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THE POTENTIAL OF ONE-CARBON COMPOUNDS AS FERMENTATION FEEDSTOCKS

J.D. LINTON AND H.G.D. NIEKUS
Shell Research Limited, Sittingbourne Research Centre, Sittingbourne, Kent ME9 8AG
and Gist-Brocades NV, Delft, The Netherlands

1. INTRODUCTION

Micro-organisms have been reported to produce a wide range of chemicals from one-carbon compounds [1-5]. These include amino-acids, organic acids like ctric, butyric and acetic as well as exopolysaccharides, single cell protein, co-factors, vitamins and enzymes. However, few attempts have been made to examine critically processes based on one-carbon feedstocks in comparison with those already operating commercially on carbohydrates. In this introductory paper to the session on "Applied Aspects of One-Carbon Metabolism" an attempt will be made to compare one-carbon feedstocks with those traditionally used in the fermentation industry on the basis of price, availability, stoichiometry of conversion and process requirements. The price-volume and price-productivity relationship for various chemicals will be examined in order to arrive at realistic estimates of the productivities and costs that will have to be attained for bioconversion from methanol to be competitive with existing processes. Both aerobic and anaerobic conversion of methanol will be discussed and the relative merits of each assessed. This general analysis will help to define the types of chemicals that offer the best prospects for bio-conversions of one-carbon compounds. The economics of production of specific chemicals will not be discussed in detail as this area will be covered by other speakers in this session.

2. CHOICE OF ONE-CARBON FEEDSTOCK FOR FERMENTATION PROCESSES

Methane, methanol and synthesis gas could all be considered as potential feedstocks for the fermentation industry. Synthesis gas is a mixture of H_2 and CO and it can be produced from coal, natural gas or biomass. The technology for the conversion of synthesis gas into a wide range of chemicals currently produced from oil is well developed. The next speaker Dr. Drent will discuss the potential and timing of synthetic processes from synthesis gas. At present, with the exception of South Africa, synthesis gas is not produced on a large scale for subsequent conversion to fuels and chemicals. It is produced on a relatively small scale (compared with that which would be required for fuel production) from natural gas and coal for the captive synthesis of methanol, acetic acid, acetic anhydride and other derivatives.

As a fermentation feedstock methanol has a number of important advantages over methane and synthesis gas. It is a commodity chemical of high purity, it is relatively cheap, easy to transport and store. Methanol is miscible with water and the hazard of explosions is considerably less than that with the gaseous feedstocks. In addition, methanol is more oxidized than methane and consequently both the oxygen demand and the amount of heat generated during aerobic fermentations will be less; both these parameters may have an important impact on process economics. For the reasons outlined above, methanol is generally regarded as the most suitable feedstock for both aerobic and anaerobic fermentations [1-7].

The current world production of methanol is approximately 12 x 10^6 t/y and the traditional outlets for methanol are only expected to show a modest rate of growth (3-5%/year) over the next 5 years [7,8]. However, there has been considerable speculation regarding the use of methanol as a fuel, either blended in gasoline as an octane booster or for use neat. The fuel related uses have not developed as anticipated and at present there is a large overcapacity for methanol production. Although a large number of plans have been shut down many new ones have come on stream and more are expected [8] so the overcapacity is likely to continue for a number of years (Figure 1). This will tend to keep the methanol price low.

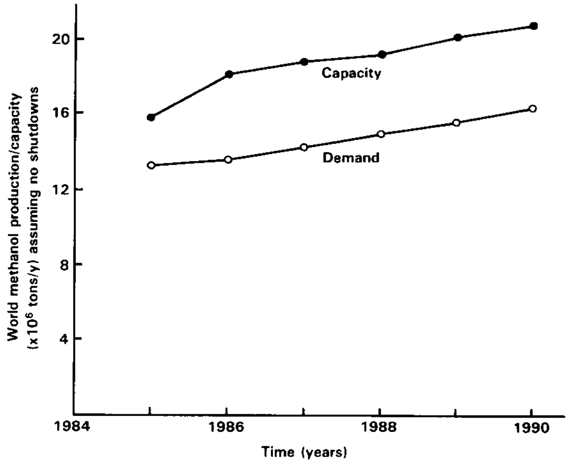

Fig 1
The imbalance between world capacity and demand for methanol assuming no further shutdowns. (based on Coxon, 1985)

Moreover, methanol prices are expected to be linked to energy prices [8] and these are also expected to remain low. It should be emphasised that a future development of fuel uses for methanol could reverse the situation completely since a 1% addition of methanol to gasoline would utilise most of the surplus capacity available [9].

