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A Laboratory and Pilot Plant Scaled Continuous Stirred Reactor Separator for the Production of Ethanol from Sugars, Corn Grits/Starch or Biomass streams.*

M. Clark Dale, Ph.D., President
Bio-Process Innovation, Inc., 226 N. 500 West, W.Lafayette, IN 47906-8505
Shuiwang Lei, Ph.D., Visiting Faculty, and Chongde Zhou, MSE, Visiting Faculty
Biochemical and Food Process Engineering Division
Agricultural Engineering, Purdue University, W. Lafayette, IN 47906-1146

Abstract

An improved bio-reactor has been developed to allow the high speed, continuos, low energy conversion of various substrates to ethanol. The Continuous Stirred Reactor Separator (CSRS) incorporates gas stripping of the ethanol using a recalculating gas stream between cascading stirred reactors in series. We have operated a 4 liter lab scale unit, and built and operated a 24,000 liter pilot scale version of the bioreactor.. High rates of fermentation are maintained in the reactor stages using a highly flocculant yeast strain. Ethanol is recovered from the stripping gas using a hydrophobic solvent absorber (isothermal), after which the gas is returned to the bioreactor. Ethanol can then be removed from the solvent to recover a highly concentrated ethanol product. We have applied the lab scale CSRS to sugars (glucose/sucrose), molasses, and raw starch with simultaneous saccharification and fermentation of the starch granules (SSF). The pilot scale CSRS has been operated as a cascade reactor using dextrins as a feed. Operating data from both the lab and pilot scale CSRS are presented. Details of how the system might be applied to cellulosics, with some preliminary data are also given.

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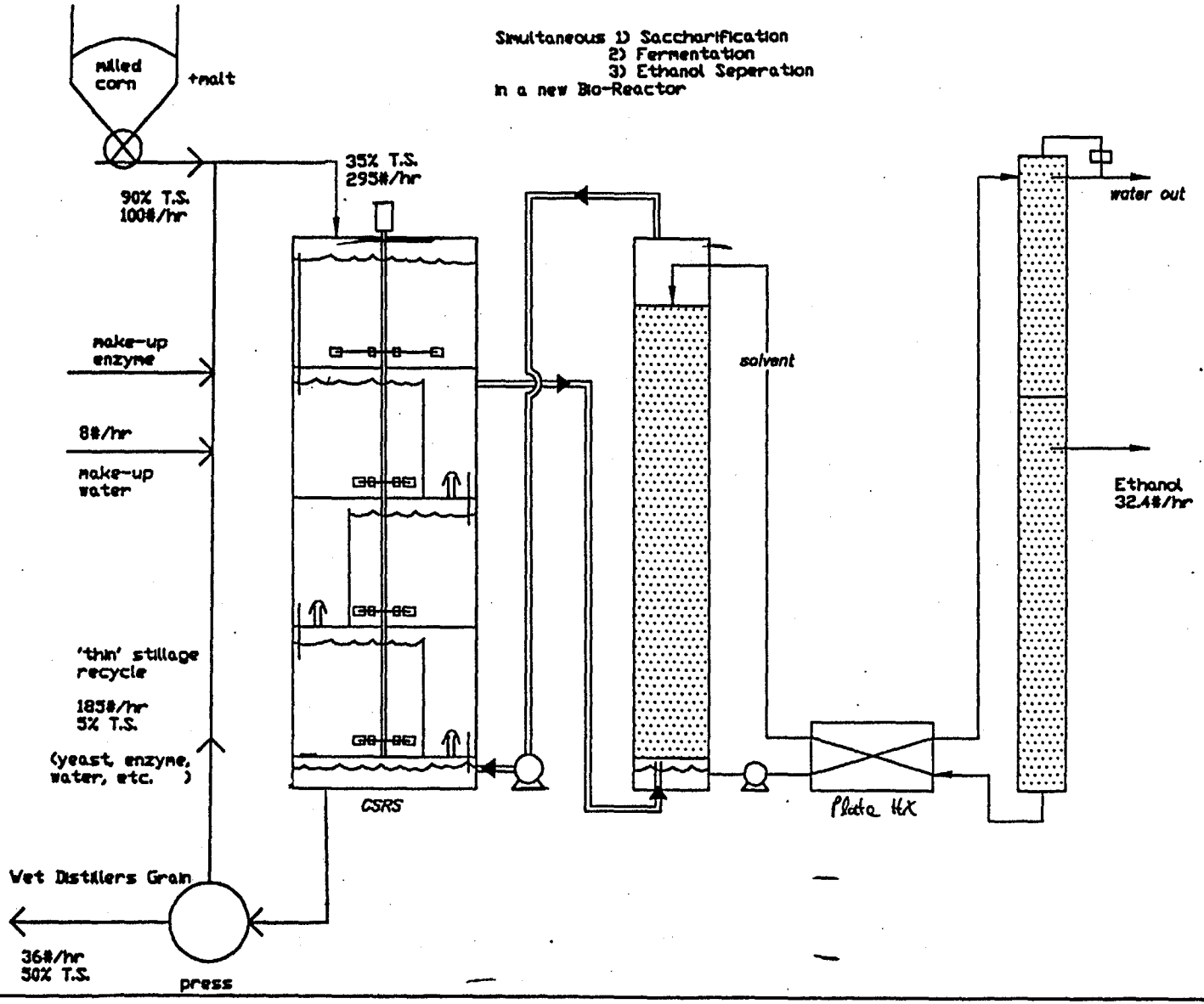
Introduction

