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The relationship between the energetic efficiency in different micro-organisms and the rate and type of metabolite over-produced

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SUMMARY

Data regarding the degree of energy conservation as determined by the $Y_{O_2}^{max}$ and the highest rates of metabolite production reported for various micro-organisms have been collated and analysed. The results have indicated that the highest rates of metabolite production occur in micro-organisms possessing low efficiencies of energy conservation. Moreover, in the case of exopolysaccharide production the oxidation state of the polymer is inversely related to the $Y_{O_2}^{max}$ value of the producing organism. In general, the rate of ATP turnover associated with exopolysaccharide production or the potential rate associated with over-production of other metabolites is inversely related to the $Y_{O_2}^{max}$ value of the producing organism. Analysis of current production rates for a range of metabolites suggests that there is scope for major improvements of existing processes by careful selection of appropriate micro-organisms.

INTRODUCTION

A great deal of research has been carried out on the biochemistry and genetics of metabolite production by microbes. This research has led to the elucidation of a large number of metabolic and genetic control systems that function to integrate the various activities of the living cell and prevent the over-production of any particular metabolite. Knowledge of these control systems has enabled the industrial microbiologist to tackle the problem of getting a microbe to over-produce and excrete a particular metabolite in a reasoned way [11,12]. There is, however, another important though largely neglected aspect, namely the energetic and physiological consequence of metabolite over-production that will be examined in this paper.

A number of obligately aerobic Gram-negative bacteria produce polysaccharides as the only or major metabolite when grown under conditions of carbon excess in continuous culture [8,40,50,56]. In

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these organisms the rate of ATP utilisation for exopolysaccharide production can amount to as much as 60–80% of that required for growth under conditions of carbon excess [32,33,37]. A study of exopolysaccharide production by *Agrobacterium radiobacter* NCIB 11883, *Erwinia herbicola* and the methylotroph *Methylophilus* sp. NCIB 12047 has revealed that as a consequence of the degree of energy conservation (ATP/O quotient) present in these organisms a significant proportion of the ATP demand for exopolysaccharide production is supplied during the synthesis of the oxidised constituents of these large molecules [32,35,37].

During nitrogen-limited growth on glucose, gluconate and xylose *A. radiobacter* produces exopolysaccharide at significantly different rates; however, the rate of ATP utilisation for exopolysaccharide synthesis was very similar. Moreover, this rate of ATP utilisation accounted for most of the respiratory activity occurring in excess of that required for cell biosynthesis (nitrogen-limited q_{O_2} – carbon-limited q_{O_2} × ATP/O quotient [35]. That is, in the production of succinoglucan from glucose, gluconate or xylose the ATP produced during the synthesis of the acid moieties of the polymer are turned over in the production of the sugar backbone so that polysaccharide production and ATP turnover are integrated. On substrates that are considerably more reduced, e.g. glycerol and ethanol, polymer production offers a poor means of ATP turnover as considerably more ATP is generated during exopolysaccharide synthesis than can be turned-over by this process, so some other means of energy dissipation must be in operation [35]. Thus very little exopolysaccharide was produced from ethanol by *A. radiobacter* [35]. A mutant of *A. radiobacter* that was unable to synthesis exopolysaccharide was found to have the same growth efficiency as the parent strain; moreover, under nitrogen limitation the respiratory activity in excess of that required for growth was close to that of the parent strain in spite of a total lack of exopolysaccharide production [35]. This suggests that respiratory activity and hence ATP turnover or energy dissipation rather than exopolysaccharide production per se is physiologically important. Similar results were found with *E. herbicola* [33]. In this paper an attempt has been made to determine whether the efficiency of growth and the rate of ATP turnover have any bearing on the type and extent of metabolite over-production.

MATERIALS AND METHODS

Rates of ATP turnover associated with metabolite production

Very little information is available concerning the efficiency of aerobic energy conservation in micro-organisms that produce exopolysaccharides or other metabolites. Difficulty was also experienced in compiling data regarding the specific rates of metabolite production as much of the data has been published in the patent literature where quantitative data concerning microbial biomass and rates of product formation are scarce. In the examples given for citric acid and glutamic acid production the microbial biomass has been estimated from the composition of the medium employed and therefore the specific rates of production are only approximate (Table 2).

