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Setup of a Microbial Hyaluronic Acid Production Process Using the BioFlo® 120 Bioprocess Control Station

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Abstract

Achieving optimal process conditions for microbial growth and production of a desired bioproduct needs hardware and software to monitor and control the bioprocess parameters, including the pH, temperature, and agitation of the fermentation broth. Intuitive bioprocess software and an easy-to-use bioprocess controller can accelerate process development, because they reduce the time needed to train staff, prepare the system, and start the process run. Researchers at Praj Industries, India used the BioFlo 120 bioprocess control station to produce hyaluronic acid (HA) in *Streptococcus zooepidemicus* at bench scale. In this application note we describe how they set up, monitored, and controlled critical process parameters, and thus present an impression of the controller's integrated, touchscreen-accessible software.

Introduction

HA is a polymer composed of repeating disaccharide units of β-1,3-N-acetyl glucosamine and β-1,4-glucuronic acid. High concentrations of HA are present in the skin, the vitreous humor, and the umbilical cord. HA is also a component of the capsules of certain microbial strains. It is industrially produced for a variety of biomedical applications and cosmetics, mainly by microbial fermentation of natural producers like *Streptococcus spec.* or recombinant bacterial strains.

Praj Industries Limited based in Pune, India, offers innovative solutions for beverage alcohol and bioethanol plants, brewery, water and wastewater treatment plants, critical process equipment and systems, and bioproducts. They tested the BioFlo 120 bioprocess control station (Fig. 1) for HA production in *Streptococcus zooepidemicus*, and in this application note describe the setup and operation of the fermentation process.

Fig. 1: The BioFlo 120 bioprocess control station equipped with fermentors for microbial applications.

Material and Methods

Bacterial strain, preculture, and inoculation

The researchers at Praj Industries used *Streptococcus equi subsp. zooepidemicus* (ATCC® 39920) to produce HA. To prepare the preculture for the fermentation process, they inoculated 50 mL of medium with a single colony grown on brain heart infusion agar and incubated the culture at 37°C for 12 to 16 hours. The medium contained 2 g/L glucose, 10 g/L beef extract, 20 g/L polypeptone, 5 g/L yeast extract, 2 g/L NaCl, 1 g/l Na_2HPO_4, and 0.12 g/L K_2HPO_4.

The main culture was inoculated with 5 % (v/v) of the preculture. The main culture medium contained 40 g/L glucose, 20 g/L polypeptone, 10 g/L yeast extract, 2 g/L NaCl, 1 g/L $MgSO_4$, and 2.5 g/L K_2HPO_4.

Cultivation in the BioFlo 120 fermentor

The researchers carried out the fermentation in a 2 L glass vessel in a working volume of 1.3 L. The culture was agitated using a direct-drive motor. The vessel was equipped with analog sensors to measure pH and dissolved oxygen, and the temperature was controlled using a heat blanket. The bioprocess system's integrated software gives an overview of these vessel parameters in the system setup screen (Fig. 2).

Process parameters were controlled with the Eppendorf bioprocess software built into the controller. The fermentation was performed at 37°C with agitation at 300 to 400 rpm. The pH was controlled at pH 7.0 by automatic addition of 5 M NaOH. Figure 3 shows the user interface to set up the pH control. The pH interface screen can be used to alter the pH setpoint for process control, the Proportional and Integral Settings for the PID controller, and the deadband used. The setpoints of other process parameters can be adjusted in a similar way.

The summary screen (Fig. 4) offers an overview of the settings for the various process parameters, showing the process value, setpoint, control mode and controller output for each of them.

With the Eppendorf bioprocess software all data measured online can be seen in a single trend screen (Fig. 5). It is simple and easy to export the data from the controller to Excel®, using a USB flash drive.

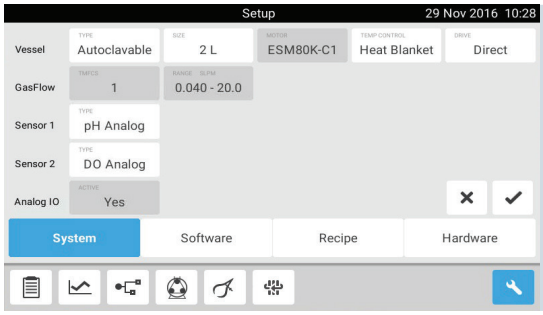

Fig. 2: The setup screen summarizes important vessel parameters*.

Fig. 3: Setup of pH control*. Complete pH control requires linking pumps for acid and base additions.

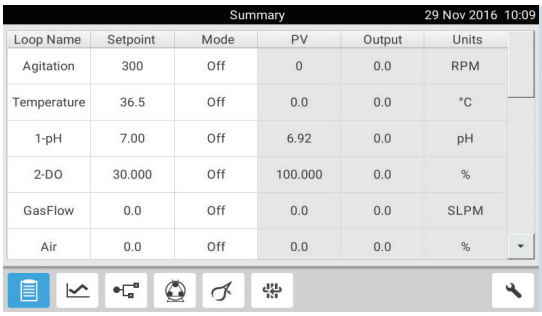

Fig. 4: The summary screen gives an overview on the process parameter settings*.

