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Fermentations of Pectin-Rich Biomass with Recombinant Bacteria to Produce Fuel Ethanol

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Abstract

Pectin-rich residues from sugar beet processing contain significant carbohydrates and insignificant amounts of lignin. Beet pulp was evaluated for conversion to ethanol using recombinant bacteria as biocatalysts. Hydrolysis of pectin-rich residues followed by ethanolic fermentations by yeasts has not been productive because galacturonic acid and arabinose are not fermentable to ethanol by these organisms. The three recombinant bacteria evaluated in this study, *Escherichia coli* strain KO11, *Klebsiella oxytoca* strain P2, and *Erwinia chrysanthemi* EC 16 pLOI 555, ferment carbohydrates in beet pulp with varying efficiencies. *E. coli* KO11 is able to convert pure galacturonic acid to ethanol with minimal acetate production. Using an enzyme loading of 10.5 filter paper units of cellulase, 120.4 polygalacturonase units of pectinase, and 6.4 g of cellobiase (per gram of dry wt sugar beet pulp), with substrate addition after 24 h of fermentation, 40 g of ethanol/L was produced. Other recombinants exhibited lower ethanol yields with increases in acetate and succinate production.

Index Entries: Recombinant bacteria; sugar beet pulp; pectin conversion; ethanol; galacturonic acid.

Introduction

Production of cellulosic biomass could supply an estimated 10 times our energy needs and 100 times our food needs on a global scale *(1)*. Woody residues termed *lignocellulose* represent the largest reservoir for renewable biomass and are typically composed of 30–60% glucose, 15–30% xylose, 10–20% lignin, and 5–30% other sugars *(2)*. Although lignocellulosic biomass constitutes the vast quantity of renewable biomass, it is heavily lignified and resistant to degradation, presenting a challenging hurdle to

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process development. Grohmann and Bothast *(3)* present a review of three pectin-rich residues generated by processing of citrus, apples, and sugar beet juice that contain significant quantities of carbohydrates with insignificant amounts of lignin. These pectin-rich processing residues are available in relatively large amounts and are already stockpiled in processing plants, which decreases transportation and collection costs.

One such pectin-rich processing residue is sugar beet pulp. In the United States, between 20 and 30 million wet t of sugar beets are produced annually *(3,4)*. On a dry weight basis, more than 1.6×10^6 t of sugar beet pulp remain after sucrose extraction. Processing of sugar beets entails extraction of sucrose with two major coproducts—molasses and residual fiber or pulp—which are a disposal concern *(5)*. In many parts of the world, production of livestock feed from molasses and beet pulp is an economically marginal part of beet sugar processing because of the low feed value and the relatively high cost of drying. In certain areas, between 30 and 40% of the overall energy cost of sugar beet processing is devoted to dehydrating and pelletizing the pulp *(6)*. In only one of several locations, in a partially processed form, more than 60,000 t of beet pulp are available per year for bioconversion to produce approx 6 million gal of fuel ethanol with our present process. This amount of ethanol is adequate to support an in-line ethanol fermentation facility provided yields are similar to those from current corn-based fermentation processes. Because of the high costs of dehydrating pectin-rich agricultural residue, there is interest in the beet sugar industry in providing an added value to its processing coproduct. The market for ethanol production from *whole* sugar beets may be self-limiting owing to competing value as food, similar to corn-based fermentations. However, the conversion of processing by-products is an attractive option for production of fuel ethanol and environmental bioremediation.

