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Technology and Economics of Commercial Scale Ethanol Production From Fodder Beets

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TECHNOLOGY AND ECONOMICS OF COMMERCIAL SCALE ETHANOL PRODUCTION FROM FODDER BEETS

BY

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ETHANOL PRODUCTION FROM FODDER BEETS

This dissertation is approved as a creditable and independent investigation by a candidate for the degree, Doctor of Philosophy, and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this dissertation does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major or minor departments.

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TABLE OF CONTENTS

TABLE OF CONTENTS
(cont'd)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES
(cont'd)

LIST OF FIGURES
(cont'd)

LIST OF FIGURES
(cont'd)

LIST OF FIGURES
(cont'd)

TECHNOLOGY AND ECONOMICS OF COMMERCIAL SCALE ETHANOL PRODUCTION FROM FODDER BEETS

ABSTRACT

WILLIAM R. GIBBONS

Fodder beets yield two to three times more fuel ethanol per hectare than corn. This increased productivity should reduce feedstock costs and, consequently, ethanol production costs. The major factor that has limited use of this crop is the lack of a proven technology to process fodder beets -- economically and energy efficiently -- to fuel ethanol on a commercial scale. Research reported herein rectifies this situation by examining the technical and economic feasibility of community scale fuel ethanol production from fodder beets using two novel, continuous fermentation systems -- solid-phase fermentation and diffusion fermentation.

Laboratory scale, batch fermentation trials were first conducted to determine optimum levels for important fermentation parameters. For the solid-phase fermentation process, these included grinding beets with a 1.27-1.91 cm hammermill screen, using a 5% (v/v) yeast inoculum, and maintaining a pulp pH of 3.0-3.5 to prevent contamination. For diffusion fermentation these parameters included using 1.91-2.54 cm beet cubes, and 0.25% potassium meta bisulfite, or 0.20% sodium meta bisulfite, or pH 2.0-2.2 to prevent bacterial contamination.

Utilizing this information, the commercial scale continuous fermentors were operated using optimum levels of each parameter. Material and energy balances, and costs were determined from the resulting operational data. The energy balance (energy output/energy input) for each novel process was 3.0, which compares favorably with ethanol production from corn (2.26). Production costs for the solid-phase process ($0.492/L ethanol) were similar to ethanol produced from corn ($0.497/L), however the diffusion process was more costly ($0.529/L). In each process, beers or pulps containing 8-10% (v/v) ethanol were produced.

Since ethanol production costs from fodder beets were equal to or higher than those for corn, it is likely that corn will remain the feedstock of choice for fuel ethanol production -- at least under present conditions. Before fodder beets can become a primary ethanol fuel feedstock, production costs must be lowered below those of corn. Only then will investors assume the increased risk of processing a new crop using a recently developed fermentation system. Future research on fodder beet production and processing hold the potential for achieving such cost reductions.

SUMMARY

Currently, the bulk of fuel ethanol produced in the United States is derived from corn (Gavett et al., 1986; Hallberg, 1984; Vaughn, 1985). There are at least two major reasons for this. First, corn is produced in large quantities over a broad geographic range and thus it is a widely available substrate for alcohol production. Second, storage of and alcohol production processes for corn are established technologies and as such are more attractive to investors.

The primary drawback to alcohol production from corn is its high cost. In 1986, alcohol fuel production costs in large scale plants (greater than 4 million liters per year [mly]) were estimated by Gavett et al. (1986) to range from $0.37 - 0.40/L, compared with wholesale gasoline prices of $0.15 - 0.20/L. Alcohol-gasoline blends are currently cost-competitive with gasoline only because of certain Federal and State motor fuel tax exemptions (Gavett et al., 1986). Even with these tax subsidies, alcohol production in smaller scale plants (<4 mly) is frequently not feasible with present grain and petroleum fuel prices (Dobbs and Hoffman, 1983; Dobbs et al., 1984b; Gibbons and Westby, 1983b; Hoffman and Dobbs, 1982; Westby and Gibbons, 1982).

Corn accounts for 30-50% of the cost of ethanol (Dobbs and Hoffman, 1983; Dobbs et al. 1984 b; Gavett et al., 1986; Hoffman and Dobbs, 1982). If an alternative feedstock could either be produced and processed at a lower cost and/or yield more alcohol per hectare

than corn, ethanol production costs would decrease and hence improve the economics of fuel ethanol (Dobbs et al., 1984a).

It is possible that alternative high-yielding biomass crops, such as fodder beets and sweet sorghum might offer greater feasibility prospects than corn (Dobbs et al., 1984a). These biomass crops offer the following advantages over corn: they yield significantly more alcohol per hectare, they don't require extensive cooking treatments, and they can often be produced with lower levels of agronomic inputs (fertilizer, etc.).

One of the major factors that has limited the use of these biomass crops in the United States is the lack of a proven technology to produce, store, and convert these crops -- economically and energy efficiently -- to fuel ethanol on a commercial scale. Research reported herein was carried out to address this situation by examining the technical and economic feasibility of community scale fuel ethanol production from biomass crops using two novel, continuous fermentation systems recently designed and assembled in the SDSU fuel ethanol plant.

Farm-community scale, continuous solid-phase and continuous diffusion fermentation systems were tested for processing a typical, high yielding biomass crop -- fodder beets -- into fuel ethanol and protein feed (PF). The results obtained, however, are directly applicable to other biomass crops, such as sweet sorghum, since they too could be processed through these systems. In this study laboratory scale batch fermentation trials were first conducted in

order to determine optimum levels of each important fermentation parameter. Following this, each fermentor was operated using these optimum set points. Mass and energy balances, and costs were determined from this in-plant fermentation data.

Results from this work clearly demonstrated that continuous solid-phase and continuous diffusion fermentation systems were both viable processes for converting fodder beets (as well as other high moisture/high fiber crops) to fuel ethanol and PF. Each process produced beers or pulps containing a distillably worthwhile 8-10% (v/v) ethanol, with an energy balance (energy output/energy input) of 3.00 This compares favorably with the conventional submerged fermentation process for corn, which produces 8-12% ethanol beers with an energy balance of only 2.26 (Gibbons, 1982; Gibbons and Westby, 1983 a and b; Westby and Gibbons, 1982).

For each of these novel fermentation systems, optimum set points for each of several important fermentation parameters were determined. For continuous solid-phase fermentation these included: 1) grinding fodder beets with a 1.27-1.91 cm hammermill screen to maximize yields and minimize fermentation time, 2) using a 5% (v/v) yeast inoculum to minimize fermentation time, and 3) maintaining a pulp pH of 3.0-3.5 to prevent bacterial contamination and maximize yeast fermentation efficiency and rate. For continuous diffusion fermentation these included: 1) using 1.91-2.54 cm fodder beet cubes to maximize ethanol yields and minimize fermentation time and energy consumption, and 2) maintaining a cube slurry pH of 2.0-2.2 to

prevent bacterial contamination and maximize yeast fermentation efficiency and rate.

With respect to the most important factor -- costs -- fodder beets compared more favorably with corn when continuous solid-phase fermentation was used and less favorably when continuous diffusion fermentation was used. Net costs of production were $0.497/L with corn (Dobbs and Hoffman, 1983; Dobbs et al., 1984b; Gibbons and Westby, 1983b; Hoffman and Dobbs, 1982; Westby and Gibbons, 1982) (costs adjusted from 1981 to 1984 levels using a 7.9% increase in the Producer Price Index), $0.492/L with fodder beets processed via solid-phase fermentation, and $0.529/L with fodder beets processed via diffusion fermentation.

Even though fodder beets were a cheaper feedstock ($0.209/L vs $0.242/L for corn) and fodder beet PF was of greater value ($0.112 to 0.14/L vs $0.08/L for corn byproduct feed), the higher capital and operating costs for the fodder beet processes negated their advantage over conventional submerged fermentation of corn. High capital costs in the solid-phase process were due to the need for a press and dryer for ethanol recovery while higher costs in diffusion fermentation were for the fermentor itself. Higher operating costs were primarily due to greater sulfuric acid useage (to control contamination in the novel fermentors) even though costs for starch hydrolyzing enzymes were eliminated.

These results indicated that, at least under present conditions, fodder beets will likely not supplant corn as the

feedstock of choice for fuel ethanol production. For this to happen, ethanol production costs for fodder beets would have to be significantly lower than costs from corn so as to justify the increased risks associated with ethanol production from this new crop. Without the possibility of higher profitability in producing ethanol from fodder beets, entreprenuers are unlikely to invest in fodder beet processing systems untested, as of yet, on a large, commercial scale.

This situation could, however, change if fodder beet production and processing costs were lowered to increase profitability. As of yet very little agronomic research has been performed on fodder beets relative to that done on corn. It is highly likely that fodder beet tuber yields and sugar contents can be increased with minimal changes in production costs. This greater productivity would thereby reduce feedstock costs and increase profitability.

Previous resarch done in our laboratory (Gibbons and Westby, 1983b; Westby and Gibbons, 1982) has shown that by modifying the "baseline" operation of our corn ethanol plant we were able to reduce production costs by up to $0.10/L and increase the energy balance by 1-1.5 units. If such modifications could be made to our fodder beet processes, operational costs might be reduced and profitability thereby increased.

INTRODUCTION

Historical Perspective of Ethanol Fuel Production in the United States

The man most commonly accepted as being the "Father of Alcohol Fuels" was Henry Ford, founder of the Ford Motor Company (Gibbons, 1982). In 1908, Ford initially designed the engine of the Model T to use ethanol (Gavett et al., 1986). Ford also saw the advantage of operating farm tractors on ethanol, noting that one year's potato crop converted to ethanol would provide enough fuel to cultivate the same field for 100 years (Gibbons, 1982). This renewable nature, coupled with ethanol's high performance and clean burning properties, endeared ethanol fuel to Ford. Unfortunately, crude oil was discovered shortly thereafter, and cheap supplies of gasoline forced ethanol off the market (Gavett et al., 1986).

In subsequent years, ethanol fuel experienced periodic resergences during times of low grain prices (Gibbons, 1982). Farmers and farm groups promoted ethanol production as a way to reduce grain surpluses by increasing demand. A gasoline-ethanol blend called "Agroll" was produced and sold in Atchison, Kansas in 1937. About that same time Cleveland Petroleum Products Company was selling an alcohol-gasoline blend in Britain called "Cleveland Discol." The U.S. government has also encouraged ethanol production, primarily during times of war (Gavett et al., 1986; Gibbons, 1982). In 1943, for example, 350 million bushels of grain were converted to 3.31 billion liters of ethanol and used mainly for

extending gasoline supplies, and manufacturing synthetic rubber and other war materials (Gavett et al., 1986; Gibbons, 1982; Stark et al., 1943b).

Prior to the late 1970's, ethanol production and use as a fuel additive was sporatic and relatively limited (Gavett et al., 1986). However during that decade the world's economy was shocked with the realization that crude oil supplies were finite, and largely held by politically unstable countries.

The first "oil shock", of 1973, occurred when the O.P.E.C. countries (Organization of Petroleum Exporting Countries) nationalized their oil production facilities and forced oil prices higher by withholding supplies. This caused gasoline prices to jump from $0.09/L ($0.35/gallon) to $0.18/L ($0.70/gallon) in a few months time.

Following a five year period of relative stability, oil and gasoline prices were pushed even higher by the Iranian oil embargo. This sudden cut-off of oil from O.P.E.C.'s largest producer caused crude oil to jump to $45/barrel. U.S. gasoline prices rapidly climbed to the $0.40/L ($1.50/gallon) level and remained high for 2-3 years. This most recent "shock" dramatically renewed interest in alternate forms of energy. The current 2.65 billion liters per year ethanol industry had its beginning during that time of upheaval. Gavett, Grinnell, and Smith (1986) provide an excellent review of the development of this industry.

More recently, crude oil prices have stabilized and fallen to pre-embargo levels as oil supplies have risen to glut the market. Conservation, increased oil exploration and production, and the switch to alternative forms of energy have combined to cause this oversupply. Dumping of oil onto world markets by O.P.E.C. countries to force other producers out of busines has also exacerbated the situation. These factors have put a severe strain on domestic ethanol producers even though corn prices have dropped from $3.00/bushel to $1.00/bushel.

The future of ethanol fuel is therefore tenous. Any further, long-term drop in gasoline prices would likely deal a fatal blow to this fledgling industry, while continuation of current price levels might only delay this demise. What the ethanol fuel industry desperately needs are higher profits, which could come from lower production costs, increased oil/gasoline prices, and/or realization of ethanol's value as an octane enhancer/lead replacer.

Benefits of Ethanol Production and Use

In the late 1970's and early 1980's an intense debate raged over the merits of ethanol production for use as a fuel. On one side were oil companies, against ethanol fuel since it competed with gasoline and could be produced independently from their control (Bernton et al., 1982). The major arguments they used against ethanol were: 1) it takes more energy to produce ethanol than is contained in the ethanol, 2) ethanol damages automobile engines, 3) use of agricultural products as ethanol fuel feedstocks will result

in food shortages and higher food prices, and 4) ethanol can never be produced in large enough amounts to totally replace gasoline.

While the last of these points was obviously true, the others have been refuted by scientific research and practical experience. New processing techniques that have reduced energy useage and/or allowed energy recovery and reuse have dramatically increased the energy efficiency of ethanol production facilities. Today, processes with energy balances (energy output/energy input) of greater than 2.5:1 are common (Gibbons, 1982; Gibbons and Westby, 1983b; Hughes, 1979; Rigelato, 1980; Westby and Gibbons, 1982).

The claim that ethanol damages car engines has best been discounted by the "2 million mile road test" conducted in Nebraska by Scheller (1980), similar tests in North Dakota (Kaufman and Klosterman, 1979) and by the billions of trouble free kilometers that U.S. motorists have driven each year on ethanol enhanced fuels. According to Vaughn (1985), more than 160 billion kilometers were driven in 1985 alone on 21.2 billion liters of domestically produced ethanol enhanced fuels.

The fallacy of the food vs fuel argument becomes apparent when one realizes that 90-95% of the U.S. corn crop goes to feed livestock (Hallberg, 1984; Meyer, 1981; Vaughn, 1985), and that ethanol fuel byproducts actually increase meat and milk production over feeding corn alone (Loosli and Warner, 1958; Merchen, 1979; Schingoethe et al., 1983; Stock and Klopfenstein, 1981). Other

studies have indicated that ethanol production will have no effect upon food production or export (Ebinger, 1985).

Instead of downgrading ethanol fuel, oil companies should have been more concerned with the environmental and economic problems with their product. According to Sneller (1986), oil spills, air pollution, seepage into ground water, and ecological degradation during exploration and production all are hidden costs of petroleum. Add to this the revenues lost as a result of tax breaks given to oil producers ($8.5 billion in 1984), and the military costs to protect foreign oil producers and shipping lanes, and the real costs of gasoline become apparent.

On the other side of the ethanol debate were farmers, ethanol producers, university researchers, and government officials. They saw ethanol production and use as an opportunity to help solve agricultural, economic, and energy problems in the U.S. Although ethanol has not been the panacea predicted by some, it has had a positive impact upon many facets of the economy. (Hughes, 1979; Von Bremen and Schmoltzi, 1986).

The most noticeable of ethanol's many benefits has been its effects on automobile fuel quantity and quality. In 1986, ethanol blended fuels were predicted to capture 7% of the domestic fuel market (Gavett et al., 1986; Sneller, 1986) thereby reducing gasoline consumption. During the previous year, domestic ethanol production reduced, by $500 million, foreign gasoline imports (Vaughn, 1985). The widespread use of ethanol enhanced fuels

translates into an even greater reduction in crude oil imports since refineries can produce a greater volume of low quality gasoline per barrel of oil than high quality gasoline. Ethanol is then simply added to bring the quality and octane rating back up to standards (Ogburn, 1980). According to a General Accounting Office (GAO) report (Anon, 1984) each liter of ethanol can thereby replace 1.2 liters of gasoline.

The high octane nature of ethanol has also made it a popular replacement for lead, a toxic additive which must be removed from gasoline, according to Environmental Protection Agency (EPA) regulations (Herman, 1983). The EPA's cost/benefit analysis found that elimination of lead from gasoline would constitute a net national benefit of $800 million annually (Vaughn, 1985). If only ethanol, an environmentally benign substitute, was used to replace lead this would create a demand for an additional 227 billion liters of ethanol over the next ten years (Sneller, 1986).

Ethanol is beneficial in terms of engine performance and wear. This is because engines operated on ethanol or ethanol-gasoline blends operate at lower temperatures than with gasoline alone (Earl, 1984; Ferfecki and Sorenson, 1983). Ethanol also acts as a solvent to keep carburetors and fuel injectors clean (Earl, 1984; Ferfecki and Sorenson, 1983). This solvent property can, however, degrade plastic parts in the fuel system of certain engines (Ferfecki and Sorenson, 1983). In general, the mileage on ethanol gasoline blends is equivalent or somewhat higher than on gasoline

since cleaner, cooler engines operate more efficiently and permit more complete fuel combustion (Hughes, 1979; Kampen, 1978). This efficiency reduces tailpipe emmissions of unburned hydrocarbons and carbon monoxide (Hughes, 1979; Kampen, 1978; Miller, 1971; Vaughn, 1985).

Agriculture is another major area benefitted by fuel ethanol production and use. In testimony before various U.S. Senate (Vaughn, 1985) and House (Hallberg, 1984) committees, past Presidents of the Renewable Fuels Association have documented these positive effects. Ethanol production provides a stable domestic outlet for U.S. agricultural production at a time when conventional markets, including export markets, are stagnating or declining. Ethanol production is a form of value added processing -- increasing the value of the raw feedstock by converting it to ethanol and high value byproduct feed. Ethanol production also establishes a shift away from policies that achieve supply/demand balance through non-productive land idling to market oriented policies dictated by providing a strong domestic market for U.S. grain.

In 1985, domestic fuel ethanol production of 2.08 billion liters created a new cash market for 210 million bushels of grain, added $750 million to farm income ($0.10/bushel increase in price multiplied by the 1985 corn crop of 7.5 billion bushels), increased agricultural exports by $100 million, and reduced farm program costs by over $650 million (Vaughn, 1985). If this industry should expand to 3.79 billion liters per year, Hallberg (1984) predicted that corn

prices would rise by $0.25 - $0.40 per bushel, net farm income would increase by $3.5 billion, millions of dollars would be saved annually in price support payments, and food prices would not be significantly affected.

In fact, the savings from the reduced cost of agricultural programs outweighs the costs of ethanol production incentives. A 1984 GAO report (Anon, 1984) stated that in 1982 the domestic ethanol industry saved the federal governement more than it cost. Counting the outlays stemming from federal exise tax exemption ($0.01585/liter gasohol or $0.1585/liter ethanol) and the 10% energy investment tax credit, the GAO found that these incentives cost the Treasury about $114 million. Fuel ethanol production and use contributed to improving the 1982 federal revenue balance in two ways. First, by reducing agricultural program outlays by $129.2 million; and second, by generating fuel ethanol import duties of $10 million, for a total of $139.2 million.

For 1985, Vaughn conducted a similar cost/benefit analysis and found that an investment of $220 million had, in effect, been made due to lost gas exise taxes and investment tax credits. However the return on investment was roughly $1.9 billion, if one includes reduced farm program costs, increased farm income, increased agricultural exports, and reduced energy imports.

Ethanol's beneficial effects upon the business sector are as significant as its effects upon agriculture. Since 1978 the domestic fuel ethanol industry has committed more than $2.0 billion

in capital outlays to construct over 150 ethanol production facilities -- ranging in size from less than 4 million liters per year (mly) to more than 190 mly (Sneller, 1986; Vaughn, 1985). Over 30,000 jobs can be directly attributed to the domestic fuel ethanol industry, with 4,300 people actually operating ethanol plants (Vaughn, 1985). The remaining jobs include construction workers, support personnel, and jobs in supply or spin-off industries. This new economic activity also provides an increased tax base for federal, state, and local government enities (Vaughn, 1985). For example, between 1981-1983 the ethanol industry increased state tax revenues by $84.5 million (Sneller, 1986).

To summarize the benefits of ethanol production on the business sector, Sneller (1986) listed the economic impacts of a 190 mly ethanol plant. Approximately 640 workers would be required for corn production, 24 workers to transport the corn to the ethanol plant, and 600 jobs would be created for plant construction and operation. This increased economic activity would have a $200 million effect annually, resulting from increased investments, new jobs, higher corn prices, etc.

A final area in which ethanol production benefits the U.S. is in the area of foreign trade and foreign affairs. To be sure, ethanol can never completely replace gasoline as an automotive fuel, however, it can extend and enhance our fuel supply. This, coupled with increased domestic oil production and conservation can and has freed us from the grip of foreign control over our energy supplies.

Concomittant with this greater energy independence, ethanol production has reduced foreign oil imports and thereby helped reduce our foreign trade deficits. A Georgetown University Center for Strategic and International Studies (CSIS) analysis of the national security impacts of ethanol production, stated that a 7.57 billion liter ethanol industry would result in a net balance of trade gain of $2.5 billion annually (Ebinger, 1985). This effect would result from reduced oil imports and the increased export of high protein co-products.

In a military vein, ethanol also has numerous advantages over conventional fuel sources which could be easily disrupted. In the same report on the national security implications of ethanol fuel, Ebinger (1985) noted that ethanol production (unlike synfuels) is available and operational now, that the raw materials for ethanol production are widely available and renewable; that rapid expansion of production capacity is possible; and that production facilities can be decentralized, thereby making disruption more difficult. Ethanol is also more storable than gasoline, which begins to degrade after relatively short-term storage (Anon, 1984; Ogburn, 1980). Some effort has been put forth to establish a strategic ethanol reserve patterned after the strategic oil reserves in Louisiana salt domes.

SDSU Fuel Alcohol Research Project

In response to the oil crisis of 1978-79, the possibility of fuel shortages, and rising fuel prices, the SDSU Administration and

Agricultural Experiment Station deemed it wise to investigate the production and use of ethanol fuel. As a result, Dr. Ray Moore, Director of the Agricultural Experiment Station, contacted various departments throughout the University in order to recruit a multi-disciplinary team to study ethanol fuel. The result was a unique combination of scientists from the fields of Agronomy, Microbiology, Agricultural and Mechanical Engineering, Animal and Dairy Science, and Economics -- charged with the responsibility of determining the feasibility of producing and utilizing ethanol fuel in the upper midwest. This group, and their associated fuel ethanol research plant, was a first of a kind research organization and provided a model which other universities were soon to follow.

From 1978-1982 the primary focus of this research group was ethanol production from corn, other grains, and cheese whey. Stampe (1982) provides an excellent review of the organization and composition of the research group during this period, and lists research goals, objectives, and responsibilities. This work resulted in numerous publications in the areas of cooking/fermentation (Gibbons, 1982; Gibbons and Westby, 1982; Gibbons and Westby, 1983 a and b; Westby and Gibbons, 1981 a and b; Westby and Gibbons, 1982; Westby and Gibbons, 1983), distillation (Lemmer, 1985; Stampe, 1982; Stampe et al., 1983; Stampe et al., 1982), ethanol fuel use (Bassett and Chisholm, 1980; Kelkar, 1981), feed byproduct use (Clark and Voelker, 1982; Schingoethe et al., 1983; Wahlstrom and Libal, 1980),

and economic feasibility (Dobbs and Hoffman, 1983; Dobbs et al., 1984b; Hoffman and Dobbs, 1982).

In 1982-83 the immediate crisis of the oil shortage was over, and had been replaced by an oil glut which was forcing gasoline prices down. A more short-sighted Administration would have likely terminated an ethanol fuel research project at that time, and that is exactly what countless Universities did. Research groups, as well as ethanol production research facilities, were dismantled as interest in ethanol fuel waned.

One of the few ethanol production research groups to remain active during these succeeding years has been the SDSU group. The initial committment made to this research in the late 1970's by SDSU was not weakened by the ensuing oil glut, as was the case with so many other programs. With the foresite that this technology would eventually be needed, and that the best time to conduct this research was before it was needed, SDSU scientists utilized their existing research program to investigate alternate feedstocks and processing technologies in order to reduce costs and energy consumption.

Justification

Consumption of ethanol blended fuels in the U.S. has increased every year since 1978. In 1986, Sneller estimated that 2.65 billion liters of domestically produced ethanol were produced and utilized, capturing a 7% share of the automobile fuel market. This trend may continue in the foreseeable future as the octane enhancing properties of ethanol become more widely recognized, and as ethanol is used to replace toxic additives, such as lead, currently used in gasoline. Increased ethanol production may also be promoted as a value-added type of processing which would create jobs, stimulate the economy, utilize agricultural surpluses, and increase the tax base (Hallberg, 1984; Vaughn, 1985). Ethanol's importance as an alternative energy source must also be considered, even though we are currently experiencing a temporary oil glut. The authors of recent reports, in which world petroleum estimates were lowered, stressed that alternative energy sources will become necessary much sooner than previously anticipated (Kerr, 1984; Raloff, 1985).

To be sure, the bulk of ethanol currently used in the U.S. is produced from corn in large (greater than 38 mly) commercial plants (Gavett et al., 1986; Hallberg, 1984; Vaughn, 1985). Economics of scale and plant locations that minimize raw material and product shipping costs allow such plants to produce ethanol at lower costs than are possible in smaller, more rural plants (Gavett et al., 1986). However, most large-scale plants depend on partial road tax exemptions for ethanol-blended fuels and on investment tax

credits for economic feasibility. Many small- or community-scale plants designed to use corn have not yet proven economical even with various tax and financial subsidies (Dobbs and Hoffman, 1983; Dobbs et al., 1984b; Hoffman and Dobbs, 1982).

Therefore it is appropriate to devote greater attention to the feasibility of producing fuel alcohol from feedstocks other than corn (Dobbs et al., 1984a). Such feedstocks, if they could be produced and/or processed more efficiently and more cheaply than corn, would provide greater economic incentive for ethanol production -- both in large and small plants. Too, they could serve as new cash crops for farmers, thereby diversifying and strengthening agriculture and rural America.

As a first step in evaluating alternate feedstocks and processing technologies, SDSU agronomists, economists, and microbiologists conducted an extensive literature review of sugar and starch crops (Dobbs et al., 1984a). This review compared feedstocks based upon agronomic, harvesting, storage, processing, and economic considerations. Cellulosic crops and residues were not included since current research indicated that, due to processing difficulties, this substrate would not be an economically viable alternative, at least for the near future.

Results of this survey (Dobbs et al., 1984a) indicated that high-yielding, sugar containing biomass crops, such as fodder beets and sweet sorghum, offered the greatest immediate potential for increasing the ethanol productivity of crop lands while reducing

ethanol production costs and energy consumption. Fodder beets were chosen for this research due to their higher ethanol yield potential (5600-7500 L/hectare) compared to sweet sorghum (5000-5500 L/hectare) (Dobbs et al., 1984a; Hills et al., 1981; Hills et al., 1983). However since sweet sorghum is more similar to other midwestern crops (corn, grain sorghum, cane) and could be incorporated into current farming practice utilizing existing farm machinery, we conducted similar research with this feedstock. Sweet sorghum results are being published elsewhere (Gibbons and Westby, 1983c; Gibbons et al., 1986; Westby and Gibbons, 1984).

Production of ethanol from these alternative feedstocks requires conversion systems significantly different from those currently used to produce ethanol from corn. This is because these crops have much higher moisture and fiber levels than corn. A limited amount of laboratory-scale research has been aimed at developing new fermentation strategies for these feedstocks; however, scale-up and optimization of these processes have yet to be accomplished. This scale-up work, which must be done before commercial plants can be constructed, is a major bottle-neck to further development of the U.S. fuel alcohol industry.

Research Objectives

The overall objectives of this study were to optimize the design and operation of two novel, farm-community scale, continuous fermentation systems for conversion of a representative biomass crop -- fodder beets -- to ethanol and protein feed (PF). Information

obtained in this study should provide valuable data on design, construction, and operation parameters for commercial ethanol plants set up to use fodder beets or similar biomass crops.

Novel continuous solid-phase and continuous diffusion fermentation systems were used in this study to process fodder beets. Research determined mass and energy balances, and costs associated with the processing of this crop into fuel ethanol and PF. The results obtained should be directly applicable to other high-yielding biomass crops, since they too can be processed through these systems.

The following were specific objectives of this study:

1) Optimize design and operation of each continuous fermentor in terms of material flow through and retention time.
2) Determine optimum levels for important fermentation parameters, including: feedstock particle or cube size, temperature, pH, potassium- or sodium-meta bisulfite, yeast inoculum size, fermentor capacity, and retention time.
3) Operate each fermentor utilizing optimum levels of each parameter listed in Objective 2. Determine mass and energy balances, and costs of ethanol production for each fermentor operated under these conditions.

LITERATURE REVIEW

During the past decade there has been a veritable explosion in the amount of information published concerning ethanol fuel. Every aspect of ethanol fuel production and use has been researched and documented as a result of the worldwide search for alternative energy sources. This information provides a solid foundation for investment decisions, as well as a basis upon which further research can be planned and conducted.

Due to this volume of information, any all-encompassing ethanol fuel literature review would be out of the question for a dissertation. In fact, such a review would be of sufficient magnitude to warrant publication in book form. Therefore, this literature review will focus primarily upon ethanol fuel use, alternate feedstocks, alternate processing technologies, and factors affecting yeast fermentation. These areas serve as a basis for the research results reported herein.

Ethanol Fuel Use

Ethanol can serve, alone or blended with gasoline or diesel fuel, as a fuel for internal combustion engines. When used by itself, either hydrated or anhydrous ethanol is satisfactory; however, minor engine modifications must be made to ensure optimum performance. These modifications include enlarging the carburetor jet size to richen the fuel/air ratio because ethanol contains less thermal energy per unit volume than gasoline, and modifying the

intake manifold to insure proper vaporization and distribution of the fuel (Anon, 1980)

The potential technical and economic advantages of using straight ethanol as a motor fuel were discussed by Kirik (1977). He also provided a brief history as to the fuel use of ethanol; experimental results from cars, trucks, and tractors running on ethanol; conversion of gasoline or diesel engines to running on ethanol; and design of true ethanol engines. Bolt (1954) and Andrews and Quick (1984) also provided general reviews regarding the production and use of ethanol as a motor fuel.

The use of straight alcohol in internal combustion engines was discussed by Earl (1984). Porter and Wiebe (1952) and Wiebe and Hummell (1954) investigated alcohol-water injection in automobile, truck, and tractor engines and reported their experimental findings. Wiebe (1954) also performed studies utilizing dual carburation with alcohol-water mixtures and alcohol blends.

Only anhydrous ethanol can be blended with gasoline, since hydrous ethanol will cause phase separation. This mixture, commonly called gasohol, will burn very efficiently in a normally aspirated, spark-ignition, internal combusion engine with no modifications. Allsup and Eccleston (1979) and Scheller (1980) each surveyed the use of ethanol, gasohol, and other ethanol-gasoline mixtures as motor fuels. Both ethanol and methanol were evaluated for use as fuels for modern cars by Keller (1980). The role of ethanol as an

anti-knock agent in automotive engines was examined by Porter and Wiebe (1952).

Experimental findings from extensive road tests using ethanol/gasoline blends in modern engines are reported by the Arizona Dept. of Transportation (Anon, 1982), Kaufman and Klosterman (1979), and Scheller (1980). Lichty and Phelps (1937) investigated emmissions from gasohol burning engines.

Diesel engines can operate on separately carbureted ethanol and diesel fuel (Cruz et al., 1982; Shropshire and Goering, 1982; Walker, 1984). The ratio of ethanol to diesel fuel is less than 25% when low quality diesel fuel is used, and 50% when the intent is to reduce "diesel smoke" and increase power (Panchapakesan, 1977). Ethanol has also been blended with vegetable oil and this combination used to directly replace diesel fuel (Faletti et al., 1984; Goering et al., 1983; Zubik et al., 1984).

Alternate Feedstocks

In 1984 the SDSU Fuel Alcohol Research Team published an extensive literature review in connection with a Title XII grant from the U.S. Agency for International Development (Dobbs et al.). That search was conducted in order to determine which sugar and starch crops showed the most promise for economical production of fuel ethanol in the northern Great Plains of the U.S. and climatically similar less developed countries.

This review indicated that fodder beets had the highest potential ethanol productivity, 5600-7500 L/hectare/yr, when

compared to other crops grown under similar conditions (Dobbs et al., 1984). Hills et al. (1981, 1983) recently completed agronomic trials which supported this conclusion. They obtained the following ethanol production levels per hectare per year: fodder beets 7572 L, sugar beets 6638 L, and sweet sorghum 5387 L. For reference, corn yields 1700-2000 L/hectare/yr (Gibbons and Westby, 1983b; Westby and Gibbons, 1982). These findings indicated that high yielding biomass crops, especially beets and sweet sorghum, should receive considerably more attention as ethanol fuel feedstocks.

In addition to yielding significantly more ethanol per hectare than starchy grain crops, these sugar crops (beets and sweet sorghum) are also advantageous ethanol feedstocks due to their carbohydrate composition (Hayes, 1981). They contain primarily sucrose, a simple disaccharide readily fermented by yeast. This eliminates the need for the energy intensive cooking-conversion step required for starchy feedstocks (Anon, 1980; Gibbons and Westby, 1983b; Stark et al., 1943b; Westby and Gibbons, 1982). This, in turn, reduces both capital and operating costs.

The fodder beet, although it is a relatively new crop to the U.S., should present farmers with no major problems agronomically. The fodder beet is closely related to the sugar beet and therefore similar soils, equipment, and farming practices can be used to grow and harvest each crop. Hayes (1981) provided more specific growing and harvesting information for fodder beets.

A number of different fodder beet varieties have and are being developed (Hayes, 1981). Desirable characteristics include good root yield, dry matter range of 17-18%, and sugar content of 13-14% or higher. The variety currently recommended by Hayes (1981) for fuel ethanol production is Monorosa. It possesses the following characteristics: root yield of 98 metric tons/hectare (43 tons/acre), 18.5% dry matter, 13.4% sugar, and 8385 L ethanol/hectare (886 gal/acre).

The fodder beet is well adapted to grow in most agricultural regions of the U.S. and in many foreign countries. It is a hardy, frost tolerant plant, resistant to most plant diseases. The primary disease affecting fodder beets is curly top virus, a disease spread by the beet leaf hopper. Sugar beets have been bred to resist this disease and plant breeders are now developing this resistance in fodder beet varieties (Hayes, 1981). Breeding programs are also attempting to increase sugar content by crossing fodder beets back with sugar beet varieties.

One of the major problems involved in the use of fodder beets is storage of this high moisture crop. Hayes (1981) suggests that the ideal storage pit is 5 m wide by 2-3 m high with the length determined by the cubic capacity required (1 m^3 will store 790 kg of fodder beets or 120-150 m^3 storage per hectare). The pits should be lined with plastic sheets and straw, there should be openings every 2 m along the length of the pit to permit heat loss, and the temperature should be maintained at 2-7^{o}C. Hayes (1981) states that

by using this storage system, beet storage for 8-9 months should be possible with minimal sugar or weight loss.

The two main factors affecting fodder beet storage are temperature and relative humidity. A study by Andales et al. (1980) was conducted to establish relationships between the individual and combined effects of temperature and relative humidity on the weight and sugar losses of beets in long-term storage. Beet samples were stored at various temperatures and humidities, and samples for weight and sugar analysis were taken during the storage period of 15 weeks.

From the results, relative humidity levels were found to have a highly significant effect on weight loss whereas temperature effects were not significant. Weight loss was higher with low relative humidity (80-85%) than with high relative humidity (95-100%). Temperature was found to have a highly significant effect on sucrose loss while humidity had little influence on it. Sucrose loss increased as the temperature was increased. Therefore it was concluded that no correlation exists between weight loss and sucrose loss as affected by temperature and relative humidity, i.e., beets can suffer weight loss during storage without losing sugar. Andales et al. (1980) further stated that in a beet storage system it is highly advisable to maintain high relative humidity as much as possible together with the optimum temperature.

The major concern in fodder beet storage is, of course, sugar loss. Reported estimates of sucrose loss using various

existing, long-term, fodder beet storage methods range from 0.087 - 0.449 kg per metric ton per day. These correspond to a sugar shrink of about 6-31% for a storage period of 100 days (29,62,98).

Wyse (1973) stated that among the many factors influencing sucrose loss, respiration has been found to be responsible for 60-70% during long-term storge. The two main factors affecting respiration in conventional beet storage are the initial condition of the beets entering storage (i.e. beet variety, handling damage, cultural practices, etc.) and the storage atmosphere (i.e., temperature and relative humidity) (Akeson, 1973; Andales et al., 1980; Pack, 1926; Wyse, 1973).