Ethanol production in the USA can offer a renewable source of liquid fuel produced within the borders of our own nation as well as providing a market for excess grain production capacity of the midwestern states. However, in order for the ethanol fuel industry to be able to expand without governmental subsidies, ethanol production costs must be reduced closer to the level of refined unleaded gasoline ($0.55-$0.75 per gallon). Ethanol production costs can be reduced via: 1) reducing costs of substrate, 2) increasing the efficiency of substrate conversion to ethanol, 3) reducing the energy costs for purifying and dehydrating the ethanol, 4) reducing the amount of effluent 'bottoms' waters which must be treated, 5) reducing the capital costs for the ethanol processing plant, 6) reducing the labor for operating the ethanol plant.

A Continuous Stirred Reactor Separator (CSRS) consisting of stirred tank type reactors operated in series, with the liquid streams moving from tank to tank contacted with a stripping gas to remove the ethanol product has been designed and tested. A patent (#5,141,861) has been issued which describes this reactor technology. Capital costs for the CSRS system have been estimated to be about $1.40/annual gal. at the 500,000 gal/yr scale, which compares to a cost of $2.70 for a batch plant at this scale. This CSRS is a new type reactor which allows simultaneous saccharification, fermentation, and ethanol separation in a combined process. Combining these reactions allows significant improvements in each operation. Combining reaction with separation allows the fermentation of highly concentrated streams of up to 50% solids. Simultaneous saccharification and fermentation of polysaccharides such as starch and cellulose can be quickly completed in this bio-reactor/separator. Saccharification (of both starch and cellulose)is sped by the reduction of sugar concentration as the sugar is fermented to ethanol. Fermentation is sped by the removal of the toxic ethanol product, and ethanol purification and concentration costs are reduced by the enrichment of the ethanol in the vapor phase. The gas stream is co-current to the tank to tank liquid flow in the enriching section, and counter-current in the stripping section. The final effluent from the CSRS is characterized by complete saccharification of all polysaccharides, complete fermentation of sugars to ethanol and complete removal or separation of the ethanol into the gas phase. A schematic of the CSRS process is shown in Figure 1 for a system incorporating complete recycle of the thin stillage.

