Ethanol production using continuous multi-staged microbe bioreactors: a laboratory study

William Bruce King

Louisiana State University and Agricultural and Mechanical College

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ETHANOL PRODUCTION USING CONTINUOUS MULTI-STAGED IMMOBILIZED MICROBE BIOREACTORS: A LABORATORY STUDY

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
In partial fulfillment of the
Requirements for the degree of
Master of Science

In

The Department of Environmental Studies

By
William Bruce King
B.S., Auburn University, 1966
May 2008
ACKNOWLEDGMENTS

Many people contributed to the successful completion of this project. I am grateful to Dr. Ralph Portier for serving as committee chair and mentor. Committee members Dr. Ed Overton and Professor Michael Wascom guided the project and provided valued advice. My classmates, Kyle Schmidt, Laura Basirico, and Chris Wootten were generous with advice and friendship. Special thanks to Kyle for providing much needed direction with lab procedures and preparation of the thesis document; to Tommy Wren for essential drawings; and to Gene Bayne of Widgett Scientific, Inc., for assisting with design and construction of the laboratory equipment. Charlotte St. Roman provided encouragement and guidance with administrate requirements.

I am also deeply grateful to my wife Betty, for her understanding and support during this project. Her sacrifice was more than anticipated and far more than fair. Thanks to the other members of our family - Beth and Stewart Serpas and children, Mitchell and Victoria; Drs. David and Karen King; and Tim and Shawna King and children, Sierra and Josh Saldaña – for their encouragement.

Most of all, thanks to Jesus Christ for giving me the desire, intelligence and health to undertake and complete this endeavor during this time in my life.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>ii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>vi</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>viii</td>
</tr>
<tr>
<td>1. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1.1 Ethanol Production and Demand</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Ethanol Production Processes</td>
<td>7</td>
</tr>
<tr>
<td>1.3 Potential Ethanol Production Improvements</td>
<td>7</td>
</tr>
<tr>
<td>1.4 Project Objectives</td>
<td>7</td>
</tr>
<tr>
<td>1.5 Description of Project Work Plan</td>
<td>8</td>
</tr>
<tr>
<td>1.6 Overview of Thesis</td>
<td>8</td>
</tr>
<tr>
<td>2. LITERATURE REVIEW</td>
<td>10</td>
</tr>
<tr>
<td>2.1 The Ethanol Market Today</td>
<td>10</td>
</tr>
<tr>
<td>2.2 Ethanol Production</td>
<td>10</td>
</tr>
<tr>
<td>2.3 Feedstocks</td>
<td>14</td>
</tr>
<tr>
<td>2.3.1 Corn</td>
<td>14</td>
</tr>
<tr>
<td>2.3.2 Sugar Cane and Sugar Beets</td>
<td>14</td>
</tr>
<tr>
<td>2.3.3 Synthesis Gas</td>
<td>15</td>
</tr>
<tr>
<td>2.4 Ethanol Economics</td>
<td>19</td>
</tr>
<tr>
<td>2.5 Environmental Considerations of Ethanol Production</td>
<td>21</td>
</tr>
<tr>
<td>2.6 Continuous versus Batch Fermentation Systems</td>
<td>22</td>
</tr>
<tr>
<td>3. MATERIALS AND METHODS</td>
<td>26</td>
</tr>
<tr>
<td>3.1 Objectives</td>
<td>26</td>
</tr>
<tr>
<td>3.2 Research Unit Design</td>
<td>26</td>
</tr>
<tr>
<td>3.2.1 Reactor Feed System</td>
<td>26</td>
</tr>
<tr>
<td>3.2.2 Microbubble Generator</td>
<td>27</td>
</tr>
<tr>
<td>3.2.3 Immobilized Microbe Bioreactors</td>
<td>29</td>
</tr>
<tr>
<td>3.2.4 Distillation System</td>
<td>32</td>
</tr>
<tr>
<td>3.3 Reactor Feed Preparation</td>
<td>44</td>
</tr>
<tr>
<td>3.4 Sampling and Analytical Methods</td>
<td>44</td>
</tr>
<tr>
<td>4. RESULTS AND DISCUSSION</td>
<td>46</td>
</tr>
<tr>
<td>4.1 Microbubble Generator Performance</td>
<td>46</td>
</tr>
<tr>
<td>4.2 Feed System Operation</td>
<td>47</td>
</tr>
<tr>
<td>4.3 Immobilized Microbe Bioreactors</td>
<td>47</td>
</tr>
<tr>
<td>4.4 Ethanol Distillation System</td>
<td>50</td>
</tr>
</tbody>
</table>
5. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS .......... 52
   5.1 Summary ........................................................................................................................................ 52
   5.2 Conclusions ...................................................................................................................................... 53
      5.2.1 Microbubble Generator ............................................................................................................. 53
      5.2.2 Immobilized Microbe Bioreactors ............................................................................................. 53
      5.2.3 Distillation Section ....................................................................................................................... 53
   5.3 Recommendations ............................................................................................................................. 54

REFERENCES ............................................................................................................................................ 55

APPENDIX: TERMS AND ABBREVIATIONS COMMONLY ASSOCIATED
WITH THE PRODUCTION OF ETHANOL ..................................................................................................... 57

VITA .......................................................................................................................................................... 69
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>United States Motor Fuel Consumption................................................................. 1</td>
</tr>
<tr>
<td>1.2</td>
<td>United States Fuel Ethanol Demand............................................................................. 2</td>
</tr>
<tr>
<td>1.3</td>
<td>Potential Ethanol Production from Sugarcane and Grains in Louisiana....................... 6</td>
</tr>
<tr>
<td>1.4</td>
<td>Potential Ethanol Production from Biomass in Louisiana............................................ 6</td>
</tr>
<tr>
<td>2.1</td>
<td>Ethanol Yields on a Common Basis ................................................................................. 19</td>
</tr>
<tr>
<td>2.2</td>
<td>Estimated Ethanol Production Costs based on Feedstocks........................................... 20</td>
</tr>
<tr>
<td>2.3</td>
<td>Estimated Greenhouse Gas Emissions............................................................................ 21</td>
</tr>
<tr>
<td>3.1</td>
<td>Column Packing Specifications....................................................................................... 41</td>
</tr>
<tr>
<td>3.2</td>
<td>Yeast Cell Count from Reactor Packing and Product..................................................... 45</td>
</tr>
<tr>
<td>4.1</td>
<td>Fermentation and Distillation Results.......................................................................... 49</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Ethanol Percent of Gasoline Pool</td>
<td>1</td>
</tr>
<tr>
<td>1.2</td>
<td>United States Fuel Ethanol Production and Demand</td>
<td>2</td>
</tr>
<tr>
<td>1.3</td>
<td>Ethanol Production in Louisiana</td>
<td>3</td>
</tr>
<tr>
<td>1.4</td>
<td>Gasohol Consumption in Louisiana</td>
<td>3</td>
</tr>
<tr>
<td>1.5</td>
<td>Entrance to Abandoned Ethanol Plant</td>
<td>4</td>
</tr>
<tr>
<td>1.6</td>
<td>Fermentation Building of Abandoned Ethanol Plant</td>
<td>4</td>
</tr>
<tr>
<td>1.7</td>
<td>Distillation System of Abandoned Ethanol Plant</td>
<td>5</td>
</tr>
<tr>
<td>2.1</td>
<td>Dry Milling Ethanol Production Process</td>
<td>11</td>
</tr>
<tr>
<td>2.2</td>
<td>Wet Milling Ethanol Production Process</td>
<td>12</td>
</tr>
<tr>
<td>2.3</td>
<td>Process for Production of Ethanol from Cellulosic Biomass</td>
<td>13</td>
</tr>
<tr>
<td>2.4</td>
<td>Flow Diagram of Syngas to Ethanol Fermentation Facility</td>
<td>17</td>
</tr>
<tr>
<td>2.5</td>
<td>Estimated Green House Gas Emissions</td>
<td>21</td>
</tr>
<tr>
<td>2.6</td>
<td>Typical Batch Alcohol Fermentor</td>
<td>23</td>
</tr>
<tr>
<td>2.7</td>
<td>Typical Batch Growth Curve for Yeast</td>
<td>24</td>
</tr>
<tr>
<td>2.8</td>
<td>Typical Continuous Multistage Fermentation Units</td>
<td>25</td>
</tr>
<tr>
<td>3.1</td>
<td>Laboratory Apparatus for Continuous Production of Ethanol</td>
<td>27</td>
</tr>
<tr>
<td>3.2</td>
<td>Microbubble Generator</td>
<td>28</td>
</tr>
<tr>
<td>3.3</td>
<td>Microbubble Generator</td>
<td>29</td>
</tr>
<tr>
<td>3.4</td>
<td>Immobilized Microbe Bioreactor for Direct Fermentation</td>
<td>30</td>
</tr>
<tr>
<td>3.5</td>
<td>Installed Immobilized Microbe Bioreactor Test Bed</td>
<td>31</td>
</tr>
<tr>
<td>3.6</td>
<td>Immobilized Microbe Bioreactor with Biocarrier Installed</td>
<td>32</td>
</tr>
<tr>
<td>Figure</td>
<td>Page</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>3.7</td>
<td>Liquid/Vapor Equilibrium Curve for Ethanol/Water Mixture</td>
<td>33</td>
</tr>
<tr>
<td>3.8</td>
<td>Typical Distillation System</td>
<td>34</td>
</tr>
<tr>
<td>3.9</td>
<td>Equilibrium Curve indicating Distillation Components</td>
<td>34</td>
</tr>
<tr>
<td>3.10</td>
<td>McCabe Thiele Diagram for Ethanol/Water</td>
<td>35</td>
</tr>
<tr>
<td>3.11</td>
<td>Distillation Section for Immobilized Microbe Bioreactor Test Bed</td>
<td>36</td>
</tr>
<tr>
<td>3.12</td>
<td>Thermal Siphon Reboiler for Distillation Section</td>
<td>37</td>
</tr>
<tr>
<td>3.13</td>
<td>Rectification Column for Distillation Section</td>
<td>38</td>
</tr>
<tr>
<td>3.14</td>
<td>Feed Preheater for Distillation Section</td>
<td>39</td>
</tr>
<tr>
<td>3.15</td>
<td>Stripper Column for Distillation Section</td>
<td>40</td>
</tr>
<tr>
<td>3.16</td>
<td>Splitter or Reflux/Product Control System for Distillation Section</td>
<td>42</td>
</tr>
<tr>
<td>3.17</td>
<td>Distillate Receiver for Distillation Section</td>
<td>43</td>
</tr>
<tr>
<td>4.1</td>
<td>Ethanol Yield versus Time</td>
<td>50</td>
</tr>
</tbody>
</table>
ABSTRACT

Alternative fuels are important for the United States to reduce their dependence on fossil fuels. Currently, ethanol is the only renewable fuel that is produced in commercial quantity. The demand for ethanol is increasing throughout the world. The production of ethanol is limited by the available feedstocks and processing technology.

Corn is the primary feedstock for ethanol in the United States. It is processed in either the wet milling or dry milling process. Both processes use either the batch or continuous fermentors. Both batch and continuous systems have operational restrictions with maintaining a good growth of yeast and preventing contamination with bacteria.

The use of Immobilized Microbe Bioreactors will provide a continuous system that maintains a growth of yeast and is resistant to bacteria. The Immobilized Microbe Bioreactors are packed with biocarrier which is a solid, porous, inorganic substance that provides a large surface area for attachment of the yeast cells. The yeast colonizes the internal surfaces as well as the external surfaces of the biocarrier. This provides a higher culture density of yeast which is resistant to bacterial contamination.

The feasibility of using the Immobilized Microbe Bioreactor was addressed by laboratory testing. Raw sugar and molasses were used as feedstocks. The Microbubble Generator was tested for the ability to saturate the feed with air to facilitate the growth of yeast. The yield of ethanol was determined by distilling azeotropic ethanol as an overhead product. The data indicated that the Immobilized Microbe Bioreactor could be an improvement to ethanol production systems.
1. INTRODUCTION

1.1 Ethanol Production and Demand

During the past decade, ethanol has been the only renewable liquid fuel made in commercial quantity with demand and production increasing throughout the world. However, the percentage of ethanol that has replaced gasoline in the United States has increased only an average of about 1.6% per year as shown in Table 1.1 and Figure 1.1 (Energy Information Administration, February 2007).