Alternate Processing Technologies

Utilizing fodder beets (or other similar biomass crops) for fuel ethanol production creates special processing problems not encountered when grain is used as the feedstock. The high moisture-high fiber content of these alternate feedstocks results in increased viscosity of beer pulps which impaires pumping and agitation (Ziobro and Williams, 1982). Therefore in order to use conventional fermentors, pulps must be diluted with water to reduce viscosity, and this in turn reduces beer ethanol concentrations and increases energy consumption and costs. Most alternate processing technologies avoid these difficulties by separating sugar or ethanol from solids either before, during, or after fermentation.

Development of the Continuous Solid-Phase Fermentation Process:

One way to process fodder beets is to separate ethanol from pulp after fermentation. Due to the aforementioned problems of handling liquid slurries of beet pulp, the only feasible option is to ferment the beets as a moist heap -- i.e., solid-phase fermentation. This type of fermentation has the following advantages according to Hayes (1981): no need for nutrient addition, no need for beet sterilization, no need for expensive sugar extraction equipment, lower capital cost than liquid phase fermentation (reduction in fermentor volume), lower production cost, less stillage for disposal, and less energy for distillation.

Solid-phase fermentation was defined by Mukhopadhyay and Pathak (1973), as any fermentation in which microorganisms act directly on a moist, solid substrate. Solid-phase fermentations have been used to produce microbial enzymes, aflatoxins, methane, animal feeds, vinegar, spores, and human foodstuffs (Aidoo et al., 1982; Hesseltine, 1977; Ralph, 1975). Aidoo et al. (1982) provided an excellent review of solid-phase fermentation and predicted its expanded use in biomass conversion to ethanol.

Solid-phase fermentation was initially used for fuel ethanol production by Kirby and Mardon (1980b) who noted that yeast could ferment sugars in pulped crops without the need for prior extraction of the sugar or agitation of the pulp. The solid-phase reactions proceeded even faster then those in the liquid phase, and the ethanol yield was essentially the same.

In their (Kirby and Mardon, 1980 a and b) laboratory scale research (1 kg beet pulp per trial), washed beets were first mechanically chopped or pulped into 3-5 mm particles. Sulfuric acid was then added to give a pH of 4.5 and a 10% dry weight (DW) suspension of dry yeast in water was added to the pulp (9 g DW/L pulp). The solid pulp was then fermented anaerobically at 25-30°C without agitation (except for that required for cooling).

Fermentation was complete in 16 h and the pulp was then pressed to separate the fiber and juice. They found that the first press removed 67% of the ethanol and that 95% could be removed by pressing twice with a small interstage wash (15% of the weight of the pulp processed). The combined liquors had an ethanol concentration of 9% (wt/wt) and contained 85% of the yeast. This juice was then centrifuged to separate the yeast (which was then recycled) from the beer (containing 9.5% wt/wt or 11.7 v/v ethanol which was then distilled). The pressed beet pulp could be used as an animal feed or subjected to anaerobic digestion with the stillage to generate methane gas. Using this process Kirby and Mardon (1980 a and b) obtained overall ethanol yields of 92% of the theoretical. The efficiency of conversion of sucrose to ethanol by fermentation with yeast was about 95% of theory, and the energy conversion was 97.5%.

In further work, Kirby and Mardon (1980a) showed that beet particle size was not a critical factor. Beet cube sizes of 0.5-0.75 mm, 1 mm, and 3 mm produced the same results, i.e., the

fermentation rate was the same, thus sugar diffusion did not limit the rate of fermentation over this particle size range. They also noted that the preferred yeast concentration of 9 g DW/L pulp should cover 75-100% of the unruptured parenchyma cells wall. In practice, ruptured cell walls also provide a surface for yeast cell deposition, decreasing the distribution, possibly, to 25-35%. Regardless of this, during fermentation the yeast cells multiply and evenly distribute themselves throughout the pulp.

Kirby and Mardon (1980a) provided an explanation for the cellular mechanics of both sugar and ethanol diffusion. They noted that the controlled continuous transfer of the concentrated sugar solution in the beet cells to the yeast cells would seem largely to overcome substrate inhibition. Counter-diffusion of ethanol back to the beet cells would be expected to swell the cell wall, thus allowing an increase in the rate of sugar diffusion to compensate for reduction in sugar concentration. This would also decrease ethanol inhibition of yeast and at the same time help explain the ease with which ethanol is pressed from the beet.

On the basis of their previous laboratory-scale research, Kirby and Mardon (1980 a and b) developed the CSIRO process, designed to process fodder beets to ethanol via solid-phase fermentation. In their process, large conventional fermentation tanks were envisioned as solid phase fermentors. Agitation was to be provided by helical screw pumps and vertical screw mixers.

Following our pilot plant experience with processing fodder beets (Gibbons and Westby, 1983c), we realized that Kirby and Mardons expectations (that conventional fermentors would work) were too optimistic. We found that mixing and material flow could not be adequately performed in a large fermentation tank. This in turn, prevented regulation and control of fermentation parameters such as temperature and pH (Ziobro and Williams, 1982). Even if this type of fermentor would work, the numerous pumps and motors for agitation would require large amounts of energy and operating and maintenance costs would be high.

The continuous solid-phase fermentation system we have designed eliminates these material handling and temperature control problems by utilizing a tubular system with one motor to continuously mix fermenting pulp and convey it through the fermentor. This system should significantly reduce ethanol production costs and increase the energy balance compared to submerged fermentation processes.

Development of the Continuous Diffusion Fermentation Process:

Diffusion cooking, a process used for decades by the sugar industry to extract sugar from beet pulp (Silin, 1957), was another technology proposed for fuel ethanol production from beets. In diffusion cooking, sugar diffuses out of the beet cell into warm water. The rate of sugar transferance (i.e., efficiency of diffusion) depends upon the area of cell wall exposed, the ease with which sugar passes through the cell wall, the difference in density

between the juice within the cell and that without, and temperature (Silin, 1957). Temperatures of 75-83°C are commonly used to increase the diffusion rate. Heat denatures the beet cell walls, permitting a more rapid extraction of the soluble sugar (Hayes, 1981). Diffusion cookers can be operated in either a batch or continuous mode.

Silin (1957) discussed operational aspects of a battery (batch) diffusion plant including design, operations, and theories. He also discussed the technology of battery diffusion and noted that in order to remove sugar from the beets with the least amount of water, the diffusion battery should be operated so that in reality the beets pass through in one direction and the water in another (i.e., counter-current).

Once the theory of counter-current diffusion was established, the next logical step was the development of a truly continuous diffusion cooker. In continuous diffusion cooking the beets are first sliced into thin pieces called cossettes (as is the case in battery diffusers). However instead of transferring beet slices batchwise, from tank to tank, the beets pass continually up against a downward flow of hot water (60-82°C) by means of twin perforated scroll flights. In this way the beet cossettes exiting the top of the cooker are practically sugar free and the hot liquid leaving the bottom of the cooker contains 12-16% (wt/wt) sugar. The beet cossettes (95% moisture) are then dewatered in a screw press and the sugar solution is cooled, inoculated with yeast, and fermented

(Hayes, 1981). Kirby and Mardon (1980a) also provide details and a diagram of such a process.

A major problem with traditional diffusion cooking is the limited sugar concentration in the diffusion liquid (Silin, 1957). Sugar concentrations of 12-16% (wt/wt) result in beers with only 6-8% (v/v) ethanol and this in near the lower limit for being distillably worthwhile (Hayes, 1981; Kirby and Mardon, 1980a). A solution to this problem is the use of hyperfiltration to concentrate the sugar in the thin juice to about 20%.

An article by Nielsen and Kristenson (1981) described a new thin film composite membrane that is chemical and temperature resistant and can work at temperatures of 60-80°C where there are no bacteriological problems. Hayes (1981) has also discussed non-fouling ultrafiltration membranes that operate at 50-100 psig, and concentrate sucrose to 30-35% or glucose to 20-25%. However, the costs for concentrating the sugar, as well as for the diffusion process itself, may make this system too costly for fuel ethanol production.

In 1979, Rolz et al. described the EX-FERM process for sugar cane fermentation. In laboratory scale experiments they mixed cane chips and water, pasteurized and cooled the mixture, then added yeast and fermented the mixture to ethanol. Spent cane chips were then removed, fresh chips added and the cycle was repeated. At least two more cycles were requried to reach an ethanol concentration of 10-11% (v/v).

Rolz (1981) noted three advantages of the EX-FERM process over conventional processes. These were a greater ethanol yield per metric ton of feedstock due to a more complete extraction of sugar, reduced need for added nutrients since proteolytic enzymes of yeast allow them to utilize the organic nitrogen present in the feedstock, and reduction in required fermentation capacity since diffusion and fermentation occur simultaneously in the same vessel. Rolz (1980) also noted that this process could be used for sweet sorghum and other hard to process biomass crops (sugar or fodder beets).

The main technical problem with the EX-FERM concept is the need to repeatedly add and then remove feedstock pieces from the fermenting broth until a high ethanol concentration is reached. Er-el et al. (1981) encountered this material handling problem when they used a pilot plant scale drum fermentor to ferment sugar cane via this process. de Cabrera et al. (1982) developed a packed-bed fermentation system to at least partially overcome this problem.

The continuous diffusion fermentation process developed at SDSU makes use of concepts embodied in both the diffusion cooking and EX-FERM processes (Gibbons and Westby, 1983). In this system, the material handling problems of the EX-FERM process are eliminated by utilizing a simple auger system similar to those used in diffusion cooking. However, the yeast-water-cube fermentation slurry employed in the EX-FERM design is used instead of the separate diffusion and fermentation steps of diffusion cooking. In the SDSU system, therefore, sugar is constantly extracted from feedstock

pieces and fermented to 8-10% ethanol beer suitable for distillation by using a simple auger system which simultaneously ferments and conveys feedstocks pieces against a flow of water and yeast cells. We theorized that this system would markedly reduce ethanol production costs and significantly increase the energy balance when compared to conventional submerged fermentation processes.

Factors Affecting Yeast Fermentation

General Information:

The alcohol fermentation industry is an outgrowth of what may be the oldest chemical process carried out by mankind (Stark, 1954). The original use of alcoholic fermentation was, of course, for preserving fruit juices, and man's first volitional use of this fermentation is lost in the pages of antiquity. Later, the fermentation was adapted to the preservation of fermented grain beverages and then distilled beverages. More recently, fermentation alcohol has been utilized as a fuel for internal combustion engines.

As a process, the fermentation of carbohydrates for the production of alcohol is dependent to a large extent on the concentration of raw materials and products in the mash or beer. Stark (1954) noted that the initial concentration of sugar in the mash governs both the final alcohol concentration and heat release per unit volume. It is, therefore, necessary to employ a sugar concentration that will not potentially result in an alcohol concentration in excess of the practical alcohol tolerance of the yeast strain. However, an unnecessarily dilute mash increases the steam

consumption for distillation and byproduct recovery and reduces plant capacity. Therefore the alcohol tolerance of the yeast is one of its most important characteristics.

However, the maximum alcohol concentration at which growth will occur is of less importance than the effect of lower alcohol concentrations on the fermentation rate (Stark, 1954). It is impractical to ferment at carbohydrate concentrations equivalent to alcohol at the maximum tolerance of the yeast strain, since the growth and fermentation rate is negligible at that point. Therefore, it is desirable to determine the fermentation rate of yeast strains at various alcohol concentrations.

The selection of suitable strains may be accomplished by means of indirect physiological studies of alcohol tolerance (Brown et al., 1981; Gray, 1941; Jimenez and Van Uden, 1985; Luong, 1985; Nosiro and Ouchi, 1962), sugar tolerance (Casey et al., 1984; Converti et al., 1985; Moulin et al., 1980), and growth and fermentation rates (Brown et al., 1981; Gray, 1941; Jones et al., 1981; Nosiro and Ouchi, 1962; Troyer, 1953). A more direct and time-saving practice is to conduct laboratory test fermentations on grain mashes (Gibbons and Westby, 1983b; Stark et al., 1943a). This results in an accurate evaluation of the yeast strain under simulated plant conditions. In general, the criteria of good distillery yeasts are rapid growth, high alcohol and sugar tolerance, efficiency in the conversion of the carbohydrates of grain mashes to alcohol, a maximum growth temperature of at least 32^{o}C, and general

hardiness to fairly extreme changes in environmental conditions, such as pH, temperature, and osmotic pressure (Jones et al., 1981; Stark, 1954).

Yeast Propagation:

The propagation of yeast for use in an alcohol fermentation plant generally involves the buildup of a large inoculum of cells over a two to three day period (Gibbons, 1982; Gibbons and Westby, 1983b; Stark 1954; Westby and Gibbons, 1982). Cultures of yeast for plant use are usually maintained in the laboratory on malt extract-agar slants. Stock cultures are commonly transferred at monthly intervals and, after incubation to secure good growth, are stored in a refrigerator. Colonies picked from these plates are then used to prepare an adequate volume of actively growing yeast culture for the plant fermentors. This is accomplished by means of serial transfers into progressively larger vessels. It is customary to use 2-5% by volume inoculum and to incubate the first two transfers at 30°C for 24 h. The final laboratory culture is used after 18 h of incubation when the culture is at peak activity. This final volume of inoculum should represent 1-3% by volume of the plant fermentor capacity.

Contamination Control:

In order to avoid contamination problems in fermentation vessels, the yeast inoculum must be propagated under strict aseptic conditions. This aim is readily achievable by using standard microbiological techniques (Costilow, 1981). In some systems, however, yeast cells are separated following fermentation and re-introduced

into fresh mash. This recycling of yeast eliminates the need for new yeast propagation, however it brings with it the increased likelyhood of contaminant buildup in the fermentors.

In order to circumvent this problem several researchers have proposed treatments to "sterilize" the separated yeast prior to re-introduction into fresh mash. Both Kirov and Leshchinskaya (1937) and Kvasnikov (1937) have noted that yeasts are not injured seriously by amounts of chlorine that would kill bacteria and that yeast will withstand relatively high acidities. They further suggested that yeast cream from the separator could be dosed with mineral acids of pH 2-3 for the purpose of inhibiting bacterial growth, if the time of treatment was not too long.

Many chemicals have differing effects upon yeast and bacteria, and these differences can be used advantageously to control or prevent contamination. Loveless et al. (1954) surveyed the effects of various chemicals on yeast and bacterial growth. Claassen (1926) found that 0.35% sulfur dioxide in raw molasses was not harmful to either yeast yield or quality. Wick (1979) noted that the addition of 125 parts per million (ppm) of potassium meta bisulfite to the mash generated sulfur dioxide gas which inhibited bacterial growth during fermentation. Schimz (1980) and Anacleto and Van Uden (1982) have also studied the effects of sulfite on yeast.

However, regardless of treatments or precautionary measures, there may arise some level of contamination in the fermentors. The Seagram group (Stark, 1954) found that 10,000 bacteria per ml

represented a level below which contamination was not a yield factor. In addition to this fact, Tenney (1954) has observed that mash has a natural resistance to many infectious microorganisms. Its low pH (4.0-5.0) is too acid for the development of most bacteria, and as the yeast rapidly consume the nutrients, denying them to less vigorous competing organisms, the alcohol and carbon dioxide formed also exert a preservative effect and the pH drops even lower. Tenney (1954) determined that lactic and acetic acid bacteria were the main contaminants in mash, however their effects were not noted unless extremely high levels of contamination existed. Haas (1960) and Bartholomew et al. (1974) discussed factors affecting contaminant control in industrial fermentation plants.

Contamination is, however, potentially a more serious problem in continuous, as opposed to batch, fermentations. This is because the long operating periods for which continuous fermentation is designed make it more liable to the occassional introduction of undesirable organisms (Hough and Button, 1972). The likelihood of serious bacterial and wild yeast infection in a continuous fermentor is determined by the nature of the contaminating organism, the number of contaminating organisms introduced, and the part of the process where infection takes place. For an infection to be serious, the foreign microorganisms must be present either in large numbers or have a considerable biological advantage over the primary organisms being cultivated (Hough and Button, 1972).

There are three possibilities with respect to the growth rate of foreign organisms in a contaminated fermentation. This growth rate can be greater than, equal to, or less than the dilution rate. If the growth rate of the contaminant is higher than the dilution rate, after a period of time the original organism will be entirely replaced by the contaminating organism. This will also eventually happen in the case where the growth rate of the foreign organism is equal to the dilution rate, but only if the foreign organism can in some way inhibit growth of the desired organism. Otherwise this situation will simply result in a mixed culture. If the growth rate of the contaminant is less than the dilution rate it will be washed from the culture. Contamination by such an organism will become serious only if its rate of entry is extremely high and its growth rate only slighly less than the dilution rate.

RESEARCH DESIGN

Framework for Ethanol Fuel Research at SDSU -- A Team Concept Utilizing a Systems Approach

Since 1979, scientists at SDSU have investigated various technical and economic factors associated with fuel ethanol production and use. The disciplines involved included Microbiology, Plant Science, Agricultural and Mechanical Engineering, Economics, and Dairy Science. A systems approach has been used throughout this multidisciplinary effort.

This systems framework has linked economic and technical studies in a continuous, interactive process (T.L. Dobbs, 1983, personal communication). Economic projections, for example, generate future crop production costs and fuel prices, and thereby suggest certain crops which might have economic potential for ethanol production. Technical studies of production, processing, and utilization of the fuel and feed byproducts from the selected crops are then conducted. Technical findings can be utilized in economic feasibility analyses from which alternative crops or technology refinements may be suggested. These results guide the next round of technical studies.

The systems framework used for conducting this research is diagrammed in Figure 1 (T.L. Dobbs, 1983, personal communication). Feedstock crop production requirements, yields, storability, and ethanol content potentials were examined in the agronomic subsystem. Alternative technologies for converting the crop products into fuel

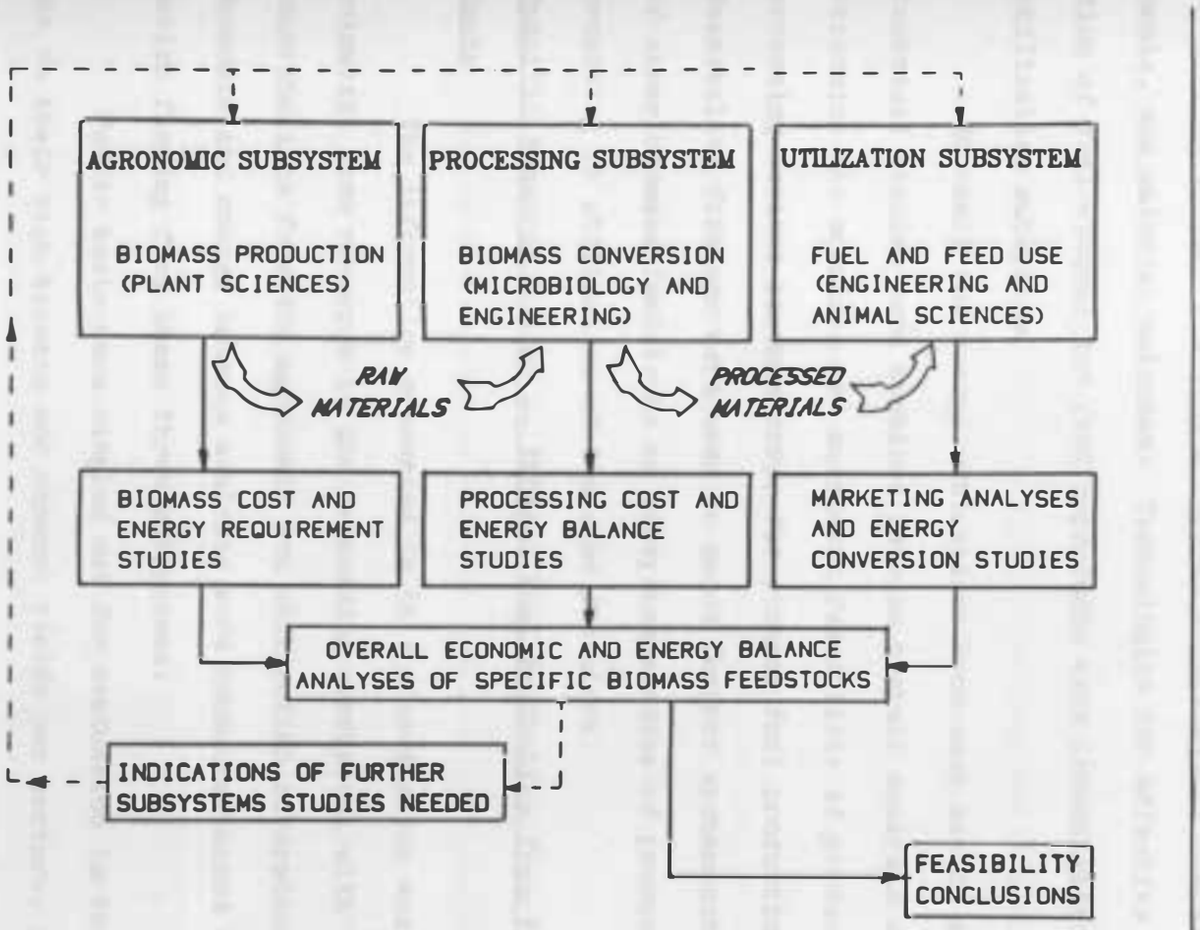

Figure 1. Systems framework for SDSU ethanol fuel research.

ethanol and usable byproducts were explored in the processing subsystem, as were processing yields, efficiencies, energy requirements, and material balances. Technologies for effective utilization of fuel ethanol and feed byproducts were investigated in the utilization subsystem.

Economic and energy evaluations from each set of subsystem technical studies were combined into an overall analysis designed to determine the economic and energetic feasibility of producing and procesing various biomass crops for ethanol fuel production. Feasibility findings were used to guide further system-wide studies of other biomass feedstocks or subsystem studies of production, proessing or utilization of the same feedstock.

Specific Research Design -- Ethanol Fuel Production from Fodder Beets

The information presented in this dissertation was derived primarily from research in the processing subsystem, with outside contributions from the agronomic and utilization subsystems. Economic and energy balance analyses were conducted based on information flowing from these three subsystems.

Fodder beets were singled out for evaluation in this study due to their high biomass and ethanol yields per hectare, potentially low production/processing costs and valuable coproducts (Dobbs et al., 1984a; Hills et al., 1981; Hills et al., 1983). Companion research was, however, simultaneously conducted with sweet sorghum due to its high yields and greater ease of incorporation into

current farming practices. Sweet sorghum results have been or will be published elsewhere (Gibbons and Westby, 1983c; Gibbons et al., 1986; Westby and Gibbons, 1984).

Since the major factor limiting ethanol production from fodder beets was the lack of an efficient and economical processing technology, this study evaluated two novel means of converting fodder beets to ethanol. These were solid-phase fermentation and diffusion fermentation. These processes represent unique adaptions of existing technologies for the purpose of producing ethanol from high moisture-high fiber feedstocks. The continuous fermentors and fermentation systems developed at SDSU for each process were novel in fermentation science and represent leading edge technology in this area.

For each fermentation process, research was conducted in three inter-related steps. The first step involved design, construction, and preliminary operation of each fermentor. The purpose here was to clearly demonstrate that each fermentor would, or could with modifications, work as expected. Of primary concern here were material flow characteristics of the fermentors and prospects for controlling contamination in them. Design and structural modifications to the fermentors were made when necessary to obtain satisfactory performance.

In the second step, various aspects of fermentor operation were individually analyzed in laboratory scale batch fermentation trials. Here optimum feedstock particles sizes, yeast inoculum

levels, and levels of various anti-bacterial chemicals for the particular fermentation system under investigation were determined.

The third step consisted of operating the larger scale continuous fermentors, using optimum levels of the individual fermentation parameters determined in step two. These large scale runs were designed to simulate actual conditions in a commercial plant. Data was collected so that energy and mass balances, and cost/return economic projections could be calculated.

MATERIALS AND METHODS

Fodder Beet Sources and Storage

Fodder beets (Beta vulgaris var. Monorosa) used in this research project were obtained from four sources, including: Mr. LaRue Sutliff (Pennsylvania), Dr. Dwayne Beck (Central Research Station, Highmore, SD), Mr. Quentin Kingsley (Northeast Research Station, Watertown, SD), and myself (SDSU Agronomy Farm). Whole fodder beets, minus the tops, were stored frozen until use. For fermentation trials, topped beets were first thawed, and then washed to remove dirt.

Microorganism, Culture Maintenance, and Inoculum Preparation

Saccharomyces cerevisiae NRRL Y-2034, used in all fermentation trials reported herein, was maintained on Difco potato dextrose agar and cultures were stored at 4°C. Inoculum for laboratory-scale trials was prepared by growing S. cerevisiae cells on a medium consisting of 5% glucose and 0.5% each of Difco neopeptone, Difco yeast extract, and Difco malt extract. Following static incubation at 30°C for 24 h, the inoculum (20,30,60, or 100 ml quantities) contained 0.5 - 1.0 x 10^8 cells/ml.

For large-scale trials in our conventional or novel fermentors, 100 ml yeast cultures were used to inoculate 5 or 19 L carboys of media consisting of 5% glucose and 0.5% yeast extract. Following static incubation at 30°C for 24 h, the inoculum contained 0.6 - 1.0 x 10^8 cells/ml.

Conventional Submerged Fermentation

Batch Fermentation Trials:

Fodder beets were sliced and loaded into a 1 KW hammermill fitted with a 1.27 cm sieve screen. The resulting ground fodder beet pulp (similar in consistency to mashed potatoes) was then weighed and 300-500 kg was loaded into a 1,300 L stainless steel tank previously filled with tap water (200-500 L). The design of this tank has been described elsewhere (Gibbons, 1982; Westby and Gibbons, 1982). The mash, with constant agitation at 40 rpm, was then heated to 90°C and held for 1 h to achieve pasteurization. At this point the mash was a homogeneous slurry of finely ground beet solids in water. Subsequently the mash was cooled to 28°C and the pH was adjusted to 4.0 by adding 36N H_2SO_4 (0.5-0.8 ml/L mash).

A 5 L inoculum of Saccharomyces cerevisiae NRRL Y-2034 was added to the homogeneous mash. Inoculated batches were incubated 48-72 h with constant agitation at 28-32°C. Concentrated NH_4OH (0.3-0.5 ml/L mash) was added at 18 h fermentation to provide sufficient nitrogen and to prevent the pH from falling below 3.4.

Distillation:

Fermented beer was distilled using a two column distillation apparatus fabricated by Arlon Industries (Sheldon, IA). The design of this column has been described elsewhere (Stampe, 1982). The products of distillation were 95% ethanol and stillage produced at rates of 83 and 830 L/h, respectively.

Centrifugation:

Separation of liquid from particulate material in stillage obtained from beet mashes was accomplished by centrifugation with a Sharples model P660 centrifuge. A bowl speed of 4326 rpm and a screw conveyor speed of 4314 rpm were used (Westby and Gibbons, 1982). Stillage was pumped into the feed nozzle of the centrifuge at a rate of 830 L/h. The products of stillage centrifugation were protein feed (PF) and stillage supernant (thin stillage).

Solid-Phase Fermentation

Batch Fermentation Trials:

To determine the optimum pulp grind size, fodder beets were sliced and passed through a hammermill fitted with screens of either 0.476, 0.635, 0.953, 1.270, or 1.905 cm (larger or smaller screens were not available). One Kg quantities of fodder beet pulp were placed in 4 L stainless steel containers, and pulp pH was adjusted to pH 3.0 by mixing in 1.4 - 1.8 ml 36N H_2SO_4/Kg pulp. Following pH adjustment, pulp was thoroughly inoculated with 100 ml of yeast inoculum. Inoculated beet pulp was incubated at 30°C for 96 h with periodic agitation. Duplicate runs at each screen size were performed and results averaged.

To evaluate the effects of yeast inoculum size, beets were first sliced and hammermilled (1.27 cm screen) and the pulp was adjusted to pH 3.0, as above, in 4 L containers. One Kg amounts of acidified beet pulp were thoroughly inoculated with 0.1, 0.5, 1.0, 5.0, 10.0, 50.0, 100.0, or 200.0 g of yeast inocula broth.

Inoculated pulp was incubated at 30°C for 96 h with periodic agitation. Duplicate runs were again performed and results averaged.

The optimum pulp pH was determined in a series of duplicate trials using hammermilled beet pulp (1.27 cm screen). Pulp pH was adjusted to the desired level by adding various amounts of 36N H_2SO_4 to each Kg of pulp in 4 L stainless steel containers. Acid useage and the corresponding pH levels were as follows: 5.3 ml (pH 1.5), 3.1 ml (pH 2.0), 2.2 ml (pH 2.5), 1.6 ml (pH 3.0), 1.2 ml (pH 3.5), 0.8 ml (pH 4.0), 0.5 ml (pH 4.5), 0.2 ml (pH 5.0), and 0 ml (pH 6.5). After acidification, pulp was inoculated with 100 ml of inoculum and pulp was incubated at 30°C for 96 h with periodic agitation. Duplicate runs were performed and results averaged.

Continuous Fermentation Trials:

The continuous, solid-phase fermentation device used in our plant is shown in Figure 2. It was constructed entirely of mild steel. The important components are: 1) a hammermill to pulp the fodder beets, 2) a non-ported, steam pasteurization chamber to destroy bacterial contaminants in fodder beet pulp that limit or prevent yeast fermentation, 3) a yeast inoculation port, and 4) a 15.25 cm diameter by 470 cm long horizontal auger tube and flighting that simultaneously conveys and mixes the fermenting pulp. The auger flighting can either be turned manually (as was the case in our experiments) or with a slow speed motor. Prior to each new run, the pasteurization chamber and auger of the fermentor were steamed

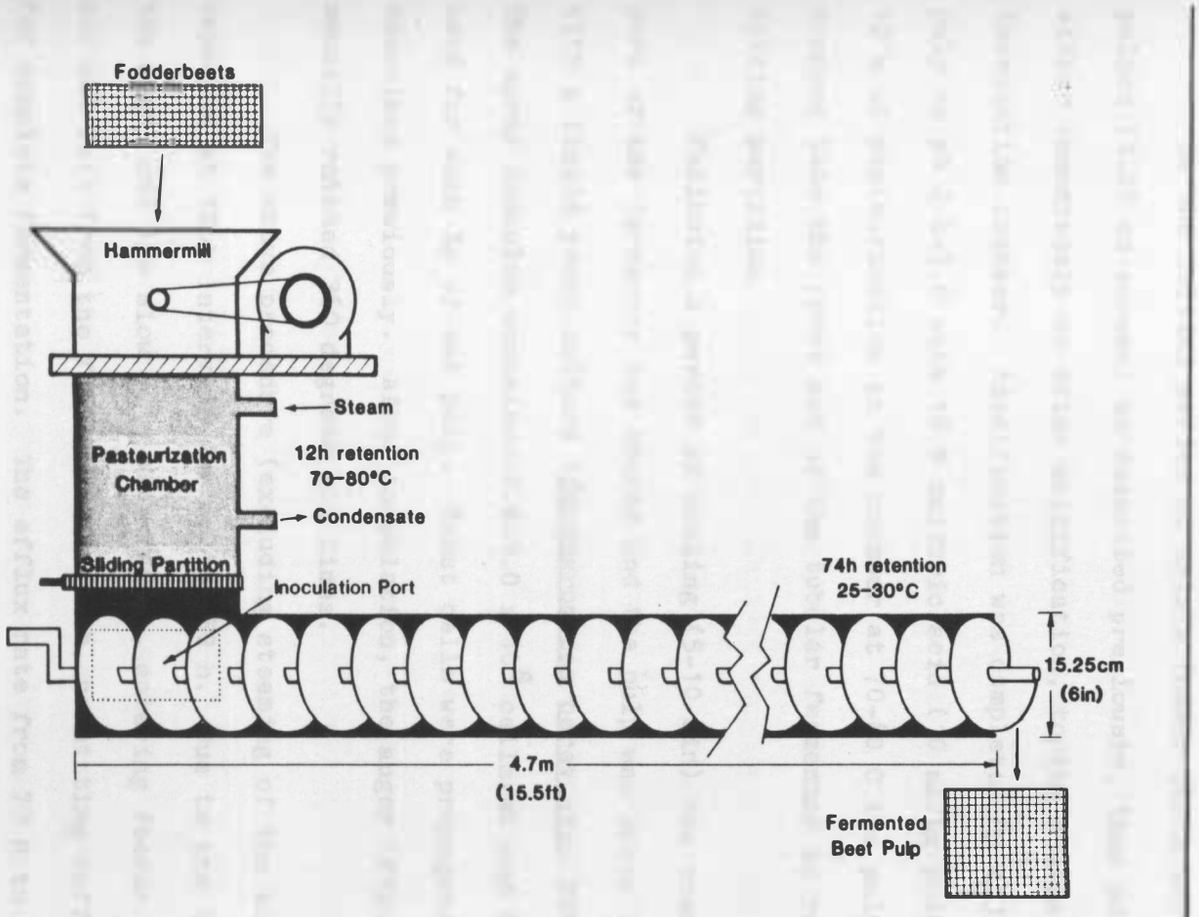

Figure 2. Continuous farm-scale, solid-phase fermentor for fodder beets and other tuberous feedstocks.

for 12-18 h to kill spoilage bacteria that may have been left over from the previous run.

In the initial series of trials fodder beets were sliced and pulped (1.27 cm screen) as described previously, then added (3 kg), either immediately or after acidification, to the steamed pasteurization chamber. Acidification was completed by adjusting the pulp to pH 2.0-3.0 with 18 N sulfuric acid (10 ml/kg pulp). After 12 h of pasteurization in the chamber at 70-80 C the pulp was dropped into the front end of the tubular fermentor by removal of a sliding partition.

Following a period of cooling (5-10 min) the inoculation port of the fermentor was opened and the pulp was spray inoculated with a liquid yeast culture (Saccharomyces cerevisiae NRRL Y-2034). The spray inoculum contained 0.6-1.0 x 10^8 cells/ml and 90 ml was used for each Kg of wet pulp. Yeast cells were propagated as described previously. After inoculation, the auger (Fig. 2) was manually rotated 360 degrees six times.

The above procedure (excluding steaming of the auger) was repeated at 12 h intervals for up to 350 h. Due to the length of the auger and its slow rate of rotation, entering fodder beet pulp did not exit from the fermentor for 72 h, permitting sufficient time for complete fermentation. The efflux rate from 72 h to the end of the run was 0.23-0.25 kg wet pulp/h.

Results from this initial series of continuous, solid-phase fermentation trials suggested several design/operational

modifications that would improve operation of the fermentor. One such modification was to jacket the auger tube with a 21.59 cm diameter by 396 cm long plastic heating/cooling shell. To maintain proper fermentation temperature (30°C) this shell was connected by 1.27 cm plastic hoses to a 19.5 L, Blue Line water bath (Blue M Electric Co., Chicago, IL). A 0.25 KW variable speed electric pump circulated water from the water bath through the fermentor jacket and back to the water bath (4 L/min).

The other major modification was to eliminate the pasteurization step and simply acidify pulp for contaminant control. Hence, for operation of the fermentor in the remaining trials, beet pulp was first mixed with sulfuric acid (2.1 ml 36N H_2SO_4/Kg pulp) to reduce pulp pH to 3.0 to 3.3. Acidified pulp was then inoculated by manually mixing in a yeast broth (100 ml inoculum/Kg acidified pulp). Inoculated pulp was loaded into the fermentor and the auger flighting was manually turned as before. This cycle was repeated at 12 h intervals, for up to 400 h.

Diffusion Fermentation

Batch Fermentation Trials:

To determine the optimum cube size to use for diffusion fermentation, fodder beets were sliced into square cubes measuring 0.64, 1.27, 1.91, 2.54, 3.18, or 3.81 cm along each side. Duplicate trials for each cube size consisted of a slurry made up of 1 L water, 1 Kg beet cubes, 5.0 g potassium meta bisulfite (PMB, used for contamination control), and 20 ml yeast inoculum. All

fermentations were incubated in 4 L stainless steel containers at 30°C, with periodic agitation, for 96 h.

To evaluate the effectiveness of PMB and sodium meta bisulfite (SMB) in controlling contamination during batch diffusion fermentation, fodder beets were first sliced into cubes of 1.27-1.91 cm. Four liter containers were then filled with 1 L water, 1 Kg beet cubes, 20 ml yeast inoculum, and 0-8 gm of either PMB or SMB. In the SMB trials the yeast inoculum contained only $4 - 6 \times 10^7$ cells/ml instead of the usual $0.5 - 1.0 \times 10^8$ cells/ml. Reported PMB and SMB concentrations were based upon the total final weight before fermentation, which was approximately 2 Kg. All trials were fermented at 30°C, with periodic agitation, for 96 h. Duplicate trials were performed for each concentration and results were averaged.

