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ORIGINAL ARTICLE

Economical fermentation media for the production of a whole cell catalyst for the treatment of Cr(VI)-containing wastewaters

Mauricio J. Alessandrello, Diana L. Vullo*

Área Química, Instituto de Ciencias, Universidad Nacional de General Sarmiento-CONICET, Buenos Aires, Argentina

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Abstract The biotechnology sector is continually seeking sustainable and more economical bioprocesses. Fermentation media produced with cheap components or wastes reduce production costs. Moreover, if wastes are used, they contribute to avoid environmental pollution. In this work, microbial growth media based on molasses or acidified glycerol as carbon sources and fertilizer as nitrogen source were tested for the production of a whole-cell catalyst that could be used in Cr(VI)-containing wastewater treatments. Results showed that the highest biomass production yield was obtained with a medium containing acidified glycerol 5% v/v and fertilizer 0.6% v/v. The biomass produced using this medium was immobilized in calcium alginate beads and used as catalyst in the biotransformation of Cr(VI) into Cr(III). The catalyst could be efficiently used for 5 reduction cycles of 40 mg/l Cr(VI) each. Cr(III) retention assays were performed to determine whether Cr(III) could be retained by the catalyst avoiding its solubilization in the supernatants. The retention capacity of the catalyst at 32 °C and pH 3.0 was 3 mg Cr(III)/g. Both an alternative and economical fermentation medium is here proposed for the optimization of Cr(VI)-containing wastewater treatment.

PALABRAS CLAVE
Bioprocesos;
Fermentación;
Biotransformación;
Cromo;
Pseudomonas

Medios de cultivo económicos para la producción de un biocatalizador a células enteras para el tratamiento de aguas residuales que contienen Cr(VI)

Resumen El sector industrial biotecnológico continuamente busca bioprocesos más económicos y sustentables. El uso de medios de cultivo producidos con componentes de bajo costo o con residuos reduce el presupuesto global del proceso y, particularmente si se utilizan residuos, se contribuye, además, a evitar la contaminación ambiental. En este trabajo se probaron medios

* Corresponding author.
E-mail address: dvullo@ungs.edu.ar (D.L. Vullo).

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t de cultivo basados en melaza de caña o glicerol ácido como fuentes de carbono y energía, y fertilizante como fuente de nitrógeno, para la producción de un biocatalizador que podría ser usado para el tratamiento de aguas residuales que contienen Cr(VI). Los resultados mostraron que el mayor rendimiento de producción de biomasa se obtuvo con un medio que contenía 5% v/v de glicerol ácido y 0,6% v/v de fertilizante. Utilizando este medio se produjo la biomasa suficiente para la biotransformación de Cr(VI) a Cr(III), luego de ser inmovilizada en alginato de calcio. El proceso pudo ser aplicado eficientemente durante 5 ciclos de reducción de 40 mg/l de Cr(VI) cada uno. Además, se realizaron ensayos de retención de Cr(III) para determinar si esta especie química podría ser removida de la solución por interacción con el biocatalizador. La capacidad de retención obtenida por el biocatalizador a 32 °C y pH 3 fue de 3 mg de Cr(III)/g. De esta manera, se propone un medio de cultivo alternativo y económico para la efectivización de un tratamiento de aguas residuales que contengan Cr(VI).

Introduction

Alternative economic microbial growth media have been intensively developed in recent years[3,19,29]. The use of wastes or useless process by-products as substrates for microbial fermentation is an ordinary practice in bioprocesses to gain sustainability[1,30,31]. Molasses and acidified glycerol are good examples of process wastes with high potential use as medium components. Molasses is a sub-product obtained from the sugar production[13,18], with high sugar and mineral contents suitable as carbon and energy sources for microbial growth. It is calculated that for every ton of processed sugar, 0.3 tons of molasses are produced with limited uses up to now[18]. Acidified glycerol is a sub-product of the biodiesel production, obtained in an early purification step that consists in the addition of HCl to crude glycerol to remove oil and other impurities[17]. This sub-product has little use nowadays and generates a problem for the biodiesel industry since it requires a mandatory treatment prior discharge, taking into consideration that for every 10 kg of biodiesel, 1 kg of glycerol is produced[14,19,26,27]. This crude glycerol excess, therefore, increases the cost of the biodiesel production process[19,25,27]. The use of crude glycerol or acidified glycerol as carbon and energy sources in microbial fermentations is a possible solution for this problem. The substitution of the commonly used nitrogen sources for others that are more economical is also a matter of great importance. There are several publications illustrating the efforts done in this direction[4,20,28,32].

Cr(VI)-containing wastewaters produced by industries[8,12,24] are a great concern because of the highly toxic effects of hexavalent chromium[6,21]. Currently used Cr(VI)-containing wastewater treatments are often inefficient and costly so new alternative treatments are being investigated[5,33]. Biological Cr(VI) reduction to the less toxic and easier to remove Cr(III) is a treatment that is under research and could provide a cost/effective solution for the industry sector.

The aim of this work is to produce biomass of *Pseudomonas veronii* 2E, a Cr(VI)-reducing bacterium[9], using economical growth media formulated with industrial by-products, immobilized in calcium alginate beads and applied as biocatalyst for the biotransformation of Cr(VI) into Cr(III). In addition, a Cr(III) retention assay was carried out to determine the possible fate of the generated Cr(III).

Materials and methods

Microorganism and strain maintenance

Pseudomonas veronii 2E, is an autochthonous bacterium isolated from polluted environments belonging to sediments associated to the Reconquista River basin (Buenos Aires Metropolitan Area). This strain was identified by 500 bp 16S r-RNA gene sequencing and is able to retain Cd(II), Zn(II) and Cu(II) and to biotransform Cr(VI) from aqueous systems, as described in previous studies[2,9,22]. *P. veronii* 2E was routinely maintained in plate count agar cultures. Growth was achieved at 32 °C for 48 h. Afterwards, the culture was stored at 4 °C until use.

Growth media

The molasses-based media (MBM) were prepared using different proportions of heat sterilized (121 °C, 15 min) commercial molasses El Trebol® (0.1–2% m/v) and a previously filter-sterilized (0.45 μm pore diameter) fertilizer solution (0–1.2% v/v; Afital® produced by Agro EMCODI S.A., located in Lanús, Buenos Aires, Argentina), whose composition is detailed in Table 1. The acidified glycerol was provided as a solution from a biodiesel plant located in Malvinas Argentinas, Buenos Aires, Argentina, which, after being sterilized, was mixed in different proportions (1–10% v/v) with the same fertilizer solution (GBM). All alternative media were neutralized at pH 7.0 with NaOH 6 mol/l. Nutrient broth (NB, Merck) was prepared by dissolving 8 g (5 g beef peptone and 3 g yeast extract) in 1 l distilled water. Dextrose was added at 1 g/l. NB was sterilized by heat at 121 °C for 15 min.