Table 1.1 - United States Motor Fuel Consumption, million gallons per year

<table>
<thead>
<tr>
<th>Year</th>
<th>Gasoline</th>
<th>Ethanol</th>
<th>Percent of Gasoline Pool</th>
</tr>
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<tbody>
<tr>
<td>2000</td>
<td>128,662</td>
<td>1,630</td>
<td>1.27</td>
</tr>
<tr>
<td>2001</td>
<td>129,312</td>
<td>1,770</td>
<td>1.37</td>
</tr>
<tr>
<td>2002</td>
<td>132,782</td>
<td>2,130</td>
<td>1.60</td>
</tr>
<tr>
<td>2003</td>
<td>134,089</td>
<td>2,800</td>
<td>2.09</td>
</tr>
<tr>
<td>2004</td>
<td>137,022</td>
<td>3,400</td>
<td>2.48</td>
</tr>
<tr>
<td>2005</td>
<td>136,949</td>
<td>3,904</td>
<td>2.85</td>
</tr>
</tbody>
</table>

Figure 1.1 - Ethanol Percent of Gasoline Pool
Table 1.2 - United States Fuel Ethanol Demand, million gallons per year

<table>
<thead>
<tr>
<th></th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
</tr>
</thead>
<tbody>
<tr>
<td>US</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Production</td>
<td>2,130</td>
<td>2,800</td>
<td>3,400</td>
<td>3,904</td>
<td>4,855</td>
</tr>
<tr>
<td>Imports</td>
<td>46</td>
<td>61</td>
<td>161</td>
<td>135</td>
<td>653</td>
</tr>
<tr>
<td>Exports</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stocks</td>
<td>-91</td>
<td>39</td>
<td>-31</td>
<td>-18</td>
<td>108</td>
</tr>
<tr>
<td>Change</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Demand</td>
<td>2,085</td>
<td>2,900</td>
<td>3,530</td>
<td>4,049</td>
<td>5,616</td>
</tr>
</tbody>
</table>

Figure 1.2 - United States Fuel Ethanol Production and Demand, million gallons per year

The domestic production of the ethanol demand decreased during the last five years. The percent of consumption of domestically produced ethanol used in the United States decreased from almost 100 per cent in 2002 to only about 86 percent in 2006, as shown in Table 1.2 and Figure 1.2 (Renewable Fuels Association, 2007).

Ethanol was produced from sugar cane in Louisiana from 1984 to 1990 with a peak production of 32 million gallons in 1986 (Figure 1.3). Production of ethanol was no longer economical after state subsidies ended in 1986 and the last plant ceased production in 1990.
Consumption of gasohol followed a similar trend as shown in Figure 1.4 (Louisiana Energy Topics, 2003).

![Ethanol Production in Louisiana](image)

**Figure 1.3 - Ethanol Production in Louisiana**

![Gasohol Consumption in Louisiana](image)

**Figure 1.4 - Gasohol Consumption in Louisiana**

An ethanol plant was constructed near Lafayette during 1990 but never became operational. Figure 1.5 is a current photo of the entrance to the plant. Nine ethanol purification units were part of the plant and the distillation columns are visible in the background. Figure 1.6 shows the fermentation building which included the control room and steam generation facilities. Details of an ethanol purification unit are shown in Figure 1.7. The purification unit includes a distillation column and dual molecular sieve beds to produce anhydrous ethanol.
Figure 1.5 – Entrance to Abandoned Ethanol Plant

Figure 1.6 – Fermentation Building of Abandoned Ethanol Plant
Essential parts of much of the equipment have been removed and the units have deteriorated to the point that the plant is no longer operational.

Data from the Energy Information Administration shown in Table 1.3 indicates that the potential production of ethanol from sugarcane and grains was 367 million gallons in 2005 (McGee, et al, 2007). This is based on crops that are currently being produced in Louisiana.
Table 1.3 - Potential Ethanol Production from Sugarcane and Grains in Louisiana

<table>
<thead>
<tr>
<th>Crop</th>
<th>2005 Total Production</th>
<th>Ethanol Conversion Factor</th>
<th>Ethanol (gallons)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugarcane</td>
<td>11,339,065 tons</td>
<td>18 gal/ton</td>
<td>204,103,170</td>
</tr>
<tr>
<td>Corn</td>
<td>44,227,116 bushels</td>
<td>2.7 gal/bu</td>
<td>119,413,213</td>
</tr>
<tr>
<td>Grain Sorghum</td>
<td>6,106,071 cwt</td>
<td>4.65 gal/cwt</td>
<td>28,393,230</td>
</tr>
<tr>
<td>Oats</td>
<td>311,422 bushels</td>
<td>0.9203 gal/bu</td>
<td>286,602</td>
</tr>
<tr>
<td>Wheat</td>
<td>5,985,589 bushels</td>
<td>2.483 gal/bu</td>
<td>14,862,217</td>
</tr>
<tr>
<td>Total Potential</td>
<td></td>
<td></td>
<td>367,058,433</td>
</tr>
</tbody>
</table>

Louisiana is also a source of biomass as a feedstock for ethanol production. Table 1.4 indicates that over 500 million gallons of ethanol could be produced from potential biomass production in the state. According to the United States Department of Agriculture, there are 390,000 acres of farmland in the Conservation Reserve Program (CRP) in Louisiana. This land is erodible and owners are encouraged to maintain a vegetative cover to prevent erosion. However, much of the land is suitable for high energy crops such as switch grass or energy cane. Data presented in Table 1.4 assumes that all existing production of biomass plus production of switch grass on one-half of the CRP land is used for production of ethanol. Solid Waste includes all the paper, paperboard, wood, food, and miscellaneous organic waste components of municipal solid waste.

Table 1.4 - Potential Ethanol Production from Biomass in Louisiana

<table>
<thead>
<tr>
<th>Biomass</th>
<th>2005 Total Production (tons)</th>
<th>Ethanol Conversion Factor (gal/ton)</th>
<th>Ethanol (gallons)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forrest Residue</td>
<td>872,000</td>
<td>60</td>
<td>52,320,000</td>
</tr>
<tr>
<td>Mill Residue</td>
<td>1,943,000</td>
<td>60</td>
<td>116,580,000</td>
</tr>
<tr>
<td>Urban Wood Waste</td>
<td>753,870</td>
<td>60</td>
<td>45,232,200</td>
</tr>
<tr>
<td>Hay</td>
<td>551,531</td>
<td>60</td>
<td>33,091,860</td>
</tr>
<tr>
<td>CRP Energy Crop</td>
<td>1,170,000</td>
<td>60</td>
<td>70,200,000</td>
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<td>Bagasse</td>
<td>1,417,400</td>
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<td>85,044,000</td>
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<tr>
<td>Solid Waste</td>
<td>1,843,009</td>
<td>60</td>
<td>110,580,540</td>
</tr>
<tr>
<td>Total Potential</td>
<td></td>
<td></td>
<td>513,048,600</td>
</tr>
</tbody>
</table>
1.2 Ethanol Production Processes

The primary commercial process for production of ethanol is direct fermentation. Corn is presently the most common feedstock in the United States. The corn is processed in either the wet milling or dry milling plants. The entire corn kernel is ground into meal in the dry milling process and then separated into various components. The meal is slurried and then converted to sugar for fermentation. The wet milling process involves soaking the corn in a water solution and then separating into the various components before fermentation and other treatment. Sugarcane is the primary feedstock for ethanol in several countries including Brazil.

Direct fermentation processes are both batch and continuous. The older and more traditional processes are batch which involve filling a vessel with feed and inoculating with yeast before a lengthy fermentation. The equipment for continuous processes is smaller and operates with continuous inoculation. Several processes for direct and indirect fermentation of cellulose are in the research phase but none are currently commercial.

1.3 Potential Ethanol Production Improvements

The primary disadvantage to the batch fermentation process is the long lag phase during which time the yeast activates its ability to synthesize enzymes. Both batch and continuous processes are subject to contamination by bacteria which can reduce yield and result in time when the unit is not operational. This study addresses the Immobilized Microbe Bioreactor which is a mechanism that reduces the potential for contamination and presents a mechanism for self-inoculation of the reactors. The Immobilized Microbe Bioreactor also provides a continuous process with much smaller equipment than the standard batch or continuous systems.

1.4 Project Objectives

The basic objective of the study was to determine the feasibility and advantages of using an Immobilized Microbe Bioreactor for ethanol fermentation. This involved designing and
installing a laboratory unit with a continuous fermentor and distillation unit to recover high purity ethanol.

An aerobic environment must be created to enable the growth of the yeast. This project will evaluate use of Microbubble Generator to create the aerobic environment for yeast growth.

Ethanol production and yield will be determined using the Immobilized Microbe Bioreactor with raw sugar or molasses as feed stock. The biorector product will be distilled using a single column distillation unit. The yield will be determined by the amount of product recovered in the distillation system overhead.

1.5 Description of Project Work Plan

To determine the feasibility and advantages of using the Immobilized Microbe Bioreactor as a continuous ethanol fermentor, needed work was performed following the work plan detailed below.

1. Review existing literature concerning ethanol production and demand, production processes and equipment and ongoing research for unique and new methods of ethanol production.

2. Design and install laboratory unit to model continuous Immobilized Microbe Bioreactor ethanol fermentation system.

3. Design and install laboratory unit to distill ethanol azeotrope.

4. Select and mix solutions to be evaluated as feedstocks for ethanol production.

5. Perform laboratory work to meet objectives.

1.6 Overview of Thesis

The first chapter is an introduction to ethanol production and demand in both the United States and Louisiana; the current ethanol production processes; potential improvements to ethanol production processes; and project objectives and work plan.
Chapter Two gives more detail concerning ethanol production, demand and processes. Potential problems dealing with ethanol production are also addressed. Alternative feedstocks such as synthesis gas are also introduced.

Chapter Three discusses the design of the research unit and research methods that were used in the project.

Chapter Four presents the results of producing ethanol utilizing the Immobilized Microbe Bioreactor. Impact of using the Microbubble Generator for aerating the feed to produce yeast growth was addressed. Using the distillation system to determine the yield of ethanol is discussed.

Chapter Five gives the summary, conclusions and recommendations for further work toward using the Immobilized Microbe Bioreactor in ethanol production.

A detailed list of abbreviations and terms used in the thesis is presented in the Appendix.
2. LITERATURE REVIEW

2.1 The Ethanol Market Today

The production of ethanol in the United States has increased from about 2,130 million gallons in 2002 to about 4,855 million gallons in 2006. There are currently 129 plants in the United States producing fuel ethanol with a capacity of about 6,843.4 million gallons per year. The 76 new plants under construction along with 9 expansions of current plants will add an additional 6,585.9 million gallons per year increasing the total capacity to about 13,429.3 million gallons per year (Renewable Fuels Association, 2007). The United States Environmental Protection Agency Act 2005 mandated a production of 4.0 billion gallons of ethanol in 2006 increasing to 7.5 billion gallons in 2012 (Kumins, 2007). Significant additional expansion of the industry is needed if ethanol is to make a meaningful contribution to our nation’s energy supply. The likelihood of this expansion is, in part, contingent on improvements in the technology used for ethanol production.

2.2 Ethanol Production

Commercial production of ethanol is currently by direct fermentation of carbohydrates or by hydration of ethylene. Direct fermentation accounts for the major production. Non-idled synthetic production was reported to be only about 50 million gallons per year in 2002 (Davenport et al, 2002). Ethylene is the major building block for synthetic production. However, Dow Chemical recently announced plans to construct a plant to produce polyethylene from sugarcane-based ethanol which counters production of ethanol from ethylene. Dow’s partner in the venture, Crystalsev, says that they will add 8 million tons of cane-crushing capability to supply ethanol needed as part of the project. Kevin McCarthy, analyst at Bank of America, is quoted as saying, “Brazil is the ideal location to commercialize this technology, as sugarcane is the low-cost route to ethanol” (Bryner, et al, 2007).
The two production processes for converting corn to ethanol are the wet milling and dry milling. The primary difference is the initial treatment of the corn. Currently approximately 82% of ethanol production is from dry mill facilities.

