CONVERSION OF EXTRACTED RICE BRAN AND ISOLATION OF PURE BIO-ETHANOL BY MEANS OF SUPERCRITICAL FLUID TECHNOLOGY

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1. INTRODUCTION AND STATE OF THE ART

The conversion of biomass to ethanol has become a new focus of interest, due to increasing costs of petrol refinery. Pure ethanol may be used as additive for fuel motors, blends from 3 – 85 % are feasible. However, in conventional processes, the final purification step of ethanol (99.9 %) from aqueous low concentrated fermentation sludge requires sophisticated distillation steps including membranes /molecular sieving technologies and others. Consequently, few bio-ethanol production technologies (Arkenol® [1] and BCI/ Organosolv® [2]) are industrially applicable. One of the main drawbacks was the need of organic acids for the catalysed hydrolysis of the cellulose/hemicellulose chains to the intermediate sugar molecules (glucose, xylose). Up to the recent times, this process was not competitive with petrol-based processes, except in some regions with unique rural conditions [3].
Process alternatives, based on the enzyme catalyzed conversion have been intensively investigated during the past decades (BPI /Logen®[4]). A process configuration of this type consists of at least four major steps: pre-treatment of raw material, including chip size reduction, sieving, (enzymatic) hydrolysis of hemicelluloses and cellulose; fermentation using a suitable yeast strain; and ethanol refining.
One of the most studied alternative methods for cellulose/ hemi-cellulose hydrolysis is the hot water pre-treatment [5]. In the temperature range above 100 °C and at pressure conditions above the temperature-affiliated vapour pressure, the hemicellulose is hydrolysed and the cellulose is made more accessible to biological attack. In the next step, cellulose may be hydrolysed by means of adapted enzymes (cellulase).
Due to its low lignin content compared to other biological waste products, milled and defatted rice bran (27% cellulose, 37 % hemicellulose, 5 % lignin) is an interesting representative of the group of lignocellulose biomass. Rice bran production is in the range of 40 megatons/year [6], defatted rice bran is mainly used as nutritional additive for cattle feed. The super-critical extraction of rice bran and the purification of the high value compounds of the extracted rice bran oil is not scope of this work, we refer to Danielski et al. [7], as well as the poster communication in this meeting.

2. THEORETICAL APPROACH:

2.1 Rice bran conversion

Chemical structure makes hemicellulose very hydrophilic. Due to the loose structure (no crystallinity at all) and the high content of –OH and –COOH groups, it acts as a sort of glue between cellulose and lignin. Hemicellulose consists of different types of polysaccharides with very complicated, but well defined, structure of repeating sequences, including xylose, arabinose, mannose. It differs thus strongly from the cellulose structure. While cellulose can

be converted from the plant material and treated to release glucose molecules, the hemicellulose can be hydrolysed to xylose. Both mono-sugars can be fermented to produce ethanol. Other intermediate products from cellulose and hemicellulose differ strongly from each other. Different side reactions of hemicellulose may lead to a non desired production of (toxic) inhibitors like furfural. In parallel, glucose (the resulting product of cellulose hydrolysis) may follow the reaction path to ethanol, but also to the side-product 5-Hydroxy-methyl furfural (HMF).
In previous works, Lissens et al. [8] and Liu [9] have studied these effects. It can be shown that it is possible to liquefy cellulose suspensions in very short times, provided the hydrolysis temperature being sufficiently high. During the residence time of the substrate in the reactor, glucose concentration reaches an intermediate maximum, before being converted to the sub-products. Process temperature can be decreased when carbon dioxide is added into the aqueous reaction fluid.