Fig. 5: The trend screen displays online process values*.

Culture feeding

Ten hours after the start of the fermentation the researchers began to feed the culture with a solution containing 60 % glucose. Using the system's integrated pump 0.416 mL of feed solution was added per minute.

Offline analytics

For offline measurements the researchers took samples every three to six hours.

They monitored bacterial growth by measuring the optical density of the culture at 600 nm (OD_{600}). The glucose

* The screenshots shown do not originate from the described experiments and values may differ.

concentration in the medium was determined using the Accu-Chek® Active glucose monitoring system (Roche®, Switzerland).
The researchers measured the viscosity of the culture to monitor HA production qualitatively. After 24 hours, they determined the concentration of HA in the culture supernatant quantitatively using a carbazole assay with D-glucuronic acid as the standard [1].

Results and Discussion

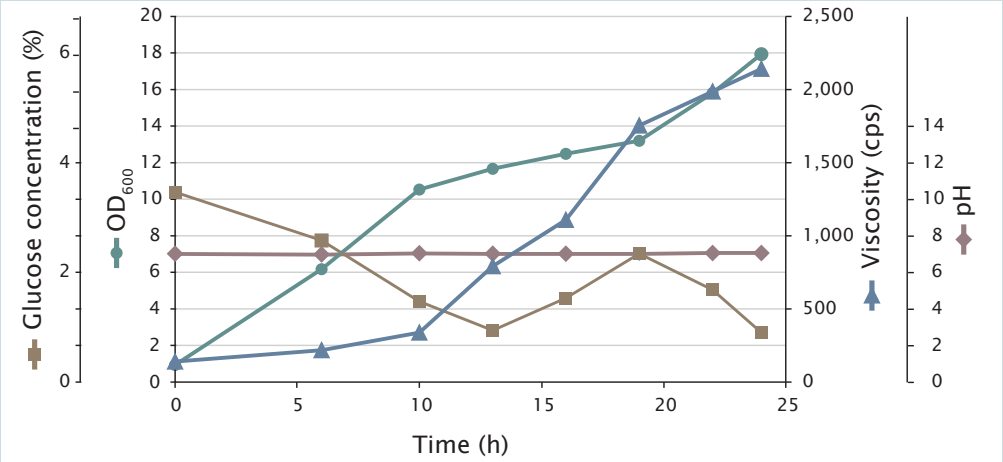

Fig. 6: Fermentation process for HA production in *Streptococcus equi subsp. zooepidemicus*. The pH of the culture was determined online. Gucose concentration, OD_{600}, and viscosity were measured offline.

Figure 6 summarizes the results of the fermentation run of *Streptococcus zooepidemicus*. Within 24 hours the culture reached an OD_{600} of 18. During the first 13 hours of the fermentation the glucose concentration in the medium decreased constantly and then increased again due to the addition of feed solution. HA production caused an increase of the viscosity of the culture up to 2142 cps at 24 hours. This corresponds to a final titer of 3.5 g/L HA.

Although the process conditions were not optimized it is clear that the BioFlo 120 bioprocess control station provides favorable conditions for microbial production of HA in a standard glass stirred-tank bioreactor.

Conclusion

Using the example of a microbial HA production process we show several software features for the setup, monitoring, and control of critical process parameters. The combination of a local touchscreen and the user-friendly Eppendorf bioprocess software has proven to make process control on the BioFlo 120 simple and straightforward.

Literature

[1] Bitter, T and Muir, HM. A modified uronic acid carbazole reaction. *Anal Biochem.* Oct; 4:330-4.1962.

Ordering information

Description	Order no.
BioFlo® 120, Advanced	
Plug type B (USA, Canada, Mexico, Japan)	B120ACS000
Plug type CEE 7/7 (EU (except UK, Ireland, Switzerland), Russia, Korea	B120ACS001
Plug type I (Australia, New Zealand, China, Argentina)	B120ACS002
Plug type J (Switzerland)	B120ACS003
Plug type G (UK, Ireland)	B120ACS004
Plug type N (Brazil)	B120ACS005
Plug type D (India)	B120ACS006
BioFlo® 120 Fermentation Vessel Bundle	
1 L, heat blanket	B120AVB000
2 L, heat blanket	B120AVB001
5 L, heat blanket	B120AVB002
10 L, heat blanket	B120AVB003
1 L, water jacket	B120AVB004
2 L, water jacket	B120AVB005
5 L, water jacket	B120AVB006
10 L, water jacket	B120AVB007

For more information on these and other configurations visit www.eppendorf.com/BioFlo120

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