Lignocellulosic biomass is readily available *(7)* and has no competing value as food; however, there are no known organisms that can rapidly and efficiently convert all biomass sugars into ethanol with high productivities. Several reviews *(2,8,9)* have described the development of genetically engineered microorganisms for ethanol production. Although engineered yeast strains *(10)* or native strains that can ferment xylose under microaerophilic conditions *(11)* have potential for xylose-rich fermentations, sugar beet pulp has very low levels of xylose (1.7% of dry wt) *(12)*. Likewise, the strains of *Zymomonas mobilis* that ferment xylose *(13)*, engineered by the National Renewable Energy Laboratories, and a second arabinose-fermenting strain *(14)*, are useful in fermentations rich with these carbohydrates; however, none can use galacturonic acid. The use of engineered bacteria is important because it permits production of ethanol from glucose, xylose, arabinose, cellobiose, and galacturonic acids *(15–20)*. Using nonedible biomass, such as cellulose *(21,22)* and sugar cane bagasse *(23)*, eliminates the uncontrollable and highly variable costs associated with food-based ethanol production by allowing the use of low-cost raw materials. Sugar beet pulp is a good source of nonstarchy polysaccharides *(24)* and has a pectin

backbone of primarily linear polymers of D-galacturonic acid *(25)* with side chains composed of neutral sugars such as arabinose *(26)*. The coupling of enzymatic hydrolysis of pectin-rich residues with ethanolic fermentations by yeasts has not been productive because galacturonic acid and arabinose are not fermentable to ethanol by conventional yeasts *(19)*. Even after sucrose extraction from beets, 70–75% of the remaining residue comprises carbohydrates, predominantly glucose (21.1%), galacturonic acid (21.1%), and arabinose (20.9%) on a dry weight basis *(27)*. Another 10% of the dry weight of sugar beet pulp is a mixture of five other sugars.

In this article, we evaluate three recombinant ethanologenic bacterial strains in fermentations using pure galacturonic acid and sugar beet pulp (in pressed and pelletized forms), with and without fungal enzyme supplementation. *Escherichia coli* KO11 is used in further fermentations with variations in substrate loading and fungal enzyme levels.

Materials and Methods

Microorganisms

Recombinant ethanologenic bacteria were provided by Dr. L. O. Ingram at the University of Florida and have been described previously *(15–18,21, 28–31)*. Two genes from *Z. mobilis* encoding alcohol dehydrogenase and pyruvate decarboxylase were introduced into three bacteria: *E. coli*, *Klebsiella oxytoca*, and *Erwinia chrysanthemi* EC 16, and resulted in the production of ethanol as the primary fermentation metabolite. Cultures were stored in 40% (w/v) glycerol at –20°C. Cultures were plated for isolation on Luria Bertani (LB) medium containing the following ingredients: 20 g/L of glucose, 10 g/L of tryptone, 5 g/L of yeast extract, 5 g/L of NaCl, and 40 mg/L of chloramphenicol solidified with 1.5% (w/v) agar. A single isolated colony was used to inoculate 1200-mL flasks containing LB medium as described with 5% glucose (w/v). Inocula were incubated for 24 h at 30°C, and then cells were harvested by centrifugation and used for fermentations at an initial cell density of 330 mg dry wt/L.

Plate Count Analysis

Standard plate counts were performed in triplicate by removing samples from Fleakers® (Fisher Scientific, Itasca, IL) at 24-h intervals during substrate concentration variation experiments. Serial dilutions were performed and aliquots plated onto LB medium with and without chloramphenicol.

Enzymes

Commercially available Pectinex Ultra SP and Celluclast 1.5L enzyme mixtures were provided by Novo Nordisk (Franklinton, NC). Pectinex Ultra SP-L (pectinase) contains approx 2335 polygalacturonase units (PGU) of activity/mL *(32)*. Celluclast 1.5L (cellulase) contains approx 102 filter paper units (FPU) of activity/mL *(33)*. Novozyme 188 (cellobiase) contains approx 250 cellobiase units (CBU)/mL (Novo Nordisk assay).

Enzyme Preincubation

Sugar beet pulp was obtained from Monitor Sugar (Bay City, MI). Pellets were ground to decrease particle size using a Krup's coffee mill. Moisture content of the sugar beet pulp was determined and appropriate weights were added to Fleakers (fermentation vessels from Fisher Scientific). Fleakers containing Teflon-coated magnets (for stirring) and sugar beet pulp were autoclaved at 121°C for 20 min. When indicated, the appropriate volume of enzymes was filter sterilized, mixed with sterile LB, and added to sterile sugar beet pulp in Fleakers. Each experiment was performed in triplicate. Enzyme preincubation was conducted (when indicated) for approx 24 h at 42°C.