In dry milling, the entire corn kernel or other starchy grain is ground into flour (meal) and is processed with separating out the various component parts of the grain. The meal is slurried with water and forms a mash. Enzymes are added to the mash to convert the starch to dextrose. Ammonia is added for pH control and as a nutrient for the yeast. The mash is processed in a high-temperature cooker to reduce bacteria levels before fermentation and then is cooled and transferred to fermentors where yeast is added. Fermentation converts the sugar to ethanol and carbon dioxide. The fermentation process takes about 40 to 50 hours during which time the mash is agitated and cooled to facilitate the activity of the yeast. After fermentation is complete, the resulting liquid (beer) is fed to the distillation system where 190 proof (95% v/v

Figure 2.1 - Dry Milling Ethanol Production Process
(Renewable Fuels Association, 2007)
ethanol) is recovered. The bottoms from the distillation system are known as sillage. Remaining water in the 95% ethanol is then removed by a molecular sieve system. A denaturant such as gasoline is then added to make the alcohol undrinkable. The sillage is then fed to a centrifuge that separates the coarse grain from the solubles. The solubles are the concentrated by evaporation resulting in syrup which are then dried with the coarse grain to produce a high quality livestock feed. The carbon dioxide released during fermentation is captured and used for carbonating soft drinks or the manufacture of dry ice.

![Figure 2.2 - Wet Milling Ethanol Production Process](image.png)

In the wet milling process, the grain is soaked or steeped in water and dilute sulfurous acid to facilitate the separation of the grain into component parts. The corn slurry is then processed through grinders to separate the corn germ. Corn oil is then extracted from the germ. The remaining fiber, gluten and starch components are further segregated using centrifugal, screen or hydroclonic separators. The steeping liquor is then concentrated in an evaporator. The concentrated product or heavy steep water is dried with the fiber and then sold as corn gluten.
feed for livestock. Heavy steep water can be used as an alternate to salt for removing ice from roads. The gluten component is filtered and dried to produce a corn gluten meal co-product which is used for poultry feed. The starch and any remaining water from the mash can be fermented into ethanol, dried into modified corn starch or processed into corn syrup. The fermentation process is similar to the dry mill process (Renewable Fuels Association, 2007).

There are various processes for fermentation of cellulosic biomass to ethanol but none of these are commercial at this time. The process flow diagram in figure 2.3 shows the basic steps in the production of ethanol from cellulosic biomass. Technologies vary in the options for pretreatment and other steps and several combine the hydrolysis and fermentation steps. One approach to utilizing this biomass resource is to modify the direct fermentation process so that, rather than fermenting the sugars present in starch, the sugars present in the cellulose and hemicellulose fractions of biomass, i.e., such as bagasse, are converted to ethanol by direct fermentation. Unfortunately, the cellulose and hemicellulose sugars are difficult to liberate and differ in composition from the sugars present in starch. Both issues lead to significant
differences between the two direct fermentation approaches.

2.3 Feedstocks

The primary feedstock for ethanol production in the United States is corn. Secondary feedstocks include sugarcane, sugar beets and cellulose. Sugarcane is the primary feedstock for ethanol in Brazil and several other countries and is emerging as a feedstock in the United States. Closely tied with cellulose is fermentation of synthesis gas derived from processing cellulose.

2.3.1 Corn

Feedstock availability is one constraint on the ability of the existing corn-based industry to make a meaningful impact on our nation’s energy supply. U.S. corn production reached 11.7 billion bushels in 2006 (National Corn Growers Association, 2007). Fuel ethanol production was responsible for consuming 2,150 million bushels, or about 18.3% of the crop for that year. The largest consuming application for corn is direct use as animal feed for domestic use, accounting for 5.975 billion bushels of consumption in 2006. One bushel of corn provides raw material to produce about 2.8 gallons of ethanol. This is equivalent to about 1.4 gallons of gasoline. Projections are that gasoline consumption in the United States will average 20.9 million barrels per day in 2007 and increase to 21.1 million barrels per day in 2008 (Energy Information Administration, 2007). Even if suitable replacements could be found for animal feed and all other uses of corn, only about 5% of the United States energy needs for gasoline type transportation fuels could be met at current corn production levels.

2.3.2 Sugarcane and Sugar Beets

The other major ethanol producing country is Brazil with over 4.0 billion gallons per year of production capacity. Brazil uses sugarcane juices/molasses as the major source of carbohydrate since they are the largest producer of sugarcane in the world. Brazil expects to achieve energy independence in the near future due to their production and use of ethanol.
Ethanol makes up about 40% of their total transportation fuel (Reel, 2006). In order to counter the energy crisis in the 1970's, the Brazilian Government created incentives such as low-interest loans to ethanol producers, set minimum prices for their products and provided tax incentives to auto makers who offered ethanol powered cars. Pure ethanol or E-25, a gasoline blend of 25 percent ethanol, is now available in nearly every filling station in the country. It has been reported that 7 out of every 10 new cars sold in Brazil are flex-fuel. Brazil has eliminated its financial support for ethanol due to the success in creating the required infrastructure for ethanol-based energy independence. The industry continues to increase productivity and lower ethanol prices. Ethanol in Brazil now costs less than gasoline (Luhnow, et al, 2006).

2.3.3. Synthesis Gas

Synthesis gas (syngas) is the basic building block for basic chemicals such as methanol, ammonia and hydrogen. The traditional method of producing syngas is by steam reforming. Currently, syngas is also produced by autothermal reforming, partial oxidation and gasification. Syngas has a high content of hydrogen, carbon monoxide and carbon dioxide.

The fact that syngas can be produced from almost any biomass material makes it of interest in the production of ethanol. Syngas fermentation is an indirect method for producing ethanol from biomass feedstocks. Syngas can be converted to ethanol using fermentation. Today’s corn-based ethanol industry is restricted to processing grain starches by direct fermentation. Direct fermentation of biomass can handle a wider variety of biomass feedstocks, but more recalcitrant materials lead to high costs. Difficult-to-handle materials, softwoods for example, may best be handled with the syngas fermentation approach. Expected yield from a grassroots biomass syngas-to-ethanol facility, with no external fuel source provided to the gasifier, are 70-105 gallons of ethanol per ton of dry biomass fed. The economics of this route appear to be competitive with today’s corn-based ethanol and projections for direct fermentation
of biomass. One report states projected cash costs on the order of $0.70 per gallon, with feedstock available at $25 per ton. Capital costs are projected at about $3.00 per gallon of annual capacity. The rational price, defined as the ethanol sales price required for a zero net present value of a project with 100% equity financing and 10% real after-tax discounting, is projected to be $1.33 per gallon. These economics would support a successful commercial project at the current ethanol sales price of $1.00-$1.50 per gallon. (Spath, et al, 2003) The syngas fermentation approach has received very modest levels of support in the past. Currently, there are no commercial plants producing ethanol from syngas and there are only a few academic groups working in this area.

Normally the syngas intermediate required for ethanol production is assumed to be generated from gasification of biomass resources such as bagasse, rice hulls, wood chips derived from forestry operations and other similar “low-cost” materials. It should be clear that other means of syngas generation could also be considered such as steam reforming of natural gas and other light hydrocarbons, reforming of anaerobic digester biogas, and gasification of other carbonaceous feedstocks such as coal, petroleum resid, coke, municipal solid waste, biomass derived fast pyrolysis oils, etc. Figure 2.4 is a simplified block flow diagram for a biomass syngas fermentation facility. The feed is first received and placed in temporary storage on-site. It is then sent to the gasifier where it is converted into a raw syngas mixture rich in carbon monoxide and hydrogen. Biomass gasification has been an area of research and development interest by governments and private industry for many years, so several technological options exist. The syngas intermediate is then converted to ethanol via fermentation. Again, this approach could be applied to a wide variety of feedstocks found in Louisiana and other sugarcane producing states/nations. The resulting fermentation broth is quite dilute, typically containing 2% or less of ethanol. The ethanol can be recovered from the broth using recovery
schemes patterned after those used in the existing corn ethanol industry (i.e. an ethanol-water mixture close to the azeotropic composition is distilled overhead and an adsorption unit is used to further dry the ethanol product to meet fuel grade specification on water content). The cell mass produced from the fermentation is not currently approved for animal feed use.

Figure 2.4 - Flow Diagram of Synthesis Gas to Ethanol Fermentation

The micro-organisms used for ethanol production from syngas mixtures are obligate anaerobes that use a heterofermentative version of the acetyl-CoA pathway for acetogenesis. Acetyl-CoA is produced from CO or H$_2$/CO$_2$ mixtures in this pathway. The acetyl-CoA intermediate is then converted into either acetic acid or ethanol as a primary metabolic product. The details of the biochemistry of acetogenesis are reviewed in Drake (Drake, 1994). Carbon monoxide is actually a preferred substrate over H$_2$/CO$_2$, since the change in free energy is more favorable. Typical CO conversions reported in the literature for laboratory scale fermentations are about 90%, while H$_2$ conversions are about 70%. Research efforts are required to improve conversions and to address issues such as mass transfer between the gas and liquid phases.

The following ratio of the heats of combustion for the products and feeds of the indicted reactions are taken from an article in the journal, Elsevier Biomedical (Roels, 1983).
Ethanol Production

\[6 \text{CO} + 3 \text{H}_2\text{O} \rightarrow \text{CH}_3\text{CH}_2\text{OH} + 4 \text{CO}_2\]  
\[\text{HHV}_{\text{Products}}/\text{HHV}_{\text{Feeds}} = 0.81\]

\[2 \text{CO}_2 + 6 \text{H}_2 \rightarrow \text{CH}_3\text{CH}_2\text{OH} + 3 \text{H}_2\text{O}\]  
\[\text{HHV}_{\text{Products}}/\text{HHV}_{\text{Feeds}} = 0.80\]

Acetic Acid Production

\[4 \text{CO} + 2 \text{H}_2\text{O} \rightarrow \text{CH}_3\text{COOH} + 2 \text{CO}_2\]  
\[\text{HHV}_{\text{Products}}/\text{HHV}_{\text{Feeds}} = 0.77\]

\[2 \text{CO}_2 + 4 \text{H}_2 \rightarrow \text{CH}_3\text{COOH} + 2 \text{H}_2\text{O}\]  
\[\text{HHV}_{\text{Products}}/\text{HHV}_{\text{Feeds}} = 0.77\]

These values represent “cold gas efficiencies” for the fermentation. It is worth noting that these values are rather low for an anaerobic fermentation. By comparison, the same ratio for production of ethanol by direct fermentation of glucose is 0.98. The ratio of ethanol to acetate produced is dependent upon the strain or the organism and the fermentation conditions. The organisms are inhibited by low pH and acetate ion concentration. When acetic acid is formed, the pH drops and the acetate ion concentration rises, so the organism switches to ethanol production to alleviate further inhibition. Typically pH is kept around 4.5 in ethanol production mode.

Many of the organisms are either mesophiles or thermophiles with temperature optimums ranging from room temperature to 90 degrees C. A fairly rich media is typically required, but high operating temperatures, low carbohydrate levels, low pH and hi CO levels (which are inhibitory to methanogens) reduce the risk of contamination (Spath, et al, 2003).

