# Ultrasonically Assisted Conversion of Lignocellulosic Biomass to Ethanol

*Maricela Toma, Osaka Prefecture University, Osaka, Japan,*
*Hiroshi Bandow, Osaka Prefecture University, Osaka, Japan,*
*Mircea Vinatoru, Institute of Organic Chemistry, Bucharest, Romania and*
*Yasuaki Maeda, Osaka Prefecture University, Osaka, Japan*

**Abstract**

In order to improve the bioconversion of lignocellulosic materials in an environmentally friendly manner, we choose to use the ultrasonic energy. Since enzymatic hydrolysis of cellulose represents a significant part of production costs, it is economically advantageous to develop a technique for reducing the cellulolitic enzyme loading. Thus, the sonication was employed in a two steps concept process: ultrasonically pretreatment and ultrasonically assisted hydrolysis of biomass. Our results show that a direct exposure of the lignocellulosic substrate to the 20 kHz high power ultrasound is in the main benefit of both pretreatment and ongoing hydrolysis.

**Introduction**

Hydrolysis of cellulose can be carried out by numerous factors of a chemical or physical nature based on the action of cellulolytic enzyme, various kind of chemicals or even steam. Enzymatic saccharification of cellulose has been employed since long time to product sugar and other products. Lignocellulosic materials are particularly attractive in this context because of their relatively low cost and plentiful supply. The main impediment to bioconversion of this important resource is the recalcitrance of cellulosic biomass. Current pretreatment processes are mostly chemically catalyzed [1].The chemically heterogeneous lignocellulosic biomass consist of high molecular weight structural components (cellulose, polyoses or hemicelluloses, and lignin), which are the major cell wall components; and of low molecular weight nonstructural components (extractives and inorganic compounds). Accessibility of the substrate to cellulase is a primary factor influencing the efficiency of enzymatic degradation of cellulose [2].

The efficiency of ultrasound in the processing of vegetal materials has been already proved [3]. The known ultrasounds benefits, such as swelling of vegetal cells and fragmentation due to the cavitational effect associated to the ultrasonic treatment, act by increasing the yield and by shortening of the extraction time. The effect of ultrasound on lignocellulosic biomass has been employed in order to improve the extractability of hemicelluloses [4], cellulose [5], lignin [6,7] or to get clean cellulosic fiber from used paper [8,9] but only few attempts to improve the susceptibility of lignocellulosic materials to biodegradation by using ultrasound power have been described. It was find out that ultrasound have a beneficial effect on saccharification processes [10]. Sonication have been reported to decrease cellulase requirements by 1/3 to 1/2 [11] and to increase ethanol production from mixed waste office paper by approximately 20% [12]. It was notice that the effect of ultrasound fragmentation of Avicel microcrystalline cellulose is similar to that of the enzymes for short incubation intervals [13].The time needed for ultrasonic treatment could be reduced when increasing the irradiation power [14].

In this work, the availability of the two approaches to enhance the cellulose hydrolysis, i.e. the pretreatment by ultrasonic irradiation of the substrate and ultrasonically assisted hydrolysis of the substrate are discussed.

**Results and Discussions**

Enzymatic bioconversion of cellulose from biomass involves the transfer of enzyme molecules from the processing liquid across the surface of the substrate and the mass transfer of the products to the bulk solution. When cavitation bubbles collapse near the surface of the solid substrate, they generate powerful shock waves. Two specific features of cavitation phenomena are especially important for bioconversion of lignocellulosic biomass, *i.e.* mass transfer intensification and an effective fine grinding together of deaglomeration. This is of great advantage since it has been reported that particle size distribution has significant implications for the rate of hydrolysis [15]

**Optimization of lignocellulosic substrate sonication.** It is well known that the sonication processes are influenced by exposure parameters such as ultrasonic frequency and intensity, exposure time and temperature. In the case of vegetal materials this process is tremendously affected also by the shape of vessel and the ultrasonic device.