Fermentation Conditions

Fermentation experiments were conducted in modified 1200-mL Fleakers containing a 500-mL working volume of substrate essentially as described *(34)*. A six-station magnetic stirrer was placed beneath a water bath and maintained at 100 rpm. The pH was maintained using a Jenco 3671 pH controller (Whatman, Hillsboro, OR). Potassium hydroxide (2 or 10 *M*) was used for pH control and siphoned into fermenters as needed. Temperature was maintained by a thermoregulator. Experiments were conducted at a pH between 5.2 and 5.5 and at 35°C *(18,21)*. Galacturonic acid fermentations (20 g/L) were conducted at a pH of 6.0 *(19)*. Experiments were conducted with and without the addition of chloramphenicol (40 μg/mL). Pressed sugar beet pulp fermentations were conducted with and without LB as indicated.

Analytical Procedures

The pH and base consumption to maintain fermentation pH were recorded. Samples (2 mL) were removed and clarified by centrifugation to remove cells and debris. Ethanol concentration was determined by gas-liquid chromatography as previously described *(15)*. Fermentation broths from galacturonic acid fermentations were examined for organic acid production by high-performance liquid chromatography with ultraviolet detection (210 nm) using an Aminex HPX-87H column (Bio-Rad Laboratories, Hercules, CA) and a mobile phase of 0.01 *N* H_2SO_4, with a flow rate of 0.8 mL/min. Quantitation was made by peak area measurement with external standards.

Results

Galacturonic Acid Fermentations

The first objective was to evaluate efficiency of galacturonic acid fermentations with three recombinant bacteria: *E. coli* KO11, *K. oxytoca* P2, and *E. chrysanthemi* EC 16 pLOI555. Fermentations with 20 g of galacturonic acid/L (in LB) indicate that *E. coli* KO11 gave the highest ethanol yield (Table 1). All strains produced some ethanol and varying amounts of acetic

Table 1
Fermentations with 20 g/L Galacturonic Acid by *E. chrysanthemi* EC 16 pLOI555, *K. oxytoca* P2, and *E. coli* KO11 at pH 6.0 and 30°C[a]

Organism	Maximum ethanol (g/L)	Ethanol yield (g EtOH/g sugar)	Acetic acid (g/g sugar)	Succinic acid (g/g sugar)
E. chrysanthemi EC 16	3.16	0.16 (0.67)	0.38 (1.24)	0.13 (0.21)
K. oxytoca P2	2.44	0.12 (0.52)	0.34 (1.10)	0.08 (0.13)
E. coli KO11	3.86	0.19 (0.81)	0.23 (0.74)	ND[b]

[a]Numbers in parentheses represent yields in moles/moles of sugar.
[b]Not detected.

acid. Recombinant *Erwinia* produced the greatest amounts of acetic acid and succinic acid. Yield for ethanol production was lowest for *K. oxytoca* P2, which also produced both acetic and succinic acid. Our galacturonic acid fermentation data for *E. coli* KO11 are quite similar to those obtained by Grohman and colleagues *(19)*, in which an equimolar amount of acetate and ethanol was produced from pure galacturonic acid. Table 1 compares ethanol, acetic acid, and succinic acid production from fermentation of galacturonic acid for each recombinant.

Fermentations with Sugar Beet Pulp: Pressed and Pelletized

Since all three recombinants produced ethanol from galacturonic acid in varying amounts, we performed fermentations with sugar beet pulp either pressed or dried and pelleted, with and without fungal enzymes. A 24-h preincubation period in which fungal enzymes were added prior to bacterial inoculation decreased the viscosity of the substrate and reduced base addition throughout the fermentation. *E. coli* KO11 had the highest yield for ethanol production, but did not possess any hydrolytic enzymes to contribute to a bioconversion process. *K. oxytoca* P2 was engineered for use in a simultaneous saccharification and fermentation (SSF) process with cellulosic biomass *(16)* and alleviates the need for an externally supplied cellobiase. Fermentations with pure cellulose (Sigmacell 50) (Sigma, St. Louis, MO) *(21)* and sugar cane bagasse *(20)* showed *K. oxytoca* P2 to be an effective biocatalyst for ethanol production from these substrates. *E. chrysanthemi* (EC 16 with pLOI555 plasmid) possesses many enzymatic activities useful in pectin degradation. Therefore, none were excluded on the basis of galacturonic acid fermentation data alone.