3.COMPARISON BETWEEN METHANOL AND TRADITIONAL FERMENTATION FEEDSTOCK PRICES

When considering methanol as a potential fermentation feedstock it is necessary to compare it with those traditionally used in the industry. The world sugar price over the past 15 years has fluctuated dramatically whereas the price of methanol has remained relatively steady (Figure 2). At first sight this looks very encouraging, because on a weight basis the price of methanol is considerably lower than that of sugar. However, most of the world sugar production (approximately 100 x 10^6 t/y) is traded directly between producer and consumer or between countries and only 30% is traded on the free market. The price of the latter is disproportionately affected by shortages and surpluses and is not a true reflection of the cost of sugar to the fermentation industry [10]. Similarly, the EEC gross intervention price for sugar is considerably higher than world prices. However, the fermentation industry in the EEC qualifies for various rebates and the actual cost of sugar will depend on the specific chemical being produced and whether or not it is exported outside the EEC.

Molasses is also widely used in the fermentation industry. On the basis of sugar content the price of molasses has been similar to that of methanol (Figure 2). However, molasses cannot be stored for long periods, its composition is variable and it contains particulate material that has to be removed from the product. The latter will add to the cost of down-stream processing.

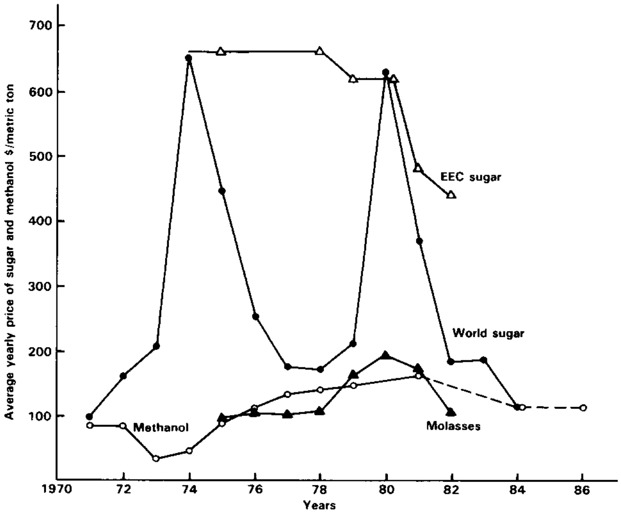

Fig 2

The price of sugar and methanol from 1970 to 1984. The international sugar agreement daily price ● was obtained from the sugar year book 1984; the EEC gross intervention price, △ (Brown, 1983); the US list price for methanol, ○ (Abshire *et al.*, 1980; and the ECN News. Molasses (cane) ▲ selling price, New Orleans (Brown, 1983), the molasses price has been adjusted assuming a 50% sugar content.

A wide range of factors may influence the price of these feedstocks and the relative price differential will vary from time to time and from country to country. The difficulties experienced in forecasting feedstock prices coupled to changes in their relative price means that it is not justifiable to plan a fermentation process based solely on methanol, because of its present price advantage. Instead, a flexible feedstock policy that allows the use of the most favourable feedstock available at a given time and location, would be advisable. The vulnerability of being tied to one feedstock is illustrated by the BP process for SCP from n-paraffins [11] and this lesson has not been lost on the fermentation industry as is clear from the policy adopted by Philips Petroleum for SCP production from a range of feedstocks including methanol, sugar and whey [6].

