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PREVIEW

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**Development, scale-up and a continuous fermentation process
for the cofermentation of cheese whey and corn for ethanol
production**

Whalen, Paul J., Ph.D.

The University of Nebraska - Lincoln, 1987

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PREVIEW

DEVELOPMENT, SCALE-UP AND A CONTINUOUS FERMENTATION PROCESS
FOR THE COFERMENTATION OF CHEESE WHEY AND
CORN FOR ETHANOL PRODUCTION

by

Paul J. Whalen

A DISSERTATION

Presented to the Faculty of
The Graduate College in the University of Nebraska
In Partial Fulfillment of Requirements
For the Degree of Doctor of Philosophy

Major: Food Science and Technology

Under the Supervision of Professor Khem M. Shahani

Lincoln, Nebraska

December, 1987

TITLE

DEVELOPMENT, SCALE-UP AND A CONTINUOUS FERMENTATION
PROCESS FOR THE COFERMENTATION OF CHEESE WHEY
AND CORN FOR ETHANOL PRODUCTION

BY

PAUL J. WHALEN

APPROVED

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DEVELOPMENT, SCALE-UP AND A CONTINUOUS FERMENTATION PROCESS

FOR THE COFERMENTATION OF CHEESE WHEY AND

CORN FOR ETHANOL PRODUCTION

Paul J. Whalen, Ph.D.

University of Nebraska, 1987

Adviser: Khem M. Shahani

Cheese whey was utilized as the liquid portion of a corn mash to supply 28% of the fermentable carbohydrate as lactose. A modified starch conversion process was developed wherein fungal alpha-amylase was used to produce maltose from corn starch. Fermentation was accomplished using Kluyveromyces marxianus followed in 8 h by Saccharomyces cerevisiae in a staggered inoculation procedure. In laboratory trials, the whey-corn process produced 9.75% ethanol v/v in 72 h. Yields equivalent to 3.63 gal/bu resulted for the whey-corn process vs. 2.63 gal/bu for corn alone. The mixing power requirement during liquefaction of the whey-corn mash was reduced 20%.

The whey-corn process was scaled-up to 6500 L runs using 5630 L of sweet whey permeate and 1243.7 kg corn per batch. K. marxianus was produced at the pilot plant in a medium of whey plus yeast extract. The whey-corn trials produced 9.3% ethanol v/v in 60 to 72 h. The pilot plant yield of 3.3 gal/bu for the whey-corn process was 96% of the expected yield while the control runs using corn alone yielded 2.6 gal/bu. The yield was increased by 27% using whey.

The whey-corn cofermentation process was further modified to use acid whey (pH 4.3). Due to the high acid content, the liquefaction was

performed at pH 5.0 and 5.5. Dextrose equivalents of 15 and 20, respectively, resulted for the starch portion of the mash, with no adverse effect observed upon fermentation. The acid whey-corn process was performed in 3800 L trials at a commercial farm-scale ethanol plant. Yields were increased by 25 to 29%.

A two-stage continuous fermentation process was developed using whey permeate and corn starch at laboratory scale. The feed rate was 120 ml/h for a dilution rate of 0.12 h^{-1} in the first vessel and 0.04 h^{-1} in the second. In the first vessel, K. marxianus fermented lactose to 3.27% v/v ethanol. The second vessel could not maintain a stable population of S. cerevisiae which washed out in 48 h. Using K. marxianus as the sole yeast produced 6.9% ethanol v/v with an overall fermentation efficiency of 86%.

DEDICATION

This dissertation is dedicated to my wife, Catherine, for her love, support, and sacrifices during the course of my studies. It was greatly appreciated. This work is also dedicated to my parents, James and Sally Whalen, for their support and insistence upon a good education.

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P.J.W.