A simple gas-sparged tank reactor, operating in batch or continuous mode, has traditionally been used for the fermentation step. While simple, this design suffers from low volumetric productivity, low gas conversion, and produces very dilute ethanol streams. Dr. Gaddy at University of Arkansas/Bioengineering Resources Inc. has studied the issue of fermentor design in detail. He suggests a two-stage fermentation system with cell recycle as a better alternative (Klasson, et al, 1991). Conditions in the first stage are selected to encourage
cell growth, while conditions in the second stage are selected to encourage ethanol production. Cells are recycled in the second stage to improve volumetric productivity and increase conversion. Mass transfer between the gas and liquid phases can limit performance of syngas fermentation designs. Dr Worden at Michigan State University has also studied this issue (Bredwell, et al, 1999).

Table 2.1 compares ethanol yields for the corn-based direct fermentation as reported in the USDA survey, the biomass-based direct fermentation process projected by NREL, and projected yields for a syngas fermentation process (Shapouri, et al, 2006).

<table>
<thead>
<tr>
<th></th>
<th>Wet Mill</th>
<th>Dry Mill, Large</th>
<th>Dry Mill, Medium</th>
<th>Dry Mill, Small</th>
<th>Direct Fermentation Of Biomass</th>
<th>Syngas Fermentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield, gal/bushel</td>
<td>2.682</td>
<td>2.688</td>
<td>2.606</td>
<td>2.588</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yield, gal/ton (dry)</td>
<td>112.7</td>
<td>112.9</td>
<td>109.5</td>
<td>108.7</td>
<td>89.7</td>
<td>70-105</td>
</tr>
</tbody>
</table>

2.4 Ethanol Economics

Ethanol conversion rates in the United States utilizing corn as the feedstock are estimated to be approximately 2.68 gallons of ethanol per bushel for a wet mill process and 2.69 gallons per bushel for a large dry mill process. The net feedstock cost during 2003-2005 is estimated at about $0.40 per gallon for a wet mill process with a total production cost of $1.03 per gallon. Net feedstock costs for a dry mill plant are estimated at $0.53 per gallon with the total ethanol production cost at $1.05 per gallon.

Sugarcane production in the United States is projected at 3,537 million tons in 2007 with sugar beet projected at 33,765 million tons (Salassi, et al, 2007). Molasses is a byproduct of sugarcane and sugar beet production. Approximately 69.4 gallons of ethanol can be made from
one ton of molasses. One ton of raw sugar would yield 135.4 gallons of ethanol and one ton of refined sugar would yield 141.0 gallons of ethanol. The cost of producing ethanol during 2003-2005 from sugarcane feedstock was approximately $2.40 per gallon with feedstock cost being 62% or $1.48 per gallon. The cost of converting sugar beets into ethanol during 2003-2005 was about $2.35 per gallon with feedstock cost being 67% or $1.58 per gallon. Estimated ethanol production cost using molasses as feedstock was $1.27 per gallon with $0.91 per gallon feedstock cost. Table 2.2 compares the estimated ethanol production cost for various feedstocks. It excludes capital and transportation costs. Brazil and European Union costs are published estimates (Salassi, et al, 2007).

<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Feedstock Costs</td>
<td>0.40</td>
<td>0.53</td>
<td>1.48</td>
<td>1.58</td>
<td>0.91</td>
<td>3.12</td>
<td>3.61</td>
<td>0.30</td>
<td>0.97</td>
</tr>
<tr>
<td>Processing Costs</td>
<td>0.63</td>
<td>0.52</td>
<td>0.92</td>
<td>0.77</td>
<td>0.36</td>
<td>0.36</td>
<td>0.36</td>
<td>0.51</td>
<td>1.92</td>
</tr>
<tr>
<td>Total Cost</td>
<td>1.03</td>
<td>1.05</td>
<td>2.40</td>
<td>2.35</td>
<td>1.27</td>
<td>3.97</td>
<td>0.81</td>
<td>2.89</td>
<td></td>
</tr>
</tbody>
</table>

The capital expenditures for a dedicated plant to process sugarcane or sugar beets are higher per gallon of ethanol than that for a corn processing plant. A 20 million gallon per year plant using sugarcane or sugar beets as feedstock would have a capital expenditure of about $2.10 per gallon of annual capacity compared to an estimate of $1.50 per gallon of annual capacity for a corn-based plant. However, the addition of an ethanol facility to an existing sugarcane or sugar beet processing plant would be much less. Therefore, location of the ethanol facility is a major factor in the economics. The use of sugarcane and sugar beet feedstock become extremely attractive if there is a shortage of corn. Development of new technology such
as Immobilized Microbe Bioreactors is important in the future use of alternative feedstock such as sugarcane.

There is some disagreement among authorities as to the exact economics of ethanol from corn as a replacement for gasoline. Dr. David Pimentel of Cornell University claims that a net loss of energy of about 29% is realized when ethanol produced from corn is used for gasoline. His calculations of energy required for production of ethanol include all energy consumptions such as transportation, cultivation, fertilizers, and production. His study reinforces the need for progress in the area of production of ethanol from cellulose and research to improve technology (Pimentel, 2003).

2.5 Environmental Considerations of Ethanol Production

The data presented in Table 2.3 and Figure 2.5 was taken from a recent article in National Geographic (Bourne, 2007). This data indicates that only a minimal reduction in greenhouse gas results from replacing gasoline with ethanol produced from corn. A much greater reduction could be realized by substituting cellulosic ethanol for gasoline.

Table 2.3 - Estimated Greenhouse Gas Emissions, lbs/gal of Equivalent Gasoline/Diesel

<table>
<thead>
<tr>
<th></th>
<th>Gasoline</th>
<th>Corn Ethanol</th>
<th>Cane Ethanol</th>
<th>Cellulosic Ethanol</th>
<th>Diesel</th>
<th>Biodiesel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lbs/gal</td>
<td>20.4</td>
<td>16.2</td>
<td>9.0</td>
<td>1.9</td>
<td>23.4</td>
<td>7.6</td>
</tr>
<tr>
<td>Reduction</td>
<td></td>
<td>22%</td>
<td>56%</td>
<td>91%</td>
<td></td>
<td>68%</td>
</tr>
</tbody>
</table>

Figure 2.5 - Greenhouse Gas Emissions
2.6 Continuous versus Batch Fermentation Systems

The total production of fuel alcohol manufactured in North America is predicted to soon exceed 3.0 billion gallons in approximately 80 production plants. About 60 percent of these plants use batch fermentation. However, these batch fermentation plants manufacture only about one-third of the total ethanol production. The distilling industry has developed and essentially converted to rapid batch fermentation with cylindro-conical or sloping bottom fermentors as plants use batch fermentation. However, these batch fermentation plants manufacture only about one-third of the total ethanol production. The distilling industry has developed and essentially converted to rapid batch fermentation with cylindro-conical or sloping bottom fermentors as shown in Figure 2.6.

The capacities of these fermentors vary between 30,000 gallons to 750,000 gallons. Key components of these batch systems include systems for cleaning, cooling, and carbon dioxide scrubbing.

An external cooling jacket system using a chilled water system is preferential to internal cooling coils which are difficult to clean with Clean-in Place (CIP) spray nozzles. Equally undesirable is external recirculation heat exchangers shared by more than one fermentor which can result in bacterial cross contamination. Both the internal cooling coils and external recirculation heat exchangers can cause microbiological problems with the fermentors. An agitator is required particularly at the start and end of fermentation. A folding action is required to ensure proper mixing and an even temperature throughout the fermentor.

Carbon dioxide is removed through a vent. The carbon dioxide is scrubbed to remove ethanol and is frequently recovered and sold. The carbon dioxide collection system is often a source of contamination and can be the mechanism for bacteria to migrate from a contaminated to a non-contaminated fermentor.
Clean-in-place (CIP) equipment is used to clean and sterilize the fermentors. A typical system has high-pressure sprayheads with automated clean cycles. A minimum cleaning cycle would be ten minutes for pre-rinse with water, 20 minutes for detergent circulation, 10 minutes for post-rinse with water and 10 minutes sterilization. Chemicals such as chlorine dioxide make ideal sterilizing agents but many operators use steam which is not as effective and is time consuming (Kelsall, et.al., 2006).

![Figure 2.6 - Typical Batch Alcohol Fermentor](image)

The lag phase or growth stage for yeast can be as long as 12 hours as shown in Figure 2.7. During the lag phase the yeast adapts to its new environment and activates its ability to synthesize enzymes. It is a period of intense biochemical activity as the yeast cells adjust from previous culture conditions to the conditions of the fresh media. There is zero cell growth during the lag phase. In addition to loss of productive time for batch fermentation units, this is an opportunity for bacteria to grow in the fermentor. Bacteria can reproduce in as little time as 20 minutes.
Continuous fermentation has an advantage over batch fermentation in that it does not have the lag phase of yeast growth. Figure 2.8 is a typical continuous multistage fermentation system.

![Figure 2.8 - Typical Batch Growth Curve for Yeast](image)

The "down time" for growing yeast in each batch vessel is eliminated. The yeast is constantly in the maximum ethanol formation phase. Cell concentration in staged continuous fermentors has been measured to be in the 150 to 300 million cells per milliliter with ethanol concentration in the 8.5 % v/v range (Chen, et al, 1990). Other advantages include long term continuous ethanol productivity, higher volumetric throughput, reduced labor cost due to steady state conditions, easier process control, savings in construction of smaller fermentors with higher output, and reduced downtime for cleaning and filling. The greatest disadvantage of the continuous fermentors is contamination with bacteria and wild yeast that upset the balance nature of the fermentors. Loss of yield of ethanol and outage of the plant results from the contamination and leads to lower than expected yields of product and less efficient conversion of sugars to ethanol (Ingledew, 2003).
Figure 2.8 - Typical Continuous Multistage Fermentation Unit
3. MATERIALS AND METHODS

3.1 Objectives

The primary objective of the study was to determine the feasibility and advantages of using an Immobilized Microbe Bioreactor for ethanol fermentation. Detail objectives included:

1. Design and install a laboratory unit with a continuous fermentor and distillation unit to recover high purity ethanol.
2. Evaluate use of Microbubble Generator to create aerobic environment for yeast growth.
3. Determine ethanol production and yield using the Immobilized Microbe Bioreactor with raw sugar or molasses as feed stock.
4. Develop distillation method and mechanism for fixing ethanol production.

3.2 Research Unit Design

The research unit consisted of three Immobilized Yeast Reactors in series to produce a low purity ethanol product. A distillation system consisting of a stripper and fractionating section recovered the ethanol/water azeotropic solution to determine overall yield of ethanol as shown in Figure 3.1.

The unit was installed in a walk-in Type 1 hood. This allows the use of flammable and toxic gases as feedstock for future research. The Type 1 hood is designed to provide at least 100 feet per minute of air face velocity. The face velocity is constantly measured and alarms will activate if the face velocity falls below 100 feet per minute.

3.2.1 Reactor Feed System

The liquid was fed to the reactors and to the distillation by a Pulsatron Series A Plus positive displacement metering pump. The stroke rate and the stroke length were adjusted to
Control the feed rate. The pump capacity is adjustable up to 0.9 liters per hour and has a maximum discharge pressure of 250 psig. The optimum feed rate to the reactors and to the distillation system was about 200 ml/hr.

3.2.2 Microbubble Generator

The reactors must be operated aerobically during the initial charging in order for the yeast to grow to an acceptable level. This was accomplished by saturating the liquid with air as it was fed through the Microbubble Generator (Figures 3.2 and 3.3). The Microbubble Generator includes a microbubble chamber packed with small inert particles through which the liquid feed
and air are admitted under pressure and followed by a venturi chamber to further reduce the size of the bubbles (Portier, et al, 1995).

Figure 3.2 - Microbubble Generator
3.2.3 Immobilized Microbe Bioreactors

Three 8 liter packed bed reactors were installed in series. The reactors are 6 inches in diameter and 18 inches long (TT) as indicated in Figures 10 and 11. The liquid capacity of each reactor is about 5 liters due to a 14 inch overflow lance installed in each reactor. A mantel heater is installed on the bottom of each reactor with a Digitrol II microprocessor-based, digital indicating, automatic temperature control based on a set temperature at the top of each reactor. The temperature at the top of the reactor was set at 30 degrees C.

