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Role of dissolved oxygen availability on lactobionic acid production from whey by *Pseudomonas taetrolens*

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ABSTRACT

The influence of dissolved oxygen availability on cell growth and lactobionic acid production from whey by *Pseudomonas taetrolens* has been investigated for the first time. Results from pH-shift bioreactor cultivations have shown that high agitation rate schemes stimulated cell growth, increased pH-shift values and the oxygen uptake rate by cells, whereas lactobionic acid production was negatively affected. Conversely, higher aeration rates than 1.5 Lpm neither stimulated cell growth nor lactobionic acid production (22% lower for an aeration rate of 2 Lpm). Overall insights into bioprocess performance enabled the implementation of 350 rpm as the optimal agitation strategy during cultivation, which increased lactobionic productivity 1.2-fold (0.58–0.7 g/L h) compared to that achieved at 1000 rpm. Oxygen supply has been shown to be a key bioprocess parameter for enhanced overall efficiency of the system, representing essential information for the implementation of lactobionic acid production at a large scale.

1. Introduction

Lactobionic acid has recently burst onto the cosmetic and pharmaceutical scene as a relevant polyhydroxy acid covering a broad spectrum of applications. In addition to its cosmeceutical use (Green et al., 2009), lactobionic acid is being increasingly employed as a potential drug carrier (Wu et al., 2009; Zhang et al., 2011), as well as in the development of carbohydrate-functionalized nanoparticles used as therapeutic agents for biomedical purposes (Lin et al., 2009). Considering that its commercial relevance is undergoing stepwise growth (Affertsholt, 2007), both the development and implementation of feasible lactobionic production systems appear as crucial key challenges to meet market demands.

Presently, emerging considerations regarding environmental regulatory frameworks are moving the dairy industry towards more sustainable practices. As a result, cheese whey has been the subject of valorization either to obtain different value-added bioproducts (Koutinas et al., 2009; Guimarães et al., 2010) or to reduce its pollution potential (Ghaly and Kamal, 2004).

Within this context, cheese whey has been proved to be a suitable, inexpensive and attractive source for obtaining lactobionic acid through a biotechnological process carried out by *Pseudomonas taetrolens* (Alonso et al., 2011). However, as submerged microbial bioprocesses involving aerobic platforms are often strongly influenced by mass transfer limitations, the availability of dissolved oxygen to microbial cells must accordingly be considered (García-Ochoa and Gómez, 2009). Both monitoring and proper control of dissolved oxygen levels represent decisive factors in aerobic systems seeing as insufficient oxygen supply may inhibit metabolism, cell growth and microbial biosynthesis. Owing to their intrinsic nature, oxygen as well as agitation regimes have been shown to play a major role in numerous submerged liquid systems involved in gellam (Giavasis et al., 2006), hyaluronic acid (Huang et al., 2006), ganoderic acid (Tang and Zhong, 2003), alginate (Peña et al., 2000), enzyme (Rahulan et al., 2011) or xantham gum synthesis (Amanullah et al., 1998). Consequently, the elucidation of suitable aeration supply schemes enables the generation of a suitable microenvironment for improved cellular proliferation patterns, as well as higher metabolite production yields.

In industrial practice, the mass transfer coefficient (K_La) as well as oxygen requirements by microbial cells (measured as the oxygen uptake rate, OUR) are used as scale-up criteria (Zou et al., 2008; García-Ochoa and Gómez, 2009) given that K_La is essentially a measure of how much oxygen can be supplied to microorganisms growing in a bioreactor (Bandyopadhyay et al., 2009). Specifically, oxygen mass transfer has been chosen as the controlling step rate for the overall behavior in oxygen-limited fermentation processes (García-Ochoa et al., 2000; Lozano et al., 2011). In fact, a concise understanding of the key oxygen parameters associated with any submerged liquid culture is crucial to enhance microbial performance (Wang et al., 2010; Xu and Zhong, 2011).

The aim of the present study was thus to investigate the relationship between dissolved oxygen and biotechnological production of lactobionic acid from whey by *P. taetrolens*, exploring for

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the first time bioconversion performance under oxygen-limited and non-oxygen-limited conditions. To this end, bioreactor cultivations were performed under different aeration and agitation rates in order to examine whether such effects would have an impact on lactobionic acid synthesis. Two bioprocess parameters were also evaluated, namely the oxygen uptake rate and the volumetric oxygen transfer coefficient, thereby enabling improved knowledge of the role of oxygen in culture performance and providing insights into the most suitable oxygen supply scheme for optimal lactobionic acid production from whey.

2. Methods

2.1. Microorganism

Pseudomonas taetrolens LMG 2336, obtained from the Belgian Coordinated Collection of Microorganisms (Gent, Belgium), was maintained frozen (in 40% [v/v] glycerol at −20 °C). This strain was subsequently subcultured on Nutrient Broth (NB, containing 1 g/L meat extract, 2 g/L yeast extract, 5 g/L peptone and 5 g/L NaCl) agar plates, incubated for 48 h at 30 °C and then preserved at 4 °C.

