow indicates present operating range. Basis: 100000 t a^{-1}; 95% ethanol from molasses.

The main cost components are the fixed costs, which are directly related to the investment, the energy costs and the substrate. In the U.K., ethanol costs are dominated by the raw material cost, which is generally greater than the value of the ethanol, except as potable spirit (table 3). However, in sugar-producing countries, carbohydrates as molasses or cane juice can be obtained at half the present price of molasses in Europe, and energy as bagasse is almost free. In this situation, the capital investment is a major part of the cost and a deterrent to further development of alcohol production. In other situations, the cost of energy is a serious deterrent.

Although the present industrial technology for alcohol production owes little to the strides that biotechnology has made over the last thirty years, it is likely that in the near future we will see new techniques enabling substantial economies in the conventional production of ethanol.

Continuous fermentation systems are being developed to increase fermenter productivity, i.e., the quantity of alcohol produced in unit volume of fermenter in unit time (Coombs *et al.* 1978; Cysewski & Wilke 1978; Rosen 1978). The basis for achieving high productivities is the retention of yeast cells, usually by separation from the product stream and recycle into the fermenter. Concentrations of yeasts of up to 100 kg m^{-3} can be achieved, which permit a high overall rate of conversion of sugar to ethanol whilst the cells grow at very low specific rates, thereby diverting little of the carbohydrate to cell mass. The rates of alcohol production that can be achieved this way in single-stage continuous fermentations are 10- to 100-fold higher than those of the conventional batch process. Batch processes generally operate at 1–4 kg ethanol per cubic metre of fermenter per hour; rates up to 82 kg m^{-3} h^{-1} have been reported for some continuous fermentations (Cysewski & Wilke 1978). Because fewer fermenters are required for a higher productivity process, the capital investment is smaller (figure 1).

Ethanol is a potent inhibitor of a number of metabolic activities. Studies in our laboratories and jointly with the University of Kent show approximately non-competitive inhibition kinetics for ethanol on fermentation rate (Brown *et al.* 1978). So there is a dilemma for the microbiologist, between high rates of production of ethanol and the high concentrations of ethanol that are needed to minimize the costs of recovery and the costs of treatment of the still bottoms. This is not a new problem for the chemical engineer, and solutions include multi-stage fermentations and plug flow systems. Fixed beds of cells in a plug flow mode theoretically give the highest productivities for a given alcohol concentration, and have been used experimentally. We have found that yeast cells were active for several months when entrapped in alginate beads (Kierstan & Bucke 1977). However, many technical problems remain to be overcome, including packing very high cell concentrations, outgrowth of cells from the support, release of carbon dioxide from the column and stability of the support.

It has sometimes been argued that fuel ethanol production could not be an economic use of resources because the energy used in the process is greater than the energy value of the alcohol produced (Anderson 1978). This is an oversimplified argument, since, generally, a low grade fuel, such as crop waste, is burnt or waste heat is used to generate a premium liquid fuel. Nonetheless, the process energy requirements restrict the production of fuel and industrial ethanol to situations in which cheap heat is available.

Studies on fuel ethanol production from a number of crops show that energy consumption in the fermentation and associated plants is at least two-thirds of the energy value of the ethanol produced. Most of the energy is required for distillation of the fermentation broth and for concentration of the residual liquor from the stills, and most of the projects for generating fuel alcohol from biomass depend on the use of crop by-products as fuel, to obtain a substantial, favourable net energy ratio (table 4).

Collection and fuel use of crop by-products is a technology well established for sugar cane 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. So, in the future, these by-products may have more valuable uses 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 of separating water from the still bottoms that are less energy-demanding than evaporation of the dilute residues.