In calculating the rate of ATP turnover associated with the production of a given metabolite the following assumptions have been made. (a) For the synthesis of a homopolymer like an extracellular glucan, 1 mol of ATP is required to produce 1 mol of glucose-1-*P*, a second is required for the generation of ADP-glucose and finally a third is required for transport of the activated glucose out of the cell and for addition to the growing chain [23,56]. Thus 3 mol ATP are required per hexose incorporated. (b) In the synthesis of a complex polymer composed of polymerised sub-units of fixed structure, e.g. xanthan, the ATP demand has been taken as 2 ATP for the generation of ADP-glucose and a further ATP per repeat unit of 5 hexoses transported during chain elongation [23]. (c) All reducing equivalents produced simultaneously with a given metabolite are oxidised to water via the electron transport system.

ATP/O quotient

Calculations on the energetics of metabolite

production are fraught with difficulties because the amount of ATP produced per O_2 consumed (ATP/O quotient) cannot be measured precisely. The ATP/O quotient of various micro-organisms have been estimated from published values of $Y_{O_2}^{max}$ (see Table 1) using the equation:

$$Y_O^{max} = Y_{ATP}^{max} \cdot ATP/O$$

For the purpose of this study Y_{ATP}^{max} values of 12.4 $g \cdot mol^{-1}$ [25] and 8.3–10.6 $g \cdot mol^{-1}$ [2] have been used for aerobic growth on glucose and methanol respectively. It is stressed that a number of assumptions have been made in calculating the ATP/O quotient from the above equation. The theoretical ATP requirement for cell biosynthesis from glucose (in mineral salts medium) is 28.8 g dry wt. $\cdot mol^{-1}$ ATP which is considerably higher than that usually estimated from growth yields and known routes of ATP synthesis [54]. This discrepancy between the theoretical Y_{ATP}^{max} of 28.8 and that derived from yield determination (12–14 $g \cdot mol^{-1}$ [25]) is an important source of error in determining the 'real' ATP/O quotient. The other assumption implied in the equation is that ATP synthesis via substrate-level phosphorylation is not important. The influence of the latter is inversely proportional to the ATP/O quotient and could result in an over-estimation by approximately 7 and 30% at ATP/O quotients of 3 and 1, respectively [53]. However, as long as organisms growing under carbon-limited conditions on the same carbon source are compared the difference in the Y_{ATP}^{max} is minimised and the ATP/O quotient obtained will be valid as a comparative measure of growth efficiency. The ATP/O quotient has only been used to estimate the rate of ATP turnover associated with metabolite production.

RESULTS AND DISCUSSION

Exometabolite production rate as a function of the growth yield from oxygen

The molar growth yield from oxygen (Y_{O_2}) is a good indicator of the degree of coupling between respiration and energy conservation in micro-organisms. The Y_{O_2} often limits the maximum productivity at which a process can operate and therefore strongly influences the economics of bioprocesses. It is therefore very surprising that so little information is available concerning the molar growth yields from oxygen of industrially important micro-organisms. From the limited data available (Tables 1 and 2) it is clear that $Y_{O_2}^{max}$ (the maximum yield from oxygen, corrected for cellular maintenance requirements) determined during carbon-limited growth for various micro-organisms is inversely related to the highest product formation rates reported for these organisms (Fig. 1). That is, the lower the growth efficiency the higher the rate of metabolite production. The highest product formation rates reported are those for *Gluconobacter oxydans* [62], an organism reported to have a $Y_{O_2}^{max}$ of 2.1 $g \cdot mol^{-1}$.

The $Y_{O_2}^{max}$ does not indicate the amount of ATP produced per 0.5 mol O_2 consumed (ATP/O quotient) or the rate of ATP synthesis that is metabolite-associated. This can only be derived from the $Y_{O_2}^{max}$ by a method that is open to criticism and debate (see Methods). However, without an estimate of the ATP/O quotient the energetics of metabolite overproduction cannot be examined further. Although the absolute values estimated for the ATP/O quotient are based on a number of assumptions, these values can be used comparatively. Such an approach has been used to assess the efficiency of exopolysaccharide production by *A. radiobacter* [32,35], *E. herbicola* [33] and *Xanthomonas campestris* [50]. These studies have indicated that once an allowance has been made for the energetic requirements of cell production the yield of exopolysaccharide from oxygen is close to the theoretical value calculated from the ATP/O quotient derived from carbon-limited cultures, which suggests that the ATP/O quotients of carbon- and nitrogen-limited cultures of these organisms are similar.