The CSRS can be coupled with a solvent ethanol recovery system to give a low energy continuous process for the production of ethanol from starch or biomass. Energy savings are attained by combining the CSRS reactor concept with solvent absorption of the ethanol from the gas stream exiting the CSRS as shown in Figure 1. The ideal solvent for absorption of ethanol from a gas stream would have the following properties: 1) low vapor pressure of the solvent, 2) solvent miscible with ethanol, 3)solvent vapor carry-over non-toxic to fermenting microbes in CSRS, 4)low solubility of water in the solvent, and 5) low solubility of solvent in water. The ideal solvent would absorb only ethanol allowing an anhydrous ethanol product to be stripped off from the solvent/ethanol stream from the absorber. However, all solvents having the ability to solvate ethanol also dissolve some



Drawing Figure 2. The BPI CSRS Fermenter Process.

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BPI Bio-Process Innovation, Inc.
226. N. 500 West
West Lafayette, Indiana 47906

water. Tedder et al. (1986) state that the solvents they tested for direct solvent extraction of ethanol have a 3 to 5% water weight fraction in the solvent phase when contacted with an aqueous phase. Each solvent can be characterized by an equilibrium distribution coefficient, K_{dc}, for ethanol between the water and solvent phase, as well as a distribution coefficient for water, K_{dw} between the aqueous and solvent phases. Dividing the ethanol distribution coefficient by the water distribution coefficient (K_{dc}/ K_{dw}) gives a separation factor, a, which describes the relative affinity of the solvent for ethanol compared to water. Dodecanol, found by Minier and Goma (1982) to be non-toxic in direct solvent extraction in-situ separation, has an ethanol distribution coefficient of .21, and an ethanol/water separation, a, factor of 21. Work by Kollerup and Daugulis, (1985) indicated that dodecanol was somewhat toxic to microbes. Research in our labs has shown that dodecanol in direct contact with immobilized cells was toxic to yeast, but when used as vapor ethanol absorber, the dodecanol vapors carried over to an immobilized cell type reactor were not toxic, with fermentation rates of immobilized cells stable over 4 to 6 days (Lee and Dale, 1991 unpublished data). By not actually contacting the cells with an organic phase, toxicity problems and solvent loss into the water phase are both minimized. An anhydrous ethanol product may be recovered from the solvent using a simple extractive distillation procedure using the same solvent used for the ethanol absorption. This process has been designed, tested, and modeled by hand calculations and using the Aspen II library using solvents with a separation factors of 50-90, which would give the energy usages shown in Table 1 for steam energy requirements to recover an anhydrous ethanol product.

Table 1. Distillation Steam Energy Requirements for Anhydrous Ethanol (MBTU/gal)

% Ethanol	Conv. Distillation/PS Dehydr.	Sol. Abs. Extr. Distillation
2.5	44	22
5.0	21	13.3
7.5	15	8.4
10.0	13	6.7
12.5	11.5	5.9

It can be seen that as the ethanol concentration in the stage from which the gas exits is allowed to increase, energy costs for ethanol recovery drop. The selection of solvents and testing of the SAED system was described in a paper given by Dale (1993).

No-cook conversion of starch to sugars-

If the cook process can be completely eliminated from the process of converting starch/grits to ethanol, energy, capital, and labor costs could be significantly reduced. This conversion, when coupled with ethanol separation may allow the simple starch to ethanol process shown in Figure 1. Glucose inhibition of enzymatic action has been noted to increase at lower temperatures. These results are similar to the kinetics determined by Matsumura et al (1987,1989) for raw starch saccharification using a glucoamylase with sweet potato starch. The use of *Aspergillus niger* on wet corn as a source of amylase

enzymes was reported by several researchers as giving good results with this type of process (Han and Steinberg, 1986; Fujio et al, 1984; Ueda et al, 1981).

