In-depth analysis of *A. niger* metabolism during industrial fed-batch fermentations

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Introduction

Aspergillus niger is used industrially to produce different products, e.g. citric acid and enzymes. For the production of glucoamylase the fungus is grown in a fed-batch process.

The process has the following characteristics:

- High initial glucose concentration
- Oxygen limitation during most of the fermentation
- Initial formation and later reconsumption of large amounts of byproducts (mainly glycerol, mannitol, erythritol, and citrate)

The aim of this study was to i) provide detailed physiological information about the glucoamylase production process and ii) examine relationships between different process parameters important for process design.

Results

A model process mimicking the industrial process was set up in 2L scale. The data was treated to remove effects of dilution and the fermentations were divided into phases based on the observed metabolism. An example of such a fermentation is shown in figure 1, and the corresponding yield data is shown in table 1.

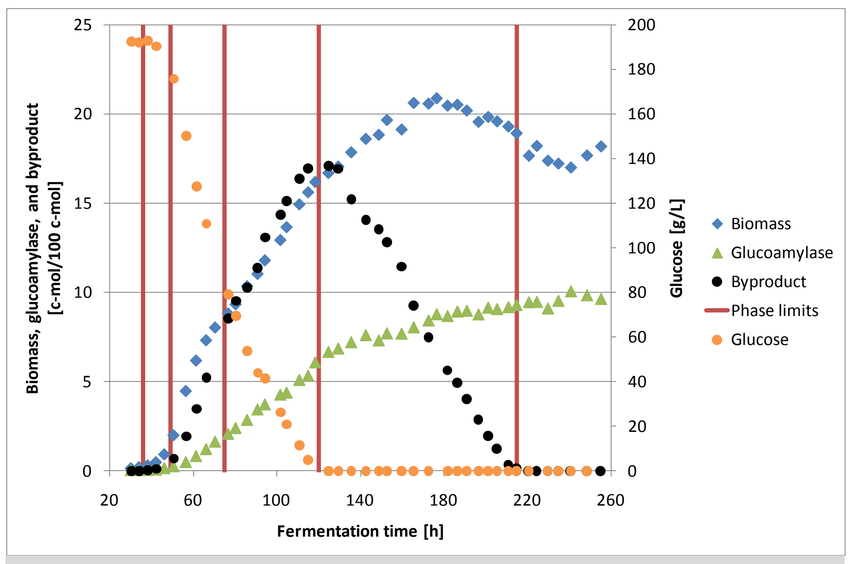
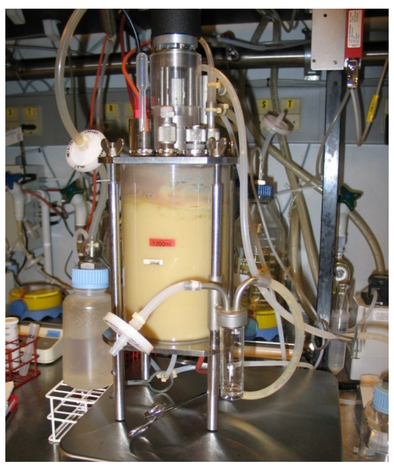


Figure 1. Model fed-batch fermentation

The phases are from left to right: Germination/lag, exponential growth, transition phase (oxygen limited), oxygen limitation (linear growth), mixed substrate utilization, and carbon limitation.



An alternative process with low glucose concentration was set up to examine the effect of osmolarity. To avoid carbon limitation the process was dosed proportional to oxygen uptake which is fairly linearly related to glucose uptake. An example is shown in figure 2, and corresponding yield data is shown in table 2.

