Bioresource Technology 133 (2013) 109–117

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Improving effect of metal and oxide nanoparticles encapsulated in porous silica on fermentative biohydrogen production by *Clostridium butyricum*

Laurent Beckers [a,*], Serge Hiligsmann [a], Stéphanie D. Lambert [b], Benoît Heinrichs [b], Philippe Thonart [a]

[a] Centre Wallon de Biologie Industrielle (CWBI), Département des Sciences de la Vie, B40, Université de Liège, B-4000 Liège, Belgium
[b] Laboratoire de Génie Chimique, B6a, Université de Liège, B-4000 Liège, Belgium

HIGHLIGHTS

- Encapsulated nanoparticles (NP) were tested for biohydrogen production improvement.
- Pd, Ag, Fe and Cu NP were added in *Clostridium butyricum* BHP tests.
- Fe NP showed an improvement of the hydrogen volume and rate of production.
- In pH controlled AnSBR, Fe NP showed +113% of hydrogen production rates.
- NP addition had no effect on the yields and metabolic pathway.

ARTICLE INFO

Article history:
Received 12 October 2012
Received in revised form 23 December 2012
Accepted 26 December 2012
Available online 4 January 2013

Keywords:
Biohydrogen
Dark fermentation
Clostridium butyricum
Encapsulated nanoparticles
Sol–gel process

ABSTRACT

This paper investigated the enhancement effect of nanometre-sized metallic (Pd, Ag and Cu) or metallic oxide (Fe_xO_y) nanoparticles on fermentative hydrogen production from glucose by a *Clostridium butyricum* strain. These nanoparticles (NP) of about 2–3 nm were encapsulated in porous silica (SiO_2) and were added at very low concentration (10^{-6} mol L^{-1}) in batch hydrogen production test. The cultures containing iron oxide NP produced 38% more hydrogen with a higher maximum H_2 production rate (HPR) of 58% than those without NP or with silica particles only. The iron oxide NP were used in a 2.5 L sequencing-batch reactor and showed no significant effect on the yields (established at 2.2 $mol_{hydrogen}$ $mol_{glucose}^{-1}$) but an improvement of the HPR (+113%, reaching a maximum HPR of 86 $mL_{hydrogen}$ L^{-1} h^{-1}). These results suggest an improvement of the electron transfers trough some combinations between enzymatic activity and inorganic materials.

1. Introduction

In the upcoming years, the population living on our planet will increase and they will have to be provided with enough energy, materials and food. Currently, our society is based on the utilization of fossil fuels as a primary energetic source leading the world to environmental, human health and macro-economic issues (Zidansek et al., 2009). The development of alternative and green energy sources is therefore regarded as a major answer aiming to lower the impact of the human industrial activity on the earth. In this context, it is believed that hydrogen will be used extensively in the future as an energetic vector to achieve a less polluting and economically more advantageous society than the current fossil fuel-based economy (Marban and Vales-Solis, 2007). Indeed, its reaction with oxygen, which produces energy and only water as a side-product, can be performed in electrochemical or combustion processes without any generation of greenhouse gases. However, currently, hydrogen is almost exclusively produced from traditional non-renewable fossil fuels in intensive chemical processes, running at elevated pressures and temperatures and releasing CO_2 in the atmosphere (Holladay et al., 2009).

The green hydrogen produced by microorganisms provides alternative routes for renewable energy production (Kothari et al., 2012). Among the several microorganisms that can convert various carbohydrate sources in hydrogen and metabolites in solution, the anaerobic fermentative bacteria have been studied during the past few years (Davila-Vazquez et al., 2008; Hallenbeck, 2009). In these microorganisms, the electrons resulting from the oxidation of the substrate are transferred to protons in order to form molecular hydrogen through the action of enzymes called hydrogenases. Among the anaerobic bacteria producing hydrogen,

* Corresponding author. Tel.: +32 (0) 4 366 28 61; fax: +32 (0) 4 366 28 62.
E-mail address: beckers.laurent@gmail.com (L. Beckers).

0960-8524/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2012.12.168

Clostridium strains are frequently characterised in highly efficient sludge for hydrogen production in mesophilic range of temperatures (Sagnak et al., 2010; Wang and Wan, 2009a). To date, the anaerobic biohydrogen process is still experimented at laboratory or small pilot scale only (Das, 2009). To make the process viable, improvements of the bioactivity of hydrogen-producing microorganisms as well as high substrate conversion yields are needed to meet economic requirements. Key factors for optimal hydrogen production such as pH, temperature, strain selection, microorganisms cell density, concentration of substrate and metabolites have been well studied to improve the kinetics and the yields (Davila-Vazquez et al., 2008; Wang and Wan, 2009a). However, further efforts and new routes have to be found to use these microorganisms more efficiently in a stable process.

Recently, the nanoscience has been involved in number of usual products and processes since the nanomaterials bring new chemical and physical properties. Indeed, due to their size between 1 and 100 nm, the nanomaterials exhibit a very large specific surface area and quantum effects start to predominate (Dinesh et al., 2012). The interest in the biological field is still increasing with practical application in many different domains since nanoparticles (NP) have recently showed interactions with microorganisms even at very low concentration. On the one hand, some NP exhibit antimicrobial activity by close contact with the microorganisms leading to membrane disruption, also raising environmental concerns about their dissemination in the nature (Neal, 2008). On the other hand, some microorganisms may take advantages of NP especially in anaerobic environment, by transferring more efficiently electrons to acceptors. Intra- or extra-cellular NP may be produced by the reduction of metal ions for the biosynthesis of nanomaterials with different chemical composition or morphologies (Korbekandi et al., 2009). Electron transfer can also occur through membrane c-type cytochromes or nanowires to electron acceptors such as polluting chemical compounds (for soil remediation applications (Jagadevan et al., 2012)), electrodes (for current generation in microbial fuel cells (Lovley, 2008)) or through interspecies electron transfer (Kato et al., 2012). In all these application fields, the NP have recently shown some advantages through their capacity to react rapidly with the electron donors leading therefore to kinetic improvement and, through their action as biocatalysts, to the enhancements of the microorganisms activity (Xu et al., 2012).