2.2 Ethanol purification.

Budich et al.[10] and Lin et al. [11] have elaborated the tools for continuous counter-current separation of ethanol-water mixtures by supercritical CO_2 (Figure 1)

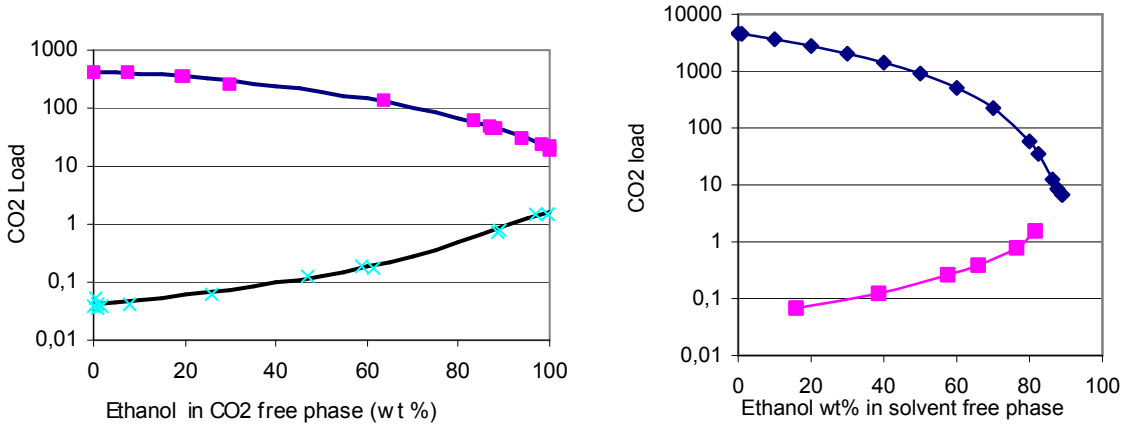

Figure 1 : Ponchon-Savarit Diagram of the system EtOH-H2O-CO2 at a) 100 bar, 60 °C[10], b) 140 bar, 60 °C [from 11]

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At process conditions of 100 bar and 60 °C, it has also been shown experimentally that a 17.5mm diameter column with Sulzer EX®-packings possesses a five - to ten times lower flooding capacity for low ethanol solutions (5- 10 % w/w) than for enriched solutions [10]. In column design, the flooding capacity would require to excessive column diameters in the stripping section. In fact, it is suggested that the problem of flooding can be evaded by joining a Mixer Settler at the raffinate outlet of a relatively short stripping section of the CC-SFE column (Figure 2). In this scenario, the counter-current column operates at 10 MPa and 60 °C, feed input is a typically a low concentrated ethanol solution resulting from fermentation in the range of 7 %. Raffinate from the CC-SFE-unit with a concentration of ethanol around 6%, will be fed to mixer settler, whereas the mixer settler works at 140 bar and 60 °C. Raffinate output of mixer settler is consequently approximately pure water. Extract from mixer settler is the enriched recycle flow with about 25 to 30% concentration in ethanol, and can directly be introduced to the original feed flow from the fermentation unit.