Bottoms water recycle

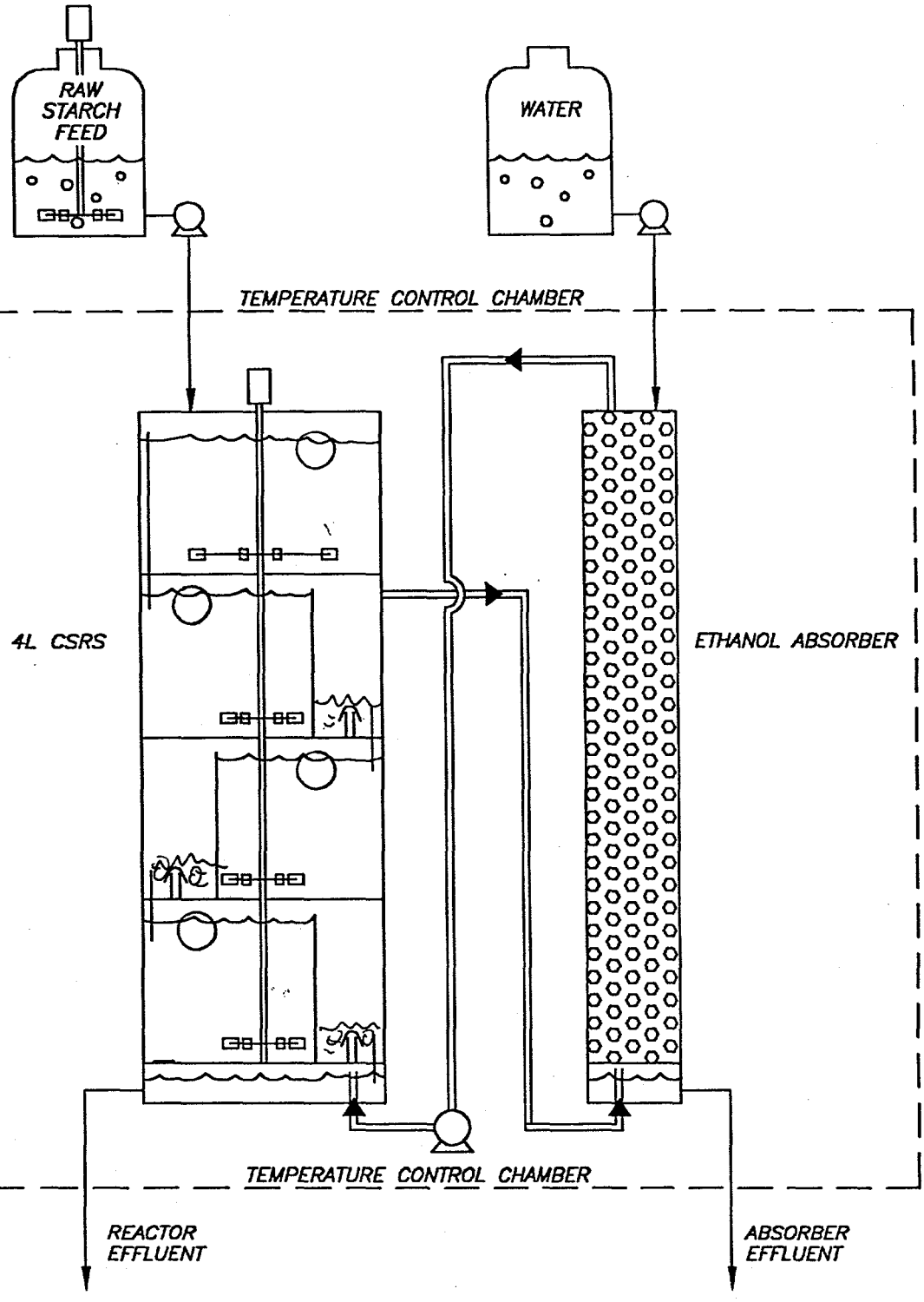
It is a common practice in the fermentation industry to recycle 20-50% of the stillage to help reduce and control the pH of the initial fermentation broth. Nofsinger et al. (1982) showed good performance with 100% recycle of stillage. However, in a recycle scheme, there will be recycling of any non-volatile fermentation products or non-fermentables in the feed. Thus for molasses (which is characterized by about 40-50% fermentable solids) a maximum stillage recycle ratio of 30% has been determined, while for high-test molasses with 85% fermentable solids, 1a 50% recycle can be used (Hodge and Hildebrandt, 1954). The major fermentation by-products that will tend to build up over time are glycerol (3.3% yield) and lactic/succinic acid (0.5-1.0% yield).