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I. INTRODUCTION

The ethanol fermentation process is one of the oldest bioconversions utilized by man. With the oil shortages of the '70s and the current instability in the Middle East, interest in ethanol as a liquid fuel has been renewed in the U.S. and other petroleum importing nations. The U.S. has a considerable fuel ethanol industry with over 30 billion gal of ethanol-supplemented fuel (gasohol) consumed since 1980. In addition, federal subsidies are in place to promote the production of ethanol. Ethanol can be produced by the fermentation of virtually any of the abundant carbohydrates found in nature. While cellulose is the most abundant carbohydrate reservoir, it is extremely resistant to breakdown and economical processes have not yet reached fruition. In the U.S., corn is the principal substrate for ethanol production, and the technology for conversion of corn starch to glucose is well established. Although corn is produced in abundance and is relatively inexpensive, the cost of the substrate comprises 50 to 75% of the cost of ethanol production. The ability to control this cost is beyond the ethanol producer. Therefore alternative substrates are of interest.

Alternative substrates range from high yield specialty crops such as sweet sorghum and Jerusalem artichokes to sugar beets and waste products such as cheese whey. Many of the crop sources are seasonal and/or require dedicated processes. Consideration of these substrates should involve the ability to utilize cereal grains in the process as well. In addition, the substrate cost must be low in order to favorably impact the economics of the ethanol process. Cheese whey meets these requirements in many ways. Whey is a waste product produced at a rate

of over 50 billion lbs/y in the U.S., of which over half is currently discarded as waste. As a waste product, whey has one of the highest polluting factors of industrial wastes (a BOD of 35,000 to 45,000 ppm) and cheese manufacturers are facing harsher restriction on its discharge. Utilization of whey for various value-added products has remained stagnant over the last 5 years while the amount of whey generated each year has increased.

As a substrate for ethanol production, whey has limited applications because of the dilute nature of its sugar content (lactose at 5%). Thus, processes based solely upon whey require concentration for transportation and fermentation. Even then, the entire waste whey in the U.S. would only produce 80 to 90 million gal of ethanol/y. However, if whey were used as the liquid portion of a grain mash, in effect supplementing the whey with starch, over 300 million gal of ethanol/y could be produced, using about 90 million bu of corn/y. However, while this combination of whey and grain appears simple enough, attempts to actually apply the process have to date proven unfeasible due to extremely long fermentation times. Clearly, the conventional approach will not suffice for a whey-corn mash and further research is required in order to take advantage of the whey addition in a grain process.

Utilization of supplemental substrates in the current starch conversion/fermentation processes requires contingencies for easily applying the process without any major changes in the process proper. In addition, a whey-corn process should also display optimum process parameters for new systems, taking full advantage of the unique properties imparted to the system and its products. Advanced fermentation

processes which maximize productivities should be investigated and explored. This includes the potential advantages produced by new microorganisms with applications to ethanol production such as Zymomonas mobilis as well as new technologies for downstream recovery.

The objectives of this study were: (1) to determine the reduction in the cost of ethanol production from corn by incorporating cheese whey into the grain ethanol process, and (2) to increase the utilization of waste whey via development of a feasible ethanol process which would encourage bulk use of this waste. In this study, process development focused on the ability to directly implement the whey-corn process in the conventional corn-based ethanol process. This study reports on the development of this process at laboratory scale and scale-up to pilot plant. Both sweet and acid whey were utilized. In addition, a continuous fermentation process was investigated for the whey-corn process.

PREVIEW

II. LITERATURE REVIEW

A. Historical

Ethanol fermentation is one of the oldest microbial processes employed by man. The incidental inoculation of yeast through the flora on the sugar-rich fruit provided the beginnings of a process ultimately steeped in tradition (Kunkee and Amerine, 1970). Present-day production methods of wine-making, brewing and distilled spirit production maintain methods for yeast strain selection and numerous analytical techniques for product evaluation. Industrial ethanol production shares many aspects of other types of alcoholic fermentations. However, the end-product is clearly more focused and, in many ways, this greatly simplifies the entire process.