Rate of metabolite-associated ATP turnover as a function of $Y_{O_2}^{max}$

The relative ATP/O quotient for a range of micro-organisms was estimated from the $Y_{O_2}^{max}$ va-

Table I

A summary of data concerning the type of exopolysaccharide, the maximum rate of production, the ATP/O quotient and the rate of ATP turnover associated with the synthesis of exopolysaccharides by different micro-organisms

Exopolysaccharide	Organism	Theoretical stoichiometry
Curdlan $\beta(1\rightarrow3)$Glc	*Alcaligenes faecalis*	Glu + 3 ATP = $[C_6H_{10}O_5]$ + H_2O + 3 ADP + 3 P_i
Glucan $\beta(1\rightarrow3)$	*Helotium* sp.	
	Cryptococcus laurentii	
Glu:Man (3:1)	*Methylophilus* sp.	6 CH_3OH + 8 ATP + 6 PQQ = $[C_6H_{10}O_5]$ + 6 $PQQH_2$ H_2O + 8 ADP + 8 P_i
Glu:Man (3:2)	*Pseudomonas polysaccharogenes*	
	Methylomonas methanolica	
2 Glu:Gal:Man:2 Py	*Methylomonas mucosa*	
Succinoglucan	*Agrobacterium radiobacter*	9.55 Glu + 17.45 ATP + 2.55 NADP + 3.65 NAD = [7 Glu:Gal:Suc:Py:0.1Ac] + 3.65 $NADH_2$ + 17.45 ADP + 17.45 P_i + 2.1 CO_2
Succinoglucan	*Pseudomonas* sp. NCIB 11264	
	Arthrobacter stabilis	
	Arthrobacter globiformis	
Man:Xyl:GluU + 7% Ace	*Cryptococcus laurentii*	
Gal:Glu:Suc:Py:Ac:UA (4.26:2.98:1.43:1.55:0.5:1.5)	*Erwinia herbicola*	11.23 Glu + 9.135 NAD + 1.435 NADP + 16.58 ATP = $[C_{63.87}H_{94.3}O_{53.15}]$ + 9.135 $NADH_2$ + 1.435 $NADPH_2$ + 3.37 CO_2 + 16.58 ADP + 16.58 P_i
Xanthan	*Xanthomonas campestris*	6 Glu + 11.5 ATP + 4 NADH + NADP = $[C_{35}H_{52}O_{29}]$ + 4 $NADH_2$ + $NADPH_2$ + CO_2 + H_2O
(2 Glu:2Man:1UA:Ac:Py)	*Xanthomonas campestris*	
Alginate	*Azotobacter vinelandii*	1.5 Glu + 2.15 NAD + 0.05 NADP + 2.9 ATP = [Ua + 0.1 Ac] + 1.0 CO_2 + 0.05 $NADPH_2$ + 2.15 $NADH_2$ + 2.9 P_i
	Azotobacter vinelandii	
Alginate	*Pseudomonas aeruginosa*	
	Pseudomonas aeruginosa (ATCC 10145)	
	Pseudomonas fluorescens	
Alginate	*Pseudomonas mendocina* (mutant)	
	Klebsiella pneumoniae	
Glu:Gal:UA:Fuc	*Klebsiella pneumoniae*	

[a] For carbon limited growth.

