**Process development for the bioproduction of L-malic acid with *Aspergillus oryzae* DSM1863**

Vanessa Schmitt1, Katrin Ochsenreither1

*1 Karlsruhe Institute of Technology (KIT), Institute of Process Engineering in Life Sciences, Section II: Technical Biology, 76131 Karlsruhe, Germany*

*\*Corresponding author: Vanessa.schmitt@kit.edu*

**Highlights**

* Repeated-Batch cultivations increase the maximal productivity compared to fed-/batch cultivations.
* The highest product titers can be achieved with calcium carbonate as neutralization agent.

**1. Introduction**

L-Malic acid is a C4-dicarboxylic acid and a potential key building block for a bio based economy. Currently, malic acid is synthesized petrochemically. It is mainly used in the food and beverages industry, in metal cleaning and pharmaceuticals. Due to its bifunctionality, malic acid can also serve as polymerization starter unit and cross linker. A sustainable alternative to petroleum based synthesis is the microbial production of L-malic acid from renewable resources [1]. As CO2 fixation is involved in biosynthesis, high yields are possible and at the same time greenhouse gases can be reduced. The mould *Aspergillus oryzae* is known for its high production capacity for L-malic acid and has received GRAS status, making it a promising candidate for industrial scale fermentations [2]. The microbial L-malic acid production with *A. oryzae* DSM1863 was evaluated in laboratory scale experiments to gain a better process understanding and to identify obstacles for the transformation into the industrial scale. Different aspects including pH regulation and cultivation strategy were investigated.

**2. Methods**

Batch, fed-batch and repeated-batch cultivations were performed in in 500 mL shake flasks with baffles and a working volume of 100 mL. A defined medium with glucose as substrate was used. Calcium carbonate was added in advance of the fermentation and as required during the fermentation for pH-regulation. Furthermore, different neutralization agents, including carbonates and hydroxides of calcium, magnesium and sodium, were tested in 2 L bench top fermenters with a working volume of 1.4 L. All fermentations were performed for at least one week and samples were taken daily. Substrate, product and by-product concentrations were measured via HPLC to determine the process characteristics (titre, yield and productivity). Additionally, pictures of the fungi were taken to document macroscopic changes of the morphology.

**3. Results and discussion**

In repeated-batch cultivations, the productivity was higher and the productive phase was prolonged compared to batch cultivations, in which the L-malic acid concentration reached its maximum after about one week. On the other hand, the productivities in the fed-batch cultivations were lower as in repeated batch cultivations.

The addition of calcium carbonate was proven to be crucial for the L-malic acid production. In reactor experiments with alternative neutralizing agents product titers were significantly lower. Hence, the positive effect of calcium carbonate cannot only be explained by the positive effect of pH-regulation. In addition, the precipitation of L-malic acid as calcium malate plays an important role in the L-malic acid production, as it lowers the acid concentration within the media, thereby preventing product inhibition.

**4. Conclusions**

In summary, the results obtained from shake flask cultivations proved the suitability of repeated‑batch fermentations for the L-malic acid production with *A. oryzae*. The high productivities in repeated-batch fermentations compared to lower productivities in batch and fed-batch cultivations suggest that product inhibition limits the L-malic acid production, while substrate inhibition is of minor importance. The next step would be the upscaling of the repeated‑batch fermentation to reactor scale. Furthermore, continuous fermentations with in situ product recovery seem to be an interesting research topic.

Calcium carbonate proved to be the best neutralizing agent for the L-malic acid production with *A. oryzae* DSM1863 in terms of product concentrations by preventing product inhibition. However, by resolving the calcium malate with sulfuric acid in downstream processing, huge amounts of gypsum are produced, generating extra costs for waste disposal. Therefore, liquid neutralizing agents like sodium carbonate or hydroxide should be considered as an alternative, despite the lower product titers observed. Additionally, the issue of product inhibition should be tackled by strain development and/or by applying in situ product removal.

**References**

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