The use of low pH to control contaminants was tested in a similar series of trials, instead that here, 0-8 ml of 36N H_2SO_4 was added to each container instead of PMB or SMB. Nine pH (termed initial pH) levels were set up corresponding to the addition (before yeast inoculation) of the following amounts of H_2SO_4: 0 ml (pH 6.42), 0.5 ml (pH 4.23), 1.0 ml (pH 2.84), 1.5 ml (pH 2.38), 2.0 ml (pH 2.35), 3.0 ml (pH 2.05), 4.0 ml (pH 1.93), 6.0 ml (pH 1.73), and 8.0 ml (pH 1.65). Fermentations were at 30°C, with periodic agitation, for 96 h, and duplicate trials at each pH level were performed.

Sequential Batch Fermentation Trials:

A series of sequential fermentation trials were performed to establish the long-term, individual effectiveness of PMB and SMB in controlling contamination. Fermentations here were conducted in 20 L stainless steel containers and beet cube/liquid slurries were incubated at 30°C for 72-144 h with periodic agitation at sampling times. Each fermentation series consisted of five individual batches, in which liquid, was transferred sequentially to a fresh batch of cubes after fermentation of the previous batch had ended. No fresh water was added after the first batch had been prepared.

In all cases the first batch of the series consisted of 3 Kg tap water, 3 Kg beet cubes (1.27-1.91 cm), 15 g PMB (approximately 0.25%, wt/wt), or 12 g SMB (approx. 0.20%, wt/wt), and 60 ml yeast inoculum. After fermentation, cubes were strained from the liquid, the liquid was weighed, and an equivalent weight of fresh cubes was added. No additional yeast inoculum was required since the liquid contained yeast from the previous batch. The batch was then allowed to ferment to completion and the process was repeated until five sequential batches of beet cubes had been fermented in the same liquid.

Four different fermentation series were conducted for both PMB and SMB. In the first, no additional PMB or SMB was added to batches 2-5. This series was termed 0% PMB (or SMB) makeup. In the second series 0.125% (wt/wt) fresh PMB (or 0.10% fresh SMB) was added to batches 2-5 and this series was called 50% PMB (or SMB)

makeup. In the third and fourth series 0.188% PMB (or 0.15% SMB) and 0.25% PMB (or 0.20% SMB) were added to batches 2-5 and these series were termed 75% and 100% PMB (or SMB) makeup, respectively.

Two similar fermentation series (5 batches per series) were conducted to determine the long-term effectiveness of low pH in controlling contaminants. In these cases the first batch of each series consisted of 1.5 Kg tap water and 1.5 Kg beet cubes (3 Kg total). To this was added 4.5 ml 36N H_2SO_4, and 30 ml yeast inoculum. After fermentation, cubes were strained from the liquid, the liquid was weighed, and an equivalent weight of fresh cubes was added. As before, no additional yeast inoculum was required. The batch was then allowed to ferment to completion and the process was repeated until five sequential batches of beet cubes had been fermented in the same liquid.

Two different fermentation series were conducted. In the first, no additional H_2SO_4 was added to batches 2-5. This series was termed 0% H_2SO_4 makeup, and as a result of repeated beet cube additions with no supplemental H_2SO_4, the pH level gradually increased. In the second series 3.5 ml 36N H_2SO_4 was added to batches 2 through 5 and this series was called 78% H_2SO_4 makeup. Here a relatively constant pH level was maintained.

Continuous Fermentation Trials:

The semi-continuous diffusion fermentor used in the SDSU plant is shown in Figure 3. It was constructed entirely of mild steel and consisted of a diagonally oriented auger tube with a

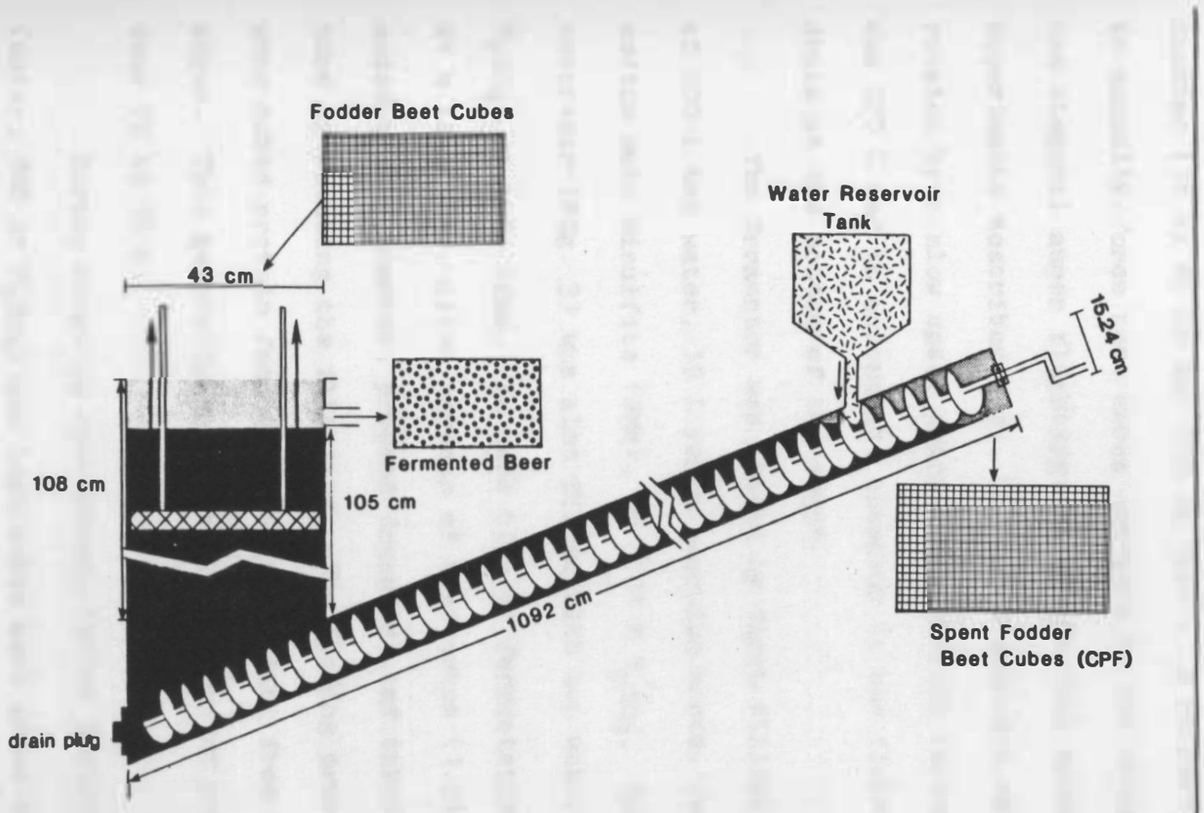

Figure 3. Semi-continuous, diffusion fermentor used to process fodder beets into fuel ethanol and cubed protein feed (CPF).

perforated flighting. The tube was 15.24 cm in diameter, 1092 cm long, and was welded at a 70° angle to a mild steel rectangular chamber (16 by 43 cm and 108 cm tall). A perforated press was used to manually force beet cubes downward in the chamber for take up by the diagonal auger flighting. The auger was turned manually for the experiments described here, but for commercial operation would be rotated by a slow speed motor. The working volume of the fermentor was 220 L and to accomodate clean-up it was fitted with a capped drain at the bottom of the auger.

The fermentor was loaded by first filling it with a mixture of 200 L tap water, 19 L yeast inoculum broth, and either 0.2% w/v sodium meta bisulfite (SMB), or 0.01 N H_2SO_4. The 200 L water reservoir (Fig. 3) was also filled with tap water, and either SMB or H_2SO_4, at this time. At zero time of fermentation and then at 12 or 24 h intervals, given amounts of beet cubes (1.91-2.54 cm) were added to the chamber, pressed downward, and taken up into the auger tube by rotating the flighting. The loading process was completed when cubed protein feed (CPF) began exiting from the top of the auger. This generally took 3 to 9 loadings of cubes and extended over 72 to 96 h.

During on-going operations, (after loading), both liquid (water, SMB or H_2SO_4) and beet cubes were added to their respective ends of the fermentor at 12 or 24 h intervals. Reservoir liquid was added first. As it flowed down the auger and back up the rectangular chamber, sugar was extracted and yeast cells were

removed from the counterflowing beet cubes. The sugar was fermented to ethanol by the yeast, consequently, beer overflowing from the top of the rectangular chamber contained up to 6-8% (v/v) ethanol. After liquid flow had ceased, (0.25 h), the same amount of beet cubes, as had been added during each previous loading interval, was augered into the fermentor, thereby discharging an equivalent weight of CPF from the other end.

The above procedure was repeated during on-going operation for up to 800 h. Flow rates of beet cubes, reservoir liquid, and beer (i.e., fermentor retention times) were varied in different trials, as were conditions of contaminant control using meta bisulfite or low pH. Duplicate trials, for each set of operating parameters, were conducted and results averaged.

Analytical Methods

In conventional batch, submerged fermentation trials, samples of mash (before inoculation) or beer (after inoculation) were withdrawn for analysis at 6 h intervals. In addition, samples of raw fodder beets and protein feed (PF) were analyzed.

For batch solid-phase fermentation trials, samples of fermenting pulp were taken at 6 h intervals. In continuous trials, samples were taken at 12 h intervals.

In diffusion fermentation trials, liquid (beer) and cubes (raw or CPF) were individually assayed, however concentrations for each component were averaged across each sampling time. Therefore each data point represents the average liquid and cube concentration

of that component at that fermentation time. In batch and sequential batch trials, samples were taken every 6 h, while they were taken every 12-24 h in continuous trials.

Components routinely assayed for included reducing sugar (primarily sucrose), ethanol, and yeast and bacterial populations. In conventional submerged fermentation trials, reducing sugar was measured by the dinitrosalicylic acid (DNS) method (Miller, 1959) following incubation of 100 ml of mash or beer for 48 h at 50°C in the presence of excess invertase. In solid-phase fermentation trials, reducing sugar was also determined by the DNS method following incubation of 20 g beet pulp in 80 ml water with an excess of invertase (48-72 h at 50°C). In diffusion fermentation trials, liquid (beer) was analyzed by the DNS method following incubation of 20 ml liquid with excess invertase for 72 h at 55°C. For cube samples, 20 g cubes and 80 ml water were homogenized in a Wareing blender and this mixture was incubated for 72 h at 55°C with excess invertase. Samples were then centrifuged and the supernatants analyzed.

Ethanol concentrations in beer, pulp, liquid, and cubes were determined by specific gravity measurements of distilled samples using AOAC procedures (Horwitz, 1980).

Yeast cell populations were determined by plate count methods using Difco potato dextrose agar and tartaric acid (Costilow, 1981; Westby and Gibbons, 1982). Bacterial contaminants were determined using unsupplemented potato dextrose agar (Westby and Gibbons, 1982). For beer, pulp, and liquid samples, 1 ml or 1 g

samples were initially diluted in 99 ml dilution blanks. For cube samples, 5 ml samples from the homogenized reducing sugar sample (prior to incubation at 55°C) were diluted initially with 95 ml dilution blanks.

Components occasionally assayed for included moisture, crude protein, crude fiber, ether extract, and ash. These latter analyses were performed by the SDSU Station Biochemistry Analysis Laboratory using AOAC procedures (Horwitz, 1980).

RESULTS AND DISCUSSION

Conventional Submerged Fermentation

Batch Fermentation Trials:

The effects of varying the fodder beet concentration upon ethanol production by S. cerevisiae in conventional submerged fermentation using a mash are presented in Table 1. Four separate fodder beet concentrations were investigated, with tap water being the diluent. Except for NH_4OH, no other ingredients were added to the beet mashes.

As the fodder beet concentration in the mash was increased, the sucrose concentration in the pasteurized mash and the ethanol concentration in the fermented beer increased accordingly. The amount of residual sucrose left in the fermented beer increased slightly with the beet concentration, as did the fermentation time. Ethanol yields remained relatively constant. These data indicate that fodder beets provide a readily fermentable source of sugar. They yield, on a weight basis, almost 70 L of ethanol/metric ton (17 gal/ton). Corn, by comparison, yields 380 L/metric ton (Gibbons and Westby, 1983; Westby and Gibbons, 1982).

Kosaric et al. (1983) have shown that yeasts other than S. cerevisiae can also ferment fodder beets. They have evidence that one such yeast, S. diastaticus, is in fact more desirable than S. cerevisiae in terms of specific growth rate and ethanol tolerance. The major problem encountered with using conventional, submerged fermentation to produce ethanol from fodder beets with S. cerevisiae

Table 1. Effects of varying fodder beet concentration upon ethanol production by *S. cerevisiae* in farm-scale submerged fermentation.[a]

Fodder beet concentration[b] (kg/L)	Pasteurized mash[c]	Fermented beer		Sucrose consumption (% of original)	Fermentation time[d] (h)	Ethanol yield[e] (L/metric ton)
	Sucrose (%)	Sucrose (%)	Ethanol [%(v/v)]			
1.11	6.95	0.43	3.50	93.81	24	67.34
1.20	7.54	0.46	3.82	93.90	30	71.08
1.41	8.53	0.44	4.25	94.84	30	71.20
1.62	9.48	0.94	4.32	90.08	30	69.87

[a] All values are averages of two trials.
[b] Values are given as wet weight, kilograms of fodder beet added per liter of water.
[c] This was accomplished by holding for 1 h at 90-93°C.
[d] Fermentation time refers to the time at which the ethanol concentration reached 96-98% of its final observed value. At this point fermentation slowed to a level which made continued fermentation uneconomical.
[e] Each ethanol yield is given as liters of 100% ethanol per 1000 kg of fodder beets.

was the disappointingly low maximum attainable ethanol concentrations in the resultant beers. The viscous nature of concentrated fodder beet mashes, necessary for high ethanol beets, caused mixing and pumping problems which limited the upper beer ethanol concentration to a maximum of 4.32% (v/v). Beers with this concentration require approximately twice as much energy for distillation as do beers with 10% (v/v) ethanol (Stampe, 1982). Beers distilled for fuel ethanol, such as good, corn mash beers, typically have 10-15% ethanol (v/v).

In addition to being difficult to agitate and pump, the high viscosity beet mashes that I examined were also hard to distill and centrifuge. Even worse, carbon dioxide tended to build up during fermentation of such mashes instead of being continuously released to the atmosphere. This created a fluffy cap which trapped more CO_2. During the vigorous part of fermentation, from 12-18 h after inoculation, fluff in the high viscosity mashes I tested invariably foamed over the top even when the tank was only 70% full. Ziobro and Williams (1982) noted similar problems when they attempted to ferment Jerusalem artichoke tuber pulp by conventional, submerged methods.

Based on these observations, and a fodder beet cost of $20.00/ton, it is likely that ethanol would cost at least $2.00-$2.50/gallon to produce using conventional submerged fermentation techniques. In addition, the energy balance for such a process

would be less than 1.0. That is, it would take more energy to produce the ethanol than was contained in the ethanol.

Table 2 lists the composition of the fodder beets we used and the make-up of the beer and protein feed (PF) obtained during submerged fermentation trials. The beet sugar and moisture values allow for an interesting comparison between this tuber crop and corn. Corn is a low moisture/high sugar crop containing 13% moisture and 72% sugar (Anon., 1980). Fodder beets, on the other hand, are a high moisture/low sugar crop. This makes the fodder beet, unlike corn, a more difficult crop to store and process by conventional means. The high moisture content is conducive for rapid physical and biological decomposition, while the low sugar content makes standard alcohol production processes unfeasible.

PF from the submerged fermentation process contained 20-21% protein on a dry matter basis. This compares with the 10-11% protein contained in dried fodder beets (calculated from Table 2) and the 30-35% protein in corn distillers' dried grain (Gibbons and Westby, 1983; Westby and Gibbons, 1982). PF is also high in crude fiber and ash when compared to fodder beets. The increased levels of protein, fiber, and ash are primarily the result of sugar removal during the fermentation process which, in effect, concentrates the unfermented components. Yeast cells produced during fermentation, however, also add to the protein level since they are removed with PF during centrifugation.

TABLE 2. Composition of fodder beets, beer, and PF obtained in farm-scale process using conventional, submerged fermentation.[a]

Parameter	Fodderbeets	Beer	Protein Feed (PF)
moisture %[b]	83.1	95.8	88.2
sucrose %	11.56	0.57	0.69
crude protein %[c]	1.84	1.07	2.41
crude fiber %	0.90	0.56	2.85
ether extract %[d]	0.04	0.04	0.06
Ash %	1.20	0.83	1.40

[a] All values are averages of the eight fermentation trials shown in Table 1 and are calculated on a wet basis (wt/wt).
[b] This includes all volatiles, mostly water.
[c] This is amine or ammonium nitrogen, mostly protein.
[d] This is mostly fat; ethanol and all other volatiles are removed before extraction.

Solid-Phase Fermentation

Preliminary Continuous Fermentation Trials:

Due to the major problems associated with conventional submerged fermentation of fodder beets, I discontinued further use of this process and, instead, concentrated my efforts on developing a continuous, solid-phase fermentation process. The device that was designed and constructed for continuous, solid-phase fermentation, and is now used in the SDSU plant, is shown in Figure 2. With this device, I investigated three different operational modes for producing fuel ethanol from fodder beets.

In the first operational mode, fodder beet pulp was neither pasteurized nor pH adjusted prior to yeast inoculation. Here I found that contaminants soon overgrew the inoculated yeast cells. High numbers of contaminants (presumably lactic and acetic acid bacteria), coupled with relatively low numbers of yeast cells, limited the ethanol concentration in the pulp to less than 1% (v/v), whereas 7-9% ethanol was expected based on fodder beet pulp sugar concentrations of 12-14%.

In the second operational mode, fodder beet pulp was pasteurized, but was not pH adjusted, prior to yeast inoculation. Even with pasteurization, certain contaminants proved to be a problem during the middle and later stages of the continuous runs. The data from one such run is shown in Figure 4.

An initially high ethanol concentration of almost 9% (v/v) in mid-run exiting pulp (108 h) eventually dropped to much less than

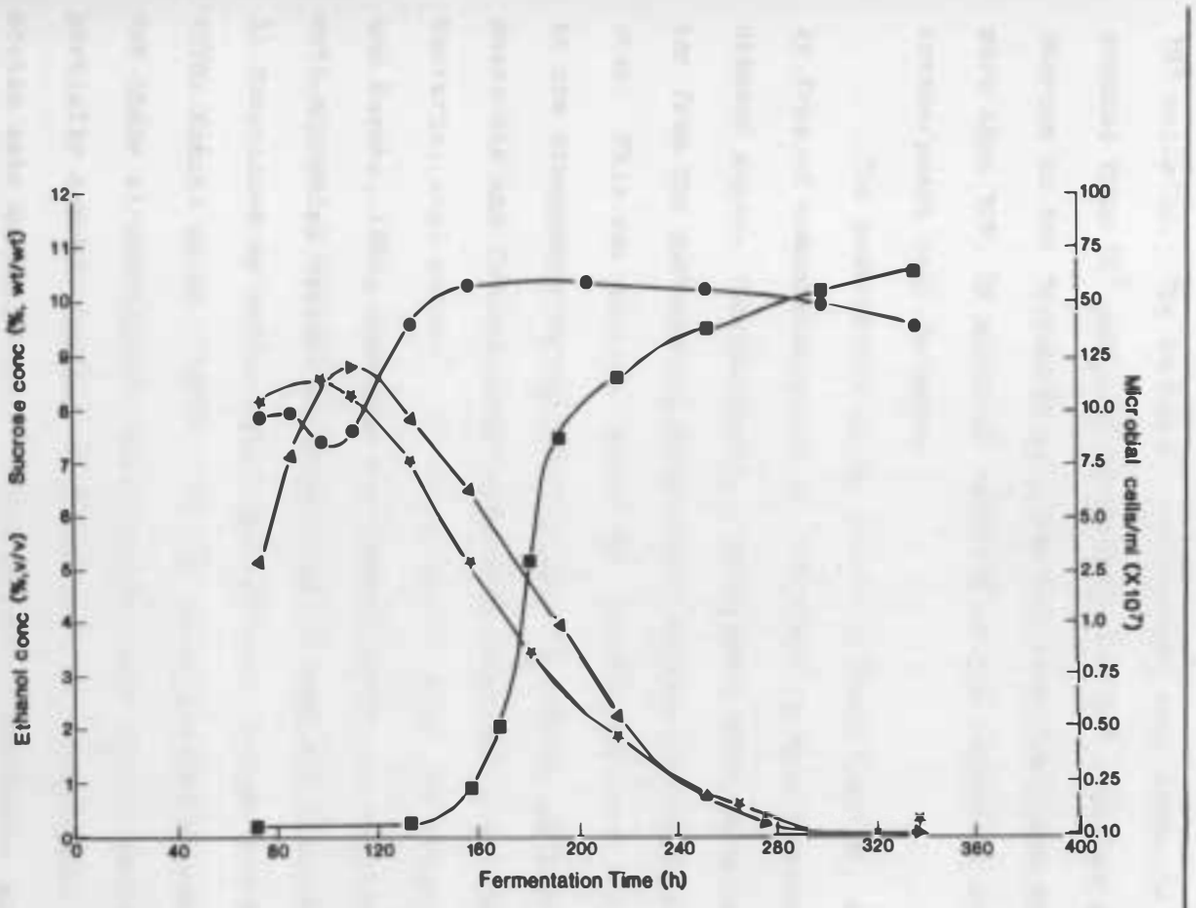

Figure 4. Ethanol production from pasteurized fodder beet pulp with a farm-scale, continuous, solid-phase fermentor. The fodder beet pulp was pasteurized before yeast inoculation but no pH adjustment was made. (■) sucrose; (▲) ethanol; (★) yeast; (●) bacteria.

1% (v/v) in later exiting pulp (320 h). At the same time the yeast cell population fell from a high of 1.5 X 10^8 cells/ml to less than 10^6 cells/ml. The bacterial contaminant population in this run increased from 10^8 cells/ml at 74 h to 5 X 10^8 cells/ml at 320 h. Sucrose in the fermented pulp rapidly rose from less than 0.5% to more than 10%, in apparent response to the bacterial contaminant increase/yeast cell decrease.

The pasteurized pulp, prior to being augered, was essentially free of contamination (< 10 cells/ml) as was, presumably, the steamed auger. Contaminants, I concluded, were entering the fermentor from the surrounding environment during the yeast inoculation step. This was possible since the inoculation port had to be opened to the atmosphere during inoculation. A likely explanation is that anaerobic and facultatively anaerobic bacteria (i.e., lactic acid bacteria, etc) either: 1) out-competed yeast for substrate (Atlas and Bartha, 1981; Harrison and Graham, 1970), 2) inhibited yeast by anti-microbial metabolite production (Kunkee and Amerine, 1970), or 3) functioned as killer cells against yeast (Beech and Davenport, 1970; Kotani et al., 1977). As the yeast population was reduced under these circumstances, the fermentor most likely became at least partially aerobic thereby allowing aerobic contaminants (i.e., acetic acid bacteria, etc) to become established with already present facultative contaminants (i.e., lactic acid bacteria, etc.).

In the third operational mode, fodder beet pulp was pasteurized and the pH was adjusted prior to yeast inoculation. By

trial and error I found, to control contamination and still permit yeast fermentation, it was necessary to add about 5 ml of concentrated H_2SO_4/kg raw pulp. This reduced the initial pH of the pulp to 2.0-2.5. Such a drastic pH lowering was necessary because the mild steel parts of the fermentor increased the pulp pH during fermentation. If pulp was only lowered to pH 4.0, I found that during fermentation, the pH increased to 6.0 and this pH, although being acceptable for yeast fermentation, was unacceptable for contamination control.

It may be possible to use less acid for acidification of the pulp if a stainless steel fermentor is used. An initial pH of only 4-5 might be acceptable in this circumstance and considerably less acid (1-3 ml H_2SO_4/kg raw pulp) would be required. Such a stainless steel fermentor would most likely be used in a commercial plant because long term reduction in operating costs (less acid) should exceed the initial increase in capital costs (fermentor).

The results from a trial, in which acidified and pasteurized fodder beet pulp was used, are shown in Figure 5. The ethanol concentration of exiting pulp in these circumstances increased to 8.5-9.0% (v/v) at 84 h of fermentation and remained there until the end of the run (400 h). This corresponds to an ethanol yield of 87.5 L/metric ton (21 gal/ton). During fermentation the sucrose concentration in the pulp was consistently below 1%, a stable yeast population of 2.5×10^8 cells/ml was maintained throughout (Fig. 5), and bacterial contaminants were not detected (data not shown).

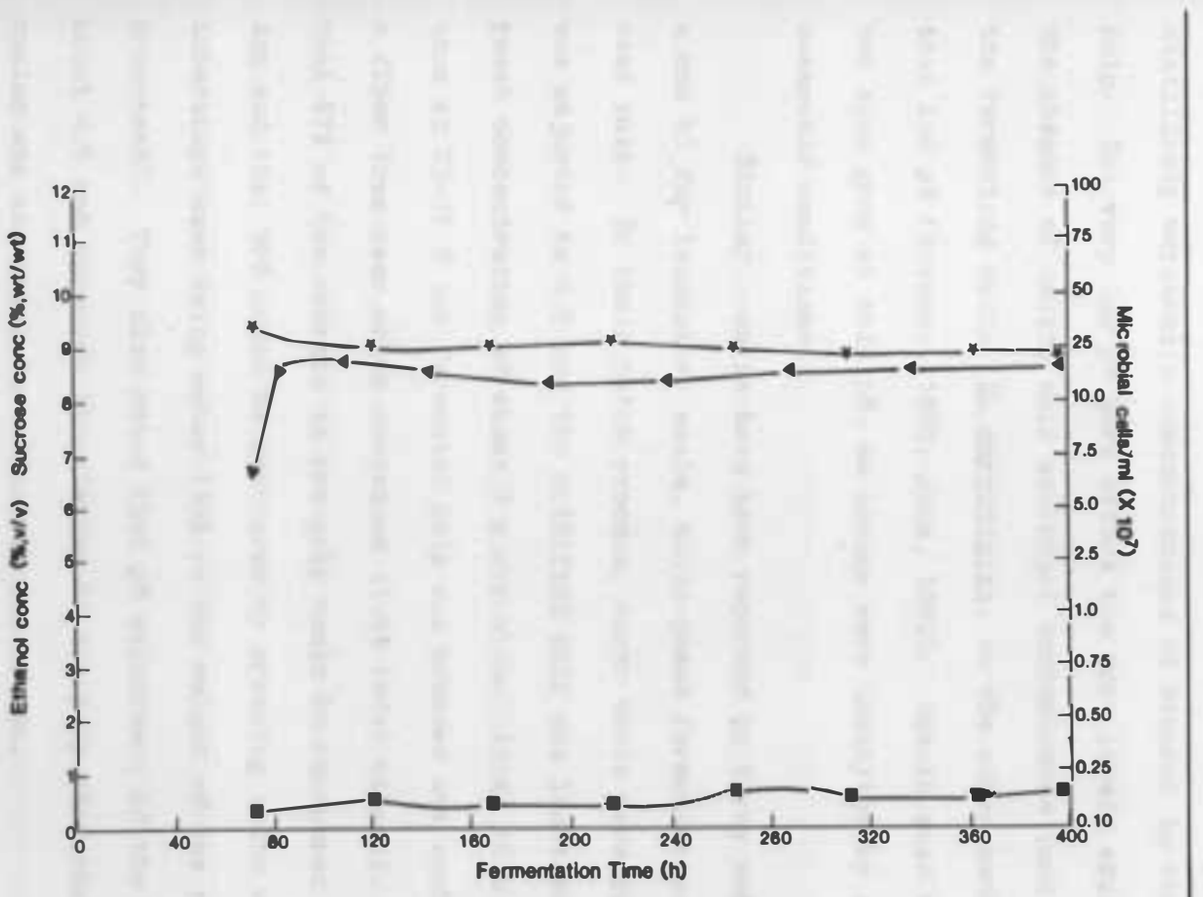

Figure 5. Ethanol production from acidified and pasteurized fodder beet pulp with a farm-scale, continuous, solid-phase fermentor. The fodder beet pulp pH was adjusted to pH 2.5-3.5 and pasteurized before yeast inoculation. Symbols as in Figure 4.

These data show that this process completely eliminates contamination problems without interfering with the production of distillably worthwhile concentrations of ethanol in the fermented pulp. The very low pH here offers the most likely explanation for the absence of lactic acid bacterial contaminants (and others) in the fermenting pulp. S. cervevisiae, on the other hand, can grow at that low pH (Burrows, 1970; Haas, 1960). Acetic acid bacteria which can also grow at this pH, we assume were inhibited by yeast created anaerobic conditions.

Similar results have been reported by Kirby and Mardon (1980 a and b) for laboratory scale, solid-phase fermentations of sugar beet pulp. In their batch process, sugar beets were pulped, the pH was adjusted to 4.5 and the acidified pulp was inoculated to a final yeast concentration of about 9 g dry wt/L. After 16 h of fermentation at 25-30 C the fermented pulp was pressed and washed to recover a fiber free beer which contained 11.4% (v/v) ethanol. They found that 67% of the ethanol in the pulp could be recovered in one pressing and that 95% could be recovered by pressing twice with a small interstage wash using water (15% of the weight of the pulp processed). They also noted that pH adjustment of the beet pulp to about 4.5 and thorough inoculation of the pulp with the yeast inoculum was essential for efficient conversion.

It may be possible to eliminate the steam pasteurization of acidified pulp (pH 2.0-2.5 with mild steel fermentor) and still prevent contamination. The lowered acidity by itself may be an

effective pasteurization device. To be workable this short cut may require inoculating the acidified pulp with a larger than normal yeast population; nevertheless, costs and energy consumption would be significantly reduced.

Batch Fermentation Trials:

In the first series of batch, solid-phase fermentation trials, I investigated the effect of varying the grind size of fodder beet pulp. Figure 6 shows the energy required to grind fodder beets using hammermill screens of various sizes. This plot shows that energy consumption rises rapidly when screens of 1.27 cm or less are used.

Contaminating bacteria were not detected in any of the batches of fermented fodder beet pulp that were examined in this study (data not shown). This was apparently due to the low initial pH (3.0) of the pulps.

The maximum yeast population in fodder beet pulp was independent of the screen size used to hammermill the beets over a broad range of screen sizes (Fig. 7). Screens used varied from 0.476 - 1.905 cm and the yeast populations remained relatively constant in the different pulps at 2.0 - 2.3 x 10^8 cells/ml.

I had initially anticipated that the smaller grinds might sustain higher yeast populations than the coarser grinds because of greater surface area, and consequently, enhanced release of nutrients from beet tissue. This did not occur and the explanation I believe lies in large part with the hammermilling process. Fodder

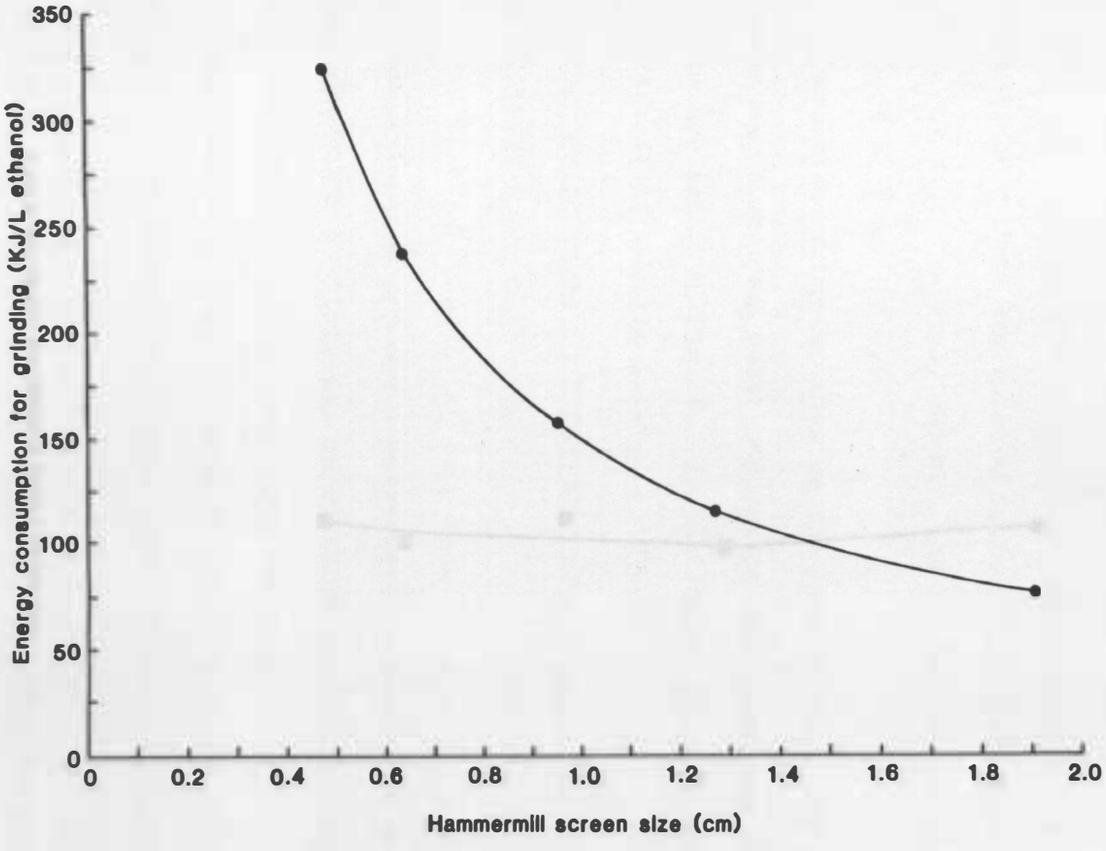

Figure 6. Energy consumption for grinding fodder beets using hammermill screens of various sizes.

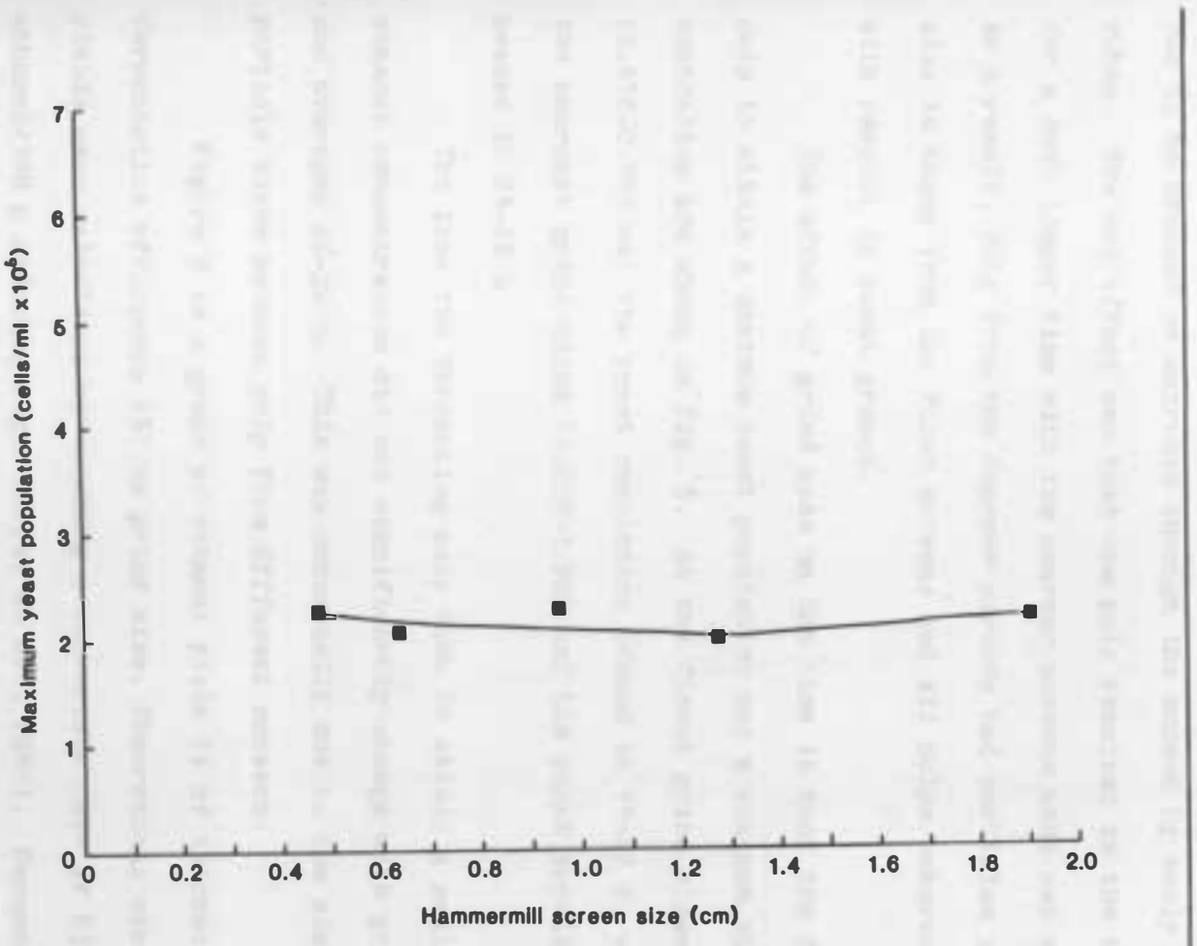

Figure 7. Effect of pulp grind size on maximum yeast population during fermentation.

beets tended to form a sticky pulp inside the hammermill which did not drop freely even through the coarser screens. Instead, the pulp had to be pressed or extruded through the screen by newly added beet cubes. The net effect was that the pulp remained in the hammermill for a much longer time with the coarser screens than was expected. As a result, pulp from the coarser screens had particles similar in size to those from the finer screens and all pulps behaved similarly with respect to yeast growth.