Glc = glucosamine; Glu = glucose; Man = mannose; Gal = galactose; Py = pyruvate; Xyl = xylose; GluU = glucuronate; Ac = acetyl; UA = uronic acid; Fuc = fucose; obs = observed; C = carbon limitation; N = nitrogen limitation.

lues of carbon-limited cultures as described in the Methods section. From the stoichiometries of metabolite production and the ATP/O quotients given in Tables 1 and 2 the rate of ATP turnover associated with metabolite production was calculated. There is a strong correlation between the $Y_{O_2}^{max}$ of a given organism and the potential rate of ATP turnover associated with metabolite produc-

ATP balance per mol product assuming ATP/O ratios:			Growth conditions or limitations	$Y_{O_2}^{max\ a}$ (g cells·$mol^{-1}O_2$)	Y_{ATP}^{max} (g cells·mol^{-1}ATP)	ATP/O quotient (mol ATP·0.5 $mol^{-1}O_2$)	Max. observed q_p ($g \cdot g^{-1} \cdot h^{-1}$)	Rate of ATP turnover for polymer production ($mmol \cdot g^{-1} \cdot h^{-1}$)	Ref.
1	2	3							
–3	–3	–3	C/N	33–50	12.4	1.3–2	0.098	1.81	45
–	–	–	batch				0.028		
							0.029		9,5
–2	–2	–2	C/N	18	8.3–10.6	0.85–1.1	0.11	5.4	33
						1.1	0.14	6.9	57
–	–	–	MeOH	18.24	8.3–10.6	0.86–1.1			13
–	–	–	batch				0.055		58
			C	40	12.4	1.6			32
–11.25	–4.9	+1.15	N				0.24	2.77	32
			N				0.21	2.4	63
			batch				0.046	0.6	4
			C	83.65	12.4	3.37			25
							0.02		24
– 5.98	+4.56	+15.3	C/N	31	12.4	1.25	0.34–0.37	3.3–3.9	33
						1.04	0.5	6.14	50
– 6.5	–1.5	+ 3.5	C/N	26	12.4				
						1.33	0.33	3.8	15,23
			C/N	33 (obs)	12.4				
–0.7	+1.5	+ 3.7	N			0.75			14,22
			C	18.6, 13	12.4				
			batch N				0.70	11.34	40
							0.44	7.13	
			C	25	12.4	1.0			Linton, unpublished
			batch	26.5 (obs)	12.4	1.06			21
			C/N	25.1		0.9	0.56	9.0	20
									Rye, unpublished
			C	49.1	12.4	1.97			25
			batch				0.07		26

tion (Tables 1 and 2, Fig. 2). The higher the $Y_{O_2}^{max}$ the lower the potential rate of ATP turnover associated with metabolite production. It is interesting that the rate of exopolysaccharide production (q_p) by methylotrophs appears to be low when plotted against the $Y_{O_2}^{max}$ (Fig. 1). However, when the q_p is plotted in terms of ATP turnover the result is no longer anomalous. Considerably more data are

Table 2

A summary of the data concerning the type of exocellular metabolite, maximum rate of production, P/O ratio of the production organism and the rate of ATP utilised or produced in association with the production of the given exocellular metabolite

Exopolysaccharide	Organism	Theoretical stoichiometry
Gluconic acid	*Penicillium stipitatum*	Glu + FAD = gluconic acid + $FADH_2$
Gluconic acid	*Gluconobacter oxydans*	Glu + PQQ = gluconic acid + $PQQH_2$
2-ketogluconic/		Gluconate + FAD = ketogluconate + $FADH_2$
5-ketogluconic mixture		2-ketogluconate + FAD = 2,5-ketogluconate + $FADH_2$
	Gluconobacter oxydans	
Citric acid		Glu + 3 NAD + ADP + P_i = citric acid + 3 $NADH_2$ + ATP
	Aspergillus niger	
	Aspergillus niger	
	Aspergillus nidulans	
	Aspergillus awamori	
	Aspergillus foetidus	
	Candida utilis	
	Bacillus licheniformis	
	Bacillus licheniformis	
Glutamic acid		Glu + NH_4 + 3 NAD + ADP = glutamic acid + CO_2 + 3 $NADH_2$ + ATP
	Corynebacterium glutamicum	
Rhamnolipid	*Pseudomonas aeruginosa*	7 Glu + 17 ATP + 0.5 NAD = R_1 rhamnolipid + 0.5 $NADH_2$ + 17 ADP + 17 P_i
	Pseudomonas aeruginosa	rhamnolipid producer (ATCC 10145)
Sophorolipid (Sob:2Ac:C18FA)	*Torulopsis bombicola*	7.5 Glu + 16.625 ATP + 6.25 NAD = sophorolipid + 6.25 $NADH_2$ 16.625 ADP + 16.625 P_i
Tripalmitin	*Candida* sp. 107	12 Glu + 6 NAD + 40 ATP = tripalmitin + 6 $NADH_2$ + 40 ADP + 40 P_i
Poly-β-hydroxybutyrate	*Methylobacter organophilum*	7 CH_3OH + 7 PQQ + 2 FP + 3 Y + 4 ATP = [PHB] + 7 $PQQH_2$ + 2 FPH_2 + 3 YH_2
	Alcaligenes eutrophus	Fru + 3 NAD = [PHB] + 3 $NADH_2$ + 2 CO_2
Extracellular protease	*Bacillus licheniformis*	For convenience, protein taken to be polyglutamate, 4.93 ATP/ glutamate incorporated.

