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Fed-Batch Biofuel Production Process Using a New Brunswick™ BioFlo® 115

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Abstract

In Brazil, it is common in the biofuel industry to utilize a biochemical process in which glucose, fructose and sucrose (derived from sugar cane juice and sugar cane molasses) are used to produce biofuel through a fed-batch fermentation process. In this experiment, *Saccharomyces cerevisiae* is used for biofuel production from sugar cane juice. The fermentation process metabolizes glucose into ethanol, and is used to produce many biofuel products in large production volumes. This strain of yeast is also widely used in other industrial applications to manufacture enzymes and proteins for beer, wine and bread. In this application note, we show that *Saccharomyces cerevisiae* can successfully be cultivated in high densities to convert sugar cane into biofuel using a BioFlo 115.

Introduction

The BioFlo 115 features a versatile and easy-to-use control station with color touchscreen monitor and built-in capability to operate in either fermentation or cell-culture mode. Switching between the operation modes automatically adjusts the control settings. Three fixed-speed pumps, temperature control, agitation control, and a rotameter with choice of gas flow ranges are available in BioFlo 115 systems. Pre-packaged kits for Basic or Advanced Fermentation and Advanced Cell Culture simplify the ordering process. Various kits can include options for direct-drive or magnetic-drive agitation, as well as water-jacketed or heat-blanketed vessels in 1 - 10 L range (approximate working volume). Ancillary equipment such as pH/DO and foam/level sensors are either included in kits, or can be added separately as options.

Materials and methods

Fermentation and cell recycling

For this application, a BioFlo 115 controller with advanced fermentation kit, direct drive and 2 L water jacketed vessel was used (Figure 1). The total fermentation process consisted of two distinct phases: An initial cell propagation and growth phase using complex medium (yeast extract) followed by sugar cane juice (growth medium) under aerobic conditions and the biofuel production phase using sugar cane juice (alcohol fermentation medium) under anaerobic conditions. The initial cell propagation phase utilized 20.0 g/L of medium substrate (dry mass) under aerobic condition, after that, the sugar cane growth media was added. Cells were recovered for use in the biofuel production phase operated under anaerobic conditions. Cell recycling was conducted through centrifugation. The cells were recovered and diluted with 500 mL of sterile water and transferred back to the bioreactor aseptically via an addition port in the headplate. Sugar cane juice feeding was performed over a four-hour period using peristaltic pump 3 (fixed flow of 6.25 mL/min) up to a final volume of 1.5 L and was maintained for two more hours to ensure uptake of accumulated sugar. Cells were recycled for three consecutive alcoholic fermentations. The cell propagation

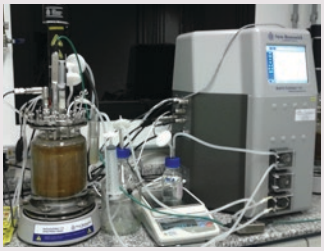

Figure 1: BioFlo 115 system used for biofuel production

phase and the biofuel production phase were both operated under fed-batch mode.

Medium

The initial cell propagation phase used complex medium as follows (per liter of de-mineralized water):

Initial complex medium composition	Concentration
K_2SO_4	6.60 g/L
KH_2PO_4	3.00 g/L
$MgSO_4$	0.50 g/L
$CaCl_2 \cdot 2H_2O$	1.00 g/L
Yeast extract	5.00 g/L

After autoclaving at 121 °C for 15 min, the medium was cooled to room temperature. The carbon source and additional supplements passed through a sterile filter were also added according to the following concentrations:

Filter-sterilized elements	Concentration
Urea	2.30 g/L
Thiamine	3.00 g/L
EDTA	15.00 mg/L
$ZnSO_4 \cdot 7H_2O$	4.50 mg/L
$CoCl_2 \cdot 6H_2O$	0.30 mg/L
$MnCl_2 \cdot 4H_2O$	0.84 mg/L
$CuSO_4 \cdot 5H_2O$	0.30 mg/L
$FeSO_4 \cdot 7H_2O$	3.00 mg/L
$NaMoO_4 \cdot 2H_2O$	0.40 mg/L
H_3BO_3	1.00 mg/L
KI	0.1 mg/L

The carbon source for growth medium and alcoholic fermentation medium were both formulated with sugar cane juice and sterilized separately at 121 °C for 15 min.

