An anaerobic bioreactor system for biobutanol production

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1 Introduction

Concerns about the greenhouse effect, as well as legislation to reduce CO_2 emissions and to increase the use of renewable energy have been the main reasons for the increased production and use of biofuels. In addition to bioethanol and biodiesel production, the research on biobutanol production has also increased during the past years. Butanol can be produced by chemical or biochemical routes. Fuel properties of butanol are considered to be superior to ethanol because of higher energy content, and better air-to-fuel ratio (Gautam and Martin 2000). Butanol is also less volatile and explosive than ethanol, has higher flash point and lower vapour pressure which makes it safer to handle (Huang et al. 2004). (Cascone 2007)

Biobutanol production is an anaerobic two-stage fermentation process where acetic and butyric acids, carbon dioxide and hydrogen are first produced in the acidogenic phase. Then the culture undergoes metabolic shift to solventogenic phase and acids are converted into acetone, ethanol and butanol. At the end of the fermentation, products are recovered from the cell mass, other suspended solids, and by-products. (Ezeji et al. 2007)

Several species of Clostridium bacteria are capable to metabolize different sugars, amino and organic acids, polyalcohols and other organic compounds to butanol and other solvents. Feedstock materials for biobutanol are diverse, including different kind of by-products, wastes and residues of agriculture and industry. Optimal fermentation conditions (pH, temperature, nutrients), products and their ratio vary with strains and substrates used. (Jones and Woods 1986, Qureshi and Blaschek 2005)

Biobutanol production has still some limitations including butanol toxicity to culture leading to low butanol yields. The product inhibition hinders the yield of butanol and acids, making integrated product separation process highly favorable. Butanol recovery from fermentation broth is expensive because of the low butanol concentration and high boiling point (118°C) (Qureshi and Blaschek 2001). Several different recovery methods are available. Membrane-based methods such as membrane evaporation, perstraction, pervaporation and reverse osmosis with high selectivity are the most promising product recovery techniques despite of the tendency for clogging and fouling (Ezeji et al. 2003, Izák et al. 2008). Process development to achieve an economical and efficient production process have been done also by genetic strain manipulation, regulation of substrate utilization and butanol production, by using cell immobilization or cell recycling, and by using different kinds of product recovery techniques. (Cascone 2007, Ezeji et al. 2007)

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2 Objectives of the research

The aim of this research was to design and built a system for the anaerobic bacteria cultivation. The purpose was to discover suitable cultivation conditions for strict anaerobic clostridia bacteria.

3 Results

An anaerobic reactor system with temperature control and pH monitoring, cell immobilization unit, and circulation of fermentation medium was developed. Higher cell concentration and shorter lag phase prior to fermentation were achieved by cell immobilization. Butanol was produced from whey supplemented with yeast extract.

3.1 Anaerobic reactor system

Cultivation system consisted of a 2.6 L jacketed glass reactor, a water bath with constant temperature at 37°C, a magnetic stirrer with agitation speed of 250 rpm, a pre-filter and an immobilization reactor, and a gas inlet-water lock system.

Parts were connected with tubings (inner diameter 1.3 cm) in the following order (Figure 1): reactor (A), three-way valve for medium changing (B), pre-filter for particle removal (C), and immobilization reactor (D). A three-way valve connecter to syringe system was used with sampling. Anaerobic environment was maintained by making the reactor air tight, and by using the nitrogen gas system. Gas outlet was done through elastic tube and 0.22 µm filter (E) ending to the water lock (f). Air tightness can be checked during the experiments by feeding nitrogen gas into the system and checking if the gas is bubbling in the water lock. Always when something is transferred in or out from the reactor, a small amount of nitrogen gas is fed in the system to push out air and toxic oxygen.

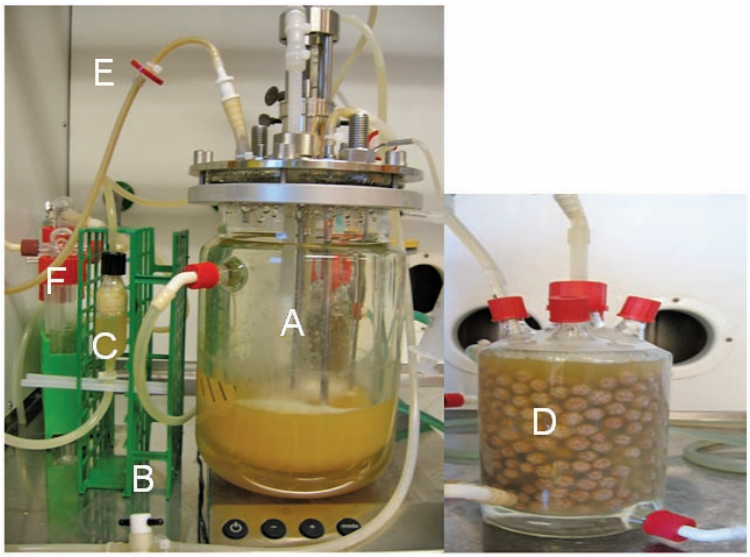

Figure 1 Anaerobic reactor system. A: Reactor, B: Three-way valve, C: Pre-filter, D: Cell immobilization unit, E: Filter used with gas-inlet and water lock tubings, F: Water lock.

