EFFECT OF CARBON:NITROGEN AND DISSOLVED OXYGEN ON THE BIOSURFACTANTS PRODUCTION FROM *Bacillus subtilis*

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Abstract
The aim of the present study was to find the effect of the Carbon and Nitrogen (C:N) ratio and dissolved oxygen (DO) concentration on biosurfactant production at flask and reactor level respectively. The biosurfactants production from *Bacillus subtilis* was evaluated by superficial activity in supernatant as well as biological activity of supernatant freeze dried at different times. It was rated the C:N ratio 2.6, 5.7, 8 and 11.6, being the cultures with C:N ratio 8 and 11.6 with 6 and 7 respectively of superficial activity and that showed hemolytic activity and antimicrobial activity against *Saccharomyces cerevisiae* and *Fusarium*. In the case of DO in batch culture, it was rated C:N ratio 8 and 11.6, the lipopeptide production at a high level of DO was 17.26 g l-1 and 14.79 g l-1 respectively in comparison with the low level of DO with 7.96 g l-1 and 2.21 g l-1. The C:N ratio 8 and 11.6 at high level of DO were favourable to produce lipopeptides with surface activity.

Key words: *Bacillus subtilis*, biosurfactant, C:N ratio, dissolved oxygen.

Introduction

Surface active-compunds produced by microorganisms are of two main types, those that reduce surface tension at the air-water interface named biosurfactants and bioemulsifiers that reduce the interfacial tension between immiscible liquids, or the solid-liquid interface. These properties are because biosurfactants and bioemsusifiers are amphipathic molecules consisting in hydrophobic and hydrophilic moieties. Biosurfactants usually exhibit emulsifying capacity but bioemulsifiers do not necessarily reduce surface tension (Karanth et al. 1999). Biosurfactants comprise a wide range of chemical structures, such as glycolipids, lipopeptides, polysaccharide-protein complexes, phospholipids, fatty acids and neutral lipids. In case of lipopeptides, their antibacterial, antifungal and antiviral activities make them relevant molecules for applications in combating many diseases and ad therapeutic agent (Rodrigues et al. 2006). In addition, biosurfactants have other applications such as bioremediation and the dispersion of oil spills, enhanced oil recovery and other possible application fields are in the food and cosmetic industries, where most biosurfactants are used as emulsifiers (Kim et al. 1997). Considering its potential in the various industries, successful production of these lipopeptides in a large-scale bioreactor is essential.
Large scale biosurfactant production costs can be reduced through process optimization (Lang et al. 2002). The culture medium, carbon source and the growth conditions can influence the yields and types of biosurfactants produced (Cooper et al. 1981, Kim et al. 1997, Wei et al. 2003, Nitschke et al. 2004, Reis et al. 2004, Batista et al. 2006). Presently, no information are available regarding the effect of carbon-nitrogen (C:N) ratio and dissolved oxygen (DO) on biosurfactant production by *Bacillus subtilis*. Therefore, in this study it was decided to determine the lipopeptide production at four different C:N ratios and two levels of dissolved oxygen in flask and reactor level respectively.

Materials and Methods

Microorganism
An isolated *Bacillus subtilis* from a commercial product was utilized for all experiments. All inoculums were prepared in 1 liter Erlenmeyer flasks with 250 ml of nutrient broth, where one disc of paper with $1.9X10^7$ spores were added. Flasks were incubated at 30 °C and 150 rpm for 12 h.

C:N ratio experiments at flask level
All experiments to evaluate the effect of C:N ratio on biosurfactant production were assayed in 1 liter Erlenmeyer flask with 250 ml of different media. The C:N ratio tested were 2.6, 5.7, 8 and 11.6. All media had the same salt composition: 3 g/l KCl, 0.2 g/l $MgSO_4 \cdot 7H_2O$, 40 mg/l $MnSO_4$, 30 mg/l $CoCl_2$,