4.DEFINITION OF TARGET AREAS FOR METHANOL CONVERSIONS

What types of chemicals offer the best prospects for methanol conversions? The most important attribute of methanol as a feedstock is its price. Consequently, methanol may be used to best effect in processes where the feedstock costs are a significant proportion of production costs. In general, the higher the cost of a given chemical the smaller the impact feedstocks costs are likely to have on process economics.

It is well known [12] that the price of a wide range of chemicals is inversely related to the volume in which they are produced. This relationship also applies to the fermentation industry [13] and it shows that virtually all fermentation products cost more than $2/kg because of the high capital and production costs associated with fermentation processes Figure 3.

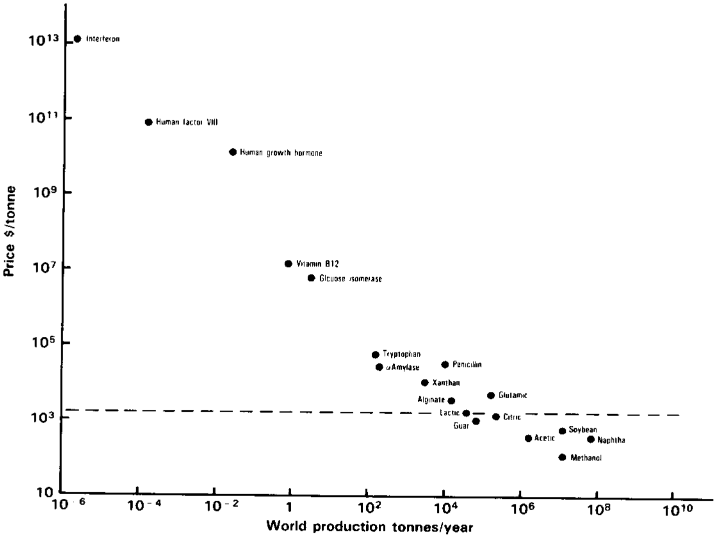

Fig 3
The relationship between the price of various chemicals and the volume in which they are produced. (Data taken from Dunnill, 1982; Tong and Cannell, 1983).

Processes favouring the use of methanol are therefore likely to be those for the production of bulk chemicals that command a low price. Competition in this area is strong from chemical synthesis for the production of simple molecules like ethanol and acetic acid. For complex molecules like natural oils and protein there is a strong competition from agriculturally-derived commodities. In the production of chemicals that have a moderate value (e.g. amino acids) the price difference between sugar and methanol has to be large in order to justify the use of methanol as a feedstock. Moreover, other factors such as product concentration, productivity and down-stream processing then assume as much significance as feedstock costs. For high-value products, feedstock costs are a minor part of overall process costs and the use of methanol for the production of these chemicals will depend on whether it offers other process advantages. For example, methylotrophs could offer advantages in the production of heterologous proteins using the powerful promoter of the methanol oxidase system in yeast.

5.BIOCONVERSIONS OF METHANOL

The microbial conversion of methanol can be carried out aerobically or anaerobically. The economic constraints of these processes differ and these will be examined separately.

5.1 Aerobic fermentation

On the basis of known biochemical pathways, stoichiometric equations can be written for the aerobic production of acetic acid, citric acid, glutamic acid, exopolysaccharide and a lipid from methanol and glucose (Table 1). The yields of these products, when expressed on a carbon-carbon basis are similar from glucose and methanol. However, the oxygen requirement for methanol conversion can be considerably higher than that from glucose. Thus, for the production of acetate from methanol the oxygen demand is higher than that for the production from glucose by a factor of 2.5, whereas this factor increases to 10 for exopolysaccharide production. The higher oxygen demand is accompanied by a corresponding increase in the heat output of these fermentations (Table 1). Consequently, in processes where maximum productivity is already limited by mass transfer and cooling, for example in the production of exopolysaccharide from glucose, these problems will be considerably more severe with methanol as the feedstock. For each product it will be necessary to determine whether the relative price advantage of methanol is sufficient to offset the disadvantages inherent in this reduced feedstock as outlined above. Examples where methanol offers an advantage for the aerobic production of specific chemicals will be covered by Professor Tani and Drs. Thill and Stroman.