[a] Biomass estimated for medium composition.

[b] Lactate.

Glu = glucose; FP = flavoprotein; Y = electron acceptor (unknown); Fru = fructose; Ac = acetate; Sob = sorbose; C18FA = 18-carbon fatty acid.

needed to establish this correlation unequivocally and a great deal of scatter is to be expected as maximum rates of q_p are plotted. Thus alginate production by *Azotobacter vinelandii*, an organism known to possess a low $Y_{O_2}^{max}$ [14,41], is considerably lower than expected; however, in this particular case the lower q_p may be attributed to the ATP requirement for nitrogen fixation that occurs in nitrogen-limited exopolysaccharide-producing cultures. Similarly the q_p for gluconate production had to be plotted on a log scale when plotted in terms of $g \cdot g^{-1} \cdot h^{-1}$ (Fig. 1). However, in terms of ATP production the relationship between the $Y_{O_2}^{max}$ and q_{ATP} is no longer anomalous (Fig. 2). These observations suggest that organisms possessing a low efficiency of aerobic energy conservation (low $Y_{O_2}^{max}$) have the capacity to turnover ATP (dissipate energy) considerably more rapidly than those possessing high

Growth conditions: continuous (C) or batch (B)	Potential ATP equivalents per mol product assuming ATP/O quotients:			$Y_{O_2}^{max}$	Y_{ATP}^{max}	ATP/O quotient	Maximum q_p ($g \cdot g^{-1} \cdot h^{-1}$)	q_{ATP} ($mmol \cdot g^{-1} \cdot h^{-1}$)	Ref.
	1	2	3						
C	1	2	2	45	12.4	1.8	1.8	H_2O_2/catalase	31
	1	1	1				7.11	3.04	
B	1	2	2				4.65	4.02 10.1	62
	1	2	2						
C				2.1	12.4	0.084			44
B	4	7	10						
C, pH 3.5							0.08		28
C, pH 2.0							0.45		
C				68	12.4	2.74			6
C				40	12.4	1.61			38
C, pH 3.4							0.1		29
				53	12.4	2.1			1
B, pH 7.0							0.025[a]	0.96	51
C				53.3	12.4	2.1			18
C	4	7	10	78	12.4	3.1			Linton unpubl.
							0.056,0.042[a]		59, 60
C	−16.5	−16	−15.5				0.09	2.3	19
C						1			Linton unpubl.
B							0.04	0.94	7
C	−34	−28	−22			2	0.035	1.6	48
C	12	–	–			1	0.033	4.6	46
C	3	6	9	65.2	8.4	3[b]	0.016	1.67	52
C	–	−4.95	–			2.14	0.040	1.53	18

efficiencies of energy conservation. ATP turnover may be achieved by exopolysaccharide production or energy may be dissipated by some other means.