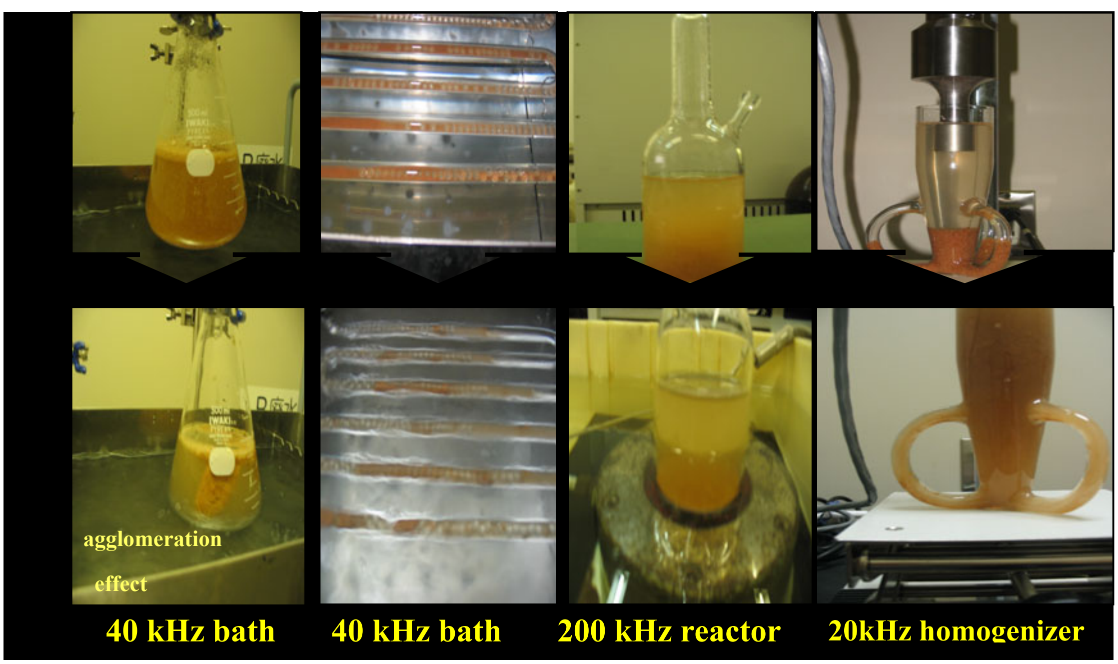


**Figure 1.** Optimization of lignocellulosic substrate sonication

Sonication can be performed as indirect sonication in a cleaning type devices or direct sonication by horn type apparatus, as can be seen in fig.1. When the aqueous suspension of vegetal material is exposed to ultrasound, it is important to achieve a uniform exposure to the sonication. But under the ultrasound, the vegetal material shows a particular behavior, such as agglomeration in cluster-like formation in positions with lower ultrasonic pressure. The air trapped into the vegetal cell play also an important role. After employing a series of different devices and vessel shapes we fund out that the 20 kHz horn type Homogenator (Bransonic Model 250/450 Sonifer) with a special design developed from the commercially available rosette type vessel is offering the optimum condition for sonication of vegetal materials. Our design (fig. 2) facilitates an ultrasonically promoted propelling of the biomass together and a better exposure of the vegetal material to the ultrasound. Four external loops continuously deliver the vegetal material under the vibrating tip of the horn. In this way we manage to avoid the agglomeration effect without employing an external propelling device.

Silent Under ultrasound

Figure 2. Our design solution for best sonication of vegetal materials

**The effect on the pretreatment of enzymatic substrate**. The ultrasonically assisted pretreatment do not seek to actually hydrolyze the biomass to soluble sugars, but rather to generate a pretreated substrate that is more easily hydrolyzed via increasing enzyme accessible surface area. Our investigations performed with a JEOL Scanning Electron Microscopes revealed that the particle size reduction of the microcrystalline cellulose MCC has been significantly reduced, especially when subjected to direct sonication (fig. 3) and wood surface has been erosion do to ultrasound exposure (fig.4)

