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Cellulolytic enzyme production and response to pH and temperature by *Trichoderma reesei*

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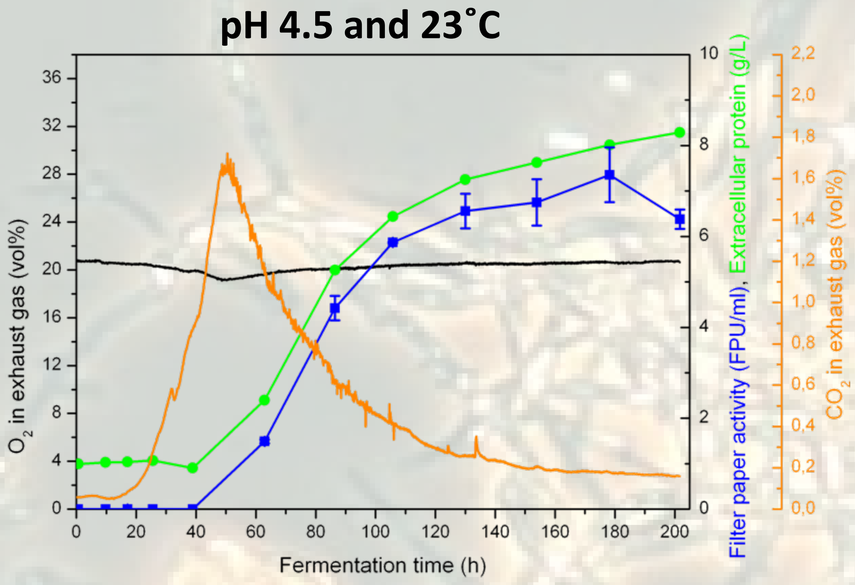
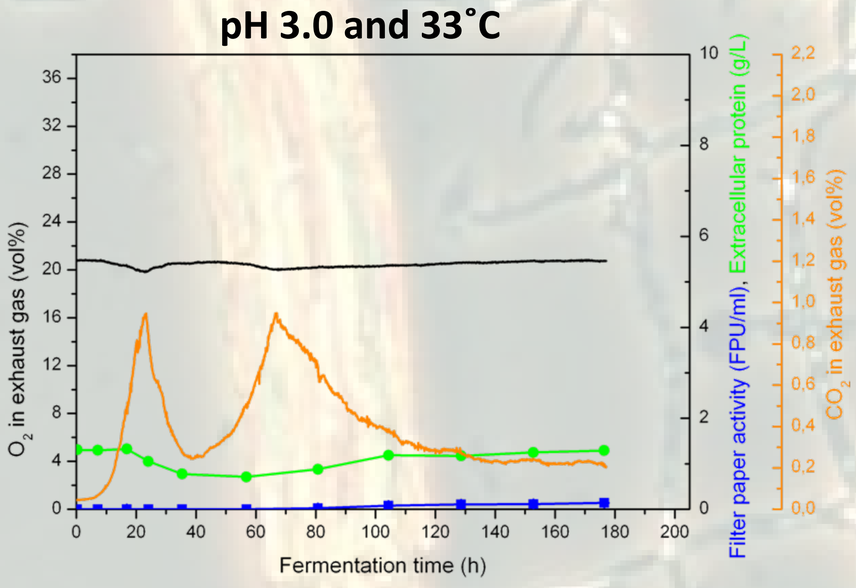
Fermentation characteristics

pH 4.5 and 23˚C

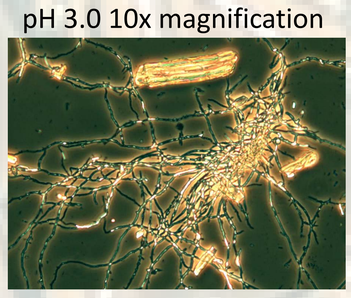


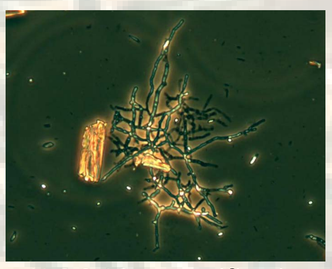
pH 3.0 and 33˚C



A series of batch fermentations of *Trichoderma reesei* RutC30 grown on Avicel (25 g/L) have been conducted at three different pH values (3.0, 4.5 and 6.0) and four different temperatures (23, 25, 28 and 33˚C). Two of the extremes for the results found are shown in the figures to the left. The results at pH 4.5 and 23˚C showed the best enzyme production which started after the peak in CO_2 production. In contrast at pH 3.0 and 33˚C enzyme production was very low and two separate growth phases were observed, the first one being growth on the peptone present in the fermentation media and the second one was growth on cellulose.

