CBP 732: Assignment 3

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1 Introduction

As the world faces new challenges such as global warming and a drive to move away from fossil fuels there is a push towards the use of more renewable resources. The use of microbes to produce chemicals previously produced from the petrochemical industries has received attention of late. The chemicals intended to be produced are carboxylic acids such as fumaric, malic and succinic. There has been a cost limitation to the production of these chemicals with microbes since the processes are not yet as efficient as the petrochemical industries. To improve the efficiencies the yield on a substrate has to be maximised, the cost of additional chemicals needs to be decreased and the amount of by-products needs to be decreased. Saccharomyces cerevisiae has a long history of being used in the baking, brewing and fuel industries, this has caused it to be well studied. As a result of this S. cerevisiae is a favourite for the production of organic acids even though it does not produce them naturally. Another factor is that S. cerevisiae does not produce any chemicals that would be dangerous for human consumption, this is important since many carboxylic acids are used in the food industry (Abbott et al., 2009).

2 Goals of microbial production

Eukaryotes have been selected for this since prokaryotic organism are not able to produce organic acids at low pH values. In order to decrease the cost of production it has been found that operating at low pH ranges is most ideal. This is as a result of the nature of the product and at these low pH ranges the carboxylic acids are in there undissociated forms. This means that additional chemicals do not have to be use in order to get the acids into this form. The product yield needs to be high, producing a product concentration of at least $1\,\mathrm{mol\,l^{-1}}$. It would also be ideal if very few other by products are produced such as ethanol in the case with S. cerevisiae. This will aid the cost as the downstream processing of the medium will be far simpler with less chemicals. A later goal of this would be the use of substrates other than glucose however the focus now is simply the production of the organic acids.

3 How is this being achieved?

As stated previously S. cerevisiae does not produce any of the required acids under normal conditions. It is therefore necessary that genetic modifications be made to the microbe that will allow it to do so. Before we explore the genetic modifications that would this possible lets explore there thermodynamics. It can be shown that the maximum theoretical yields of organic acids (fumaric and succinic acids) can be quite high however this does not mean that these processes are possible since no metabolic pathways have been taken into account (Malherbe et al., 2003).

Figure 1 shows that at low pH values the fumaric acid is in an undissociated form and can also be found in a solid form. This is useful as a solid form of the acid could potentially be removed from the reactor during the reaction and promote the forward reaction. This is also beneficial since the osmotic and ionic stress is low at low pH values.

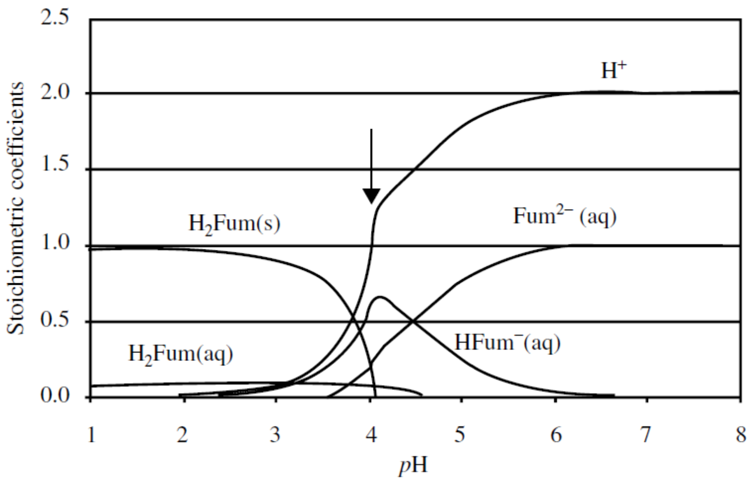

Figure 1: Stoichiometry of fumaric acid species and H as a function of pH(Jamalzadeh et al., 2012)

Transporters All organic acids are produced on the inside of the cell, for these acids to be moved to the medium in which the cells are suspended, transporters have to be used. There are three types of transporters namely a symporter, uniporter and an antiporter. All three of these operate at different pH ranges and have different energy costs.