2.2. Inoculum and seed culture preparation

A loopful of *P. taetrolens* from a fresh Nutrient Broth agar plate was used to inoculate a 500 mL Erlenmeyer flask containing 100 mL of NB medium. This flask was incubated on an orbital shaker (Infors HT, model Flyer Aerotron, Switzerland) at 250 rpm and 30 °C for 10 h. Actively growing cells from this culture were then harvested by centrifugation at 11,000×*g* for 10 min and re-suspended in a 500 mL Erlenmeyer flask containing 100 mL of whey. This flask was incubated at 250 rpm in an orbital shaker at 30 °C for 12 h and subsequently employed as seed culture in the bioreactor experiments containing sweet whey.

2.3. Sweet whey preparation

Cheese sweet whey (provided by ILAS, Asturias, Spain) was 1-fold diluted with distilled water (1:1) and adjusted to pH 6.5 (by adding NaOH 6 N) prior to sterilization using a tangential microfiltration device equipped with a PVDF membrane-cassette of 0.22 μm pore size (Millipore, Massachusetts, USA).

2.4. Batch cultivations in stirred tank bioreactor

Batch cultivations were performed in a 2-L bioreactor (BioFlo 110, New Brunswick Scientific Co., New Jersey, USA) with 1 L of whey as working volume. Bioreactor experiments with an inoculation level of 10% (v/v) were conducted at 30 °C. The bioreactor was equipped with a pH meter (Mettler Toledo, Switzerland) and a polarographic dissolved oxygen electrode (InPro 6830, Mettler Toledo, Switzerland) in order to measure pH values on line and continuously monitor dissolved oxygen tension (DOT), respectively. Excessive foam formation was prevented by automatic addition of diluted (1:10) Y-30 emulsion (Sigma–Aldrich, Steinheim, Germany). An efficient two-stage pH-shifted bioconversion strategy was adopted as previously described (Alonso et al., 2011): pH was controlled above 6.5 (pH was left uncontrolled above this value during the growth phase and subsequently maintained at 6.5) by means of computer-controlled peristaltic pumps via automatic addition of 2 M NaOH. These prior conditions were applied to all cultivations unless otherwise specified. Cultivations were carried out in duplicate as independent experiments.

2.5. Influence of oxygen deprivation after the growth phase

Process performance was analyzed by combining a two-stage batch cultivation carried out at a medium agitation rate (350 rpm): an initial aerobic growth phase (1 Lpm during 0–10 h) followed by an oxygen supply deprivation stage after the exponential growth phase (10–60 h).

2.6. Effect of agitation rate on biomass and lactobionic acid production from whey

In order to examine the influence of the agitation rate on bioprocess parameters, bioreactor cultivations were carried out at 150, 350, 500, 700 and 1000 rpm with the aeration rate kept at 1 Lpm.

2.7. Effect of aeration rate on bioprocess parameters

The effect of different aeration rates (0, 0.5, 1, 1.5 and 2 Lpm) on bioprocess parameters was studied in batch cultivations carried out at 350 rpm.

2.8. Effect of dissolved oxygen concentration under high-cell density conditions

In order to gain insight into how the system and bioprocess parameters are affected by the availability of different dissolved oxygen concentration, high-cell density cultivations were performed under several DOT control levels during the exponential growth phase. The DOT level was left free or controlled at 20%, 40% and 60% (percentage of air saturation) by cascading the agitation speed (from 350 up to 500 rpm). For the cell density conditions, cultivations were conducted with an inoculation level of 30% (v/v) at 1 Lpm as aeration rate.

2.9. Determination of the oxygen uptake rate (OUR) and volumetric oxygen transfer coefficient (K_La)

The oxygen uptake rate (OUR) and volumetric oxygen transfer coefficient (K_La) were determined via the dynamic method (Bandyopadhyay et al., 2009; García-Ochoa and Gómez, 2009). The concentration of dissolved oxygen under steady state conditions can thus be formulated as Eq. (1):

$$\frac{dC}{dt} = K_La(C^* - C_L) - q_{O_2}X = 0 \quad (1)$$

where q_{O_2} is the specific oxygen consumption rate, X is the concentration of biomass, while the first term corresponds to the mass transfer rate. Accordingly, the OUR was obtained from the slope of the plot of dissolved oxygen concentration (C_L) versus time following a momentary interruption of air supply to the bioreactor. After aeration was re-established, K_La was calculated from the slope of Eq. (2) by plotting C_L versus $(q_{O_2}X + dC_L/dt)$:

$$C_L = C^* - \frac{1}{K_La}\left(q_{O_2}X + \frac{dC_L}{dt}\right) \quad (2)$$

2.10. Analytical methods

Bacterial growth was measured spectrophotometrically as optical density at 600 nm (Shimazdu, UV 1203 model) after centrifugation of culture samples at 16,000×*g* for 5 min. Optical density data were converted to cell dry weight (expressed in grams per liter) using the corresponding calibration curve obtained previously.

The lactobionic acid and lactose content of cell-free culture samples were measured by high performance liquid chromatography