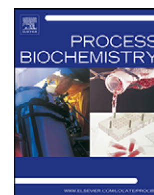
Process Biochemistry 44 (2009) 975–978

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio



Lipopeptide overproduction by cell immobilization on iron-enriched light polymer particles

Frédérique Gancel [a], Ludovic Montastruc [a], Tao Liu [b], Ling Zhao [b], Iordan Nikov [a,*]

[a] Laboratoire ProBioGEM EA 1026, Polytech'Lille, Université Lille 1, Lille, France
[b] State Key Laboratory of Chemical Engineering, East China University of Science and Technology, Shanghai, PR China

ARTICLE INFO

Article history:
Received 19 September 2008
Received in revised form 25 February 2009
Accepted 25 April 2009

Keywords:
Surfactin
Fengycin
Iron polymer
Biofilm
Light carriers

ABSTRACT

The study concerns surfactin and/or fengycin batch production by immobilized cells of *Bacillus subtilis* ATCC 21332. Light carriers designed for a three phase inverse fluidized bed biofilm reactor (TPFIBR) were used. With respect to the biofilm reactor development, a new support based on iron grafting onto polypropylene foams has been proposed. A suspension solid-state grafting process was applied to graft ferric acetylacetonate onto polypropylene (PP) foams with a density of 0.3–0.7 g/cm^3. The iron contents grafted onto PP increased with the reaction time and then it tended to level off. The iron contents at 7.5 and 10 h are 0.74 and 0.75 wt%, respectively. It was specified that the equilibrium was reached at 7.5 h. Influence of particles on lipopeptide production was analyzed in two kinds of experiments: preliminary colonization step of particles, followed by production step in modified culture medium (named in this work colonization step) or direct addition of pellets in culture medium (named production step). All PP+ iron pellets promoted biomass enhancement. The production yield was modified for all types of PP supports, containing respectively 0, 0.35 and 0.75% of iron. The immobilized cultures produced 2.09–4.3 times more biosurfactants than planktonic cells. In production experiments addition of carriers seemed to modify the ratio between surfactin and fengycin with an enhancement of the fengycin production. The highest concentration of fengycin was obtained with addition of pellets containing 0.35% of iron.

© 2009 Published by Elsevier Ltd.

1. Introduction

In literature, numerous studies concerning free cells productivities of biosurfactants have been reported. Several parameters such as carbon sources, temperature, amount of inorganic ions, and addition of solid carriers may trigger both strain growth and lipopeptide synthesis. In the present work, we focused on evaluating various bioreactor parameters and systems with respect to efficient lipopeptide production by *Bacillus subtilis* ATCC 21332 immobilized bacteria on light carriers designed for use in three phase inverse fluidized bed biofilm reactor (TPFIBR). The *B. subtilis* ATCC 21332 is known to produce a single surfactin lipopeptide [1,2], but this strain was recently proved to be a co-producer of surfactin and fengycin [3]. However, the lipopeptide surfactin is one of the most powerful biosurfactants, while fengycins show more strong antifungal activity in combination with surfactins [4]. The use of immobilized bacteria, leading to high cell concentrations within the reactor, seemed a promising method for improving reactor performance and selectivity [5,6–8]. With respect to the cell's immobilizing performance various parameters such as solid supports, culture medium, oxygen transfer and ions concentrations of metals are object of analysis. According to Wei and Chu [9,10], surfactin concentration was enhanced when iron concentration in the medium raised 4 μM and when Mn^{2+} was added. Wei et al. [11] determined with experimental design plan that Mg^{2+}, K^{+}, Mn^{2+} and Fe^{2+} were the more significant factors affecting production of surfactin. Yeh et al. [12], Davies et al. [1] and Lee and Kim [13], studied the influence of oxygen concentration on the synthesis of the biosurfactant. Yeh et al. [14] found that the oxygen volumetric mass transfer coefficient (K_La) was highly correlated with the performance of surfactin production. For planktonic cultures, limitation of O_2 enhances the production while surfactin is no more produced under static conditions. The highest surfactin productivity was achieved when the fermentation was carried out at a K_La value of 0.0132 s^{-1}. The focused inverse fluidized bed reactor shows high mass transfer at relatively low gas flow rate ($K_La > 0.016$ s^{-1} [15,16]). This type of reactor has found a privileged place in biotechnology and various industrial-scale applications do exist: ethanol production, aerobic and anaerobic wastewater treatment [17–19]. The main difficulty during the production process in aerated and stirred bioreactors lies in the high foaming properties of the surface-active compounds. Yeh et al. [12] showed that activated carbon or expanded clay added to the liquid medium led

* Corresponding author. Tel.: +33 03 28 76 74 10; fax: +33 03 28 76 74 01.
E-mail address: Iordan.Nikov@polytech-lille.fr (I. Nikov).