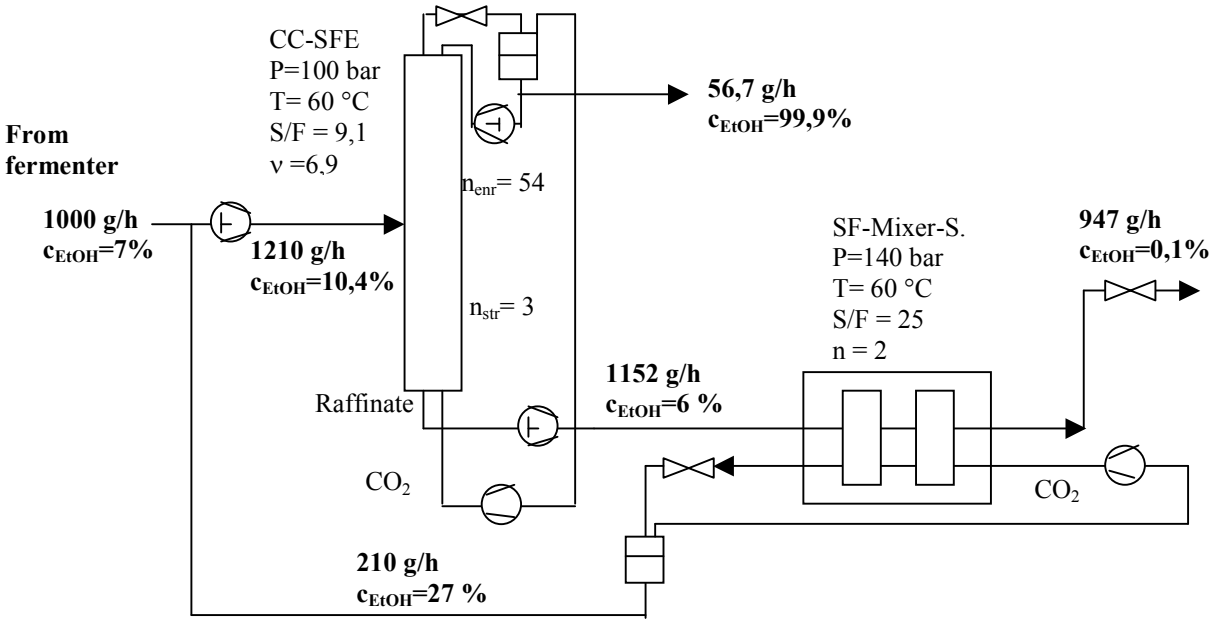


Figure 2 : Scetch of a supercritical purification unit for low concentrated ethanol solutions, data calculated with Ponchon-Savarit method

3. MATERIALS AND METHODS

1. Rice bran has been furnished by Oryza, Hamburg. Pre-treatment methods contained a) milling (particle size < 180 μm) b) pelletizing in a laboratory pelletizer, c) defatting by Supercritical Fluid Extraction (P=250 bar, T=60°C, S/F= 22 kg/h.kg, t = 3h),
2. Hydrolysis was performed in a piston reactor (50 mL autoclave) which is tempered inside the oven of a "Spe-ed" SFE unit (Applied Separations, Allentown, US). All conversion experiments were done with an initial rice bran concentration of 5 wt-% in the feed suspension. The reaction kinetics were assumed to be independent of the solid concentration and the evaluation in terms of residence time was performed using the properties of pure water. Carbon Dioxide has been added to the flow optionally.
3. Autoclave output has to be converted with enzymes in a Batch Reactor at ambient pressure. To be economically profitable both cellulose and sugar molecules (pentoses and hexoses) must be hydrolyzed and fermented in the process. DEPOL 692 L® from Biocatalyst, and commercial available bakers' yeast is used for the hydrolysis of cellulose and co-fermentation. The working temperature for enzymatic hydrolysis is 50-60°C, and for fermentation is 30-35°C, both having in the pH range of 4-6. A trial amount of enzyme would be about 2% Celluclast and 0.2% Cellobiase on a weight/weight ratio to available cellulose and 40 gm per 500-1000 gm of fermentable sugar solution.
4. The determination of carbohydrates is done by HPLC. Equipment specification is pump L-7100 (Merck-Hitachi), column oven (Techlab), RI-detector (Agilent 1100), Datasytem Kroma (Bio-Tek). Column is Nucleogel Sugar Na, 300*7.8 mm, Eluent is deionized water, further conditioned by ion-exchange, charcoal adsorbance and bacteria filtration. Sample volume is 20 μL, flow is 0,5 mL/min, temperature is 70 °C, detection through Refractive Index (RI) was done according to external standard.
5. Countercurrent SFE of Ethanol Water mixtures has been studied in a lab scale 7m x 17,5 mm i.d. column with Sulzer EX packings. SF Mixer settler unit consists of five tempered mixing cells (side-channel pumps) with negligible void volume, each of them being connected with upstream diffusor/cyclone units.

