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Production of biofuels from pretreated microalgae biomass by anaerobic fermentation with immobilized *Clostridium acetobutylicum* cells

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ABSTRACT

The purpose of this work was to study the possible use of pretreated biomass of various microalgae and cyanobacteria as substrates for acetone–butanol–ethanol (ABE) fermentation by *Clostridium acetobutylicum* cells immobilized into poly(vinyl alcohol) cryogel. To this end, the biochemical composition of photosynthetic microorganisms cultivated under various conditions was studied. The most efficient technique for pretreating microalgal biomass for its subsequent conversion into biofuels appeared to be thermal decomposition at 108 °C. For the first time the maximum productivity of the ABE fermentation in terms of hydrogen (8.5 mmol/L medium/day) was obtained using pretreated biomass of *Nannochloropsis* sp. Maximum yields of butanol and ethanol were observed with *Arthrospira platensis* biomass used as the substrate. Immobilized *Clostridium* cells were demonstrated to be suitable for multiple reuses (for a minimum of five cycles) in ABE fermentation for producing biofuels from pretreated microalgal biomass.

1. Introduction

Microalgae are attracting interest not only as a possible source of biodiesel (Amaro et al., 2011), but also as a source of other types of biofuels: hydrogen, butanol and ethanol (Brennan and Owende, 2010; Parmar et al., 2011; Varfolomeev et al., 2010; Varfolomeev and Wasserman, 2011).

Hydrogen is an alternative source of clean and renewable energy (Park et al., 2009). No atmospheric pollutants such as carbon, nitrogen and sulfur oxides are produced when it is used as a fuel. However, the industrial hydrogen production methods known today are either environmentally harmful or costly (Tsygankov, 2007). In view of this, biological methods of hydrogen production attract enormous interest (Das and Veziroglu, 2008).

There are a number of known methods for producing hydrogen using biotechnology with photosynthetic microorganisms as biocatalysts (Eroglu and Melis, 2011; Varfolomeev and Wasserman, 2011). However, the efficiency of hydrogen production in all these processes is lower than in the case of acetone–butanol–ethanol fermentation (ABE fermentation) catalyzed by *Clostridium* bacteria (Dürre, 2005; Wang and Wan, 2009). In addition to hydrogen, ABE fermentation provides butanol and ethanol (Dürre, 2005; Wang and Wan, 2009). Both these alcohols are very promising energy sources. They can be used as a fuel; specifically, ethanol can be used in rocket engines or internal combustion engines in the pure form. Butanol can be added to gasoline as its energy output is comparable to that of gasoline (Pfromm et al., 2010).

Glucose is the most widely used substrate for ABE fermentation by *Clostridium* bacteria. However, these cells are well known to be sensitive to butanol, which accumulates in the medium and causes a drop in process efficiency. The use of bacterial cells in the immobilized form in media containing substrates other than glucose allows to harness the new industrial potential of ABE fermentation to produce biofuels.

Immobilization of bacterial cells allows to evenly distribute biofuel producers throughout the reactor volume, reducing the negative impact of metabolites accumulated in the process of ABE fermentation, as well as to enhance cell stability (Lee et al., 2008; Tripathi et al., 2010). In view of optimizing this process, an efficient biocatalyst based on *Clostridium acetobutylicum* cells immobilized in poly(vinyl alcohol) (PVA) cryogel was recently developed (Nikolskaya et al., 2012).

PVA cryogel is a promising cell carrier that can be successfully applied for immobilizing the cells of various microorganisms. What makes this support unique is its macroporous structure

Abbreviations: ABE fermentation, acetone–butanol–ethanol fermentation; PVA, poly(vinyl alcohol); DMSO, dimethyl sulfoxide; SDS, sodium dodecyl sulfate.

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