The biocarrier used in the packed bed reactors consists of beads of diatomaceous material
previously inoculated with the standard yeast, *Saccharomyces cerevisiae*. This biocarrier is in the solid phase, porous and inorganic in nature. The porous nature of the biocarrier provides a large surface area to the yeast for attachment. The yeast clings to the external surfaces and colonizes the internal surfaces as well. This leads to attainment of higher culture densities within the porous matrix. The internal or protected areas provide biomass reserves for recolonization of the reactor in the event of a system upset.

Figure 3.4 - Immobilized Microbe Bioreactor for Direct Fermentation
Figure 3.6 is a photograph of the Immobilized Microbe Bioreactor with the biocarrier installed. The biocarrier occupies about 3.5 liters of the 6.5 liters or about 54% of the volume of the reactors.
3.2.4 Distillation Section

A customized distillation section as shown in Figure 3.7 was used to recover an azeotropic ethanol/water mixture. Ethanol forms an azeotrope with water. Azeotrope is a term used to describe a constant boiling mixture. This means that an azeotrope is a mixture of two or more pure compounds in such a ratio that its composition cannot be changed by simple distillation. This is because when an azeotrope is boiled, the resulting vapor has the same ratio of constituents as the mixture of liquids. The composition of the ethanol-water azeotrope is 95.6% ethanol and 4.4% water (by weight). Ethanol boils at 78.4 degrees C, water at 100 degrees C and the azeotrope at 78.1 degrees C. Figure 3.7 is a liquid/vapor equilibrium curve for the ethanol/water mixture at atmospheric pressure which shows the azeotrope. It shows mole percent ethanol in the liquid (x-axis) versus mole percent of ethanol in the vapor (y-axis).
Data from the liquid/vapor equilibrium curve as indicated in Figure 3.7 is the basis for design of a distillation system such as is shown in Figure 3.8. This system is typical for separating two component feed into relatively pure overhead product containing the lower boiling component and bottoms product containing primarily the higher boiling component of the feed. The lower boiling component is the ethanol/water azeotrope and the higher boiling component is water in the ethanol/water system. The part of the packed tower below the feed point is the stripper. The stripper is the hottest section of the tower and the ethanol rich stream is vaporized and goes up the column. The ethanol rich vapor continues to rise up the rectifying section of the column where it contacts and increases the purity of the richer ethanol stream as it comes down the column. The overhead is the lowest boiling stream, which is the azeotrope in this case. This results in a constant temperature of about 78 degrees C in the overhead. Figure 3.9 is a vapor/liquid equilibrium curve which depicts the structure of the distillation process by dividing the vapor/liquid equilibrium information into distinct zones of process and equipment.
requirements. These zones are stripping, rectifying and dehydration. This division is the basis for the design of equipment and systems to perform the recovery of ethanol. The dehydration step was not part of this study but will be discussed later.

Figure 3.8 - Typical Distillation System

Figure 3.9 - Equilibrium Curve indicating Distillation Components
Separation of the components in a distillation column requires a certain amount of vapor and liquid contact. This contact is created by stages or theoretically ideal trays. These stages are determined by a graphical solution utilizing the vapor/liquid equilibrium curve. This curve is commonly called a McCabe-Thiele diagram, named after the developers. Figure 3.10 is a vapor/liquid equilibrium stage analysis for an ethanol/water distillation.

![McCabe-Thiele Diagram for Ethanol/Water Separation](image)

The system that was chosen for our study uses a packed tower to provide the stages required for separation. The packing for the column was 0.16 inch Protruded packing further described in Table 3.1.

The primary components of the laboratory distillation section are a Thermal Siphon Reboiler with Liquid Level Control; Stripper Column; Feed Section; Rectification Column; Reflux/Product Separator; Distillate Condenser and a Distillate Collection Vessel with Cold Trap.
The Thermal Siphon Reboiler (Figure 3.12) is electrically heated with control by a Glas-Col Minitrol and Minitwin power controls (manually adjusted power controls of percentage-timer design). They are designed to provide full-line voltage to load from 5 1/2% to 100% of time up to a maximum load of 1800 Watts (15 amps). The controls are set manually to maintain 100 degree C vapor leaving the reboiler and 79 degrees C on the column overhead. The reboiler must be initially heated very slowly to prevent excess "bumping". The level in the reboiler is set manually.
The feed to the Distillation Section enters through a Preheater (Figure 3.14) above the Stripper Column (Figure 3.15) and below the Rectification Column (Figure 3.13). This prevents
"shocking" the Stripper with cold feed and improves the thermal efficiency of the distillation. The Stripper is a two inch (ID) glass column packed with 8 inches of 0.16 inch Protruded Packing.

Figure 3.13 - Rectification Column for Distillation Section
Figure 3.14 - Feed Preheater for Distillation Section
Figure 3.15 - Stripper Column for Distillation Section
Table 3.1 - Column Packing Specifications Provided by Supplier

<table>
<thead>
<tr>
<th>Material</th>
<th>Fabricated from 316SS metal ribbon ¼ inch wide and 0.003 inch thick</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shape</td>
<td>Half-cylinder with two corners bent slightly upward</td>
</tr>
<tr>
<td>Size</td>
<td>0.16 inch in diameter and 0.016 inch in length</td>
</tr>
<tr>
<td>Number of holes</td>
<td>1024 per square inch</td>
</tr>
<tr>
<td>Size of holes</td>
<td>Approximately 0.40 by 0.37 millimeters</td>
</tr>
<tr>
<td>Pieces per cubic foot</td>
<td>800,000</td>
</tr>
<tr>
<td>Packing density</td>
<td>27.6 pounds per cubic foot</td>
</tr>
<tr>
<td>Surface area</td>
<td>576 square feet per cubic foot</td>
</tr>
<tr>
<td>Per cent free space</td>
<td>94</td>
</tr>
<tr>
<td>Packing factor</td>
<td>693</td>
</tr>
</tbody>
</table>

The supplier also states that test data obtained with n-heptane and methycyclohexane test mixture indicates a Height of Theoretical Plate of about 1.10 inch at atmospheric pressure and total reflux. A McCabe Thiele diagram indicates that approximately 8 stages are required for stripping the ethanol from the feed.

The Rectification Column is a one inch (ID) column packed with 12 inches of 0.16 Protruded Packing with specifications as shown in Table 3.1. The supplier also states that test data obtained with n-heptane and methycyclohexane test mixture indicates a Height of Theoretical Plate of about 1.03 inch at atmospheric pressure and total reflux. A typical McCabe Thiele diagram indicates that approximately 12 stages are required in the rectifying section. The small size of the Stripper and Rectification Column resulted in excessive cooling of the column and preventing the top of the column from reaching the required temperature (79 degrees) which would allow the ethanol to leave the column as a vapor. Insulating columns were installed around the stripper and rectifying columns.

A Distillation Splitter or Reflux/Product control (Figure 3.16) was installed on the make from the Rectification Column. An electro-magnetic coil moves the discharge nozzle to the "product" position if activated. The nozzle returns to the "reflux" position when the coil is...
deactivated. The time of each sequence can be manually adjusted from total reflux to total product make.

![Figure 3.16 - Splitter or Reflux/Product Controls for Distillation Section](image)

Carbon dioxide is generated during fermentation and about one percent of ethanol can leave the system through the gases vented from the system. This could significantly impact the
yield of ethanol. The loss of ethanol in the vent was reduced by installing a cold trap packed with dry ice on the vent from the Distillate Receiver as indicated in Figure 3.17.

Figure 3.17 - Distillate Receiver for Distillation Section

The full impact of this recovery was not achieved on the laboratory unit in that the reactors were not "tied in" with the distillation section but were operated separately. The feed rate, reflux rate and heat input required balancing in order to maintain the proper temperature of the overhead and bottoms. Removing more ethanol from the overhead than was injected into the feed would cause water to move up the column resulting in high overhead temperature and low purity product. "Bumping" was experienced in the thermal siphon reboiler if heat up was too fast. It was necessary to hold the setting on the heating controls on three for about 30 minutes and then increase slowly to about the normal operating setting of seven.
3.3 Reactor Feed Preparation

Approximately 500 grams of sugar were dissolved in one liter of distilled water resulting in a solution with Brix Number of 22 to 28 degrees. One gram of ammonium nitrate was added for each 10 grams of sugar. Yeast growth is enhanced by an acid pH and its optimum pH is 5.0 to 5.2. The optimum pH for ethanol formation is about 4.0. The pH of the feed was adjusted using 1.0 N HCL (Russell, 2003).

3.4 Sampling and Analytical Methods

The sugar content and pH of the feed was measured and recorded after thoroughly mixing. A Brix Meter (Westover Model RHB-32 ATC) was used to measure the sugar content. The Brix Meter was also used to measure the sugar content of the final product and the contents of each reactor at various stages of the fermentation. The pH of the sugar solution was measured with pH paper and adjusted to the proper pH.

The Distillation Section was used as the primary tool for measuring the ethanol concentration in the product from the fermentors. Approximate two liter samples were taken for feed to the distillation system. The feed rate, as well as the heat input and reflux rate, was adjusted until the column stabilized. Both the overhead collection vessel and the bottoms collection vessel were emptied to begin recording data. The amount of ethanol solution in the overhead collection vessel and amount of bottoms were recorded after a given time. The percent of ethanol in the product from the fermentors was calculated by dividing the amount of solution in the overhead collection vessel by the total of the solutions in the overhead collection vessel and the bottoms collection vessel and adjusting for the azeotrope composition and multiplying by 100.

Samples of the biocarrier from each reactor and the product were taken for determination of yeast cell count. Approximately ten pellets were taken from each reactor, crushed and
injected into a bottle of saline solution. A sample of liquid from each reactor was also injected into a bottle of saline solution. Liquid sample from each bottle was put into a Yeast Agar and allowed to incubate resulting in the cell count reported in Table 3.2.

Table 3.2 - Yeast Cell Count from Reactor Packing and Product

<table>
<thead>
<tr>
<th>Source</th>
<th>Reactor Feed</th>
<th>Cell Count, Millions/ml</th>
<th>Cell Count, Millions/ml</th>
<th>Cell Count, Millions/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactor #1 - Pellets</td>
<td>Sugar Solution</td>
<td>130</td>
<td>330</td>
<td>450</td>
</tr>
<tr>
<td>Reactor #1 - Pellets</td>
<td>Molasses Solution</td>
<td>890</td>
<td>220</td>
<td>100</td>
</tr>
<tr>
<td>Reactor #1 - Product</td>
<td>Molasses Solution</td>
<td>120</td>
<td>420</td>
<td>690</td>
</tr>
<tr>
<td>Reactor #2 - Pellets</td>
<td>Molasses Solution</td>
<td>980</td>
<td>1320</td>
<td>887</td>
</tr>
<tr>
<td>Reactor #2 - Product</td>
<td>Molasses Solution</td>
<td>920</td>
<td>670</td>
<td>428</td>
</tr>
<tr>
<td>Reactor #3 - Pellets</td>
<td>Molasses Solution</td>
<td>760</td>
<td>820</td>
<td>112</td>
</tr>
<tr>
<td>Reactor #3 - Product</td>
<td>Molasses Solution</td>
<td>580</td>
<td>840</td>
<td>976</td>
</tr>
</tbody>
</table>
4. RESULTS AND DISCUSSION

4.1 Microbubble Generator Performance

The Microbubble Generator seemed to perform well in spite of having several operational problems such as plugging, leaking and breaking. The generator was initially packed with 1.0 mm glass beads. The packing plugged with residue and growth from the feed within an hour or two when feeding molasses or dirty raw sugar which was near the bottom of the container. This often prevented use of the Microbubble Generator during the entire run. The plugging did not occur when clean sugar was used as feed. The cell growth in each reactor was measured during the run with molasses and indicated a good growth of cells in the reactors. After several trials the packing was changed from 1.0 mm glass beads to 1.5 mm glass shapes in an effort to reduce plugging.

The generator leaked and internal parts broke when an attempt was made to tighten fittings to stop the leakage. The small packing appeared to get into the threads of the fitting resulting in the leaking and breakage. This problem disappeared when the larger packing was used. It is recommended that the size of the generator components be reviewed and revised prior to construction of additional units.

