**Influence of Different CO2 Concentrations in Aeration Air During Thermophilic Fermentation Of *Bacillus Caldolyticus.***

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**Highlights**

* Optical dissolved CO2-probe at 70 °C
* Fermentation of thermophilic bacterium
* Beneficial effect of increased CO2 concentrations
* Increased yield by reduced aeration rate

1. **Introduction**

In fermentation processes, the concentration of CO2 is a relevant parameter for the control of the metabolic function of microorganisms in the bioreactor. In most cases, CO2 is determined in the exhaust-gas-stream as a sum parameter of the metabolic activity in the bioreactor. In large scale bioreactor inhomogenities exist, which can influence product yield and product quality. Whereas small amounts of dissolved CO2 have advantageous effects on the production of amylase and proteases (Popovic *et al*., 2009 [1]; Bader *et al*., 2015 [2]), increased CO2 concentrations result in a decreased pH value in the cells followed by altered metabolic activity. Hence, it is beneficial to measure directly in the fermentation broth, instead of using the exhaust-gas-analysis. A slight increase of CO2-concentration can be achieved by a reduction of the aeration rate combined with a pressurized fermentation. Hence, aeration costs may be reduced and the yield can be increased simultaneously (Popovic et al. 2014 [3]).

1. **Methods**

Fermentation of *Bacillus caldolyticus* DSM 405 was performed in stirred tank reactor Biostat E (Sartorius AG, Germany) at 70 °C in batch mode. Amylase and protease activities were determined according to Manning and Campbell (1961) [4] and Strydom *et al.* (1986) [5]. The amount of dissolved carbon dioxide was calculated by the exhaust air composition (Sidor, Sick Maihack GmbH, Reute, Germany). CO2 measurement by in-situ probe was carried out by using YSI 8500 CO2 Monitor (YSI Inc., Yellow Springs, USA).

1. **Results and discussion**

In the Biostat E bioreactors a congruent response of in-situ probe and exhaust gas analysis could be observed (Figure 1).

Figure 1. Comparison of the CO2-quantification by in-situ probe (black dot) and exhaust gas measurement (blue diamond) (Bader *et al*., 2015) [2])

At atmospheric CO2 concentration, an amylase concentration of 5.5 U/mL was observed after 300 minutes whereas the increase in CO2 concentration to 5% (v/v) resulted in a maximal amylase concentration of 6 U/mL after only 180 min. Comparable effects of the increased CO2 concentration were observed for the protease production. Without increased CO2 concentration, 1.2 U/ml of protease were observed. At 5% (v/v) CO2, 2 U/ml were secreted after 210 min.

These results indicate to importance of reliable CO2 measurement during fermentation processes. Especially in large scale bioreactors these effects have to be taken into account due to high hydrostatic pressure and known inhomogenities.

1. **Conclusion**

In the presented work, the reliability of an optical dissolved CO2-probe was evaluated during the fermentation of the thermophilic microorganism *Bacillus caldolyticus* at a temperature of 70 °C over a period of 10 hours. The results were compared with exhaust-gas-analysis measurement. The applied optical dissolved CO2-probe showed comparable and reliable results during the whole fermentation process. This indicated the possibility of using thisin-situ measurement even under high temperatures.

**References**

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