In a previous work, only gold NP at very low concentration (10^{-8} mol L^{-1}) were tested to observe effects on the biohydrogen production. An enhancement of the performances of about 56% was achieved (Zhang and Shen, 2007). The authors concluded that gold NP would operate as "electron sinks" due to their affinity for electrons, which allows to further reduce protons to hydrogen. They acted in parallel on hydrogenases that naturally achieve this reaction in the metabolism of the cell. Other metal are known to interact with microorganisms in environmental conditions. Ag and Cu are often cited as metal having interaction with the bacteria for their antimicrobial activity (Bagchi et al., 2012; Sotiriou and Pratsinis, 2010). Pd is a metal involved usually for its strong interactions with molecular hydrogen in chemical processes (Klavsyuk et al., 2011). Finally, iron is known to be an important element as a cofactor for hydrogenases or for its role in environmental processes (Grieger et al., 2010; Lee et al., 2001; Xu et al., 2012).

In this work, the effect of nanoparticles (NP) of about 2–3 nm of three metals (Pd, Ag, Cu) and one iron (Fe) oxide was investigated with pure *Clostridium butyricum* cultures. These NP were encapsulated in a porous silica (SiO_2) matrix. The SiO_2 matrix without NP was also tested in the same conditions. To synthesize the catalyst (NP + SiO_2 = catalyst), a one-step sol–gel process was applied to obtain NP finely dispersed in the porosity of a silica matrix (Heinrichs et al., 2008; Lambert et al., 2004). In such catalysts, in order to reach active sites, reactants must first diffuse through large pores located between aggregates of SiO_2 particles and then through smaller pores between those elementary particles inside the aggregates. Finally, they diffuse through micropores inside the silica particles. It was shown that there are no limitations of mass transfer at each of the three levels (Heinrichs et al., 2001).

These NP were experimented in Biochemical Hydrogen Potential (BHP) tests. The most efficient conditions were further investigated in a stirred 2.3 L Anaerobic Sequenced-Batch Reactor (AnSBR). The production of hydrogen and metabolites was monitored in the cultures and the Gompertz model was applied on the volumetric production curves.

2. Methods

2.1. Microorganism and culture medium

The strain used as hydrogen-producing microorganism was *C. butyricum* CWBI1009 (denoted *C. butyricum*) and was previously isolated and identified by the authors (Masset et al., 2010). It was conserved by sterile monthly transfer of 1 mL from previous pure culture in a hermetically sealed 25 mL tubes containing "MDT" medium and incubated at 30 °C. The MDT culture medium contained, per litre of deionized water: glucose monohydrate (5 g), casein peptone (5 g), yeast extract (0.5 g), Na_2HPO_4 (5.1 g), KH_2PO_4 (1.2 g), $MgSO_4$. $7H_2O$ (0.5 g) and L-cysteine hydrochloride (0.5 g). The MDT culture medium was used in biochemical hydrogen potential (BHP) batch serum bottles test and in 2.5 L stirred tank reactor driven in anaerobic sequenced-batch mode (AnSBR).

For the preparation of fresh inoculum, the transfer in new MDT tubes was repeated twice a week before being used in the culture vessel. Purity tests were performed by spreading 100 μL of culture on sterile PCA (Plate Count Agar) Petri dishes before incubation at 30 °C for 24–48 h. The PCA medium contained glucose monohydrate (1 g), casein peptone (5 g), yeast extract (2.5 g) and agar (15 g) per litre of deionized water. The absence of bacterial growth after incubation for 48 h incubation confirmed the absence of any facultative anaerobic contaminants.

2.2. Preparation and characterization of encapsulated nanoparticles

Four metallic salts (Pd, Ag, Cu and Fe) have been used for preparing the nanoparticles (NP). To encapsulate these NP inside a porous silica matrix, the cogelation method was used as described by Lambert et al. (2004) and by Heinrichs et al. (2008). The samples are denoted Pd/SiO_2, Ag/SiO_2, Cu/SiO_2 and Fe/SiO_2 cogel (Table 1). The cogelation method allows doping an inorganic matrix with cations, in one step at the molecular scale. The process is based on the simultaneous hydrolysis and condensation of two alkoxysilanes: an SiO_2 network-forming reagent such as tetraethoxysilane (TEOS, $Si(OC_2H_5)_4$) and an alkoxysilane-functionalized ligand of the type $(RO)_3Si–X–L$, in which the ligand L, able to form a complex $–L_nM$ with a cation of a metal M (M = Pd, Ag, Cu, Fe, etc.), is connected to the hydrolysable alkoxide group $(RO)_3Si–$ via an inert and hydrolytically stable spacer X. The concomitant hydrolysis and condensation of such molecules, i.e. their cogelation, results in materials in which the catalytic metal cation is anchored to the silica matrix.

In Table 1, a second Fe/SiO_2 sample, called Fe/SiO_2 dissol, is presented. This sample was prepared by the dissolution method (Heinrichs et al., 2008), which consists of dissolving the iron salt in the initial homogenous solution of silica gel precursor. Moreover, the porous silica matrix without NP, denoted SiO_2, was also synthesized by the sol–gel process (Lambert et al., 2004) to check if SiO_2 plays a significant rule for the biohydrogen production.