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# Propionic acid production of *Propionibacterium acidipropionici* in a BIOSTAT® A


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turning science into solutions

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**Introduction**

Traditionally derived from fossil fuels, propionic acid (PA) is widely used in the food and pharmaceutical industries. PA is used as a preservative and increasingly, in the synthesis of monomers. Recently, the market for PA has grown to over 410,000 tonnes per year with a steady 3 % increase per annum. Mounting environmental concerns have encouraged end-users to look for sustainable PA alternatives, opening a niche market for biological production of PA (Liu et al., 2012). Bio-production of PA addresses many environmental concerns and offers a sustainable alternative for the production of C3 chemicals including propylene, propanol and vinyl propionate. *Propionibacterium acidipropionici* (*P. acidipropionici*) is a gram-positive, anaerobic, rod bacteria that naturally produce PA as the main fermentation product through the Wood-Werckman cycle (Liu et al., 2012; Parizzi et al., 2012). Natively, PA is produced along other organic acids such as lactate, succinate and acetate.

The anaerobic fermentation of *P. acidipropionici* is sensitive to environmental, physicochemical, and hydrodynamic conditions, including temperature, pH, dissolved oxygen, and shear stress. These conditions need to be tightly controlled during the fermentation. *P. acidipropionici* uses a complex mixture of nutrients to produce optimally PA. This generates a dynamic metabolism through the different growth phases. In this study, we demonstrated the robustness of the BIOSTAT® A to ferment *P. acidipropionici* cells. The equipment was able to control the pH successfully despite the large production of PA. The pH control parameters had to be adjusted to have an adequate pH control. Nitrogen was constantly fed into the reactor to ensure anaerobic conditions. The temperature was controlled with the thermo-jacket surrounding the vessel and the recirculation chiller. The chiller also helped to control evaporation by cooling the exhaust gases. The hydrodynamic conditions were maintained using two Rushton impellers.

**1. Material and Methods**

**1.1 Bacteria and media**

*P. acidipropionici* ATCC 55737 selected from a collection of 17 strains (Stowers, Cox, & Rodriguez, 2014). The strain was kept at -80°C using glycerol (20%) as cryoprotector. The culture media (PAM) was made from (g/L): yeast extract (10), trypticase soy (5), $K_2HPO_4$ (0.05), $MnSO_4$ (0.05), and glucose (75). Media components and the carbon source were sterilized separately for 20 min at 121°C.

**1.2 Cultivation**

Glycerol stock cultures were revived in 1.5 mL Eppendorf tubes containing 1 mL of PAM media inoculated with 0.8% (v/v) of the glycerol stock. This culture grew for 24 hours at 32°C. The culture was transferred to a 15 mL Falcon tube containing 14 mL of PAM media and allowed to grow for an additional 24 hours. 5% (v/v) of this culture was used to inoculate a 250 mL serum bottles containing 100 mL of PAM media which grew for an additional 24 hours. Cells from the serum bottles in mid-exponential phase were used to inoculate the fermenter at an initial $OD_{600nm}$ of 0.3. The fermentation was performed using a 1 L BIOSTAT® A fermenter. The fermenter was equipped with standard probes and standard controllers, controlling pH, dissolved oxygen, temperature, and agitation. The agitation rate was kept constant at 300 rpm. The pH was controlled at 6.4 using 10 M NaOH. The temperature of the culture was maintained at 32°C using an electric jacket and a recirculation chiller. The exhaust gases condenser was set to 20% to avoid evaporation media. Prior to inoculation, the fermenter was sparged with $N_2$ for at least 15 minutes. A constant $N_2$ flow was kept for the entire fermentation at a flow rate of 300 ccm using a correction factor for the mass flow controller of 0.992.

### 1.3. Analytical methods

Optical density of the culture was measured at 600 nm using a Biochrom Libra S12 UV/Vis Spectrophotometer. Organic acids and carbohydrates were quantified by ion-exclusion chromatography using an Agilent 1200 HPLC system and an Agilent Hiplex H column (300 × 7.7 mm, PL1170-6830) with guard column (SecurityGuard Carbo-H, Phenomenex PN: AJO-4490). Sugars were monitored using a refractive index detector (Agilent RID, G1362A) set on positive polarity and optical unit temperature of 40°C, while organic acids were monitored at 210 nm (Agilent MWD, G1365B). 30 µL of sample was injected onto the column using an auto-sampler (Agilent HiP-ALS, G1367B) and column temperature kept at 65°C [70°C] using a thermostatted column compartment (Agilent TCC, G1316A). Analytes were eluted isocratically with 4 mM $H_2SO_4$ at 0.6 mL/min for 26 min. Chromatograms were integrated using ChemStation (Rev B.03.02[341]).