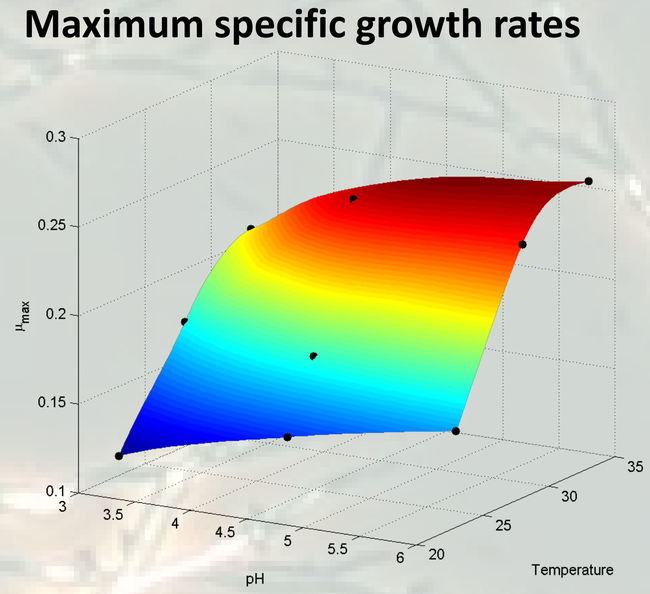
pH 3.0 10x magnification





pH 6.0 10x magnification

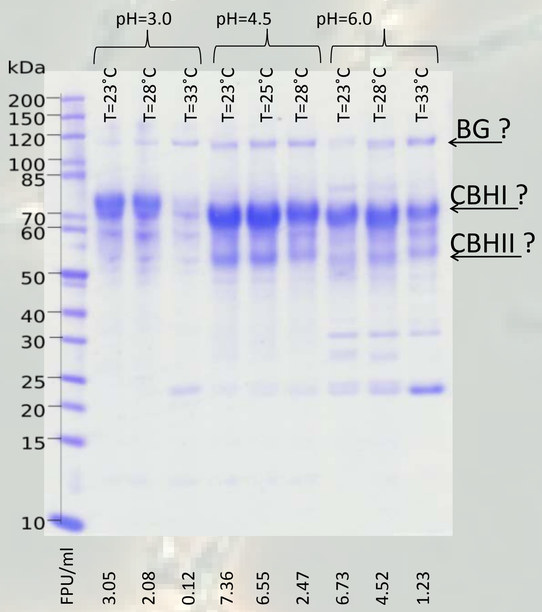
Maximum specific growth rates



The maximum specific growth rate (μ_{max}) was calculated based on the CO_2 production. In this case μ_{max} reflects *T.reesei* growth on peptone rather than cellulose. From the figure above, it is seen that growth rate increases with increasing temperature. The optimal pH for growth is between 4.5 and 6.0.
The morphology which is seen in the figures to the left consisted of long hyphae with regular branching. However, at pH 6.0 the hyphae were short and highly branched and sporulation was observed after a few days cultivation. This was consistent with extensive foam production.

Enzyme production is dependent on pH and temperature

SDS-PAGE of protein samples

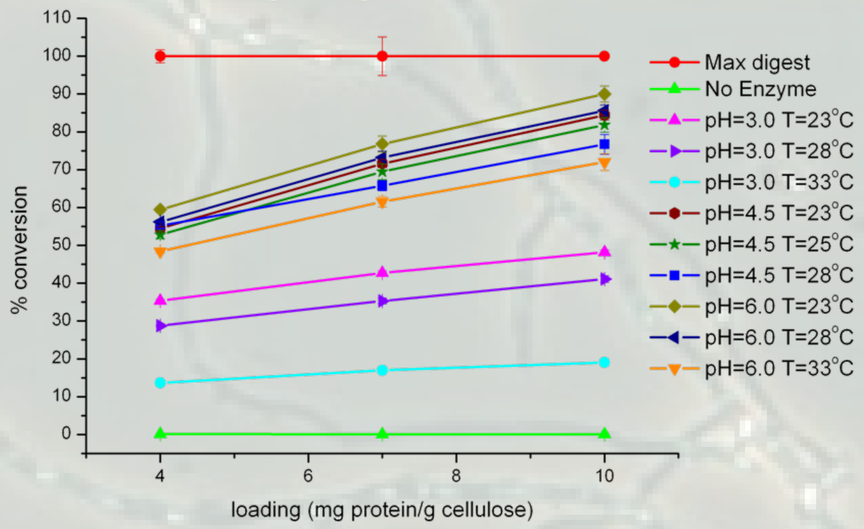


Filtered fermentation samples were analysed by SDS-PAGE and showed the highest amount of what is thought to be CBHI and CBHII from the fermentations performed at pH 4.5. Only at pH 6.0 were proteins around 30 kDa observed.

pH	T	FPU/ml	Protein (mg/ml)	FPU/CO_2
3.0	23˚C	3.05	4.91	0.09
3.0	28˚C	2.08	4.59	0.05
3.0	33˚C	0.12	1.25	0.004
4.5	23˚C	7.36	8.02	0.16
4.5	25˚C	6.55	8.03	0.14
4.5	28˚C	2.47	3.85	0.05
6.0	23˚C	6.73	6.29	0.02
6.0	28˚C	4.52	5.65	0.08
6.0	33˚C	1.23	2.69	0.02

An overview of the filter paper activity (FPU/ml), total protein production and FPU/ml compared to the CO_2 production in mol/L confirms the best performance from fermentations conducted at pH 4.5 and 23˚C. The value of FPU/CO_2 is an indication of how much enzyme has been produced compared to growth.

Hydrolysis of PCS



To test the performance of the fermentation broths pretreated corn stover (PCS) was hydrolyzed for 72 h at 50˚C. The assay was performed in a volume of 1 ml with 5% (w/v) of washed and ground PCS. Samples were filtered and analyzed by HPLC for glucose, cellobiose and xylose. A sample for max digest was provided by Novozymes, Davis, CA. The best performance was seen with fermentations conducted at pH 4.5 or 6.0 and temperature of 23˚C.

Conclusions

- The best fermentation conditions for enzyme production are pH 4.5 and 23˚C
- The performance of the enzymes produced was best at fermentations conducted at the lowest temperatures and pH 4.5 or 6.0, however for the latter there was excessive foaming during fermentation

Acknowledgements

I would like to thank KC McFarland and David Osborn from Novozymes, Davis, CA for helping me with the PCS hydrolysis assay.