The effect of grind size on the time it took the fermenting pulp to attain a maximum yeast population and a maximum ethanol concentration are shown in Fig. 8. At the finest grind sizes (0.476-0.953 cm) the yeast population peaked in 19-22 h, while with the coarsest grind sizes (1.270-1.905 cm) the yeast population peaked in 24-28 h.

The time the fermenting pulp took to attain a maximum ethanol concentration did not significantly change with grind size and averaged 28-30 h. This was undoubtedly due to the similarity in particle sizes between pulp from different screens.

Figure 9 is a graph of ethanol yield (% of theoretical) and fermentation efficiency (%) vs grind size. Theoretical ethanol yields were calculated by assuming a conversion rate of 53.8 g ethanol/100 g reducing sugar (Bryan et al., 1981). Fermentation efficiencies were calculated by dividing the reducing sugar consumed during fermentation by the initial reducing sugar and multiplying the result by 100.

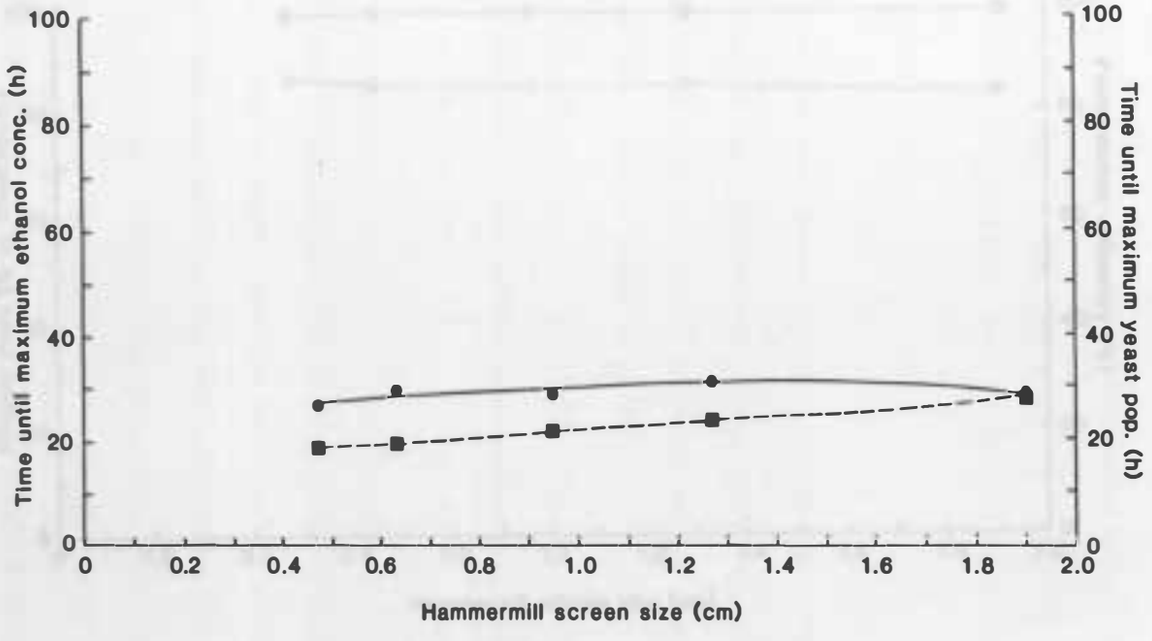

Figure 8. Effect of pulp grind size on fermentation time; (●) fermentation time to reach maximum ethanol concentration, (■) fermentation time to reach maximum yeast population.

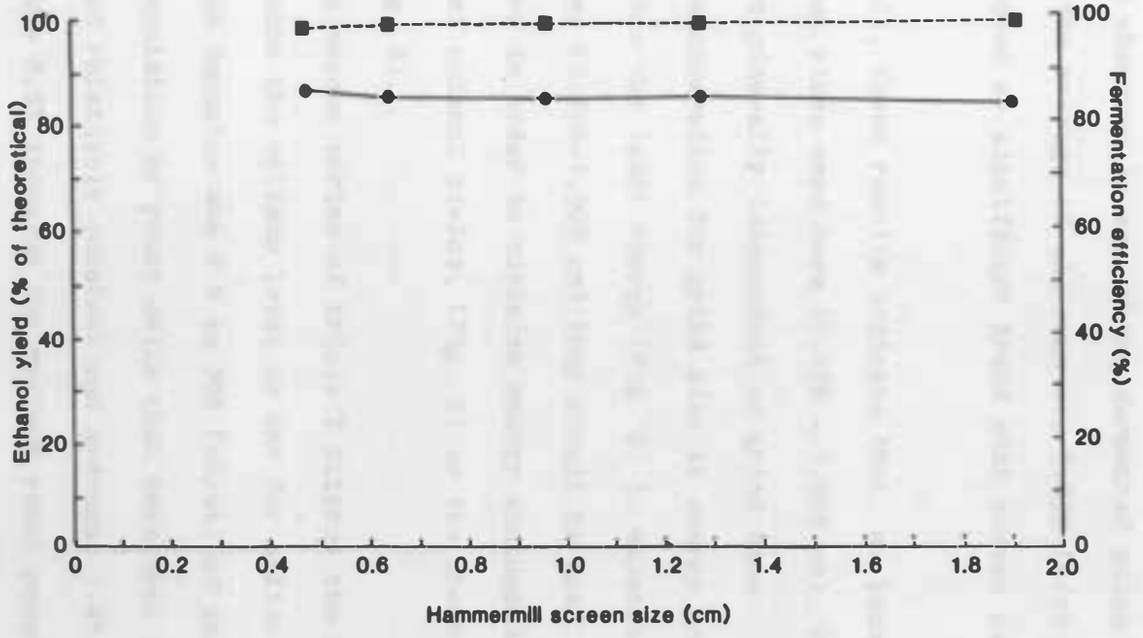

Figure 9. Effect of pulp grind size on (●) ethanol yield and (■) fermentation efficiency.

The results show that ethanol yields dropped slightly with increasing screen size. The finest screens yielded 85-87% while coarsest screens yielded 83-84% of theoretical. The maximum ethanol concentration observed in any of the fermented pulps was 7.96% (v/v), while the average of all runs was 7.63% (v/v). Fermentation efficiency showed no significant trend with screen size and averaged 98-99%.

Overall, these results indicate that, at least over the range of screen sizes used here (0.476 - 1.905 cm), fermentation parameters are generally independent of grind size. This means that the primary consideration for grind size is energy consumption for grinding. Since the least energy (Fig. 6) is expended with the largest screens (1.270-1.905 cm) they should be used instead of the smaller screens in order to minimize energy consumption. This still permits maximal ethanol yields, (Fig. 9) in the shortest fermentation time (Fig. 8).

In the second series of trials I altered the yeast inoculum size to determine the optimum level to use for solid-phase fermentation. When the inoculum was 0.5 to 20% (wt/wt) of the beet pulp, the maximum population of yeast cells that developed during fermentation remained relatively constant and averaged 1.85×10^8 cells/g (Fig 10). Below 0.5% inoculum the maximum yeast population gradually decreased to 9.4×10^7 cells/g at the 0.01% level. The average variability of yeast populations across replications for all trials was $\pm 1.8 \times 10^7$ cells/g.

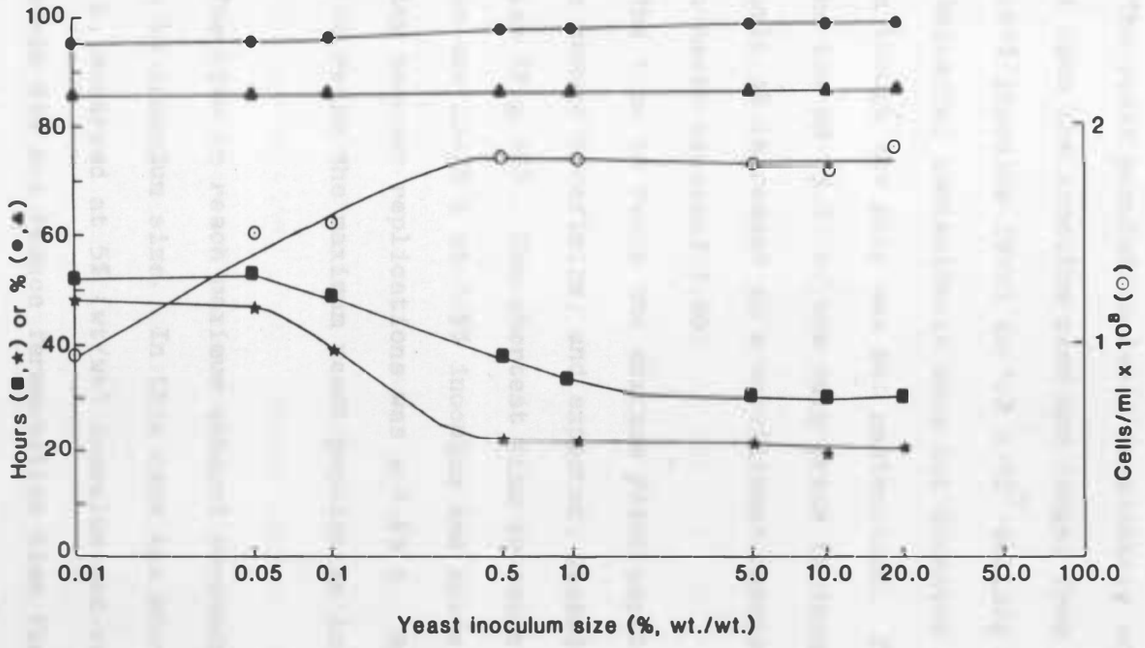

Figure 10. Effect of S. cerevisiae NRRL Y-2034 inoculum size on ethanol and yeast cell production during solid-phase fermentation of fodder beet pulp. The symbols are: (■), the time required for maximum ethanol concentration; (★), the time required for maximum yeast population; (●), fermentation efficiency as percent; (▲), ethanol yield as percent of theoretical; (⊙), maximum yeast population.

A high yeast population in the fermenting pulp is advantageous because it allows for shorter fermentation times and higher protein concentrations in the feed byproduct (Gibbons et al., 1986). The yeast population level immediately after inoculation was dependent upon the inoculum used and ranged from 1.35×10^4 cells/g at the 0.01% inoculum level to 1.2×10^7 cells/g at the 20% level.

Bacterial contaminants were not detected in any of the trials, even though the pulp was not pasteurized. This was presumably due to the low pH (3.0) of the pulp prior to inoculation. In all trials, pulp pH increased in a curvilinear fashion during fermentation, but never exceeded 3.60.

The time to reach the maximum yeast population during fermentation showed a definite, and expected, dependency on the inoculum size (Fig 10). The shortest time to reach the maximum yeast population was 20-22 h at 0.5% inoculum and above. The average variability between replications was ± 1.69 h. Below 0.5% inoculum the time to reach the maximum yeast population increased rapidly to 46-48 h.

The time to reach maximum ethanol concentration also responded to inoculum size. In this case the shortest fermentation time, 30 h, occurred at 5% (wt/wt) inoculum and above. Higher inoculum levels did not reduce fermentation time further. At 5% inoculum the initial yeast cell population was $3.8\text{-}4.2 \times 10^6$ cells/g. Here average variability between replications was ± 1.75 h. Below

5% inoculum, fermentation time gradually increased to 53 h at 0.05% inoculum.

The ethanol yield was independent of inoculum size and averaged 86% of theoretical (Fig 10) with an average variability of + 0.75%. The maximum ethanol concentration that developed during solid phase fermentation of fodder beet pulp inoculated with at least 5% inoculum was 9.13% (v/v), and the average of all trials was 8.89% (v/v).

Fermentation efficiency decreased slightly from 99% at 5.0% inoculum and above, to 95-96% at 0.1% inoculum and below (Fig 10). Average variability for all trials was ± 0.57%. The apparent explanation for this effect was that fewer yeast cells grew at lower inoculum levels (Fig 10) and thus less sugar was converted to cell biomass, even though ethanol yields remained constant.

Overall, these results suggest that a 5% (wt/wt) inoculum (4×10^6 yeast cells/g wet weight pulp) is the minimum amount necessary to ensure both maximum ethanol and yeast levels in a reasonably short fermentation time (30 h). Higher inocula (10-20%) provide no greater benefits, and would require higher investments in capital and operating costs to produce the greater quantity of inoculum.

In the final series of batch trials I determined the optimum pulp pH to use during solid-phase fermentation. As can be seen in Figure 11, the initial pulp pH had a very definite effect on the size of yeast and bacterial populations found during fermentation. Bacterial contaminants were not detected in fermenting pulp

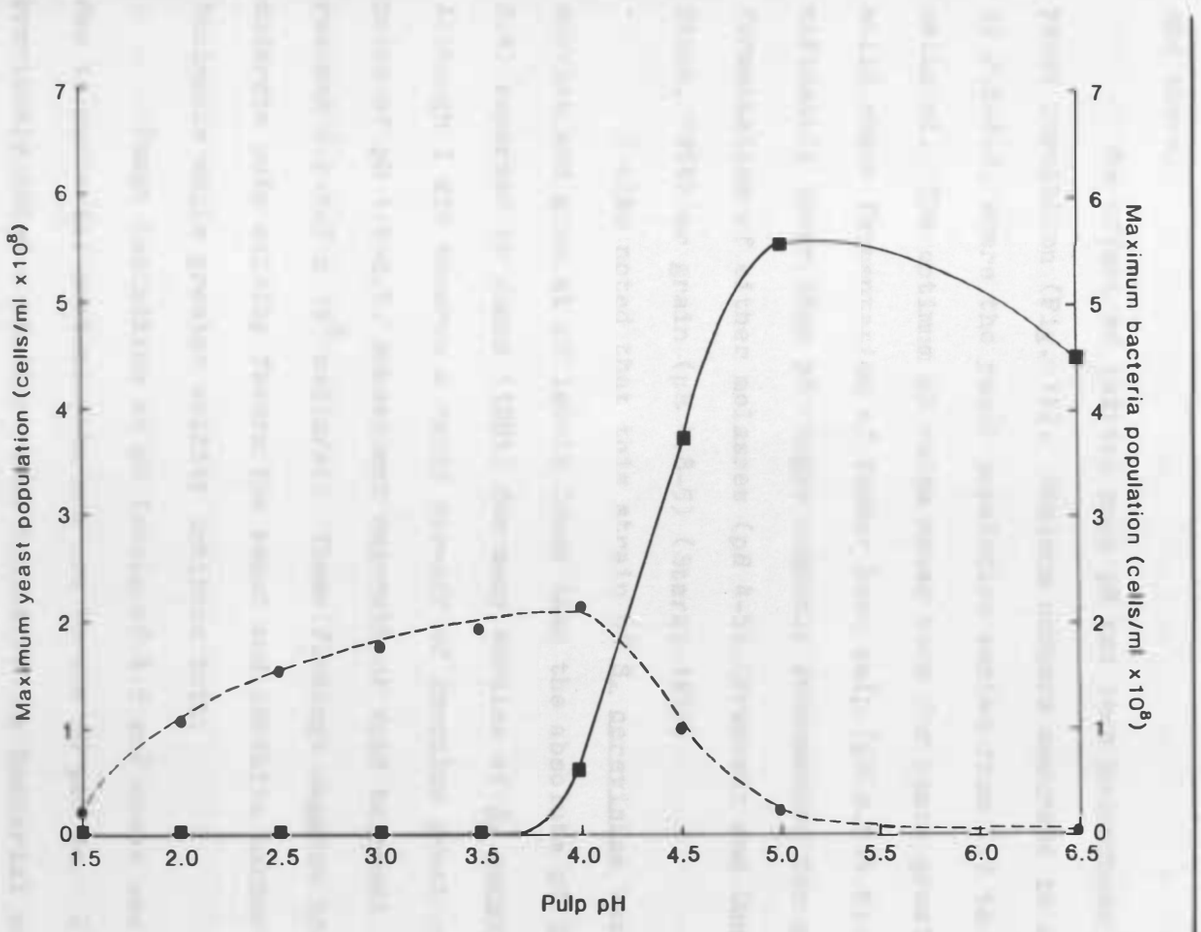

Figure 11. Effect of pulp pH on maximum yeast (●), and maximum bacterial (■), populations during fermentation. The pH of the pulp was adjusted just prior to yeast inoculation.

initially adjusted to pH 3.5 or below, whereas high bacterial numbers, of up to 5.6 x 10^8 cells/ml, developed in pulps of pH 4.0 and above.

The effect of initial pulp pH was less pronounced on the yeast population (Fig. 11). Maximum numbers occurred in a pH range of 2.5-4.0, where the yeast population varied from 1.7 to 2.2 x 10^8 cells/ml. The optimum pH range noted here for yeast growth during solid phase fermentation of fodder beet pulp (pH 2.5-4.0) was significantly lower than pH ranges commonly recommended for submerged fermentation of either molasses (pH 4-5) (Prescott and Dunn, 1949; Stark, 1954) or grain (pH 4.8-5) (Stark, 1954).

I also noted that this strain of S. cerevisiae was able to survive and grow at pH levels lower than the absolute pH limit (pH 2.4) reported by Jones (1981) for many strains of S. cerevisiae. Although I did observe a rapid die-off of inoculum yeast cells in pulps of pH 1.5-2.5, subsequent regrowth of acid tolerant survivors reached 0.2-1.5 x 10^8 cells/ml. These findings suggest that moderate pulp acidity favors the yeast and inhibits bacterial contaminants while greater acidity inhibits both.

Yeast inhibition at pH levels of 4.5 and above was likely due to bacterial contaminants and not to the pH per se. I have previously noted a similar yeast inhibition by bacterial contaminants during continuous solid-phase fermentation of fodder beet pulp (Fig. 4).

Figure 12 shows the effects of initial pulp pH on the time it took fermenting pulp to reach both maximum yeast population and maximum ethanol concentration. The yeast population peaked after only 24 h of fermentation when the initial pH was 2.5 - 5.0 but peaked considerably later at pH levels on either side of this range. The shortest fermentation time for maximum ethanol concentration was 30 h and this occurred at a pulp pH of 3.5. Fermentation took somewhat longer (36-39 h) at starting pulp pHs of 3.0, 4.0 and 4.5, but outside of this range the fermentation time increased dramatically.

Jones et al. (1981) have noted that yeast sugar fermentation rates are relatively insensitive to pH values between 3.5-6.0. My findings seem to indicate that the low end of this range (3.5) appears optimum, at least for solid phase fermentation of fodder beet pulp using this S. cerevisiae strain.

In Fig. 13 is plotted the ethanol yield (% of theoretical) and fermentation efficiency vs. initial pulp pH. The results indicate that both ethanol yields (78 - 85%) and fermentation efficiencies (97-99%) remained at high levels during batch fermentation of pulp initially set at pH 2.5-5.0. The maximum ethanol concentration observed in pulps within this pH range was 10.15% (v/v); with an average of 8.91% (v/v). At pHs below and above this range, yields and efficiencies dropped as yeast became inhibited by acid and bacteria, respectively. Here ethanol concentrations only reached an average of 4.37% (v/v).

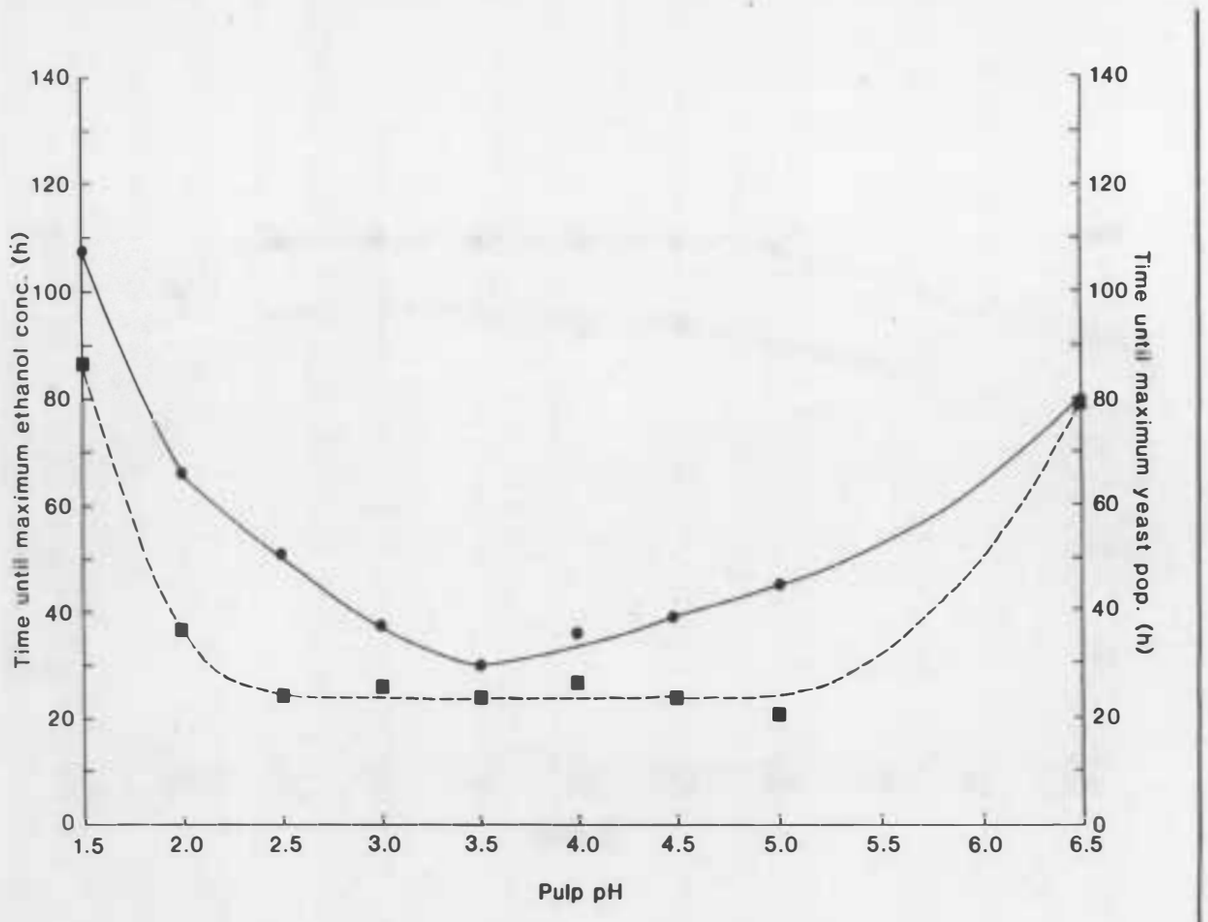

Figure 12. Effect of pulp pH on fermentation time to reach maximum ethanol concentration (●), and maximum yeast population (■). The pH of the pulp was adjusted as in Figure 11.

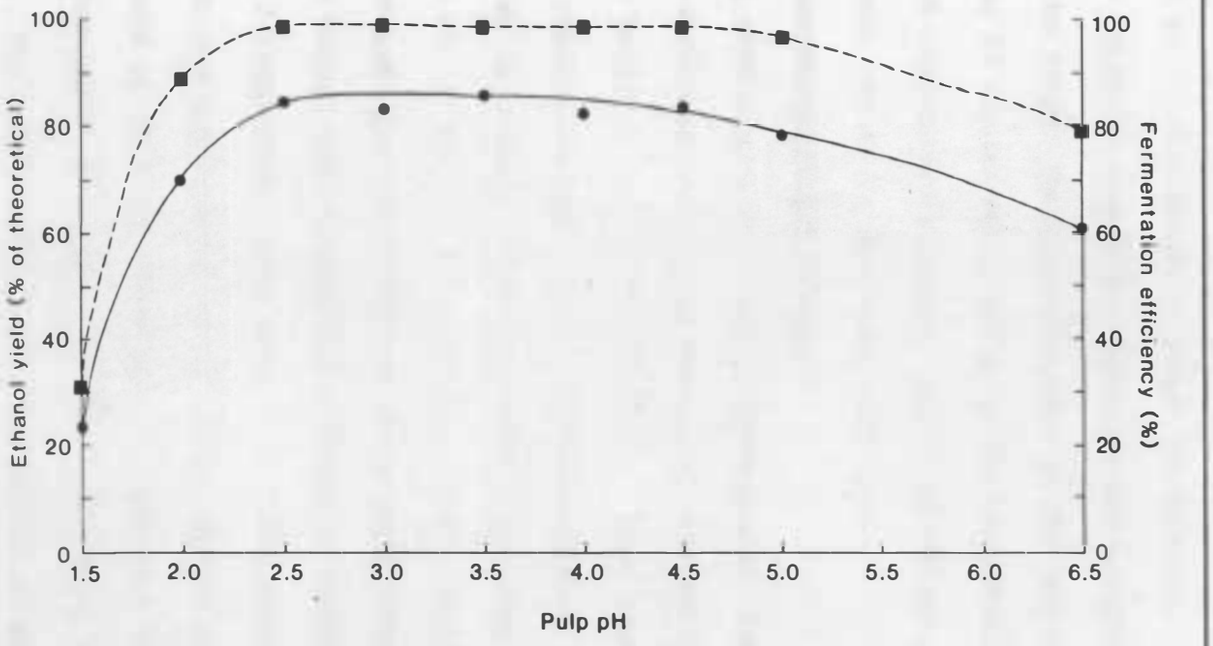

Figure 13. Effect of pulp pH on ethanol yield (●), and fermentation efficiency (■). The pH of the pulp was adjusted as in Figure 11.

Based on the fermentation parameters described herein, it can be concluded that fodder beet pulp used to make ethanol should be adjusted to pH 3.0-3.5 prior to yeast inoculation. This pH range 1) effectively inhibits bacterial contamination without pasteurization, 2) permits larger yeast populations to develop over a shorter period of time, 3) allows rapid and complete fermentation of beets to high ethanol concentration pulps, and 4) minimizes acid useage while maintaining the above mentioned benefits.

Final Continuous Fermentation Trials:

During preliminary continuous fermentation trials I found that both unpasteurized and pasteurized pulp eventually became contaminated with bacteria during fermentation. This contamination reduced yeast populations and ethanol concentrations. As a result, I tested the use of acidified and pasteurized pulp and found that this treatment was effective in controlling contamination.

I theorized that acidification alone might also control contamination and tested this concept in a series of batch fermentation trials. Here I found that a pulp pH of 3.0 - 3.5 would prevent contamination. To confirm whether low pH would control contamination in the continuous solid-phase fermentor, I conducted trials in which the pulp was initially adjusted to pH 2.9 - 3.2 using 2.0 ml 36N H_2SO_4/Kg pulp. The fermentor was operated at 26% of capacity with a retention time of 96 h.

Acidification alone was effective in controlling contamination of continuously fermenting fodder beet pulp. Both

freshly inoculated and fermented pulp, that had been acidified, contained approximately 2.0 $X10^7$ bacterial cells/ml, indicating that the acid level used was bacteriostatic. The lack of a bacteriocidal effect was due to the mild steel of the fermentor, which interacted with the acid, gradually increasing the pH from inhibitory levels (pH 2.9-3.2) in the inlet section of the fermentor, to non-inhibitory levels (pH 4.5-5.5) in the middle and outlet sections. Commercial fermentors envisioned for the future would most likely not experience this problem because they would be constructed of stainless steel (Gibbons et al., 1986).

The bacteriostatic levels of acid used in the fermentor were not inhibitory to the yeast strain used. Yeast levels increased from an average of 5.8 $X10^6$ cells/ml in freshly inoculated pulp to 9.84 $X10^7$ cells/ml in fermented pulp. This was paralleled by an increase in the ethanol concentration from 0% to an average of 7.21% (v/v), and a drop in the reducing sugar level from 12.44% (wt/wt) in raw and inoculated pulp to 0.44% in fermented pulp.

In the next series of experiments, I varied the amount of pulp entering the fermentor in order to determine its optimum operating capacity. Three operating capacities were tested--26, 65, and 92%. Pulp retention time was maintained as before at 96 h, and the pulp was acidified to control contaminants.

In all three sets of trials, bacterial contaminants in the fermented pulp remained at or below the 2.0 $X10^7$ cells/ml level observed before, confirming the effectiveness of pulp acidification

(pH 2.9-3.2) in controlling these contaminants. I also observed a slight bacteriocidal effect in the pulps that were run at the higher operating capacities. This effect apparently occurred because there was a lower pH in pulps run at the high capacities (eg., pH 4.74 at 92% capacity) than pulp run at the low capacity (eg., pH 5.03 at 26% capacity). This was undoubtedly due, to the fact that when a greater volume of pulp was run through the fermentor, a lower percentage of it contacted the mild steel of the fermentor than when a lesser amount was used and this minimized pulp pH neutralization.

Yeast populations were unaffected by increasing the operating capacity of the fermentor. From initial counts of 5.8 to 7.7 $X10^6$ cells/ml, yeast increased to the 10^8 cell/ml level during fermentation. Beet pulp reducing sugar concentrations declined here from an average of 12.5% (wt/wt) to 0.24% during fermentation, while ethanol concentrations increased to an average of 8.02% (v/v).

The only problem encountered during this series of trials was a slight amount of liquid leakage through the auger flighting handle bearing. This occurred only during runs at 92% of capacity, and indicated that this capacity was slightly above the maximum level the fermentor could be operated at.

Following the pH and capacity tests, experiments were run to determine the minimum time required to completely ferment the beet pulp. Four retention times were selected--12, 24, 48, and 96 h. Pulp was acidified, as before, to control bacterial contaminants,

and the fermentor was operated at only 90% of capacity to prevent juice leakage.

At all of the retention times tested, bacterial contaminants were held to below the 2.0 $X10^7$ cells/ml level previously observed and yeast populations increased to the 10^8 cells/ml level (average was 2.6 $X10^8$ cells/ml). Thus the reduced fermentation times did not significantly affect the final numbers of yeast and bacteria that occurred.

No similar parallel was seen with respect to changes in levels of ethanol and reducing sugar. In the 24, 48, and 96 h retention trials, the final ethanol concentration averaged 8.42% and the reducing sugar 0.25% (wt/wt); whereas in the 12h trial the final ethanol concentration was only 6.33%, and the reducing sugar was a high 1.82%. This data indicated that the optimum retention time for maximizing yields and minimizing fermentor size was at or near 24 h.

The technical findings described above allowed me to set baseline operational parameters for the continuous, solid-phase fermentation system described in Figure 14. For each parameter, conservative estimates were used in making the settings since I felt this would match "real-world" plant operating conditions more closely. The parameters included 1) initially adjusting the pulp pH to 2.9-3.3, 2) utilizing a fermentation time of 24 h, 3) operating the fermentor at 75% of capacity, and 4) obtaining a fermented beet pulp with 8% (v/v) ethanol. The settings for each parameter were used to calculate material and equipment costs.

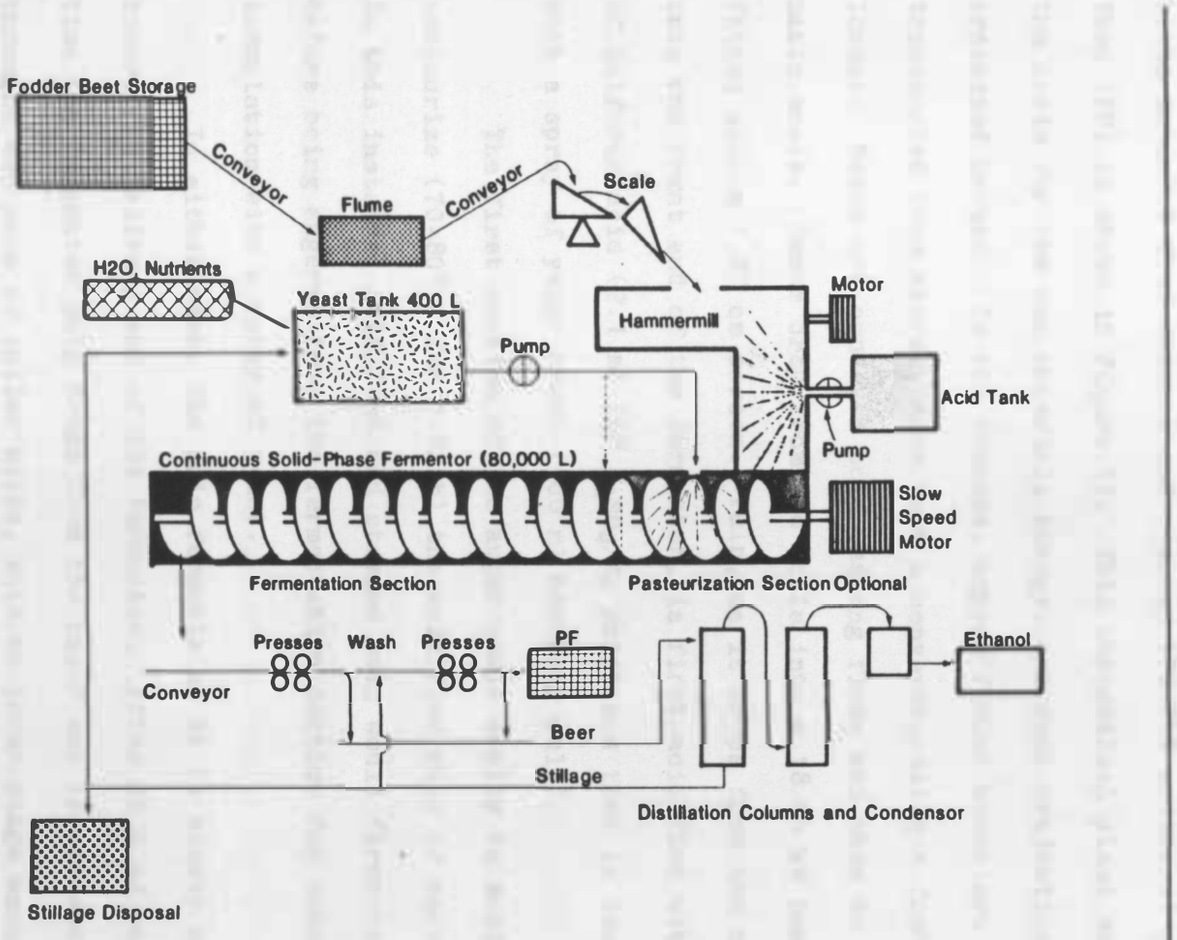

Figure 14. Design and operation of theoretical, community-scale ethanol plant for conversion of fodder beets to fuel ethanol and protein feed (PF).

Continuous, Solid-Phase Fermentation Plant Design:

A diagram of a plant designed to continuously process fodder beets into 95% fuel ethanol and pressed (70-75% moisture) protein feed (PF) is shown in Figure 14. This theoretical plant serves as the basis for the raw material, energy, and cost projections presented herein. In the process, topped fodder beets are transported from storage bins onto a conveyor, using a front end loader. Beets are conveyed to a washing flume and then to an automatic scale. Beets drop from the scale into a 18.65 kW hammermill fitted with a 1.27 cm screen. Pulp, as it drops from the hammermill into the front end of the fermentor, is first acidified with a spray of sulfuric acid (2.1 ml 36N H_2SO_4/Kg pulp) and then is inoculated with a spray of yeast broth (100 ml broth/Kg pulp).

The first section of the auger could easily be modified to pasteurize (70-80° C for 3-12 h) the acidified pulp if necessary. In this instance, acidified and steamed pulp would first be cooled before being augered into the fermentation section for subsequent inoculation with a spray of yeast.

In either case, the pulp ferments as it is slowly augered toward the delivery end of the fermentor. After 24 h of retention time the fermented pulp drops from the auger and is conveyed throught two sets of roller mills, with an inter-stage wash. This separates the beer from the PF. The beer is subsequently distilled to 95% (v/v) ethanol and the stillage is either reused in the

process (wash water or yeast propagation medium) or is disposed of as waste.

