Continuous Production of Ethanol in High Productivity Bioreactors Using Genetically Engineered *Escherichia coli* FBR5: Membrane and Fixed Cell Reactors

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Biochemical reactor design plays a major role in determining the economics of fuel and chemical production. Reactors that result in continuously high productivities can significantly reduce the cost of the final product. With this aim, five different reactor systems were evaluated for ethanol production from xylose using genetically engineered E. *coli* FBR5, a strain developed to produce ethanol in high yield from xylose and other pentose sugars. The reactor systems evaluated were batch free cell, continuous free cell, continuous adsorbed cell, continuous entrapped cell, and continuous membrane cell recycle bioreactors. All of the five reactor systems were fed with a medium containing xylose 100 g/L, yeast extract 5 g/L, tryptone 10 g/L, and NaCl 5 g/L.

The batch reactor resulted in a maximum ethanol production of 43.5 g/L with a yield of 0.47-0.50 and a maximum productivity of 0.90 g/L.h. Following this a free cell continuous reactor was operated with a culture volume of 1L. The reactor was operated at dilution rates ranging from 0.014 to 0.08 h^{-1}. At a dilution rate of 0.014 h^{-1} the reactor produced 35.2 g/L ethanol resulting in a productivity of 0.49 g/L.h, while at a dilution rate of 0.08 h^{-1} the reactor produced 22.1 g/L ethanol with a productivity of 1.77 g/L.h. The reactor was operated at a low dilution rate (0.014 h^{-1}) to obtain a maximum ethanol concentration in the effluent.

Further, *E. coli* cells were adsorbed onto clay brick and packed in a reactor. The reactor was fed at the bottom thus getting product at the top. This reactor was operated at dilution rates ranging from 0.08 to 0.24 h^{-1}. At a dilution rate of 0.08 h^{-1} the reactor produced 21.9 g/L ethanol resulting in a productivity of 1.75 g/L.h. At a dilution rate of 0.24 h^{-1} the reactor produced 18.9 g/L ethanol with a productivity of 4.54 g/L.h. The cell concentration inside the adsorbed cell reactor varied with time from 0.17-70.2 g/L reactor volume.

Next, cells were immobilized by entrapment in calcium alginate gel. The entrapped cell reactor contained 39.3 g cell/L bead volume. The cell slurry that was used to fix the cells contained 59.0 g cell/L slurry (dry weight). In the entrapped cell reactor, a maximum ethanol concentration of 37.1 g/L was obtained at a dilution rate of 0.022 h^{-1}.

In this reactor, a productivity of 2.00 g/L.h was obtained at a dilution rate of 0.08 h^{-1}. At this dilution rate, the ethanol concentration in the effluent was 25.1 g/L.

Membrane cell recycle reactors are known to offer high reactor productivities. In order to achieve this, an ultrafiltration membrane was used to recycle *E. coli* cells to the bioreactor. Initially, the membrane was characterized for flux and fouling studies using water, fermentation medium, and fermentation broth. Upon characterization, the membrane was connected to recycle cells. The reactor was operated at dilution rates ranging from 0.075 h^{-1} to 0.80 h^{-1}. At a dilution rate of 0.075 h^{-1} the reactor produced 26.9 g/L ethanol with a productivity of 2.01 g/L.h. At a dilution rate of 0.80 h^{-1} the reactor produced 31.5 g/L ethanol thus resulting in a productivity of 25.20 g/L.h which is the highest of the five reactor systems. The cell concentration in the reactor ranged from 12.0 to 23.0 g/L. It is anticipated that further increase in cell concentration will improve reactor productivity drastically. In membrane cell recycle reactors, cell concentrations in excess of 100 g/L can be achieved. During these studies no signs of fouling the membrane were observed. The results on further improvement in productivity will be presented. At the time of submission of this abstract, studies on membrane cell recycle reactor are continuing in the author's laboratory.

It should be noted that studies were also performed with optimizing medium where batch reactors were fed with commercial media such as 100 g/L xylose supplemented with CSL. Some of the commercial media resulted in equally good performance (as above) of the system in terms of ethanol concentration, and reactor productivity. However, these studies will not be reported at this time.