3.2 Cell immobilization unit

The cell immobilization system was used to reduce the time needed for cultivations, and to achieve better cell mass concentration and reactor productivity. A jacketed 600 mL mini-fermenter was used as an immobilization reactor (Figure 1D). The reactor was filled with spherical and porous sintered quartz granules (Eheim Substrat Pro Biological Filter Media) in which cells can attach and grow. Cell broth was circulated through the reactor to allow cells to attach. For the cell broth outlet, a glass tube was placed in one of the side openings to make airtight connection for medium circulation. Other side openings of the reactor were used for the jacket water circulation.

Cell broth was pumped through the pre-filter (Figure 1c) before the immobilization reactor to make sure that any bigger particles do not get into the immobilization reactor and block it. Pre-filter was made of glass tube filled with 2–3 mm thick layer of Ehfi Synth fine filter wadding.

After inoculation of the main reactor, bacteria were allowed to grow until the cell concentration reached the optical density of ~ 4.0. Cell immobilization was started by circulating fermentation broth through the whole system. The pumping rate was approximately 25 mL/min to allow cells to immobilize onto the quartz granule matrix in the immobilization reactor. Later the circulation speed was raised to 100 mL/min.

3.3 Achieved results

Cell growth was monitored by optical density measurements while sugar and metabolite concentrations were determined with high performance liquid chromatography. Without any further optimization, 2.1g/L of butanol was produced by *C. acetobutylicum* ATCC 4259 from whey permeate supplemented with yeast extract. Simultaneously, formation of butyric acid was 5.8 g/L and that of acetic acid 3.2 g/L, suggesting that the acidogenic phase was working more efficiently compared to the solventogenic phase. A two-step fermentation was also tested, in which acidic products from *C. tyrobutyricum* fermentation were fed to the *C. acetobutylicum*.

4 Relevance of the research

The research work is part of the Academy of Finland funded SUSFUFLEX project (New, innovative sustainable transportation fuels for mobile applications: From biocomponents to flexible liquid fuels). The project is performed in cooperation with the Department of Chemistry of Oulu University (coordinator); Microelectronics and Material Physics Laboratories of Oulu University and the Laboratory of Industrial Chemistry and Reaction Engineering at Åbo Akademi University.

Our contribution to the project is to evaluate old and novel procedures for the microbiological production of biobutanol, higher alcohols and oxygenates to be used as fuel substitutes in order to find the most efficient and feasible processes.

Anaerobic fermentation system will be further developed and optimized, and experiments with whey and other substrates will be done. A product removal system will also be included into the fermentation system.

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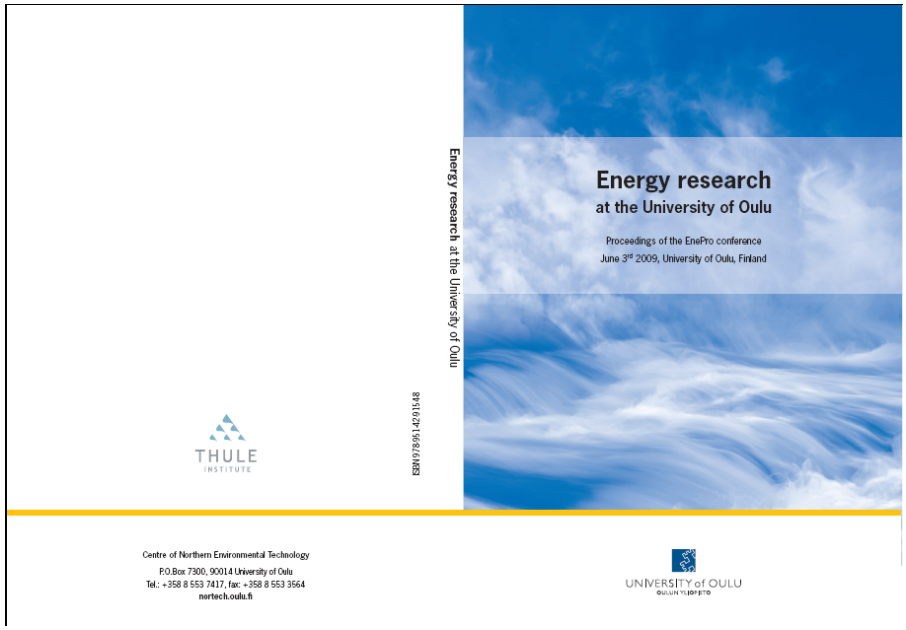

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