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Commentary on Optimizing Alternative Substrate for Simultaneous Production of Surfactin and 2,3-Butanediol by *Bacillus subtilis* LB5a

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Review Article

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ABSTRACT

In this short commentary, we put a light on the white technology, in which different wastes can be used to compose an optimum culture medium for the simultaneous production of surfactin and 2,3-butanediol. In addition, we combined two types of 2,3-butanediol analysis: solid-phase microextraction and liquid-liquid extraction. The simultaneous production of biochemicals is one of the most interesting strategies which could lead to the commercial viability of production.

INTRODUCTION

The white biotechnology is an important branch of green chemistry concept. It deals with novel roductions/applications or the replacement of chemical by biochemicals. Thus, biotechnological processes of enzymes, peptides, biosurfactants, etc., at industrial scale are increasing worldwide. Some theories point out the reasons for biosurfactant production by microorganisms: (i) to inhibit the growth of other cells, (ii) to store energy, (iii) to regulate the cell membrane attachment and detachment, (iv) to solubilize hydrophobic compounds, (v) to increase membrane permeability, and (vi) to protect (layer) the microorganism against high ionic strength [1,2]. Among the types of biosurfactant, surfactin is one of the most well-known, which is a heptapeptide (cyclic) linked to β–hydroxy fatty acid (12-16 carbons) [3,4]. Surfactin shows powerful surface activity (e.g. water: from 72 to 27 mN/m at a concentration as low as 10 mg/L) and also has biological properties such as antiviral, anti-tumorigenic, etc. [3,4].

Other remarkable biochemical is the 2,3-butanediol, which can be used in the production of printing inks, perfumes, fumigants, moistening, softening agents, explosives, antifreeze agent [5]; flavoring agents, precursors of polyurethane [6]; cosmetics [7]; food, pharmaceuticals [5,7]; plasticizers [5,6]; and rubber, [5-7].

During any biotechnological production (fermentation) many compounds are synthesized (e.g. enzymes, flavours, etc.), in which some of these biochemicals may have high-added value. Therefore, the simultaneous production always should be investigated. The simultaneous production does not require any implementation (facilities), however, it worth noting that high productivities of both (or more) biochemicals must be reached (sometimes the optimum condition of production are not the same for both biochemicals). In addition, an entire new purification process will need be set. In our previous study, it was proved the technical feasibility of simultaneous production of surfactin and 2,3-butanediol by *Bacillus subtilis* LB5a, in which the culture medium was composed by cassava wastewater, whey (2 industrial wastes) and activated carbon.

Production of Surfactin Using Industrial Wastes as Culture Medium

It is predicted that culture medium represents ≈ 30% of cost production [8,9]. Thus, the use of industrial waste as culture medium will significantly impact on the commercial viability of production. Some industrial wastes were already investigated, as culture medium, for the surfactin production, and many reports have described in details the production of surfactin using cassava wastewater as substrate.

The recovery of surfactin by foam overflow was already reported by Andrade et al. [8]. The foam overflow is an advantageous strategy for biosurfactant recovery since it is a quite simple technique and recovery high rates of biosurfactants [8]. The presence of activated carbon in the culture medium during the production of surfactin will affect the foam overflow because activated

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carbon adsorbs surfactin micelles [10]. On the other hand, activated carbon enhances (stimulation of cell growth as a biofilm) the surfactin production (36-fold).

Production of 2,3-Butanediol Using Industrial Wastes as Culture Medium

The production of 2,3-butanediol is a growth-associated process [11,12]. High yield as 100 g/L is usually reach, as showed by Zhang et al. [11] in their study where the production of 2,3-butanediol by *Serratia marcescens* H30 was using sucrose as carbon source. The authors described that the highest concentration was 139.92 g/L (acetoin and 2,3-butanediol). As already mentioned, the use of industrial wastes in biotechnological process is a quite interesting approach. In this sense, Wong et al. [12] described the production of 2,3-butanediol by *Klebsiella* sp. Zmd30 using hydrolyzed rice straw as culture medium. The authors reported that the highest production of 2,3-butanediol was ≈ 25 g/L.

Simultaneous Production of Biochemicals

The simultaneous production is one of the most potential strategies to overcome the production cost. However, this topic has not been much investigated. Most of researches has focused on the simultaneous saccharification and fermentation, for instance Li et al. [13] used *Platanus orientalis* leaves to produce hydrogen through simultaneous saccharification and fermentative by bacteria and He et al. [14] that described a semi-simultaneous saccharification and fermentative using Napier grass to produce acetone–butanol–ethanol (ABE fermentation).

Several other studies exclusively aimed the simultaneous production of two biochemicals, as an example Wu et al. [15] that detailed the biopolymers simultaneous production of extra cellular sphingan Ss and intracellular poly (R-3-hydroxybutyrate (PHB) by the bacteria *Sphingomonas sanxanigenens* NX02 and also Alonso et al. [16] that studied a culture medium composed by glucose and whey for the simultaneous production of lactobionic and gluconic acid. To the best of our knowledge, our previous report was the first simultaneous biotechnological production that deals with surfactin.

Simultaneous strategy generates (in terms of productivity) less residues in comparison with two bioprocesses: one for the production of surfactin and another for the production of 2,3-butanediol. In addition, there are no environmental issues related to the potential harmful effects of surfactin and 2,3-butanediol. Therefore, the waste disposal procedures should be focus on the biomass (bacteria)-sterilization of culture medium after the bioprocess.

Regarding the culture medium, cassava wastewater is known to contain cyanide, which is hazardous to environmental. Thus, the thermal pretreatment of cassava wastewater is essential for the cyanide removal and subsequent use of cassava wastewater. In this sense, whey does not have any specific hazardous compounds. However, whey and also cassava wastewater have high biochemical oxygen demands, which implies in environmental issues as well.

It is worth noting that the cassava crop cultivation and whey production (cheese making process) are carried out throughout the year, in which the variation in their composition is negligible (advantage supply). This characteristic allows a well-controlled bioprocess, even using two agro-industrial residues as substrate.

CONCLUSION

The simultaneous production of any bioprocesses should be investigated. This type of strategy could lead to the commercial viability of production since it is expected to reduce the cost of production. In this sense, the use of industrial wastes as culture medium is very aligned to the green chemistry concept (white industry) and does also favor the commercial viability. In our previous study, the optimized composition of culture medium for both surfactin and 2,3-butanediol was whey (27.7 g/L), activated carbon (25 g/L) and cassava wastewater (74 g/L). Therefore, both high-value biochemicals were produced using low value-added substrates.

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