BIOLOGICAL CONVERSION OF GLYCEROL INTO VALUE ADDED PRODUCTS

By

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DISSERTATION TITLE

Biological Conversion of Glycerol Into Value Added Products

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BIOLOGICAL CONVERSION OF GLYCEROL

INTO VALUE ADDED PRODUCTS

John Henry Austin Amery, Ph.D.

University of Nebraska, 2004

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Glycerol is an underutilized byproduct from the chemical processing of biological origin fats and oils. Aspects of the biological conversion of glycerol into value added products were investigated in this dissertation.

- The first aspect investigated was the nutrients required for the *Clostridium butyricum* fermentation of glycerol to 1,3 propanediol. The nutrient supply was minimized, and unnecessary and rich components were eliminated. Batch fermentations with this nutrient minimized media resulted in a 90% substrate utilization, converting 51 % (w/w) into product, with a maximum specific productivity of 0.2% (w/w) per hour.
- A packed bed / fluidized bed reactor insert, novel to use in fermentation studies, was also designed that can be used in stirred tank reactors that allowed the study of immobilized biocatalyst performance in the typical stirred tank reactor configuration. Alginate beads were not sufficiently stable to be useful for the *C. butyricum* fermentation of glycerol, but expanded vermiculite was found to be a suitable immobilization matrix.

- Enzymatic techniques for the conversion of glycerol were investigated. The extent of the enzymatic conversion of glycerol to 3-hydroxypropionaldehyde was found to be limited by substrate induced enzyme inactivation. Inactivation was not due to enzyme thermal denaturation, coenzyme limitation, or oxidative events. This inactivation event was observed to be due to the time of exposure to the substrate and not due to the number of reactions performed by the enzyme.
- A naturally solvent tolerant bacteria, *Providencia rettgeri* OF011, was shown to be genetically modified using *Escherichia coli* techniques and materials. This solvent tolerant organism could prove useful for increased solvent concentration in fermentations, or for solvent / solvent extractions of fermentations.

The technique that currently shows the most promise for converting glycerol into a value added product, in an industrially relevant manner, is the fermentation route to 1,3 propanediol.

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Chapter 1

Introduction to Biological Conversion of Glycerol to Value Added Products

1.1.0.0 Introduction

The desire to derive chemical feedstocks from renewable resources has been an intensely studied topic in recent years. Whether this interest is due to trying to end the dependence of the United States on foreign oil, to create a larger market for domestic farm products, or for the production of inherently "green" products, is up for debate. However, the one driving force that does not change is the need for a cost competitive product.

One of the categories of feedstocks obtained from natural products are the triglycerides, also known as fats or biological oils. These raw materials of the oleochemical industry may be readily obtained from plant seeds such as soybeans, corn, or canola, or from animal fats such as beef tallow. Regardless of the source, the triglyceride consists of three fatty acid units and one glycerol unit. The fatty acid composition will vary from source to source, but the glycerol is uniform amongst the different sources.

Recent and past work has focused on converting the fatty acid portion into products such as soap and biodiesel and fatty acid alcohols and amines. While these products efficiently use the fatty acid portion of the triglyceride, the crude glycerol coproduct portion has historically not been the product from these conversions that is driving the market. If the conversion of triglycerides into biodiesel becomes prevalent,

which at most would supply 10% of a 55 billion gallon per year diesel market, this could result in the overproduction of crude glycerol for which no current market exists. Current usage and growth rates estimate that in the year 2011, 350 million gallons of biodiesel will be sold, compared to the 6.2 million gallons sold in 2000 (Wilson, 2002).

The fatty acid industry is viewed as growing at rates similar to the growth of the gross domestic product. Although the raw materials and products of this industry are viewed as environmentally friendly, the industry is not growing by leaps and bounds (McCoy, 1999). This industry works on thin margins, and the price of raw materials and products are subject to fluctuations, which is one factor that influenced the substantial corporate restructuring that occurred in recent years in this industry. The coproduct of fats and oils splitting, glycerol, is notoriously volatile with respect to market value because its production is dictated by demand for fatty acids. Purified glycerol prices typically vary between $0.40 and $1.00 per pound (McCoy, 2000).