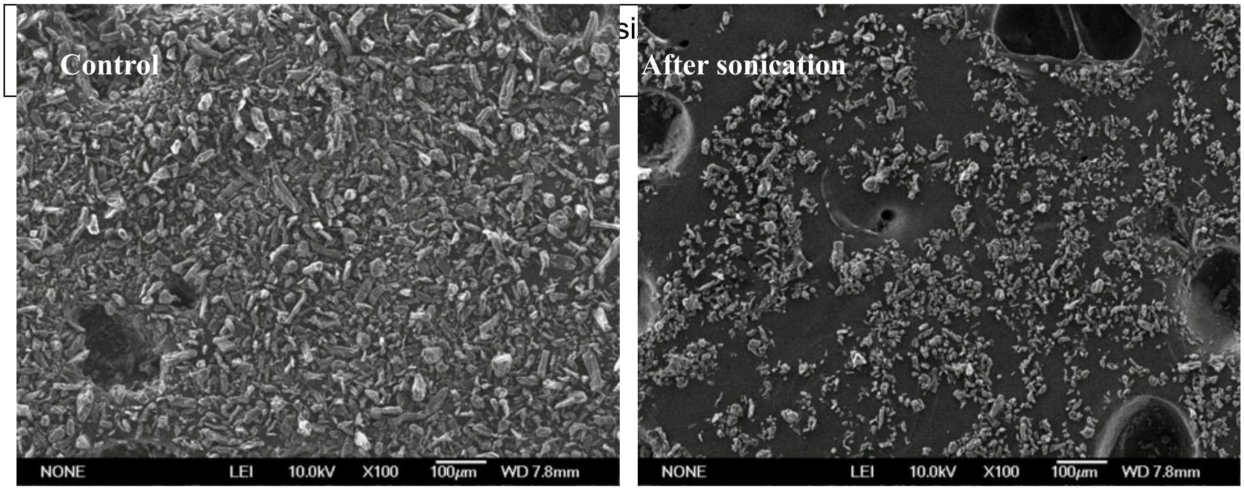


**Figure 3.** The MCC microstructure before and after sonication

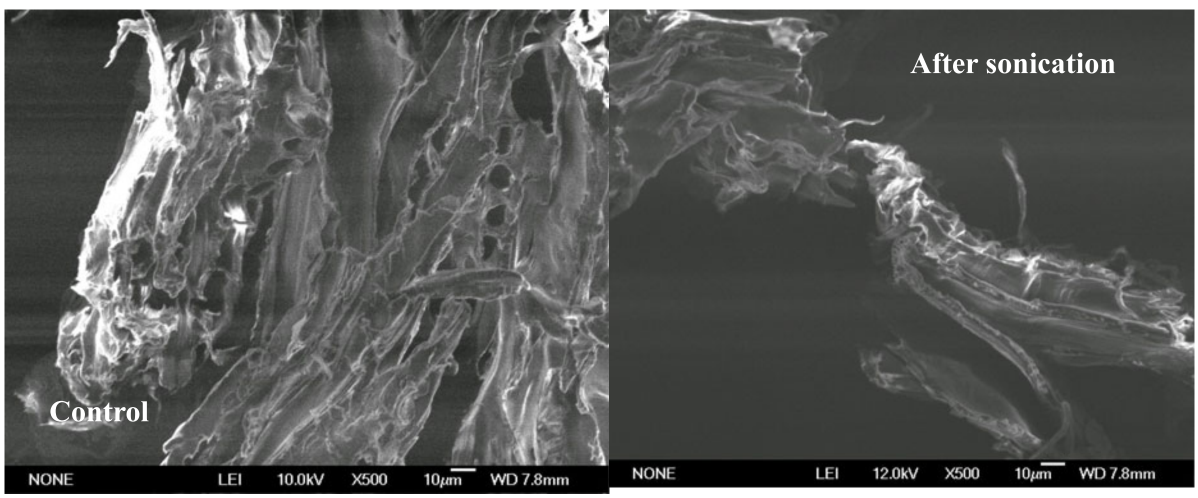


**Figure 4.** The wood microstructure before and after sonication

When exposed to ultrasonic pretreatment, the lignocellulosic substrate is subjected to an advance milling process due to the shock waves generated by the cavitational collapse. The measurements are reported in table 1. This is accompanying the increasing of total surface area of the wood sawdust to be exposed to enzymatic attack.

| | 20 kHz<br>60 min | 20h stirr.+ 20 kHz<br>60min | 20 kHz<br>30 min | 40 kHz<br>180 min |
|---|---|---|---|---|
| up 60 mesh | 64.408 | 64.758 | 66.893 | 86.542 |
| up to 80 mesh | 22.167 | 21.9 | 23.001 | 11.5654 |
| up to 100 mesh | 3.425 | 3.09 | 3.744 | 0.821 |
| under 100 mesh | 9.999 | 10.251 | 6.36 | 1.071 |

**Table 1.** The effect of ultrasounds on particle size reduction of wood sawdust

**The effect of ultrasound on the ongoing hydrolysis**. We also demonstrated that a gain in efficiency of cellulose hydrolysis due to the ultrasound exposure is possible by employing ultrasound for the promotion of the ongoing hydrolysis. Fig.5 shows a comparison of low (20 kHz horn type sonicator) *vs*. high frequency ultrasound effect on the hydrolysis of a standard substrate ($\alpha$- cellulose).