4. RESULTS AND DISCUSSION

The treatment of cellulose and hemicellulose in rice bran in water at 200 bar and in the temperature range of 160°C - 220°C shows a remarkable degree of liquefaction (Figure 3a). The graph shows that the rate of reaction increases with increase of temperature and a maximum conversion slope achieved between 20 to 40 minutes of residence time, which clearly indicates that higher temperature will be beneficial for the increase in degree of liquefaction. When the effluents of this thermal treatment in water were subjected to HPLC for the carbohydrate analysis, it gives the indication of formation of some by-products :

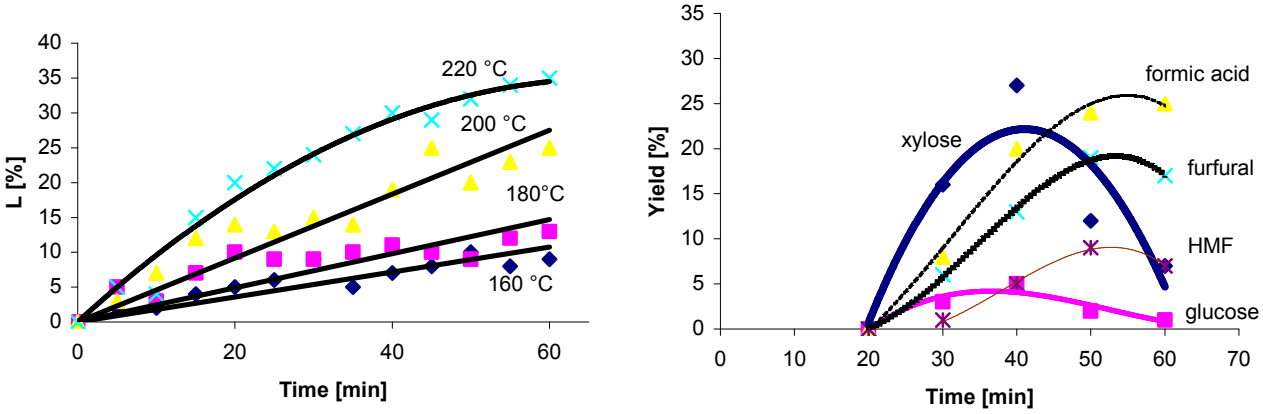


Figure 3 : a) Hot Water Rice Bran liquefaction in a piston reactor at a pressure 200 bar
b) Production of side products in function of time

Figure 4 shows the first results concerning the addition of carbon dioxide in the flow, there is just a slight increase of liquefaction yield, however the concentration of carbon dioxide was very low. The results can related to former experiments from other researchers (hydrolysis of corn starch,[9]), which confirm the enhancing impact of carbon dioxide environment to hot water hydrolysis.