Conversion of cellulose and hemicellulose to ethanol-

Biomass in the form of corn stalks, wood chips, waste paper, and grass clippings offers an enormous and low cost source of sugars for ethanol production. Xylose fermentation techniques, and the simultaneous saccharification and fermentation of cellulose are being studied as an application for the CSRS reactor system. The two stage fermentation of biomass, following a basic extrusion solubiliziaton of lignin and hemicellulose (the Xylan delignification process (Tyson et al, 1995)) is being developed. We are hoping to demonstrate a 5 stage CSRS system with a two stage xylose fermentation followed by a 3 stage SSF of cellulose (Dale, 1994).

Methods and Materials

A four liter, four stage lab scale CSRS was constructed using rectangular stages as per Figure 2.The ethanol vapors were absorbed into an isothermal water stream flowing 4 times faster than the feed to the reactor. Stage 1 has a liquid volume of 1,100 ml and stages 2-4 have a hold up of 900 ml. A single trough type bubble cap contactor was used as the gas-liquid contacting device after stages 2,3 and 4. This reactor was used for starch, sucrose, and molasses fermentations. Batch fermentations were performed in 250 to 500 ml Er. flasks using a magnetic stirrer placed in an environmental temperature controlled cabinet. Organisms used included strains of *S. pombe, S. cerevisae, K. marxianus,* and *A. niger* obtained from the NCAUR and CBS culture collections. Standard nutrients used in these fermentations were a YEP supplementation, with yeast extract, malt extract, and peptone added at 3 g/l each unless otherwise noted. A mix of 8% malt and 200 to 300 DU/# starch L-200 glucoamylase from Solvay was used in our no-cook fermentation studies. In the continuous reactor experiments, pH was maintained at between 3.5 and 4.2 via addition of ammonium hydroxide. Degree of starch conversion was determined by mass balance in the CSRS experments, and by glucose release from the fermentation broth in a post experiment cook with alpha-amylase followed by glucoamylase at 60 C (Solvay, 1991).



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Results

Batch 'No-cook' fermentations.

12 and 20% starch concentrations were fermented using a flocculant yeast strain. Comparisons of the fermentation of cooked starch treated with alpha-amylase were run versus a raw starch fermentation. Figure 3 show the results of this comparison with a 12% raw starch feed. It can be seen that ethanol generation is slightly quicker with the cooked medium as compared with the raw starch medium. Glucose levels in the raw starch feed were maintained at near zero levels. The release of glucose was rate limiting. At a 20% starch feed, the cooked medium showed a faster fermentation again during the first 20 hours, after which the ethanol levels in the no-cook medium reached the same level at 25 hours of 82 g/l. Conversion efficiency (based on residual starch) was determined to be 96% and 86% for the cooked medium at 12 and 20% starch as compared to 92% and 84% for the raw starch medium at 12 and 20% starch mediums respectively. The possibility of generating amylase enzymes insitu was investigated using *Aspergillus niger* co-cultured with yeast. Good, but somewhat slower starch breakdown was noted when compared to commercial gluco-amylase added to the same raw starch medium (12%) as shown in Figure 4. A lower final starch conversion rate with the co-culture broth was noted after 100 hours (92 vs 96%).

Continuous 'No-cook' fermentation of starch in the lab CSRS

The lab scale CSRS was operated on raw starch for a period of 37 days using concentrations of 10%, 15%, 20% and 30% TS sequentially. Enzymes for starch granule hydrolysis were a commercial gluco-amylase and ground malt at an 8% level (Dale et al, 1991). The CSRS was operated at 38C using an adapted temperature tolerant strain of yeast (*K. marxianus*, NRRL 2415). Profiles of glucose and ethanol on the stages are given in Table 1 for a 20% starch feed. It is difficult to determine starch concentration directly due to the starch and yeast granules being mixed together, however, the conversion efficiency can be determined based on average ethanol and glucose yields (1 g starch ->1.0 g glucose-> .46 g ethanol). Using these conversions, a conversion efficiency of 79% was determined for the data shown in Table 2 at a residence time of 40 hours. Yeast density of 1.5 e 9 was measured on this day. When it was attempted to feed 30% starch, clogging of the bubble troughs caused pressure drop of the gas phase through the reactor to increase and the reactor was shut down for cleaning.