One approach is to increase the concentration of ethanol produced by the organisms and so decrease the amount of water used in the process. Present technology is mostly in the range 40–90 g l^{-1} in the fermentation broth (figure 2). However, some yeasts are capable of fermentation at ethanol concentrations up to 250 g l^{-1}, albeit slowly and for short periods of time. 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 levels, and so save energy. 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, 200 g l^{-1} of ethanol requires a feed at over 400 g l^{-1} of carbohydrate, so we then approach a new set of limitations: the preparation of the carbohydrate raw material at concentrations above the 200–300 g l^{-1}, which can be made, at present, from starch, without using evaporation or other energy-demanding techniques.

Table 4. Energy (GJ) used per tonne of alcohol produced from biomass

	sugar cane				
crop	†	‡	cassava†	sorghum†	maize§
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

† From Da Silva *et al.* (1976).
‡ From P. Rawlings, C.S.I.R.O. (personal communication).
§ From Scheller (1977).

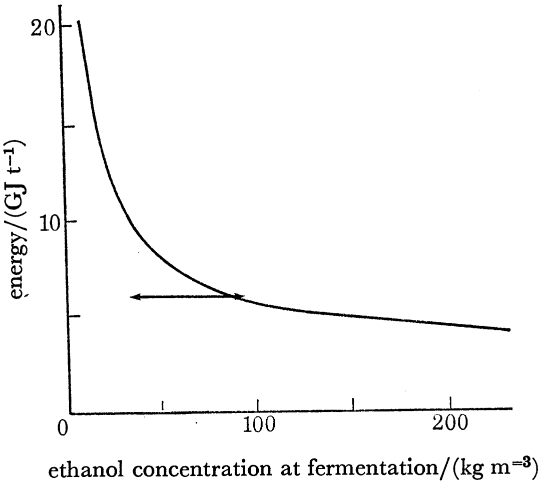

Figure 2. Effect of ethanol concentration on energy used in distillation to 95% (Bungay & Walsh 1978). Arrow indicates present operating range.

For every tonne of alcohol distilled, some 10–20 t of stillage is separated; this contains the non-metabolized components of the raw material and yeast products. It has a high biological oxygen deficit and presents a major waste treatment problem. Evaporation to a thick sludge or dry powder is practised in many distilleries and provides a low-value animal feed and fermentation nutrient, but the process consumes extra energy. Two treatment schemes are being developed that contribute energy back into the process: anaerobic digestion and incineration. The large volumes of stillage mean that high rate digestion processes will be required for economic treatment (Donnelly 1978; Righelato 1978).

The developments that have been discussed above will lead to lower investment costs and lower energy costs in making fermentation alcohol. They can be expected to hasten the present trend to distil more fuel and industrial alcohol from sugars. In the longer term, more dramatic technological changes could widen the scope for alcohol production. An important limitation of the ethanol fermentation is that the yeasts used are capable of metabolizing only a small range of carbohydrates: starch and cellulose; the major carbohydrates in most plant biomass cannot be used without prior hydrolysis. Work currently in progress in a number of laboratories involves the use of an ethanol-producing themophile, *Clostridium thermocellum*. Results of Weimer & Zeikus (1977) show that, during growth on cellulose, ethanol, hydrogen, acetic acid and CO_2 were produced in the approximate molar ratio 1:1:0.5:1. Extensive work with this system, by Cooney & Wang and their coworkers at the Massachusetts Institute of Technology, has shown that the proportion of ethanol can be greatly increased (unpublished). This system presents the possibility of making ethanol from the whole of the plant biomass and from cellulosic crop waste. Thermophilic ethanol-producing organisms would be particularly valuable at temperatures around 70 °C, at which fermentation and distillation could take place simultaneously without the application of high vacuum.

Another group of bacteria, *Zymomonas spp*., produce 2 moles ethanol from each mole of glucose, by a route that yields one ATP per mole of glucose, and they exhibit maximum rates of fermentation several times higher than those of the yeasts (A. J. Forage, unpublished data). However, the substrate range of *Zymomonas* is limited, though extension of the range by gene transfer would probably be more readily achieved than with yeasts. So this organism, too, offers another route for improving ethanol production.