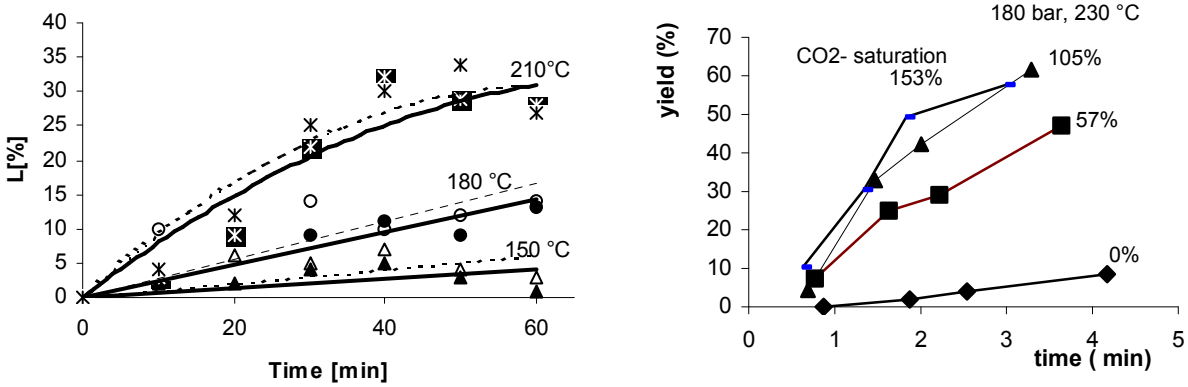
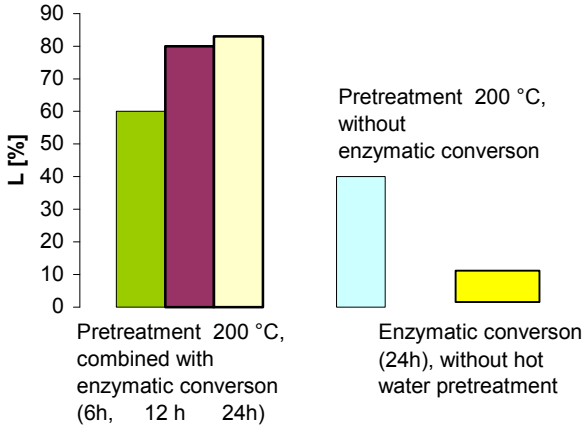


Figure 4: Conversion of Biomass with pure water and Water + CO_2
a) this work, liquification of rice bran, at P = 200 bar ,influence of temperature
dotted lines : $CO_2 + H_2O$, CO_2-saturation 10 % ; bold lines : pure H_2O
b) data [8] on hydrolysis of water- insoluble corn starch, glucose yield at P = 180 bar, T = 230 °C
influence of CO_2-saturation (0 → 153 %)

The effect of liquid hot water treatment on the efficiency of enzymatic hydrolysis has also been analyzed, it clearly shows that the enzymatic hydrolysis without pre-treatment is nothing just a waste of enzymes.



	Glucose Yield	Xylose Yield
Hot Water Hydrolysis 200 °C	5%	35 %
Hot Water Hydrolysis with subsequent enzyme treatment	70 %	70 %

Figure 5 : Influence of hot water hydrolysis on the liquefaction of rice bran and yield of xylose and glucose

The table in Figure 5 confirms also the fact that the isolated hydrolysis is efficient for xylose conversion from hemicellulose, but glucose yield is still quite poor. However, the pre-treatment gives favorable conditions for the subsequent enzyme-catalysed conversion of cellulose.

Although the degree of liquefaction increases with increase of temperature and both glucose and xylose are the main constituents to produce ethanol, so there is a need to optimise the process parameter for this thermal pre-treatment. The results shows that in the temperature range of 180 °C to 220 °C within the residence time of 20 to 30 minutes, we can hydrolyse the hemicellulose up to 30% and the rest of conversion can be done through enzymatic hydrolysis, in order to prevent the solution from by products.

Lab scale experiments on ethanol/water purification have been performed to show the feasibility of enriching ethanol above the aceotrope point. The experimental unit which has been described in [10] has actually been loaded with a feed mixture of 80 % EtOH, process conditions were 100 bar and 60°C. Extract purity above 99% EtOH has been achieved. Raffinate purity was in the range of 14- 20 %.
Mixer Settler experiments have been performed with a feed input of 5% EtOH. Process conditions were 140 bar and 60 °C. Although simulation suggested the necessity of only 2 mixing plates, the apparatus was operating with 5 cells. A pure water raffinate output (99.6% water) was achieved at S/F = 74, mixer-settler extract contains consequently 20 – 30 % ethanol.

The calculation of the theoretical stages in the counter current extraction unit as well as in the mixer settler unit is described in figure 6.

