Malic acid production by *Aspergillus oryzae*: the role of CaCO₃

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The petrochemical industry has been the focus of research and development for many years and is the norm for many chemical processes. The potential to produce bulk valuable chemical precursors, like malic acid, from microbial bioconversion has been proposed for many years but is still being researched to a lesser extent. Malic acid is a speciality chemical that is mainly used in the food and beverage industry. *Aspergillus oryzae* has shown to be a natural producer of malic acid using various renewable sugar substrates and nitrogen sources. Before venturing into this avenue for biomass-based production of malic acid, the influence of process parameters needs to be investigated. CaCO₃ is the go-to buffer in biochemical research since it not only controls the pH but also releases CO₂ which is a valuable co-substrate in the TCA cycle. However, the problem occurs in downstream processing where the fermentation broth has to be acidified in order to remove the valuable metabolites. This requires time and resources which lowers the economic viability of this biochemical route. Investigating the role CaCO₃ plays in the metabolic pathway of *A. oryzae* will allow researchers to further optimise this process.

The objective of the current study was to determine the influence of CaCO₃ concentration on the ability of *A. oryzae* to produce malic acid. The microorganism used was wild-type *A. oryzae* strain NRRL 3488. Powdered CaCO₃ was added to the medium at 3 different concentrations: 20 g/L, 80 g/L and 120 g/L. The experiments were performed in shake flasks using a one-stage fermentation approach (traditionally growth and acid-production performed separately) for 216 hours. The concentrations of metabolites were measured with an HPLC fitted with a refractive index detector.

It was found that 120 g/L CaCO₃ performed the best with 89 % of the glucose consumed where 45 % was converted to malic acid. Following is 80 g/L CaCO₃ with 78 % glucose consumption (35 % to malic acid) and lastly 20 g/L CaCO₃ with 68 % of the glucose consumed (19 % to malic acid). It was also found that that up to 4 times more biomass was produced with 80 g/L and 120 g/L CaCO₃ when compared to 20 g/L. Of interest, it was decided to add 80 g/L powdered CaCO₃ at the start of the fermentation, after 24 h and in pellet form. It was found that this did not significantly improve the amount of malic acid produced but did influence the morphology of the fungus as seen in Figure 1 below.

The results from these experiments confirm that the amount of CaCO₃ in the system influences the malic acid capabilities of *A. oryzae*. This suggests that CaCO₃ plays a more significant role than just controlling the pH. When large quantities of CaCO₃ is present, it forms a boundary layer around the cells which could, in turn, inhibit the diffusion of oxygen into the cell. This suggests that the mechanism for malic acid production is not, in fact, nitrogen starvation but a combination of excess CO₂ and limited O₂.
Figure 1: (A) Filamentous morphology seen when CaCO3 added from start, (B) Fungal pellets when CaCO3 added after 24 h.