The air was injected through the generator to the bottom of the first reactor in a series of three. The results of cell count from material from each of the reactors conclude that all three had good cell growth which indicates that air was "carrying through" the reactors. Future designs should incorporate a method of injecting air to each reactor when needed to maintain maximum yeast growth.

The results of using the Microbubble Generator to saturate the feed with oxygen and experience in operating the Microbubble Generator will be useful in future work with other systems. This will be important during research concerning fermentation of synthesis gas. The
first required achievement in this research will be getting the gases into the liquid containing the enzymes necessary for fermentation of the gas.

4.2 Feed System Operation

The same positive displacement pump was used to feed both the reactors and the distillation unit. This necessitated completing the fermentation run before beginning distillation. It is recommended that future installations have two pumps so that distillation can be conducted at the same time that the reactors are operating. This would result in the system operating more like a commercial system.

The minimum consistent operating rate for the pump was about 32.3 milliliters per hour. The pump would "vapor lock" with air below this rate and stop pumping. However, this seemed to be a good feed rate for both the reactors and the distillation unit. A pump with a different operating mode should be considered for future work.

4.3 Immobilized Microbe Bioreactors

A bed of inoculated diatomatious pellets about 14 inches deep was installed in each Immobilized Microbe Bioreactor. The total void space in each reactor was about 2.8 liters or about 24% of the total space. This means that each reactor was 76% full of pellets.

Each reactor was filled in series with the first reactor overflowing to the bottom of the second and the second overflowing to the bottom of the third. The third reactor overflowed to a holding vessel.

The study was conducted in three runs - first, with raw sugar from a sugar mill; second, with molasses from a sugar mill and lastly, with purchased food grade raw sugar. The first part of run one was quite successful with minimum problems. The sugar was taken from a "clean" section of the storage drum and was mixed and filtered to remove contaminants. Difficulty was encountered with reading the Brix number of the sugar mixture due to solids contained in the
mixture. The percent ethanol in the fermented product indicated by distillation varied between 8% and 10% v/v. The second part of the run was a failure resulting from excessive solids in the feed mixture because the sugar was taken from the bottom of the storage drum. In addition to the problems reading the Brix number, the indicated percent ethanol in the product was about 5%. Difficulty was experienced in distilling the small amount of product with such low ethanol content. This problem will be addressed in the distillation discussion.

Results were not obtained for run two using molasses feed. The Brix number for the feed and product was almost impossible to read due to solids in the solution. The molasses solution almost immediately plugged the Microbubble Generator requiring direct injection of air into the reactor for growth of yeast. However, measured cell count for crushed pellets and product from the reactors was as high as 1.3 billion/ml. Consistent and more thorough air injection would probably improve the count.

The most pronounced problem for the run was buildup of material on the pellets and indication of almost no conversion of sugar to ethanol. After aborting the run, the pellets and reactors were washed in-situ with a 10% ethanol mixture to remove any bacteria that may have been present. This was done to protect future runs. The cleaning of the pellets and reactors was quite successful and should be a guideline for future and commercial operations. In addition to not having enough ethanol in the product to adequately reflux the distillation column, the bottom material in the reboiler became very thick and viscous. The boiling actually began "rolling" to the extent that it seemed unsafe to operate the system, therefore, no yield data was taken for the run using molasses as feedstock.

The feedstock for run three was food grade raw sugar. This feed did not have the impurities that caused problems for the previous runs. The Brix Numbers were measured carefully and the pH controlled at an average level of 4.5. As indicated in Table 4.1, the once-
through run with a hydraulic retention time of 26 hours resulted in a percent ethanol as high as 16.5 with an average about 15 v/v or 12.0 g/l. This means that the overall volumetric productivity was only about 0.6 g/l-hr. Reported batch volumetric productivity is about 1.0 g/l-hr and staged continuous systems have reached as high as 10.0 g/l-hr (Chen, *et al*, 1990). It is highly probable that a larger and fine-tuned Immobilized Micro Bioreactor can reach this performance. The existing multi-stage continuous industrial processes require equipment pasteurization/sterilization, addition of antiseptics, and recycle or seeding with new yeast. The Immobilized Microbe Bioreactor will eliminate or simplify these operations.

<table>
<thead>
<tr>
<th>Feed</th>
<th>Feed Brix No.</th>
<th>Rx 1 Brix No.</th>
<th>Rx 2 Brix No.</th>
<th>Rx 3 Brix No.</th>
<th>Product Brix No.</th>
<th>Initial Run, Hrs.</th>
<th>Recycle, Hrs.</th>
<th>Incub, Hrs.</th>
<th>Hold, Hrs.</th>
<th>Total Time, Hrs.</th>
<th>Distl Feed</th>
<th>Percent Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw Sugar</td>
<td>Prod</td>
<td>8.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw Sugar</td>
<td>24.0</td>
<td>23.0</td>
<td>16.0</td>
<td>18.8</td>
<td></td>
<td>Prod</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10.8</td>
</tr>
<tr>
<td>Raw Sugar</td>
<td>24.0</td>
<td>24.0</td>
<td>18.8</td>
<td></td>
<td></td>
<td>Prod</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.6</td>
</tr>
<tr>
<td>Raw Sugar</td>
<td>24.0</td>
<td>13.0</td>
<td>13.0</td>
<td></td>
<td></td>
<td>Rx 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10.6</td>
</tr>
<tr>
<td>Raw Sugar</td>
<td>24.0</td>
<td>24.0</td>
<td></td>
<td></td>
<td></td>
<td>Rx 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12.1</td>
</tr>
<tr>
<td>Raw Sugar</td>
<td>22.8</td>
<td>21.0</td>
<td></td>
<td></td>
<td></td>
<td>Prod</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.2</td>
</tr>
<tr>
<td>Raw Sugar</td>
<td>22.8</td>
<td>21.0</td>
<td>21.0</td>
<td>69.0</td>
<td>90.0</td>
<td>Prod</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.8</td>
</tr>
<tr>
<td>Raw Sugar</td>
<td>16.2</td>
<td>16.2</td>
<td>16.8</td>
<td>15.4</td>
<td>21.0</td>
<td>148.0</td>
<td>169.0</td>
<td>Rx 1</td>
<td>5.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sugar</td>
<td>25.0</td>
<td>24.0</td>
<td>22.0</td>
<td>21.0</td>
<td>18.0</td>
<td>26.0</td>
<td></td>
<td></td>
<td></td>
<td>26.0</td>
<td>Prod</td>
<td>14.3</td>
</tr>
<tr>
<td>Sugar</td>
<td>25.0</td>
<td>22.0</td>
<td>20.0</td>
<td>16.0</td>
<td>16.0</td>
<td>26.0</td>
<td>20.0</td>
<td></td>
<td></td>
<td>46.0</td>
<td>Prod</td>
<td>15.3</td>
</tr>
<tr>
<td>Sugar</td>
<td>25.0</td>
<td>18.0</td>
<td>17.0</td>
<td>14.0</td>
<td>18.0</td>
<td>26.0</td>
<td>20.0</td>
<td>78.0</td>
<td>124.0</td>
<td>Rx 1</td>
<td>16.0</td>
<td></td>
</tr>
<tr>
<td>Sugar</td>
<td>25.0</td>
<td>18.0</td>
<td>17.0</td>
<td>14.0</td>
<td>14.0</td>
<td>26.0</td>
<td>20.0</td>
<td>82.0</td>
<td>128.0</td>
<td>Rx 3</td>
<td>16.5</td>
<td></td>
</tr>
</tbody>
</table>
The data in Table 4.1 and Figure 4.1 do not indicate significant additional ethanol yield by increasing fermentation time during recycle, holding or incubation. In fact, the yield after incubation actually decreased during second run with raw sugar but this can be attributed to measurement variance. Run three, with purchased sugar, showed a slight, consistent, increase after recycle and holding but it was too small to be significant. Activity in the reactors during holding was evident by "bubbling" caused by carbon dioxide evolution.

4.4 Ethanol Distillation Section

A distillation section was designed to recover a solution containing 90 to 97% ethanol. The amount of ethanol recovered was used to determine the yield of ethanol from the reactors. Operations data from the evaluation can also be used as design data for future lab distillation units, distillation pilot plant units and commercial units.

The columns seemed to provide the separation desired and showed no evidence of improper operation. However, control of the distillation section was difficult at times. It was necessary to balance the feed and heat input and the reflux to maintain the desired separation. The bottoms temperature, measured at the outlet of the reboiler, was maintained at the boiling
point of water, 100 degrees C. The overhead temperature was held at the boiling point of the ethanol/water azeotrope, about 78 degrees. Sufficient reflux was necessary to maintain the overhead temperature at 78 degrees C. Feed input was adjusted to prevent too much cooling of the bottom temperature and give enough ethanol input to provide sufficient reflux to the column. The rate of heat input was restricted during the startup due to "bumping" in the reboiler. An hour or more was often required to start the unit. Uncontrolled bumping could damage the system by dislodging the fittings.

Accurate timing of taking data was somewhat questionable. It was necessary to have ethanol in the system to begin operation and to leave some ethanol after taking data. Experience showed that no data should be taken before the distillation column operated stable for at least 20 minutes and that feed should be stopped at least 20 minutes before finishing taking data.

As indicated earlier, this study only addressed recovery of the ethanol/water azeotrope, not anhydrous ethanol as is required for fuel. Recovery of anhydrous ethanol requires a dehydration system as is indicated on Figure 3.9. Further purification of the ethanol would require additional distillation or mechanical recovery. Additional distillation can be extractive distillation in which a third component is added to "break the azeotrope" and allow distillation and separation of a dryer product. Use of molecular sieve drying is the most common mechanical recovery system. Molecular sieves usually employ the pressure-swing concept in which the water is adsorbed at a high pressure and the sieve is regenerated by rapidly lowering the pressure.
5. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary

Ethanol has emerged as the strongest candidate for replacing fossil fuels and is now the only renewable fuel made in commercial quantity. The production of ethanol in the United States has more than doubled during the past four years with a projected doubling again in the next six years. Improvements in technology for ethanol production are necessary for this increase.

Ethanol production in the United States is primarily the direct fermentation of corn. The entire corn kernel is ground into meal in dry milling. The meal is then separated into components and the starch is converted into sugar for fermentation. Approximately 82% of the ethanol production is by dry milling. The corn is soaked in water and dilute acid during the wet milling process. The corn slurry is then ground to separate the corn components and fermentation proceeds similar to the dry process.

A significant amount of research is now being conducted to determine and refine processes to convert cellulosic biomas into ethanol and other alcohols. Conversion of the biomas into synthesis gas is being considered as well as direct fermentation. The synthesis will be a feedstock for a special fermentation system.

The primary feedstock for ethanol production in other countries such as Brazil is sugarcane. Brazil expects to achieve energy independence in the near future with its 4.0 billion gallons per year ethanol production capacity.

The fermentation units are either operated in the batch or continuous mode. About 60 per cent of the units in North America are batch. These units have inherent operating problems due to long lag time in developing the yeast growth and potential for bacteria contamination. The continuous units do not have the lag problem but are plagued with contamination problems.
A research laboratory unit was designed and installed to evaluate the use of the Immobilized Microbe Bioreactor as continuous fermenters. The unit was used to gather data on the fermentation of raw sugar and molasses. A distillation system was installed to recover high purity ethanol and determine yield of ethanol from the sugar solutions.

5.2 Conclusions

5.2.1 Microbubble Generator

The Microbubble Generator appeared to sufficiently dissolve air into the liquid to create yeast growth in the reactors. This was verified by the rather large and consistent yeast cell count in the three reactors in series. The air was only injected in the liquid feed to the first reactor but carried through to the other two reactors.

5.2.2 Immobilized Microbe Bioreactors

The biocarrier in the Immobilized Microbe Bioreactor was instrumental in maintaining a consistent, active source of enzymes for the fermentation process. The overall fermentation continuous process took less time than a standard batch fermentor and did not require inoculation resulting in a lag phase.