5.2 Anaerobic fermentation

There are two groups of micro-organisms capable of utilizing methanol anaerobically. The first group are the methanogens that convert it into methane and the second group, which are of more interest from the point of view of chemicals production, are the acidogens. The latter group have only been discovered comparatively recently [15] and the physiological and energetic requirements for cell

Table 1 - Stoichiometry of metabolite production from glucose and methanol showing the relative oxygen demand, free energies and yields of various chemicals.

Product	Carbon source	Overall Stoichiometry (Assuming that excess reducing equivalents are oxidised to water)	$-\ G^{0'}$ (kJ)	Yield Y substrate ($g\ g^{-1}$)	YO_2 ($g\ g^{-1}$)
Acetate	Methanol	$3CH_3OH + 2.5O_2 = CH_3COOH + CO_2 + 4H_2O$	1254	0.625	0.75
	Glucose	$0.5C_6H_{12}O_6 + O_2 = CH_3COOH + CO_2 + H_2O$	531	0.67	1.88
Citrate	Methanol	$6CH_3OH + 4.5O_2 = C_6H_8O_7 + 8H_2O$	2280	1.0	1.33
	Glucose	$C_6H_{12}O_6 + 1.5O_2 = C_6H_8O_7 + 2H_2O$	829	1.07	4.0
Glutamate	Methanol	$6CH_3OH + NH_3 + 4.5O_2 = C_5H_9O_4N + CO_2 + 9H_2O$	-	0.76	1.02
	Glucose	$C_6H_{12}O_6 + NH_3 + 1.5O_2 = C_5H_9O_4N + CO_2 + 3H_2O$	-	0.82	3.06
Hexose-exopoly-saccharide	Methanol	$6.14CH_3OH + 3.21O_2 = [C_6H_{10}O_5] + 0.14CO_2 + 7.28H_2O$	1550	0.82	1.58
	Methane	$12CH_4 + 18O_2 = [C_6H_{10}O_5] + 6CO_2 + 19H_2O$	7760	0.84	0.28
	Glucose	$1.05C_6H_{12}O_6 + 0.3O_2 = [C_6H_{10}O_5] + 0.3CO_2 + 1.3H_2O$	146	0.86	16.3
Lipid (palmitic acid)	Methanol	$24CH_3OH + 13O_2 = C_{16}H_{32}O_2 + 8CO_2 + 32H_2O$	7029	0.33	0.62
	Glucose	$4.16C_6H_{12}O_6 + 1.97O_2 = C_{16}H_{32}O_2 + 8.97CO_2 + 8.97H_2O$	1682	0.34	4.06

synthesis of these organisms are still poorly understood. The only metabolites produced by the various acidogens are acetic acid, butyric acid or both and these products are excreted as a consequence of the energy metabolisms in these organisms. The amount of energy harnessed by these organisms during the conversion of methanol to acetic or butyric acid is small (Table 2). Thus the yield of cells per mol of methanol consumed is low, but the product yields are high. Moreover, because CO_2 is incorporated together with methanol into the product, the yield of acetic acid from methanol exceeds 1 g/g (Table 2) and most of the energy of the substrate is conserved in the acidic product. This results in a large reduction in heat production compared with the aerobic production of acetic acid (Table 1). Most of the above mentioned advantages of using methanol as a feedstock would also apply to the anaerobic production of other well-known fermentation products like ethanol, acetone or butanol. However, no reports of methylotrophic anaerobes capable of producing these products from C_1 compounds have been published.

Table 2 - Yields on a weight or energy basis and free energy of the reactions ($G^{0'}$) from methanol to acetic and butyric acid.