The recombinant bacteria we have described were used in identical fermentations to determine which biocatalyst should be used in future studies. Fermentations were conducted using sugar beet pulp loadings of 106 g/L of dry wt sugar beet pulp (sbp) and run at 35°C and pH 5.5. Samples were removed from the fermenters every 24 h and analyzed for ethanol content. Table 2 gives the amount of ethanol produced from sbp with and without the addition of fungal enzymes. Although *Erwinia* and *Klebsiella*

Table 2
Ethanol Production from Sugar Beet Pulp
in Pelletized, Ground Form and Wet/Pressed Form[a]

Organism	Pellet/ wet[b]	Fungal enzymes[c]	Maximum ethanol (g/L)	Ethanol yield (g ethanol/g dry wt sugar beet pulp)
E. chrysanthemi EC 16	P	–	7.10	0.067
	W	–	10.00	0.094
	P	+	16.00	0.151
	W	+	17.26 (19.74)	0.163 (0.186)
K. oxytoca P2	P	–	5.42	0.051
	W	–	7.55	0.071
	P	+	15.51	0.146
	W	+	18.33 (21.06)	0.173 (0.199)
E. coli KO11	P	–	1.97	0.019
	W	–	3.50	0.033
	P	+	24.79	0.234
	W	+	25.50 (26.00)	0.241 (0.245)

[a]Fermentation was performed with 106 g/L of dry wt sbp with and without fungal enzymes (Celluclast Ultra SP at 0.57% [v/v] and Pectinex at 0.28% [v/v]) at pH 5.5 and 35°C. Numbers in parentheses refer to values obtained with preincubation of sbp with fungal enzymes at 42°C for 24 h with pH adjustment to 5.5 before inoculation.
[b]P, pellet; W, wet.
[c]–, absent; +, present.

produced more ethanol than *E. coli* when fungal enzymes were not added, the amount of ethanol produced was low. Enzymatic saccharification (preincubation) at 42°C for 24 h before inoculation (and adjustment of pH to 5.5 and at 35°C) decreased viscosity and base consumption during the fermentation. However, this enzyme preincubation increased maximum ethanol concentrations only slightly (Table 2). Figure 1 illustrates the ethanol production curves for the recombinants when supplemented with cellulase (5.25 FPU/g of dry wt sbp) and pectinase (60.2 PGU/g of dry wt sbp) at pH 5.5 and 35°C in LB medium with enzyme preincubation as described. Pressed pulp fermentations were repeated with fungal enzymes and no supplementation with LB medium. Ethanol values remained the same or were decreased by <2 g/L per time point (data not shown).

Variation in Substrate Concentration

While keeping the enzyme-to-substrate ratio constant (5.25 FPU and 60.2 PGU/g of dry wt sbp), experiments were conducted to determine the effect on cell mass and ethanol production using *E. coli* KO11 as the biocatalyst. Table 3 presents ethanol production from 5, 7.5, 10, and 11.5% (w/v) of sugar beet pulp. Ethanol concentrations increased with substrate concentrations until 10% (w/v) (27.12 g/L). Further increasing the substrate concentration to 11.5% (w/v) did not result in additional ethanol production.