No indication of bacteria contamination was seen during the fermentation process. This eliminated shutdown time for cleaning and decontamination. However, it was demonstrated that, if needed, the reactors were very easy to clean and decontaminate in place. This was done using a weak solution of ethanol in water.

5.2.3 Distillation Section

High purity ethanol was adequately recovered by the distillation section. The measurement of yield was based on volume of overhead product versus amount of bottoms from the column. The start and stop times for the measurements were decisions by the operator. This
could have compromised the accuracy of the measurements. However, the overall basic operation and yield was verified.

5.3 Recommendations

1. Research and evaluation of the Immobilized Microbe Bioreactor for production of ethanol be continued in the laboratory.

2. The laboratory unit should be modified to include larger thermal siphon reboiler and holdup capacity above the reboiler. Additional pumps should be installed to allow simultaneously operation of reactors and distillation unit.

2. The research and evaluation of the Immobilized Microbe Bioreactor for production of ethanol be expanded to include the use of gases for the feedstock. The Microbubble Generator would be an integral part of this evaluation.

3. The research using the Immobilized Microbe Bioreactor for production of ethanol be extended to a pilot plant study. This is a necessary step for commercialization.

4. The use of distillation for determining the yield of ethanol should be refined. Other more accurate methods of determining ethanol concentration reactor product and distillation products should be employed.
REFERENCES


Energy Information Administration, Biofuels in the U.S. Transportation Sector, February 2007.


Louisiana Energy Topic. Louisiana Department of Natural Resources/Technology Assessment Division. April 2003.


APPENDIX: TERMS AND ABBREVIATIONS COMMONLY ASSOCIATED WITH THE PRODUCTION OF ETHANOL

**Acetic acid**  A colorless liquid with a pungent odor which is flammable at high concentrations. Chemical formula is CH$_3$COOH. Acetic acid may be produced from ethanol by acetobacter bacteria under aerobic conditions such as when a completed fermentation is agitated or aerated excessively.

**Acetobacter**  A genus of gram-negative aerobic bacterial comprising ellipsoidal to rod-shaped cells as singles, pairs or chains. They are able to oxidize ethanol to acetic acid and may be responsible for loss of yield in ethanol production if a fermented mash is agitated or aerated excessively.

**Acetogenesis**  A process through which acetate is produced by anaerobic bacteria from a variety of energy and carbon sources.

**Acidity**  A quantitative measure of the amount of acid present.

**Acid-acid Process**  Term used in starch processing when acid hydrolysis is used to accomplish both the initial liquefaction and the final saccharification to simple sugars.

**Acid-enzyme Process**  Term used in starch processing when acid hydrolysis is used to accomplish the initial liquefaction and an enzyme is used for the saccharification to simple sugars.

**Acid Hydrolysis**  The hydrolysis of a polymer using acid. For starch hydrolysis, acids may be used as an alternate to enzymes in either or both the liquefaction or saccharification processes.

**Acid Washing**  A process in which yeast recovered from a finished fermentation is acidified to pH 2.2 for about 20 minutes using an acid to reduce the level of bacterial contamination prior to recycling into a new fermentation.

**Active Dry Yeast**  A yeast preparation made from compressed yeast by careful and controlled removal of moisture to about 5% w/v such that 10 to 50 billion yeast/gram remain viable.

**Alcohol**  A member of a class of organic compounds containing carbon, hydrogen and oxygen. The principal alcohol in fuel and beverage use is ethanol or ethyl alcohol. Methanol is a lower molecular weight alcohol and is more toxic than ethanol.

**Alcohol Fuel Permit (AFP)**  A permit issued by the Bureau of Alcohol, Tobacco and Firearms allowing the holder to engage in the production of ethanol solely for fuel use.

**Aldehyde**  Class of organic compounds considered to be derived by the removal of hydrogen atoms from an alcohol. Aldehydes tend to be produced as congeners or by-products of fermentation.
Alkali  A compound capable of neutralizing hydrogen ions, usually by generating hydroxyl ions which combine with hydrogen ions to form water.

Alkalinity  A measure of the total bicarbonate and carbonate content in water determined by titration to an endpoint using various indicators such as phenylphthalein, methyl orange, etc.

Alpha-amylase  An enzyme used in the liquefaction of starch in the grain mashing process prior to saccharification and fermentation. Alpha-amylase hydrolyses the long-chain starch molecules into short-chain dextrin which are more suitable for subsequent saccharification by other enzymes to fermentable glucose. In fuel ethanol production the enzyme is obtained solely as a bacterial product. The enzyme molecule contains a calcium atom which is essential for its activity.

Amylase  The name given to any enzyme that hydrolyzes amyllose, which is the major component of starch.

Amyloglucosidase  An enzyme, also known as glucoamylase, which hydrolyzes amyllose into its constituent glucose units.

Amylopectin  A major component of starch. The molecule is composed of large, branched chains of thousands of glucose units.

Amylose  A major component of starch. The amylose molecule is composed of straight chains of hundreds of glucose units.

Anaerobic  Literally means "without air", the opposite of aerobic


Anhydrous  The term for a substance that does not contain water.

Azeotrope  The term to describe a constant boiling mixture of two or more components with a lower boiling point than either component alone.

Azeotropic distillation  A distillation process in which a liquid compound (entrainer) is added to the mixture to be separated to form an azeotrope with one or more of the components.

B

Bacteria  Microscopic organisms of the kingdom Monera usually characterized by small size, vigorous biochemical activity and the lack of a true nucleus.

Bacterial contamination  The condition occurring when undesirable bacteria become established in a fermenting mash and reduce the ethanol yield.

Barrel  A liquid measure equal to 42 US gallons or 5.6 cubic feet.
**Batch fermentation**  
The fermentation of a set amount of mash in a single vessel in a discontinuous operation.

**Beer**  
The product of alcohol fermentation.

**Benzene**  
A colorless, flammable, aromatic hydrocarbon liquid used as an entrainer for the dehydration of ethanol by azeotropic distillation.

**Biomass**  
Any renewable organic matter such as agricultural crops, crop waste residue, wood, animal and municipal wastes, aquatic plants, fungal growth, etc.

**Boiling Point**  
The temperature at which the vapor pressure of a liquid equals the total pressure of the atmosphere above it.

**Brix**  
A scale used to measure the specific gravity of a liquid in relation to that of a solution of sugar in water. Each unit is equivalent to 1% by weight of sugar. A hydrometer, refractometer, or density meter is used to take the measurement.

**By-products**  
Products that are secondary to the principal product of a process. In ethanol production, carbon dioxide and distillers dried grains are normally considered by-products but in certain circumstances they may be viewed as co-products, in that they may contribute significantly to the overall process economics.

C

**Carbon dioxide**  
A colorless non-flammable gas. It is produced by various means, notably the combustion of fuels in an excess of air and is a by-product of yeast fermentation. It may be recovered from fermentations and compressed to a liquid or sold (dry ice).

**Cell recycle**  
The process of recovering yeast from fermented beer to return it to the starting vessel in a continuous fermentation or to a new vessel in a batch fermentation system.

**Cellulase**  
An enzyme capable of hydrolyzing long-chain cellulose molecules into simple sugars or short-chain polymers.

**Cellulose**  
The principal polysaccharide in living plants. It forms the skeletal structure of the cell wall, hence the name.

**Centrifuge**  
A machine for separating insoluble liquids or solids from liquids by the application of centrifugal force.

**CIP**  
Abbreviation for cleaning-in-place system.

**Cleaning-in-place system**  
A system designed to permit process equipment to be cleaned without disconnecting or dismantling.
Cleaning-out-of-place  The manual disassembly, inspection and cleaning of processing equipment and systems.

Column  A vertical cylindrical vessel containing a series of perforated plates, packing, or other contact devices through which vapors may pass to effect a separation of liquid mixtures by distillation.

Condensate  Liquid condensed from vapor in a condenser.

Condenser  A heat exchange device connected to the vapor discharge pipe of a column to permit the vapor to be cooled and condensed to a liquid.

Conservation Reserve Program (CRP) Land  Highly erodible land which the government encourages (with payments) landowners to maintain a vegetative cover to prevent erosion. Much of this land can support high energy crops such as switch grass and energy cane.

Continuous fermentation  A system into which mash, sugar or molasses solution, or gases may be fed continuously to be fermented and then discharged to be fed to a distillation system.

Continuous distillation  A process using specially-designed equipment to permit a volatile component to be separated by distillation from a continuous flow of a solution.

COP  The abbreviation for cleaning-out-of-place.

Dehydration  The process of removing water from a substance, particularly the removal of most of the remaining 5% of water from 190 proof ethanol in the production of absolute or anhydrous ethanol.

Denaturant  A substance added to ethanol to make it unfit for human consumption so that it is not subject to taxation as beverage alcohol.

Distillate  That portion of a liquid removed as a vapor and condensed during a distillation process.

Distillation  The process by which the components of a liquid mixture are separated by differences in boiling point by boiling and recondensing the resultant vapors.

Dry milling  In the ethanol production industry, dry milling refers to the milling of whole dry grain, where, in contrast to wet milling, no attempt is made to remove fractions such as germ and bran.

Enzymatic hydrolysis  The hydrolysis of a polymer by the use of enzymes.
**Enzyme**  Any of a class of complex proteinaceous substances produced by living organisms that catalyze chemical reactions without being destroyed.

**Ethanol**  A clear, colorless, flammable oxygenated hydrocarbon. Chemical formula: $\text{C}_2\text{H}_5\text{OH}$. Has a boiling point of 78.5°C in the anhydrous state. Forms a binary azeotrope with water with a boiling point of 78.15°C at a composition of 95.57% by weight ethanol.

**Extractant**  A substance such as ethylene glycol or glycerol used in extractive distillation processes for dehydration of ethanol.

**Extractive distillation**  A process when an extractant is added to a mixture being distilled to change the volatility of one or more components.

**Exoenzyme**  An enzyme restricted to acting on the outer end of large polymeric molecules and cleaves molecules one by one.

**F**

**Facultative anaerobe**  Term used to describe a microorganism (such as a yeast) that is essentially aerobic (or oxygen requiring) but can also thrive under anaerobic (or oxygen free) conditions.

**Feed plate**  The plate or tray onto which the distilland (liquid to be distilled) is introduced into a distillation tower. A plate or tray can be a certain height of packing in a packed tower. It is the point in a tower above which enrichment or concentration occurs and below which stripping occurs.

**Feedstock**  The raw material used in a process. Corn, molasses, sugar, etc., can be used as feedstocks for ethanol production.

**Fermentable sugars**  Simple sugars such as glucose and fructose that can be converted into ethanol by fermentation with yeast.

**Fermentation**  The enzymatic transformation by microorganisms of organic compounds such as sugars.

**Fermentation efficiency**  The measure of the actual output of a fermentation product such as ethanol in relation to the theoretical yield.

**Fermentation ethanol**  The term used to distinguish ethanol produced by fermentation from synthetic ethanol produced from ethylene.

**Fossil fuel**  Any naturally-occurring fuel of an organic nature that originated in a past geologic age such as coal, crude oil or natural gas.

**Fractional distillation**  A process of separating mixtures such as ethanol and water by boiling and drawing off the condensed vapors from different levels of the distillation tower.
**Fructose**  
A fermentable monosaccharide (simple sugar) of the chemical formula $C_6H_{12}O_6$.

**Fuel ethanol**  
Anhydrous ethanol that has been denatured by addition of 2-5% unleaded gasoline and is intended for use as an automotive fuel in blends with gasoline.

**G**

**Gasoline**  
A volatile, flammable liquid hydrocarbon mixture suitable for fuel in internal combustion engines.

**Gasoline extender**  
The term used to describe ethanol when it is simply used as a partial replacement for gasoline without any consideration for its value as an octane enhancer or oxygenate.

**Gay Lussac**  
The name given to a scale of the concentration of ethanol in mixtures with water where each degree is equal to 1% by volume.