1359-5113/$ – see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.procbio.2009.04.023

to increase of surfactin production up to 2150 and 3300 mg L^{-1}, respectively (22- and 33-fold increase when compared to the control experiment). Diimitrov et al. [7] were tested different solids surfaces, including polypropylene (PP), with respect to propose a method to improve the biofilm growth on different polymer materials by modifying their surface properties. The ability of two aerobic bacteria strains: *Pseudomonas aeruginosa O1* and *B. subtilis CIP 5265* to grow on various non-coated and coated polymer materials were investigated also. It was found that supports with modified surface show higher biofilm development rate and better surface colonization. The influence of the surface free energy on the detachment force and correspondingly on the biofilm formation was demonstrated.

According to these previous results, we choose in this work to study the effect of light PP solid carriers coated with Fe^{2+}. A batch stirred tanks reactors were used as a model and also a control. The particles have densities lower than the liquid phase, generally for TPFIBR, between 0.3 and 0.7 g/cm^3. Moreover, the particles should support a temperature of 130 °C which permit sterilization. Polypropylene, one of the general plastics, is capable of being used at high temperature repeatedly but the mass density of original PP pellets is 0.9 g/cm^3, higher than the required densities of the biofilm carriers. Xu et al. [20] showed that the mass densities of polypropylene could be regulated easily to 0.3–0.7 g/cm^3 by using a supercritical CO_2 assisted foaming process while retaining the thermal stability of polypropylene.

2. Materials and method

2.1. Bacterial strain and culture conditions

B. subtilis ATCC 21332 from the collection of ProBioGem Laboratory and the same, recently obtained from the American Type Culture Collection, was used. The strain ATCC 21332, was recently proved to be a co-producer of surfactin and fengycin [3] The strain was cultivated in modified Landy base medium at pH 7 [21] buffered with MOPS 100 mM without or with glutamic acid (respectively named Lm1 and Lm2 media). Cells were cultivated overnight in Lm2 medium at 30 °C then washed and concentrated 10 times by centrifugation. The main cultures were inoculated into 1000 mL flasks containing 200 mL of Lm1 (colonization experiment) or Lm2 (production experiment) medium, in order to obtain 0.5 as initial optical density (at 600 nm) In colonization experiments, Lm1 medium was first used to avoid the synthesis of lipopeptides. Particles were allowed to be coated by the strain at 40 rpm for 48 h. The supernatant was then discarded and both free cells and coated beads were put in 200 mL of Lm2 medium for 48 h at 140 rpm, 30 °C to produce biosurfactants. In production experiments, pellets were directly added in Lm2 medium and lipopeptides were produced as explained above during 48 h. All experiments were achieved in batch conditions. With respect to reproduce hydrodynamic and mass transfer conditions of TPIFB [5,15], the concentration of particles was equal to 1 g per 15 mL of culture medium and the aerobic fermentation, namely the production of surfactin, was realised under O_2-sufficient conditions using 1000 mL flasks containing 200 mL of medium. All samples were triplicate.

2.2. Quantitative analysis of biomass

At the end of the production, pellets were sonicated with 4 mL of NaOH (0.1 M) during 5 min at 20 kHz, and proteins were quantified by Lowry method. After centrifugation, planktonic biomass was quantified by the same method.

2.3. Quantitative analysis of lipopeptides

Preliminary tests showed that *B. subtilis* ATCC 213332 produced two kinds of lipopeptides, surfactin and fengycin. At the end of the culture, 1 mL of supernatant was purified on C_{18} column (Extract - clean SPE 500 mg, Altech) and eluted in methanol.

The surfactin concentration was determined by reverse phase C_{18} HPLC (600 s, Waters, USA) equipped with a Merck C_{18} column (5 μm, Merck, Germany) as previously described [8,21,22]. The standard of surfactin was purchased from Sigma (USA). The Fengycins were eluted during 40 min under a gradient ACN/H_2O/TFA from 45/55/0.1 to 55/45/0.1 at 0.6 mL/min. The standard of fengycins was kindly provided by Dr M. Deleu from the Agricultural University of Gembloux (Belgium) and gave peaks between 10 and 25 min. Peaks were selected through calibration and spectra were analyzed using values of second derivative which give two major peaks at 213 and 236 nm associated with a minor peak at 290 nm [21,23].