The growth medium contained 129 g/L of total reducing sugar (TRS). The sugar content is shown in the table below:

Sugar cane juice composition	Concentration
Sucrose	102.51 g/L
Glucose	10.99 g/L
Fructose	10.01 g/L

The alcoholic fermentation medium contained 171.65 g/L of total reducing sugar (TRS):

Sugar cane juice composition	Concentration
Sucrose	133.01 g/L
Glucose	16.79 g/L
Fructose	14.85 g/L

Inoculum

The *Saccharomyces cerevisiae* strain used in this work was an unnamed strain cultivated at the Brazilian Bioethanol Science and Technology Laboratory. It was originated from the department of Food Engineering, State University of Campinas, Brazil. The strain was maintained on agar plates prepared as follows (per liter of de-mineralized water):

Inoculum composition	Concentration
Yeast extract	10.00 g/L
Peptone	20.00 g/L
Glucose	20.00 g/L
Agar	20.00 g/L

Before the inoculation, the strain was transferred from agar plate to a liquid complex medium containing the following (per liter of de-mineralized water):

Liquid complex medium composition	Concentration
Yeast extract	10.00 g/L
Peptone	20.00 g/L
Glucose	20.00 g/L

The inoculum was cultured in shake flask for 24 hours using a shaker set to 33 °C at 250 rpm.

Fermentor control conditions for cell propagation phase

During the cell propagation phase, the Dissolved Oxygen (DO) was controlled to 60 % using the cascade feature through agitation and air control. Temperature was controlled to 33 °C throughout the run and pH was controlled to 5.0 via the acid and base assigned to peristaltic pumps 1 and 2. These parameters were maintained until the biofuel production phase (alcohol fermentation phase) was initiated. When the cell propagation phase was completed, the medium was transferred to a 2 L flask using an external pump (Watson Marlow®). The medium was then placed inside the laminar flow cabinet and transferred into a centrifuge (Beckman Coulter® centrifuge with JLA-9.100 rotor) and was spun down at 8.000 rpm/4 °C for 10 minutes. Setpoints are listed below:

BioFlo® 115 setpoints	
Agitation	Cascaded range at 250 – 600 rpm
Temperature	33 °C
pH	5.0
DO	> 60 %
Gas (supplied by sparge)	Cascaded range at 0.5 – 1.0 SLPM (Air)

Fermentor control conditions for biofuel production phase

During the biofuel production phase, the process was changed from aerobic to anaerobic fermentation, and DO was monitored between 0 % and 2.3 %, but not controlled. Agitation was set to auto mode and held at 100 rpm. Temperature controlled to 33 °C throughout the run. pH was controlled to 5.0. These parameters were maintained until the alcoholic fermentation/biofuel production phase was initiated. Gas flow was shut-off and the alcohol fermentation medium was introduced so that the *Saccharomyces cerevisiae* could start anaerobic fermentation and produce ethanol. Setpoints are listed below:

BioFlo® 115 setpoints	
Agitation	100 rpm
Temperature	33 °C
pH	5.0
DO (monitored only)	0 – 2.3
Gas	None

DO calibration

The DO sensor was calibrated using a standard two-point calibration method: 0 % (often referred to as the zero point) and 100 % (often referred to as span). The electronic zero method was performed by disconnecting the sensor from the cabinet, allowing the value to stabilize, and then reconnecting the sensor to the cable. The span was achieved by bringing the vessel filled with medium to all of the operational setpoints to a stable value and then spanning the DO sensor. DO should be calibrated post-autoclave as part of the pre-inoculation setup. The sensor was allowed to polarize for a 6 hour period. After calibration, DO should remain around 100 % until after inoculation.

pH calibration

The pH sensor was calibrated prior to the autoclave cycle outside the vessel using a two-point calibration method with two standard pH buffers. The pH 7.0 buffer was used to zero the sensor and the pH 4.0 was used as the span.

pH control

The pH parameters were maintained by adding a sulfuric acid solution (H_2SO_4) via pump 1 (assigned as "acid") to lower the pH and adding potassium hydroxide solution (KOH) via pump 2 (assigned as "base") to raise the pH. The dead-band implemented for pH control was 0.02.