Exopolysaccharide production

In general, it appears that organisms possessing a high degree of energy conservation (high $Y_{O_2}^{max}$, carbon-limited) produce exopolysaccharides at relatively low specific rates (Fig. 1, Table 1). Moreover, the oxidation state of the exopolysaccharide is also inversely related to the $Y_{O_2}^{max}$ (carbon-limited). Thus few eukaryotes (organisms that would be expected to have $Y_{O_2}^{max}$ values in the range 45–80 $g \cdot mol^{-1}$) produce exopolysaccharides containing acids or oxidised sugar moieties at rates above 0.1 $g \cdot g^{-1} \cdot h^{-1}$. Most eukaryotes produce unsubstituted exopolysaccharides [16], for example *Aureobasidium pullulans, Sclerotium rolfsii, Aspergillus niger, Peni-*

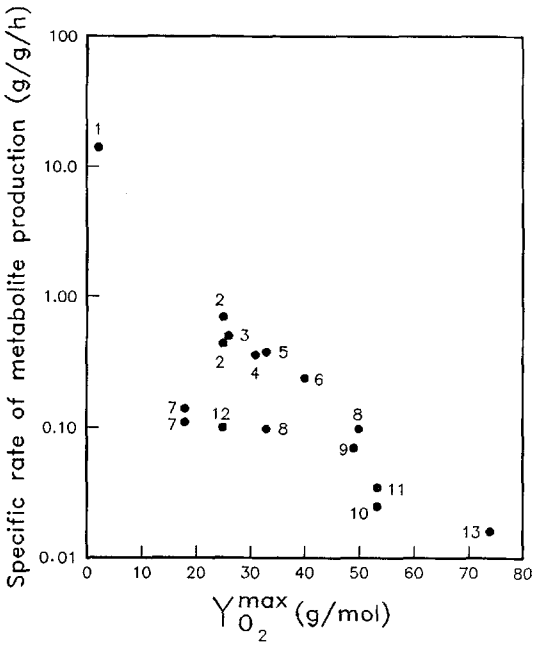

Fig. 1. The highest specific rates of metabolite production reported as a function of the $Y_{O_2}^{max}$ of the producing organism. 1, total gluconate [62]; 2, alginate [40]; 3, xanthan [50]; 4, succinogalactan [33]; 5, xanthan [15]; 6, succinoglucan [32]; 7, glucomannan [37,57]; 8, curdlan [45]; 9, *Klebsiella pneumoniae* exopolysaccharide [26]; 10, citric acid [51]; 11, exoenzyme [18]; 12, rhamnolipid [19]; 13, polyhydroxybutyrate [52].

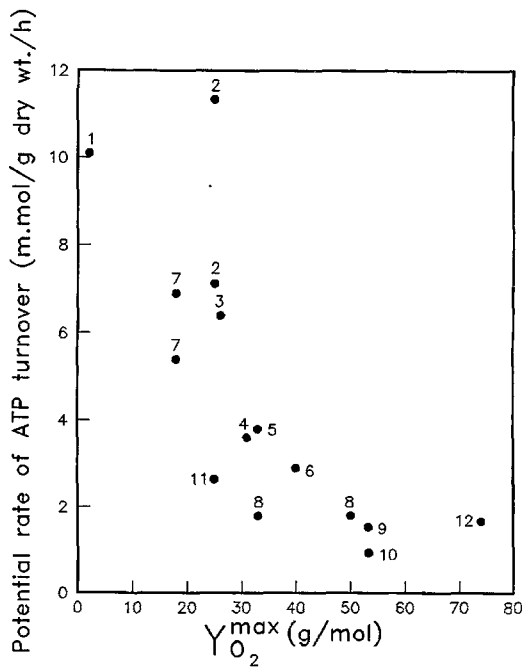

Fig. 2. The potential rate of ATP turnover associated with metabolite production as a function of the $Y_{O_2}^{max}$ of the producing organism. 1, total gluconate [62]; 2, alginate [40]; 3, xanthan [50]; 4, succinogalactan [33]; 5, xanthan [15]; 6, succinoglucan [32]; 7, glucomannan [37,57]; 8, curdlan [45]; 9, exoenzyme [18]; 10, citric acid [51]; 11, alginate [10]; 12, rhamnolipid [19].

cillium charlesii, *Monilia fructicola*, *Calviceps fusiformis*, *Dictyostelium discoideum*, *Polyporus tumulosus*, *Saccharomyces cerevisiae*, *Hansenula, capsulata*, *Candida* sp., *Rhodotorula glutinis*, *Schizophyllum commune*, *Microsporium qunckeanum*, *Trichosporium citaneum*, and *Plectania occidintalis*. This relationship is not absolute, since a number of eukaryotes produce oxidised extracellular polysaccharides; however, the rates of production are exceedingly low (Table 1). Similarly some bacteria do produce homo-polysaccharides from glucose; e.g. curdlan is produced by *Alcaligenes faecalis* [47] and glucomannan from methanol by *Methylophilus* sp. [37]. These observations may be explained in terms of exopolysaccharide production acting as a means of ATP turnover when carbohydrate uptake is not controlled to a sufficiently low value during conditions of carbon excess.