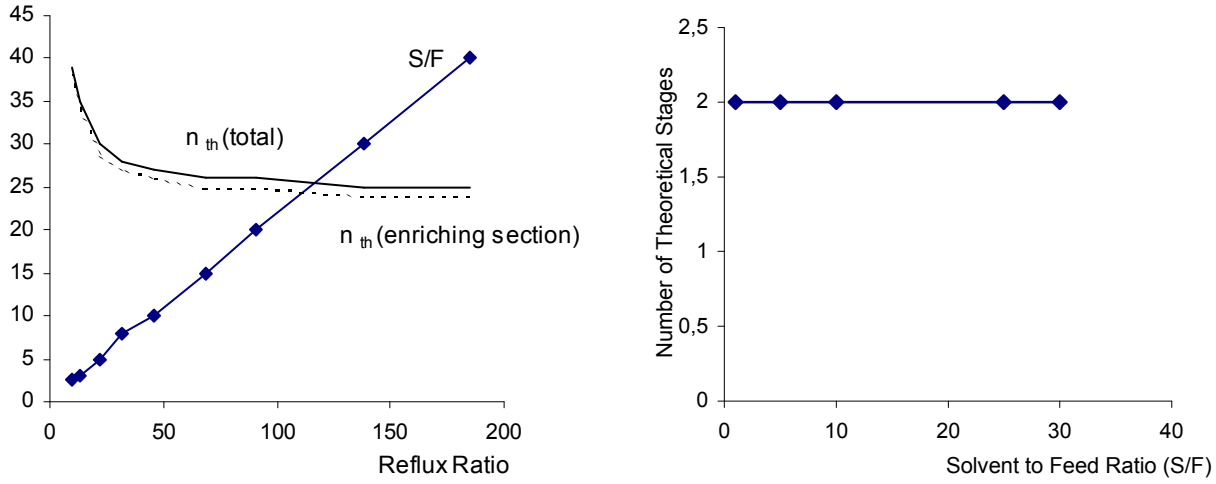


Figure 6: Calculation of the number of theoretical plates for separation of EtOH-H2O
a) CC-SFE at 100 bar, 60 °C,
EtOH concentrations : Feed input 7%, Extract 99,9 %, Raffinate 6 %
b) SF- Mixer Settler at 140 bar, 60 °C,
EtOH concentrations : Feed input 6%, Extract 27 %, Raffinate 0,1 %

5. CONCLUSION

In this work the technological feasibility of converting defatted rice bran to glucose and xylose as well as the subsequent separation of the fermented ethanol solution has been studied. It is in fact possible to pre-treat rice bran with hot water at relative moderate conditions, so that a) the hemicellulose is already considerably converted to xylose, and b) the subsequent enzymatic conversion gives elevated yields in cellulose hydrolysis.
The fermentation by-product carbon dioxide from can be used in the following for the separation of the produced ethanol solution to pure ethanol and pure water by means of countercurrent SFE and a supercritical Mixer-Settler unit.

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REFERENCES

[1] CUZENS et al., Renewable Energy, Vol.10, **1997**, 285-290
[2] ROSSEL et al., International Sugar Journal, Vol 107, **2005** , 192-195
[3] FNR : Schriftenreihe " Nachwachsende Rohstoffe" , Bd . 23, **2003** : „Ethanol aus Biomasse"
[4] DALE et al. , BPI Press information , www.bio-process.com
[5] VAN WALSUM, App. Biochemistry & Biotechnology Vol .57/58., **1996**,157-170,
[6] HERNANDEZ, **EPSCoR Conference 2005,** Morgantown, WV
[7] DANIELSKI et al., Journal of Supercritical Fluids, Vol 34, **2005**, 133-141
[8] LISSENS et al., Biodegradation, 15, **2004**, 173-183
[9] LIU: PhD Thesis, TUHH, 2000
[10] BUDICH et al. , Journal of Supercritical Fluids Vol. 25, **2003**,45-55
[11] LIM et al., Journal of Supercritical Fluids, Vol 7, **1994**, 219-230