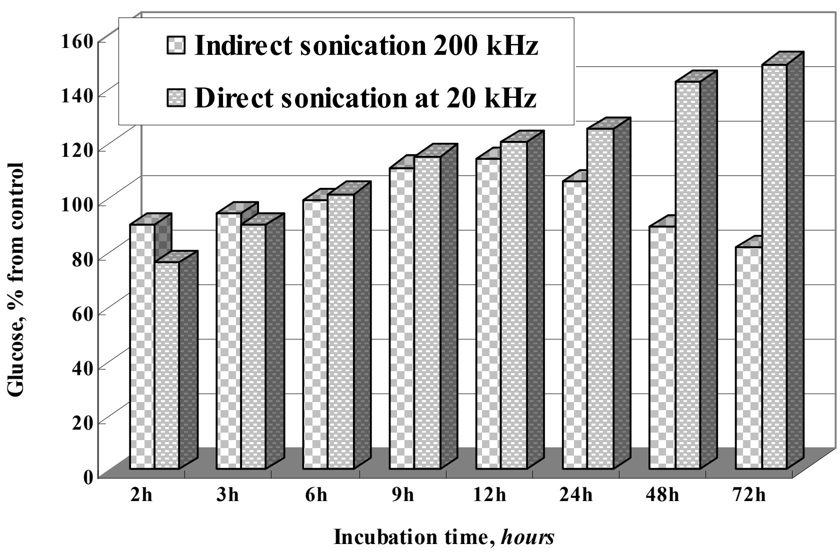


**Figure 5.** The Effect of Ultrasounds on the Ongoing Hydrolysis

## Conclusions

This presentation summarizes the performance characteristics of ultrasonically assisted processing of lignocellulosic biomass when the sonication was performed by using a special designed glass vessel that facilitate an ultrasonically promoted propelling of the biomass with an horn type sonicator. Our experimental results shows that:

-Ultrasounds energy is thought to affect both the chemical and physical characteristics of the lignocellulosic biomass.

-The saccharification of cellulose was enhanced efficiently by ultrasonic pretreatment.

## References

1. Kim K.K., K.K. Jun-Suk, K.K. Oh, S.W. Kim, Y.S. Jeong, S.I. Hong. Ethanol production

from lignocellulosic biomass by simultaneous saccharification and fermentation employing the reuse of yeast and enzyme. J. Microb. Biotechnol. 1999;9 (3):297–302.

2. Nazhad, M.M., L.P. Ramos, L. Paszner, J.N. Sadler. Structural constraints affecting the initial enzymatic hydrolysis of recycled paper. Enz. Microb. Tech. 1995;17: 68-74.
3. Vinatoru M, M. Toma, T.J. Mason. Ultrasonically assisted extraction of bioactive principles from plants and their constituents, in: T.J. Mason (Ed.), Advances in Sonochemistry, vol. 5, JAI Press. 1999:209–248.
4. Ebringerová A , Z. Hromádková. Effect of ultrasound on the extractibility of corn bran hemicelluloses. Ultrason. Sonochem. 2002;9(4): 225-229.
5. Pappas C, P. A. Tarantilis, I. Daliani, T. Mavromoustakos, M. Polissiou. Comparison of classical and ultrasound-assisted isolation procedures of cellulose from kenaf (Hibiscus cannabinus L.) and eucalyptus (Eucalyptus rodustrus Sm.). Ultrason. Sonochem. 2002;9(1):19-23.
6. Sun R.C., J. Tomkinson. Comparative study of lignins isolated by alkali and ultrasound-assisted alkali extractions from wheat straw. Ultrason. Sonochem. 2002;9(2): 85-93.
7. Fengel D., G. Wegener. Wood: chemistry, ultrastructure, reactions. Walter de Gruyter, Berlin. New York. 1984:52.
8. Scott W.E., P. Gerber. Using ultrasound to deink xerographic waste. TAPPI J. 1995;78:125-130.
9. Sell, N.J., J.C. Norman, D. Jayaprakash. Deinking secondary fiber using ultrasound. Progress in Paper Recycling. 1995; 28-34.
10. Rolz, C. Ultrasound Effect of Enzymatic Saccharification. Biotech. Letters 1986;8(2):131-136.
11. Ingram; L.O., B.E. Wood. Ethanol production from lignocellulose United States Patent 6333181.
12. Wood B.E, H.C. Aldrich, L.O. Ingram. Ultrasound stimulates ethanol production during the simultaneous saccharification and fermentation of mixed waste office paper. Biotechnol Prog. 1997;13 (3): 232-237.
13. Gama, F.M., M.G. Carvalho, M.M. Figueiredo, M. Mota. Comparative study of cellulose fragmentation by enzymes and ultrasound. Enzyme and Microb. Technol. 1997;20:12-17.
14. Imai, M., K. Ikari, I. Suzuki, High-Performance Hydrolysis of Cellulose Using Mixed Cellulase Species and Ultrasonication Pretreatment, Biochem. Eng. J., 2004;17, 79-83.
15. Lynd L. R, P. J. Weimer, W. H. van Zyl, I. S. Pretorius. Microbial Cellulose Utilization: Fundamentals and Biotechnology. Microb.and Molec. Biology Rev. 2002;66:506–577.
16. J. Mason. Sonochemistry: (Oxford University Press, Inc., New York ).1999:65.