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Sugar-cane as an Energy Crop

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Introduction

Sugar-cane is well known as a source of a wide variety of food and beverage products including white and speciality sugars, molasses and rum, as well as other fermentation products (Paturau, 1982). The term energy, as applied here to crop, is used (as so often) in a loose manner. Hence, the title of this review would perhaps be more correctly expressed as 'The potential of sugar-cane as a source of raw material for the production of high-grade fuels for transport, heat or power applications.' This in turn raises the question of why sugar-cane might be singled out in this context and what is the relevance to the rapidly expanding fields of biotechnology and genetic engineering. If these questions are considered in the reverse order, then it might be anticipated that developments in biotechnology will follow two eventually divergent paths: the group taking the first path is related to the production of relatively small quantities of high-value speciality chemicals and health-care products; the group taking the second path is concerned with the large-scale production of fuels and chemical feedstocks from renewable resources. As far as the first group is concerned, the cost and local availability of suitable substrates will not be of prime importance. On the other hand, as far as bulk chemicals and fuels are concerned, it is already clear that the major consideration which will decide the eventual size and geographical location of any new biotechnical energy industries will relate first to the nature, availability and cost of raw materials and second to the competitive advantages of biological conversion technologies over thermochemical alternatives. For instance, processes based on biological raw material (biomass) must compete now with oil and natural gas, and in the longer term with coal as raw material. In Europe it has been estimated (Anonymous, 1983) that the total energy which might be derived from biomass sources in the year 2000 will be of the order of 85×10^6 tonnes oil equivalent, i.e. of about the same order of magnitude as the present stockpile of coal in the UK. In the same way, if all the molasses available in Europe were converted to alcohol it would not be sufficient to provide even a 10% blend with petroleum as car fuel. As far as technology is concerned, the production of methanol from wood (not to mention natural gas or coal) has both a greater weight yield and a better energy efficiency than the

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biological hydrolysis of cellulose and fermentation to ethanol of the sugars so released.

The challenge to biotechnology and genetic engineering in this area is to cause significant increases in production of energy crops, by increasing the yield per unit area of land harvested each year, and also to improve biological conversion processes so that fuels produced through biological routes can become competitive in real terms. The true assessment of such competition is important. Systems can be shown to be economically viable, at present, where raw materials represent biological wastes from agriculture or industry. Fermentation alcohol is being produced in a number of countries at a price which makes it competitive with alcohol derived from natural gas. However, this is due to a distortion of prices by artificial economic factors, resulting from surpluses of agricultural products in the USA and Europe, coupled with European (EC) prices which are maintained at about twice world prices for sugar and starch. Alternatively, in the less-developed regions of the world, apparently viable programmes aimed at the production of fuel alcohol may be based on very low incomes in the agricultural sector.

The term 'biomass energy system' has been adopted to describe systems where the raw materials are derived from plants, through fixation of carbon dioxide using solar energy in the process of photosynthesis. The use of plant material as a source of fuel is, of course, not new. Before the industrial revolution it was the major energy source throughout the world, and it remains so in rural regions of most tropical developing countries, representing over 15% of the world's total energy supply (Hall, Barnard and Moss, 1981). In many developing countries the use of such biological materials as fuel may represent over 80% of the total use: even in the USA, with the highest fuel consumption expressed both on a national and on a per capita basis, biological materials represent over 3% of the current energy use. However, until the last decade, little attention was paid either to improvements in such use of biological energy, or to considerations of true costs, energy balances, effects on the environment, rural populations or national economies. This situation changed during the 1970s in response to real or anticipated shortages of oil-derived fuels, as well as to rapid price increases. As a result, many research programmes were initiated and feasibility studies carried out, reaching a peak of activity at the end of the last decade. Peak activity to find alternative fuels coincided in 1979 with the peak of oil consumption (about 64 million barrels per day) (Anonymous, 1981a). Since then, demand for oil has dropped, resulting in both a decrease in the price of oil in real terms and a decrease in the amount of activity being devoted to schemes for the production of alternative fuels. This lull in activity permits an evaluation of existing biomass-based fuel programmes, as well as an assessment of what systems might be developed in order to meet future crises which may arise.

At the national level, the only significant use of biomass as a fuel is by direct combustion of wood, of agricultural residues, and of domestic solid waste (which may be regarded as a biomass energy resource because of its large wood and paper content). There is a world-wide increase in the use of such materials as a substitute for oil for the production of heat, and to a lesser extent for the production of power—this may be considered as a biomass activity rather than as biotechnology. In addition, in a few countries there is a significant production of ethanol by fermentation of sugar and starch (derived in the main from sugar-cane, molasses and maize) to provide material for use as a liquid transport fuel. The major fuel alcohol programmes are in Brazil (based on sugar cane: Rothman, Greenshields and Calle, 1983), in USA (based on maize: OTA, 1980) and in Zimbabwe and elsewhere (based on molasses and cane juice: Kovarik, 1982b). Anaerobic digestion, resulting in the production of methane, receives considerable attention and may be of importance in China in terms of the number of people who obtain some light and heat from the 7 million digestors which have been built there (Chen, 1980). However, in the rest of the world, both the number of digesters which have been built (about 500 in the EC, for example, discounting sewage digesters) and the amount of energy generated, are negligible. Hence, at present the only biotechnical energy system of any significance is ethanol production by fermentation.

Fuel alcohol programmes have already been subjected to extensive criticism on the basis of a wide range of factors, some related to economics, some to energy balances and some to environmental and social aspects. Numerous studies have shown that alcohol is expensive to produce in comparison with the use of oil: in many systems more oil is consumed in the production of alcohol than is gained, and with current technology there is competition between the use of agricultural raw materials for fuel or food production (Brown, 1980; Flaim and Hertzmark, 1981; Levinson, 1982). Where such food/fuel competition exists it has been suggested that crops for fuel could be produced by clearing more land, or by using marginal land—causing further concern about deforestation, desertification and other environmental issues, including worries about the effects of discharge of large volumes of stillage from the alcohol factories where suitable treatment is not available, or not economic. Above all, it is still not clear whether fuel alcohol can be produced on a sustainable basis, resulting in a significant net gain of energy, at a realistic cost under circumstances which also give a reasonable financial return to the grower. Hence, it would be of interest to consider an optimal system from a biological viewpoint and to see what are the limitations, to what extent these are biological, and where biotechnology might have an input which could help to realize the optimum potential.

Under ideal conditions, sugar-cane (for many reasons which will be detailed below) represents at present both an optimal agricultural system for trapping solar energy into plant biomass (Hudson, 1975; Thompson, 1978; Coombs, 1980; Lipinsky and Kresovich, 1982) and an ideal raw material for the biological production of higher-value fuels, because it can produce both fermentation substrate in the form of a solution of fermentable sugars in the juice, and a combustible fuel in the form of the fibrous parts of the plant (bagasse). In addition, breeding programmes, agricultural expertise, extensive research experience, available technology for both production and conversion, and established plantations capable of producing and processing very large quantities (approaching 10×10^3–15×10^3 tonnes/day in extreme cases) already exist. However, to quote from a recent publication from within the sugar industry (Bennett, 1983): 'It is simply a matter of fact that in the US, faced with an opportunity to produce and market fuel alcohol, the corn industry has been successful while the sugar industry has failed.' This is in spite of the fact that considerable opposition to the use of corn for this purpose, on the basis of energy considerations in particular, was voiced in the US.

The failure of the sugar industry (other than in Brazil) to exploit the fuel alcohol market in the late 1970s can be linked with a general depression in the sugar industry world-wide. This depression has been greater and more prolonged than that of the general world recession, and led to ill-advised projects (such as that in Kisumu, Kenya; Kovarik, 1982a) and inaccurate or exaggerated claims by some sectors of the sugar construction industry in an attempt to obtain contracts during the period of depression. At the same time, significant changes have occurred in the structure of the world sugar markets as a result of nationalization of previously colonial sugar estates following granting of independence of many nations in Africa and the Caribbean in particular, followed by periods of national instability and political problems. At the same time, entry of the UK into the European Economic Community led to the reshaping of the previous Commonwealth Sugar Agreement and its replacement by the Lohme Agreement, resulting in a reduction of about 50% in cane sugar imports to Europe and a disruption of sugar markets for Australia, followed by an increase in European beet sugar production and a slump in world sugar prices. Hence, it would be quite unrealistic to try to deal with the potential of sugar-cane as an energy crop in the abstract, considering only aspects of photosynthesis, fermentation and genetic engineering, without a realistic assessment of both the industry and the capability of producing countries to make the contributions necessary to enable such production to occur.

The sugar industry

Sugar-cane is grown in more than a hundred different countries located between latitudes of about 40 degrees North and South of the equator. The distribution reflects the availability of water and the need cane has for a high annual solar input averaging about 200 $\mathrm{W m^2}$. The major producers of cane sugar include Brazil, India, Cuba, China, Mexico, the Philippines, South Africa, Australia and the USA, as well as many smaller nations in the Caribbean, Latin America, Africa, the Far East, the Pacific and even in Europe at the southern tip of Spain and Portugal. Climatic differences, soil types and variations in farming practice result in crops with widely differing yields grown for anything from 9 months to almost 2 years between harvests. Yields, expressed in terms of tonnes (t) of green (natural moisture content) cane per hectare vary from as low as 20 t to over 200 t. This is material 'as harvested', which may represent about 60% of the above-ground biomass. An annual yield of 100 green tonnes (equivalent to 30 dry tonnes) would be regarded as a good average for almost any region, with current national averages of all developed countries at about 80 green tonnes per hectare and developing countries averaging about 54 tonnes per hectare (FAO, 1981); such yields have been more or less static over the last decade. This contrasts dramatically with the situation in respect of almost all other important agricultural crops, including sugar beet and the major grains, where steady annual rates of increase of about 2·5–3·5% have been recorded. As a result, increased production of cane reflects increased land area use rather than increased yield, again in contrast to most other crops, where yield increases reflect the introduction of new varieties, mechanization, and increased inputs in terms of fertilizer, irrigation and crop protection.