Exopolysaccharide synthesis as a means of ATP turnover

If polymer synthesis operates as a means of ATP turnover [32,35] then the nature of the carbon source and the ATP/O quotient will determine to what extent ATP turnover and polysaccharide production are matched. For example, if an organism with an ATP/O quotient of 3 produces an oxidised polymer like succinoglucan at a rate similar to that produced by *A. radiobacter* (Table 1) then the amount of ATP generated during the production of the oxidised constituents of the polymer would be considerably higher than that which is utilised during synthesis of the total molecule and therefore expolysaccharide production would not serve as an efficient means of ATP turnover [35]. However, for organisms with ATP/O quotients of approximately 1.5 this polymer serves as a good means of ATP

turnover as the ATP generated in the synthesis of the oxidised moieties of the molecule is utilised during the polymerisation of the hexose backbone [33,35]. Thus in organisms possessing a high degree of energy conservation (high $Y_{O_2}^{max}$) exopolysaccharide production can only be invoked as an effective means of turning over ATP if a homo-unoxidised polysaccharide is produced. In these organisms alternative means of turning over ATP must be operational and these may also operate in organisms capable of rapid rates of exopolysaccharide production. For example, the respiratory activity in excess of growth requirements (N-limited q_{O_2} minus C-limited q_{O_2}) in *A. radiobacter* and *E. herbicola* grown under nitrogen limitation is the same in polymer-producing wild type and polymer-negative mutant [33,35]. However, in both cases the rate of substrate uptake by the mutant is considerably lower than that of the wild type. Thus energy dissipation or ATP turnover is occurring at the same level in both the wild type and the mutant; however, in the latter this is dissociated from exopolysaccharide production. Therefore not all organisms possessing low $Y_{O_2}^{max}$ values will necessarily exhibit high rates of exopolysaccharide production from glucose. The potential q_p for exopolysaccharide production will depend on other factors such as the extent to which substrate uptake is regulated.

Mutation for increased rates/altered structure of exopolysaccharides

The correlation between the $Y_{O_2}^{max}$, rate of ATP turnover and the oxidation state of the exopolysaccharide produced suggests that mutation programmes aimed at selecting organisms that produce polymers with altered chemical composition could result in a major impact on the rate of production if the energetic balance of polymer production is changed significantly. This has indeed been found to be the case. The amount of a truncated form of xanthan gum lacking uronic acid produced by genetically engineered strains of *Xanthomonas campestris* is two orders of magnitude lower than that observed for the wild type producing xanthan gum [3,61]. Exopolysaccharide production may be regarded as an integrated metabolite-excreting system where ATP production and utilisation is co-ordinated. This may explain why the structures of exopolysaccharides appear to be strongly conserved [33,35,41]. The excretion of mixtures of organic acids as well as a homo-polysaccharide may be regarded as a similar system; e.g. *Acetobacter aceti* produces cellulose and acetic acid from glucose. Mutation of producers of complex oxidised exopolysaccharides so that the oxidised moieties are still produced but excreted separately may provide a means of producing homo- or truncated polymers at considerably faster rates than can be achieved currently because the energetic balance of synthesis is maintained.

Energy dissipation during metabolite production

It is proposed that, under conditions of glucose excess, all obligate aerobic micro-organisms are capable of dissipating energy to an extent that is inversely proportional to their $Y_{O_2}^{max}$. The extensive excretion of partially oxidised metabolites appears to be confined to organisms with poor $Y_{O_2}^{max}$ values. With exopolysaccharide production the ATP produced as a result of the synthesis of the oxidised moieties is utilised during polymer production and there is a strong correlation between growth efficiency and the type of exopolysaccharide produced. With other metabolites, e.g. gluconic acid or citric acid, where metabolite production is associated with a net production of energy, some other form of ATP turnover or energy dissipation mechanism must be operational and it is the dissipation of this potential energy that influences the rate of metabolite excretion. It has been previously suggested [30,55] that the rate of production and yield of such metabolites may be increased if the producing organism is provided with a means of dissipating the concomitant energy produced. If this is the case then the addition of uncouplers to aerobic cultures excreting significant levels of organic acids (not exopolysaccharides or fermentative end-products) would be expected to cause an increase in the rate of their production. This has been shown to be the case: addition of the uncoupler 2,4-dinitrophenol to nitrogen-limited cultures of *Klebsiella aerogenes* resulted in very high rates of 2-ketogluconate production [43]. In certain