Community-Scale Alcohol Plant Parameters:

Table 3 lists the annual raw material requirements, the capacity, and the rate of production for a theoretical plant producing fuel ethanol and a pressed PF from fodder beets using the continuous, solid-phase fermentation process shown in Figure 14. An ethanol yield of 87 L/metric ton of fodder beets (21 gal/ton) is assumed.

Energy Balance:

Table 4 gives the energy inputs, energy outputs, and energy balances (energy output/energy input) for a theoretical solid-phase plant producing fuel ethanol and PF from fodder beets, with and without pasteurization. In determining the energy balances, the output energy of the PF and the input energy for planting, growing, harvesting and storing the fodder beets were not considered. As can be seen in Table 4, omission of pasteurization eliminates a high energy input and significantly increases the energy balance.

Costs:

Table 5 gives the capital, operating, and other fixed costs associated with ethanol production from fodder beets in the theoretical plant shown in Figure 14. Costs are provided both for the pasteurization/acidification mode of operation and for the non-pasteurization/acidification mode. A credit for protein feed (PF) is included.

TABLE 3. Raw materials, Rates and Products in Theoretical Fuel Ethanol Plant for Fodder Beets[a]

Component	Amount/yr[b]	Rates
Raw materials		
fodder beets[c]	7.48×10^6 Kg	990 Kg pulp/hr[e]
water[d]	1.0×10^5 L	83 L 95% ethanol/hr[e]
H_2SO_4[f]	40,000 L	
yeast[g]	1,400 Kg	
Products		
ethanol with denaturant[h]	658,854 L (or 596,106 L anhydrous)	
PF[i]	5.55×10^6 Kg	

[a] Plant design and operation are based on preliminary research findings.
[b] A 45 wk work year is assumed.
[c] An ethanol yield of 87 L/metric ton of fodder beets (21 gal/ton) is assumed here.
[d] Water is used in fodder beet cleaning, yeast inoculum preparation, washing fermented pulp, and condensing ethanol vapor.
[e] The process uses an 80,000 L capacity, stainless steel, continuous, solid-phase fermentor as illustrated in Figure 14. It is assumed that the fermentor is filled to 75% capacity and is operated as is shown in Figure 14.
[f] 3-6 ml concentrated H_2SO_4/Kg pulp used here.
[g] Yeast is obtained commercially in a dried, powder form with about 10^7 cells/gm.
[h] Using 5 L of unleaded gasoline/100 L of 95% (v/v) ethanol.
[i] PF is assumed to be 70-75% moisture.

TABLE 4. Energy Balance of Ethanol Production from Fodder Beets

Parameter	KJ/L[a]	
	Acidified and Pasteurized Pulp	Acidified and Non-Pasteurized Pulp
Energy Inputs[b]		
Conveying and Cleaning[c]	64	64
Grinding[d]	805	805
Pasteurization[e]	2995	0
Fermentation[f]	15	15
Pressing[g]	97	97
Distillation[h]	6,078	6,078
Total	10,054	7,059
Energy Output[i]	21,192	21,000
Energy Balance[j]	2.11	3.00

[a] KJ/L are kilojoules/L of denatured 95% (v/v) ethanol.
[b] Energy input values are based whenever possible (eg. pasteurization) on actual plant findings obtained from replicate runs with fodder beets. The values for distillation are extrapolated from earlier work on corn (Westby and Gibbons, 1982). Values for acidified and pasteurized pulp are all theoretical but are based on solid phase and submerged fermentation experience in the plant with fodder beets and corn, respectively.
[c] Two 0.37 kW motors are required for conveying and one 0.75 kW motor is required for pumping water.
[d] One 18.65 kW motor attached to hammermill is required here.
[e] Steam heat is used to pasteurize pulp at 70 C for 6 h.
[f] One 0.37 kW motor is required for rotating auger.
[g] One 0.37 kW motor is required for conveying and two 0.75 kW motors are required for pressing.
[h] Three 0.75 kW motors are required for operating pumps; the remainder of the energy for distillation is in the form of steam.
[i] This is the energy content of 190 proof (95%, v/v) ethanol (Stampe, 1982).
[j] Energy balance is energy output divided by energy input.

TABLE 5. 1983 Costs of Ethanol Production from Fodder Beets

Parameter	Cost/L[a]	
	Acidified and Pasteurized Pulp[b]	Acidified and Non-Pasteurized Pulp[c]
Capital Costs[d]	$0.042 ($0.160)	$0.041 ($0.155)
Operating Costs		
Feedstock[e]	$0.251 ($0.952)	$0.251 ($0.952)
Other	$0.212 ($0.803)	$0.207 ($0.785)
Other Fixed Costs[f]	$0.041 ($0.153)	$0.041 ($0.153)
Total Costs	$0.546 ($2.068)	$0.540 ($2.045)
Credit for PF[g]	$0.079 ($0.30)	$0.079 ($0.30)
Net Cost of Denatured Ethanol	$0.467 ($1.768)	$0.461 ($1.745)

[a] The costs, which have been derived in most cases from Hoffman and Dobbs' (1982) cost breakdown of the plant, are given in U.S. dollars per liter of denatured 95% (v/v) ethanol. The figures in parentheses are costs in U.S. dollars per gallon.

[b] Data are from SDSU ethanol plant operation.

[c] These are theoretical data based on experience at SDSU ethanol plant with pasteurized fodder beets.

[d] All capital items are amortized at a rate of 15% over their useful lifetime.

[e] This assumes fodder beet cost of $22.05/metric ton ($20.00/ton). The feedstock cost is based upon an alcohol yield of 87 L/metric ton (21 gal/ton).

[f] This includes insurance, maintenance, and property taxes.

[g] PF contains 20% protein on a dry basis.

When possible, costs were determined by comparing the plant (Figure 14) to a similarly sized corn-based ethanol plant economically characterized previously by Hoffman and Dobbs (1982). So, for example, whenever similar equipment or supplies were used in each process, the data of Hoffman and Dobbs (1982) were used to make estimates for the fodder beet-based ethanol plant. Fodder beets were assumed to cost $22.05/metric ton ($20.00/ton). This compares to estimated production costs in one recent study of $19.85 to $26.46/metric ton, depending on whether "experimental" or "adjusted" crop yields were used (Hills et al., 1983).

Hoffman and Dobbs' previous estimates for producing ethanol from corn are contained in other articles (Gibbons and Westby, 1983; Hoffman and Dobbs, 1982; Westby and Gibbons, 1982). Using a similarly sized plant and a corn cost of $3.00/bushel, the ethanol production cost was $0.52/L ($1.97/gallon). It was $0.47/L ($1.78/gallon) with corn priced at $2.50/bushel. By comparison, this preliminary estimate of fodder beet-based ethanol production was $0.46 to $0.47/L ($1.74 to $1.77/gallon), when beets were priced at $22.05/metric ton.

Several assumptions were made in arriving at cost estimates for ethanol derived from fodder beets. Some of the important ones concerning capital costs included the following: 1) the boiler required for the non-pasteurization/acidification mode of operation was smaller than that required in our studies of corn-based ethanol production, since less steam was required; 2) a solid-phase

fermentor would cost about the same as a conventional fermentation system; 3) conveyors and a flume needed in a fodder beet-based ethanol plant would cost as much as the grain handling system in a corn-based plant; 4) the press for handling fodder beet stillage would be less expensive than a centrifuge used for corn stillage. With respect to operating costs, important assumptions included these: 1) although enzymes were not required in the fodder beet-based ethanol production processes, 10 times as much acid was required as in corn-based processes; 2) less ammonium hydroxide was required in the fodder beet processes; 3) less fuel was required in the non-pasteurization/acidification mode of operation than in corn-based ethanol production, because of the lower steam requirement; and 4) only about one-half as much water was required in the fodder beet processes as in our studies of ethanol production from corn.

The fodder beet feedstock cost was $0.25/L (when fodder beets cost $22.05/metric ton), compared to a corn feedstock cost of $0.24/L (when corn is $2.50/bushel). More attention needs to be given to the cost of the fodder beet feedstock and to the byproduct credit associated with fodder beet-based ethanol production. Growing conditions in different regions will influence the feedstock cost, making fodder beets more appropriate in some areas than others as an ethanol crop.

Regarding the byproduct credit, I assumed in this preliminary fodder beet economic analysis that the credit is the same for fodder beets as for corn-- $0.079/L ($0.30/gallon) of ethanol. The

corn byproduct credit was computed on the basis of the byproduct, distillers wet grain (DWG), being used as a protein supplement in livestock rations. Although fodder beets have a much lower protein content on a percentage of fresh weight basis than does corn, there is a substantially larger quantity of fodderbeet byproduct per gallon of ethanol produced. As a consequence, the quantity of protein per gallon of ethanol is similar for fodderbeet and corn feedstocks. Thus, as a first approximation, using the same byproduct credit for both feedstocks is not unreasonable. However, more detailed analysis of overall feeding, handling, and marketing characteristics of the byproduct of fodder beet-based ethanol production is needed to more precisely estimate its value. If nutritional, handling, or other characteriatics cause the fodder beet-based byproduct to be less marketable than corn DWG, the byproduct credit shown in Table 5 could turn out to be too high.

In response to the limitations inherent in any preliminary economic analysis, Habash (1985) and Dobbs and Habash (1986) have reported findings from a more extensive economic analysis of ethanol production from fodder beets using a plant designed similarly to that shown in Figure 14. The three major differences between the preliminary and final plant designs were that: 1) the two stage PF presses with interstage wash were replaced by a press and a dryer in the new plant (i.e., PF dried to 5% moisture), 2) beer from the press and vapors from the dryer are both routed to the distillation columns, and 3) a heating/cooling jacket was added to the fermentor

to maintain proper fermentation temperature. In addition, the production capacity of the plant was slightly increased to 662,725 L/yr of denatured 185 proof ethanol and 936 metric tons of PF (5% moisture).

Habash (1985) and Dobbs and Habash (1986), used various assumptions and estimates from both sugar and fodder beet production data to estimate a 1984 cost of $19.25/metric ton for producing fodder beets in east central South Dakota, assuming non-irrigated conditions and a yield of 56.8 metric tons/ha. Assuming a conservative yield of 87.44 L of 185 proof ethanol per metric ton of fodder beets, the feedstock cost per liter of denatured ethanol was $0.209. Other operating costs were calculated to be $0.278/L, for total operating costs of $0.487/L. The capital cost of the plant was estimated to be $0.09/L, other fixed costs added $0.056/L, for total capital and fixed costs of $0.146/L. All costs added together came to $0.63/L (rounded from ($0.633). Subtracted from this was a credit for PF ($0.14/L), thereby leaving a net cost of production of $0.49/L. This was slightly more than the $0.46/L preliminary cost estimate.

Dobbs and Habash (1986) also performed a series of sensitivity analyzes on key parameters. The most optimistic assumptions included: 1) a high ethanol yield of 96.19 L/metric ton; 2) a low annual interest rate of 10%; 3) a 12-month beet storage and alcohol processing period; and 4) a low feedstock cost of

$15.40/metric ton. With this set of assumptions, production costs net of the byproduct credit came to $0.34/L ($1.29/gal).

Diffusion Fermentation

Batch Fermentation Trials:

Prior to committing significant time and material resources to large-scale trials in the continuous diffusion fermentor, several series of batch (BDF) and sequential batch diffusion fermentation (SBDF) trials were performed to determine optimum levels for important fermentation parameters. These parameters included fodder beet cube size, potassium (PBM) and sodium meta bisulfite (SMB) concentrations, and pH.

Figure 15 shows the effect of fodder beet cube size on the maximum yeast population that developed in fermenting slurries (cubes in liquid). As can be seen, the yeast population varied only slightly (1.3 - 1.8 x 10^8 cells/ml) when cubes of 0.64 - 3.18 cm were used. This indicates that sufficient surface area was available for adequate sugar diffusion and hence yeast growth. When larger cubes (3.81 cm) were used, however, the maximum yeast population only rose to 0.94 x 10^8 cells/ml. In this case, the reduced surface:volume ratio provided less surface area for yeast growth, and might have inhibited penetration of yeast cells into the beet cubes (Er-el et al., 1981). Yeast growth might also have been reduced if sugar diffusion from the larger beet cubes was restricted. Cubes smaller than 0.64 cm were not utilized due to the difficulty of finely slicing beet tissue, the increased energy consumption for slicing, and the obvious lack of any yield or fermentation time advantage.

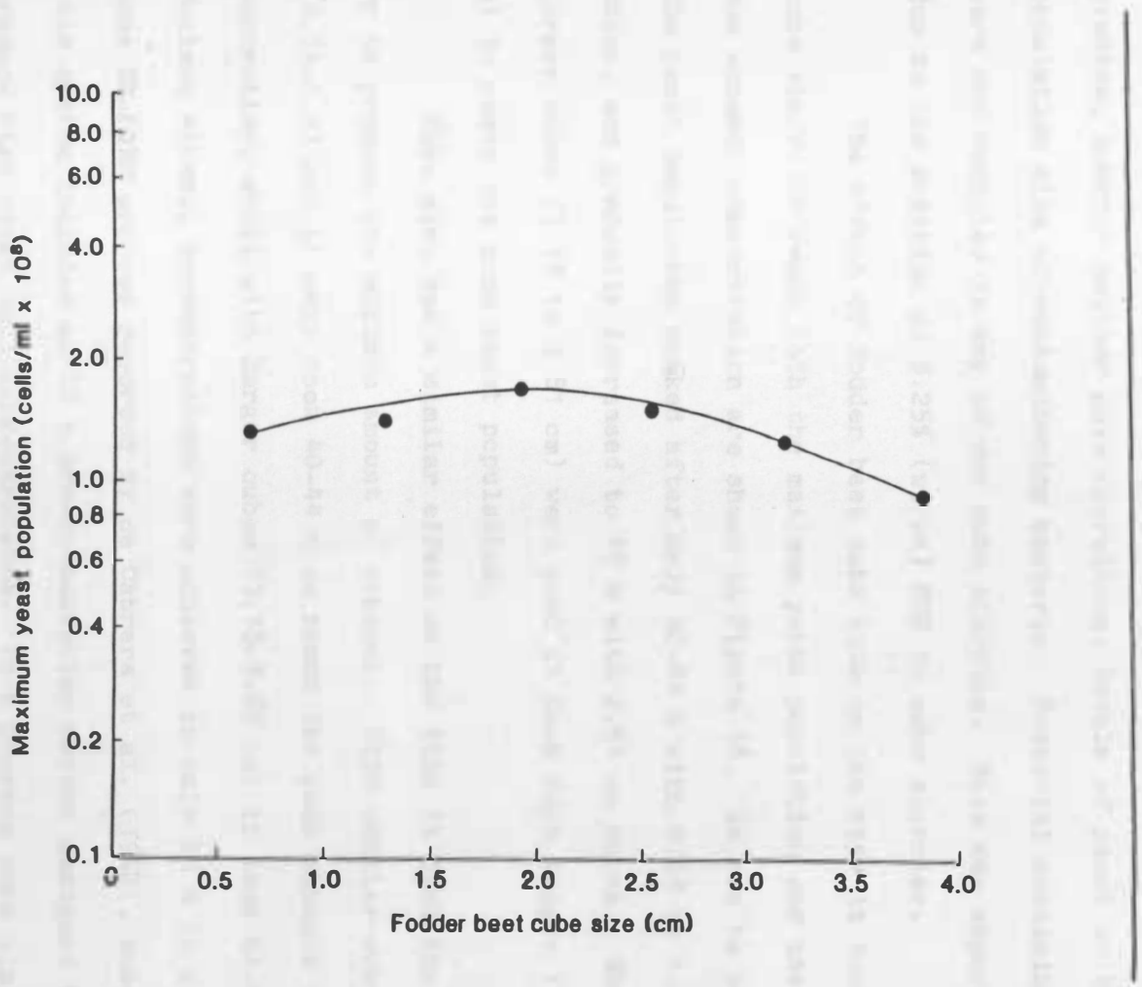

Figure 15. Effect of fodder beet cube size on maximum yeast population during diffusion fermentation (●). Fodder beet cubes are square and size indicated is the length of one side.

Er-el et al. (1981) and de Cabrera et al. (1982) investigated sugar cane billet sizes of 0.5-2.2 cm using the EX-FERM process, however neither gave operational levels of yeast or the population size of contaminating bacteria. Bacterial contaminants were not detected in any of our cube slurries. This was apparently due to the addition of 0.25% (wt/wt) PMB to cube slurries.

The effect of fodder beet cube size on the time it took a cube slurry to reach both the maximum yeast population and the maximum ethanol concentration are shown in Figure 16. As can be seen, the yeast population peaked after only 42-44 h with 0.64 to 1.27 cm cubes, and gradually increased to 49 h with 2.54 cm cubes. When larger cubes (3.18 to 3.81 cm) were used it took much longer (59-69 h) to reach the peak yeast population.

Cube size had a similar effect on the time it took the slurry to produce the maximum amount of ethanol. With smaller cubes (0.64-1.91 cm) it only took 40-44 h to reach the peak ethanol concentration, while with larger cubes (3.18-3.81 cm) it took 61-72 h. Maximum ethanol concentrations were achieved in only 24 h in a sugar cane EX-FERM process reported by de Cabrera et al. (1982), however, this system included an 18 h pre-fermentation period designed to produce high yeast cell concentrations. In a process more similar to ours, Er-el et al. (1981) used a 12% (v/v) yeast inoculum and obtained maximum ethanol concentrations in 35 h using sugar cane billets fermented by EX-FERM. Sugar cane billets of 0.5-2.2 cm and 1 cm were used in those studies, respectively.

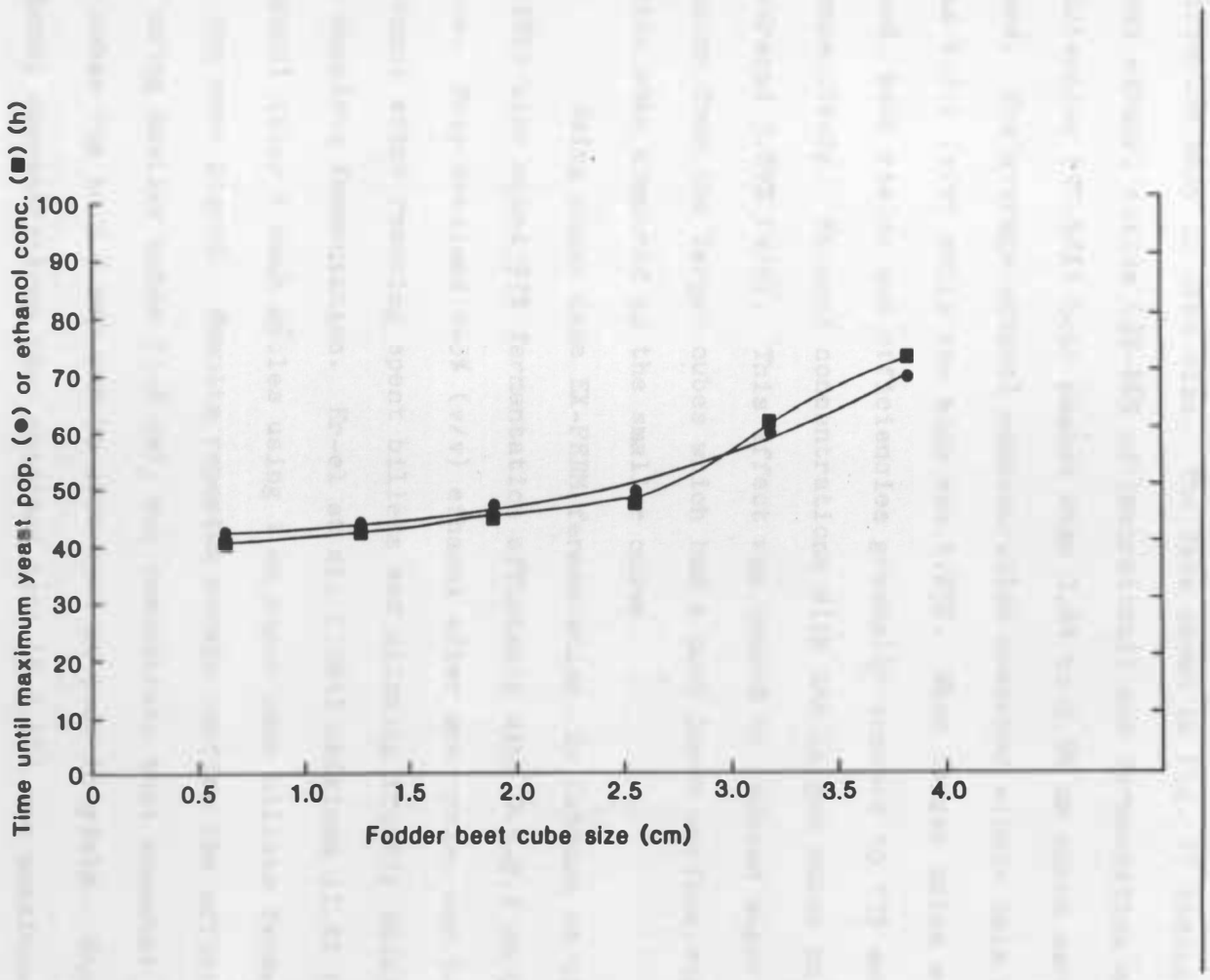

Figure 16. Effect of fodder beet cube size on fermentation time to reach maximum yeast population (●), and maximum ethanol concentration (■) during diffusion fermentation.

In Figure 17 are plotted the ethanol yields (% of theoretical) and fermentation efficiencies of beet cube slurries differing only in cube size. The data shown in Fig. 17 indicate that ethanol yields (83-86% of theoretical) and fermentation efficiencies (97-98%) both peaked when 0.64 to 2.54 cm cubes were used. The average ethanol concentration observed within this range was 4.21% (v/v) while the high was 4.83%. When larger cubes were used, both yields and efficiencies gradually dropped to 73% and 90%, respectively. Ethanol concentrations with the larger cubes only averaged 3.89% (v/v). This effect was caused by reduced sugar diffusion from the larger cubes which had a much lower surface:volume ratio when compared to the smaller cubes.

Using sugar cane EX-FERM fermentation, de Cabrera et al. (1982) also noted 97% fermentation efficiency with 0.5-2.2 cm billets. They obtained 4-5% (v/v) ethanol after one cycle, and 5-7.6% ethanol after removing spent billets and allowing freshly added ones to complete fermentation. Er-el et al. (1981) obtained 11.4% (v/v) ethanol after 4 such cycles using 1 cm sugar cane billets fermented in the same liquid. Results reported herein confirm the efficiency of using smaller cubes (1-2 cm), but demonstrate that somewhat larger cubes (up to 2.5 cm) can be used with no loss in yield. Higher ethanol concentrations than obtained here (4-4.5%) are possible when 2-4 sequential fermentation cycles are conducted in the same liquid, as evidenced by the EX-FERM experiments (de Cabrera et al., 1982; Er-el et al., 1981).

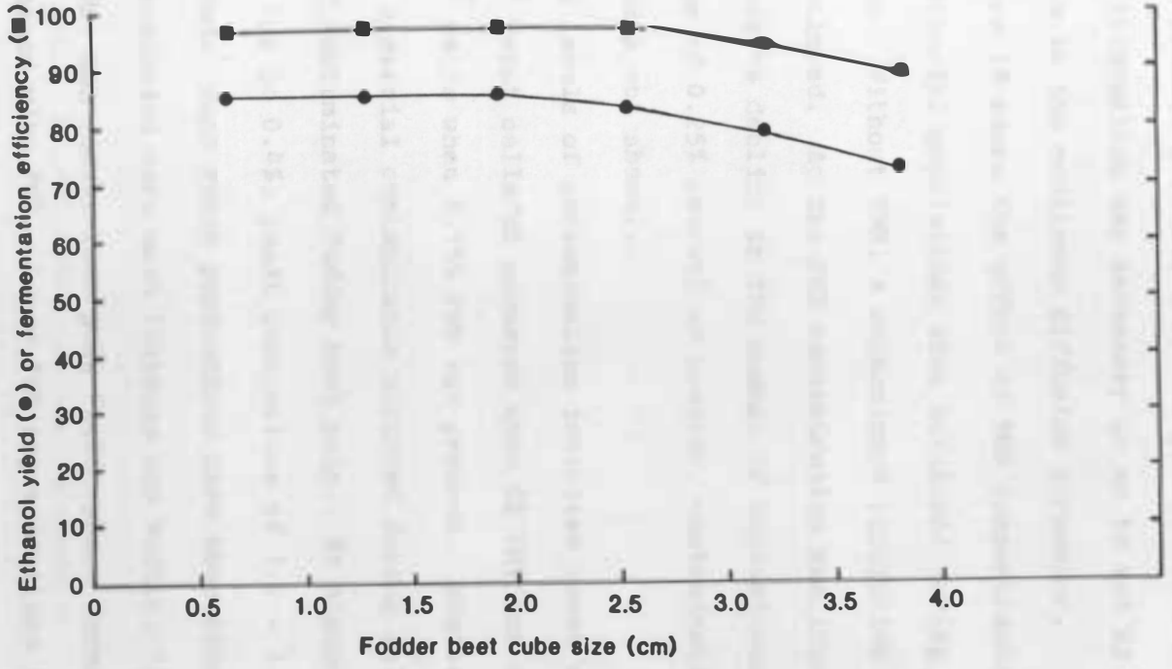

Figure 17. Effect of fodder beet cube size on ethanol yield (●), and fermentation efficiency (■) during diffusion fermentation. Ethanol yields are given as % of theoretical.

To determine the minimum PMB concentration that would prevent bacterial contamination of fermenting beet cubes, and yet permit rapid ethanol production by the yeast, the BDF process was used. This information was necessary so as to set up proper start-up conditions in the continous diffusion fermentor.

Figure 18 shows the effect of PMB concentration on maximum yeast and bacterial populations that developed during BDF of beet cube slurries. Without PMB, a contaminant population of 1.15×10^9 cells/ml developed. As the PMB concentration was increased there was a progressive decline in the number of contaminants, and at PMB concentration of 0.25% (wt/wt) or greater, contamination was prevented (data not shown).

High levels of contamination inhibited yeast reproduction as only 3×10^7 yeast cells/ml occurred when 0% PMB was used, compared to 2.2×10^8 cells when 0.15% PMB was present. Similar yeast inhibition by bacterial contaminants occurred during solid-phase fermentation of contaminated fodder beet pulp. At higher PMB concentrations (up to 0.4%) yeast populations of $1.2 - 1.8 \times 10^8$ cells/ml occurred. Such yeast populations have been observed previously in uncontaminated corn mash (Gibbons and Westby, 1983; Westby and Gibbons, 1982) and fodder beet pulp (solid-phase fermentation trials) not treated with PMB. Lower PMB concentrations (0.01-0.03%) than utilized here have reportedly prevented contamination during submerged ethanolic fermentations of glucose or grape must (Amerine and Kunkee, 1968; Wick, 1979) These feedstocks were, however,

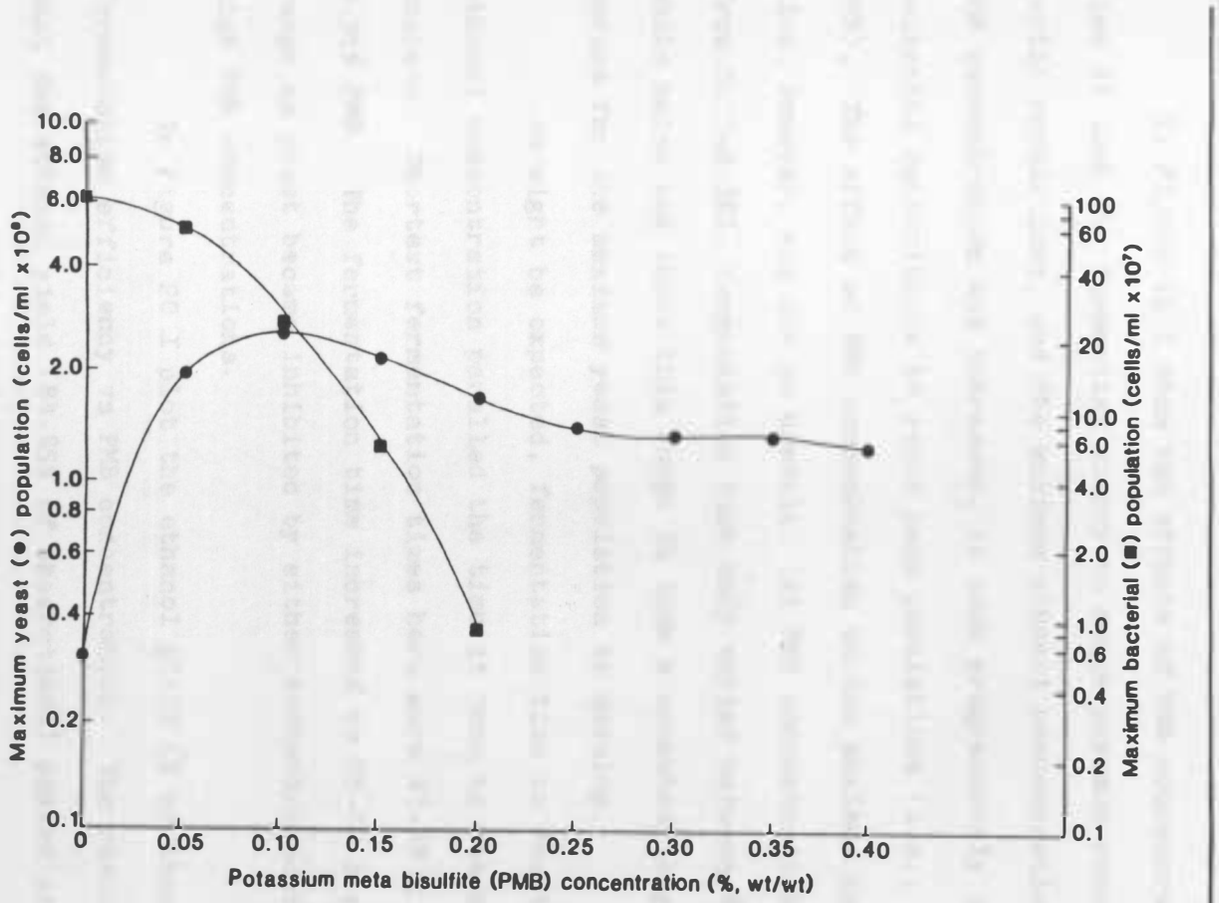

Figure 18. Effect of potassium meta bisulfite (PMB) concentration on maximum yeast (●), and maximum bacterial (■), populations during batch diffusion fermentation (BDF) of fodder beet cubes.

relatively free of bacterial contaminants, as opposed to fodder beet cubes.

In Figure 19 I show the effects of PMB concentration on the time it took the fermenting slurry to reach maximum yeast and bacterial populations, and the maximum ethanol concentration. As the PMB concentration was increased, it took progressively longer for bacterial contaminants to reach peak populations (i.e., 89 h at 0.2% PMB). The effect of PMB concentration on the maximum yeast population, however, was not as drastic. At PMB concentrations ranging from 0.15-0.30%, fermentation time only varied between 48-53 h, while below and above this range it took a somewhat longer 57-75 h period for the maximum yeast population to develop.

As might be expected, fermentation time to reach the maximum ethanol concentration paralled the time it took to reach peak yeast numbers. Shortest fermentation times here were 41-48 h at 0.10 - 0.35% PMB. The fermentation time increased to 55-72 h outside this range as yeast became inhibited by either bacterial contaminants or high PMB concentrations.

In Figure 20 I plot the ethanol yield (% of theoretical) and fermentation efficiency vs PMB concentration. The results indicate that the ethanol yield (84-85% of theoretical) peaked at PMB concentrations of 0.20 - 0.30% (wt/wt), and fell rapidly to 53-67% outside this range. The maximum ethanol concentration observed in trials between 0.20-0.30% PMB was 4.72% (v/v), and averaged 3.68% (v/v) outside this range. Fermentation efficiency remained at 96-97% for

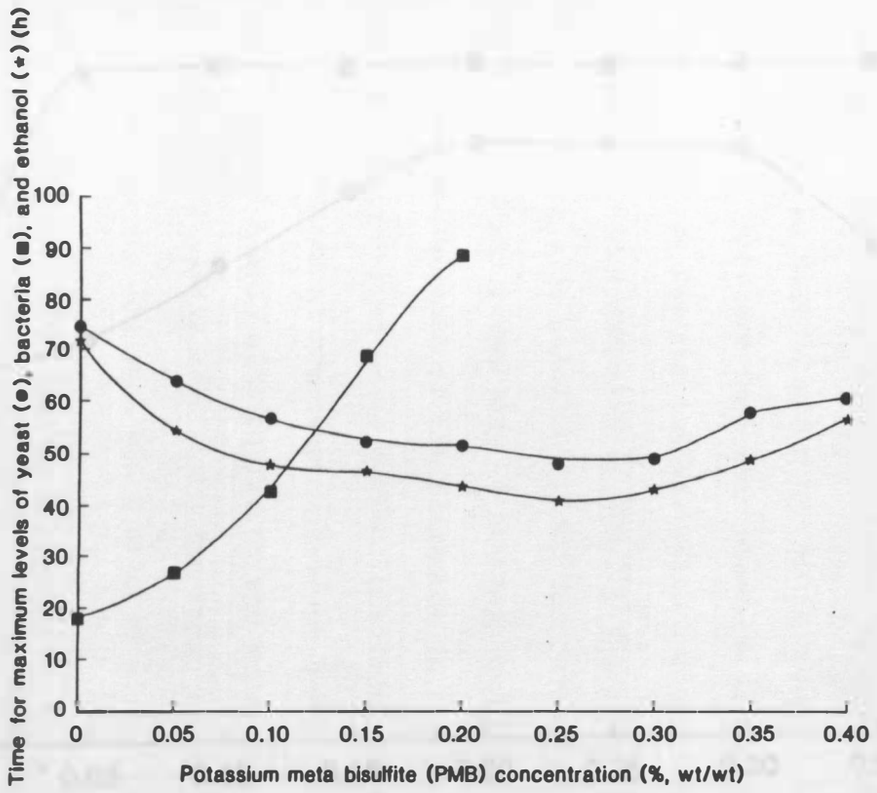

Figure 19. Effect of PMB concentration on fermentation time to reach maximum yeast (●), and maximum bacterial (■), populations; and maximum ethanol concentration (★), during BDF of fodder beet cubes.

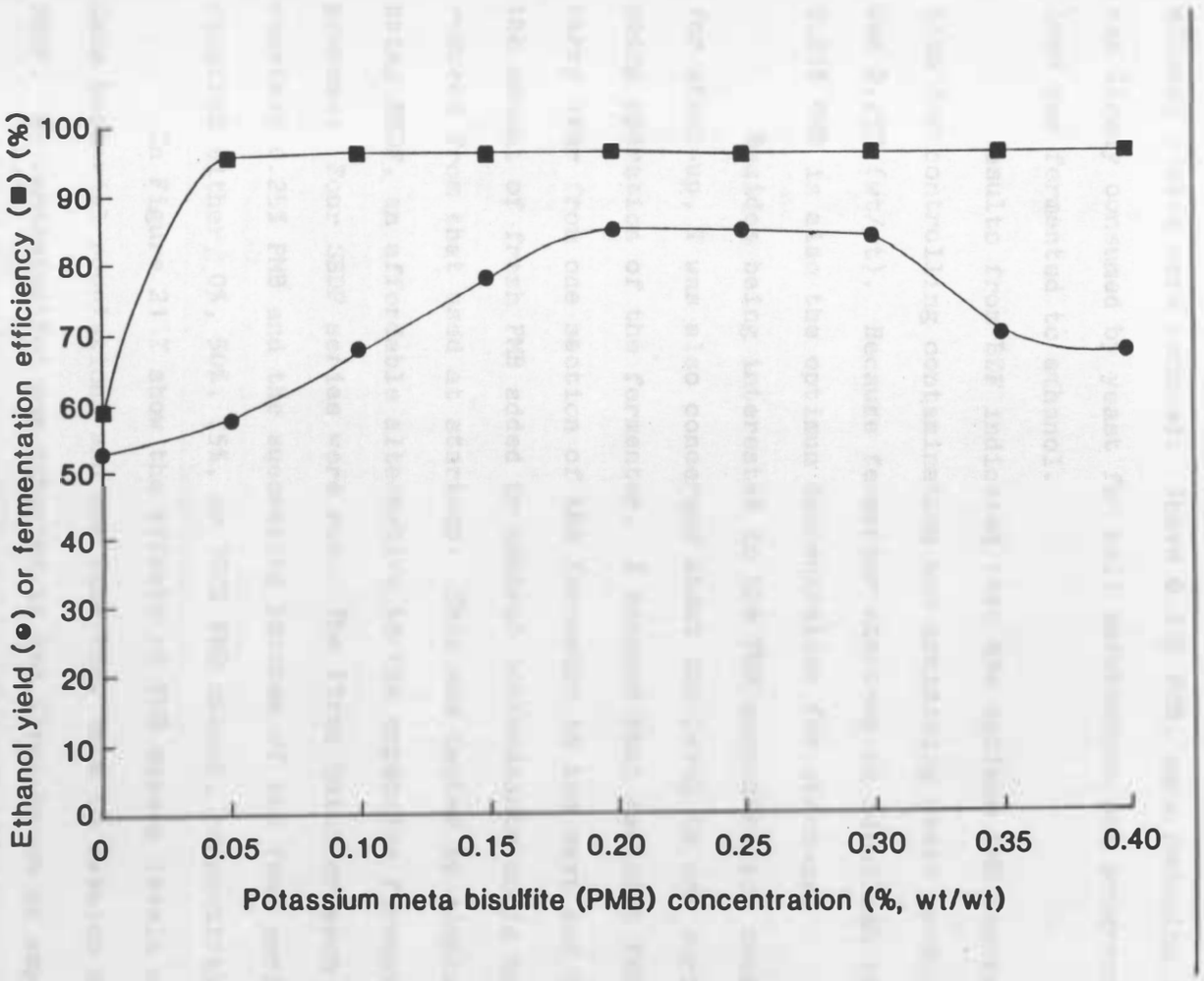

Figure 20. Effect of PMB concentration on ethanol yield (●), and fermentation efficiency (■) during BDF of fodder beet cubes. Ethanol yeilds are given as % of theoretical.

all trials except 0% PMB. In trials below 0.20% PMB, bacteria evidently consumed a significant portion of the reducing sugar since ethanol yields were reduced. Above 0.30% PMB, more reducing sugar was likely consumed by yeast for cell maintenance and progressively less was fermented to ethanol.