Table 2. Compositions (g/l) in the CSRS with Raw Starch Feed (Day 10)

Stage	Glucose	Ethanol
1	22	17.8
2	2.8	31.1
3	2.1	33.3
4	0.1	26.7
Abs. effl.	0	8.9

Figure 3. Batch Fermentation Comparison of Cooked versus Raw Starch

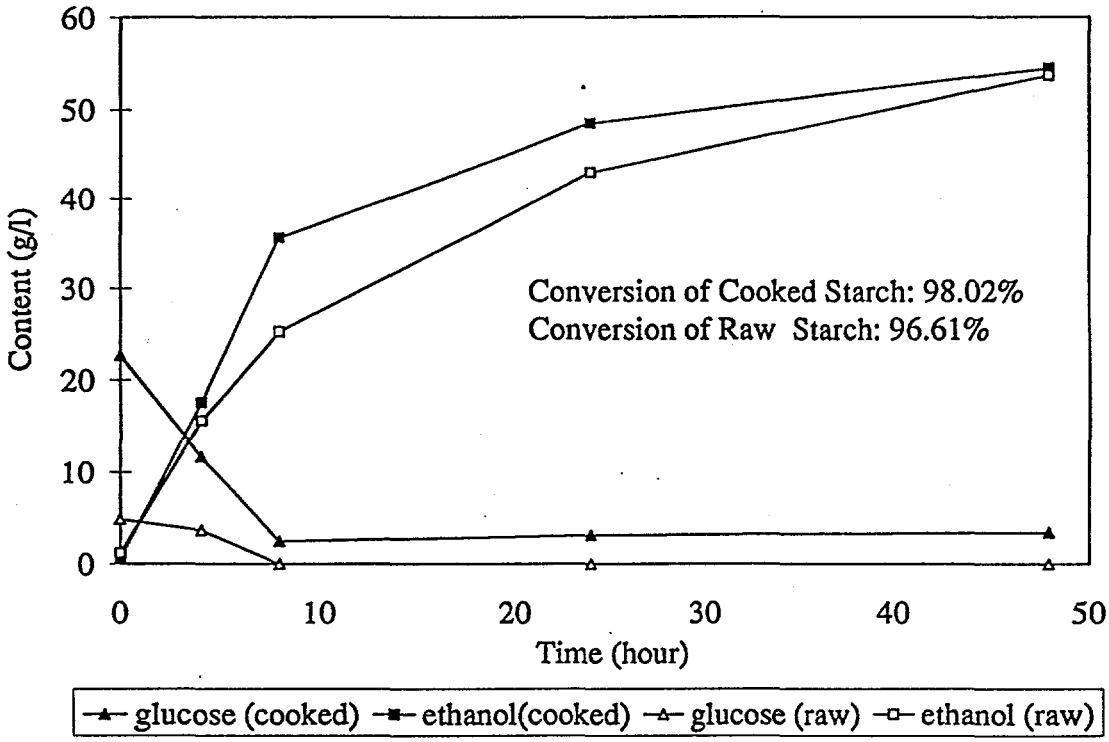
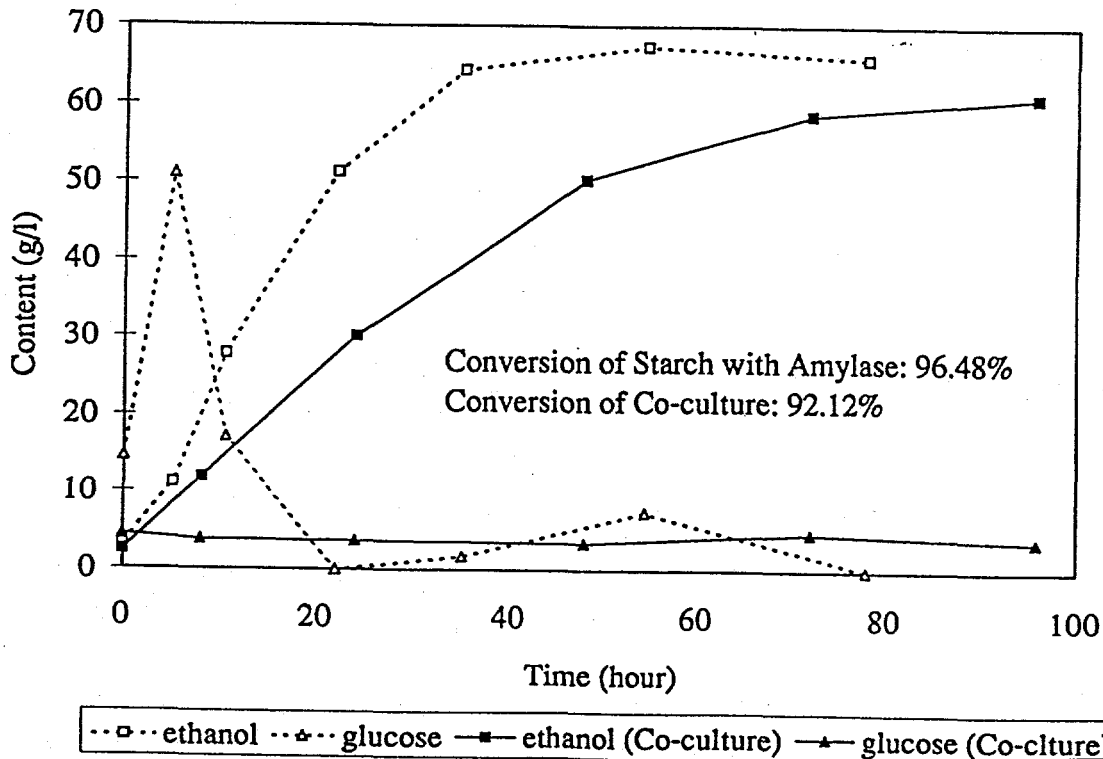


