Review

Cell PRESS

Applications and perspectives of multi-parameter flow cytometry to microbial biofuels production processes

Teresa Lopes da Silva, José Carlos Roseiro and Alberto Reis

Laboratório Nacional de Energia e Geologia, I.P., Unidade de Bioenergia, Estrada do Paço do Lumiar 22, 1649-038 Lisboa, Portugal

Conventional microbiology methods used to monitor microbial biofuels production are based on off-line analyses. The analyses are, unfortunately, insufficient for bioprocess optimization. Real time process control strategies, such as flow cytometry (FC), can be used to monitor bioprocess development (at-line) by providing single cell information that improves process model formulation and validation. This paper reviews the current uses and potential applications of FC in biodiesel, bioethanol, biomethane, biohydrogen and fuel cell processes. By highlighting the inherent accuracy and robustness of the technique for a range of biofuel processing parameters, more robust monitoring and control may be implemented to enhance process efficiency.

Biofuel processing

In the midst of the energy crisis, second-generation biofuels (derived from lignocellulosic agriculture and forest residues and from non-food crop feedstocks) and third generation biofuels (derived from microbes and microalgae) are considered to be viable fuel alternatives. In order for these fuels to be sustainable, appropriate conversion and process management technologies need to be optimized [1–3]. Until recently, biofuel process monitoring has been done by conventional analyses such as dry cell weight or serial dilution methods. These data are usually only available a considerable time after the sample is taken. Other methods such as optical density or capacitance monitoring, though faster, only provide average data for the microbial population [4–7].

An alternative analytical technique, FC, is used to both qualitatively and quantitatively assess biological and physical characteristics of individual cells almost in real time. FC can be used in bioprocess monitoring to obtain information from heterogeneous and complex microbial samples faster and more accurately than with conventional microbiological methods. Complex bioprocesses require almost real time insight to develop dynamic process control strategies for improved performance, quality, productivity and process yield [5]. This review explores the current shortcomings in biofuel processing and introduces FC as a potential tool to optimize production and conversion processing.

Corresponding author: Silva, T.L.d. (teresa.lopesilva@lneg.pt).
Keywords: biodiesel; bioethanol; biomethane; biohydrogen; microbial fuel cells; bioprocess monitoring.

Current biofuel processing shortcomings

Biodiesel processing

Oils and fats are the main raw material for biodiesel (Box 1) production. Thus, identification of high lipid-producing microbial strains is a prerequisite for sustainable and economically-viable fuel production from microorganisms [8,9]. Growth conditions strongly influence cellular lipid content in microorganisms [10] so that cellular lipid content should be monitored throughout bioprocess development to optimize control strategies and enhance yields. At present, most of the available literature discussing biodiesel production from microorganisms has used conventional microbiology techniques to monitor microbial lipid content [11–15]. These are time-consuming methods that generate high amounts of waste (organic solvent) that are harmful to the environment if not recycled by distillation. In addition, these methods require large amounts of biomass for sufficient subsequent lipid extraction [16]. For example, at least 20 mg of lipid per sample should be extracted and gravimetrically evaluated. Thus, assuming an average 20% (w/w) lipid content, at least 100–200 mg dry weight biomass is required.

Oleaginous microorganisms, which normally contain more than 20% oil (w/w), are of interest in biotechnology as an alternative source of biodiesel [10]. Unfortunately, independent of the organism used, lipid content data is usually only available a considerable time after the sample is taken during the microbial lipid production, too late to alter process controls. Therefore, quick and accurate estimates of oil content are needed to optimize oil production or to expand biofuel production bioprocesses.

Bioethanol processing

Bioethanol (Box 1) production is similar to brewing and wine production. In yeast (*Saccharomyces cerevisiae*) fermentation, yeast cells are subjected to adverse conditions: limited nutrients, high temperature, ethanol toxicity and osmotic stress from substrate sugars [17]. These nutrient deficiencies are intensified by the microenvironments formed in large scale fermentors as a result of inefficient mixing. As cells circulate within the bulk of a large-scale