Discussion

Ethanol is the largest volume fermentation product made today, mostly for drinking. Historically it has been used as a fuel in times of crisis in a number of countries, and this role is now being reexamined worldwide. The overall contribution that fermentation of home-produced biomass could make to the energy economy of a densely populated, highly developed country like Britain is small. For instance, liquid fuel for transport, which accounts for *ca*. 15 % of U.K. energy consumption, is about 1.3×10^9 GJ/a; to make this from ethanol from home-grown starch or sugar would require most of our arable land. Production of power ethanol from European surpluses of beet and grains could generate a few million tonnes of fuel, but the irregular nature of surpluses would present problems in manufacture and in distribution. Much more probably, in Europe, can we expect a steady increase in the fermentation of industrial ethanol and other microbial chemicals as oil prices continue to rise more than agricultural costs. But the major impact will be in countries with high photosynthetic productivities, which could become world suppliers of chemicals based on carbohydrates, and indigenous fuel producers from biomass.

There are, however, a number of questions that blur the horizon.

(i) Fermentation is not the only way of making reduced products from biomass: pyrolysis of biomass generates carbon monoxide and hydrogen, which can be used directly as a gaseous fuel or can be converted to methanol and to hydrocarbons. This old technology will gain added impetus from the developments currently in progress on coal gasification and pyrolysis of urban waste.

(ii) Ethanol may be superceded as an energy source by other microbial products, such as methane, esters or some lipids, which are more easily separated from the fermentation broth and could be cheaper to produce.

(iii) Extensive use of carbohydrates as fuel or chemical feedstocks in Europe will be facilitated by access to the agricultural produce of tropical countries, where photosynthate productivities are higher; this will require major changes in E.E.C. agricultural policy.

(iv) Alternatively, technological developments may occur, either giving very much higher agricultural yields of fermentable carbohydrates or permitting the economic use of the cellulosic components of the biomass, effectively doubling the agricultural yield.

(v) Lastly, chemical technologies may be developed in the longer term that make more use of carbohydrate and other biomass products as building blocks, rather than the naphtha-derived intermediates, so that in the next century it may be considered a wasteful use of resources to degrade carbohydrates to generate intermediates that fit into today's petrochemical technology.

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Discussion

F. W. Hayes (*37, Sussex Square, London, W2 2SP, U.K.*). In assessing the merits of various 'energy crops', one inevitably meets comparisons with sugar cane on a yield basis. There is a serious flaw in much of the reasoning, particularly when talking of cassava, which has never been grown on a large commercial scale under intensive cultivation conditions such as those that obtain in the cane fields in sugar production. Yet one sees calculations with enormous scale-up factors based on yield results for cassava grown on relatively small experimental plots. These figures are then compared with yields of cane that represent *average* returns for a large district or even a whole country. While experimental plots of cassava have yielded 40–60 t/ha per year under ideal conditions, small trials with cane have, under the same circumstances, produced up to 250 t/ha per year. In any case, under modern agronomical practice, one is quite safe in assuming *average* yields of cane of 100 t/ha per year.

Similarly, I find that production costs of fermentation alcohol seem to be saddled with the burden of archaic technology, particularly with regard to the figures used for steam and power. We must remember that, for fuel ethanol, we have to deal with the 'whole cane concept'. Cane will be grown for its yield of total fermentable carbohydrate. Therefore we will be utilizing not only the soluble constituents in the juice, such as the sugars, but also all the components of the 'fibre', including the hemicelluloses, the pentosans, the lignin and the gums and pectins, as well as the basic cellulose. In the established sugar industry procedure of burning the 'bagasse', i.e. the total fibre, to produce steam and power for the process work, it must now be recognized that the fibre is regarded as having no greater value than its use as a fuel. With today's escalating prices for oil and gas, this may well be correct, but the fact remains that the fibre can be the source of valuable products, including fermentable sugars, by hydrolysis and other process means.