In spite of the apparent constant global production of cane sugar, very marked changes in regional production can be seen. Cane production has increased in Brazil, linked with the alcohol programme discussed in more detail below; most other major producers have remained static. However, dramatic decreases in production are seen in many of the smaller island states which were formerly colonies of European countries. Although these decreases may not be significant in terms of total world production, in many cases cane was a major agricultural crop in terms of land area harvested, and a major source of income to the islands. These falling production figures reflect major changes in the world pattern of sugar trade during the last decade.

The sugar market as a whole can be divided into three parts: (1) that intended for internal consumption by the producer; (2) that which is preferentially traded between countries at fixed price under specific quotas; (3) that part subject to free trade on the world market. This last part has been controlled by a series of International Sugar Agreements, aimed at reducing fluctuations in the world sugar price which arise as a result of periods of over-production or under-production. However, in spite of such agreements sugar prices rose in an uncontrolled manner in late 1974, to approach £500/t. This had serious consequences because it stimulated production of beet sugar within the European Community, resulting in over-production, flooding the free market and depressing prices to below costs of production in many tropical countries. The depression in the sugar industry was increased by the advent of immobilized enzyme technology leading to the development of high-fructose corn syrup in the USA, which has now displaced around 11 million tonnes of sucrose (*see* Chapter 5).

These more recent events followed on from important changes in sugar supply patterns which started to evolve in the the 1950s. Prior to this, most cane sugar was produced in colonies and exported thence as raw sugar for refining in Europe and the USA, often being re-exported back to the tropics as white sugar. However, since then there has been a gradual shift towards greater self-sufficiency, with the ratio of exports to production for such countries falling from 54% to 39%, resulting in a reduction in foreign exchange for purchase of goods and of oil-based fuels in particular. At the same time, problems of establishing stable management systems (both for cane plantations and for islands themselves, following independence) has contributed further to the decline. For instance, in 1971 cane sugar production in Barbados and in Trinidad were about 146×10^3 t and 229×10^3 t respectively. In June 1983 the estimated crop in Barbados is less than 86×10^3 t and the crop in Trinidad is expected to be around 70×10^3 t, with the cost of production substantially in excess of the current price. There is thus a paradoxical situation, that those areas which have lost foreign exchange income needed for fuel-oil purchase also have a depleted industry unable to take advantage of the possibilities of using cane without outside support. As a result, numerous feasibility studies have been carried out, funded by aid programmes and development banks, which tend to support the use of sugar cane as a source of fuel alcohol. However, the number of projects which have been initiated remain low.

Sugar cane

The world-wide distribution of cane is a result of man's intervention, as is the occurrence of present varieties with thick stems and high sugar content at maturity. Sugar cane is a large grass of the genus *Saccharum*, belonging to the family Gramineae in the tribe Andropogoneae. It is of relatively recent evolution with an origin in South-East Asia. As with most members of the tribe, the sugar cane is highly specialized and well adapted to dry subtropical savannahs. The subtribe Saccharineae includes *Saccharum* and the related genera *Erianthus*, *Sclerostachya* and *Narenga* (the so-called Saccharum complex), the common features of which are large size (with vegetative stems of over 5 metres tall), prominent nodes with root initials which will readily propagate, and the ability to form intergeneric crosses. The similarity of form, plus the ability to form hybrids and the fact that the initial distribution by man took place 200–300 years ago, can lead to problems in the identification of different types of 'sugar-canes'. Further difficulties have arisen from the splitting of what were different species into different genera and the division of what was the genus *S.officinarum* L. into various species or genera. Sugar-cane plants that have been selected for high sugar are in general natural or purpose-bred hybrids (Mukherjee, 1957).

It is probable that the major ancestor of the present cultivated cane is *S. spontaneum*, probably arising from *Erianthus* with germplasm contributions from *Miscanthus* originating in Indo-China (Simmonds, 1976). No true *Saccharum* species, or even a distant relative, has ever been found in the New World; it is believed that it was introduced to America by Columbus. On the other hand, the diversity of cane germplasm is greatest in Melanesia, with *S. robustum* as the dominant species. This species was cultivated by primitive man, in the region of New Guinea, on the basis of characteristics such as sweetness and thickness, for the purpose of chewing. As the commercial cane industry developed, the need for cane with characteristics such as increased resistance, higher sugar content, and erect habit, led to further hybridization with *S. spontaneum*, because this species was resistant to attack by mosaic virus. Two other forms, *S. sinense* and *S. bakeri*, which are probably natural hybrids, have also been used for breeding purposes. Early European-controlled cultivation in the West Indies was based on the Creole cane, a *sinense* derivative. Although questions of origin and nomenclature may appear to be academic, cultivated cane (like so many of the world's major crops) is based, in some areas, on very narrow germplasm resources. An understanding of these, coupled with an opportunity to introduce new genetic material from diverse natural populations by conventional crossing, remains the major method of genetic recombination, certainly in the near term at least, and is thus of paramount importance. Within the various species of *Saccharum*, wide variations of both sugar and fibre content occur. For instance Bull and Glasziou (1963) obtained a range of sucrose content, based on fresh cane weight, from 4% in *S. spontaneum*, through 8% in *robustum*, 14% in *sinense* and 17% in *officinarum*. Fibre content showed an opposite trend, dropping from around 35% in *spontaneum* to less than 10% in *officinarum*. This wide variation offers the opportunity to breed cane for either high sugar content or high fibre, as discussed below (page 332).

Cane production

Cane is a perennial crop taking 8–20 months to mature, depending on the region, after being planted as stem cuttings or sett pieces (Barnes, 1974). The first 'plant crop' is taken after about a year; thereafter, regrowth is followed by 'ratooning' until a reduction in yield indicates the need for replanting. Exactly how many crops are taken, the length of time between crops, and harvesting techniques, vary widely throughout the cane-growing regions. The highest sugar yields are obtained with a long warm growing season followed by a cooler and drier ripening period, free from frosts. Ripening (i.e. accumulation of sugar in the lower portion of the stem) may also be encouraged by deprivation of water, by low nitrogen or, under some circumstances, by application of plant growth regulating chemicals ('cane ripeners'). Ripening can thus be regarded as a 'stress' response because it is favoured by conditions which restrict vegetative growth. For this reason, if cane were bred and grown for total biomass, rather than for sucrose content and juice purity as at present, higher yields of total dry matter might be expected. However, to achieve high yields, considerable application of fertilizer is needed. A crop of 70 t/ha may require 100 kg N, 60 kg P_2O_5 and 300 kg K_2O. Part of this may be re-released into the field by burning dead leaves and trash on the crop before harvest, as well as returning boiler ash and/or filter mud to the field from factory operations. Cane will grow on a wide variety of soil types as long as adequate water is available (about 150 cm/year); the amount of water needed per year is about 1 t/kg sugar produced.

Production systems vary from vertically integrated plantations with agriculture, transport and processing controlled by a single management system—as often found in the developed countries and their previous colonies—to small groups of farmers selling to a central processing station. Traditionally, cane was harvested by hand and cane production was labour intensive. Over the last 10–15 years there has been a shift towards mechanical harvesting, but the cost of machinery is high. In 1980 it was estimated that about 15% of the 8×10^6 ha grown world-wide were being harvested mechanically and that to reach a figure of 25% of cane mechanically harvested by 1985 would take an investment of over US $\$1{\cdot}5 \times 10^9$.

Over the last 10 years, considerable attention has been paid to questions of the cost of cane production, yields and agricultural energy ratios. The reason for this is twofold: first, rising costs and other problems have, as detailed above, caused a decline in many of the traditional cane-producing regions and new methods of production will be necessary if the industries are to recover; second, such considerations are of particular relevance if sugar cane is to be used as a source of substitute fuel.

Because of the wide range of living conditions in the various countries which produce sugar cane, production costs may vary from less than US $10 t to over $60 t (Nathan, 1978; Anonymous, 1980a, 1980c; Bohall *et al.*, 1981).

the difference reflecting both labour costs (or farmer incomes) and the extent of inputs into the agriculture, in terms of fertilizer, crop protection and irrigation. The total energy input per hectare varies from the subsistence level to over 1500 MJ/t of cane produced. In the more developed regions the average input is about 40 GJ/ha for irrigated cane with the major inputs being fertilizer and fuel (Austin *et al.*, 1978). Of particular interest is the fact that studies both of highly mechanized systems in South Africa (Donovan, 1978) and of low-input systems in North Brazil (Khan and Fox, 1982) have indicated that high inputs do not necessarily give high yields in return. For instance, in the South African study a low-energy input group (at about 230 MJ/t yielded an average of 69 t/ha/year. In contrast, the high-energy group (at 1470 MJ/t) gave only 60 t/ha. Conditions on farms vary and in some cases added inputs represent an attempt to increase acreage to land which is not very suitable for cane growing. However, at the same time it would appear that, before the increase in fuel and fertilizer costs in 1973, insufficient attention may have been paid to such considerations, or to the alternative possibilities of breeding cane more suited to a specific use, soil type or climatic region.

Cane processing

The production of white refined cane sugar evolved as a two-stage process with raw sugar manufacture in the country of origin, followed by refining by European, North American, Japanese or other importers. Both stages of processing are similar, in that impurities are removed from the juice or melted raw sugar by precipitation following addition of various chemicals such as lime and carbon dioxide (carbonatation), phosphoric acid (phosphatation) or sulphite (sulphitation). (Baikow, 1982). Sucrose is then recovered by crystallization from a thick syrup, derived by concentration of the clarified and decolorized liquor under vacuum. Once the crystal sugar has been removed from the mother liquor by centrifugation, the residual syrup may be recycled until it is not economic to remove further sugar. This liquid residue—which contains the concentrated impurities, considerable ash derived from the original crop plus process chemicals, and varying amounts of residual sucrose as well as invert sugar (glucose and fructose)—becomes molasses when concentrated to about 80% solids. As far as the cane-sugar industry is concerned, molasses may be of three types: (1) raw factory (or blackstrap) molasses; (2) refinery molasses; (3) high test molasses. The third category represents concentrated, partly inverted cane juice from which sucrose has not been removed. This is of interest where cane is considered as an energy crop, because juice may be stored in this concentrated form for use outside the normal cane-harvesting season, or transported to other processing localities.

