**METHODS**

**Understanding Pretreatment**
In order to release maximum amount of sugars, the optimum pretreatment condition needs to be identified. Pretreatment needs to be harsh enough to degrade the sugars, but not so harsh so that it is damaging to the material. Different pretreatment conditions were used for six different sets of data. Each had triplicates of both corn fiber and sweet sorghum bagasse, giving a total of 18 specimens.

**Lipid production by Cryptococcus curvatus on hydrolysates derived from corn fiber and sweet sorghum bagasse following dilute acid pretreatment**
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**BACKGROUND**
There is an international demand and concern for an alternative fuel and energy source, due to the fact that natural resources such as petroleum are being rapidly depleted. As it stands, current efforts towards a sustainable renewable are being directed towards ethanol production. Ethanol production appears to be an economical, efficient solution as an alternative fuel source. It utilizes corn fiber biomass which is produced in mass quantities by the corn industry, but not suitable for animal feed. This minimizes agricultural waste. Ethanol may be a good initial attempt at producing an alternative fuel source; however it is not the solution to the problem.

Ethanol presents too many problems in terms of high water solubility, flammability, and low energy content. It is difficult to transport and there are obstacles that need to be addressed for gasoline. Therefore, this is not a sustainable solution. A promising resolution, as an alternative to ethanol, is the production of biodiesel. Biodiesel is a better option as an alternative to ethanol. It has a higher energy density and the opportunity to be very cost efficient. Cellulose is an important component of the biocatalytic process. Cellulose can be found in lignocellulosic materials, combined along with two other carbohydrates, hemicellulose and lignin. For this reason, lignocellulosic materials are the target choice for substrates.

**DISCUSSION**
Theoretically, severe pretreatment tends to disrupt the lignocellulosic structures more than those done under mild conditions. But severe pretreatment may also lead to degradation of released sugars from cellulose and hemicellulose. As indicated by Fig. 2 (not included), most of TRS was released during the first 24 h. Samples with SFE of 1.87 had the highest TRS release compared with those with SFE of 1.2 and 1.28. Thus, for SSF, the lowest severity treatment resulted in maximal release of sugars. In this case, a fast and simple dilute acid pretreatment (0.5% sulfuric acid) at 121°C for 1 h is enough to release more than 98% of potentially available sugars from SSF. The remaining materials which include undehydrolyzed cellulose, hemicellulose and lignin can be processed further through thermochemical processes to produce bio-crude.

The lowest SFE for corn fiber of 1.05 resulted in a recovery of 83.2% of theoretically available sugars just by pretreatment. Pretreatment of corn fiber with SFE of 1.64 and 2.24 led to sugar yield of 90.7% and 67.5%, respectively. Pretreatment having the highest SFE had the lowest sugar yield. For corn fiber, pretreatment at 121°C for 1 h using 5% of sulfuric acid is enough to unlock most of sugars out of the feedstock.

Hydrolysates which were the supernatant of slurries after pretreatment and centrifugation of those derived from the two lower SFEs for SSF and corn fiber were used to ferment C. curvatus. In this study, no detoxification of any hydrolysates were carried out. Once yeast cells were added to the hydrolysates, they started to grow immediately with no lag phase. After 2 days, the dry cell dry weight content was measured to be 40%. With regard to hydrolysates derived from corn fiber, no significant cell growth was detected though nutrients in a mineral medium and a spore’s solution were provided (data not shown). For this hydrolysate, detoxification may be needed to remove any residual compounds to yeast fermentation.

Regarding the non-sugar compounds that were also identified. For SFE 1.02, among the non-sugar compounds, HMF had the highest concentration of 1.5 g/l which is lower than the threshold of 3 g/l above which significant inhibition on C. curvatus growth and lipid production will take place. However, though HMF was consumed by C. curvatus in our study, this chemical remained unchanged at a concentration around 0.4 g/l throughout the 7-day fermentation period using the same yeast strain (Yu et al., 2011). Some kind of chemicals were observed in hydrolysates from SSF with SFE of 1.84.

Comparing sugars in hydrolysates of SSB obtained from SFE of 1.02 and 1.87, concentrations of glucose and arabinose were basically the same. Concentration of xylose in hydrolysate from SFE = 1.87 was 41.2% of that from SFE = 1.02. Thus, it indicated that severe pretreatment degraded xylose. As a result of this degradation, higher concentration of formic acid (1.6 g/l) was measured in hydrolysates from SSF of 1.87 than that (0.1 g/l) from SSF of 1.02. In addition to formic acid, hydrolysis from SFE of 1.84 contained levulinic acid at a concentration of 5.0 g/l which was much higher than that (0.3 g/l) from SFE of 1.02. Consequently, under strongly acidic condition (Du et al., 2010), thus it seemed that glucose was released under the harsher pretreatment condition, but owing to glucose degradation, higher concentration of glucose was identified in comparison. From these two liquids, it is obvious that the high concentration of levulinic acid may be the reason for slow growth and fermentation.

To understand why C. curvatus did not grow on corn fiber hydrolysates, we profiled the chemicals in these hydrolysates, too through use of HPLC. Corn fiber hydrolysates contained more xylitol than glucose, especially for those obtained from SFE of 1.02 and 1.84, and higher concentration of arabinose than those in SSF. This is consistent with the fact that corn fiber has a high content of hemicellulose (27%) which includes xylo-oligosaccharides while SSF contains 17.8% of hemicellulose which is mainly xylan. The presence of high concentration of xylose and arabinose may limit cell growth as they are not the preferred sugars for C. curvatus. Thus, it is apparent that harsher pretreatment increased the formation of non-sugar compounds could inhibit growth of C. curvatus. But the exact reason for why C. curvatus did not grow on corn fiber hydrolysates is not certain at this point since other chemicals that were not detected and quantified by HPLC could result in growth inhibition. In this case, to utilize corn fiber hydrolysates as substrates for cultivating C. curvatus or other yeast strains, detoxification through overlying and/or absorption is needed.

**RESULTS**

**Flowchart of basic process used**
An inoculum of C. curvatus was set up by adding frozen stock of this yeast to a medium. After 3 days, this yeast culture was used to inoculate hydrolysates of corn fiber or SSF. An inoculum size of 10% of final volume was directly added without any nutrient supplementation. All cultures were maintained at room temperature.

**Flowchart continued after fermentation**
YEAST FERMENTATION

1.5 ml samples taken daily (5 days)

Chlorogenic acid water extracts for determination of chlorogenic acid

**REFERENCES**


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