Fed-batch

Filter-sterilized media were fed to the vessel through pump 3.

Monitoring

Sucrose, glucose and fructose concentrations were detected by high-performance liquid chromatography (HPLC) using an Agilent® 1260 Infinity with RI detector through an Aminex® column (HPX-87P, 300 mm x 7.8 mm) at 60 °C. EMD Millipore® Milli-Q® water was used (column flow rate 0.5 mL/min) for the eluent phase. Ethanol concentration was determined by HPLC as well, using a Thermo Fisher Scientific® Dionex® UltiMate® 3000 with RI detector (Shodex RI-101) via an Aminex column (HPX-87H 300 mm x 7.8 mm) at 50 °C. Sulfuric acid, 5 mM at a rate of 0.5 mL/min, was used for the eluent phase. Dry weight mass measurements were carried out in triplicate using an analytical balance. Cell propagation was monitored on line through an Aber® capacitance probe as well as by taking samples for optical density measurements at 600 nm using a spectrophotometer.

Results and discussion

Figure 2, right, shows plots of the three key process parameters monitored during the biofuel production phase: total cell mass, X (kg/m³); substrate, S (kg/m³); and ethanol, P (kg/m³). All three are important factors in monitoring ethanol production from metabolized sugar cane juice. The cell concentration profile presented in Figure 2 (A) and the substrate profile presented in Figure 2 (B) illustrate the typical results in a fed-batch configuration. For the first 3 hours of the biofuel production phase, the initial density of yeast cells (44 kg/m³) decreased due to the dilution factor by sugar feeding. In the meantime, the sugar concentration (S) started to accumulate. After necessary sugar feeding, the cell concentration stabilized and the sugar concentration started to decrease and was completely consumed over time. The ethanol, as illustrated in Figure 2 (C), was produced according to available sugars in the medium and the production increased over time to reach a final concentration of approximately 56 kg/m³.

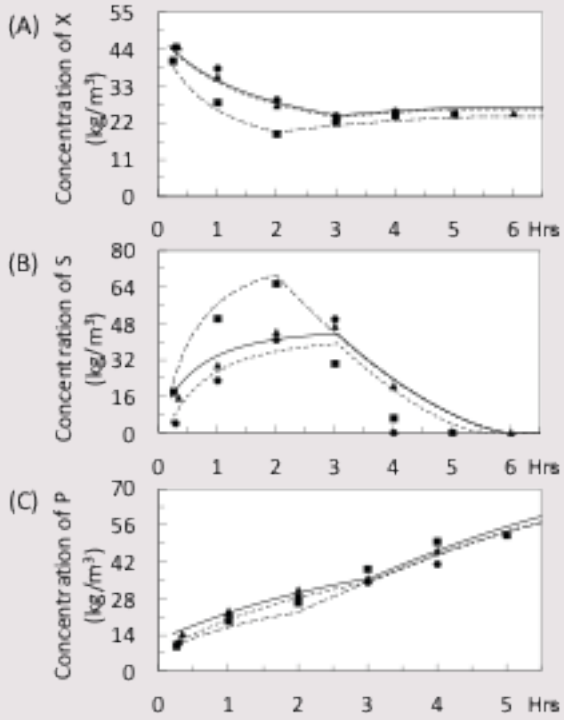

Figure 2: Experimental (Ferm. 1 (■); Ferm. 2 (▲) and Ferm. 3 (●) for concentration of (A) Cell, (B) Substrate and (C) Ethanol

Ordering information

Description	N. America Order no.	International Order no.
New Brunswick™ BioFlo® 115 Master Control Station w/Thermal Mass Flow Controller (TMFC)	Call	Call
Add-a-Vessel Advanced Fermentation Kit 3 L (Water Jacketed)	M1369-1612	M1369-1612

Your local distributor: www.eppendorf.com/contact
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