The raw sugar process is outlined in *Figure 1*, which also indicates an approximate mass balance for conventional sugar cane treatment. However, much of the process used for sugar production is of little relevance to the use of cane as an energy crop, because pure sugar does not represent a suitable fuel. Although it has been suggested that sugar surpluses might be converted to fuel alcohol, such direct use does not make sense from the energy viewpoint because the

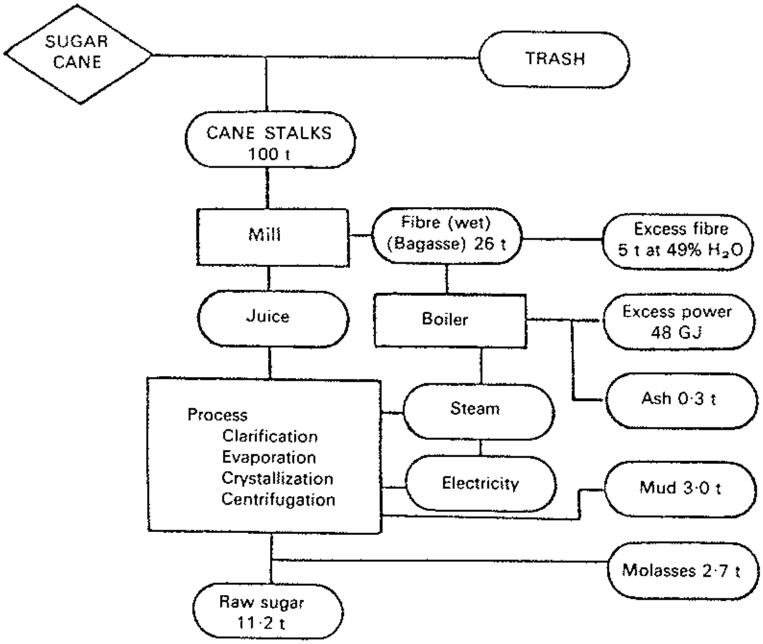

Figure 1. Processing of sugar cane.

energy input of the major process is associated with the removal of water (either in alcohol distillation or in sugar crystallization). There is thus no point in removing water to produce solid sugar and then adding it back to produce the liquid fermenter feed stream, other than as a means of reducing transport or storage costs. The use of high test molasses for this purpose has the advantage that the syrup will also contain invert sugar derived from the crop, and hence the total fermentable carbohydrates are recovered, rather than just sucrose.

Where possible, if the objective is to produce fuel alcohol from sugar-cane, it is better to ferment the juice directly. Thus, from the energy viewpoint, interest lies in the 'front end' of the process—juice extraction. Most cane is extracted by cutting and crushing the cane by passing it through large rollers (mills), which squeeze out the juice, residual sugar being removed by addition of water at the final stage. In addition, some cane is processed using counter-current diffusion techniques. Publicity has also been given to the Tilby separator, which separates the pith from the rind and wax; this was developed in order to obtain pith for cattle food (Pigden, 1974; James, 1975). It has been claimed that this technique has advantages in the processing of cane for energy purposes (Lipinsky, 1981). However, problems exist in extraction of the pulp, maintenance of the high-speed cutters, and the need to feed the machine with uniform straight billets of cane. These disadvantages probably outweigh any advantages which might accrue from an initial separation of rind and pith, as far as the use of cane for energy purposes is concerned; nevertheless, where the objective is to produce board or paper from the rind, the separator may be of value.

The mills and other equipment may be driven by steam or by electricity derived from bagasse-fed boilers and in-house electricity generation. The choice of power used to drive the mills may depend on the overall steam and energy balance of the factory. Where sugar is produced, process steam is condensed in the vacuum pans used in crystallization. However, where alcohol is the only product, surplus low-pressure steam may be available. In the same way, if all the bagasse available is burnt at high efficiency, then surplus electricity may be available for sale to outside consumers.

The objective in cane processing for sugar production is to obtain a sugar solution of high purity, without diluting the juice by addition of too much water (causing hydrolysis of sucrose), and without extracting unripe cane, which would contribute more impurities and colour precursors. In processing cane for energy, the objectives may be quite different: juice quality and purity can be sacrificed in order to obtain a higher yield of fermentable material. Nevertheless, the bagasse should have a low moisture content because the effective calorific value decreases as water content increases (Atchison, 1978; Paturau, 1982). However, because mechanical methods are the most energy-efficient for initial dewatering of cane, even if it is grown as a fibre crop it is probable that the initial stages of processing will remain the same (Alexander, 1980), and the juice produced will be used for fermentation or production of high test molasses. However, the efficiency with which juice can be extracted from high-fibre cane decreases with fibre content (*Figure 2*). Hence, an optimum balance exists between the total energy which can be recovered and the process sequence used.

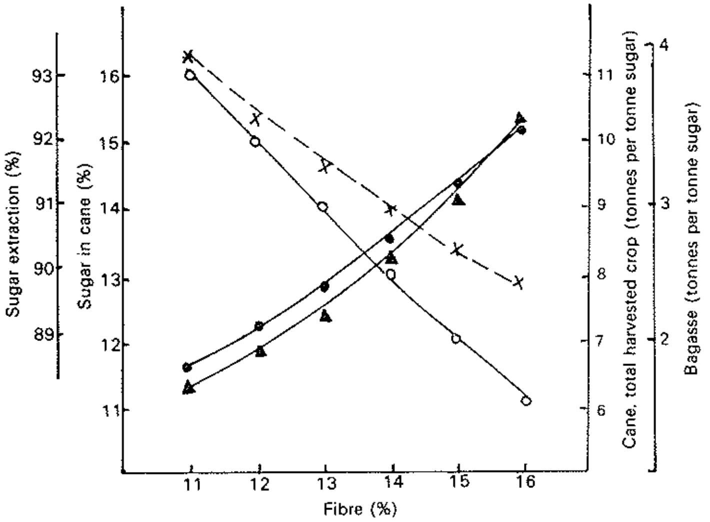

Figure 2. Effect of fibre content on the efficiency of sugar extraction in a mill. × - - - ×, sugar extraction (%); ○——○, sugar in cane (%); ●——●, cane, total harvested crop (tonnes per tonne sugar); ▲——▲, bagasse (tonnes per tonne sugar).

High-energy products

The only major raw materials available from cane are fermentable carbohydrate (as juice or molasses) and bagasse. The amount of fibre which can be harvested may be increased by inclusion of cane tops and leaves. The energy content available reflects the yield of millable cane, plus that of cane tops, so that a crop of 100 green tonnes could give between 650 and 750 GJ/ha of which about 40% would be in leaves and trash, 25% in each of fermentable solids and bagasse fractions, and the rest in molasses and waste streams. However, recoverable yields of power, electricity, alcohol or other up-graded fuels will be much lower, because of the relative efficiencies of conversion processes.

At the conventional assumed moisture content of 49%, bagasse as it comes from the mills has a gross calorific value of about 10 GJ/t (Atchison, 1978), and at the boiler efficiency of 60–70% of typical factory installations, will produce 2–2·5 kg of steam at 300°C per kg of bagasse. In a sugar factory, about 450 kg steam may be used per tonne of cane processed so that the ratio of fibre used to cane processed is about 0·1 to 1. Thus, with cane containing 13% fibre there would be a surplus of about 20% of bagasse: in an alcohol factory where the demand for steam is less, up to 30% bagasse may be surplus. This surplus may be used to generate electricity for the grid at a rate of about 0·25 kW/kg bagasse: alternatively, it may be used as fuel in order to process alternative sources of fermentable carbohydrate, such as cassava or maize, during the period when cane is not available, although storage may cause problems (Cusi, 1980) which may be overcome by pelletization (Bouvet and Suzor 1980).

Traditionally, the cane-sugar industry in Hawaii has supplied electricity to the local grid. In 1979 Murata reported that the plantations, with a total capacity of approximately 180 MW of electricity-generating capacity, generated a total of 669×10^6 kW, of which about 187×10^6 kW were sold to local utilities under various contractual arrangements. This electricity was produced by burning most of the bagasse and some additional leafy trash from a total of 14×10^6 t of cane grown on about 45 000 ha. This produced 9×10^6 t of prepared cane, yielding $2{\cdot}8 \times 10^6$ t of wet bagasse, 1×10^6 t of sugar and 310 000 t of molasses. Since then the efficiency of electricity generation has been improved by installation of bagasse driers powered by recycling flue gas as a means of increasing the calorific value of the bagasse as burnt, resulting in the provision of about 7% of the Hawaiian States total electricity use.

Although world-wide many factories sell some electricity, opportunities to do this vary, depending both on local demand and on the amount, seasonality and quality of bagasse produced on the other. Hawaii is in a unique position, with very high yields of cane because of the longer growing season and high inputs, as well as having an extended harvest with milling for 40 weeks of the year. Elsewhere, climatic restrictions on crop production and length of the milling season can result in a less favourable energy balance. This balance also depends on the range of products being manufactured in any particular factory, i.e. the proportions of raw sugar, factory white sugar, alcohol and/or molasses.

As far as the production of alcohol is concerned, the following alternatives exist using cane as the original raw material: (1) fermentation of molasses with an external fuel supply (e.g. European production from imported molasses); (2) fermentation of molasses in a distillery added on to an existing cane sugar factory, using bagasse as fuel; (3) an autonomous distillery fermenting cane juice using bagasse as fuel; (4) an integrated alcohol system in which cane is used to provide fermentable juice for part of the year and surplus bagasse is used as fuel with starch feedstocks obtained from cassava or maize for the non-crop season; (5) possible future systems in which the amount of ethanol produced would be increased by also using part of the bagasse, following hydrolysis, as fermentation substrate; (6) factory complexes in which the juice is fermented to ethanol, but the bagasse is converted to methanol via synthesis gas following thermal gasification.

At present, commercial fuel alcohol production is restricted to the first three categories, with the major activity in Brazil.