## 2. Results

*P. acidipropionici* produces organic acids which generates a strong pH dynamic which needs to be controlled. Figure 1 shows the control parameters in the bioreactor using the BIOSTAT® A. As illustrated, the pH was maintained constant via the addition of base using the integrated peristaltic pump which supplied the required 10 M NaOH. The PID control parameters were adjusted to P: 300%, I: 0%, and D: 0%. $N_2$ was continuously supplied to maintained the anaerobic environment in the fermenter. The temperature and agitation were also controlled and monitored.

Figure 2 shows the growth kinetic profiles of *P. acidipropionici*. PA was the main fermentation product along with minor quantities of acetic acid and succinic acid. As can be observed in the figure, the growth stopped after 40 h. However, PA the production continued until the end of the fermentation, resulting in an increase in the addition of NaOH until the 96$^{th}$ h.

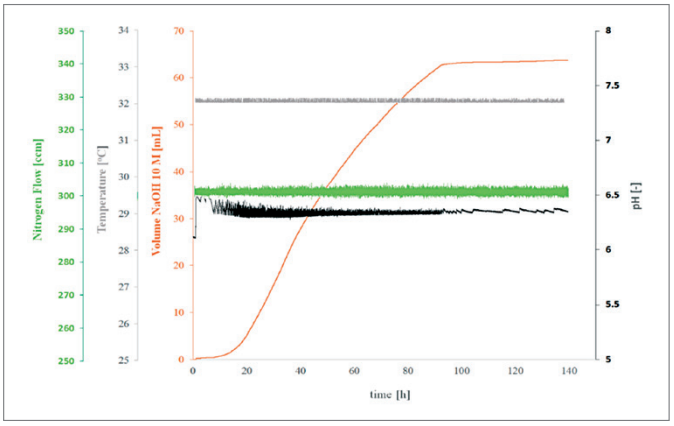
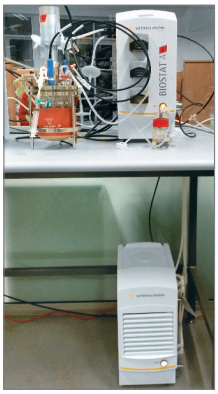
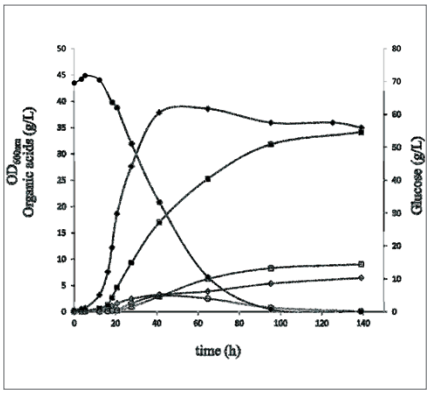



**Figure 1:** BIOSTAT® A control parameters during the fermentation of *P. acidipropionici*. Nitrogen flow, temperature, pH, and volume of NaOH 10 M are shown.



(a)




(b)

**Figure 2:** Growth kinetic of *P. acidipropionici* fermentation in a BIOSTAT® A.

(a) BIOSTAT® A equipment.
(b) Growth and production kinetic.
Optical Density: Black line and ♦;
Propionic Acid: Black line and ■;
Glucose: Black line and ●;
Succinic Acid: Grey line and □;
Acetic Acid: Grey line and ◇;
Pyruvate: Grey line and ○.

## 3. Conclusion

*P. acidipropionici* fermentation for the production of PA can be performed in a BIOSTAT® A. The most important parameter to control was the pH, which was controlled by changing the PID. In conclusion, BIOSTAT® A can be used to ferment anaerobic microorganisms for the production of more than 30 g/L of propionic acid.

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