BACKGROUND

Biofuels, fuels produced from biomass, represent an environmentally friendly alternative for energy production. Due to the prospects of replacing fossil fuels, biodiesel production has continuously grown in the last decade. Biodiesel, is synthesized by conversion of vegetable oil into their (m)ethyl esters via transesterification. Biodiesel industries are facing a surplus of its main byproduct, glycerol, which represents 10% (w/w) of the final ester. Supported by governments to increase energy independence and meet the rising energy demand, the biodiesel market is expected to reach 37 billion gallons by 2016, an average growth of 42% per year. This will result in a production of 4 billion gallons of crude glycerol that year, saturating the glycerol market.

Industrial application of crude glycerol in food, pharmaceutical and cosmetics industries, its main markets, requires a costly refining process in order to achieve a necessary high purity. In the last years many research projects have been conducted with the aim of finding a new utilization for raw glycerol. In addition to new applications in the food industry, polymer industry, glycerol has also been considered as a feedstock for new industrial fermentations.

OBJECTIVES

The objectives of this proposal are:

1) Maximization of PHA production in a two stage aerobic process
2) Elucidation of metabolic pathways involved in biopolymers production using crude glycerol as substrate
PHAs are biodegradable polyesters with market capacity to replace some of the more commonly used elastomeric/thermo plastics. These biopolymers are naturally synthesized and stored inside the cells by several microbial species. Commercialization of bacterial PHA is still restricted to the use of pure cultures fermentations and high cost synthetic substrates making their price, in average, two times higher than conventional plastics (i.e PVC) (Chanprateep 2010). In recent years, research has focused on the development of alternative PHA production processes, including the use waste/surplus based feedstocks and mixed microbial cultures (MMC). The main problems associated with those strategies are the lower PHA content and the lower volumetric productivities achieved when compared with the ones reported for pure culture and synthetic substrates. A critical step in this strategy is the selection of a stable culture with a high PHA storage capacity. This can be achieved by subjecting microbial cultures to alternate periods of short carbon availability followed by a long unavailability, designated as aerobic dynamic feeding (ADF, also known as feast/famine).

Previous work demonstrated the feasibility of PHB production by a mixed microbial community using crude glycerol as substrate. A two-step process was used, comprising (1) selection of a PHA-accumulating culture under ADF conditions, and (2) batch PHA accumulation using the selected culture. The selected culture had the ability to consume both glycerol and methanol fraction present in the crude. However, glycerol seemed to be the only carbon source contributing for the two biopolymers stored: poly-3-hydroxybutyrate (PHB) and glucose biopolymer (GB). In this previous work the culture reached a maximum PHB content of 47% (cdw).

This work proposal will be directed towards the maximization and elucidation of the metabolic pathways used for PHA or glucose polymer production from crude glycerol components. Crude glycerol and glycerol and methanol will be used as substrates, the two last particularly when considering the utilization of labeled substrates for NMR experiments (either in vivo or ex vivo). Apart from the observation of the interchange between the external substrate into PHA using the different possible metabolic pathways also the importance and turnover of the produced glucose polymer (glycogen?) will be clarified. The understanding of carbon drift to this second polymer and its possible involvement on PHA production is crucial for further maximization of the PHA yield on substrate, and ultimately the PHA content inside cells.