The Brazilian alcohol programme

The Brazilian national alcohol programme (Programa Naccional do Alcool) is at present by far the largest attempt to use biomass-derived liquid fuel as a substitute for petroleum products. The objective is to reduce foreign exchange spending; with current inflation running at about 160% and the largest national debt of any Third World country, the incentive to do this is great. Before initiation of the present programme in 1975, alcohol was produced in significant amounts as a by-product of the cane sugar industry. However, the programme had the specific objective of using alcohol to displace Brazil's petroleum demand, both by use of alcohol in blends and as a fuel in its own right in cars developed to run on hydrated (95%) alcohol. The target for 1985 is the production of about 11×10^9 ℓ, mostly derived from sugar cane (Anonymous, 1979c; Pimental, 1980; Anonymous, 1981d; Rothman, Greenshields and Calle, 1983).

The growth of alcohol production in Brazil during the last decade is shown in *Figure 3*, which shows both annual production (inset *a*) and monthly production for both the central and north-east region (inset *b*). The ability to produce the very large amounts of cane reflects the large areas of land available, but at the cost of destruction of wide areas of forest (inset *c*). The concentration of the industry in the central region can be seen from this Figure, as well as the variation in the length and season of the harvest campaign depending on the latitude, with a maximum of around 200 days of harvest of cane for alcohol production which contrasts with the shorter harvest of cane for sugar manufacture of around 160 days per annum.

Brazilian sugar cane production is currently approximately 153×10^6 t, with a low average yield of about 55 green tonnes per hectare grown over an area of some $2{\cdot}8 \times 10^6$ ha. This is equal to about one-fifth of all the land used to grow cane throughout the world. To achieve self-sufficiency in auto-fuel production will require almost five times as much land to be used; to reach even the 1985 objective will require 10% of all the Brazilian crop land to be devoted to cane.

Initial increase in alcohol production was based on the use of molasses in

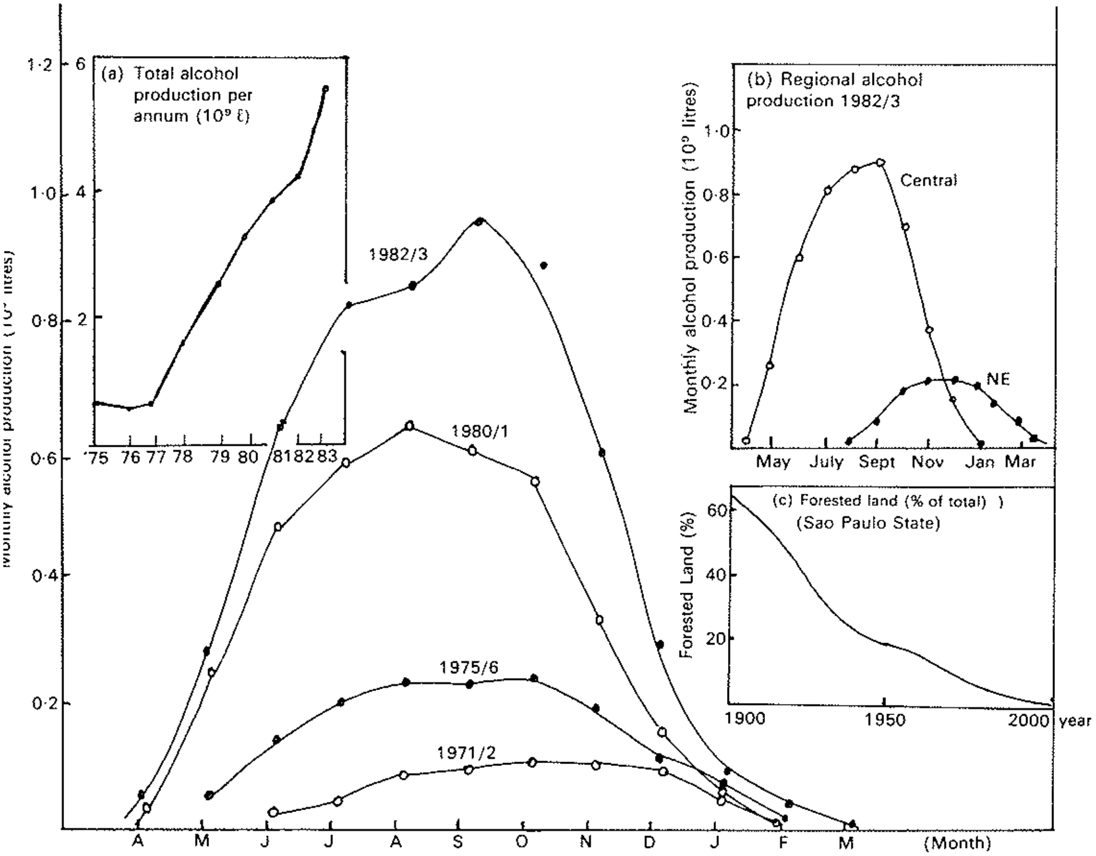

Figure 3. Growth of the production of fuel alcohol in Brazil: Source of data F. O. Licht, GmbH, International Molasses report, volume 12 (1983).

annexed distilleries. However, the rapid growth phase has been achieved by construction of several hundred autonomous distilleries with production rates of 30–250 m^3 per day. In general, the technology used has been conventional, based on traditional cane mills, followed by batch fermentation and distillation. Most of the equipment, for both agricultural production and processing, has been manufactured in Brazil, resulting in an expanding manufacturing industry which includes the production of specifically designed cars to run on alcohol fuel.

The programme has been widely criticized in terms of economics, environmental effects, diversion of food to fuel products, energy balances and lack of benefit to the poor rural work force. These arguments will not be repeated in detail here. Considerations of energy balance—a central theme of this chapter—

are dealt with on pages 327–329. Environmental issues relate in part to the effects of destruction of forest (*see Figure 3*, inset *c*) and in part to the effects of discharge of large amounts of stillage (*see* page 338). The economics of the programme are complex. If the objective is to produce foreign exchange savings, but at the same time an overseas market exists for sugar, then the programme is running at a net loss of revenue to Brazil. According to Kovarik (1982b) it would have been 30% cheaper to export sugar and buy oil on the proceeds in 1979, when sugar stood at a low of US $0·22 per kilo and oil was US $25 per barrel (160 ℓ). By 1981, with sugar prices up to US $0·33 per kilo and oil at US $40 a barrel, the sugar from one tonne of cane would be worth about $30 whereas the alcohol produced would directly replace only $17 worth of oil. Two other factors complicate the issue. First, substitution of ethanol for petroleum results in an imbalance within the oil refineries and a surplus of light fractions which have to be re-exported. The situation is made worse by the fact that alcohol is not suitable for use in diesel engines—diesel being the main fuel for agricultural machinery, road haulage and public transport. Second, the growth of distribution facilities and public demand has not matched the growth of production, resulting in stockpiles and the need also to export alcohol.

At the same time, if all the Brazilian cane were used to produce sugar, this would be equivalent to about 15% of the world total production of sugar (cane plus beet sugar), and equal to the amount traded on the free non-preferential markets. Hence, the international market would collapse if even a fraction of the Brazilian cane at present being used to produce alcohol were to be released on the world market. At present, therefore, an increasing proportion of the alcohol produced is being diverted to the chemical industry.

The Brazilian programme is characterized by having a single product (ethanol) derived from cane grown at relatively low yield and processed using traditional technology. The important questions thus relate to the extent to which innovations based on biotechnological advances might have an effect on the overall process and improve economics, energy balances and environmental effects. At present the greatest cost relates to cane production; at the same time, since the factory process can be energy self-sufficient, the major energy inputs which affect the net energy production also lie in the agriculture sector. Thus, what is needed is higher agricultural yields at lower energy inputs and costs.

Energy yields

Energy yields within the cane system may be considered from a number of viewpoints, which include the following: (1) the yield of total biomass, expressed in terms of the available solar radiation falling on the crop; (2) the yield of harvestable cane; (3) the yield of alcohol obtained by fermentation; (4) the yield of all fuels produced, i.e. for an alcohol distillery, not only alcohol but also surplus electricity and any excess bagasse. The energy content of the end-products (fuels) may also be expressed in terms of the energy content of the raw materials used, to give the efficiency (in terms of the ratio between fossil fuels used and the high-grade biological fuels produced, to give the net energy balance) or—in terms of all outputs divided by all inputs—to give the overall energy efficiency in thermodynamic terms. The problems with all such estimates lie both in definitions and in methods used to estimate the size of a given component within the system. A further problem arises from the fact that quite often the actual balance in question is not defined and it is often difficult, therefore, to compare data published in various sources. For this reason the various possible approaches to defining yields are discussed briefly, and some typical values for the complete process—from trapping of solar energy by photosynthesis to final alcohol production—are then considered.

THE EFFICIENCY OF TRAPPING OF SOLAR ENERGY

The total energy trapped into standing biomass before harvest may be defined in terms of the total energy incident on a unit area of land (hectare) during one growth season (which may be greater or less than one year) multiplied by the efficiency of conversion of solar energy to biomass. Because both land area and average annual solar irradiance are constants, the amount of energy trapped reflects the product of photosynthetic efficiency and effects of such factors as nutrient deficiency, water stress, pests and diseases which may prevent this theoretical efficiency being attained.

BIOMASS YIELD

Not all the biomass produced in the field will be harvested. The fraction used, or harvest index, may be expressed in terms of biomass harvested divided by total biomass. However, only some of this biomass will be recovered in the form of fuel. Hence, the gross fuel production can be derived from the weight of biomass harvested, multiplied by its average calorific value and by a factor which reflects the efficiency with which the harvested material is converted to the fuel under discussion.

NET FUEL GAIN

The net gain in fuel will be equal to the energy content of the final fuel product, less the energy put into the system for agriculture, processing and transport. These parameters may be further divided to distinguish, for instance, agricultural fuels, fertilizer, irrigation, transport of raw material to the factory, process fuels and so on. In general the inputs are considered in terms of fossil fuels only, because it is assumed that biomass feedstocks are free whether used as fuel or as raw material for the actual production of fuel exported from the factory. Such energy balances can be changed dramatically if an energy value is given to by-products or co-products formed at the same time. Such materials may include surplus bagasse, methane generated by anaerobic digestion of stillage, animal feed produced from yeast generated during fermentation or surplus electricity. Again, such by-products may be ascribed an energy value which reflects their actual calorific value, or they may be defined in terms of an energy equivalence. For example, the value given to animal feed need not be expressed in terms of the calorific value as cattle feed, but may be expressed in terms of the energy which would be needed to produce an equal protein source by growth of soybeans. Problems in interpretation of such data are compounded where the results are expressed in terms of ratios, often referred to as net energy ratios (NER).

