Rev Argent Microbiol. 2015;47(4):344–349

REVISTA ARGENTINA DE MICROBIOLOGÍA

www.elsevier.es/ram

ORIGINAL ARTICLE

Control of agitation and aeration rates in the production of surfactin in foam overflowing fed-batch culture with industrial fermentation

Shulin Yao, Shengming Zhao, Zhaoxin Lu, Yuqi Gao, Fengxia Lv, Xiaomei Bie*

College of Food Science and Technology, Nanjing Agricultural University, Key Laboratory of Food Processing and Quality Control, Ministry of Agriculture of China, 1 Weigang, Nanjing 210095, PR China

Received 4 December 2014; accepted 7 September 2015

KEYWORDS
Surfactin;
Agitation rate;
Aeration rate;
Foam overflowing;
Fed-batch culture;
Industrial fermentation

Abstract *Bacillus amyloliquefaciens* fmb50 produces a high yield of surfactin, a lipopeptide-type biosurfactant that has been widely studied and has potential applications in many fields. A foam overflowing culture has been successfully used in the combined production-enrichment fermentation of surfactin. In this study, the agitation and aeration rates were found to have relationships with foam formation and surfactin enrichment. A maximum surfactin concentration of 4.7 g/l of foam was obtained after 21 h of culture with an agitation rate of 150 rpm and an aeration rate of 1 vvm in fed-batch culture. By controlling the foam overflow rate (f_{out}) of a fed-batch culture, surfactin concentration in the foam was continuously maintained above 4 g/l.

PALABRAS CLAVE
Surfactina;
Tasa de agitación;
Tasa de aireación;
Espuma desbordante;
Cultivo *fed-batch*;
Fermentación industrial

Control de las tasas de aireación y agitación en la producción de surfactina en un cultivo alimentado *(fed-batch)* en espuma desbordante con fermentación industrial

Resumen *Bacillus amyloliquefaciens* fmb50 produce gran cantidad de surfactina, un biosurfactante de tipo lipopeptídico que ha sido objeto de estudios pormenorizados y tiene aplicaciones en muchos campos. El cultivo en espuma desbordante se ha utilizado con éxito en la fermentación combinada de producción-enriquecimiento de surfactina. En este estudio, se halló que las tasas de aireación y agitación tienen relación con la formación de espuma y el enriquecimiento de la surfactina. Se obtuvo una concentración máxima de surfactina de 4,7 g/l de espuma después de 21 h de cultivo con una tasa de agitación de 150 rpm y una tasa de

* Corresponding author.
E-mail address: bxm43@jiau.edu.cn (X. Bie).

http://dx.doi.org/10.1016/j.ram.2015.09.003

aireación de 1 vvm en un cultivo alimentado *(fed-batch)*. Al controlar la tasa de espuma desbordante (f_{out}) de un cultivo *fed-batch*, la concentración de surfactina en la espuma se mantuvo continua por encima de 4 g/l.

Introduction

Biosurfactants are amphiphilic molecules widely produced by a variety of microorganisms, which have been considered as an alternative to chemical surfactants. Lipopeptides are one of the major types of biosurfactants[14]. As the most effective biosurfactant that has been found so far, surfactin can lower the surface tension of water from 72 to 27 mN/m[5,12]. Surfactin exhibits antibacterial and antiviral properties and biodegradability, and appears to be promising for applications in areas such as bioremediation and oil recovery[1,2,15]. However, after almost 50 years, surfactin is not yet a viable alternative to chemical surfactants because of the low yield in bioreactors[13], its relatively high medium cost, and severe foaming in aerated and stirred bioreactors[16]. Therefore, the production of surfactin is still limited to laboratory scale[5]. To cope with these problems, renewable substrates and foam overflow fermentation were used in recent years[4,9].

Some studies on fed-batch culture of surfactin have been undertaken in the past few years[4,5,16]. In foam overflowing fed-batch culture (FOFC), the flow rates of the feed and of the overflow foam were equal, and thus the broth volume in the bioreactor was kept constant, and continuous enrichment of surfactin in foam overflow was accomplished[6]. It has been shown that by controlling the agitation and aeration rates, maximum surfactin productivity could be achieved when the oxygen volumetric mass transfer coefficient (k_La) value was 0.0132/s[16]. However, the effect of agitation and aeration rates on surfactin enrichment in the foam has not been reported.

In our previous studies, we identified a strain of *Bacillus amyloliquefaciens* as producer of five surfactin homologues by using high performance liquid chromatography (HPLC) and electrospray ionization mass spectrometry methods[13]. A high yield of surfactin from strain *B. amyloliquefaciens* fmb50 was obtained by genome shuffling, and a method for surfactin determination was established[17].

In this work, a semi-defined medium, which is of low cost and high yield compared with the commonly used Landy medium, was initially combined with foam overflow batch culture. The effect of agitation and aeration rates on the foam overflowing rate (f_{out}) and surfactin enrichment in the batch culture process were studied. By using fed-batch culture, a continuous, high concentration enrichment of surfactin could be achieved. This novel fermentation technology has a potential for the industrial application of surfactin production.

Materials and methods

Microorganism and culture media

B. amyloliquefaciens fmb50 (CGMCC No. 6249), the surfactin producer used in this study, is registered by the China Committee for Culture Collection of Microorganisms.

The seed medium (BPY) consisted of: beef extract 5.0 g/l, peptone 10.0 g/l, yeast extract 5.0 g/l, glucose 10.0 g/l and NaCl 5.0 g/l (pH 7.0).

The semi-defined medium (IBM) used for fermentation was optimized by the Taguchi method in our previous work, and consisted of: corn powder 35 g/l, ammonium nitrate 15 g/l, urea 6 g/l, KCl 1.47 g/l, NaH_2PO_4 20 mmol/l, $MnSO_4$ 0.5 mmol/l, $MgSO_4$ 0.1 mmol/l, $CuSO_4$ 12.8 μmol/l, $FeSO_4$ 1 μmol/l and $CaCl_2$ 0.5 μmol/l (pH 7.0).

Flask and bioreactor culture conditions

In the primary inoculum, a loop of colonies from a fresh potato dextrose agar-slant was transferred into 50 ml BPY medium in shaken flasks (250 ml) and cultured at 37 °C and 180 rpm for 12 h. For the secondary inoculation, 10 ml of the primary culture was inoculated into 200 ml BPY medium in shaken flasks[11], and cultured in the same conditions as those in the primary inoculum.

Bioreactor cultivation was performed in a 19 l bioreactor containing 12 l of medium (L1523, Bioengineering AG, Switzerland); the temperature was controlled at 32 °C and the pH was maintained at 7.0 with the automatic addition of 4.0 mol/l NaOH. Three control strategies were adopted in batch cultivation to investigate the effects of agitation and aeration rates on f_{out} and surfactin enrichment in foam from overflowing cultures. The agitation and aeration rates were controlled as shown in Table 1. In control 1, the agitation and aeration rates were controlled at 200 rpm and at 0.66 vvm respectively. In control 2, the agitation and aeration rates were controlled at a relatively high level; the agitation rate was 300 rpm and the aeration was increased from 1.2 to 2.66 vvm as the foam overflowed and the medium volume in the bioreactor was reduced.

Analytical methods

The colony forming units (CFU) were calculated using plate colony-counting methods. Dry cell weight (DCW) was obtained by collecting the fermentation broth with different