Glycerol is not only produced as the coproduct of fats and oils splitting operations. Dow Chemical makes glycerol synthetically from epichlorohydrin. Glycerol is also a coproduct produced during industrial ethanol fermentation, which has seen increasing applications in recent years. In a process purification patent issued in 1993 (U.S. Pat. No. 5177009), an ion exclusion based purification is described that allows the purification of glycerol. It is anticipated that a pound of glycerol can be obtained for every gallon of ethanol produced, and 99.7% pure glycerol could be produced for as little as $0.15 per pound (McCoy, 2000).

An approach to utilizing the glycerol coproduct is to refine it into reagent grade glycerol. The oldest branded product made by Procter and Gamble is Star Glycerin, and

it has been made for more than 100 years (McCoy, 2002). Unfortunately traditional purification processes for glycerol are expensive and there is a limited market for this product even though there are thousands of uses for glycerol. A second strategy for utilizing this glycerol would be to convert the crude coproduct glycerol into a different product that is worth more money, which could then be purified and sold. One of the products that glycerol can be biologically converted into is 1,3 propanediol (1,3 PD). As with any process, the conversion to a new product and subsequent purification is driven by the requirement that the cost of the conversion process not be excessively large with respect to the price commanded by the final product.

Dupont is particularly interested in 1,3 propanediol, as it is polymerized with terephthalic acid to form the polymer polytrimethylene terephthalate (PTT) (Tullo, 2000; CENEAR, 2000). It is estimated that the demand for this polymer will reach 2 billion pounds by year 2010. DuPont markets this stain resistant PTT polyester under the name of Sorona. These polymers have been known for over 50 years, but were never widely used because the route for synthesizing 1,3 PD was not economical. Synthetic routes to produce 1,3 PD include the Degussa-Hüls process, recently acquired by DuPont, which makes 1,3 PD from the hydration of acrolein (U.S. Pat. No. 5093537). Another route recently developed by Shell, the only other producer of PTT in the world, marketed with the name Corterra, involves the reaction of ethylene oxide with carbon monoxide in the presence of a cobalt catalyst to form 1,3 PD (U.S. Pat. No. 5463144, 5463145, 5463146, 5545767, and 5684214). Both of these chemically synthetic production methods rely on the use of components listed as EPA hazardous air pollutants and extreme reaction conditions to produce 1,3 PD (Ritter, 2003).

An alternative to the chemical synthesis of 1,3 propanediol is the anaerobic fermentation of glycerol to this product. This fermentation has been known for over a century, and was patented in 1993 (U.S. Pat. No. 5254467). Recent work by DuPont and Genencor (Ritter, 2003) has involved genetically constructing a pathway into a bacterial strain that allows the fermentation of glucose into 1,3 PD (U.S. Pat. No. 5686276). This process has the potential to be a lower cost route to 1,3 PD when compared to the synthetic routes (McCoy, 2003). This new pathway consists of the conversion of glucose into glycerol, which is ultimately converted into 1,3 PD. DuPont demonstrated the process at a pilot plant operated by A.E. Staley, a subsidiary of Tate & Lyle, in Decatur Illinois. They have started construction on a "large-scale" fermentation facility in Loudon, Tennessee in collaboration with the carbohydrate processor Tate & Lyle. This facility is expected to begin operations in 2006 (Ritter, 2004).

The anaerobic conversion of glucose into glycerol requires a reducing equivalent (NADH) as does the conversion of glycerol into 1,3 PD. When only a single carbon source is available for fermentation, the glucose fermentation to 1,3 PD has a maximum carbon efficiency of 33% forming product, while the glycerol fermentation has 66% forming product. If it is assumed that 1,3 PD will command a market value similar to that of 1,4 butanediol ($0.55 to $0.70 per pound) (Chemical Market Reporter), a diol used for similar terephthalate polyesters, and applying the "rule of thumb" that product cost should be at least 4 times the raw material cost, we can infer that the price of crude glycerol and the nutrients necessary to ferment it to 1,3 PD should be no more than $0.10 to $0.15 per pound of glycerol substrate.