NET ENERGY RATIOS

The simplest way to define the net energy ratio is in terms of the calorific value of the fuel produced divided by the energy content of all external (fossil) fuel inputs to the system. This may be defined as a simple ratio, or in terms of the number of joules put into the system in order to produce one joule of product. The problem here is that in the first case a number greater than one reflects an efficient system whereas in the second type of expression net fuel production will result in a value of less than one: i.e. an NER of 3 means that 0·33 joules of fossil energy are expended per joule of product energy achieved. Again, the end-product may be expressed in 'equivalence' terms rather than true calorific value. For example, the efficiency with which alcohol will serve as a fuel in a spark ignition engine is greater for a blend (where it confers an octane-boosting benefit) than for use in a 'pure ethanol' engine. However, even when used neat, the efficiency of the performance on a volumetric mileage basis is still higher than would be expected from a direct comparison of calorific value (remembering that the pure alcohol car is in fact running on 95% ethanol: water mix). Again, where the objective is petrochemical substitution and coal, hydroelectric or off-peak nuclear energy are available, these may not be regarded as significant inputs.

Considerable attention has been paid (particularly in the USA) to NER for alcohol production from grain, which may indicate an overall energy loss. However, NER should be combined with consideration of the overall process efficiency, as well as of the efficiency of use of the biomass raw materials.

EFFICIENCY OF RAW MATERIAL USE

The efficiency with which raw material is used may be defined in terms of the energy content in the final product as a fraction of the energy content of the biomass as brought to the factory, whereas the efficiency of the overall process can be derived by adding the energy content of all inputs to the denominator. The importance of looking at systems in this way can be demonstrated by considering the case of alcohol production (methanol or ethanol) either by fermentation of sugar, starch or cellulose, or by catalytic synthesis following thermal degradation to synthesis gas, a mixture of carbon monoxide and hydrogen. If wood is used both as fuel and as raw material for ethanol production, a positive NER is obtained; however only 18% of the biomass is recovered in fuel, and the overall efficiency on all energy inputs is 0·16. In contrast if wheat is used, combined with coal, 60% of the biomass energy ends up as fuel, with an overall efficiency of 0·34. The NER of the thermal chemical conversion system is lower, but the efficiency of the overall system is comparable to that of fermentation using sugar or starch as raw material. Hence, the 'best' route will reflect the relative availability and economic cost (rather than market price) both of the biomass raw material and of alternative energy inputs available at any particular site.

Overall cane energy balance

Estimates of the solar energy available to plants (photosynthetically active radiation, PAR, of wavelength about 400–700 nm), coupled with knowledge of the basic photochemical and biochemical processes and their thermodynamic efficiency, enable the theoretical maximum productivity at any given level of incident radiation to be calculated on a unit leaf area basis. Assuming that a leaf is fully formed and metabolically active, the maximum efficiency with which it can form carbohydrate can be estimated by giving numerical values to a series of constraints (Loomis and Williams, 1963; Bassham, 1977; Bolton and Hall, 1979; Coombs, 1983). The series of constraints usually considered are as follows: (1) the proportion of light which comprises PAR (given the value 0·43–0·5); (2) the proportion of PAR which is involved in useful work (0·7–0·9); (3) loss due to degradation of the absorbed quanta to excitation at 700 nm (given the value 0·86); (4) loss due to conversion of excitation energy to chemical energy of D-glucose. The value of this last factor varies depending on whether a value of 8 quanta per molecule of carbon dioxide reduced is assumed, or whether the more probable value of 10 is taken, giving factors of 0·26–0·33. Multiplication of these factors together gives an efficiency of production of carbohydrate of about 10%, which is further reduced by the need to use metabolic energy to convert carbohydrate to more reduced components such as protein, lipids or nucleic acids, so that the optimum efficiency of conversion of solar energy into plant biomass for a crop such as sugar cane is approximately 6%. However, this efficiency must be superimposed on the natural growth and development cycle of the crop. In this respect, the most important factor is the rate at which the developing crop forms a closed canopy capable of intercepting the available radiation at high efficiency and—once the crop has developed to its mature form—the availability of storage tissue. In general, the form of growth will follow the classic sigmoid growth curve, with the initial rate of the exponential phase depending on the size of the original seed material, and the final size reached reflecting the genotypic characteristics of the particular plant. An optimum 'ideotype' for biomass production has been suggested by Coombs, Hall and Chartier (1983) as one in which the plant has prolonged vegetative growth, indeterminate growth habit, and arises from a large initial root stock. The canopy should be erect in order to obtain a high leaf area index resulting in efficient solar energy trapping. In addition, an effective translocation system capable of removing assimilated carbon from the leaves at a high rate is necessary in order to reduce feedback inhibition of photosynthesis resulting from accumulation of sugars in the chloroplast. Sugar cane can be shown on this basis to be capable of very high rates of dry matter accumulation compared with other land plants, if grown under optimum conditions of nutrients, water and pest control (Alexander, 1973).

The overall flow of energy in a sugar-cane-based system producing ethanol is shown in *Table 1*. In this, average figures from various sources (da Silva *et al.*, 1978; Hopkinson and Day, 1980; Essien and Pyle, 1983; Johnson, 1983) have been rounded off. This Table can be considered in terms of the overall balance for trapping of solar energy into biomass, i.e. photosynthetic efficiency; agricultural energy inputs; process energy inputs.

Table 1. Energy balance for one hectare of sugar-cane receiving 200 W/m^2 per year.

(a) *Solar energy harvested by the crop*	(GJ/ha)	%
Total irradiation	30000	100·0
Available as photosynthetically active radiation	15000	50·0
Intercepted by canopy	13000	43·0
Converted through photosynthesis to carbohydrate	3000	10·0
Trapped as plant material, allowing for respiratory losses	1500	5·0
After losses due to water stress, nutrient deficiency and pests.	750	2·5
Material harvested	500	1·7
Fermentable carbohydrate	200	0·7
Ethanol	170	0·6

(b) *Agricultural energy inputs* (GJ/ha):

	Yield (t/ha/year)		
Input	Subsistence 60	Low 80	High 100
Machinery	1·4	2·2	10·0
Nitrogen	—	5·4	11·0
Fuel and labour	6·2	8·0	10·0
Other chemicals	0·5	1·5	2·0
Seed cane	1·1	2·5	2·5
Irrigation	—	—	15·0
Total	9·2	19·6	50·5

(c) *Energy equivalence of factory machinery:* 5·6 to 10 GJ/ha per year

Table 1a indicates the conversion of solar radiation at 200 Wm^2 per hectare of a hypothetical cane crop, which would receive approximately 30 000 GJ per annum of solar radiation. At the suggested optimum efficiency of 6%, this would give a total biomass yield of 1800 GJ or 100 dry tonnes (300 green tonnes). Theoretical maximum productivity could, in fact, be higher than this because for many regions where cane grows, light intensities may be higher than 200 Wm^2, with PAR of full sunlight approximately 400 Wm^2 (1800 μE/m^2/s). However, as is well known, actual yields are much lower than these optimum values because of the effects of water stress, lack of nutrients (nitrogen in particular), temperature, pests and disease as well as ineffective management of the production systems, in many cases.

The cane crop produces about 80 t of millable cane per hectare at the factory gate. This would represent about 60% of the standing above-ground biomass, which would thus be equivalent to approximately 500 GJ, or a 1·7% efficiency of solar energy capture (*Table 1a*); this can be compared with record high values for the efficiency of cane photosynthesis of about 4%. The energy content of alcohol produced from this cane (at 90% extraction efficiency and 90% fermentation efficiency) would be about 170 GJ. Assuming an agricultural energy input of 20 GJ/ha (*Table 1b*) and a factory machinery energy equivalence (based on a 20-year life time) of 6 GJ, the NER (ethanol; non-biomass energy inputs) would be 6·5, with a surplus of bagasse of 80 GJ/ha, having consumed 100 GJ of bagasse as process energy. It is important to note that most process energy is consumed in milling the cane. However, the high-pressure steam used to generate electricity may subsequently be used at lower pressure for distillation. If this energy comes from bagasse, then fuel imports are restricted to start-up and the energy equivalence of the capital plant manufacture, discounted over its life time. As discussed on page 318, it has been estimated that energy inputs into cane production, for example for irrigation, fertilizer, fuel, may be as high as 150 GJ/ha. The extent of such inputs is a critical factor as far as alcohol production is concerned. Under these high inputs there will not be a significant net energy gain even if the process is fuelled using bagasse (*Figure 4*). Hence, the key factor in such systems is a high biomass yield at low agricultural energy input. This can be achieved if the best areas (in terms of such factors as soil, and water availability) are used for energy crops. However, it is often suggested that such crops are produced on marginal lands. Under such conditions marginal yields are to be expected, unless inputs into the system are increased, or unless the cane is bred specifically to survive adverse conditions.

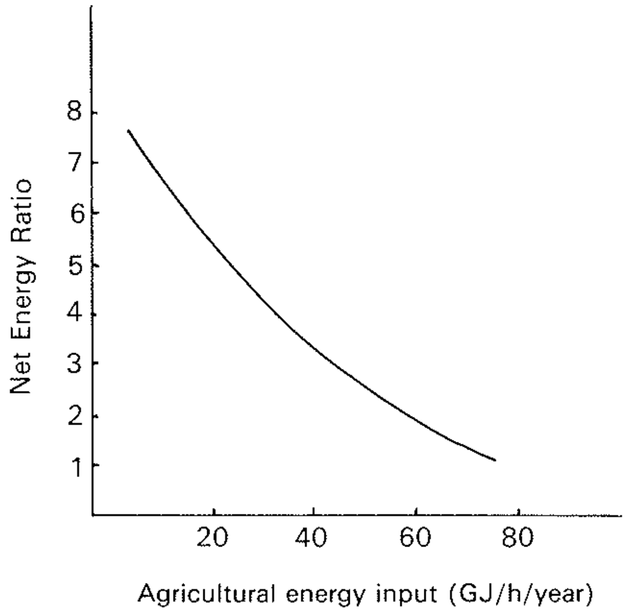

Figure 4. Decrease in Net Energy Ratio for alcohol production in a bagasse-fired distillery as a function of the agricultural energy input.

