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Immobilization of Cells for Erythritol Production

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Abstract

Today's generation, commercial erythritol is produced through free-cell fermentation with fungi in liquid media containing high concentrations of pure carbon sources. Alternative fermentation techniques, such as cell immobilisation, could result in an economic and energy improvement for erythritol-producing factories. The current study describes for the first time the feasibility of achieving cell immobilisation during erythritol production. Moniliella pollinis cells were successfully immobilised on a cotton cloth and placed inside a 2-L bioreactor, where they were fed red grape must supplemented with yeast extract. The similar results obtained for free and immobilised cells demonstrate the effectiveness of the immobilisation system. As a result, the proposed method for erythritol bioproduction eliminates the need for continuous preparation of fungal inocula before each fermentation batch,lowering reagent and energy costs.

Keywords: Immobilization • Winery surplus • Erythritol

Introduction

Erythritol is a four-carbon polyol that is commonly used in foods as a sweetener, flavour enhancer, humectant, stabiliser, and thickener. Erythritol's main benefits are its low glycemic response (suitable for diabetics), non-cariogenic effect, low calorie content, natural origin, and good digestibility. The US Food and Drug Administration classifies erythritol as GRAS (Generally Recognized as Safe), but the European Food Safety Authority advises not exceeding a daily erythritol intake of 0.71-0.78 g/kg body weight to avoid laxative effects. This polyol has the potential to be used as a platform chemical in the synthesis of butadiene, 1,4-butanediol, 2,5-dihydrofuran, and tetrahydrofuran. Commercial erythritol is primarily produced through the microbial transformation of liquid media containing high concentrations of pure carbon sources such as sucrose,glucose,fructose, or glycerol. When an excess of sugar, glycerol, or salts in the extracellular medium causes a high level of osmotic pressure on the microorganism, these carbon sources are converted into erythritol by various fungal strains of the genera Candida,Yarrowia,Moniliella, or Pseudozyma via the pentose phosphate pathway. In theory,1 mol hexose yields 1 mol erythritol, while 3 mol glycerol yields 2 mol erythritol.

Literature Review

Although some bacteria, such as Fructilactobacillus florum and Leuconostoc oenos, can produce erythritol at low concentrations, fungal strains are preferred for bulk erythritol synthesis due to their higher productivity and proven safety. In fact, the European Union's legislation specifically mentions the use of Moniliella pollinis and M. megachiliensis for erythritol production for food applications. Erythritol production from alternative substrates, such as agricultural, food, and industrial by-products and surplus, has recently received a lot of attention in order to reduce reagent costs associated with using pure sugars or pure glycerol as feedstocks. Sugarcane molasses, beet molasses, surplus grape must, and crude glycerol, for example, have all been considered for erythritol biosynthesis. Certain process innovations, such as the use of cellular debris from erythritol-producing fungi, have also been proposed.

Cell immobilisation techniques, on the other hand, have been successfully applied to other bioprocesses, which offers advantages in terms of stability and allows for the reuse of the biocatalyst. Cell immobilisation is the process of keeping a microorganism inside or on the surface of a supporting material for an extended period of time in order for it to grow and reproduce normally while interacting with the medium and performing the expected metabolic activities of industrial interest. Immobilized microorganisms can be easily recovered, recycled, and used in subsequent fermentation batches. Furthermore, no new microbial seeds are required to inoculate each batch because the microorganisms are already present (immobilised) in/on the supporting material and can colonise the new fermentation broth.

Discussion

The current study seeks to evaluate erythritol production using a cell-immobilization system and surplus grape must as a feedstock. First, five different fungal strains were compared to determine which one produced the most erythritol from grape must. The optimal C/N ratio was determined by optimising the addition of yeast extract (nitrogen source) to grape must. Because yeast extract is a costly reagent, the partial replacement of yeast extract by (NH4)2SO4 was also tested for cost-cutting purposes by comparing different combinations of both nitrogen sources. Finally, the fungal cells were immobilised on a cotton cloth and placed inside a bioreactor to determine their ability and stability to produce erythritol over multiple fermentation batches, The biofilm attached to the cotton towel served as a source of microorganisms, which colonised the liquid medium (i.e., the grape must) whenever a new fermentation batch began, allowing glucose and fructose to be converted into erythritol. During the immobilisation experiments in the bioreactor, the microbial cell densities reached 0.50-1.75 108 cells/mL in the liquid broth at the end of the exponential growth phase, which was comparable to the cell densities recorded for free-cell fermentations [1-5].

Conclusion

The efficient cell immobilisation system described herein is an intriguing alternative for erythritol production because it eliminates the need for continuous fungal cultivation (inocula preparation) prior to each fermentation batch, lowering reagent and energy costs. The immobilisation was performed on a cotton cloth in this study, but other immobilisation supports or mechanisms

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could be investigated in the future. Over four fermentation cycles, the proposed erythritol production method performed flawlessly. Nonetheless, the process must be improved before it can be carried out on a large scale, particularly in terms of aeration and agitation in order to achieve erythritol concentrations. Although the system has been tested with grape must as a feedstock, it is expected to function properly when fed with other suitable substrates high in sugars or glycerol, regardless of the nature of the carbon source (pure or byproduct-related ones). In any case, as previously stated, preferential use of by-products over pure substrates results in financial savings. Furthermore, the establishment of biorefineries using agri-food by-products or surplus products as substrates (such as grape must) could revitalise the depressed rural areas where these feedstocks are generated from agricultural activities, providing farmers with additional income.

Acknowledgement

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Conflict of Interest

There are no conflicts of interest by author.

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