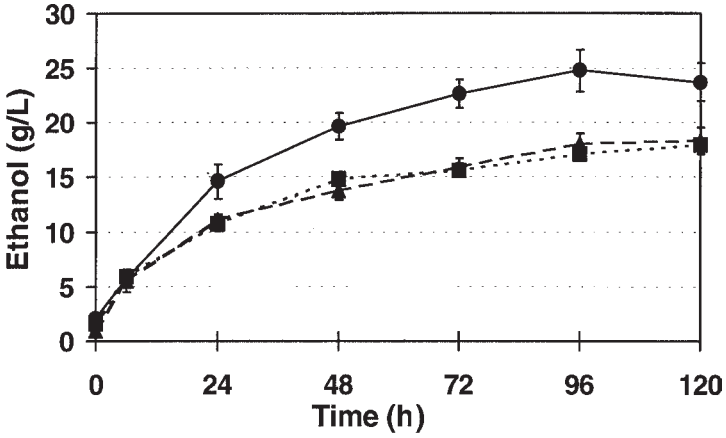

Fig. 1. Ethanol production from sugar beet pulp (106 g/L of dry wt sbp) using *E. coli* KO11, *K. oxytoca* strain P2, and *E. chrysanthemi* EC 16 pLOI 555, supplemented with fungal enzymes Celluclast Ultra SP (0.58% v/v) and Pectinex (0.27% v/v) at pH 5.5 and 35°C in LB medium. Sugar beet pulp was preincubated with enzymes at 42°C for 24 h, with titration to pH 5.5 before inoculation. ●, *E. coli* KO11; ■, *E. chrysanthemi* EC 16 pLOI 555; ▲, *K. oxytoca* P2.

Table 3
Parameters Analyzed During Variations in Substrate Concentration Fermentations

Sugar beet pulp concentration (% w/v)	Maximum ethanol (g/L)	Acetic acid at maximum ethanol (g/L)	Yield (g ethanol/g dry wt sugar beet pulp)[a]
5.0	15.59	3.68	0.32
7.5	20.61	5.12	0.29
10.0	27.12	6.39	0.28
11.5	26.21	4.88	0.27

[a]Values corrected for dilution by acid or base acetic acid concentration at inoculation was approx 2 g/L.

Plate count analysis was conducted to estimate cell mass produced during fermentations. Colony-forming units (CFU) produced during fermentations were greatest for all substrate concentrations at 24 h, with numbers decreasing slightly afterward. For all time points examined (except at the time of inoculation, when all were equal), CFU were lowest for a substrate concentration of 11.5% (w/v) (data not shown). Ethanol yields and acetic acid concentrations at maximum ethanol were similar for all conditions and are presented in Table 3 along with maximum ethanol values.

Increase in Enzyme Loading

E. coli KO11 fermentations of sugar beet pulp (106 g/L of dry wt sbp) using cellulase (5.25 FPU/g of dry wt sbp) and pectinase (60.2 PGU/g of dry wt sbp) produced an average of 25.5 g of ethanol/L by 96 h, with a yield

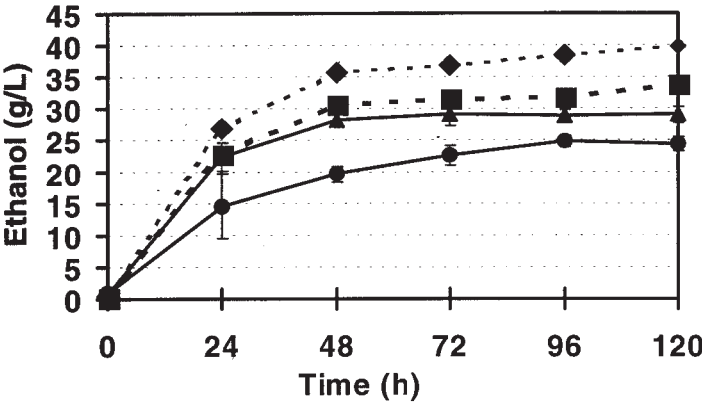

Fig. 2. Ethanol production from pressed sugar beet pulp by *E. coli* KO11 with varying enzyme loads and substrate concentrations. Solid lines indicate sugar beet pulp concentration of 106 g/L of dry wt sbp and dashed lines of 122 g/L of dry wt sbp. ●, 5.25 FPU, 60.2 PGU/g of dry wt sbp; ▲, 5.25 FPU, 60.2 PGU, 6.4 CBU/g of dry wt sbp; ■, 10.5 FPU, 120.4 PGU, 6.4 CBU/g of dry wt sbp; ◆, 106 g/L of dry wt sbp with 16 g/L of dry wt sbp added after 24 h fermentation (10.5 FPU, 120.4 PGU, 6.4 CBU/g of dry wt sbp).