Photosynthesis and physiology of sugar cane

Some of the figures quoted above will have indicated the high productivity of sugar cane compared with that of many temperate crops. When one considers parameters such as maximum recorded yields of dry matter per year, net rate of carbon assimilation, and rates of short-term carbon dioxide fixation for all the major cultivated crops, sugar-cane will be among the leaders. Other species showing high productivities include the related grasses sorghum and maize. It is now known that these high productivities are associated with a number of specific anatomical, ultrastructural, physiological and biochemical characteristics which together make up what has been referred to as the C4 syndrome (Laetsch, 1974). The discovery of the mechanism of C4 photosynthesis was a direct result of work carried out within the cane sugar industry, in Hawaii (Kortschak, Hartt and Burr, 1965), Australia (Hatch and Slack, 1970) and the UK (Coombs, 1976). In Hawaii and Australia this interest in photosynthesis followed detailed studies on the mechanism of sucrose synthesis and translocation (*see* Alexander, 1973), which established the highly efficient mechanism of sucrose accumulation during ripening. In particular, C4 plants show low compensation points (the compensation point being the equilibrium concentration of carbon dioxide reached in a closed atmosphere in the light, that is when carbon dioxide output from respiration equals carbon dioxide assimilation by photosynthesis), and do not lose carbon dioxide in the light, through photorespiration.

It is now clear that most temperate plants (now known as C3 species) do lose a significant proportion of the carbon fixed in the light, because of the process of photorespiration. This may result in an overall loss of yield, in crops such as wheat, of 10–30%, depending on factors such as light intensity and temperature. The reason for this is that the enzyme (ribulosebisphosphate carboxylase, EC 4.1.1.39) which catalyses the initial fixation of carbon dioxide in the reductive photosynthetic cycle is inhibited by oxygen in a competitive manner (Lorimer, 1981). As a result of this oxygen-dependent reaction, a two-carbon compound, glycollate, is produced. This contrasts with the normal carboxylation reaction in which two molecules of phosphoglyceric acid (a three-carbon compound, hence the term C3) are formed (Gibbs and Latzko, 1979): these are then reduced to three-carbon sugars, two molecules of which form fructose 1, 6-bisphosphate. In order to recover some of the carbon lost in photorespiration, glycollate is recycled through the C2 pathway (Tolbert, 1971) during which the three-carbon phosphoglycerate is re-formed with the loss of a molecule of carbon dioxide. As a result, in normal atmospheric concentrations of oxygen, photosynthesis in C3 plants is inhibited, in part because of loss of carbon from the carbon reduction cycle, and in part because of loss of carbon dioxide from the plant.

Sugar-cane and other C4 plants do not show photorespiration. Hence, it may be concluded that the high productivity of sugar-cane is associated with a distinct mechanism of photosynthesis which has evolved in order to overcome deficiencies associated with photorespiration. This is of particular importance in the tropics because the ratio of photorespiration to photosynthesis increases with increase in temperature and light intensity; the effects are therefore more deleterious in tropical latitudes and any species which develop a mechanism to overcome these effects have a competitive advantage.

The mechanism of C4 photosynthesis is of particular interest as far as improving productivity of C3 crops is concerned. The differences between C3 and C4 plants are sufficient to confirm the benefits of reduced photorespiration. For this reason extensive searches have been made for C3 plants with reduced photorespiration, lower compensation point, or a ribulosebisphosphate carboxylase which has a greater resistance to inhibition by oxygen (Somerville and Ogren, 1982). These studies include attempts to select mutants defective in enzymes of the C2 pathway, but so far without success. In the same way, attempts to obtain hybrids with higher photosynthetic capacity by crossing C3 and C4 species of *Atriplex* have also been unsuccessful (Bjorkmann, Gauhl and Nobbs, 1969). It seems, therefore, that it will not be easy to incorporate the characteristics of the C4 plants into other species: the reason for this can be seen if the actual mechanism of C4 photosynthesis is considered in more detail.

In the C4 plants, photosynthetic tissue is arranged in two layers around the vascular bundles, comprising an inner bundle sheath and an outer mesophyll layer. Chloroplasts in the outer layer appear to be normal: however, in the most advanced C4 plants such as sugar cane the bundle sheath chloroplasts lack some of the characteristic structures found in other higher plants, and also lack the ability to produce oxygen. Ribulosebisphosphate carboxylase is confined to this inner cell layer. The outer layer of photosynthetic cells lack this crucial enzyme of the carbon reduction cycle, but contain high levels of a second carboxylase (phosphoenolpyruvate carboxylase, EC 4.1.1.31) which catalyses the formation of the four-carbon acid, oxaloacetic acid (hence the term C4 plant). Oxaloacetic acid is reduced to malate in the mesophyll cells, and transported to the bundle sheath where decarboxylation results in release of carbon dioxide. This is refixed into phosphoglyceric acid which is then reduced to a sugar (dihydroxyacetone phosphate), using reductant derived from the activity of the mesophyll cells which possess complete non-cyclic photophosphorylation, are capable of splitting water, and do evolve oxygen. By this means the oxygen-sensitive reactions are separated in space from the light-dependent water-splitting reactions which generate oxygen. At the same time the C4 cycle acts as a pump to maintain a higher internal carbon dioxide concentration and thus to decrease the competitive inhibition by oxygen.

Although comparatively little work has been carried out on the molecular genetics of sugar cane, considerable information is now available concerning the structure of genes coding for the synthesis of ribulosebisphosphate carboxylase of maize (Link *et al.*, 1978). This enzyme is of particular interest as it is composed both of large and of small subunits (Ellis, 1976), the former coded within the chloroplast and the latter being coded for by nuclear genes. Genes for both subunits have now been cloned and expressed in yeast. It is therefore possible (although not very probable) that some engineering of the genes controlling the reaction centre may bring about changes in the relative affinity for oxygen and carbon dioxide. However, the complex cytogenetics of sugar-cane offer not only advantages for breeding a wide variety of canes, but also the disadvantage of providing a very complex base on which to attempt to use *in vitro* genetic recombinant techniques.

Sugar-cane breeding

In the past, sugar-cane breeding has been directed towards varieties which produce good yields of juice of high purity, are resistant to major diseases and are of reasonable stem diameter and erect habit in order to facilitate harvesting. Experience has shown that the most profitable cane to grow is not necessarily that with the highest total carbohydrate content, but rather one in which a sweet juice with a very high percentage of sucrose and a low content of all other organic impurities occurs; this will reduce processing costs and the amount of residual molasses. However, if cane is to be bred for the production of fuels, a different approach may be taken: if the primary objective is the manufacture of ethanol, then breeding can be directed towards high production of total fermentable solids; if the objective is the production of combustible material, then cane can be bred for high fibre (Alexander, 1980; Giamalva, Clarke and Bischoff, 1981; Giamalva and Clarke, 1982). In an extensive programme aimed at production of 'energy cane' in Puerto Rico, Alexander has selected for high fibre cane which also gives high total dry-matter yields of approximately 80 ha. This has been achieved in part by breeding and selection, in part by increased inputs and in part by different management techniques (Alexander and Allison, 1981). In spite of the high fibre content, the actual yield per hectare of fermentable carbohydrate produced in the form of high test molasses from these canes is greater than that produced by local cane grown for the production of sugar. The variation in fibre and sugar content of the different *Saccharum* species has been discussed on pages 316–317. This wide variation gives ample scope for the production of cane suited to specific uses. However, cane breeding is complicated by factors related to high chromosome numbers (reflecting polyploidy), daylength responses of the various varieties, and low seed viability resulting from the complex cytogenetic base.

Chromosome numbers in cultivated canes vary between 60 and 200 (Stevenson, 1965). The basic chromosome number (x) of the Andropogoneae is 10. In fact no diploids ($2n = 2x = 20$) of *Saccharum* varieties are known. In addition, there is a large degree of variation in chromosome number, not only between varieties of the same species or hybrid parentage, but also between cells within the same plant, a considerable degree of aneuploidy being common. Of the various named species, *S. officinarum* is more stable at $2n = 80$; ranges for the other recognized species are as follows: *S. robustum*, $2n = 60$ or 80 in general but range from 63 to over 200; *S. spontaneum*, $2n = 40$–128; *S. sinense*, $2n = 82$–124. In addition, wild hybrids of *spontaneum* × *robustum* of $2n = 80$–101 and intergeneric hybrids of *Saccharum* × *Miscanthus* of $2n = 114$–205 are also known. A wide range of interspecific hybrids have also been produced, at the Indian cane breeding station in particular: these include crosses between *Saccharum* and *Erianthus*, *Miscanthus*, *Narenga*, *Imperata*, *Sorghum* and *Zea* (maize).

Sugar canes are wind pollinated and show inbreeding depression. Hence, crossing techniques are those characteristic of outbreeding clonally propagated crops (Simmonds, 1979). The progeny of a desired cross are planted out in large numbers and offspring are selected by visual inspection, survival, pest resistance, chemical analysis, etc., in order to obtain a single plant suitable for cloning through subsequent propagation of sett pieces derived from each mature node. The canes are daylength sensitive and flower in response to light periods greater than 12 hours, assuming that other conditions of nutrients, water supply and temperature are correct. The extent of flowering also depends on the variety, species and growth cycle in relation to season and latitude. Flowering is greater in the middle tropics and decreases towards the equator. In some regions, breeding and, in particular, interspecific crosses, may be facilitated if the parent material is subjected to the required conditions to promote flowering by use of chambers with artificial illumination, temperature control etc.

Crossing of noble canes with wild (*spontaneum*) types results in an unusual restitution of chromosome number; if a female of a noble variety is crossed with a wild-type male cane the viable progeny have the somatic complement of the female parent (80 chromosomes) plus the gamete number of the male. On the other hand, self-fertilization or crosses between noble canes yield progeny with 80 chromosomes. Restitution occurs for only two generations, but not at the third, so there is a limit to the increase in chromosome number, resulting in the higher values of about 200 mentioned previously.