	Yield		
	Weight basis (g/g)	Energy basis	$-\ G^{0'}$ (kJ)
$1.33CH_3OH + 0.67CO_2 \longrightarrow CH_3COOH + 0.67H_2O$	1.41	0.91	71
$3.33CH_3OH + 0.67CO_2 \longrightarrow CH_3CH_2CH_2COOH + 2.67H_2O$	0.83	0.90	177

5.3 Prospects for the microbial production of acetic acid

Acetic acid is a bulk chemical and therefore its production from methanol meets the criteria described previously concerning economic targets for bioconversions from methanol. As methanol can be converted into acetic acid in high yield by anaerobic methylotrophs it is instructive to examine the prospects for the biotechnological route to this chemical. In order to be competitive with the synthetic route the fermentation process has to be greatly improved in a number of ways. From the relationship between product price and fermentation productivity (Figure 4) it can be concluded that the fermentation process will have to attain a productivity in the region of 25-80 g/l.h. These productivities are exceptionally high for fermentation processes, but they are not impossible. In a recent patent [14] volumetric productivities as high as 14.3 g acetic acid/l.h were reported for immobilized *Clostridium thermoaceticum* cells using glucose as the substrate. Daicel Chemical Industry, the major manufacturer of acetic acid in Japan, has started research on acetic acid production from $H_2 + CO_2$ using anaerobic micro-organisms. However, the best productivity reported was 1.9 g/l.h [18]. This productivity could potentially be improved by using a cell re-cycle system to maintain a high level of biomass in the reactor. The Japanese interest in this area may also be gathered from a large number of patent applications issued on behalf of the Japanese Agency of Industrial Science and Technology dealing with the production of a variety of fermentation products by autotrophic and methylotrophic (facultative) anaerobes. The main factor limiting productivity is the sensitivity of these organisms to their own acidic metabolic products. A theory recently proposed concerning acetic acid toxicity [19] suggests that there may be physiological limits to the extent of the improvement likely to be achieved by mutation or adaptation. The other important area in need of marked improvement is in the recovery of acetic acid

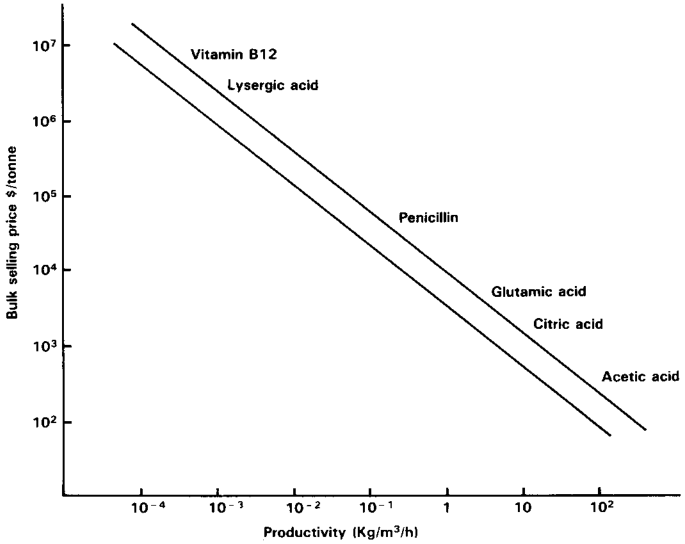

Fig 4
The relationship between the price of various chemicals and the productivity at which they are operated. (based on Andrew, 1983).

from dilute aqueous fermentation broths, (preferably as the acid rather than as a salt). Recovery is directly linked to productivity and a cheap and efficient means of continuously removing acetic acid could be of considerable help in a developing high productivity fermentation. A number of new methods of recovering chemicals from aqueous fermentation broths are being developed, for example super-critical CO_2 and membrane extraction methods. If these new methods are successful then the fermentation process to acetic acid could become competitive with the synthetic route.

6.CONCLUSIONS

Methanol is a relatively cheap commodity chemical and is likely to remain so for the foreseeable future. It can be converted into a wide range of chemicals by micro-organisms. The most important economic incentive for using methanol is its cost relative to traditional fermentation feedstocks, but the extent of the price advantage varies with time and location. Because of its relatively low price methanol may offer advantages over carbohydrate feedstocks for the production of bulk chemicals where feedstock costs represent an important part of the overall costs. However, there is strong competition from both the chemical industry and agriculture in this area. Consequently specific markets have to be identified. The rational for using methanol for the production of high value chemicals is more likely to be based on the special metabolic attributes of methylotrophs than on the relative price of C_1 feedstocks.

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