Figure 4. Batch Co-culture of S. Cerevisiae & A. Niger for Ethanol from Raw Starch



Continuous fermentation of sucrose in the lab CSRS with 80% bottoms water recycle

The effects of bottoms water recycle were studied in the CSRS. An adapted flocculant S. cerevisae strain (NRRL Y265) was added to the CSRS. 4 liters of bottoms water was used to reconstitute 1,200 g of sucrose, after which nutrients were added and the volume adjusted to 6 liters with deionized water. The reactor was operated on a continuous basis for 120 days. at a feed rate of 100 ml/hr. Settled yeast from the effluent was also recycled. A steady state cell density of 14 to 18 g/l was maintained over this period. High rate conversion of the sucrose was noted as shown in Table 3. Sugar utilization rates of 10 to 19 g/l hr were routinely achieved in stage 1. Glycerol levels of between 17-54 g/l. were noted over the test period. The average glycerol levels were 22-28 g/l. Even at the highest glycerol levels, high rates of fermentation were noted.

Table 3 CSRS Compositions (g/l) on Day 32-Sucrose Feed

Stage	Glucose	Fructose	Lactic A.	Glycerol	Eth.	Brix
1	117	96	2.5	22.5	21.9	20
2	51	66	2.7	24.1	56.2	16.2
3	4.8	16	2.5	22.5	53.7	10.2
4	0	0.5	2.6	23.1	33.7	7.1
Recycle	0	0	3.1	27.9	5.0	7.0

Continuous fermentation of cane molasses in the lab CSRS with bottoms water recycle