**Gay Lussac equation**  
The equation for the fermentation of sugar by yeast to carbon dioxide and ethanol.

**Glucose**  
A fermentable sugar otherwise referred to as dextrose. Glucose is the ultimate product in the hydrolysis of starch and cellulose, which are both polymers of glucose units.

**Glucose isomerase**  
An enzyme that converts glucose into fructose.

**Gram stain**  
A microbiological staining technique that aids in the identification and characterization of bacteria. Bacteria are described as Gram-positive if their cell walls absorb and retain the stain and Gram-negative if they do not.

**H**

**Hammer mill**  
A type of impact mill or crusher with hammers revolving rapidly in a vertical plane within a steel casing and is used for grinding corn as a fermentation feedstock.

**Hemicellulose**  
Non-cellulosic polysaccharide components of plant cell walls.

**Hydrolysis**  
Means the breakdown, destruction or alteration of a chemical substance by water.

**Hydrometer**  
An instrument for measuring the density, specific gravity or other similar characteristic of liquids.

**Hydrous ethanol**  
Term used for ethanol that has not been dehydrated.
Hydroscopic Property of absorbing moisture from the air. Anhydrous ethanol is hygroscopic and its exposure to moist air should be minimized.

Hydroxyl group A combination of one atom of oxygen and one atom of hydrogen (OH) that form an essential part of any alcohol.

I

Inoculum The portion of a culture of yeast or bacteria used to start a new culture.

Isomer One of a series of two or more molecules with the same number and kind of atoms and hence the same molecular weight, but differing in respect to the configuration of the atoms. Glucose and fructose have the same formula but different molecular structure.

Isomerase An enzyme that can convert a compound into an isomeric form.

Isomerization Process of converting a chemical compound into its isomer such as converting glucose to fructose.

K

Karl Fischer titration A method to chemically determine the amount of water present in a sample of ethanol or other substance.

Kerosene One of the three permissible denaturants for fuel ethanol.

Kubierschky process The first patented process for continuous dehydration of ethanol with benzene.

L

Lactase An enzyme that hydrolyzes lactose into glucose and galactose.

Lactic acid The organic acid produced in the fermentation of carbohydrates by Lactobacillus bacteria.

Lactobacillus A genus of bacteria that produces lactic acid as a major product in the fermentation of carbohydrates. Lactobacilli are found extensively in fermenting food products such as souring milk and in grain dust. They are the principal cause of loss of yield in ethanol fermentations. They are generally Gram-positive and are controlled with penicillin or other antibiotics.

Lactose The principal sugar in milk and cheese whey.
**Lag phase**  
Lag phase, when applied to yeast propagation, refers to the initial period in which yeast inoculum becomes adapted to the mash prior to the logarithmic phase or the rapid increase in cell numbers.

**Lignin**  
A polymeric, non-carbohydrate constituent of wood that functions as a support and binder for cellulose fibers. Its presence in wood is a major barrier to the hydrolysis of cellulose to sugars.

**Lignocellulose**  
Woody materials made up largely of lignin, cellulose and hemicelluloses. The chemical bonding between the constituents makes it resistant to hydrolysis.

**Liter**  
Metric measure of volume defined as the equivalent of 1000 cubic centimeters.

**Logarithmic Phase**  
Period in which cell numbers increase at an exponential rate after the initial lag phase when applied to yeast propagation.

**M**

**Mash**  
A mixture of milled grain or other fermentable carbohydrate in water used for the production of ethanol.

**McCabe-Thiele diagram**  
A graphic method for calculation of the number of theoretical plates required in a distillation column to achieve a desired separation of two components.

**Meal**  
The granular product resulting from milling or grinding of cereal grains.

**Metabolism**  
The chemical processes in living cells by which energy is derived for vital processes, growth and activities.

**Methane**  
A colorless, odorless, tasteless, combustible, asphyxiating, lighter-than-air gas. It is produced by the decaying of vegetation and other organic matter.

**Methanol**  
A colorless, poisonous liquid with essentially no odor and very little taste. It is the simplest alcohol with the formula, CH₃OH.

**Microorganism**  
Collective term for microscopic organisms including bacteria, yeasts, viruses, algae and protozoa.

**Molasses**  
The thick liquid remaining after sucrose has been removed from the mother liquor in sugar manufacture.

**Molecular sieve**  
A microporous substance composed of materials such as crystalline aluminosilicates belong to a class known as zeolites. The size of the pores in the substance may vary with its chemical structure. Since the material has a very precise pore size, it is possible to separate smaller molecules from larger ones by a sieving action. The term molecular sieve is often used to describe the entire ethanol dehydration apparatus holding the
beads of sieve material and the equipment and controls necessary to regenerate them when saturated with water.

**Monomer**
A single molecule of a substance of relatively low molecular weight and simple structure, which is capable of conversion to polymers by combination with other identical or similar molecules.

**Monosaccharide**
Sugar monomer, the simplest forms of sugar.

**Mother yeasting**
System of yeast propagation frequently used for molasses fermentations in which the propagator is not emptied entirely when inoculating a fermentor and the portion retained is used for starting another yeast propagation cycle.

**N**

**Normal solution**
A solution containing one equivalent weight of a dissolved substance per liter.

**O**

**Obligate anaerobe**
An organism that cannot grow in the presence of oxygen.

**Octane**
A flammable liquid hydrocarbon of chemical formula, C8H18. One of the eighteen isomers of octane, 2,2,4-trimethylpentane is used as a standard in assessing the octane rating of fuels.

**Octane enhancer**
Any substance such as ethanol, methanol, benzene, etc., that will raise the octane rating when blended with gasoline.

**Octane rating**
A laboratory assessment of a fuel's ability to resist self-ignition or "knock" during combustion in a spark-ignition engine.

**Oxygenated fuels**
Literally meaning any fuel substance containing oxygen but the term is commonly used to cover gasoline-based fuels that contain such oxygen containing compounds such as ethanol, methanol, MTBE, etc.

**P**

**Packed distillation column**
A column filled with a packing designed to increase the surface area for contact between liquids and vapors.

**Pentose**
Sugars with five carbon atoms per molecule such as xylose and arabinose, which are the constituents of hemicellulose.

**pH**
A value measuring the acidity or alkalinity of an aqueous solution. Defined as the logarithm of the reciprocal of the hydrogen ion concentration.
Plate  A contacting device placed horizontally at intervals within a distillation column.

Polysaccharide  Polymer composed of numerous sugar monomers or mono-scaccharides.

Propagation  Process of increasing numbers of organisms by natural reproduction.

R

Reboiler  A device for supplying heat to a distillation column without introducing live steam.

Rectification  Process of concentrating and purifying ethanol or other materials in a rectifying column.

Rectifying column  That portion of a distillation column above the feed tray in which rising vapor is enriched by interaction with a countercurrent descending stream of condensed vapor.

Reflux  The portion of the condensed overhead vapors returned to a distillation column to maintain the liquid-vapor equilibrium.

Reflux ratio  The ratio of the amount of condensate refluxed to the amount withdrawn as product.

Refractometer  An instrument used to measure the refractive index of liquids and liquid solutions. In the ethanol industry, refractometers are calibrated as degrees Brix.


Reverse osmosis  A technique used in water purification and wastewater treatment in which pressure is applied to the liquid in a suitable apparatus to force pure water through a membrane that does not allow the passage of dissolved ions.

Roller mill  A mill for crushing or grinding grain or other solid material by passing it between two or more steel rollers.

S

Saccharification  The process of converting a complex carbohydrate such as starch or cellulose into fermentable sugars such as glucose or maltose.

Saccharomyces  A genus of unicellular yeasts of the family Saccharomycetaceae distinguished by the general absence of mycelium and their facility to reproduce asexually by
budding. This genus includes the species Saccharomyces cerevisiae, which is the yeast most commonly used by brewers.

**Specific gravity**  
The ratio of the density of a material to the density of a standard reference material such as water at a specific temperature.

**Starch**  
A mixture of two carbohydrate polymers (amylose and amylopectin), both of which are composed of glucose monomers linked by glycosidic bonds.

**Stoichiometric yield**  
The theoretical yield of a product of a chemical reaction as calculated from the chemical reaction equation.

**Stoichiometry**  
The branch of chemistry that deals with the quantities of substances that enters into and is produced by chemical reactions.

**Stover**  
The dried stalks and leaves remaining from a crop after the grain has been harvested. It is of interest as a potential source of cellulose feedstock for ethanol production.

**Stripping column**  
The portion of a distillation column below the feed tray in which the descending liquid is progressively depleted of its volatile components by the introduction of heat at the base.

**Sucrose**  
Common table sugar, derived from beet or cane sources.

**Sugar**  
Any of a class of water-soluble, simple carbohydrate, crystalline compounds.

**Synthetic ethanol**  
Ethanol produced by any of several synthetic processes such as the catalytic hydration of ethylene, the sulfuric acid hydration of ethylene and the Fischer-Tropsch process, in which it is a major by-product of the synthesis of methanol.

**V**

**Vaporization**  
The conversion of a chemical substance from a liquid or solid state to a vapor or gaseous state.

**Vapor pressure**  
The saturation pressure exerted by vapors when in equilibrium with their liquid or solid forms.

**Vent condenser**  
The final condenser in a series of two or more connected to the overhead vapor line of a distillation column.

**Volatility**  
The tendency of a solid or liquid to pass into the vapor state at a given temperature.
W

Wet milling A process in which corn is first soaked in water containing sulfur
dioxide to soften the kernels and loosen the hulls.

Y

Yeast Any of certain unicellular fungi. Many yeasts are capable of
producing ethanol and carbon dioxide by fermentation of sugars.

Yeast autolysis The disintegration of yeast cells by the action of their own
enzymes.

Yeast strain A pure culture of yeast derived from a single isolation.

Z

Zymomonas A genus of the Psutomonadaceae family of bacteria which are
distinguished by being Gram-negative and non-spore-forming. The genus Zymomonas is
distinguished by its fermentation of sugar to ethanol and is being examined commercially for
fuel ethanol production.
VITA

William Bruce King was born in Macon County, Alabama, on May 27, 1939, to the late Wilmer Bruce King and Bessie Mae Thomas King. He graduated from Macon County High School in 1957. He entered the U.S. Naval Reserves while a student in 1956 and entered active duty in 1959. While serving in the Navy, he was selected to participate in the Naval Enlisted Scientific Education Program (NESEP) and received a Bachelor of Science degree in chemical engineering from Auburn University in 1966. Scholastic honors at Auburn University included membership in Phi Lambda Upsilon (Honorary Chemical Society) and recognition by Tau Beta Pi (Honorary Engineering Society) as the chemical engineering student with the highest grade point average in his graduating class.

He was commissioned as an Ensign, U.S. Navy, in 1966, and continued on active duty until 1970, serving onboard destroyers and with the U.S. Marines in Viet Nam. Upon release from active duty he was commissioned in the U.S. Naval Reserve and was retired as a Captain, Engineering Duty, in 1996.

Bruce has over 37 years experience in process and project engineering in chemical, petrochemical and environmental organizations. He is a Licensed Professional Engineer and a Fellow in the American Institute of Chemical Engineering. He was the recipient of the Charles E. Coates Award for Chemical Engineering in 2004.

During his work with an environmental company, he met and was very impressed with Dr. Ralph Portier and his work with bioremediation. This was instrumental in Bruce enrolling in Louisiana State University and pursuing a Master of Science degree in environmental studies which will be conferred at the May 2008 commencement. His grandson, Mitchell will receive a Bachelor of Science degree in chemical engineering at the same commencement.
Bruce and his wife, Betty, recently celebrated their 50th wedding anniversary. They are extremely proud of their family – Beth and Stew Serpas with children, Mitchell and Victoria, of Baton Rouge; Drs. David and Karen King of Little Rock, Arkansas; and Tim and Shauna King with children, Sierra and Joshua Saldaña, of Bowie, Texas. Beth, Stew and David are Louisiana State University graduates and Mitchell and Victoria are current students at Louisiana State University.