of 0.245 g of ethanol/g of dry wt sbp (Fig. 1, Table 2). When Novozyme 188 (cellobiase) was added (6.4 CBU/g of dry wt sbp) to analogous experiments, ethanol concentration increased to approx 29 g of ethanol/L by 72 h (Fig. 2), with a yield of 0.294 g of ethanol/g of dry wt sbp. Further increases in enzyme loading did not result in significantly higher ethanol concentrations at this substrate concentration.

Increasing Enzyme Load and Substrate Concentration

Adding substrate to fermentations with cellulase (5.25 FPU/g of dry wt sbp), pectinase (60.2 PGU/g of dry wt sbp), and cellobiase (6.4 CBU/g of dry wt sbp) did not increase ethanol concentrations (data not shown). Starting with a higher substrate concentration (122 g/L of dry wt sbp) and doubling the enzyme load (10.5 FPU cellulase/g of dry wt sbp, 120.4 PGU pectinase/g of dry wt sbp) produced an average of four more grams of ethanol per liter. Adding cellobiase to this fermentation (6.4 CBU/g of dry wt sbp) further increased ethanol production by only 1 g/L.

To increase ethanol production without further increases in enzyme loading, sugar beet pulp was added to fermentations in stages. Fermentations with 106 g/L of dry wt sbp and cellulase (10.5 FPU/g of dry wt sbp), pectinase (120.4 PGU/g of dry wt sbp), and cellobiase (6.4 CBU/g of dry wt sbp) were conducted for 24 h (Fig. 2). Then 16 g/L of dry wt sbp was added to the fermentation mixture, and fermentation continued for a total of 120 h. Adding the sugar beet pulp in two stages resulted in production of 40 g of ethanol/L between 96 and 120 h of fermentation (Fig. 2).

Discussion

Enzymatic processes are currently expensive, but they can operate with high yields and generate few inhibitory side products. Complete enzymatic hydrolysis of only one component of beet pulp, cellulose, to glucose requires several classes of enzymes and represents a major hurdle for commercialization of cellulose to ethanol processes *(35,36)*. Enzymes secreted by the fungus *Trichoderma reesei* have been successfully utilized to hydrolyze cellulose molecules to glucose *(37–39)*. If enzymatic hydrolysis is employed, the fermentation and hydrolysis stages could be combined in a single step. In this process, known as SSF, fermenting organisms consume the simple sugars as they are produced by the enzymes effectively preventing end-product inhibition of the cellulases. This process was developed and patented in 1976 by Gulf Oil using conventional yeasts as the biocatalyst *(40)*. The SSF process remains the best available technology for biomass conversion to ethanol, although the patent has now expired *(41)*. With modifications such as replacement of the biocatalyst and optimization of conditions, this may become a more cost-efficient process. Enzymatic hydrolysis of pectin-rich processing residue such as sugar beet pulp appears to be much more amenable to enzymatic degradation than lignified cellulosic substrates *(3)*. Also, harsh chemomechanical treatments required for effective enzymatic hydrolysis of lignocellulose do not appear to be required with pectin-rich residues (*[3]*; Table 2).

Note that the sugar beet pulp fermentations described herein have been conducted using enzymatic digestion alone with no chemical pretreatment. More than 25 g of ethanol/L have been obtained using an enzyme load of 0.85% (v/v) with pressed beet pulp (5.25 FPU cellulase and 60.2 PGU pectinase/g of dry wt sbp). *E. coli* KO11 appears to show the most promise for a biocatalyst, even though it does not produce any enzymes on its own for the degradation of plant material. *E. chrysanthemi* and *K. oxytoca* both possess enzymes in the native state that could help degrade plant material; however, these enzymes do not appear to confer any advantage to these organisms in our process. Therefore, not surprisingly, the addition of fungal enzymes is required for significant levels of ethanol to be produced.

