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High-cell-density poly (3-hydroxybutyrate) production from sucrose using *Burkholderia sacchari* culture in airlift bioreactor

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ABSTRACT

Burkholderia sacchari IPT 189 poly (3-hydroxybutyrate) (P3HB) production in airlift bioreactor were investigated in batch and fed-batch culture using sucrose as carbon source. In batch experiments it was observed that during the growth phase *B. sacchari* IPT 189 might display exponential growth even at very low carbohydrate concentration, as long as NH_4^+ concentration was above 190 mg l^{-1}. The onset of accumulation phase took place when NH_4^+ concentration dropped below this value and continued as long as carbohydrate was in excess, even with dissolved oxygen concentration at 0.0% of air saturation. In the fed-batch experiments, nitrogen limitation was used to induce P3HB biosynthesis in a two-phase process. In the first phase, an initial batch followed by a limited sucrose fed regime led to a growth with low-P3HB-content (less than 13%) and up to 60 g l^{-1} of biomass concentration in *c.a.* 25 h. In the second phase, nitrogen concentration limitation induced P3HB accumulation up to 42%, raising the biomass concentration to *c.a.* 150 g l^{-1}. Calculated parameters for the experiments were P3HB productivity = 1.7 g l^{-1} h^{-1} and P3HB yield factor from sucrose = 0.22 g g^{-1}.

1. Introduction

Polyhydroxyalkanoates (PHA) are biodegradable synthesized and stored by numerous prokaryotes. They can be produced in large quantities from renewable resources by means of well known fermentation processes such as batch, semi-batch and continuous operation and the imposition of particular limited culture conditions such as nitrogen, phosphorus and oxygen (Anderson and Dawes, 1990). PHA has properties similar to those of some polypropylene and may substitute these petrochemical plastics in several applications. A major drawback to the commercialization of PHA is its still higher production cost compared with petrochemical-based synthetic plastic material. Main costs of production are investments costs and carbon source raw material (Lee and Choi, 1998; Choi and Lee, 1999, 2000). To reduce costs and optimize production, extensive studies have been done to develop high-cell-density protocols up to pilot scale. Biomass concentrations above 100 g l^{-1} and PHA productivity superior to 1 g l^{-1} h^{-1} has been reached by *Cupriavidus necator* (formerly *Ralstonia eutropha*) fermentation in mechanical stirred tank bioreactor using cheap inverted sugarcane sucrose syrup as carbon source (Bueno Netto et al., 1993; Nonato et al., 2001; Rossell et al., 2006) in order to incorporate this product in a sugarcane biorefinery. In this sense, lignocellulosic biomass carbohydrate (xylose and glucose) might also be very interesting carbon sources used by *Burkhloderia* sp. to produce PHA, aggregating value in sugarcane biorefinery (Silva et al., 2004). Recently, Rocha et al. (2008) demonstrated the potential to produce PHA by *Burkhloderia sacchari* fermentation since this microorganism may produce large amount of this biopolymer at high biosynthesis rate directly from sugarcane sucrose syrup in stirred tank fermentor but at low cell density culture. On the other hand, airlift bioreactors have been investigated as an alternative to stirred tank bioreactor to produce numerous products. Their main characteristics are low shear stress, simplicity of design and construction and low energy requirement for mass transfer (Chisti, 1989). In addition, Tavares et al. (2004) showed that this type of bioreactor is also an attractive economical alternative to mechanical stirred tank bioreactor for PHA production with *C. necator* using nitrogen and/or oxygen regime limitation, even though utilizing low cell density cultivation.

In the present work, we explored the utilization of airlift bioreactor to produce PHB in high-cell-density culture using *B. sacchari* and sucrose syrup as carbon source.

2. Methods

2.1. Microorganism

It was utilized the microorganism *B. sacchari* IPT 189, mutant of *B. sacchari* IPT 101 (=LMG 19450 = CCT 6771) deficient in the

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oxidation of propionate, which is empowered to achieve high conversion of this substrate to 3-hydroxyvalerate units (Brämer et al. 2002). This strain was maintained by cryopreservation at −80 °C.

2.2. Inocula preparation

A stock culture suspension was inoculated in Erlenmeyer flasks (0.1 ml l^{-1}) with Nutrient Broth media (Table 1) and incubated for 15 h, at 30 °C and 250 rpm. Ten percent (v/v) of this culture was inoculated in the seed culture media (Table 1) and incubated for 15 h, at 30 °C and 250 rpm. A volume of about 2 l of this culture, enough to obtain 0.4 g l^{-1} of initial biomass concentration was transferred to the airlift bioreactor containing 8 l of the culture media (Initial Batch, Table 1). The same procedure was done for the *limitation experiments* or for the growth phase of the *High-cell-density experiments*. All the experiments had the temperature maintained at 32 °C and pH controlled at 7.0 with 4 N H_2SO_4 solution and NH_4OH (v/v 1:5) or 4 N NaOH solutions. Dissolved oxygen concentration (pO_2) was measured as a percentage of air saturation with two polarographic DO probes (Ingold, Switzerland) installed one at the top and another at the bottom of bioreactor vessel. The bioreactor utilized was a B. Braun model E10 (B Braun, Germany) modified to maximize oxygen transfer rate and minimize mixing time. The main features of the equipment are represented in Fig. 1.