industrial processes conditions have developed empirically that effectively achieve the same ends. For example, in citric acid production the growth and production phases are quite distinct. The former is controlled at pH values near 5 whereas the latter occurs at pH 2.5–3.5. At pH values near the pK_a value of an organic acid the proportion of the undissociated acid is at its maximum and this species diffuses into the cell and may cause uncoupling between respiration and ATP production [22,31,34]. Thus the rate of citric acid production is markedly influenced by the pH value and within a single organism a change from pH 3.5 to 2 caused a 5-fold increase in the rate of citric acid production [29] (Table 2). Indeed, the addition of organic acids to fermentations results in increased rates of citric acid production [39]. Citric acid production using bacteria at pH 7.0, e.g. *Bacillus licheniformis*, results in very poor rates of production (Table 2). This probably occurs because this organism has a relatively high growth efficiency [18] and furthermore the organism does not tolerate pH values as low as 2.5; consequently energy dissipation by uncoupling respiration at low pH values cannot be achieved. On the basis of the arguments outlined, it may be expected that high rates of organic and amino acid production (indeed, any metabolite that leads to the net production of ATP or reducing equivalents) could be achieved by an organism such as *Bacillus acidocaldarius*, a thermophilic acidophile capable of growth at pH 3.0 and exhibiting very low growth yields from oxygen [17]. For these metabolites the ability to tolerate low pH values and the possession of a low ATP/O quotient to facilitate energy dissipation appears to be a prerequisite. In the production of gluconic acid, the process organism, *Gluconobacter oxydans*, has precisely these attributes [44] (Table 2). Similarly, high rates of gluconic acid production can also be achieved by eukaryotes [31] via the glucose oxidase/catalase system which facilitates rapid energy dissipation.

The maximum rate of production of any given metabolic product can be estimated if the energetics of synthesis and the $Y_{O_2}^{max}$ value of the producing organism are known. Industrial strains that produce organic acids and polymers at high rate fit into the category of those possessing low $Y_{O_2}^{max}$ values. A systematic examination of other organisms that possess these properties (low $Y_{O_2}^{max}$ values) may lead to the identification of new process organisms. As pointed out earlier, not all organisms with low ATP/O quotients will necessarily excrete large amounts of metabolic products. What is suggested is that these organisms have the capacity to turnover ATP (dissipate energy) at much higher rates than tightly coupled organisms. This ATP turnover can be achieved either by the complete oxidation of glucose to CO_2 or by the partial oxidation of glucose to metabolic products to achieve the same level of ATP turnover. Thus it will be necessary to mutate some organisms to achieve rapid rates of partial oxidation of glucose to the desired metabolite.

The results reported here suggest that there may be considerable scope for improving existing processes by using different micro-organisms. This is particularly so in the case of amino-acid production where bacteria (pH 7) have been used and the use of an organism with a low $Y_{O_2}^{max}$ value coupled with the ability to tolerate low pH values could result in a significant improvement of production rates. In other cases like bio-surfactant and lipids the production of the lipid moieties results in a large requirement for ATP and even at low relative ATP/O quotients this results in poor rates of production. For these, a supply of preformed lipids will reduce the ATP requirement for synthesis and could result in a significant improvement in production rates.

Similar arguments apply to the use of microbes to carry out various chemical transformations. If the particular chemical transformation is associated with a net production of reducing equivalents or ATP some means of dissipating this energy must operate before rapid rates of transformation can occur. Here again organisms with poor $Y_{O_2}^{max}$ values would be expected to carry out these reactions at considerably faster rates than those that are highly coupled.

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