The effect of enzymes on the fermentation of sugar beets

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Introduction

- As the name indicates, sugar beets contain large amounts of different sugars (about 75% of the dry matter is sugar), so they are easily fermented
- Sugar beets grow during the winter, so they provide an excellent addition to switchgrass as feedstock for biofuel, which grows during the summer
- Polysaccharides compose the majority of sugar beet dry matter, including cellulose, hemicellulose, lignin and pectin
- Pectin, a polymer of D-galacturonic acid, is present in cell walls and the middle lamella between plant cells to bind them together
- The enzyme pectinase breaks pectin down into sugars and galacturonic acid, while the enzyme cellulase breaks cellulose down into β-glucose

Figure 1. Sugar beet

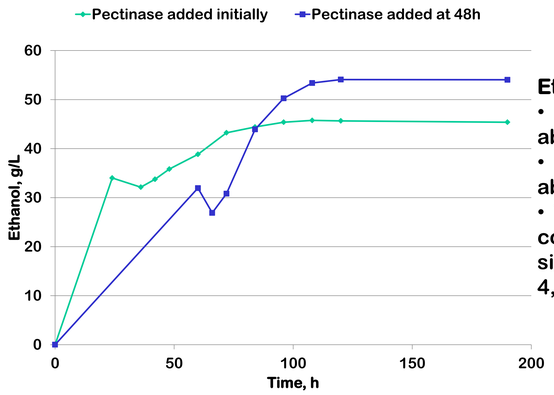
Methods and Materials

- The sugar beets are ground into a pulp using a food processor
- 100 g beet pulp added to each of 6 flasks
- 200 μL cellulase (Accelerase 1500) added to all flasks
- 30 μL pectinase (Pectinex Ultra SP-L) added to flasks 4 – 6
- Flasks incubated at 50°C at 250 rpm

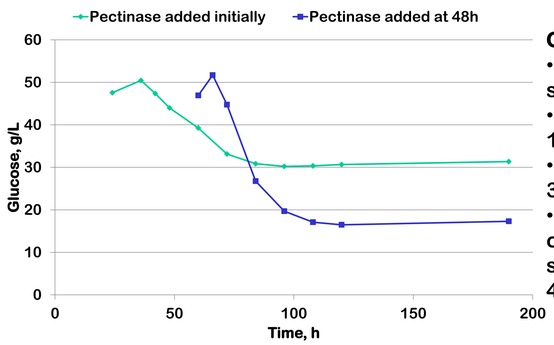
Figure 2. Sugar beet pulp before and after incubation

- 0.2 g dry yeast added to all flasks and allowed to ferment at 37°C at 200 rpm
- Samples taken every 6 hours for the first 48 hours, then every 12 hours
- Flask 1 – 3 do not liquefy, so 1 mL additional cellulase is added; no liquefaction is observed, so 10 μL pectinase added at 48 hours and pulp liquefies within 12 hours
- Sugars and ethanol measured by HPLC

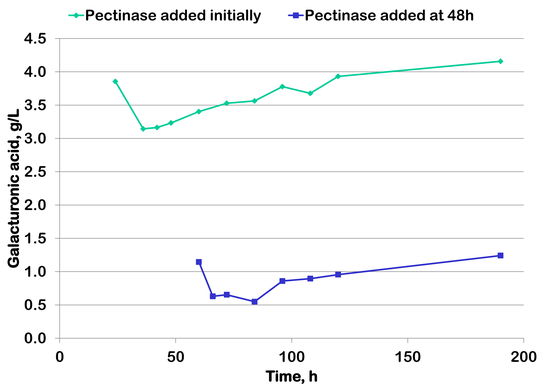
Results



Ethanol Concentration
- Flasks 1, 2, & 3 – increase from 0 to about 54 g/L
- Flasks 4, 5, & 6 – increase from 0 to about 45 g/L
- The final average ethanol concentration of flasks 1, 2, & 3 is significantly higher than that of flasks 4, 5, & 6, indicating more fermentation

