Available online at www.sciencedirect.com
ScienceDirect
Chemosphere 70 (2008) 967–973
CHEMOSPHERE
www.elsevier.com/locate/chemosphere

Dibenzothiophene desulfurization by *Gordonia alkanivorans* strain 1B using recycled paper sludge hydrolyzate

Luís Alves [a], Susana Marques [a], José Matos [a], Rogério Tenreiro [b], Francisco M. Gírio [a,*]

[a] *INETI, Departamento de Biotecnologia, Estrada do Paço do Lumiar, 22, 1649-038 Lisboa, Portugal*
[b] *Universidade de Lisboa, Faculdade de Ciências, Centro de Genética e Biologia Molecular and Instituto de Ciência Aplicada e Tecnologia, Portugal*

Received 18 June 2007; received in revised form 8 August 2007; accepted 9 August 2007
Available online 25 September 2007

Abstract

Enzymatic hydrolyzates of recycled paper sludge were tested as suitable feedstock for biological desulfurization by *Gordonia alkanivorans* strain 1B. Only the hydrolyzate obtained after enzymatic mixture dialysis (dialyzed hydrolyzate) allowed dibenzothiophene (DBT) desulfurization, in spite of faster bacterial growth did occur on non-dialyzed hydrolyzate. For dialyzed hydrolyzate, 250 μM DBT was consumed after 96 h displaying a maximum specific productivity of 2-hydroxybiphenyl of 1.1 μmol g^{-1}(dry cell weight) h^{-1}. A comparison of the kinetics of biodesulfurization was assessed according to the type of hydrolyzate supplementation. Complete consumption of DBT was observed upon the addition of only phosphates and ammonia although further addition of zinc did increase the 2-hydroxybiphenyl production by 14%. Strain 1B was able to desulfurize a model oil containing DBT, 4-methylDBT and 4,6-dimethylDBT, reducing by 63% the total sulfur content in 168 h.

Keywords: Biodesulfurization; Dibenzothiophene; *Gordonia alkanivorans*; Biocatalyst; Industrial wastes; Recycled paper sludge

1. Introduction

The rising consumption of fossil fuels around the world, due to the growing industrial activity, provokes an increase on waste products causing atmospheric pollution. These wastes products are particulates and various gases such as sulfur dioxide, nitrogen oxides and volatile organic compounds that are produced due to impurities in the fuels (Gupta et al., 2005). Thus, environmental authorities along the world have recognized this problem and are imposing increasingly stringent restrictions on the maximum sulfur concentration allowed in the fossil fuels. The process currently utilized in refineries to remove sulfur from these fuels is called hydrodesulfurization. However, heterocyclic sulfur compounds such as substituted dibenzothiophenes (DBT) are very difficult to desulfurize by hydrodesulfurization.

Biological desulfurization of fossil fuels may offer an alternative process to remove the recalcitrant sulfur. One of the limiting factors to apply this process on an industrial scale is the cost associated to the production of biocatalysts, mainly due to the costs associated to the culture media formulation. At present, there is no economically suitable method for large-scale preparation of biocatalysts (Ma et al., 2006). The utilization of alternative carbon sources derived from agro-industrial by-products or wastes may thereby represent an opportunity to cheaper culture media. These alternative substrates have widely been used as feedstock for several fermentation processes such as for the production of lactic acid (Bustos et al., 2005), polyhydroxybutyrate (Hu et al., 1999), ethanol (Karimi et al., 2006), pullulan (Israilides et al., 1998), xanthan gum (Yoo and Harcum, 1999), bacterial cellulose (Bae and Shoda, 2005), and xylanase (Nascimento et al., 2003). However, studies involving the DBT bacterial desulfurization have been carried out only using reagent-grade sugar-containing media. The utilization of alternative carbon sources by

* Corresponding author. Tel.: +351 21 0924721; fax: +351 21 7163636.
E-mail address: francisco.girio@ineti.pt (F.M. Gírio).

0045-6535/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2007.08.016

desulfurizing bacteria might raise a problem associated to the presence of readily bioavailable sulfur compounds. Desulfurization of DBT is completely inhibited in the presence of several sulfur compounds in the culture medium. The *dsz* promoter has been characterized from *Rhodococcus erythropolis* strain IGTS8 and it was found that the *dsz* genes expression is strongly repressed by sulfate or other sulfur compounds even in the presence of DBT (Li et al., 1996). Therefore, it is important to search for low-cost feedstocks containing low residual concentration or even a null content of sulfur.