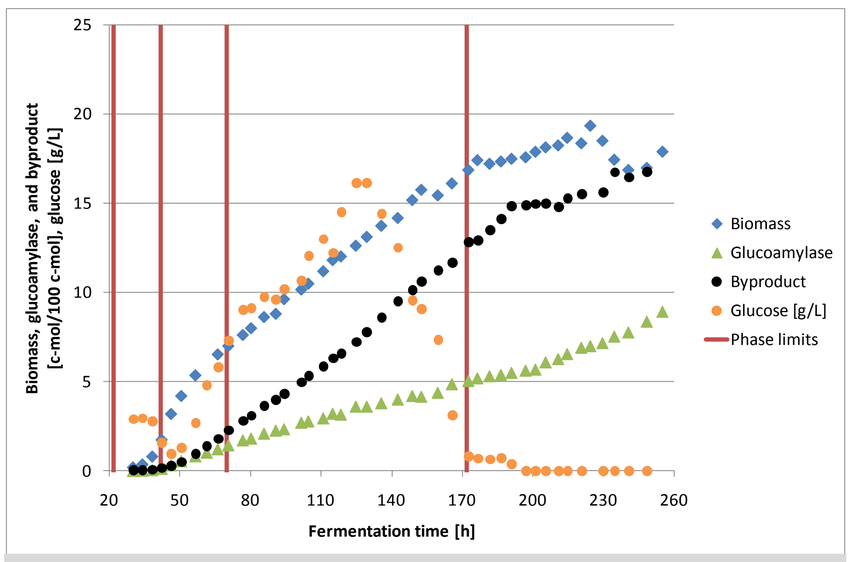


Figure 2. Alternative fed-batch fermentation

The phases are from left to right: Germination/lag, exponential growth, transition phase, oxygen limitation (linear growth), and mixed substrate utilization.

Table 1. Average yields from the model process.

	Exponential growth	Transition to oxygen limitation	Oxygen limitation (linear growth)	Mixed substrate utilization[1]
Y_{SO_2}	29.5	24.8	27.6	78.5
Y_{SCO_2}	35.7	28.4	31.5	79.4
Ysx	43.6	28.0	21.0	6.0
Ysp	4.5	5.5	11.2	8.5
Ysbyproduct	16.1	24.6	25.3	-52.1
Yscitrate	0.0	4.6	0.6	-4.4
Ysmannitol	0.0	1.0	11.6	-13.4
Ysarabitol	0.0	0.3	0.4	-0.7
Yserythritol	0.0	2.6	4.4	-8.5
Ysglycerol	16.1	16.0	8.0	-24.2
Ysfumarate	0.0	0.1	0.2	-0.3
Balance	100.0	86.5	89.0	93.9

[1]Based on total carbon uptake (glucose + metabolites).

Table 2. Average yields from the alternative process.

	Exponential growth	Transition to oxygen limitation	Oxygen limitation (linear growth)	Mixed substrate utilization
Y_{SO_2}	25.2	28.3	32.5	62.4
Y_{SCO_2}	28.5	31.6	36.6	66.6
Ysx	46.5	33.0	24.2	5.8
Ysp	3.5	8.3	8.2	9.8
Ysmetab	3.6	11.3	23.7	8.5
Yscitrate	0.0	0.4	1.3	2.2
Ysmannitol	0.0	5.2	16.7	6.9
Ysarabitol	0.0	0.4	0.4	0.6
Yserythritol	0.0	1.8	1.8	1.6
Ysglycerol	2.7	3.4	3.4	-2.8
Ysfumarate	0.0	0.1	0.1	0.0
Balance	82.1	84.1	92.7	90.6

Summary

Byproduct formation is by default expected to lead to lower yield of the desired product. This was shown not to be the case for the glucoamylase process. During oxygen limitation of the model process a lot of byproducts were formed. However, the glucoamylase yield (Ysp) was at it's maximum, table 1. Hence, it was seen that phases with high byproduct yields also featured high glucoamylase yields. Furthermore, the formed byproducts were recycled and turned into product in a later phase.

Glycerol formation is a response to high osmolarity[1]. Reducing glucose concentration decreased the formation rate of byproducts, but because all other rates decreased as well and mannitol production substituted glycerol production the byproduct yield remained unchanged. Product yield was lower at low glucose concentration. It therefore did not seem to be straight forward to reduce byproduct formation without reducing product formation as well.

In carbon-limited chemostats the glucoamylase productivity has been correlated to growth[1]. In the present study no growth relation was observed. Reduction in growth and even autolysis did not affect product formation noticeably. In the model process the glucoamylase productivity was reduced when glucose was depleted, which was expected from known induction characteristics of the promoter[2].

Acknowledgement

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References:

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[2] E.g. Ganzlin, M., Rinas, U., 2008. In-depth analysis of the Aspergillus niger glucoamylase (glaA) promoter performance using high-throughput screening and controlled bioreactor cultivation techniques. J Biotechnol 135, 266-271.