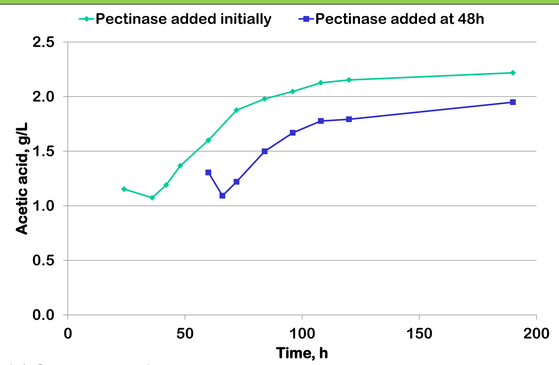


Glucose Concentration
- Initial values unknown due to solid state of beet pulp
- Flasks 1, 2, & 3 – decrease to about 17 g/L
- Flasks 4, 5, & 6 – decrease to about 31 g/L
- The final average glucose concentration of flasks 1, 2, & 3 is significantly lower than that of flasks 4, 5, & 6, indicating more fermentation



Galacturonic Acid Concentration
- Initial values unknown due to solid state of beet pulp
- Flasks 1, 2, & 3 – increase to about 1.2 g/L
- Flasks 4, 5, & 6 – increase to about 4.2 g/L
- The final average galacturonic acid concentration of flasks 4, 5, & 6 is significantly higher than that of flasks 1, 2, & 3 due to higher volume of pectinase added to hydrolyze pectin

Results Continued



Acetic Acid Concentration
- Initial values unknown due to solid state of beet pulp
- Flasks 1, 2, & 3 – increase to about 1.95 g/L
- Flasks 4, 5, & 6 – increase to about 2.2 g/L
- The final average acetic acid concentration of flasks 4, 5, & 6 is slightly higher than that of flasks 1, 2, & 3 – although acetic acid can be produced by yeast, the higher concentration in flask 4, 5, & 6 indicates that it was most likely produced as a byproduct of the hydrolysis of pectin

Conclusions

In order for the flasks to be mixed properly and for samples to be taken, the beet pulp must liquefy. The original plan was for pectinase to be added to only flasks 4, 5, & 6 and there was no incubation step, but there was no liquefaction observed in any of the flasks. In order for the enzymes to act at their optimum conditions, the temperature was increased to 50°C and the shaking speed was increased to 250 rpm. Flasks 4, 5, & 6 liquefied within 12 hours, but flasks 1, 2, 3 still showed few signs of liquefaction. 1 mL of cellulase was added to these flasks, but no change was observed. 10 μL was added to these flasks, and liquefaction was finally observed 12 hours later. Therefore, pectinase is essential for the liquefaction of sugar beet pulp in order for it to be fermented.

When considering the extent of fermentation, several factors must be observed:

1. How much does the concentration of glucose decrease?
2. How much does the concentration of ethanol increase?

According to the data, more fermentation occurred in flasks 1, 2, & 3 than in flask 4, 5, & 6. This result is most likely due to the higher volume of cellulase added to flasks 1, 2, & 3, which would break down more cellulose and render more simple sugars for fermentation.

The data also indicates that at some point something caused fermentation to stop. The most likely cause of this is that the production of different acids lowered the pH of the sample to a point that the yeast were killed. Another reason why more fermentation occurred in the first 3 flasks might be because since more acid was produced in flasks 4, 5, & 6, the pH was lower and could have killed the yeast more quickly than in flasks 1, 2, 3. Since our results show that both cellulase and pectinase are important for the fermentation of sugar beet pulp to occur, further investigations into this process should control the volume of one and observe how changing the volume of the other effects the extent of fermentation and vice-versa, in order to find the optimum balance of the two enzymes to produce a maximum yield of ethanol. Also, a way to control the pH must be included so that it does not become too low and kill all of the yeast.

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