

Process Development for Silage Inoculants

Optimization of Fermentation for *Lactobacillus sp.*

The Challenge

Silage is an animal feed ingredient produced by controlled fermentation of crops with high moisture content. The main objective of ensiling is the achievement of anaerobic conditions as quickly as possible, which causes the inhibition of undesirable microorganisms such as *clostridia* and *enterobacteria*. Thus, the nutritional value of the original crop is preserved. The optimum conditions can only be guaranteed by quick filling and proper sealing of the silo, in order to provide the necessary conditions for the following fermentation process. The fermentation process can be accelerated and improved by the addition of homofermentative and/or heterofermentative lactic acid bacteria (LAB).

The following application note partly describes the fermentation process development of *Lactobacillus sp.* for the application as silage inoculant using the DASGIP Parallel Bioreactor System. For the experiments eight reactors were used simultaneously to test the growth parameters.

≡ Biomin[®] ≡ (<http://www.biomin.net>) is a research-oriented company whose task is to improve animal health and the economic production of animals. Our core business is the development and manufacturing of innovative and natural feed additives and preservatives to stabilise feed materials.

Goal

The main goal of the process development was to determine the optimum parameters which lead to a maximum yield of active cells. These parameters were pH, temperature, agitation, consumption of base, the media components and their concentrations.



The DASGIP Parallel Bioreactor System allowed testing of different parameters in parallel fermentations at the same time. Therefore a sufficient amount of data could be generated for statistical evaluations to optimize medium composition and growth conditions. In this application report the optimization of the temperature and the pH is described.

Setting Up & Procedures

All experiments were carried out using various LAB strains in media containing for example glucose, yeast extract, peptone and salts. The fermentation time depended on the glucose concentration in the media.

The experiments were done with the DASGIP Parallel Bioreactor System containing eight 1500 mL fermentation vessels. The initial fermentation volume was 500 to 1000 mL. Subsequently scaling-up experiments were done in a 20 L lab fermenter to verify the optimized parameters in pilot scale. The key parameters during fermentation like pH, temperature, agitation and most importantly base consumption were controlled online and documented with the DASGIP Control 4.0 software. The same parameters were also measured in the pilot

Process Development for Silage Inoculants

Optimization of Fermentation for *Lactobacillus sp.*

scale fermentation allowing correlating the results of both systems.

A benefit of the DASGIP Parallel Bioreactor System was the simultaneous calibration of the pH and redox sensors and the pumps which saved a lot of time during the preparation of the experiments.

Results

Fermentation process development could be performed successfully with the DASGIP Parallel Bioreactor System. All figures and data were obtained and analyzed with the software DASGIP Control 4.0.

The first figure exemplarily shows the results of a parallel fermentation of *Lactobacillus sp.* for optimizing growth temperature. Base consumption was used as proportional indicator for the growth dependent acidification of the fermentation broth. The strain was cultivated at different temperatures ranging from 29°C to 37°C and the fermentation was finished after base consumption had stopped.

For a detailed insight into the bacterial growth, the colony forming units (CFU) for strains obtained at different temperatures (29 °C, 30 °C, 33 °C, 34 °C and 37 °C) are illustrated in table 1.

Temperature [°C]	CFU/ml Ferm. broth
29	1.16E+10
30	1.18E+10
33	1.32E+10
34	1.59E+10
37	1.74E+10

Table 1

Considering these values a fermentation temperature of 37°C turned out to be the most suitable temperature for cultivation of the used *Lactobacillus sp.*, verifying the results shown in Fig. 1.

In the next development step the optimum pH value was determined, again by using eight bioreactors in parallel. Figure 2 shows the base consumption during the fermentation at different pH values.

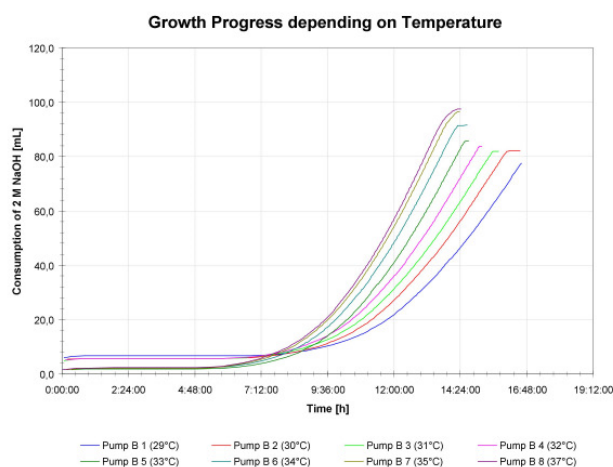


Fig. 1

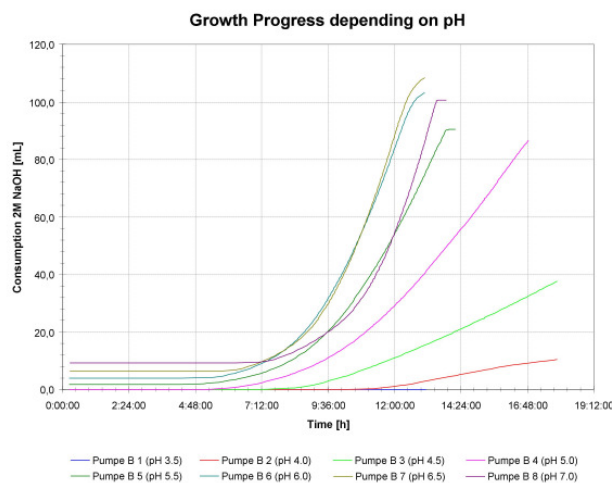


Fig. 2

Process Development for Silage Inoculants

Optimization of Fermentation for *Lactobacillus sp.*

After fermentation the CFU in each bioreactor were determined. The optimum was obtained at a pH value of 5.5. This result correlated with the base consumption (Fig. 2), which was also very high for pH 5.5. In the following table (table 2) the numbers of CFUs in the fermenters with different pH values are shown:

pH-value	CFU/ml Ferm. broth
3.50	2.85E+09
4.00	1.18E+10
5.50	1.69E+10
6.00	1.54E+10
7.00	1.02E+10

Table 2

Finally the process developed with the DASGIP Parallel Bioreactor System was successfully transferred to 20 L pilot scale fermentation, demonstrating the reliable scalability properties of the DASGIP System.

Benefits of a DASGIP

The DASGIP Parallel Bioreactor System is very suitable for the first steps in fermentation process development of both, aerobic and anaerobic microorganisms.

A particular advantage of the system is that it allows optimization of fermentation processes very effectively as different levels of certain parameters like pH, redox potential, oxygen concentration and temperature can be tested at the same time.

The results obtained in the eight reactors are comparable among each other and show good reproducibility between different runs. Additionally, results are compa-

ble to larger scale and therewith can be used for the efficient design of scaling-up experiments.

The whole set-up of the system is very user-friendly, especially the software, which is self-explanatory.

The only malfunctions which can be reported so far can be found in the software, especially after having received new updates, but the support from DASGIP is always on the spot and helps to handle this kind of problems.

Finally our users like the very attractive design of the system.

Structural Data

August Kreici and Florian Strohmayer are the operators of the DASGIP System at the BIOMIN Research Center.

Company: BIOMIN Holding GmbH;
Tulln, Austria

Business Function: Research Associates

Educational Background: Biotechnologists

Bioreactor experience: for more than 3 years