During the breeding of a new variety, the rate of build-up of new clonal propagation material is limited by the number of sett pieces which can be obtained from each mature stem. Hence, the rate of multiplication of a new variety determines the time taken before it can be used commercially on a large scale. Because of the odd cytogenetic behaviour and low seed viability, similar material cannot be produced through a further cross of the same parents, nor will seed produced in the same crossing result in similar progeny. For this reason propagation by tissue culture has obvious attractions. A considerable amount of work has been carried out on *in vitro* culture of both callus and suspension tissues of cane, following the initial work of Nickell (1964). It has been suggested that such cultures are of value in studies of cane physiology and nutrition (Nickell and Maretzki, 1969), but that they are not suitable as a means of cloning, because of the wide variation which occurs in chromosome number of individual cultured cells, resulting in plants of diverse character. On the other hand, this diversity lends the technique both to production and to selection of new varieties (Heinz and Mee, 1969). Nevertheless, as pointed out by Heinz *et al.* (1977), 'the problems arising from the identification of disease and pest resistant clones that are also high-yielding are similar whether selecting from subclones derived from callus or from clones obtained by conventional breeding techniques ... the greatest gains in the use of tissue culture will be realized when directed genetic changes can be coupled with effective cellular selection methods'. Using *in vitro* techniques these authors were successful in screening for resistance to three diseases, namely eyespot disease (caused by *Helminthosporium sacchari*); Fiji disease (caused by a virus); downy mildew (*Sclerospora sacchari*), but not for smut (*Ustilago scitaminea*). Subclones derived from cell cultures produced about 80 t of cane per hectare, in one clone giving 12 t of sugar, which was similar to the parent cane. However, other clones were either less resistant, or gave a lower sugar yield than the parents. Although physiological responses to materials such as disease toxins can be screened at the cell level, other characteristics such as sucrose content, fibre, ash, juice purity, and acceptability for mechanical harvesting or milling, can be determined only after propagation of suitable quantities by conventional use of sett pieces and prolonged growth to maturity in the field.

Nitrogen fixation

The total energy input used in growing sugar-cane varies very considerably between the various countries where it is grown, and between subsistence level farms and modern fully mechanized plantations. Khan and Fox (1982) describe the energy cost of nitrogen fertilizer as 5·4 GJ/ha out of a total agricultural input of 16 GJ. However, this use is low: in some regions over 500 kg/ha (38·5 GJ) of ammonium nitrate may be applied, representing well over 50% of the total agricultural energy input. Such inputs of nitrogen represent an additional financial burden for those regions where fertilizer has to be imported. At the same time, such high inputs results in a significant reduction of the overall net energy balance. The observation of Dobereiner (1959), that populations of the nitrogen-fixing organism *Beijerinckia* were higher in the region of sugar cane roots (rhizosphere) than in the soil between the rows, is of particular significance; furthermore, these organisms were active in nitrogen fixation, as demonstrated by the acetylene reduction technique (Dobereiner, Day and Dart, 1972). Subsequently, various species of *Azotobacter*, *Beijerinckia*, *Derxia*, *Caulobacter Vibrio* and *Clostridia* have been isolated from free-living associative interactions with the roots of various grasses. Both acetylene reduction tests and the assimilation of heavy isotopic nitrogen (^{15}N) have been used in order to determine the significance of such fixation. Ruschel, Henis and Salati (1975) obtained rates of fixation of N_2 in sugar-cane roots of 523 μg N per gram of root per day, equivalent to about 20 g of nitrogen per hectare per day (Ruschel *et al.*, 1978). Sugar-cane, as well as a number of other C4 grasses, can also possess associative nitrogen-fixing organisms (e.g. *Spirillum lipoferum*) located within the root cortex (Dobereiner and Day, 1976) although *S. lipoferum* may be more abundant on the surface of the roots than within the tissue (Burris, Okon and Albrecht, 1978). The latter authors showed that sterile maize seeds could be inoculated with *Spirillum* and cultured in such a way that nitrogen reduction occurred and viable cells could be recovered; however, they reported that no benefit from such infection could be detected in field trials. At present there seems little doubt that an association can be formed between nitrogen-fixing organisms and roots of non-leguminous crops (Evans and Barber, 1977), in as much as organic material produced by the plant and excreted through the root represents a substrate for microbial growth. However, the extent to which any nitrogen assimilated by these bacteria is transferred to the plants appears to be small. It is possible that plants better suited to take advantage of the association could be selected or, alternatively, more effective bacterial strains could be selected from natural populations, or developed using genetic manipulation.

Fermentation

Because fermentation of sugars to ethanol remains the only important commercial process for the conversion of biomass to liquid fuel, it has been given particular attention. Actual or potential improvements may result from changes either in the organisms used or in the design of fermentation systems. The objectives are to improve efficiency (in terms both of substrate and of energy use), volumetric productivity and final net yields. However, fermentation cannot be looked at in isolation, as is sometimes done, because it represents only a small part of the overall capital and energy investment in its own right. Of greater importance are interactions between the techniques used for the fermentation step and their effect on the design, cost and energy balance of the system as a whole; this extends to the nature of primary raw materials, pretreatment to produce the fermenter feed stream, and downstream processing (including product separation, effluent treatment and recovery of by-products with cash or energy value).

Where the main substrate is soluble sugars, the organism used is a yeast (*Saccharomyces*), generally *S. cerevisiae*, which has the ability to use sucrose, glucose, fructose, maltose and maltotriose. This organism has the advantage that selection over hundreds of years in the beverage industry has resulted in known strains with stable behaviour attributable to their polyploid nature and low frequency of sporulation (Stewart and Russell, 1981). This is an advantage from the commercial viewpoint, but a disadvantage as far as genetic modification is concerned. The attraction of yeast as a means of converting biomass to a fuel of higher value lies in the fact that although the theoretical weight yield of alcohol is about 50%, over 90% of the energy in the substrate is retained in the ethanol, resulting in an increase from 16 kJ/g for glucose to 30 kJ/g for ethanol (Coombs, 1981a). In practice the yield is less, because part of the substrate is used for cell growth and part is diverted to by-products. Furthermore, as the final product is in dilute aqueous solution, considerable energy has to be expended to separate and dehydrate it. In batch fermentation systems, productivity is low on a volumetric basis and the actual yield may be only 80–85% of the theoretical yield. On a volumetric basis, productivity depends both on cell concentration and on substrate concentration and thus varies from approximately 1 g ethanol per litre of reactor volume per hour to over 40 g/ℓ/h for systems with cell recycle or as high as 80 g/ℓ/h for some experimental systems (Wilke *et al.*, 1983).

Most commercial processes, in developing countries, use batch fermentation or a cell recycle system in which each fermenter is inoculated with a high cell concentration obtained from washed yeast from the previous run. The objectives of the large number of experimental systems which have been investigated are as follows:

1. To increase efficiency of substrate utilization by removing the need to produce cells for each run, and decreasing the amount of by-products formed;
2. To increase productivity on a volumetric basis, in order to decrease capital cost;

3. To produce continuous systems more amenable to automatic control, in order to decrease labour costs;
4. To increase concentrations of alcohol in the product stream and to work at higher temperature, to improve energy efficiency by reducing the need for fermenter cooling and decreasing the energy needed for product recovery;
5. To extend the range of substrates which can be used, to cellulose in particular;
6. To decrease sensitivity of the organisms used to inhibition by alcohol and other metabolites.

As far as yeasts are concerned, factors which affect alcohol production include oxygen concentration, substrate concentration, cell density, ethanol concentration, temperature and the ratio of metabolism associated with cell growth to that associated with cell maintenance, alcohol production and the amount and nature of by-products. Where the cells are harvested by sedimentation or flotation, the flocculation characteristics are also important. Where practicable, substrate levels at 16–25% solids, giving alcohol concentrations of 8–12%, are desirable. Present yeasts work best at 28–34°C: thus, for alcohol production from cane, because of the problem of obtaining suitable fermenter cooling water in the tropics, a particular advantage would be availability of yeasts capable of the same alcohol tolerance and production rate at higher temperatures.

Thermophilic organisms are also of interest because they possess the following attractive characteristics: (1) higher cell growth and metabolic rates; (2) low cell biomass yields; (3) higher stability of enzymes and cells, in the absence of oxygen; (4) ability to use a wider variety of substrates. In particular, many thermophilic anaerobes have the ability to degrade lignocellulose or its hydrolysis products, to varying extents. These advantages in turn result in increased ethanol production rates, increased product recovery and improved energy balances. The problems associated with the use of organisms such as *Clostridium*, *Thermoanaerobacter*, or *Bacillus stearothermophilus*, relate to lower alcohol tolerance and continued production of by-products such as acetate. However, considerable improvements both in alcohol tolerance and in reduction in by-product formation have been made using mutagens and selection techniques (Wang *et al.*, 1981; Hartley *et al.*, 1983). At the present level of performance it is thought by Hartley and colleagues that the thermophilic process has advantages over the best-yielding yeast-based systems using cell recycle and vacuum fermentation (*see* Wilke *et al.*, 1983).

It is possible that further advances in development of these bacteria could be made using genetic engineering techniques. However, at present this approach has additional associated problems because little is known about the metabolism, metabolic pathways, life cycles or genetics of many of the cellulolytic fungi or bacteria which might be used. For yeasts, however, the life cycle is well established, as are details of the seven chromosomes and location of about 200 genes (Stewart and Russell, 1981) although the polyploid nature of commercial yeasts makes conventional genetic analysis and crossing difficult. On the other hand *in vitro* techniques of protoplast fusion and recombinant DNA technology (Hollenberg *et al.*, 1976; Beggs, 1978; Hinnen, Hicks and Fink, 1978) are being used with yeasts world-wide. In the short term these techniques can be used to determine further details of metabolic control and of the nature of alcohol tolerance, and to study the genetics of yeast in more depth. In the longer term it may be possible to incorporate genes coding for a greater range of hydrolytic enzymes and to obtain strains with ability to hydrolyse starch and cellulose. The use of plasmid vectors in yeasts is established (Beggs *et al.*, 1980). However, it remains to be seen whether introduced hydrolytic enzymes can be expressed in an efficient manner, and whether addition of such abilities would, in fact, confer a genuine commercial advantage. With regard to the use of cane, an advantage could be obtained if such genetic changes led to an efficient system for the hydrolysis of lignocellulose and fermentation of the sugars thus produced, including the pentoses derived from hemicellulose.

