Journal of the Taiwan Institute of Chemical Engineers 43 (2012) 347–353

Contents lists available at SciVerse ScienceDirect

Journal of the Taiwan Institute of Chemical Engineers

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

Microfiltration characteristics of *Bacillus subtilis* fermentation broths

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ARTICLE INFO

Article history:
Received 18 September 2011
Received in revised form 14 December 2011
Accepted 17 December 2011
Available online 31 January 2012

Keywords:
Microfiltration
Bacillus subtilis
Membrane filtration
Bio-separation
Cake properties

ABSTRACT

The microfiltration characteristics of *Bacillus subtilis* broths under different culture conditions were studied. In the no medium addition culture condition the major component in the broth was sole *B. subtilis* cells. The filtration curve of dt/dv vs. v (reciprocal of filtration flux vs. filtrate volume) can be divided into three regions. After a short relaxation time the tangent slope increases drastically. This implies that significant cell deformation and cake compression occurs in the second region. The average specific cake filtration resistance will reach the maximum value at the filtration curve inflection point. The cake compressibility gradually decreases in the third region because most solid compressive pressures are depleted by the formed compact cake. In the medium addition culture condition the *B. subtilis* cells and extracellular polymer substances (EPS) flocs concentrations in the broth increase markedly with culture time. The filtration curve tangent slope increases continuously during the entire filtration period because of the highly compressible cake. All proteins in the extra-cellular polymeric substances are retained by the filter cake and membrane during a filtration, while most polysaccharides have the opportunity to penetrate into the filtrate when the culture time exceeds 1 day. Polysaccharide rejection increases with increasing filtration pressure for the 1-day culture broth. However, the filtration pressure has a trivial effect on the polysaccharide rejection for longer culture times. The filter cake resistance formed by *B. subtilis* cells and EPS flocs plays an important role in determining the overall filtration resistance. A longer culture time and lower filtration pressure are beneficial for protein/polysaccharide separation using microfiltration.

1. Introduction

Bacillus subtilis is a rod-shaped bacterium that secretes numerous enzymes to degrade a variety of substances during metabolism [1]. In addition to being a cell factory for pharmaceutical proteins, *B. subtilis* has many industrial and environmental applications. For instance, biosurfactant production from *B. subtilis* bioconversions has created great potential for biotechnological and pharmaceutical applications in recent years [2–4]. The *B. subtilis* isolated from fermented food also showed inhibition to various fungi [4]. *B. subtilis* was also reported effective for biological control of multiple plant diseases caused by microbial infections [5]. Vijayalakshmi and Raichur [6] studied the utility of *B. subtilis* as a bioflocculant to improve particle settling. They found that the adhesion of *B. subtilis* to fine coal particles was independent of the pH, while enhanced by adding electrolytes. Toledo et al. [7] showed that *B. subtilis* was able to emulsify organic solvents, mineral oil and crude oil as a bioemulsifier for bioremediation applications. Yang et al. [8] reported that *B. subtilis* exhibited the ability to enhance nitrogen removal from ammonium-rich wastewater under fully aerobic conditions.

Membrane filtration has been used for recovering surfactin from *B. subtilis* fermentations. Surfactin is a natural bacterial lipopeptide produced by *B. subtilis*. The surfactin separation efficiency from *B. subtilis* fermentation broth is the essential issue in developing commercial scale processes. Isa et al. [9,10] used a two-step tangential membrane filtration process for surfactin purification from *B. subtilis* fermentation broth. They found that the transmembrane pressure had no significant effect on the filtration rate and surfactin rejection. A polyethersulfone membrane with a molecular weight cut-off of 10 kDa was suitable for surfactin purification due to the high recovery rate. Chen et al. [11] used cross-flow ultrafiltration to purify acid-based pre-treated *B. subtilis* fermentation broths using a polyethersulfone membrane with a molecular weight cut-off of 100 kDa. They indicated that the filtration rate increased with increasing cross-flow velocity, but decreased with increasing initial surfactin concentration and transmembrane pressure. Dead-end ultrafiltration of *B. subtilis* fermentation broths was also carried out by the same authors [12]. The broth was pre-treated by centrifugation under a centrifugal effect of 10,000 × *g*. They reported that the filtration resistance due to solute concentration polarization dominated the flux decline, while the sum of resistances due to cake formation and solute adsorption contributed below 3% of the overall resistance.

In recent years, microfiltration has been increasingly used in the pretreatment of biotechnology products. Microbial cells are

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doi:10.1016/j.jtice.2011.12.009

Nomenclature

A	empirical constant defined in Eq. (3) [–]
c	dry cake mass per unit received filtrate volume [kg/m^3]
$C_{o,s}$	polysaccharide concentration in broth [kg/m^3]
$C_{p,s}$	polysaccharide concentration in filtrate [kg/m^3]
n	cake compressibility [–]
ΔP	filtration pressure [N/m^2]
R_c	cake filtration resistance [m^{-1}]
R_m	membrane filtration resistance [m^{-1}]
$R_{rej,s}$	polysaccharide rejection [–]
t	filtration time [s]
v	filtrate volume per unit area [m^3/m^2]
w_c	cake mass per unit area [kg/m^2]