Pulp and paper industry generates large amounts of waste throughout the year (Thomas, 2000). Concentrated sludge generated by the wastewater treatment facilities of recycled paper plants is currently a major disposal problem concerning the paper industry and it has to be urgently solved (Oral et al., 2005). Recycled paper sludge (RPS) (after neutralization) is approximately made up of 35% cellulose, 10% xylan and 20% lignin (on a dry-weight basis), being the remaining mainly inorganic ash. Due to this high polysaccharide content RPS appears as a promising feedstock for formulation of inexpensive culture media (Van Wyk and Mohulatsi, 2003) providing their polymeric carbohydrates are broken down into fermentable monosaccharides. This hydrolysis can be carried out by chemical or enzymatic methods. The latter is advantageous since it is more specific, it allows milder operation conditions leading to reduced production of biologically inhibitory compounds (such as sugar and lignin degradation products) and the biocatalysts are potentially reusable (Wen et al., 2004).

In this context, the aim of the present work was to evaluate the performance of the hydrolyzates obtained by enzymatic saccharification of RPS, as nutrient source for low-cost DBT desulfurization by *Gordonia alkanivorans* strain 1B. To our knowledge, this is the first report on the utilization of alternative raw materials as substitutes for refined substrates (namely glucose) in biodesulfurization studies.

2. Materials and methods

2.1. Substrate

2.1.1. Recycled paper sludge

The present study used pressed RPS consisting of the solids resulting from wastewater treatment facility of the paper-recycling mill of Renova (Torres Novas, PT).

2.1.2. Enzymatic hydrolysis

After neutralization with hydrochloric acid (to reduce the carbonate content) RPS was suspended in 50 mM sodium citrate buffer, pH 5.5, for an initial consistency of 7.5% (w/v), expressed in terms of total carbohydrate mass, and it was steam sterilized by autoclaving (at 121 °C, 101.3 kPa, for 15 min). This sludge suspension was incubated with the filter-sterilized enzyme solution containing a mixture of two commercial enzyme preparations (cellulolytic and xylanolytic, from Novozymes, Denmark): Celluclast® 1.5 L, applied on a dosage of filter paper activity (FPase) of 10 U g^{-1} carbohydrate; and Novozym® 188, 0.4 ml g^{-1} carbohydrate on sludge. The hydrolysis was carried out at 35 °C in an orbital shaker (150 rpm) for 120 h, under aseptic conditions. A control enzyme mixture was subjected to the same hydrolysis conditions but in the absence of sludge.

In an alternative approach in order to remove any sulfur compounds present in the commercial enzymatic mixture, this mixture was dialyzed overnight (cut-off = 12–14 kDa; Spectra/Por membranes, Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA) against milli-Q water at 4 °C.

The hydrolyzates obtained were filter sterilized and analyzed for sugar composition or used for supplementation of culture media.

2.2. Bacterial strain and growth conditions

2.2.1. General conditions

G. alkanivorans strain 1B, originally isolated from oil-contaminated soil (Alves et al., 2005), was used in all cultivation assays. Unless otherwise stated, strain 1B was cultured in sulfur-free mineral (SFM) medium supplemented with a trace elements solution as described by Alves et al. (2007). DBT, 4-methyl DBT (4-mDBT), and/or 4,6-dimethyl DBT (4,6-dmDBT), dissolved in dimethylformamide, were added as a source of sulfur. All *G. alkanivorans* liquid cultivations were carried out in duplicate shake-flasks, at 30 °C and initial pH = 7.5, with 150 rpm (orbital shaking). Harvested samples were analyzed for cell growth, organic compounds involved in desulfurization and sugar contents.

2.2.2. DBT desulfurization on RPS hydrolyzate

The RPS hydrolyzates obtained either with dialyzed enzymes (dialyzed hydrolyzate) or non-dialyzed enzymes (non-dialyzed hydrolyzate), were used as carbon source in a concentration of 10 g l^{-1} glucose in a medium containing 0.25 mM DBT. Growth controls were carried out using reagent-grade glucose, xylose or cellobiose on a concentration of 10 g l^{-1}.

2.2.3. Effect of supplementation of RPS hydrolyzate

In order to investigate the possibility of using the RPS hydrolyzate obtained with dialyzed enzymes as the component of culture medium (on the concentration previously described), cultivations were carried out on the following formulations: (1) RPS hydrolyzate; (2) RPS hydrolyzate + phosphates (KH_2PO_4 and $Na_2HPO_4 \cdot 2H_2O$); (3) RPS hydrolyzate + ammonia (NH_4Cl); (4) RPS hydrolyzate + phosphates + ammonia; (5) RPS hydrolyzate + phosphates + magnesium ($MgCl_2 \cdot 6H_2O$); (6) RPS hydrolyzate + phosphates + ammonia + magnesium; (7) RPS hydrolyzate + phosphates + ammonia + magnesium + Zinc