

Catalytic and Enzymatic Conversion of Lignocellulosic Sunn hemp fibres to Biofuel

Abstract

Identifying non-food energy crops that can produce lignocellulosic fuels is an undeniable tool for fighting against climate change in our planet today. In our work, we have identified a new-non-edible bioenergy crop, namely Sunn hemp fibre (*Crotalaria juncea*), as the potential substrate for lignocellulosic biofuel production. It is widely grown in the subtropics of Brazil, India, Bangladesh, Pakistan, Russia, Sri Lanka, Uganda, USA, etc. Sunn hemp fibre produces 19 MJ of energy/kg dry biomass with 2268 kg dry biomass/acre in only 9-12 weeks. The global production of Sunn hemp fibre is 130,000 MT/year. The energy content of this fibre is 2.5×10^9 MJ/year. The annual production of Sunn hemp fibre in India was around 70,000-75,000 tons from almost 2 lakh hectares of land during mid-sixties. Presently, 156,463,000 hectares of arable land are present in India, and 25-27 tons of Sunn hemp fibre can be generated per hectare of land. However, physiochemical analysis shows that it has high crystallinity of 80.17%, high degree of polymerization of 650, and low porosity of 1.58%. These characteristics make Sunn hemp fibres recalcitrant and resistant to the cleaving of the β -(1-4)-glycosidic bonds in their cellulose polymers. The Composition analysis shows that it contains 75.6% cellulose, 10.05% hemicelluloses, 10.2% lignin, 3.6% moisture, and 0.43% ash. We aim to find ways of overcoming these challenges by exploring various catalytic and enzymatic hydrolysis processes with disparate timescales (ranging from minutes to hours to days) for converting the long-chain cellulose molecules in Sunn hemp fibres to monomeric sugar.

We perform a microwave-assisted ionic liquid ([Bmim]Cl) based copper chloride catalyzed conversion of Sunn hemp fibre to biofuel precursors such as glucose, 5-hydroxymethylfurfural (HMF), levulinic acid (LA), and formic acid (FA). The ionic liquid quickly ionises to form a Lewis acid catalyst with copper chloride and water to form a supramolecular complex with cellulose. The polar supramolecular complex aligns in the microwave's electromagnetic field. Polar molecules are efficient absorbers of microwave energy and undergo molecular rotation known as dipole rotation, leading to collisions of the supramolecule with its adjacent molecules. As the electromagnetic field changes its polarity by millions time per second, the supramolecule also rotates millions time per second, which causes frequent dipole rotation and intermolecular collision, leading to dissipation of the electromagnetic energy as friction and heat in the reaction mixture. Microwave irradiation raises temperatures uniformly across the reactor, enhances reaction rates, and rapidly breaks the glycosidic bonds of the cellulose polymer in 36 to 51 minutes. Only 46 minutes of optimized reactions at 160-200°C and 5 bar pressure with water addition of 5% to 90% (wt % of ionic liquid) result in up to 78.7%, 26.8%, 44.9%, and 10.8% yields of glucose, HMF, LA, and FA, respectively. This process also provides us with the optimum reaction

temperature for each maximizing the yield of each product – 160°C for glucose, 180°C for HMF and 200°C for LA and FA. The yields of glucose, LA and FA can be increased by optimizing the water addition to 40%, while that of HMF is maximized at 25% water addition at their respective optimal temperatures. This method allows us to control the product distribution and obtain a certain desired product by manipulating water concentration and temperature during hydrolysis process.

We experimentally demonstrate how to employ non-equilibrium dissipative chemical structures formed via autocatalytic pathways and significantly enhance lignocellulosic biofuel production. The cross-catalyst water is added at a rate of 25-75 $\mu\text{l/gm}$ of ionic liquid/h and chaotic temporal oscillations with fractal dimensions and positive Lyapunov exponents appear at water addition rates ranging from 25 to 42.8 $\mu\text{l/gm}$ of ionic liquid/h for all the product concentrations. We observe that the highest yields of biofuel precursors such as glucose, fructose, HMF, LA and FA are maximum for the case where fractal dimensions and Lyapunov exponent are highest. High fractal dimension and Lyapunov exponent suggest that higher the system's capacity of chaos and more is its sensitivity to initial condition, higher is the product yields. The addition of 37.5 $\mu\text{l/gm}$ of ionic liquid/h of water addition maximizes product concentrations at 5 hours with the peak non-equilibrium yields being 69%, 12.6% and 5% for glucose, LA and FA, respectively. Maximum HMF yield of 4.7% is observed at 5 hours for 28.33 $\mu\text{l/gm/h}$ water addition. We show that any further increase in water (cross catalyst) concentration leads to the disappearance of the temporal oscillations and the onset of equilibrium. The equilibrium yields of glucose, HMF, LA and FA are decreased by 9%, 0.5%, 9%, 3.6%, respectively, at water addition rate of 45 $\mu\text{l/gm/h}$ compared to non-equilibrium yields of glucose, HMF, LA and FA at water addition rate of 37.5 $\mu\text{l/gm/h}$.

In enzymatic hydrolysis of cellulose extracted from Sunn hemp fibre, we show that reactor mixing does not alter the non-competitive nature of product inhibition. We observe the effects of mixing and mass transfer on the kinetics of enzymatic hydrolysis for cellulose loading of 10-50 mg/ml and mixing speed of 0-150 rpm. The kinetic parameters (K_m , V_{max}), and glucose inhibition constant (K_x) are experimentally determined at a function of mixing speeds (0-150 rpm). The experimental values of K_m , V_{max} , and K_x are fitted to algebraic expressions that quantify their exponential decrease with increasing mixing speed. Reactor mixing at 150 rpm reduces the glucose yield by 5.6-6.4% and the reducing sugar yield by 6-7% for cellulose loading of 10-50 mg/ml, respectively, when compared to no mixing after 3 days of hydrolysis. Faster mixing facilitates convective transport of enzymes to solid celluloses while enhancing non-competitive product inhibition by rapidly homogenizing the inhibitors (glucose, cellobiose) in the reactor. Faster mixing transforms the hydrolysis system from being mass-transfer limited to being kinetic limited, the soluble sugar yields decrease as stronger product inhibition overpowers the rate-enhancing effects of mass transfer in well-mixed reactors. We show that batch hydrolysis performed under mass-transfer limitations maximizes sugar yields by reducing product inhibition.

We also quantify the effect of mixing, temperature, initial sugar concentration, cell concentration on the yield of bioethanol by performing *Saccharomyces cerevisiae* mediated batch fermentation of experimentally produced glucose. We find that cell concentration increases and glucose concentration decreases, both exponentially, with fermentation time. Optimization of the fermentation process shows the optimal temperature, mixing speed, initial cell concentration, initial sugar concentration and fermentation time for maximum bioethanol production to be 30°C, 40 rpm, 0.57 mg/ml, 12 mg/ml and 15h, respectively. The maximum bioethanol yields (in gm of bioethanol produced/100 gm cellulose) are obtained as 30.4%, 25.5%, and 21.8%, respectively, for the above three hydrolysis processes, suggesting microwave-assisted hydrolysis for 46 minutes to be the best process.

Keywords: Lignocellulosic Biofuel; Sunn hemp fibre; Ionic Liquid; Microwave-assisted Catalytic Hydrolysis; Temporal Oscillations; Chemical Chaos; Fractal Dimension; Lyapunov Exponent; Enzymatic Hydrolysis; Reactor Mixing; Non-competitive Inhibition; Microbial Fermentation