Importance of oxygen budgets in fermentations

It is of crucial importance to have indications of the amounts of oxygen required per substrate utilized and product formed. As examples, 3 products (lactic acid, 2,3-butanediol and Single Cell Protein) have been chosen in highlighting this aspect when produced from either of the three one-carbon compounds methane, methanol or trioxane. Current large-scale fermentations for ethanol or for lactic acid require very little input of oxygen. It makes these processes successful. Fermentations requiring oxygen input such as the production of Single Cell Protein are struggling because of oxygen requirements. It indeed is very well known that oxygen budgets in commodity production processes are a key aspect in their overall economics.

Data are presented in the Table below in order to demonstrate the superior properties of trioxane over the two other one-carbon componds in this overriding aspect.

Oxygen budgets either per product or substrate can be seen in the columns O2 per product and O2 per substrate. The outcomes readily demonstrate that trioxane is by far more suitable than either methane or methanol in each of the three situations.

Product	Feedstock	Yield product/feedstock (w/w)	Input oxygen/product (w/w)	Input oxygen/feedstock (w/w)
Lactic acid	Trioxane	1.00	0	0
Lactic acid	Methanol	0.94	0.53	0.50
Lactic acid	Methane	1.88	1.07	2.00
2,3-butanediol	Trioxane	0.50	0.18	0.09
2,3-butanediol	Methanol	0.47	1.24	0.58
2,3-butanediol	Methane	0.94	2.31	2.17
SCP	Trioxane	0.56	0.64	0.36
SCP	Methanol	0.57	1.47	0.83
SCP	Methane	1.05	2.60	2.67

Indicative oxygen budgets

In the Table on oxygen budgets 2 fermentation end products (lactic acid and 2,3-butanediol) are given as representative products to be produced under (semi-)anoxic conditions. Single Cell Protein is given as an example of an oxic process.

In the case of lactic acid and butanediol, no increase in biomass is assumed in order to have a simplified picture. However, biomass will have to be produced which will change the picture, but the basic principle remains unchanged. An important aspect here is if cells have ample energy (ATP) available for their growth. In the case of organisms employing the RuMP-pathway, ATP supply seems warranted. Quayle and Ferenci in 1978 in their review clearly described the ATP yield under various assumptions.

If an organism employs the RuMP-route, then

1) 6 formaldehyde + 2 ADP + 2 Pi + 2 NAD = 2 pyruvate + 2 ATP + 2 NADH2

which compares to the glycolysis:

2) 1 glucose + 2 ADP + 2 Pi + 2 NAD = 2 pyruvate + 2 ATP + 2 NADH2

By adopting the equation 1) and by taking

3) 2 pyruvate + 2 NADH2 = 2 lactic acid + 2 NAD

or

4) 2 pyruvate + 1 NADH2 = 1 butanediol + 2 CO2 + 1 NAD

it follows

5) 6 formaldehyde + 2 ADP = 2 lactic acid + 2 ATP

and

6) 6 formaldehyde + 2 ADP + 1 NAD = 1 butanediol + 2 CO2 + 2 ATP + 1 NADH2

In the cases of methane and methanol it is assumed that per molecule of substrate 1 O2 or 0.5 O2 is required, respectively, to oxidize these compounds to the intermediary formaldehyde. For the oxidation of each 1 NADH2 in excess to NAD, 0.5 O2 is taken.

For SCP, it is by far more complex to arrive at indicative oxygen budgets as affected by the nature of the growth substrate. The yield of biomass per methane or per methanol has been a long-standing topic starting with a paper on theoretical grounds by Van Dijken and Harder (1975). Experimental work on substrate consumed as affected by the nature of the growth substrate dates back to 1972 when Harwood and Pirt obtained a balance for biomass production under methane-limited conditions in chemostat. Experimental approaches have continued up until recently, for instance by Gilman et al 2015. In the methanol field, experimental biomass yields are equally a cumbersome topic. Anthony (1982) has discussed this aspect, also while presenting experimental results. Others have presented both theoretical and experimental results, for instance Leak and Dalton (1986). In a recent paper by Carnicer et al 2016, on an organism containing an NAD- rather than a PQQ-dependent methanol dehydrogenase, interesting results on biomass yields were presented. It was made explicit that growth rate had a great effect on the yield.

It is beyond the scope of this website to provide detailed data on yields either on methane, methanol or trioxane. For the sake of simplicity, a balance for growth on glucose is taken from a paper by Tempest and Neijssel who quote for their organism and for the conditions employed a balance of:

7) 1 $C_6H_{12}O_6$ + 1 NH_3 + 2 O_2 = 1 $C_4H_7O_2N$ + 2 CO_2 + 4 H_2O

The cell yield here is rather high which can be explained by the high growth rate of 0.85 h^{-1} which will be lower for C1-utillizing bacteria. Since only figures for comparison are sought here, these values are taken nevertheless as they allow a convenient starting point for calculations. When extrapolated to the formaldehyde situation, it follows:

8) 6 formaldehyde + 1 NH_3 + 2 O_2 = 1 $C_4H_7O_2N$ + 2 CO_2 + 4 H_2O

The 6 formaldehyde are to be seen as 2 trioxane in the above equation.

As before, in the cases of methane and methanol it is assumed that per molecule of substrate 1 O_2 or 0.5 O_2 is required, respectively, to oxidize these compounds to the intermediary formaldehyde. Furthermore, no ATP yield from methane into formaldehyde is assumed. For methanol, some additional useful energy generation is assumed, which probably holds especially for the NAD-dependent methanoldehydrogenase-containing bacteria. Some 5% yield increase on methanol over formaldehyde is assumed.

The comparative calculations are based on the same growth rates, same medium composition, etc. In reality, it will be difficult to obtain relatively high growth rates especially on methane but also on methanol. The higher the growth rate, 1) the less maintenance requirement (higher yield) and 2) importantly a higher volumetric productivity. These two aspects place trioxane in a by far better position than anticipated from the values as given in the Table.

References

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