Since pelletization and dehydration is a concern of the processing plants owing to high energy costs, sugar beet pulp in pressed form (75% moisture) was compared with results from dry ground pellets (10% moisture). Table 2 compares the amount of ethanol produced using pellets to that produced using wet pulp for each of the recombinants (equivalent dry weight). Pressed sugar beet pulp (particle size in the centimeter range) yielded equivalent or slightly better results in terms of ethanol production than experiments using the dehydrated pelletized form (millimeter range).

Our goal for an economically feasible distillation process is to reach a minimum of 40 g of ethanol/L within a 96-h period with as low an enzyme loading as possible. Fermentations with a sugar beet pulp loading of 106 g/L

of dry wt sbp have reached 25.5 g of ethanol/L by 96 h using cellulase at 0.57% (v/v) and pectinase at 0.28% (v/v) (Fig. 1, Table 2). However, this concentration of ethanol may be too dilute for cost-efficient distillation. An enzyme preincubation at 42°C, followed by cooling to 35°C and pH adjustment to 5.5 before inoculation, facilitated mixing but increased ethanol yields only by 0.5–3 g of ethanol/L (Table 2).

When Novozyme 188 was added (6.4 CBU/g of dry wt sbp) to experiments with 106 g/L of dry wt sbp, ethanol concentration increased from 25.5 to 29 g of ethanol/L by 72 h (Fig. 2) at lower enzyme concentrations. In an effort to increase substantially final concentrations of ethanol, experiments with additional substrate were conducted.

Increasing the substrate concentration to 122 g/L of dry wt sbp at an enzyme loading of 5.25 FPU cellulase, 60.2 PGU pectinase, and 6.4 CBU cellobiase/g of dry wt sbp did not increase ethanol concentrations. Doubling this enzyme load resulted in concentrations of ethanol at 34.0 g/L. To determine whether this substrate concentration was creating osmotic stress for *E. coli* KO11, fermentations were conducted with sugar beet pulp at 106 g/L of dry wt sbp, with more substrate (16 g/L of dry wt sbp) added after 24 h of fermentation (Fig. 2). Even though the total amount of sugar beet pulp used was the same, the concentration of solids in the fermenters at any one time was less than if it were all present at the time of inoculation. The decrease in osmotic pressure coupled with better mixing resulted in ethanol yields of 0.340–0.352 g of ethanol/g of dry wt sbp (40 g of ethanol/L). Although these yields are reasonable, this enzyme load may be too high for use on an industrial scale. Current studies are being conducted to determine the optimum ratio and quantity of enzymes necessary for an economically feasible sugar beet pulp to ethanol process. In addition to enzymatic hydrolysis, chemical pretreatments are also being examined in an effort to reduce the amount of enzymes required.

Plate count analysis during the substrate concentration experiment revealed the *E. coli* KO11 strain to be quite stable under the conditions of the experiment, and sugar beet pulp does not appear to contain strong inhibitors of growth or ethanol production for this recombinant. When assayed on medium with and without chloramphenicol, there was no significant difference in cell counts obtained at each 24 h interval (120 h total). During experiments with and without the addition of antibiotic during fermentation, there was no difference in ethanol yield. By contrast, Lawford and Rousseau *(42,43)* have reported phenotypic instability for this strain in continuous culture indicating declining ethanol and increasing lactic acid production. Dumsday and colleagues *(44)* have shown that *E. coli* KO11 gives high stable ethanol yields in continuous culture with glucose without the addition of antibiotic. Since this organism is efficient at converting arabinose and galacturonic acid to ethanol, further studies addressing its stability are being conducted. For this process to proceed eventually to an industrial scale, strain stability and enzyme loading are of paramount importance.

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