Industrial ethanol is ethyl alcohol for non-beverage uses such as for solvent, pharmaceutical, chemical feedstock or fuel purposes (Jacobs, 1950). Ethanol was produced primarily via fermentation through World War II, during which emergency expansion of production was made by both the U.S. (for munitions and rubber production) and Germany (for fuel) (Stark, 1954; Underkofler, 1969; Scheller, 1981). That ethanol could serve as a fuel (referred to as "power alcohol") for internal combustion engines was recognized as early as 1897 (Maercker, 1907). Ethanol was recognized as a renewable fuel by Henry Ford to power early automobiles (Vollmar, 1980). This concept was rapidly replaced with the advent of cheap, abundant oil discoveries and the associated refinery technology. Likewise, it was the petroleum industry's production of synthetic ethanol from ethylene that replaced the distilleries of WWII. Cheap ethylene produced as a by-product of natural gas and gasoline

processing drove fermentation ethanol from the market (Hacking, 1986). The situation continued as long as cheap, plentiful petroleum sources remained available. The U.S. and other industrial countries outstripped home reserves and became dependent on imported oil to meet the majority of our petroleum needs (Vollmar, 1980). In 1974 and 1979, the Oil and Petroleum Exporting Countries (OPEC) made their power felt in western and industrial nations by exerting an embargo, causing greatly increased oil prices and fuel shortages (Jenkins, 1981; Scheller, 1981; Hacking, 1986). Alternative fuels became of paramount importance along with conservation measures. As the gasoline shortages of the day testified, the oil embargo and control executed by OPEC concerned liquid fuels for internal combustion engines. While numerous alternative energy sources were suggested--such as coal, solar and nuclear energy--none of these attended to the liquid fuel needs of a nation for transportation purposes. Thus, ethanol was re-introduced as a strong candidate for liquid fuel production in the U.S. as well as other countries (Anderson, 1979; Ember, 1980).

In the U.S., the production of fuel alcohol from carbohydrate reserves of the grains--maize (corn), wheat and sorghum--could potentially supplement gasoline in a blend of 10% ethanol and gasoline called "gasohol" (Scheller, 1981). The 1980 Energy Security Act and associated legislation established tax and other incentives to encourage ethanol production. Tax exemptions on ethanol fuels were accompanied by state exemptions and producer incentives as well (Reidy, 1986). The move back towards ethanol as a supplemental fuel was consistent with a need to

increase utilization of starchy grains which are chronically over-produced in the U.S. (Chattin and Doering, 1984).

Almost immediately, a number of opposing issues challenged the prospects for the development of fermentation ethanol from grain. Economists' questioned the economics of the process (Reilly, 1979), engineers questioned the energy balance, market issues of food vs fuel were raised (Anon., 1981) and automobile manufacturers predicted fuel line problems. In each case, responses have been made or answers borne out which have clarified fuel ethanol's place in the market (Essien and Pyle, 1983; Esser and Schmidt, 1982; Gill, 1987; Lyons, 1982). For example, economists' initial assessment of the fuel alcohol production process was based on beverage processes (Reilly, 1979)--not at all appropriate for fuel ethanol production (Scheller, 1981). Techniques such as BTUs upon combustion of the corn were employed as well as ignoring the fuel efficiency of ethanol in an internal combustion engine and engine modifications. Food vs fuel (Tyner, 1982) was hardly an issue when the ethanol process itself yields an animal feed (Turhollow and Heady, 1986) and direct human food use (or other industrial use) of corn in this country is ~13% of the crop (Hull, 1987). Considering the worldwide waste of grains due to storage losses because of over-production, increased use of grain for fuel has been welcomed in the agricultural sector where lack of parity and a failing subsidy program have not improved the farmers' situation. In addition, alcohol-supplemented fuels now have a history of performance in automobiles with ~30 billion gallons consumed in the U.S. since 1980 (Gill, 1987). Thus, fuel ethanol from grain is beginning to acquire some longevity as a fuel