Greek symbols

α_{av}	average specific cake filtration resistance [m/kg]
μ	viscosity of fluid [kg/s m]

retained by the filter membrane to form a filter cake, while some smaller soluble components, *e.g.*, polysaccharides, proteins, *etc.*, will penetrate through the cake and membrane into the filtrate. Since most microbial cells are deformable and compressible, the biological cakes always exhibit high compressibility and high specific filtration resistance [13–16], often resulting in a low filtration rate. Hwang et al. [15] studied the dead-end microfiltration of a *Pseudomonas* suspension. Their results showed that a compact cake layer was formed next to the membrane surface in the early filtration periods. McCarthy et al. [17] characterized the cake structure and compressibility in the dead-end filtration of different morphological yeasts using the mean cell aspect ratio. The cake compressibility increased with the increase in mean cell aspect ratio and cell deformability. Hwang and Yang [16] studied the role of polysaccharides on the dead-end microfiltration of microbial cells. They found that the filter cake became more compressible and exhibited much higher filtration resistance when polysaccharides co-existed in the cake structure. A cake compression-and-resistance model was also derived to explain the relationship between the average specific cake filtration resistance and filtration pressure. Therefore, to understand how the broth culture conditions, suspension composition and operating conditions affect the filtration performance is an essential step in improving filtration performance and designing an efficient microfiltration apparatus.

In this study, *B. subtilis* was cultured in two conditions, with no culture medium and with medium addition. The cell concentrations and extracellular polymer substances (EPS) in the broth under different culture conditions were measured. The filtration curves of dt/dv vs. v in *B. subtilis* dead-end microfiltration were analyzed to understand the filtration characteristics under various conditions. The average specific cake filtration resistance, cake compressibility and the protein and polysaccharide rejections under different culture conditions and filtration pressures were also compared and discussed.

2. Materials and methods

2.1. Bacillus subtilis culture and analysis methods

Bacillus subtilis white powder was purchased from Advanced Green Biotechnology Inc. in Taiwan. The powder was suspended uniformly in distilled water with a concentration of 6 g/L before culture. A 10 mM buffer solution prepared using sodium phosphate (Na_2HPO_4) and sodium hypophosphite (NaH_2PO_2) was used to keep the suspension at pH 7.3. Two culture conditions, no culture medium and medium addition, were selected in this study. A culture medium with a composition of 5 g/L yeast extract, 10 g/L tryptone, and 10 g//L NaCl was used in the later condition. The *B. subtilis* broth was then cultured in a shaker under 180 rpm at 37 °C for various setting culture times (1–4 days).

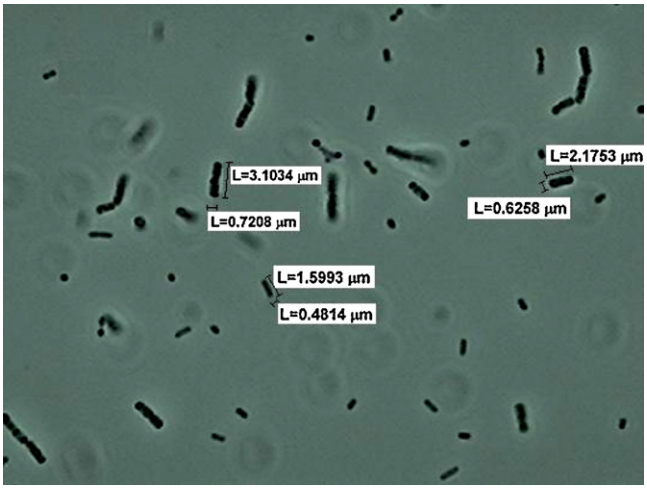

Fig. 1. The observation of *B. subtilis* cells in 3-day culture broth with no medium addition using *PIA System*.

In the no medium culture condition the major component in the broths was *B. subtilis* cells due to the difficult environment. The *B. subtilis* cells in a 3-day culture broth with no medium were observed using a MDS-3600 Microscope and *Power Image Analysis* (*PIA*) *System* produced by *Ching Hsing Computer-Tech Ltd.* in Taiwan and shown in Fig. 1. The *B. subtilis* cells had a rod-like shape with a diameter of 0.5–0.7 μm and a length of 1–5 μm. No evident increase in cell number was observed. The cell concentrations were counted to *ca.* 2.93×10^7 cells/L for 1-day culture, 3.39×10^7 cells/L for 2-day culture, and 4.11×10^7 cells/L for 3-day culture. In the medium addition condition the cell concentrations and related OD_{660} (optical density at 660 nm) values at different culture times are listed in Table 1. The cell number grew very quickly. The number became 50-fold on the second day and was double that the following next two days. The cell concentration could also be detected using an UV/visible spectrometer (Model: Helios β, *UNICAM* Co., UK). The measured absorptions using a wavelength of 660 nm were indicated as OD_{660} and shown in Table 1. The suspension in the broth after 1-day culture with medium addition was observed using the *PIA System* and is shown in Fig. 2. In addition to the cell growth extracellular polymeric substances (EPS) were formed due to cell metabolism. Some cotton-like flocs composed of EPS and cells were formed in the broth, as shown in the upper part of Fig. 2. The extracellular polymeric substances were composed primarily of proteins, polysaccharides, carbohydrates and a few nucleic acids, lipids and humic substances. The protein and polysaccharide concentrations in the broth and the

Table 1
The concentration and OD_{660} of *Bacillus subtlis* at different culture times.

Culture time (day)	Cell number/l	OD_{660}
1	1.14×10^7	0.107
2	5.60×10^8	0.164
4	1.21×10^9	0.214