2.4. Biofilm carriers

Foamed polypropylene pellets were used as biofilm carriers in this work.

2.4.1. PP foaming process using supercritical carbon dioxide

The polypropylene (PP) pellets with an average diameter of 3–4 mm and a mass density of 0.9 g/cm^3 were supplied by Shanghai Petrochemical Company. The mass-average molar mass of the PP was 188,700 g/mol. Its polydispersity index and crystallinity were 5.1 and 47%, respectively.

In order to reduce the mass density of original PP pellets (from 0.9 to 0.3–0.7 g/cm^3), an environmental-friendly technique previously described was used to prepare PP microcellular foams using supercritical carbon dioxide (scCO_2) as a foaming agent [20]. The CO_2 (purity 99.9%) was obtained from Air Product Co., Shanghai, China. Briefly, a high-pressure stainless steel vessel with internal volume of 100 cm^3 was used. About 20 g PP pellets were placed in the high-pressure vessel and then washed with low-pressure CO_2. Thereafter, the high-pressure vessel was immersed in a oil bath with a controlling accuracy of ±0.2 °C and rapidly heated to 157 °C. A given amount of CO_2 was charged to a pressure of 15 MPa, which was measured with accuracy of ±0.01 MPa by a pressure transducer of Shenzhen MSI/JL Electronics Co., China. The CO_2 loading was achieved by a DZB-1A syringe pump of Beijing Satellite Instrument Co., China, with an accuracy of 0.01 cm^3. After the sorption of CO_2 into the PP pellets reached at equilibrium, the CO_2 in the high-pressure vessel was released from the foaming pressure to the ambient one. The average depressurization rate was controlled at 15 MPa/s. The foamed PP pellets were then taken out for subsequent analysis and application.

2.4.2. Physical–chemical characterization of carriers

The cell morphologies of the foamed PP samples were characterized by a JSM-6360LV scanning electron microscopy (SEM, Fig. 1). The samples were immersed in liquid nitrogen for 10 min and then fractured. The SEM scanned fractured surfaces. The average cell size and cell density was calculated through the analysis of the SEM photographs by the software Winroof.

The mass densities of foamed PP samples ρ_f were measured according to ASTMD792-00 involving weighing polymer foam in water using a sinker. And ρ_f was calculated as follows:

$$\rho_f = \frac{a}{a + w - b}\rho_{\text{water}} \tag{1}$$

where *a* is the apparent mass of specimen in air without sinker, *b* the apparent mass of specimen and sinker completely immersed in water and *w* is the apparent mass of the totally immersed sinker.

2.4.3. Grafting process of ferric acetylacetonate onto PP

Three types of particles were used containing 0, 0.35 and 0.74% of iron (w/w) benzoyl peroxide (BPO) (chemical-grade) was purchased from Shanghai Chemical Co. and purified twice in chloroform before use. Ferric acetylacetonate {[$CH_3COCH{=}C(O-)CH_3$]$_3$Fe} with a purity of 97% was purchased from Sigma–Aldrich Co.

A suspension solid-state grafting process was applied by using water as a dispersion medium, BPO as an initiator and a small amount of toluene as a swelling agent. In the typical procedure, reaction was carried out in a 250-mL three-necked

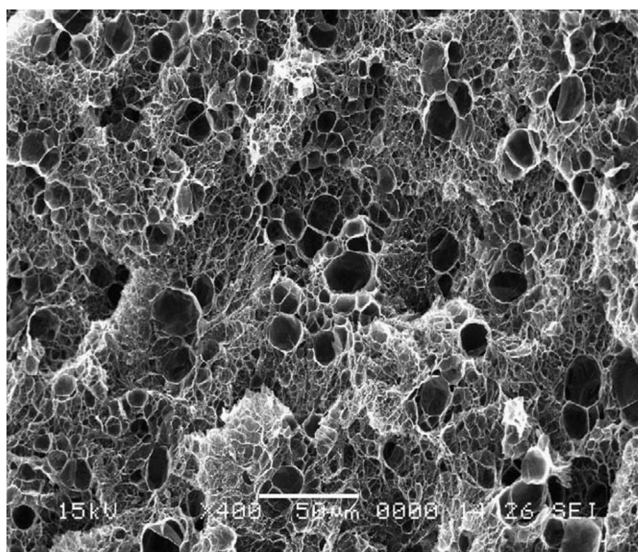


Fig. 1. SEM micrographs of foamed PP.

Download English Version:

https://daneshyari.com/en/article/35333

Download Persian Version:

https://daneshyari.com/article/35333

Daneshyari.com