Results from BDF indicated that the optimum PMB concentration for controlling contamination and maximizing yeast productivity was 0.25% (wt/wt). Because fermentor start-up is identical to BDF, 0.25% PMB is also the optimum concentration for start-up.

Besides being interested in the PMB concentration necessary for start-up, I was also concerned about the level to use during on going operation of the fermentor. I assumed that residual PMB might carry over from one section of the fermentor to the next and that the amount of fresh PMB added to control contaminants could be reduced from that used at start-up. This was tested by simulation using SBDF, an affordable alternative to the expensive fermentor process. Four SBDF series were run. The first batch of each series received 0.25% PMB and the succeeding batches of the four series received either, 0%, 50%, 75%, or 100% PMB makeup, respectively.

In Figure 21 I show the effects of PMB makeup levels on maximum bacterial populations and the time they took to develop during SBDF. No contamination was detected in the first batch of any of the fermentation series, as was expected, since each batch here contained 0.25% PMB.

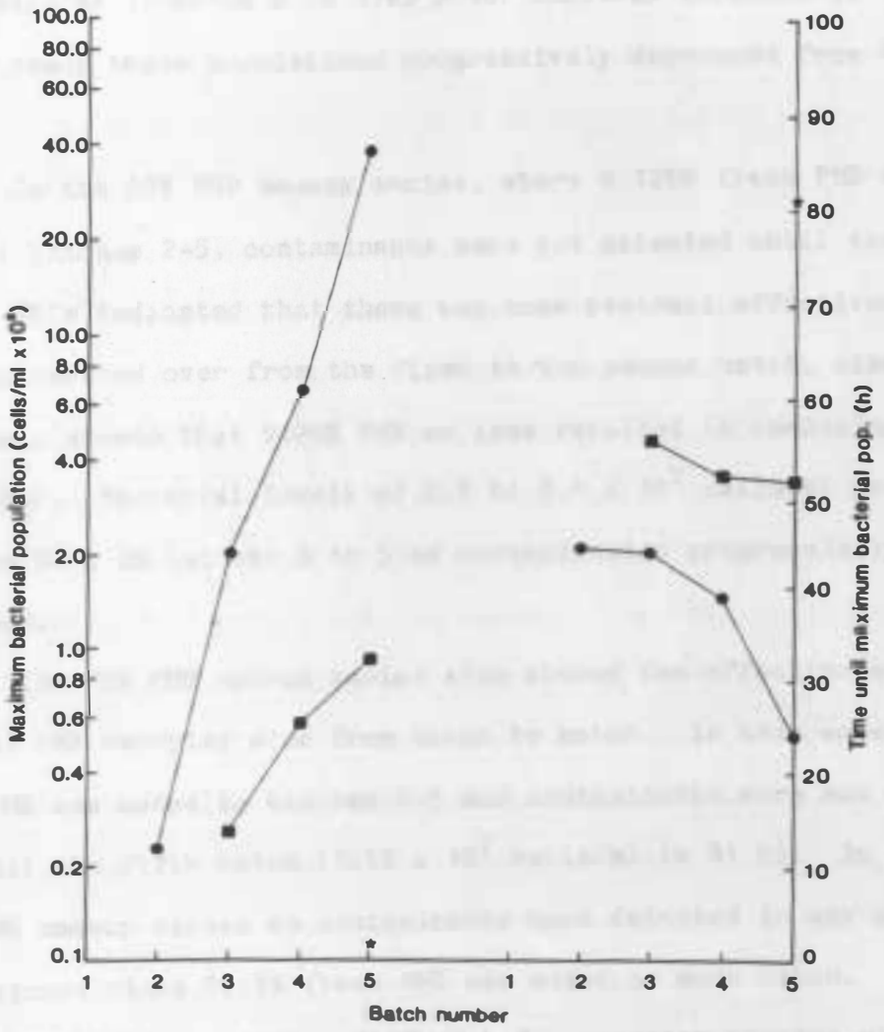

Figure 21. Effect of PMB makeup level on size of maximum bacterial population and the time to reach it during sequential batch diffusion fermentation (SBDF) of fodder beet cubes. SMB makeup levels are: (●) 0% makeup, (■) 50% makeup, and (★) 75% makeup.

In the 0% PMB makeup series no fresh PMB was added to batches 2-5. As a result, bacterial populations increased from 2.3 x 10^7 cells/ml in batch 2 to 3.85 x 10^9 cells/ml in batch 5. The time to reach these populations progressively decreased from 44 to 24 h.

In the 50% PMB makeup series, where 0.125% fresh PMB was added to batches 2-5, contaminants were not detected until the third batch. This indicated that there was some residual effectiveness of PMB that carried over from the first to the second batch, since I previously showed that 0.20% PMB or less resulted in contamination during BDF. Bacterial levels of 2.6 to 9.4 x 10^7 cells/ml developed in 56 to 52 h in batches 3 to 5 as contamination progressively increased.

The 75% PMB makeup series also showed the effectiveness of residual PMB carrying over from batch to batch. In this case 0.188% fresh PMB was added to batches 2-5 and contaminants were not detected until the fifth batch (1.15 x 10^7 cells/ml in 81 h). In the 100% PMB makeup series no contaminants were detected in any of the five batches since 0.25% fresh PMB was added to each batch.

Utilizing a similar diffusion fermentation process with sugar cane billets and high initial yeast populations, de Cabrera et al. (1982) and Er-el et al. (1981) noted no serious contamination problems after 2 and 4 sequential batches, respectively, had been run under non-aseptic conditions without PMB. In these trials, however, juice pH averaged 3.1-3.5 and this, according to the

authors, was most likely what prevented massive contamination. The antibacterial effect of low pH during solid phase fermentation of fodder beet pulp was noted previously. In these SBDF series the juice pH stabilized between 4-5, and this pH range was not effective in preventing contamination of fodder beet pulp during solid-phase fermentation.

In Figure 22 shows how PMB makeup levels affected maximum yeast populations and the time it took to reach them during SBDF. In the 0% PMB makeup series maximum yeast populations remained fairly stable in the first three batches (1.25 - 1.4 x 10^8 cells/ml developing in 43-46 h) but progressively dropped in the fourth and fifth batches (2.1 x 10^7cells/ml in 49 h in fifth batch) as the level of bacterial contaminants increased. The 50% and 100% PMB makeup series showed a similar pattern in the yeast population decline which occurred after the second batch. In the former case this is attributable to bacterial contamination (see Fig. 21) and in the latter case to PMB residue build-up, possibly enhanced by increasing intra - and extra-cellular ethanol concentrations (Brown et al., 1981; Loureiro and Ferreira, 1983; Schimz, 1980).

The 75% makeup series showed relatively stable yeast numbers throughout all five batches (1.4 - 1.7 x 10^8 cells/ml) and fermentation times only varied from 45-53 h. This data is consistant with the conclusion that there occurred little or no contamination through the fifth batch (Fig 21) and no PMB inhibition of the yeast.

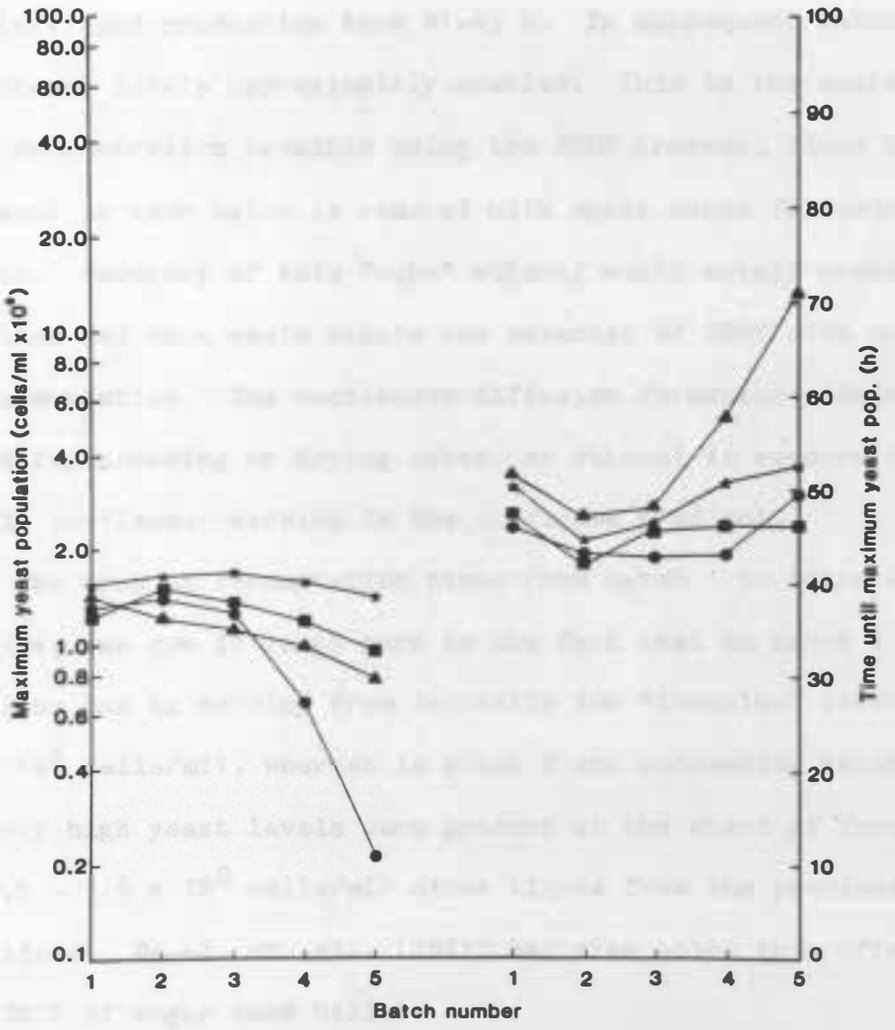

Figure 22. Effect of PMB makeup level on size of maximum yeast population produced and time to reach it during SBDF of fodder beet cubes. PMB makeup levels are as indicated in Fig. 21. 100% makeup is given as (▲).

The effects of PMB makeup level on ethanol production are shown in Figure 23. In the first batch of all four series, where 0.25% PMB was used, maximum ethanol concentrations ranged from 4.35 - 5.8% (v/v) and production took 41-45 h. In subsequent batches (2-5) ethanol levels approximately doubled. This is the maximum ethanol concentration possible using the SBDF process, since half the ethanol in each batch is removed with spent cubes following fermentation. Recovery of this "cube" ethanol would entail pressing or drying them and this would negate any advantge of SBDF over solid phase fermentation. The continuous diffusion fermentor eliminates the need for pressing or drying cubes, as ethanol is removed from cubes via continuous washing in the diffusion gradient.

The drop in fermentation times from batch 1 to batch 2, for all series, was due in large part to the fact that in batch 1 yeast populations had to develop from initially low "inoculum" levels (0.6 - 1.0 x 10^6 cells/ml), whereas in batch 2 and succeeding batches, relatively high yeast levels were present at the start of fermentation (0.5 - 1.0 x 10^8 cells/ml) since liquid from the previous batch was utilized. Er-el, et. al., (1981) has also noted this effect during SBDF of sugar cane billets.

In the 0% PMB makeup trial ethanol levels increased through the third batch to reach 7.77% (v/v), while fermentation times decreased from 42 to 35 h. In the remaining two batches, however, ethanol concentrations dropped to 7% and fermentation times increased to 57 h as bacterial populations increased (Fig. 21) and

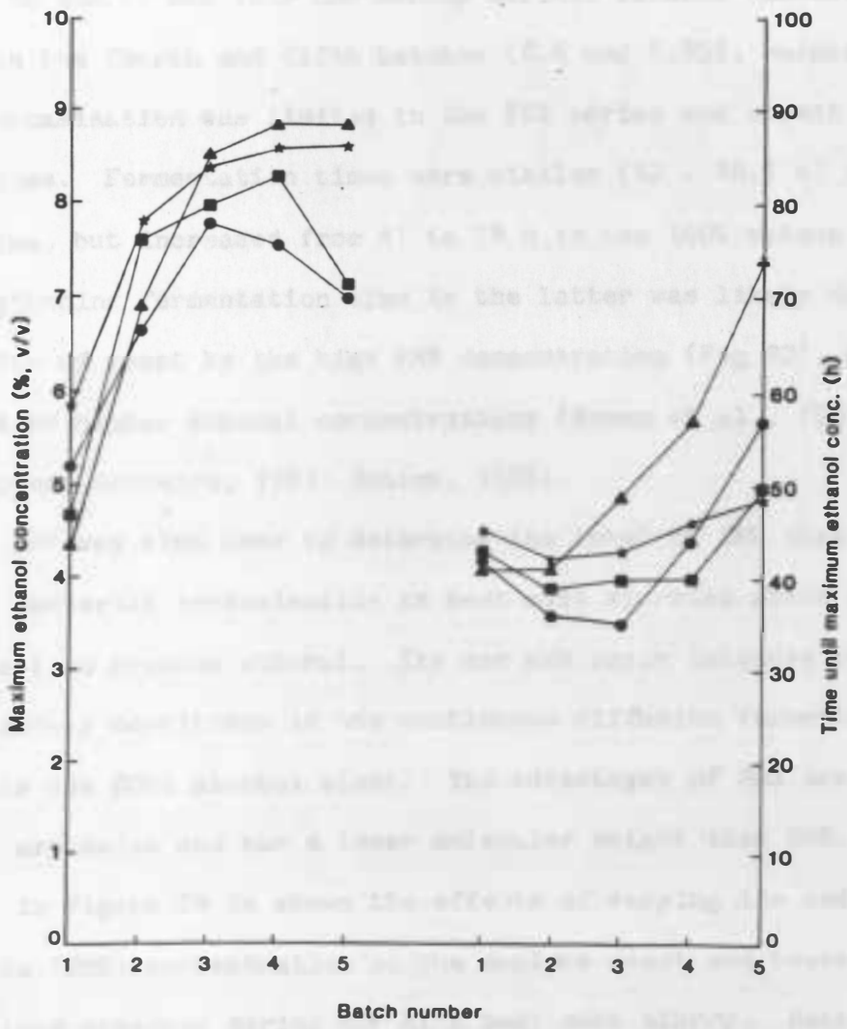

Figure 23. Effect of PMB makeup level on maximum ethanol concentrations produced and time to reach it during SBDF of fodder beet cubes. PMB makeup levels are as indicated in Figs. 21 and 22.

yeast levels decreased (Fig. 22). The 50% PMB makeup series showed a similar response to contamination in the fifth batch.

In the 75 and 100% PMB makeup series, ethanol concentrations peaked in the fourth and fifth batches (8.6 and 8.85%, respectively) since contamination was limited in the 75% series and absent in the 100% series. Fermentation times were similar (42 - 48.5 h) in the 75% series, but increased from 41 to 74 h in the 100% makeup series. The lengthening fermentation time in the latter was likely due to inhibition of yeast by the high PMB concentration (Fig 22), possibly enhanced by higher ethanol concentrations (Brown et al., 1981; Loureiro and Ferreira, 1983; Schimz, 1980).

BDF was also used to determine the level of SMB that would prevent bacterial contamination in beet cube slurries while permitting yeast to produce ethanol. Its use was again intended to simulate start-up conditions in the continuous diffusion fermentor housed in the SDSU alcohol plant. The advantages of SMB are that it is less expensive and has a lower molecular weight than PMB.

In Figure 24 is shown the effects of varying the sodium meta bisulfite (SMB) concentration on the maximum yeast and bacterial populations observed during BDF of a beet cube slurry. Bacterial contaminants were not detected in either juice or cubes when at least 0.20% SMB was used (data not shown). High bacterial numbers of up to 9.8×10^8 cells/ml, however, were observed in juice and cubes when the SMB concentration was 0.15% or less.

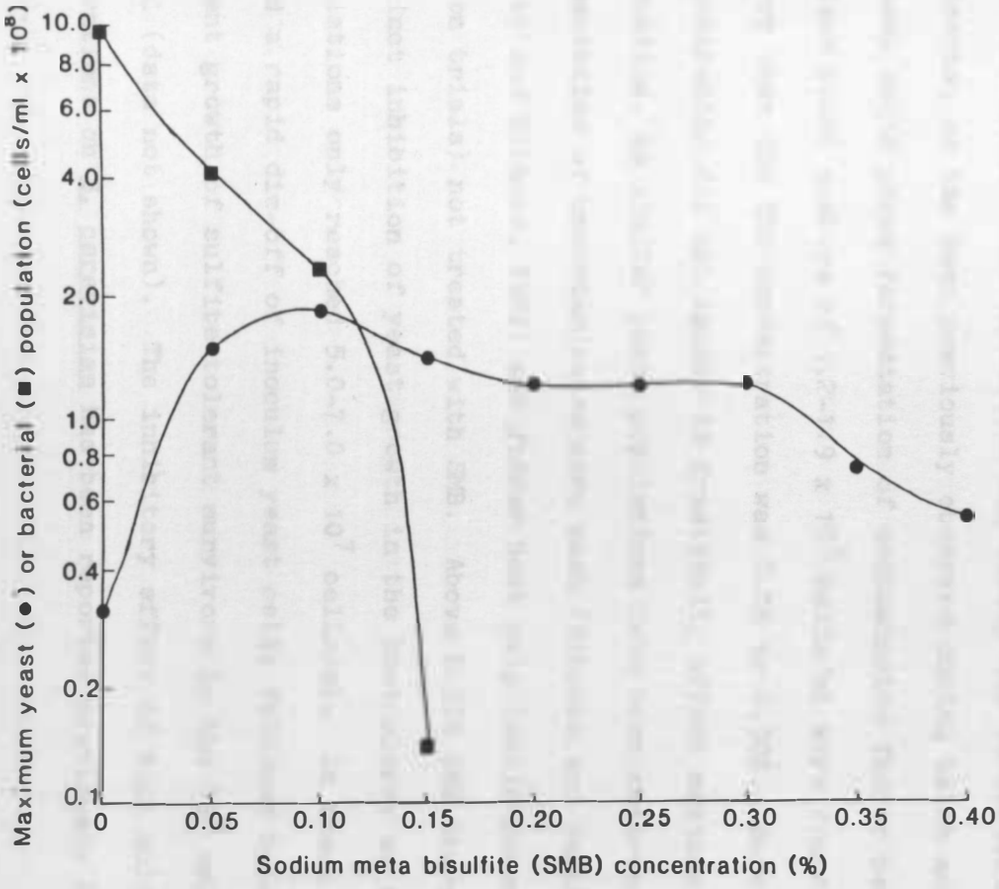

Figure 24. Effect of sodium meta bisulfite (SMB) concentration on maximum yeast (●), and bacterial (■), populations produced during batch diffusion fermentation (BDF) of fodder beet cubes.

The SMB concentration also had a marked effect on the maximum yeast populations that developed in the slurry during fermentation. At 0% SMB the yeast population only rose to approximately 3.0 x 10^7 cells/ml. This inhibition was likely due to bacterial contaminants, as has been previously observed during batch and continuous solid phase fermentation of contaminated fodder beet pulp. Maximum yeast numbers of 1.2-1.9 x 10^8 cells/ml were found in the slurry when the SMB concentration was 0.05 to 0.30%. Here the SMB concentration did not appear to drastically affect maximum yeast production, as similar yeast populations have been observed during fermentation of uncontaminated corn mash (Gibbons and Westby, 1983; Westby and Gibbons, 1982) and fodder beet pulp (solid-phase fermentation trials) not treated with SMB. Above 0.30% SMB there was a distinct inhibition of yeast growth in the beet slurry as maximum populations only reached 5.0-7.0 x 10^7 cells/ml. In these trials I noted a rapid die-off of inoculum yeast cells followed by a subsequent growth of sulfite tolerant survivors to the 10^8 cell/ml level (data not shown). The inhibitory effect of high sulfite concentrations on S. cerevisiae has been reported previously (Schimz, 1980).

In the previous PMB study I determined that a PMB concentration of 0.25% (wt/wt) would prevent bacterial contamination and allow optimum ethanol yields during BDF of fodder beet cubes. Other researchers have reported that PMB concentrations lower than these and ranging from 0.01-0.03%, were effective in controlling

contamination during submerged ethanolic fermentations (Amerine and Kunkee, 1968; Wick, 1979). In those cases, however, the initial feedstock (glucose or grape must) was free of large scale contamination. Raw fodder beets used in these tests, on the other hand, had a significantly higher bacterial population to be eliminated due to adhering soil particles, mechanical damage received during harvest, and microbial growth during transport and storage.

Increasing the SMB concentration of beet cube slurries only slightly reduced initial pH values. Over the SMB concentrations tested, initial pH values ranged from 5.60-5.85, with an average of 5.43. After fermentation, pH ranged from 3.71-4.37, with an average of 4.08. The pH decline during fermentation resulted from the metabolic activity of yeast and/or contaminating bacteria. These pH's were, however, not low enough to control contamination alone. In previous sections I determined that a pH range of 3.0-3.5 was required to control contamination in fodder beet pulp.

In Figure 25 I show the effects of SMB concentration on the time it took the beet slurry to attain both maximum yeast and bacterial populations and maximum ethanol concentration. As can be seen, it took progressively longer (18 to 41h) for bacterial contaminants to reach a peak population as the SMB concentration increased. This increasingly inhibitory effect eventually prevented bacterial growth or survival at SMB concentrations of 0.20% or higher (data not shown).

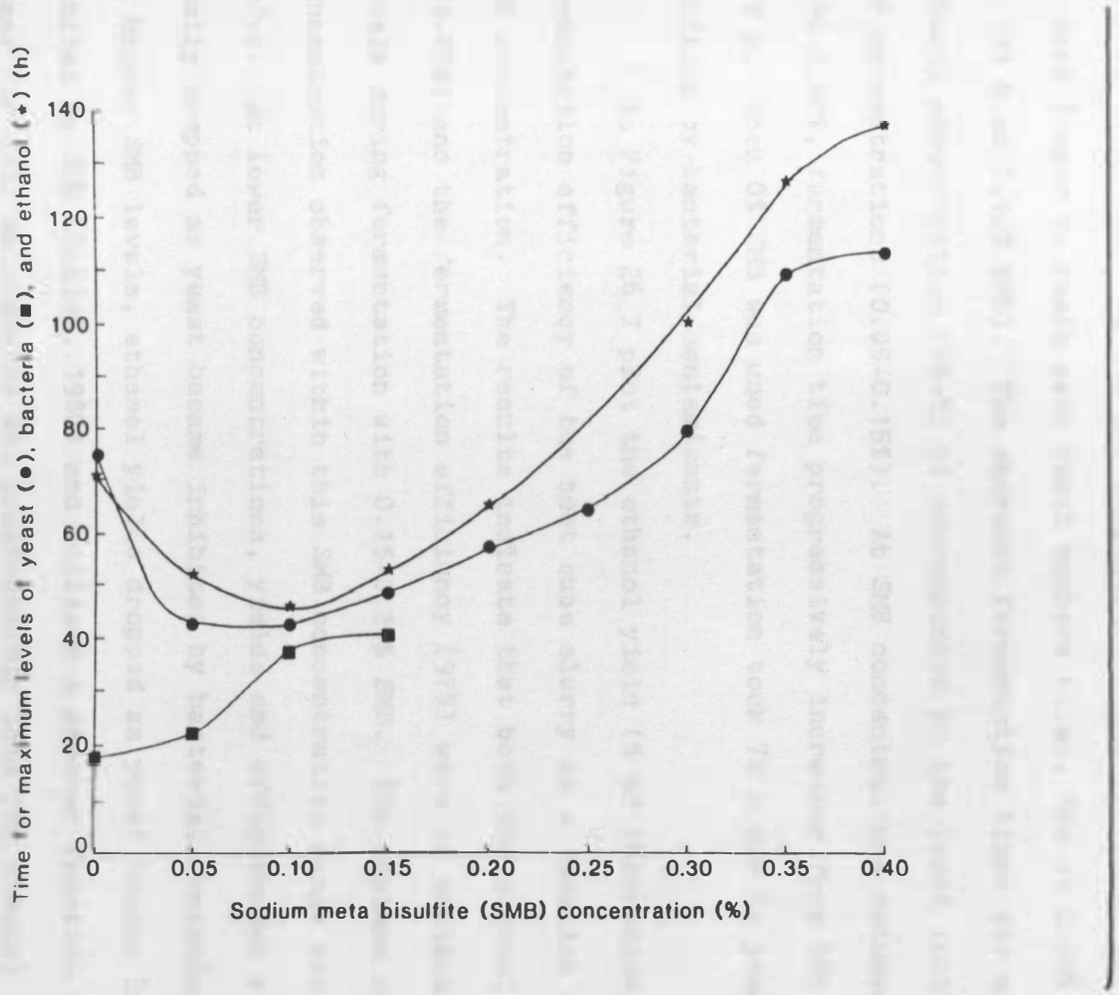

Figure 25. Effect of SMB concentration on the time to reach maximum yeast (●), and bacterial (■) populations; and maximum ethanol concentration (★) in BDF of fodder beet cubes.

The yeast population peaked after only 43 h of fermentation at 0.05-0.10% SMB. As the SMB concentration was increased further, it took longer to reach peak yeast numbers (i.e., 58h at 0.20% SMB to 114 h at 0.40% SMB). The shortest fermentation times for maximum ethanol concentration (46-53 h) corresponded to the least inhibitory SMB concentrations (0.05-0.15%). At SMB concentrations between 0.20-0.40%, fermentation time progressively increased from 66h to 138 h. When 0% SMB was used fermentation took 72 h due to yeast inhibition by bacterial contaminants.

In Figure 26 I plot the ethanol yield (% of theoretical) and fermentation efficiency of the beet cube slurry as a function of the SMB concentration. The results indicate that both the ethanol yield (83-85%) and the fermentation efficiency (97%) were at maximum levels during fermentation with 0.15-0.20% SMB. The maximum ethanol concentration observed within this SMB concentration range was 4.74% (v/v). At lower SMB concentrations, yields and efficiencies eventually dropped as yeast became inhibited by bacterial contaminants. At higher SMB levels, ethanol yields dropped as yeast became inhibited by SMB (Schimz, 1980) and utilized a greater fraction of the sugar for cell maintenance and progressively less for ethanol production.

Results from BDF of fodder beets indicated that 0.2% SMB prevented bacterial contamination and at the same time permitted an 84% ethanol yield at 97% efficiency. Unfortunately, the amount of alcohol produced, 4.69% (v/v), was not distillably worthwhile and

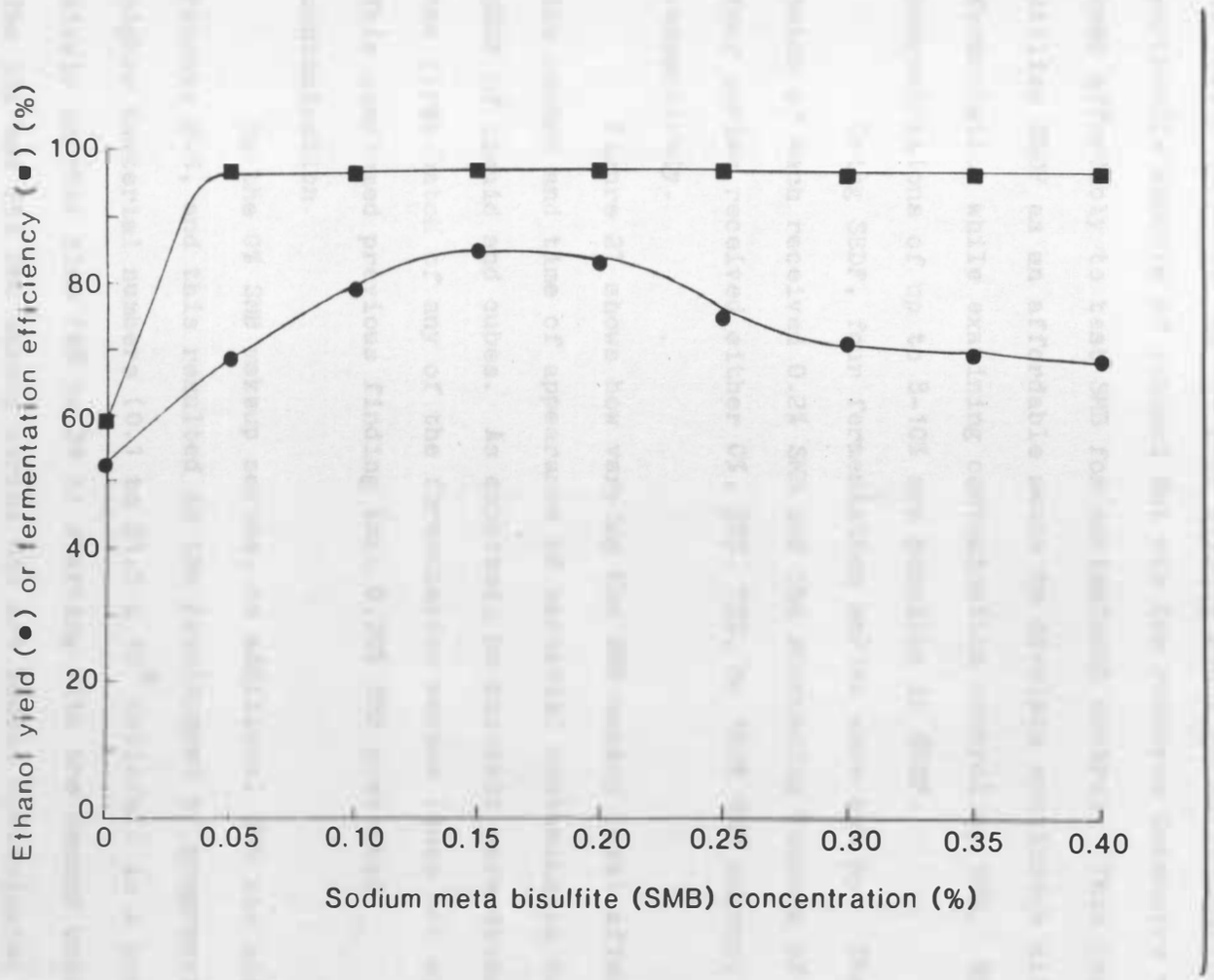

Figure 26. Effect of SMB concentration on ethanol yield (●), and fermentation efficiency (■) during BDF of fodder beet cubes. Ethanol yields are given as % of theoretical.

hence the BDF process is not commercially viable. The continuous diffusion fermentor in the SDSU alcohol plant permitted distillably worthwhile amounts of ethanol but was too resource intensive to be used affordably to test SMB for contaminant control. This led me to utilize SBDF as an affordable means to simulate continuous diffusion fermentation while examining contamination control by SMB. Ethanol concentrations of up to 8-10% are possible in SBDF.

Using SBDF, four fermentation series were set up. The first batch of each received 0.2% SMB and the succeeding batches of the four series received either 0%, 50%, 75%, or 100% SMB makeup, respectively.

Figure 27 shows how varying the SMB makeup level affected the number and time of appearance of bacterial contaminants during SBDF of liquid and cubes. As expected, no bacteria were detected in the first batch of any of the fermentation series (data not shown). This confirmed previous finding that 0.20% SMB prevented contamination.

In the 0% SMB makeup series, no additional SMB was added to batches 2-5, and this resulted in the development of progressively higher bacterial numbers (0.1 to 21.5 x 10^8 cells/ml) in a progressively shorter time (48 to 24 h) starting with the second batch. The 50% and 75% SMB makeup series did not become contaminated until the fourth batch, and then it took 55 and 82h, respectively, to reach peak bacterial populations (5.2 and 1.0 x 10^7 cells/ml, respectively). When 100% SMB makeup was used (0.20% SMB added to

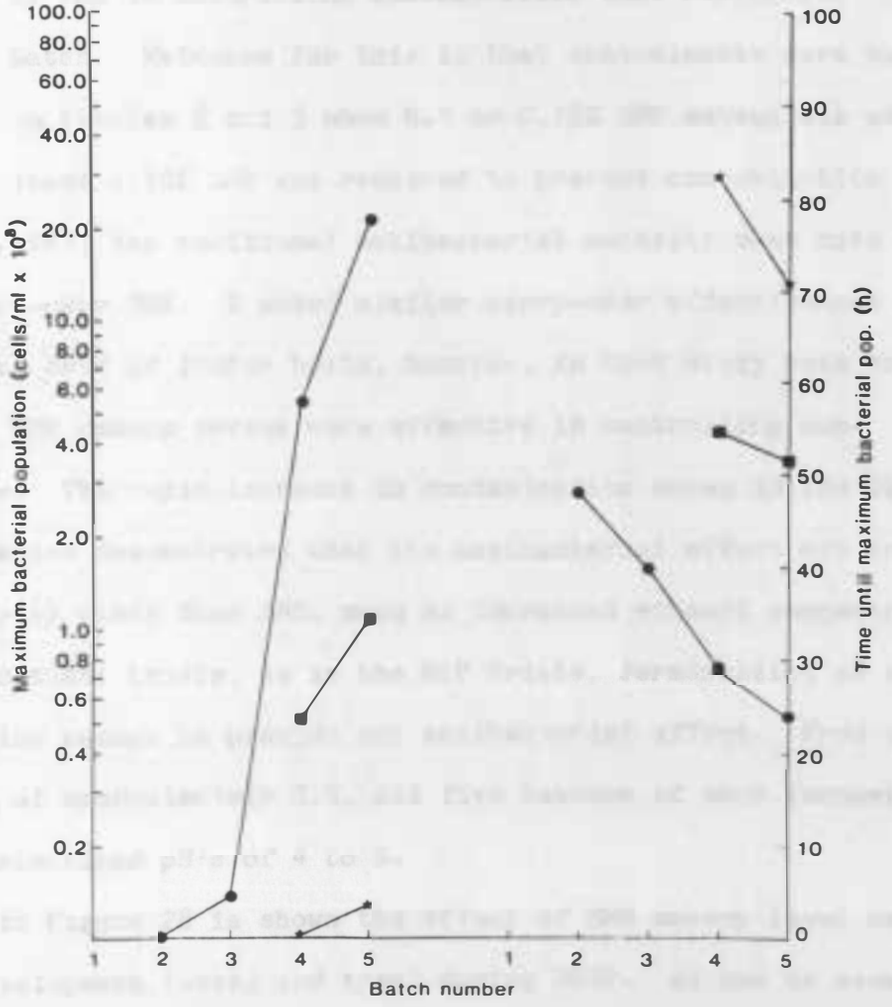

Figure 27. Effect of SMB makeup level on size of maximum bacterial population and the time to reach it during sequential batch diffusion fermentation (SBDF) of fodder beet cubes. SMB makeup levels are: (●) 0% makeup, (■) 50% makeup, and (★)75% makeup.

batches 2-5) no contaminants were detected in any batch (data not shown).