Cane molasses from the Savannah Sugar company was fermented in the CSRS . The molasses was diluted to the desired brix using a mix of 80% bottoms water and 20% fresh DI water. Feed to the system was maintained at 100 ml/hr. Good conversion was noted on day 1 with a 34 brix feed reduced to 13 brix in stage 4. By day 11, however, brix in stage 4 had increased to 21. Brix drop in stage 1 dropped from 6 to 2 over the 17 day test, stage 2 showed a drop from 5 to3 brix drop, while stage 3 stayed relatively constant at 4-5 brix drop. After 17 days operation a lower brix feed of 17% was next fed for 5 days. Cell density was observed to drop from an average density of 6.5 g/l on day 3 with low brix feed to under 2 g/l on day 5 even though it was attempted to recycle the yeast. The molasses substrate seemed to have some inhibitors which caused floc breakdown and loss of fermentation activity over time, so that either continuous addition of yeast, recycle of yeast or further research is needed to apply the use of the floc yeast to molasses.

Construction of a 24,000 Liter pilot scale CSRS system.

A 24,000 liter CSRS bioreactor was constructed during 1994 and installed at Permeate Refining Inc's ethanol plant site in Hopkinton, IA. This system will be similar to the diagram shown in Figure 1, and a photograph of the installed reactor/solvent absorber system is shown in Figure 5. This system has been operated as a continuous cascade reactor during the first quarter of 1996. Feed rates have been varied between 2 and 5 GPM (18 -40 hour residence time) with near complete utilization of a 20% feed sugar stream. Some trouble shooting during this period of time has been accomplished including; 1) redesigning and replacing seals around the stirring shaft, 2) continuous addition system and

Figure 5 A Vertical View Down from the 24,000 L Pilot Scale CSRS

formulation of nutrients/enzymes , and 3) introduction and maintenance of an adapted flocculant yeast strain in the bioreactor has been a. A feed of dextrins (Amaizo, AMP, Hammond IN) is being fed with simultaneous saccharificiation of the dextrins to glucose using glucoamylase and fermentation of the glucose occurring in the bioreactor. The Solvent Absorption Extractive Distillation system is scheduled for installation during June of 1995 to allow in-situ removal of ethanol from the bio-reactor.

Conclusions

Our basic objective in this work has been to demonstrate feasibility a process as outlined in Figure 1. This includes 1) maintenance of a high cell density yeast in the bioreactor, 2) the no-cook conversion of starch granules to sugars and ethanol, and 3) the inclusion of a high amount of recycle waters from the bottom of the reactor. Each of these objectives were met as described in our experimental section. Use of a flocculant yeast strain in the bioreactor allows continuous high rate fermentations without the cost and complexity of a cell recycle system. Cell densities of 20-40 g/l have been regularly observed in our long term tests. This will allow residence times to be reduced to as low as 6 to 12 hours in the CSRS system. Raw starch can be converted to ethanol with a high conversion efficiency (>95%) in batch fermentations of 30 to 48 hours, but current results show less efficient (80%) yields in the CSRS when residence times are under 20 hours. Enzymatic breakdown of the starch granule is rate limiting, with glucose levels held low in both batch and continuous fermentations. The use of the no-cook technology will have to be economically compared to starch cooking technology on a site by site basis, with either a longer reactor hold time or incomplete starch utilization being factored into the comparison of the no-cook technology with conventional cooking of the starch/grits. The lab scale conversion of sucrose with an 80% recycle of bottoms water showed some build-up of glycerol over time, but no significant inhibition of fermentation rates. The use of up to 80% recycle of bottoms water as per Figure 1 can thus be confidently implemented and will have the further benefit of recycling of some nutrients and enzymes remaining in the broth.

We thus see no obstacles to implementations of the CSRS technology as outlined in Figure 1 on grains or to any other fermentable substrates, and are in the process of demonstrating the technology using the 24,000 L pilot plant (design capacity of 0.3-0.5 million gal/r scale) in Hopkinton IA presently. It is our goal to develop and demonstrate technology to allow the economical production of ethanol on a smaller scale (0.5 -10 million gal/yr) for on-site production of ethanol at 1)cow or chicken feeding operations, 2) cheese making facilities, 3) corn refining operations, 4) food/candy processing operations.

Acknowledgments

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