2.3. Studies of nitrogen and oxygen limitation

The experiments were performed at two different superficial air velocity, V_{sg} = 1163 m h^{-1} and V_{sg} = 2326 m h^{-1}. The analysis of the ammonium and dissolved oxygen concentration throughout the experiments were performed in order to verify the nitrogen and/or oxygen limitation as proposed by Tavares et al. 2004. Experiments were run with the Initial Batch culture media (Table 1) designed to attain biomass concentration up to 12 g l^{-1}.

2.4. High-cell-density experiments

Two high-cell-density cultivations (run 01 and 02) were carried out to confirm reproducibility of the fermentation process. A two-phase P3HB production comprising a growth phase followed by an accumulation phase was performed. In the growth phase the Initial Batch culture media (Table 1) was balanced in order to get approximately 12 g l^{-1} of residual biomass. High-cell-density growth was attained using a fed-batch procedure with constant volumetric flow of 120 ml h^{-1} of a 700 g l^{-1} sucrose solution. This fed was designed in order to fit with the maximum bioreactor oxygen transfer capacity as proposed by Diniz et al. (2007). The dissolved oxygen concentration was maintained above 10% of air saturation, and nitrogen addition was carried out by the pH automatic control in order to have a non-limited NH_4^+ level. These values are defined by the Limitation experiments (see Section 3). A mass of 17.125 g of $MgSO_4{\cdot}7H_2O$ was supplemented to the bioreactor in two equal portions: at the beginning of the fermentation and at the beginning of the fed-batch period. Also, a mass of 81.27 g of KH_2PO_4 was supplemented in three equal portions: at the beginning of the fermentation, at the beginning of fed-batch period and at 15 h of culture.

Table 1
Composition of culture media used to produce P3HB from sucrose.

Component	Nutrient Broth (gl^{-1})	Seed Culture (gl^{-1})	Initial Batch (gl^{-1})	Fed Batch
Sucrose	–	10.0	30.0	700 gl^{-1}[(b)]
KH_2PO_4	–	1.50	1.29	17.125 g
Na_2HPO_4	–	4.45	–	–
$(NH_4)_2SO_4$	–	3.00	1.83	–
$MGSO_4.7H_2O$	–	0.20	0.55	81.27 g
$CaCl_2.2H_2O$	–	0.01	0.02	–
Ammoniac citric ferric	–	0.06	0.05	–
TES[(a)](ml l^{-1})	–	1.00	2.00	–
Meat extract	3.0	–	–	–
Peptone	5.0	–	–	–

[(a)] The Trace Elements Solution (TES) composition was (per liter): 0.3g, H_2BO_4; 0.2g, $CoCl_2.6H_2O$; 0.1g, $ZnSO_4.7H_2O$; 0.03g, $NaMoO_4.2H_2O$; 0.02g, $NiCl_2.6H_2O$; 0.01g, $CuSO_4.5H_2O$; 0.03g, $MnCl_2.6H_2O$.
[(b)] Concentration of sucrose fed solution during fed-batch procedure.

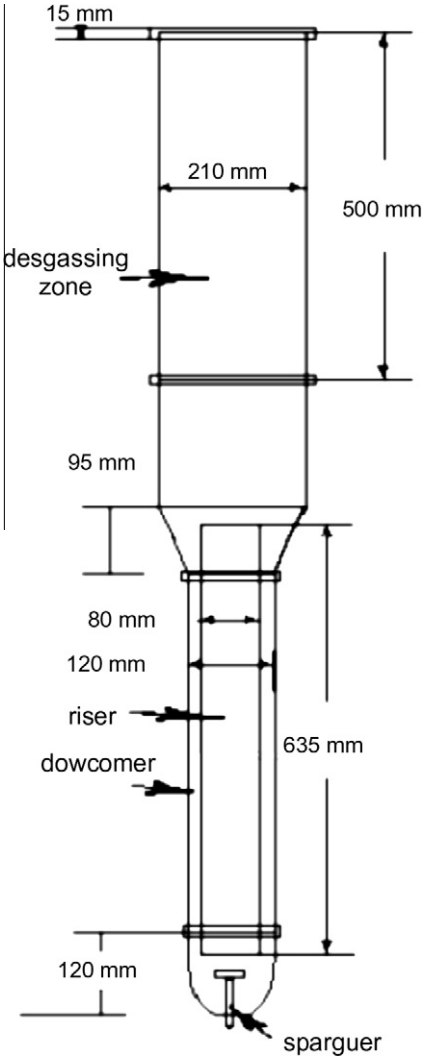

Fig. 1. Airlift bioreactor configuration. D_T: diameter of the tank (riser and downcomer sections) = 0.12 m; H_T: height of the tank = 1.00 m; H_L: height of the liquid = 0.70 m; D_R: diameter of the riser section = 0.08 m; sparger type: perforated ring; number of sparger hole: 32; diameter of sparger hole: 0.8 mm.

2.5. Analytical procedures

Total cell concentration (Xt) was determined by measuring the dry cell weight after centrifugation at 9220*g* (10 min, 4 °C) followed by filtration using 0.45 μm pore membrane and oven drying at 104 °C until constant weight. The P3HB concentration was determined by gas chromatography. Sucrose, glucose and fructose concentrations were determined using high performance liquid chromatography (Tavares et al., 2004). Nitrogen concentration was measured using a specific NH_4^+ electrode (Orion Ion Analyzer, USA). The percentage of P3HB content was defined as the ratio of