With use of sugar cane as a feedstock, the process steam requirements for the production of ethanol, including the milling of the cane, sterilization of juice etc., distillation and evaporation, will still leave an excess of bagasse. Market conditions will determine whether the production of materials from the pentosans (furfural etc.) or the lignin, is justified.

Under no circumstances can the bagasse be described as a 'waste product'. If burned in the boiler furnaces it is still a source of valuable carbon and therefore it must not be wasted, any more than we should waste petrol by using badly tuned motor car engines. Efficient combustion, the generation of steam at high pressure and temperature, and use of the heat drop in back-pressure or pass-out turbines for the generation of power, with the back-pressure steam being used for process needs, are all mandatory elements in plant design. This principle can be one of the biggest factors in bringing down the production cost of ethanol and making it a practical proposition as a gasoline extender or substitute.

On the vexed question of the treatment of the spent wash or 'stillage', once again the availability of low-cost steam is the deciding factor. Many years ago, when I was with National Chemical Products Ltd in South Africa, fermenting molasses for a variety of products, we developed a 'spray dryer-cum-incinerator' that produced an ash that contained 35% K_2O and was sold to the fertilizer industry, mainly as a source of potash. After the spent wash from the stills had been evaporated to a 40% solids concentrate, the organic matter present was able to support its own combustion without additional fuel. Although this process solved the effluent problem, it was later abandoned in favour of evaporating and then spray drying the spent wash to produce animal feed supplements, which were sold on a world-wide basis. Thus you will see that *the market* dictated the final process. I maintain that the same principle should hold for bagasse, or the cane fibre. So long as there is not a better financial return to be obtained by using bagasse as a feedstock for chemical products, it can be used as a fuel.

I might add that none of the above operations was subsidized in any way by Government or other authorities.

Surely, in what I have described, we are just applying the same principles for the utilization of our photosynthetic carbon from crops as that used for the carbon in our fossil petroleum materials, and which we should also apply to coal.

Anon. Would Dr Righelato like to comment on the possible role of marine biomass as a source of feedstock?

R. C. Righelato. This is possible, but there are more exciting prospects for marine systems, such as hydrogen production. In any case, the technology of enclosed marine systems is very complex and is not yet sufficiently well developed.

A. N. Emery (*Department of Chemical Engineering, University of Birmingham, P.O. Box* 363, *Birmingham B*15 2*TT, U.K.*). Sugar cane is the only biomass crop that includes sufficient waste material to supply all the energy required for its processing. Therefore, for other sources of biomass we must look for alternative sources of energy. We cannot afford to continue to throw away potential resources. The utilization of wood, which requires the degradation of lignins and hemicelluloses in addition to cellulose, provides a microbiological challenge that must be solved.

R. C. Righelato. What about pyrolysis for the treatment of cellulosic wastes, or even for treatment of the primary product?

A. N. Emery. Pyrolysis is as good a way as any to recover energy from residual materials.

W. J. Cuthbertson (*Glaxo Group Research Ltd, Greenford Road, Greenford, Middlesex UB*6 0*HE, U.K.*). In certain economies it is more profitable to use wood by pyrolysis rather than by fermentation. You can recover a proportion of the energy directly as liquid fuel (methanol) without the need for hydrolysis. The residue is charcoal, which can readily be further processed. Has this been investigated?

A. N. Emery. Yes, in many locations this does apply; however, in the tropics, fermentation is more attractive, and will be so, at least for the next few years.

J. T. Worgan (*National College of Food Technology, University of Reading, St Georges Avenue, Weybridge, Surrey, U.K.*). Surely, in view of the enormous areas of land that would be required to grow sufficient sugar cane to provide the biomass to satisfy world energy demands, the prospects are poor since increasing amounts of agricultural land are required for food production?

R. C. Righelato. I agree, but in the short term it is a viable process for such countries as Brazil.