The results indicated that there was some residual effectiveness of SMB in controlling contamination that was passed from batch to batch. Evidence for this is that contaminants were not detected in batches 2 and 3 when 0.1 or 0.15% SMB makeup was added. Since at least 0.20% SMB was required to prevent contamination in BDF (Fig. 24), the additional antibacterial activity must have come from carry-over SMB. I noted similar carry-over effectiveness of PMB during SBDF of fodder beets, however, in that study both the 75 and 100% PMB makeup series were effective in controlling contaminants. The rapid increase in contamination shown in the 0% SMB makeup series demonstrated that the antibacterial effect was not due to factor(s) other than SMB, such as increased ethanol concentration. In these trials, as in the BDF trials, fermentation pH never dropped low enough to provide any antibacterial effect. From starting pH's of approximately 5.5, all five batches of each fermentation series maintained pH's of 4 to 5.

In Figure 28 is shown the effect of SMB makeup level on yeast development (level and time) during SBDF. As can be seen, yeast populations were higher and developed in a shorter time in the first batch of each SBDF series (Fig. 28) when compared to BDF using 0.20% SMB (Fig. 24 and 25). This was due to the difference in inoculum yeast populations.

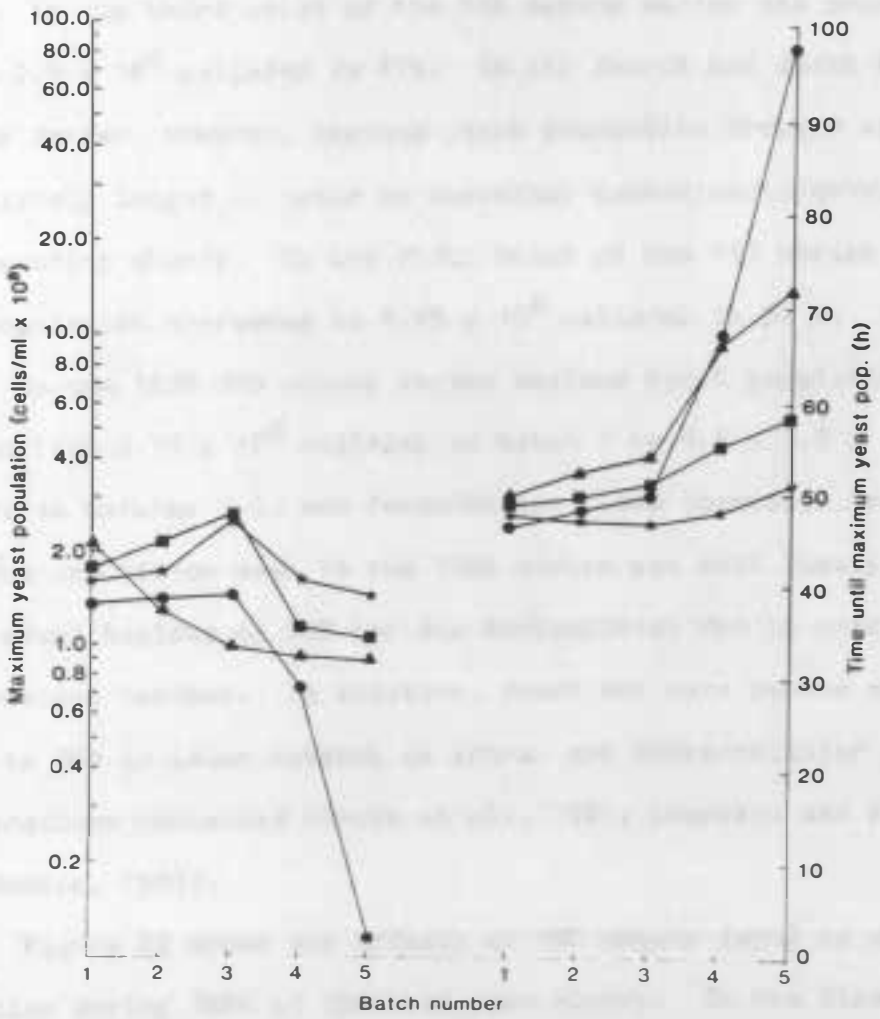

Figure 28. Effect of SMB makeup level on size of maximum yeast population produced and time to reach it during SBDF of fodder beet cubes. SMB makeup levels are as indicated in Fig. 27. 100% makeup given as (▲).

In the 0, 50, and 75% SMB makeup series, maximum yeast populations progressively rose in size through the first three batches while the fermentation times were relatively short. So, for example, in the third batch of the 75% makeup series the population rose to 2.5 x 10^8 cells/ml in 47h. In the fourth and fifth batches of these series, however, maximum yeast production dropped and took progressively longer to occur as bacterial contaminants developed in the fermenting slurry. In the fifth batch of the 75% series the yeast population decreased to 1.45 x 10^8 cells/ml in 52 h.

In the 100% SMB makeup series maximum yeast populations declined from 2.15 x 10^8 cells/ml in batch 1 to 9.8 - 8.9 x 10^7 cells/ml in batches 3-5, and fermentation times increased from 50 to 72h. The inhibition seen in the 100% series was most likely caused by a gradual buildup of SMB (or its derivatives) due to carry-over from previous batches. In addition, yeast may have become more sensitive to SMB in later batches as intra- and extra-cellular ethanol concentrations increased (Brown et al., 1981; Loureiro and Ferreira, 1983; Schimz, 1980).

Figure 29 shows the effects of SMB makeup level on ethanol production during SBDF of the beet cube slurry. In the first batch of all four series, where 0.2% SMB was used, the maximum ethanol concentrations ranged from 4.2-5.2% (v/v) and production took 50-58 h. During subsequent batches (2-5) ethanol levels increased until the concentration approached twice what it was in the first batch (i.e., 8-9% v/v). This is the maximum ethanol concentration

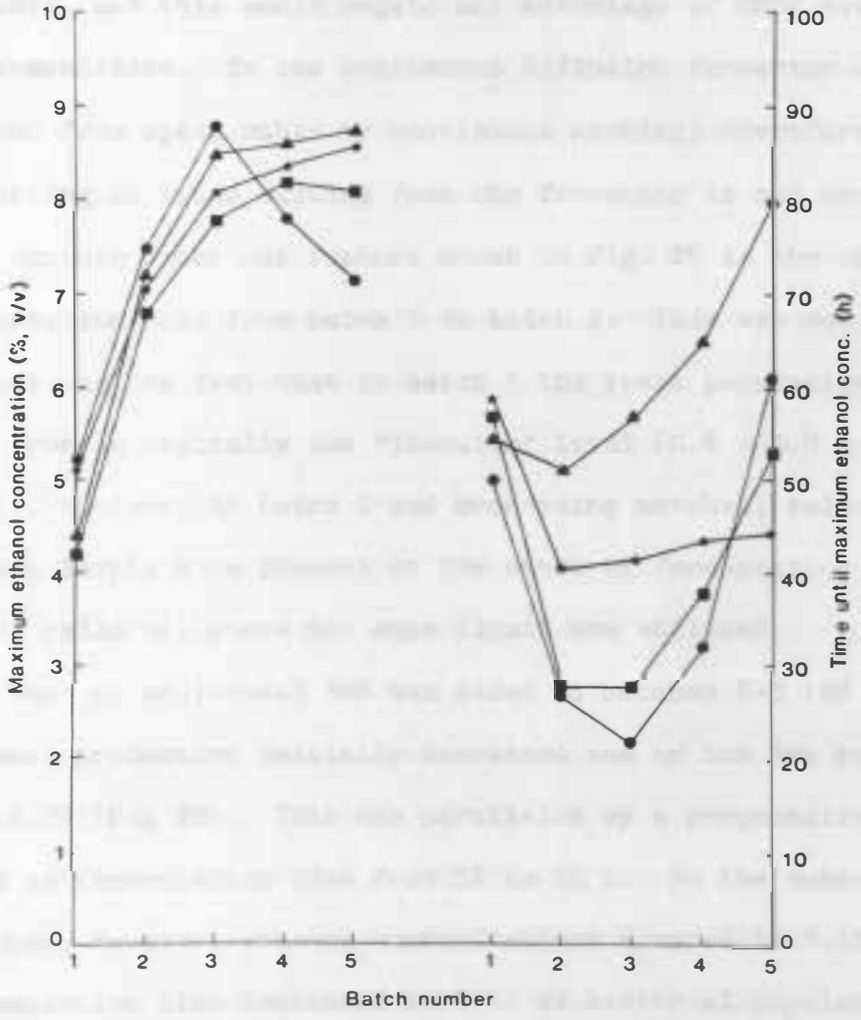

Figure 29. Effect of SMB makeup level on maximum ethanol concentration produced and time to reach it during SBDF of fodder beet cubes. SMB makeup levels are as indicated in Figs. 27 and 28.

possible using the SBDF process, since approximately half of the ethanol in each batch is removed with spent cubes at the end of each batch. Recovery of ethanol in the cubes would involve pressing or drying them, and this would negate any advantage of SBDF over solid phase fermentation. In the continuous diffusion fermentor ethanol is removed from spent cubes by continuous washing, therefore pressing or drying of cubes exiting from the fermentor is not necessary.

Another important feature shown in Fig. 29 is the rapid drop in fermentation time from batch 1 to batch 2. This was due, in large part, to the fact that in batch 1 the yeast population had to develop from an initially low "inoculum" level (0.6 - 1.0 x 10^6 cells/ml), whereas, in batch 2 and succeeding batches, relatively high yeast levels were present at the start of fermentation (0.5 - 1.0 x 10^8 cells/ml) since the same liquid was utilized.

When no additional SMB was added to batches 2-5 (0% makeup) the ethanol production initially increased and by the 3rd batch reached 8.8% (Fig 29). This was paralleled by a progressive decrease in fermentation time from 52 to 22 h. In the subsequent two batches, however, ethanol concentrations dropped to 7.15% and the fermentation time increased to 61 h as bacterial populations increased (Fig 27) and yeast levels decreased (Fig 28). The 50% SMB makeup series showed a somewhat similar response for the same reasons.

In the 75 and 100% SMB makeup series, ethanol production continued to rise through the fifth batch (8.6 and 8.75%,

respectively). This was due to the limited amount of contamination in the 75% series and absence of contamination in the 100% series. Fermentation times remained relatively stable at 40-44 h during batches 2-5 of the 75% makeup series but increased from 51 to 80 h in the 100% makeup series. The latter was evidently due to inhibition of the yeast by the high SMB concentration, (Fig 28), possibly enhanced by higher ethanol concentrations.

To determine if there was a pH range that would prevent bacterial contamination of beet cube slurries, yet permit yeast fermentation, I added different levels of H_2SO_4 to nine test slurries. BDF was employed to simulate start-up conditions in the fermentor. Acidified cube slurries varied in pH from 4.23 to 1.65. When no acid was added the inital pH was 6.42.

Figure 30 shows how initial pH values of the cube slurries changed during and after fermentation. As can be seen, pH 3.3 to 3.5 was a break-point for pH values during and after fermentation. That is, cube slurries with initial pH values less than this range tended to increase in pH during and after fermentation toward that level, while slurries with initial pH values greater than pH 3.3 to 3.5 decreased in pH toward that level. This buffering effect was caused primarily by beet cube components that were released during fermentation. In the case of fermentations carried out at pHs above 3.5, this may have been abbetted by acid production from bacterial contaminants.

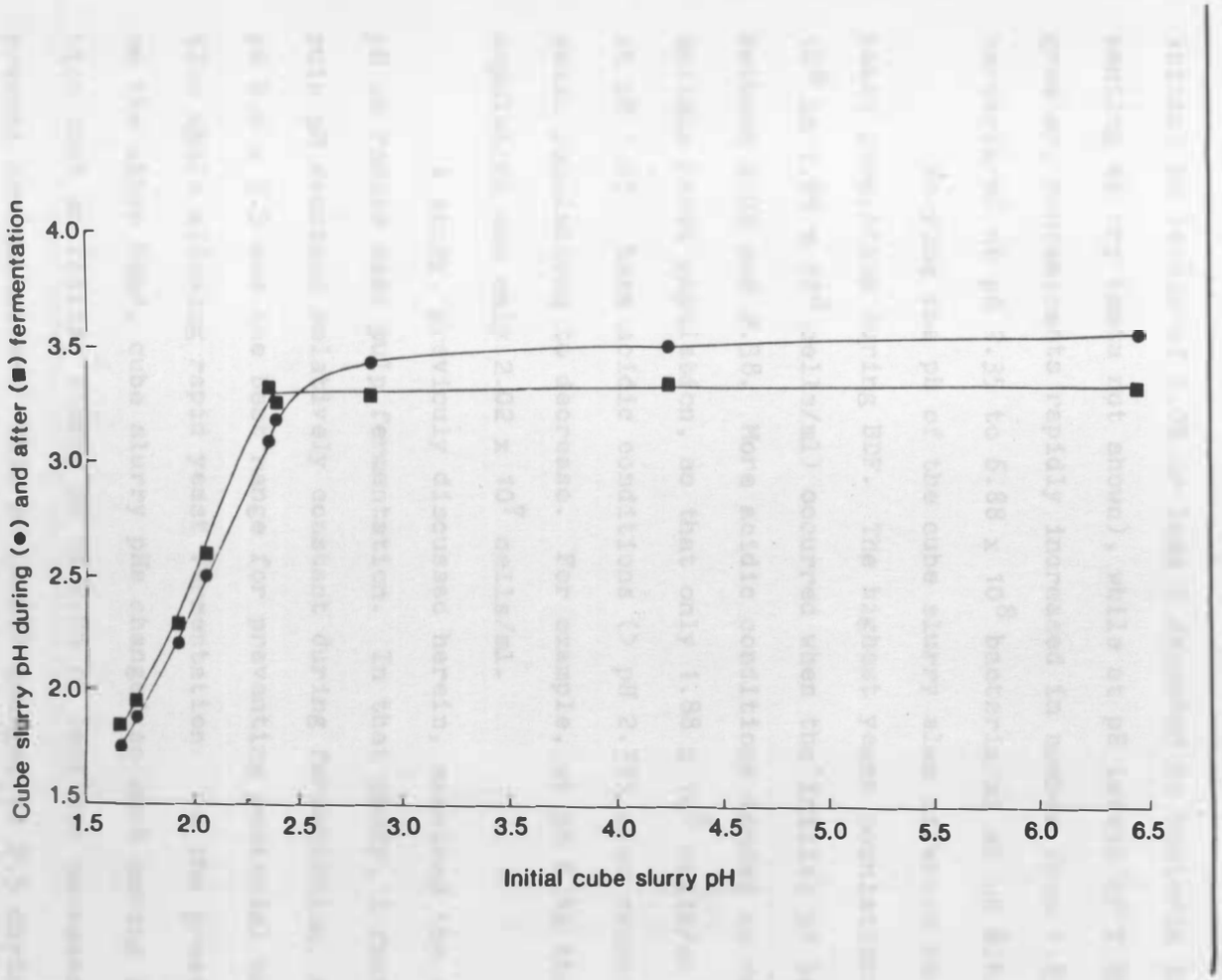

Figure 30. Effect of initial fodder beet cube slurry pH on the pH that developed during (●) and after (■) batch diffusion fermentation (BDF). The initial pH of the slurry was that just prior to yeast inoculation. The pH value during fermentation was that between 10 and 20 h after yeast inoculation. The pH value after fermentation was that after fermentation had ceased.

In Figure 31 I show how the initial cube slurry pH affected maximum levels of yeast and bacteria that developed during BDF. At initial pH levels of 2.05 or less I detected no bacteria in the fermenting slurry (data not shown), while at pH levels of 2.35 and greater, contaminants rapidly increased in number from 1.33×10^7 bacteria/ml at pH 2.35 to 6.88×10^8 bacteria/ml at pH 6.42.

Varying the pH of the cube slurry also affected the maximum yeast population during BDF. The highest yeast populations (1.25×10^8 to 1.95×10^8 cells/ml) occurred when the initial pH level was between 2.05 and 2.38. More acidic conditions tended to reduce the maximum yeast population, so that only 1.88×10^6 cells/ml occurred at pH 1.65. Less acidic conditions (> pH 2.38) also caused maximum yeast populations to decrease. For example, at pH 6.42 the yeast population was only 2.02×10^7 cells/ml.

A study, previouly discussed herein, examined the effect of pH on fodder beet pulp fermentation. In that study, I found that pulp pH remained relatively constant during fermentation, and that pH 3.0 - 3.5 was the best range for preventing bacterial contamination while allowing rapid yeast fermentation. In the present study, on the other hand, cube slurry pHs changed so much during fermentation that an initial slurry pH of 2.05 or less was necessary to prevent contamination. This pH (2.05) changed to 2.5 during, and 2.6 after fermentation. These pH levels are much lower than those commonly recommended for submerged fermentation of either molasses

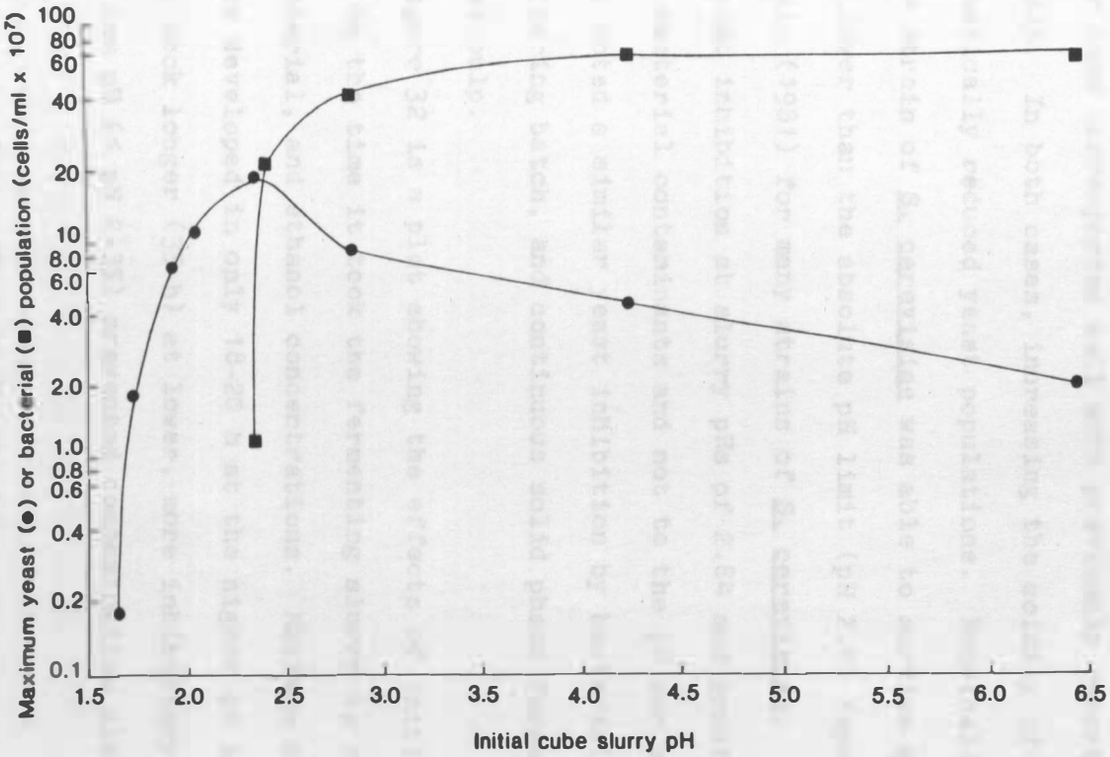

Figure 31. Effect of initial fodder beet cube slurry pH on maximum yeast (●), and bacterial (■) populations that developed during BDF. The pH of each slurry was adjusted as in Figure 30.

(pH 4-5) (Prescott and Dunn, 1959; Stark, 1954) or grain (pH 4.8-5) (Stark, 1954).

The maximum yeast populations observed in cube slurries of pH 2.05 or less corresponded well with previously reported research on beet pulp. In both cases, increasing the acidity of the substrate drastically reduced yeast populations. Nonetheless, in both cases this strain of S. cerevisiae was able to survive and grow at pH levels lower than the absolute pH limit (pH 2.4) reported by Jones et al. (1981) for many strains of S. cerevisiae.

Yeast inhibition at slurry pHs of 2.84 and greater was likely due to bacterial contaminants and not to the pH per se. I previously noted a similar yeast inhibition by bacterial contaminants during batch, and continuous solid phase fermentation of fodder beet pulp.

Figure 32 is a plot showing the effects of initial cube slurry pH on the time it took the fermenting slurry to reach maximum yeast, bacterial, and ethanol concentrations. Maximum bacterial populations developed in only 18-20 h at the higher pH levels (>pH 4.24), but took longer (35 h) at lower, more inhibitiory pHs. Extremely low pH (< pH 2.35) prevented contamination altogether (Fig. 31).

For most of the pHs tested, the time to reach the maximum yeast population was quite similar to the time it took to reach the maximum ethanol concentration. The shortest times here (22-28 h) occurred when the initial slurry pH was 2.05 - 4.24. On either side

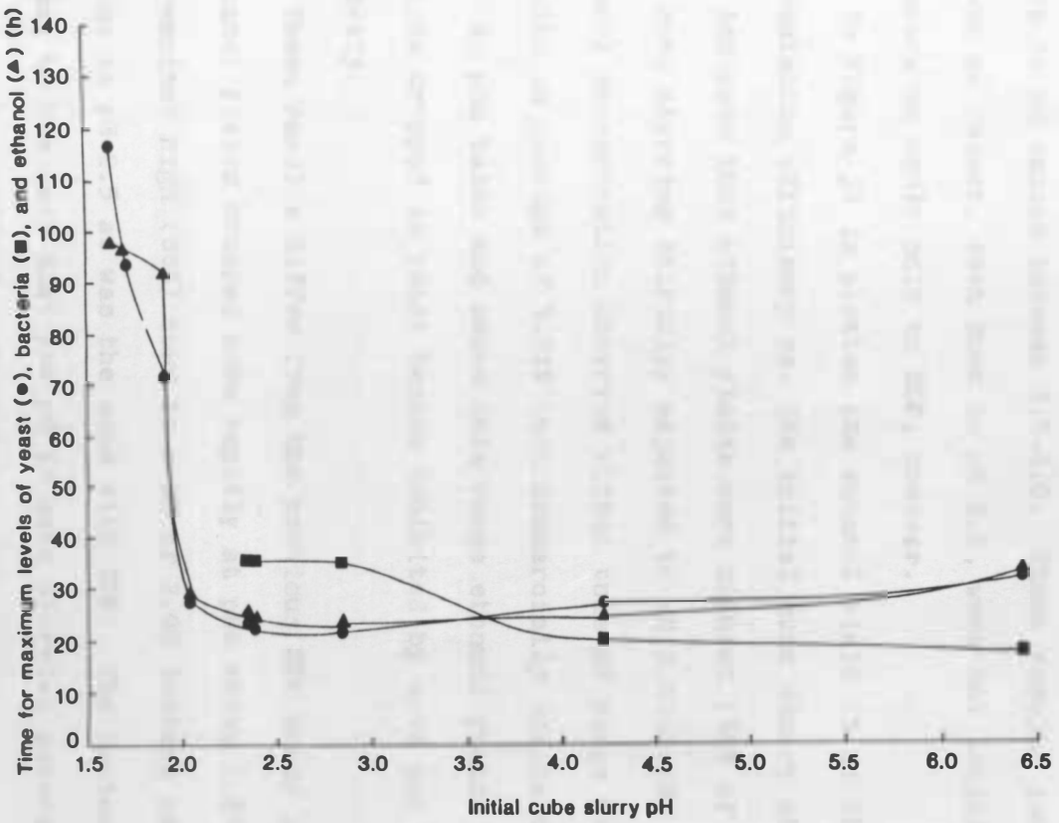

Figure 32. Effect of initial fodder beet cube slurry pH on fermentation time to reach maximum yeast (●), and bacterial (■) populations, and maximum ethanol concentration (★) during BDF. The pH of each slurry was adjusted as in Figure 30.

of this pH range, fermentation times were longer. In the case of the lower pHs this was attributable to acidic conditions, and in the case of the higher pHs, to bacterial contaminants. Jones et al. 1981 noted that yeast sugar fermentation rates were relatively insensitive to pH values between 3.5-6.0. These results indicated that lower pH values, even down to pH 2.0, were not inhibitory. This appears to apply only to BDF, however.

In Figure 33 is plotted the ethanol yield (% of theoretical) and fermentation efficiency vs. the initial cube slurry pH. The results indicate that ethanol yields were highest (86% of theoretical) in cube slurries initially adjusted to pH 2.05-2.38. The maximum ethanol concentration observed within this pH range was 3.84% (v/v), with an average of 3.31% (not commercially viable concentrations). At pHs below and above this range ethanol yields and concentrations dropped as yeast became inhibited by acid and bacteria, respectively.

These results differ from the previous SPF study in that here ethanol yields dropped more rapidly at pHs above 2.84. Also, yields remained high (86%) down to a pH of 2.05 instead of beginning to decline at pH 2.5 as was the case with SPF. The latter was most likely due to the fact that low pH in cube slurries adversely affected the yeast less than low pH in pulp.

Fermentation efficiencies were approximately 98% when the pH was 2.05 or greater, but fell off to 21% at pH 1.65. Reduced efficiencies at low pHs paralleled the drop in ethanol yield. The

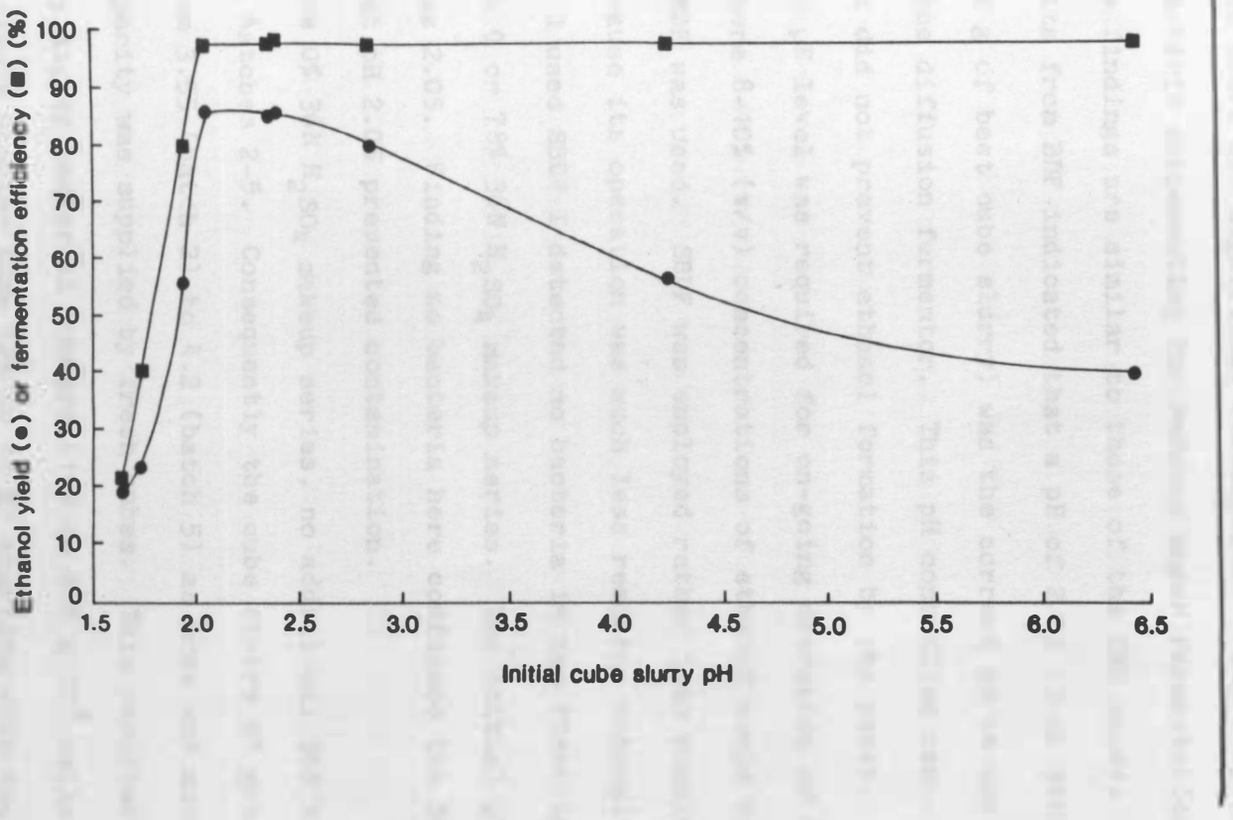

Figure 33. Effect of initial fodder beet cube slurry pH on ethanol yield (●), and fermentation efficiency (■) during BDF. The pH of the slurry was adjusted as in Figure 30.

high fermentation efficiencies observed at the high pHs (Fig. 33) indicates that there was significant consumption of sugar by contaminating bacteria compensating for reduced sugar fermentation by yeast. These findings are similar to those of the SPF study.

Results from BDF indicated that a pH of 2.05 (3 ml 36N 36N H_2SO_4 in 2000 g of beet cube slurry) was the correct pH to use for start-up of the diffusion fermentor. This pH controlled contaminants but did not prevent ethanol formation by the yeast. To find out what pH level was required for on-going operation of the fermentor, where 8-10% (v/v) concentrations of ethanol could be generated, SBDF was used. SBDF was employed rather than running the fermentor because its operation was much less resource intensive.

When I used SBDF I detected no bacteria in the first batch of either the 0 or 78% 36N H_2SO_4 makeup series. The initial pH in both cases was 2.05. Finding no bacteria here confirmed the BDF observation that pH 2.05 prevented contamination.

In the 0% 36N H_2SO_4 makeup series, no additional 36N H_2SO_4 was added to batches 2-5. Consequently the cube slurry pH gradually increased from 3.55 (batch 2) to 4.2 (batch 5) as more and more buffering capacity was supplied by fresh cubes. This resulted in progressively higher bacterial numbers (1.9 to 9.6 x 10^8 cells/ml) in a progressively shorter time (24 to 18 h) starting with the second batch. In the 78% 36N H_2SO_4 makeup series 3.5 ml 36N 36N H_2SO_4 was added to each batch in order to maintain a consistent pH

of 2.0-2.2. No contaminants, as expected, were detected in any batch.

The EX-FERM process (de Cabrera et al., 1982; Rolz, 1980; Rolz, 1981; Rolz et al., 1979) is similar to SBDF and some valid comparisons would therefore seem to be in order with respect to contamination problems. Unfortunately, EX-FERM has not yet been used to process fodder beets, and bacterial contaminant levels have not yet been measured with the feedstock that has been used, sugar cane billets. Observations made so far with cane billets indicate, in general, that low pH prevents bacterial contaminants from gaining a foothold. A pH less acidic (3.1 to 3.5) than required for fodder beets (2.0 to 2.2), in fact, was effective. The less acidic requirement was undoubtedly due to the fact that fodder beets, being a root crop, carry a greater contaminant load into fermentation than do sugar cane billets, a stalk crop.

In Figure 34 I show the effect of 0 and 78% 36N H_2SO_4 makeup on yeast development (level and time) during SBDF. In the 0% 36N H_2SO_4 makeup series, maximum yeast populations progressively increased in size through the first three batches while fermentation times dropped. Thus in the third batch, the yeast population rose to 2.8×10^8 cells/ml in 17 h. In the forth and fifth batches of these series, however, maximum yeast levels dropped and took progressively longer to develop as bacterial contaminants caused increasing inhibition. In the fifth batch the yeast population

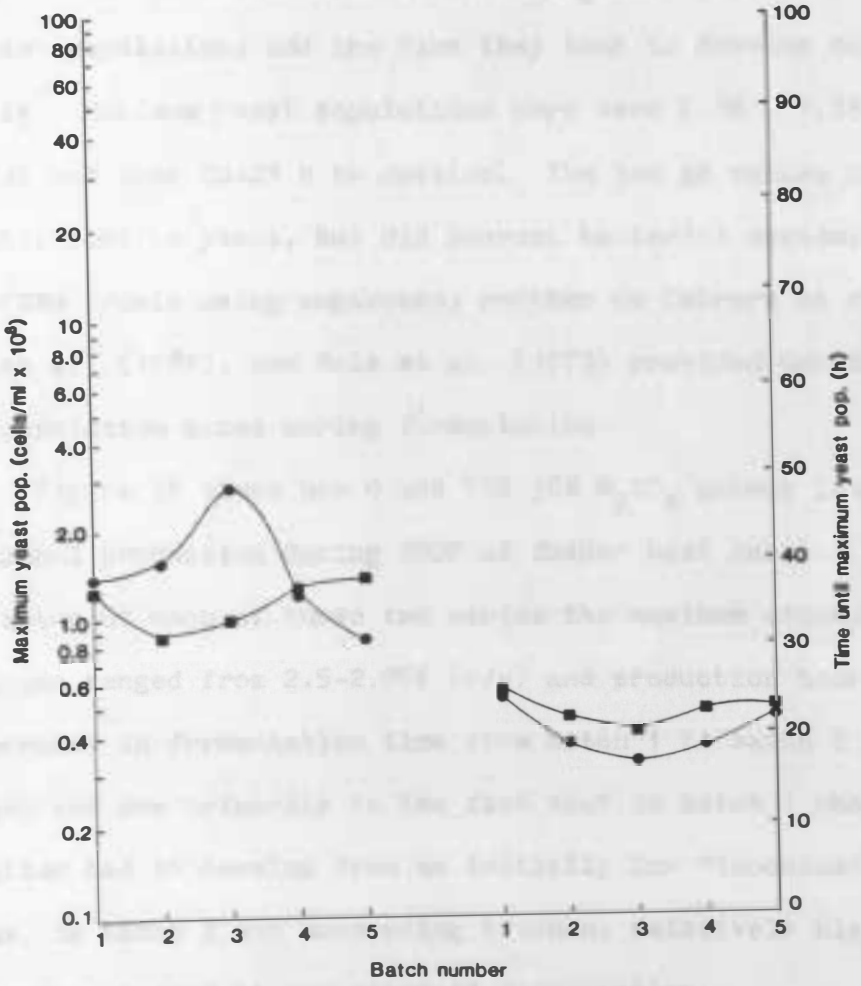

Figure 34. Effect of H_2SO_4 makeup level on yeast development (level and time) during SBDF of fodder beet cubes. (●) refers to 0% and (■) refers to 78% H_2SO_4 makeup series.

dropped to 8.3 x 10^7 cells/ml in 22 h. These are similar to previous findings using BDF (Fig. 31) and SPF.

When constant pH levels of 2.0-2.2 were maintained throughout all five batches (78% 36N H_2SO_4 makeup series) both maximum yeast populations and the time they took to develop only varied slightly. Maximum yeast populations here were 0.88 - 1.35 x 10^8 cells/ml and took 20-25 h to develop. The low pH values used were not inhibitory to yeast, but did prevent bacterial contamination. In EX-FERM trials using sugarcane, neither de Cabrera et al. (1982), Er-el et al. (1981), nor Rolz et al. (1979) provided details on yeast population sizes during fermentation.

Figure 35 shows how 0 and 78% 36N H_2SO_4 makeup levels affected ethanol production during SBDF of fodder beet cubes. In the first batch of each of these two series the maximum ethanol concentration ranged from 2.5-2.95% (v/v) and production took 34-36 h. The decrease in fermentation time from batch 1 to batch 2 (8-11 h shorter) was due primarily to the fact that in batch 1 the yeast population had to develop from an initially low "inoculum" level whereas, in batch 2 and succeeding batches, relatively high yeast levels were present at the start of fermentation.