It has been found that there is a minimum concentration gradient between the acid on the inside and outside of the cell. This concentration is shown by the dashed line on Figure 2. On this graph is can be seen that the highest achievable concentration at a pH range where the acid will be in its undissociated form is below the minimum concentration. This concentration can be seen to be produced by an antiporter. If an antiporter could be encoded into the cell that had a higher energy cost than a regular antiporter the production of fumaric acid at low pH values may be possible.

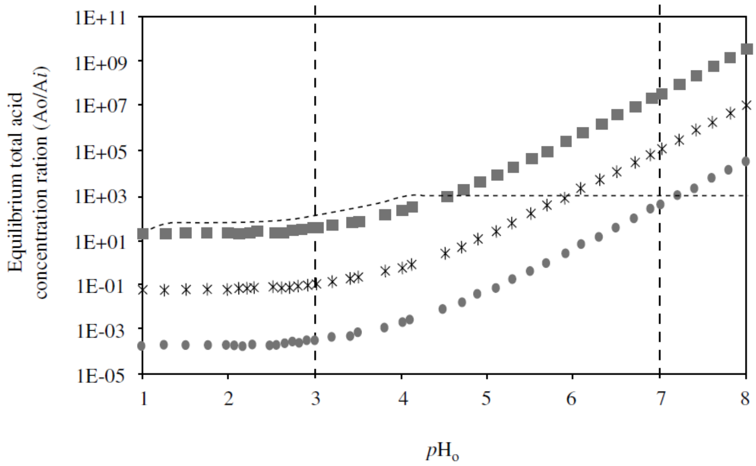

Figure 2: Outin equilibrium total acid concentration ratio (AoAi) as a function of pH_o forantiporter (squares), uniporter (stars), and symporter (circles) (Jamalzadeh et al., 2012)

4 Genetic modifications

In order to satisfy all the requirements it is necessary to genetically modify S. cerevisiae to produce the organic acids in the required concentrations. However, wild strains have been found that produce some of the citric acid cycle products the yields are not satisfactory. As a result of the research already done with S. cerevisiae genetic modification are relativly simple. In an effort to produce malate, they overexpressed the pyruvate carboxylase and malate dehydrogenase as well as the expression of the malate transporter. It was found that this achieved a high malate titer of $59\,\mathrm{g\,l^{-1}}$. This shows that it is possible to get S. cerevisiae to produce the required organic acids however it is not a simple process as many different reactions have to be taken into account.

Another goal of genetic modifications to yeast is to allow yeast to ferment on substrates other than glucose. It has been found that if a bacterial xylose isomerase is expressed in yeast, yeast has the ability to convert xylose into ethanol (Brat et al., 2009). This suggests that it is possible for yeast to be genetically modified to consume a variaty of substrates. This will help decrease the cost of production since cheaper feed stocks can then be used.

References

Abbott, DA, RM Zelle, JT Pronk, and AJA Van Maris (2009). "Metabolic engineering of Saccharomyces cerevisiae for production of carboxylic acids: Current status and challenges". In: *FEMS Yeast Research* 9.8, pp. 1123–1136. ISSN: 15671356. DOI: 10.1111/j.1567-1364.2009.00537.x.

Brat, D, E Boles, and B Wiedemann (2009). "Functional expression of a bacterial xylose isomerase in Saccharomyces cerevisiae". In: *Applied and Environmental Microbiology* 75.8, pp. 2304–2311. ISSN: 00992240. DOI: 10.1128/AEM.02522-08.

Jamalzadeh, E, H Taymaz-Nikerel, JJ Heijnen, WM van Gulik, and PJT Verheijen (2012). "A thermodynamic analysis of dicarboxylic acid production in microorganisms BT - Thermodynamics in biochemical engineering". In:

Malherbe, DF, M Du Toit, RR Cordero Otero, P Van Rensburg, and IS Pretorius (2003). "Expression of the Aspergillus niger glucose oxidase gene in Saccharomyces cerevisiae and its potential applications in wine production". In: *Applied Microbiology and Biotechnology* 61.5-6, pp. 502–511. ISSN: 01757598. DOI: 10.1007/s00253-002-1208-0.