USE OF CELLULOSE AS FERMENTATION SUBSTRATE

Because of the extensive availability and low cost of waste wood, paper and agricultural residues, considerable research has been devoted to obtaining liquid fuels, by fermentation, from lignocellulose (Stone and Marshall, 1980). However, in spite of much excellent research, no viable commercial process exists which could be applied to excess bagasse from a cane distillery in order to increase the output of ethanol. What is needed is a process with the following characteristics: (1) high yield of alcohol per tonne of bagasse: (2) low capital cost; (3) low energy input; (4) either no necessity for, or ease of efficient recovery of, expensive chemicals, solvents or enzymes; (5) no production of large amounts of solid wastes, such as are formed during acid neutralization; (6) high NER and high efficiency overall.

Various partial solutions to such problems have been achieved, (Levy *et al.*, 1981; Wilke *et al.*, 1981; Tsao *et al.*, 1982; Ladisch *et al.*, 1983), but at present extensive pre-treatment to reduce particle size of raw materials must be followed by acid or enzyme hydrolysis, resulting in dilute impure feed streams with considerable loss or destruction of sugars if acid systems are used, or high enzyme costs if this route is followed. In spite of substantial increase in the specific activity of enzymes, from the fungus *Trichoderma ressei* in particular, specific activities of cellulases are still several orders of magnitude lower than those of commercial amylases used in the production of alcohol from corn.

Process plant

In the production of fuel alcohol from cane, batch fermentation systems associated with distillation and azeotropic dehydration are universal. In spite of the development of a large number of improved fermentation systems (Wilke *et al.*, 1983) these have not been accepted by the cane-based alcohol industry to any great extent, although pilot and demonstration plant have been run by a number of major engineering and sugar companies such as John Brown Engineering (Alcon Biotechnology), Alpha Laval, Tate and Lyle, Uhde, W. S. Atkins, etc. In the same way, the belief that distillation contributes a major part of the energy demand in a fuel alcohol system has led to extensive research into alternative methods of separation, including the use of selective membranes, adsorption, supercritical liquids and reverse osmosis. However, at the same time developments in the efficiency of steam distillation by techniques such as multiple effect evaporators and vapour recompression have improved steam consumption so that demand may be less than 1–2 kg steam per litre of alcohol (Keim, 1983). The variation reflects the way in which stillage is handled. With present plant, the use of energy in crushing sugar-cane in order to produce the juice is about twice that of distillation, although the development of alternatives for the former procedure attracts much less attention than that for the latter. One approach which has been suggested is the direct fermentation of fresh pith or chopped cane (Rolz, de Cabrea and Garcia 1979), in packed-bed reactors. However, this remains at the development stage.

The problem with distillation is the large amount of waste liquor produced at stillage. At an 8% alcohol concentration in the stream leaving the fermenter, 12 volumes of stillage will be produced per volume of alcohol; in other words, by 1985 Brazil will produce 110×10^9 litres of stillage of high biological oxygen demand (BOD). This can be spread on the land, but discharge into rivers or leaching can cause problems. Alternatives include drying for use as animal feed or fuel, aerobic treatment, or anaerobic digestion, the relative costs and merit of which have recently been reviewed by Maiorella, Blanch and Wilke (1983). The lower protein content in stillage from a cane distillery, compared with one based on maize as raw material, makes the production of animal feed of less interest. However, if an effective anaerobic digestion process can be developed, this would have the advantage of also providing some process energy. In spite of extensive research on anaerobic digestion and the development of a wide variety of systems (Callander and Barford, 1983) aimed at the treatment of industrial effluents of various strengths and composition cost (*see* Chapter 10), effective systems have yet to be proved for use with cane stillage.

As an alternative approach to the use of sugar-cane as an energy crop, attempts are being made in Brazil to produce methane by anaerobic digestion of entire cane. This has an advantage that the high-value fuel product (methane) does not have to be separated from the liquor. However, if the gas is to be used for generation of electricity, or in larger installations fed to pipe lines or compressed for use as a transport fuel, gas cleaning (removal of hydrogen sulphide and carbon dioxide) and compression costs may negate this advantage. A further problem relates to the long generation times needed in order to digest the lignocellulose (bagasse) fraction of the entire cane. Digestibility of agricultural residues such as straw can be increased by mechanical or chemical treatment, but the costs are again, high (Mardon, 1981).

ECONOMICS

Detailed site-specific studies have been carried out for many countries or regions in attempts to determine the actual costs of producing ethanol as a liquid transport fuel (e.g. Japan: Anonymous, 1981b; Italy: Anonymous, 1979b; India: Anonymous, 1979a; New Zealand: Harris *et al.*, 1979; Australia: Stewart *et al.*, 1979; USA: Nathan, 1978; Anonymous, 1980a; OTA, 1980; South Africa: Ravno

1979; Sweden: Anonymous, 1980b; Brazil: Anonymous, 1979c; Rothman, Greenshields and Calle, 1983; developing countries: Anonymous, 1980c; Thailand: Anonymous, 1981c). In general, the costs of producing ethanol by fermentation of feed derived from sugar or starch crops exceed the alternative cost of buying oil. In cost terms, value of the crop as a source of food or animal feed can be compared with the value of the volume of petrol (gasoline) which can be saved if ethanol is used as substitute. However, this is justified only for small countries which do not have their own oil refineries, because substitution of the light end of the barrel causes disruption of established petroleum technology. For this reason, ethanol looks most attractive as an alternative octane booster for use in unleaded petrol.

At present it is claimed that the cost of fermentation ethanol produced from maize in the United States is less than that of ethanol produced from natural gas (Ng *et al.*, 1983). Again, this comparison relates the selling price of the alcohol from the two sources, but does not take into account the underlying economic reasons. In the USA, corn prices are low because of over-production; it is also possible to produce a valuable by-product in terms of animal feed protein which lowers the actual raw material costs to less than $1 per bushel (about $40/t). A considerable proportion of maize-based protein feed is currently being sold into the European Communities (EC), where it commands a higher price due to the effects of the Common Agricultural Policy (Pearce, 1981), which results in grain and sugar prices which are twice those of the world market. These high prices have, in turn, encouraged the over-production of beet sugar, resulting in the export of EC sugar on to the world market and depressing the price of sugar, molasses and, eventually, the value of sugar-cane-based ethanol, if considered in terms of international trade. However, the production of alcohol can be justified, for any specific country, on the basis of self-sufficiency, foreign exchange savings and improvement of rural economies. Such alternatives may be viewed as shown in *Figure 5*. However rigorous application of such a flow chart analysis (Coombs, 1981b) in most countries at present gives a negative answer as far as the true value of producing fermentation alcohol as fuel is concerned.

In general, the greater proportion of the cost of alcohol production relates to the production of raw materials. Where inputs are low, sugar cane may be produced at fairly low cost, but also at low yield. If local supplies of fertilizer are not available, import costs may outweigh the benefits of fuel production. The prime objectives of any biotechnological programme therefore should be to improve productivity and to decrease the need for inputs of synthetic nitrogen.

Conclusions

Sugar cane represents one of the most efficient land-based systems for converting solar energy into biomass. This biomass can provide both a solid fuel for combustion to produce process heat, steam or electricity (as well as power or heat which may be sold), and an easily fermentable juice which may be upgraded to ethanol which is of value as a liquid transport fuel and, in particular, as an octane booster for use in lead-free petrol. Fibre and juice may be separated easily by established mechanical means; both agricultural and process expertise

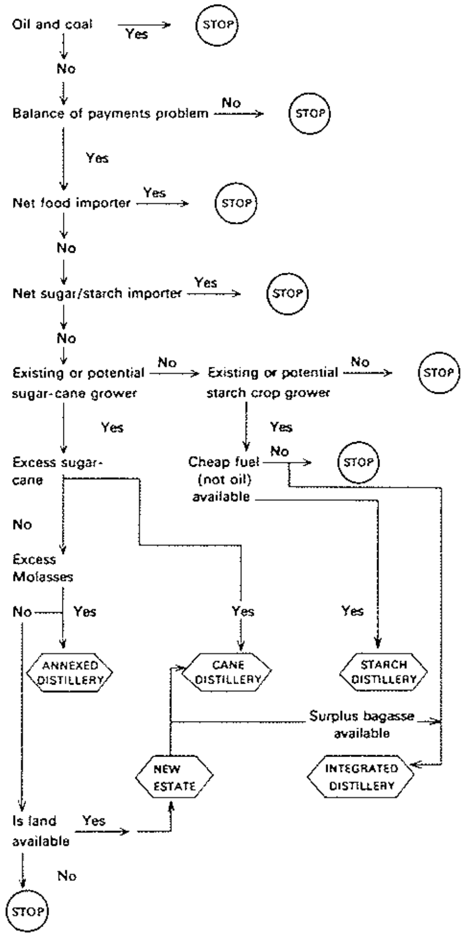

Figure 5. Options for the production of fuel alcohol from sugar and starch crops, using bagasse as fuel. An annexed distillery is one associated with a sugar factory: an integrated distillery is one which uses more than one feedstock.

exist to enable 10 000–15 000 t/day of raw material to be handled and, as long as fertilizer inputs are not excessive, the system will show a positive net energy gain. However, at present, developments of such systems are restricted by the economics of cane production in those countries with established plantation systems. Where cane is produced on a smaller scale by peasant farmers, apparent economic systems may reflect low farmer incomes. If biotechnology is to have a significant effect on the use of cane as an energy crop, it will have to relate to the overall production process, including cane breeding and waste treatment, rather than to fermentation alone. In the short term, it is possible that the most rapid advance will be in the growing of high-fibre canes for use as combustion fuel, rather than in the continued production of alcohol.

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