When no additional 36N H_2SO_4 was added to batches 2-5 (0% makeup) ethanol concentrations increased to 5.15% by the third batch (Fig. 35), while fermentation time decreased from 36 to 25-28 h. Over the next two batches, however, the ethanol concentration dropped to 3.85% and the fermentation time increased to 31 h as

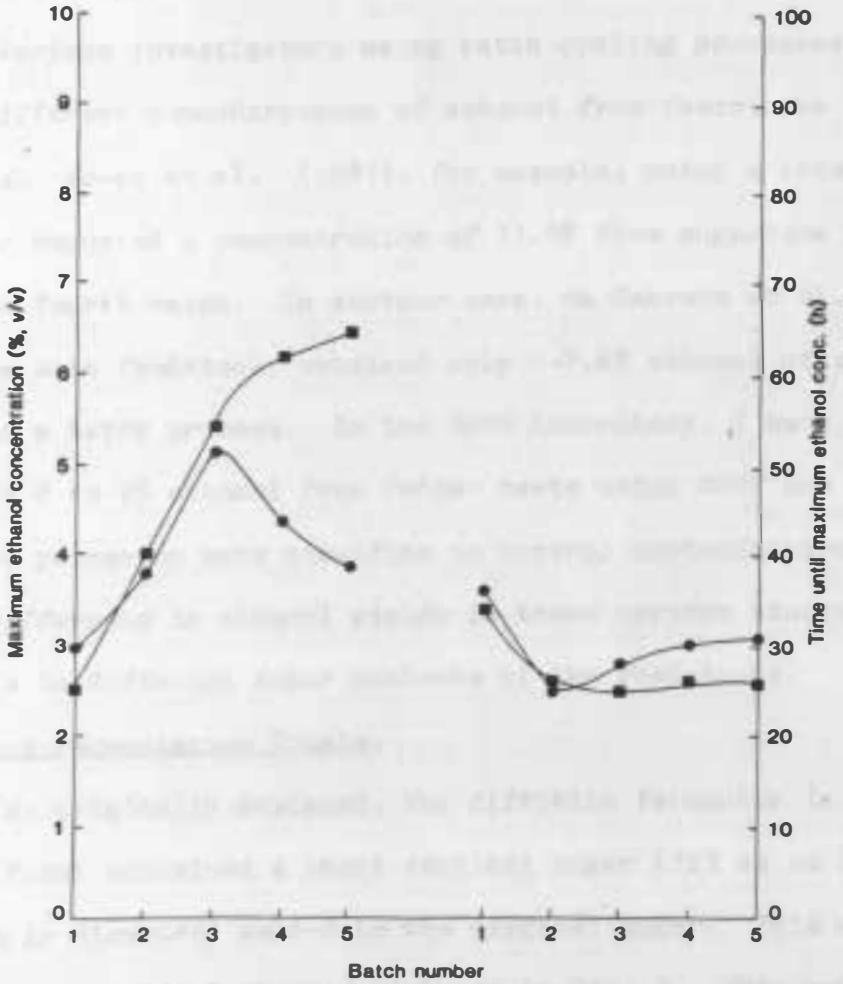

Figure 35. Effect of H_2SO_4 makeup level on maximum ethanol concentrations and the time it took to reach them during SBDF. Symbols are as in Figure 34.

bacterial populations increased and yeast levels decreased (Fig. 34). In the 78% 36N H_2SO_4 makeup series, ethanol concentrations continued to rise through the fifth batch (6.45%, v/v), while fermentation times stabilized at 26 h.

Various investigators using batch cycling processes have obtained different concentrations of ethanol from feedstocks like fodder beets. Er-el et al. (1981), for example, using a rotating drum fermentor reported a concentration of 11.4% from sugarcane billets after the fourth batch. In another case, de Cabrera et al. (1982), using the same feedstock, obtained only 5-7.6% ethanol after two cycles of a batch process. In the SDSU laboratory, I have been able to obtain 8 to 9% ethanol from fodder beets using SBDF and employing sodium or potassium meta bisulfite to control contamination. Much of the difference in ethanol yields in these various studies in traceable to differing sugar contents of the feedstocks.

Continuous Fermentation Trials:

As originally designed, the diffusion fermentor in the SDSU Alcohol Plant contained a short vertical auger (123 cm in length and 15.24 cm in diameter) welded to the diagonal auger. This was in place of the vertical chamber pictured in Fig. 3. This original arrangement proved unsatisfactory, however, primarily because inadequate take-up of cubes from the vertical auger into the diagonal auger caused cubes to form a plug at the junction between the two augers. This plugging severly restricted movement of cubes through the fermentor. The vertical chamber with its perforated press

solved this take-up problem by exposing a greater section of the diagonal auger (43 cm vs. 15.24 cm) to downflowing cubes.

I found that when control measures were not taken, bacterial contamination was as much a problem in the fermentor (Fig. 3) as it had been before in 4 and 20 L containers used, in preliminary tests, to simulate the fermentor. I also found, for on-going operation of the fermentor, that continuous anti-bacterial treatment of raw materials was necessary. Thus loading the fermentor with treated liquid and cubes and properly inoculating them with yeast was not sufficient to control contamination, unless I also treated the reservoir liquid and cubes entering the fermentor later. For example, when 0.2% SMB was used to control bacteria in the loaded fermentor, but on-going treatment was omitted, the ethanol concentration in the beer rose slightly at first (to 2.9% v/v after 108 h) but then later declined to undetectable levels as bacterial contaminants overwhelmed the system (8.8×10^8 cells/ml).

To test whether initial and on-going SMB treatments would satisfactorily prevent fermentor contamination, I conducted trials in which the loaded fermentor initially contained 0.2% SMB. Then during on-going operation, 0.4% SMB was added with both incoming beet cubes and reservoir liquid (Fig. 36). Fermentor loading took the first 96 h of fermentation and involved adding 9 Kg of beet cubes every 12 h to the yeast-SMB slurry. During on-going operation (96-396 h), 9 Kg quantities of beet cubes with 0.4% SMB, and 11 L quantities of a 0.4% SMB reservoir solution were added every 12 h.

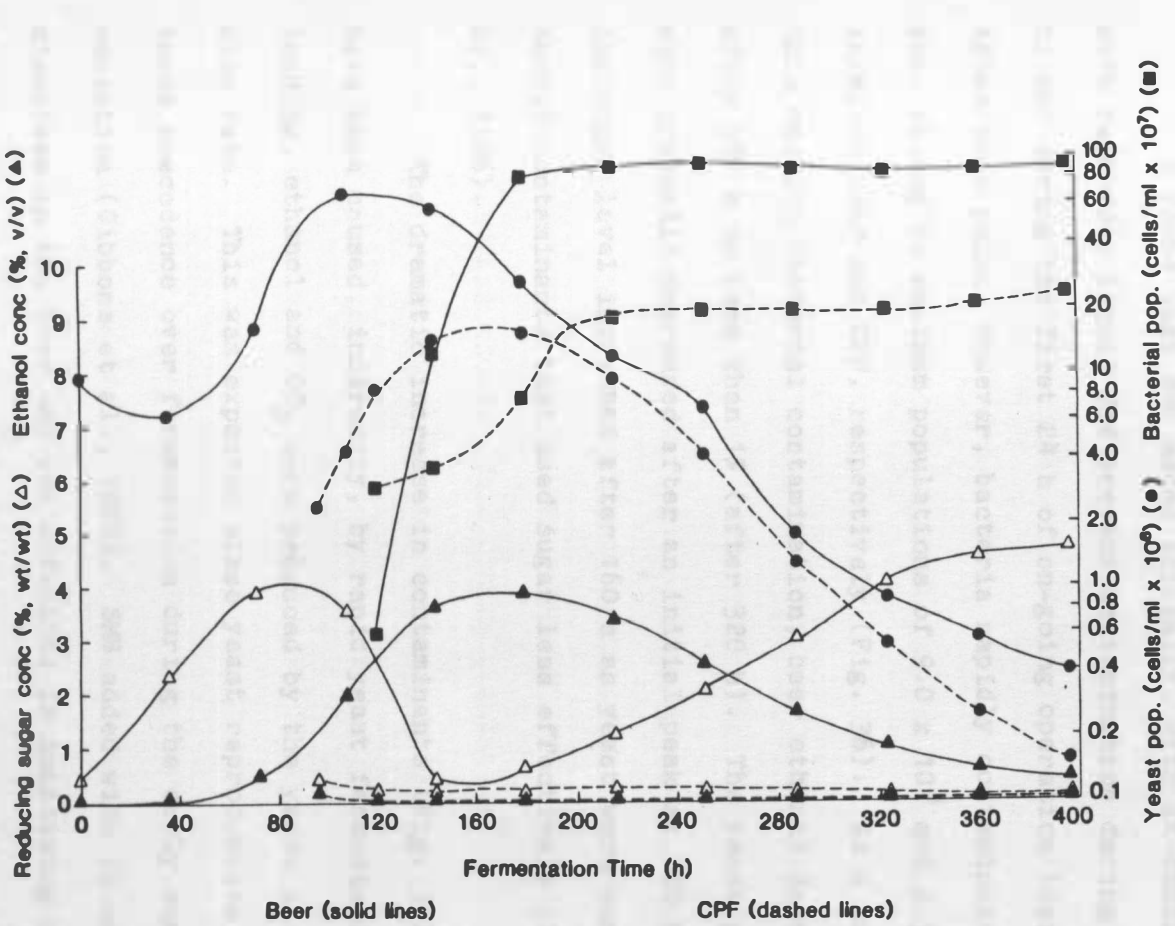

Figure 36. Semi-continuous diffusion fermentation of fodder beet cubes for fuel ethanol and cubed protein feed (CPF) production using SMB to control contaminants. The retention time for beet cubes was 60 h and for beer it was 196 h. Symbols are: (△) reducing sugar, (▲) ethanol, (●) yeast, and (■) bacteria. Solid lines correspond to levels of these components in beer, dashed lines to levels in CPF.

Retention times were 60 h for beet cubes and 196 h for beer that had passed through the fermentor.

I found that SMB added initially, with in-coming cubes, and with reservoir liquid did prevent contamination during loading (0-96 h) and during the first 24 h of on-going operation (data not shown). After that point, however, bacteria rapidly contaminated the fermentor, rising to maximum populations of 9.0×10^8 and 2.3×10^8 cells/ml in beer and CPF, respectively (Fig. 36). As a consequence of this massive bacterial contamination, beer ethanol levels declined after 180 h to less than 1% (after 320 h). The yeast population also gradually decreased after an initial peak at 100 h. The reducing sugar level increased after 160 h as yeast were replaced by bacterial contaminants that used sugar less effectively (Gibbons et al., 1986).

The dramatic increase in contaminants (Fig. 36) appears to have been caused, indirectly, by rapid yeast fermentation. During loading, ethanol and CO_2 were produced by the yeast at a relatively slow rate. This was expected since yeast reproduction generally takes precedence over fermentation during the early stages of fermentation (Gibbons et al., 1986). SMB added with in-coming cubes dissolved in the beer and was effective in inhibiting bacteria. However, during the first 84 h of on-going operation (96-180 h of fermentation), large amounts of CO_2 were produced in the fermentor as the yeast more actively fermented the sugar to ethanol. When either granular or a liquid concentrate of SMB were added to the

fermentor with in-coming cubes, CO_2 percolating up through the cube slurry in the vertical chamber caused the meta bisulfite to quickly bubble off as sulfur dioxide (SO_2) gas. Because of this, SO_2 contact time with the slurry was limited and its antibacterial effectiveness was greatly reduced. As a result, contaminant levels rapidly increased. Because of this problem I discontinued use of meta bisulfite for contamination control in the fermentor and switched, instead, to using low pH.

In preliminary tests with 4 and 20 L containers that simulated fermentor conditions, I had found that a start-up pH of 2.05 and an on-going pH of 2.0 to 2.5 prevented contamination. Therefore I tested these pHs in the fermentor itself for contaminant control. This was done, first by adjusting the pH of the fermentor to 2.05 before cubes were added. Then, 20 Kg quantities of cubes were added every 24 h for 72 h to fill the fermentor. Finally, 20 Kg quantities of acidified cubes (2.65 ml 36 N H_2SO_4/Kg wet wt. cubes) and 15 L quantities of reservoir acid (0.26 N H_2SO_4) were augered and dispensed, respectively, into the fermentor every 24 h during on-going operation. The pH range of the slurry at the beer outlet, during on-going operation, was 2.4 to 2.6, while the pH range at the cube outlet was 2.1 to 2.2. Retention times were 72 h for the cubes and 264 h for the beer. Fermentor additions were made on a 24 h basis (instead of 12 h) for convenience.

I found, using H_2SO_4, that when low pH levels (pH 2.1-2.6) were continually maintained throughout the fermentor, bacterial

contamination was prevented (data not shown). Yeast cells in the beer were not significantly affected by the low pH, however, as maximum populations of 2.2 - 3.6 x 10^7 cells/ml occurred after 24 h (Fig. 37). Yeast cells associated with beet cubes, on the other hand, were severely reduced in number with only 4.5 to 5.4 x 10^4 cells/ml occurring after 240 h fermentation. This reduction was apparently caused by the extremely low pH of the reservoir liquid (< pH 1.5) used to diffuse sugar from the cubes. Such a low pH in the reservoir was necessary because beet cubes neutralized the beer as they passed through the fermentor. When less acid was used, the beer pH rose and little antibacterial activity was observed.

Ethanol concentrations in the beer steadily rose until approximately 500 h fermentation, and remained relatively stable at 8.3 - 8.7% (v/v) thereafter (Fig. 37). This is a distillably worthwhile amount. The start of this stable period coincided with the point at which the beer had been completely replaced twice with reservoir acid, thus indicating steady-state conditions had been achieved. Ethanol in the spent cubes also increased during fermentation, and stabilized after 400 h at 2.6%. This ethanol level is higher than expected and indicates that the fermentor will have to be lengthened in the future in order to expand the concentration gradient between the outlet beer and the spent cubes. Ideally the cubes should contain little or no ethanol so that pressing and/or drying them for ethanol recovery are not necessary.

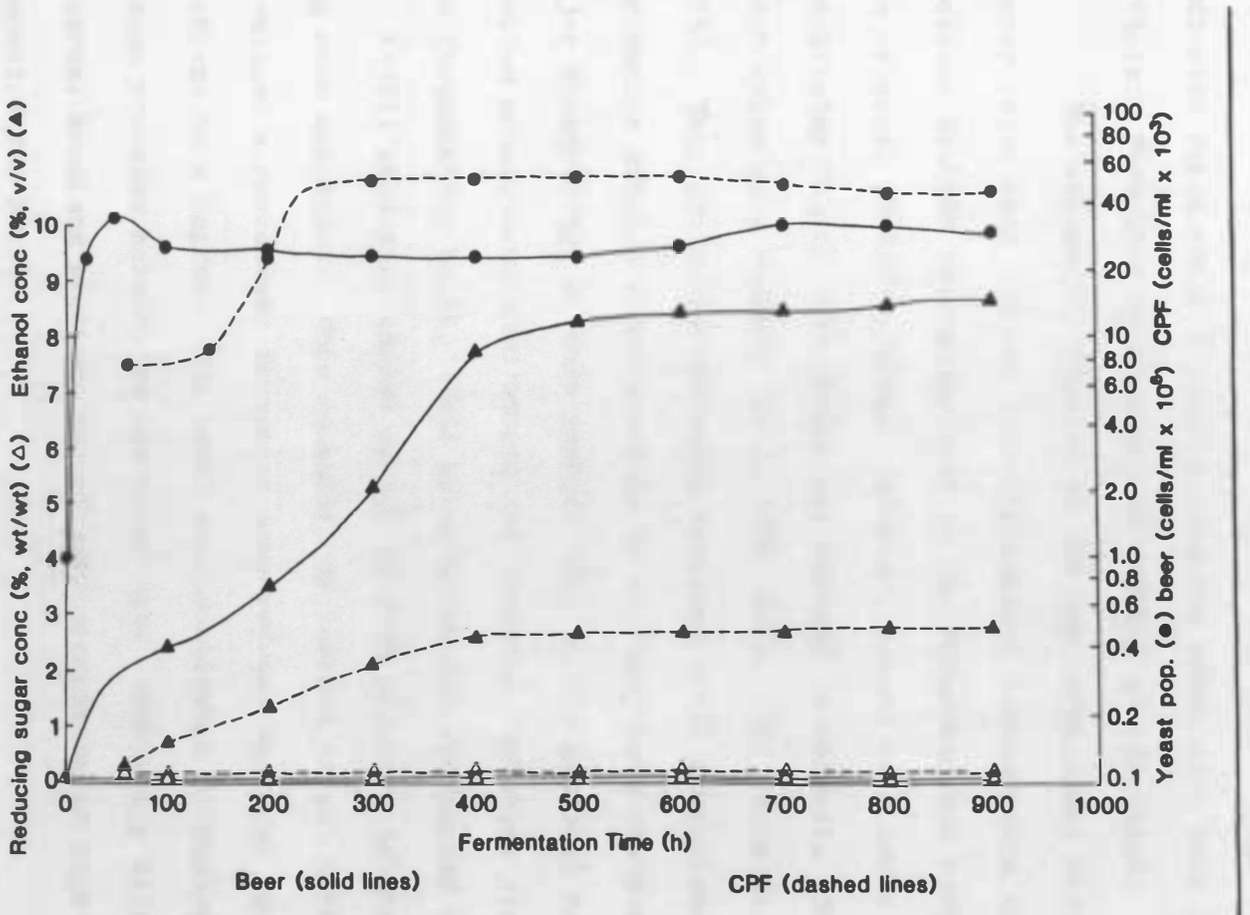

Figure 37. Semi-continuous diffusion fermentation of fodder beet cubes for fuel ethanol and CPF production using H_2SO_4 to control contaminants. The retention time for beet cubes was 72 h and for beer it was 264 h. Symbols are the same as in Fig. 36.

Less than 0.2% (wt/wt) reducing sugar was present in beer and spent cubes throughout the fermentation cycle (Fig. 37). This indicated rapid sugar diffusion from the cubes into beer followed by efficient fermentation of sugar to ethanol by the yeast.

The successful testing of the semi-continuous diffusion fermentor (with acid control of contaminants) demonstrated that the modified EX-FERM technology used in the fermentor was workable. In the original EX-FERM process, feedstock pieces were added to a yeast/water slurry, the system was allowed to ferment, and then spent cubes were removed (Rolz, 1980; Rolz, 1981; Rolz et al., 1979). This cycle was thereupon repeated until distillably worthwhile ethanol concentrations in the beer were reached. The major disadvantage of this process lies in the material handling problem encountered when adding and removing feedstock pieces from the fermentation broth. This problem was not eliminated by Er-el et al. (1981) when they scaled up the EX-FERM process, in their rotating drum apparatus. More recently, de Cabrera et al. (1982) developed a packed-bed fermentor that reduced material handling problems to a degree. The novel semi-continuous diffusion fermentor design provided herein, on the other hand, completely eliminated material handling problems and allowed production of high ethanol concentration beers.

Besides material handling, the other major concern I had during the development of this fermentation process was contamination control. I was concerned about contamination because

I had previously experienced severely reduced ethanol yields and concentrations as a result of it. This occurred during solid-phase fermentation experiments (Gibbons et al., 1986) and during laboratory-scale diffusion fermentation trials, with both fodder beets and sweet sorghum. Interestingly enough, little or no mention has been made of contamination during previous EX-FERM research. This may have been due to the fact that in batch or sequential batch fermentation trials, the effects of contamination may not be evident unless severe contamination develops rapidly. However, in a continuous or semi-continuous processes, such as the SDSU process, no level of contamination is acceptable because the more rapidly growing bacteria will eventually overwhelm the yeast and result in reduced yields and longer fermentaion times.

Rolz et al. (1979) added boiling water to the first EX-FERM batch of sugar cane billets to partially pasteurize the mixture before yeast were added. Measures to control contamination during subsequent batches, however, were not given. Er-el et al. (1981) also conducted EX-FERM tests under non-aseptic conditions and found no effect on ethanol yields. In this case, starting pHs were 5.0-5.5 and the pH ranged from 3.2-3.5 in subsequent batches. A similar low pH of approximately 3.1 was observed by de Cabrera et al. (1982) during packed-bed EX-FERM fermentations of sugar cane billets, and they attributed contamination control to naturally occurring acidic conditions. My findings indicate that slightly lower pHs (2.1 to 2.6) are necessary to control contamination during

semi-continuous diffusion fermentation of fodder beets, and that acidified reservoir liquid and acidified cubes must be used to maintain contamination control.

Continuous Diffusion Fermentation Plant Design:

Figure 38 shows the design and operation of a theoretical, community-scale plant to continuously produce fuel ethanol and CPF from fodder beets. The design is for a scaled-up (X1,400) fermentor based upon the smaller fermentor described herein. A continuous, rather than semi-continuous, mode of operation was assumed for this commercial plant.

In the process, topped fodder beets are conveyed from storage, first into a washing flume, and then to an automatic scale. From here the beets drop into a slicer where they are cut into cubes of 1.91 to 2.54 cm. Cubes then drop into the vertical section of the fermentor along with a steady flow of sulfuric acid (2.66 ml 36N H_2SO_4/Kg cubes wet wt.). The acid is used to maintain a stable pH level. Acid additions at points along the length of the fermentor might also be required. Beet cubes drop (or are forced by pressing using rotating screens or multiple perforated scroll flights) down to the diagonal auger where the rotating flighting screw picks them up and transports them to the top of the diagonal auger (72 h retention). Spent cubes (CPF) are partially dewatered in the top section of the diagonal auger, as it lies above the water line.

While cubes are being augered upward, a dilute H_2SO_4 solution or a combination of acid and stillage (pH 2.0-2.1), enter

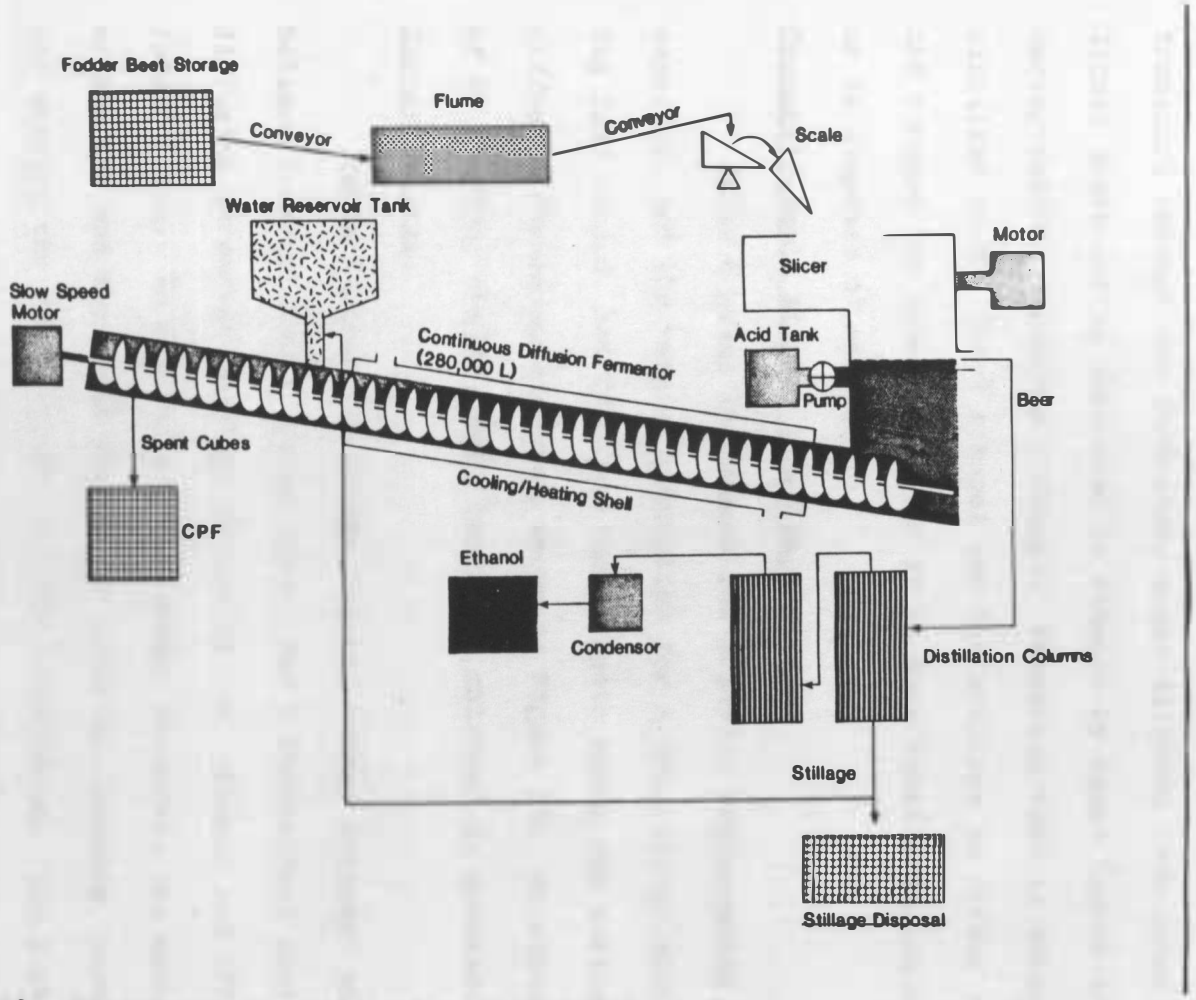

Figure 38. Design and operation of a theoretical, community-scale ethanol plant for conversion of fodder beets and other tuberous crops to ethanol and CPF. Fermentor volume is calculated on the basis of a 264 h retention time for beer and 72 h for fodder beet cubes. Yeast are continuously cultivated within the fermentor, however, for start-up and maintenance, powdered yeast inoculum can be added to the fermentor.

from the reservoir, flow down the length of the fermentor, and exit near the top of the vertical section (264 h retention). During transport through the fermentor, sugar diffuses from cubes into the liquid phase and is fermented to ethanol by yeast (yeast added during initial fermentor filling). Fermented beer is subsequently distilled to 95% (v/v) ethanol and the stillage is either reused in the process (as reservoir fluid or to make yeast propagation medium) or is disposed of as waste.

Community-Scale Alcohol Plant Parameters:

Table 6 gives the annual raw material requirements, the capacity, and the rate of production for a theoretical plant producing fuel ethanol and CPF from fodder beets using the continuous, diffusion fermentaion process shown in Figure 38. An ethanol yield of 87 L/metric ton of fodder beets (21 gal/ton) is assumed.

Energy Balance:

Table 7 gives the energy inputs, energy outputs, and energy balance (energy output/energy input) for a theoretical continuous diffusion fermentation plant producing fuel ethanol and CPF from fodder beets. In determining the energy balances, the output energy of the CPF and the input energy for planting, growing, harvesting and storing the fodder beets were not considered. The 3.04 energy balance value for continuous diffusion fermentation of fodder beets is slightly higher than the 3.00 energy balance value for solid-phase fermentation of this feedstock indicating the former is slightly more energy efficient.

Table 6. Raw materials, rates, and products in a theoretical fuel ethanol plant with fodder beets.[a]

Component	Amount (per year)[b]	Consumption or production rates (per h)
Raw materials		
fodder beets[c]	7.48×10^6 Kg	990 Kg cubes[d]
water[e]	2.5×10^6 L	
H_2SO_4[f]	40,337 L	
Yeast[g]	1400 Kg	
Products		
Ethanol with denaturant[h]	658,854 L (or 596,106 L anhydrous)	83 L 95% ethanol[d]
PF[i]	5.55×10^6 Kg	

[a] Plant design and operation are based on preliminary research findings.
[b] A 45-week work year is assumed.
[c] An ethanol yield of 87 L/metric ton of fodder beets (21 gal/ton) is assumed.
[d] The process uses an 280,000 L capacity, stainless steel, continuous diffusion fermentor as illustrated and operated in Figure 38. Retention times for beet cubes and beer are 72 h and 264 h, respectively.
[e] Water is used for fodder beet cleaning, yeast inoculum preparation, diffusing sugar/ethanol from beet cubes and condensing ethanol vapor. It is assumed that at least 50% of the total water used can be recycled in the process.
[f] A total of 64.5 ml concentrated H_2SO_4 per L ethanol produced are used in the process. Part is added with beet cubes (2.66 ml/Kg cube) and the remainder is added with inlet water (7.3 ml/L water).
[g] Yeast is obtained commercially in a dried, powder form with about 10^7 cells/g. Yeast inoculum is added to fermentor only periodically when needed, as the fermentation process continually generates yeast.
[h] Using 5 L unleaded gasoline/100 L of 95% (v/v) ethanol.
[i] PF is assumed to be 70-75% moisture.

TABLE 7. Energy balance of ethanol production from fodder beets.

Parameter	Energy (kJ/liter) produced or consumed[d]
Energy inputs[b]	
conveying and cleaning[c]	64
slicing[d]	805
fermentation[e]	15
distillation[f]	6,078
TOTAL	6,962
Energy output[g]	21,192
Energy balance[h]	3.04

[a] Values are expressed as kilojoules per liter of denatured 95% (v/v) ethanol.

[b] Energy input values are based whenever possible on actual plant findings obtained from replicate runs with fodder beets. Some values are taken from our previous work with fodder beets (Gibbons and Westby, 1983 c). The value for distillation is extrapolated from earlier work on corn (Stampe, 1982). The energy required for growing fodder beets is not included.

[c] Two 0.37 kW motors are required for conveying and one 0.75 kW motor is required for pumping water.

[d] One 18.65 kW motor attached to slicer is required here.

[e] One 0.5 kW motor is required for rotating auger.

[f] Three 0.75 kW motors are required for operating pumps; the remainder of the energy for distillation is in the form of steam.

[g] This is the energy content of 190 proof [95% (v/v)] ethanol The energy content of PF is not included.

[h] Energy balance is energy output divided by energy input.

Costs:

In Table 8 I list the capital, operating, and other fixed costs associated with ethanol production from fodder beets in the theoretical plant shown in Figure 38. Total costs before and after deducting a credit for the CPF byproduct are shown.

Costs were estimated by comparing the theoretical plant (Fig. 38) with a similarly sized solid-phase fermentation ethanol plant, also designed to process fodder beets, that was economically characterized by Dobbs and Habash (1986) and Habash (1985). Whenever identical equipment or supplies were used in each process, the data of Dobbs and Habash (1986) were used directly. When input components were not identical, the costs of Dobbs and Habash were modified and then used in estimating costs. This same procedure was used previously for estimating the ethanol production costs of fodder beet and sweet sorghum pulps (Gibbons et al., 1986).

The fodder beet feedstock cost that I used, $19.25/metric ton ($17.50/ton), was determined by Dobbs and Habash (1986). It assumes a yield of 57 metric tons/hectare (25 tons/acre). A breakdown of variable and fixed costs for fodder beet production was also available from Dobbs and Habash (1986). The feedstock cost is somewhat lower than that used in the previous solid-phase fermentation section ($22.05/metric ton), and lower than the range reported by Hills et al. (1981, 1983) ($19.85 - $26.46/metric ton). I feel, however, that this new cost estimate is more representative of actual production costs for the north central United States.

TABLE 8. 1986 costs of ethanol production from fodder beets

Parameter	Cost[a] ($/liter)	Cost[a] ($/gallon)
Capital costs[b]	$0.099	($0.373)
Operating costs		
Feedstock[c]	$0.209	($0.792)
Other	$0.277	($1.048)
Other fixed costs[d]	$0.056	($0.213)
Total costs	$0.641	($2.426)
Credit for PF[e]	$0.112	($0.424)
Net cost of denatured ethanol	$0.529	($2.002)

[a] The costs have been derived in most cases from Habash's (1985) and Dobbs and Habash's (1986) cost breakdown of the plant. Data are from SDSU ethanol plant operation.

[b] All capital items are amortized at a rate of 15% over their useful lifetime.

[c] This assumes a fodder beet cost of $19.25/metric ton ($17.50/ton), based upon a yield of 57 metric tons/hectare (25 tons/acre). An ethanol yield of 87 L/metric ton (21 gallons/ton) is used to calculate feedstock cost. A more detailed fodder beet production cost breakdown is given elsewhere (Habash, 1985; Dobbs and Habash, 1986).

[d] This includes insurance, maintenance, and property taxes.

[e] PF contains 20% protein on a dry basis.

Storage costs for fodder beets were included in the capital and operating cost estimates for the ethanol plant, rather than in the feedstock cost estimate.

The projected credit for fodder beet CPF (spent cubes at 70% moisture) was estimated from the credit, given to fodder beet pulp protein feed (PF) (Dobbs and Habash, 1986; Habash, 1985). In determining the credit for the PF, Dobbs and Habash (1986) used the AGNET "Feedmix" program to estimate the value of the PF as a protein source in least-cost beef or dairy cattle rations. Their analysis set the feed byproduct credit at $0.14/L ethanol produced ($0.53/gallon ethanol). Chemical analysis data for fodder beet PF are given previously in the solid-phase fermentation section.

CPF contains 70% moisture, therefore it should have less value than the dried byproduct on a per liter of ethanol basis due to increased transportation, storage, and handling costs associated with the moisture. A conservative estimate (T.L. Dobbs, personal communication, 1986) is that it may have only 60 to 80% of the value of the dry byproduct. The difference here largely depends upon the marketing area and marketability. If the CPF is worth $0.112/L ethanol, the net ethanol production cost will be $0.529/L; whereas if the CPF is discounted further to $0.084/L, the ethanol costs rise to $0.557/L.

My most optimistic cost estimate for producing 95% fuel ethanol and CPF from fodder beets using the continuous diffusion fermentation process is $0.529/L ($2.002/gal). This is higher than

the production cost from corn ($2.50/bushel) using the dry-milling process (Gibbons and Westby, 1983; Hoffman and Dobbs, 1982; Westby and Gibbons, 1982), which was $0.497/L ($1.88/gal) when adjusted for inflation. It is also higher than the estimated production costs from sweet sorghum ($0.47/L using feedstock priced at $51.16/metric ton) (Gibbons et al., 1986), and fodder beets ($0.49/L using feedstock priced at $19.25/metric ton) using the solid phase fermentation process.

Previous research with corn has demonstrated that ethanol production costs can be reduced by as much as $0.13/L (below $0.497/L), if certain modifications are made in plant design and/or operation (Gibbons and Westby, 1983; Westby and Gibbons, 1982). Future research might also show ways to reduce ethanol production costs from fodder beets using the continuous diffusion fermentation process.

CONCLUSIONS

This research has demonstrated the effectiveness of both the continuous solid-phase fermentor and the continuous diffusion fermentor in processing fodder beets to fuel ethanol and PF or CPF. Each process produced either pulps or beers, respectively, that contained distillably worthwhile amounts of ethanol (8-10% [v/v]). A high value protein feed (PF or CPF), which contained approximately 20-21% protein, was also produced. For reference, conventional submerged fermentation of corn yields beers with 8-12% ethanol, and the DWG produced contains 28-34% protein (Gibbons and Westby, 1983; Westby and Gibbons, 1982).

For both novel processes, optimum levels of important fermentation parameters were determined. Solid-phase fermentation parameters were:

1) 1.27 - 1.91 cm hammermill screens for pulping beets
2) 5% (v/v) yeast inoculum
3) pH 3.0 - 3.5

Diffusion fermentation parameters were:

1) 1.91 - 2.54 cm fodder beet cubes
2) 0.25% potassium meta bisulfite
3) 0.20% sodium meta bisulfite
4) pH 2.0-2.2

Approximately three times more energy was contained in the ethanol produced in each novel process, compared to the amount of energy required to produce the ethanol. The energy balance (energy

output/energy input) for processing corn is only 2.26 (Gibbons and Westby, 1983; Westby and Gibbons, 1982). The advantage of fodder beets is that the energy intensive cooking process to convert corn starch to sugar is not needed for fodder beets, which contain a readily fermentable sugar, sucrose.

Even though fodder beets were a cheaper feedstock than corn ($0.209/L ethanol produced vs $0.242/L) and fodder beet PF/CPF was of greater value than corn DWG ($0.112 to 0.14/L vs $0.08/L), capital and operating costs for the fodder beet processes were higher than those for conventional submerged fermentation of corn (Gibbons and Westby, 1983; Westby and Gibbons, 1982). Higher capital costs were due to the addition of a press and dryer for ethanol recovery in the solid phase process, and for the fermentor itself in diffusion fermentation. Higher operating costs were primarily due to increased sulfuric acid useage to control contamination in the novel fermentors, even though costs for starch hydrolyzing enzymes were eliminated.

The result of all these cost differences was that ethanol produced via continuous solid-phase fermentation ($0.492/L) cost about the same as ethanol produced from corn by conventional means ($0.497/L) (Dobbs and Hoffman, 1983; Dobbs et al., 1984 b; Gibbons and Westby, 1983 b; Hoffman and Dobbs, 1982; Westby and Gibbons, 1982). The cost of corn ethanol was adjusted for inflation using a 7.9% increase in the Producer Price Index. Ethanol produced via

continuous diffusion fermentation ($0.529/L) was slightly more expensive than ethanol produced from corn.

On the basis of this cost data, it appears unlikely that fodder beets will soon replace corn as the feedstock of choice for fuel ethanol production. For this to happen, lower production costs for fodder beet produced ethanol is necessary, so as to show increased profitability over corn. Only then will investors assume the increased risks of developing this new feedstock and novel processing technologies.

Relatively little research has been conducted on increasing the biomass and ethanol yields of fodder beets, compared to that done on corn. Likewise, ethanol processing technologies for fodder beets are in their infancy relative to decades of research on grain fermentation. Therefore future research holds great promise for increasing productivity and reducing costs of ethanol production from fodder beets. If such developments do occur, fodder beets could become the most desirable feedstock for fuel ethanol production.

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