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Recent Research Efforts in the Area of Biotechnology for Fuels and Chemicals

Poster Session Papers

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There were 57 papers presented at the poster session of the Symposium covering a wide spectrum of current biotechnological research activities (*1*). They focused mostly on ethanol production and methane generation from biomass material via microbial processing, as well as on enhanced hydrogen yield from algae. Several of the posters dealt with the pretreatment of cellulosic materials, and enzyme production/characterization, whereas a good number of papers displayed research efforts on bioremediation, photosynthesis, production of various useful chemicals from biomass by bioprocessing, and on other miscellaneous subjects. One of the papers treated a very interesting topic of cellulose–cellulase complexes. Many of the poster papers are included in this volume, and a synopsis of all the poster papers presented is the subject of this article.

Studies were conducted on xylose and arabinose fermentation to ethanol by *Pichia stipitis* with regard to initial ethanol concentration and temperature effects on yeast growth and ethanol production (*2*). Newsprint and paper wastes were hydrolyzed and fermented to ethanol by cocultures of thermophilic celluloytic bacteria and pentose fermenting bacteria (*3*). Distillers dried grains solubles from corn dry-milling (to produce ethanol) were hydrolyzed by fungal cellulases in a simultaneous saccharification and fermentation (SSF) process that fermented both the hexose- and pentose-based fractions (*4*). Attempts were made to remove extraneous chemicals from sugarcane bagasse hydrolyzate that have been found

to inhibit yeast growth and fermentation of the feedstock to ethanol (*5*). A commercial plant for ethanol production from municipal solid waste was designed and evaluated (*6*).

Genetically improved thermotolerant strains of *Saccharomyces, Candida,* and *Kluyveromyces* genera were tested to optimize the SSF of biomass to ethanol (*7*). Characterization of β-D-glucosidase (an essential enzyme for the cellobiose hydrolysis to glucose) from *Aspergillus niger* separated the enzyme to two distinct species (*8*). Cultures of a self-aggregating strain of *Saccharomyces uvarum* were used in a tower fermenter for continuous ethanol production, and based on this experimentation, a mathematical model of a plug flow and a continuous stirred-tank reactor (CSTR) in series was proposed to describe the tower fermenter and predict final ethanol concentration and productivity (*9*). The isolation of the anaerobic bacterium *Clostridium ljungdahlii* that produces ethanol from CO and H_2O or CO_2 and H_2 was reported, and results on improving reaction kinetics and ethanol yields were shown (*10*).

A design involving continuous fermentation of cellulosic substrates in a partially mixed mode and two biosystems: SSF using yeast and fungal cellulase and direct microbial conversion (DMC) using *Clostridium thermocellum,* was presented and evaluated (*11*). pH-Controlled batch fermentations were conducted with both softwood and hardwood "spent sulfite liquor" (SSL; sulfite untreated pulp mill effluent) by *Escerichia coli* B (a genetically engineered strain from ATCC) for ethanol production, and sugar-to-ethanol efficiencies of 84% for hardwood SSL and 77% for softwood SSL (of theoretical maximum) were reported (*12*). The effect of acetic acid on ethanol production by a *Zymomonas mobilis* strain (ATCC 29191) fermenting glucose in a batch-fed reactor was examined and preliminary results were presented (*13*). An analysis of the interactive double-substrate limited growth and inhibition of microbes along with the associated dynamic behavior of multiplicity, stability, and bifurcation was presented (*14*).

A computerized simulation to obtain an optimization of a percolation reactor performance using acid pretreated hardwood (hybrid poplar) feedstock to hydrolyze and recover hemicellulose sugars for ethanol production, helped to make direct comparisons for the key parameters and the sugar yield between this reactor and those of other conventional reactors (*15*). Comparative studies were made between four anaerobic fungi for their release of cellulolytic and xylanolytic enzymes during their growth on a range of carbohydrates, and it was pointed out that the *Piromyces* strains excreted the highest amounts of extracellular enzymes used (*16*).

Experimental work indicated that a gel-form cellulose–zinc complex subjected to cellulose (from *Aspergillus niger* and *Trichoderma viride*) activity was more quickly hydrolyzed than the amorphous-form of cellulose (*17*). Attaching polyethylene glycol or dinitrophenol groups to certain reducing enzymes, including alcohol dehydrogenase, modified these enzymes and

enhanced their solubilization and activity in organic solvents (*18*). Genetically improved strains of *Aspergillus niger* increased glucose oxidase production when their conidia were exposed to UV irradiation (*19*).

A current understanding of the structure of native cellulose and cellulase enzyme components, as well as the possible mechanisms of complete hydrolysis of cellulose to glucose, were presented and discussed (*20*). A cellobiohydrolase II core protein from *Trichoderma reesei* cellulase was shown to increase the rate of cellulose hydrolysis and dispersion of cellulose fibers (*21*). A β-mannanase free of cellulase or xylanase activity was isolated from culture filtrates of *Trichoderma harzianum*, and its activity at certain pH, temperature, and time ranges was investigated (*22*). This valuable enzyme could be used in the selective processing of β-mannans in wood pulps and other cellulosic wastes.

Work focused on the enzymatic (cellulase, hemicellulase, and pectinolytic enzymes) depolymerization of polysaccharides (pectins, arabinogalacturans, xylans, and cellulose) in citrus processing residues (*23*). An investigation on the effect of bubbling CO_2 in liquid containing cellulase indicated that the enzyme is concentrated on the bubble-foam surfaces and this was enhanced with pH levels less that 5 (*24*). The adsorption and recovery of *Trichoderma* cellulases from lignaceous residues after complete enzymatic hydrolysis of dilute-acid-pretreated hardwood were studied and some results were presented (*25*). A study of the role of organic acid chelators in manganese regulations of the lignin-degrading system of *Phanerochaete chrysosporium* indicated that regulation occurred only when Mn was in solution, lignin peroxidase was repressed as long as the Mn—organic acid complex was stable, and mineralization and depolymerization of synthetic lignins were followed in the presence and absense of chelators and low and high Mn levels (*26*).

To thoroughly understand the efficiency and benefits of high-solids MSW anaerobic digestion, comparative studies were conducted between high-solids and low-solids bioconversions to methane (*27*). Studies with a novel high-solids MSW reactor under mesophilic conditions indicated a linear relationship of increasing total biogas production with increasing organic loading rate to the process, and this loading rate was close to 4 times greater than that which may be obtained with comparable low-solids artaerobic reactor technology (*28*).

Experimentation with a fluidized-bed reactor converting cassava starch (liquefied with α-amylase) into glucose with amyloglucosidase immobilized in controlled pore silica, determined the flow axial dispersion, and indicated that the fluidized-bed reactor displayed a behavior similar to that of a plug-flow reactor (*29*). Immobilized cells of an *Acetobacter* strain on particles made of α-alumina added to the aqueous solution of sodium alginate, were used in a circulating three-phase fluidized-bed bioreactor to oxidize ethanol to acetic acid. The use of this biocatalyst for cell immo-

bilization enhanced the oxygen tranfer and acid production. Experiments focused also on the effect of certain key parameters on acetate yields (*30*). The adsorption isotherms of bovine serum albumin on ferromagnetic material (Ni) for the potential bioseparation within a magnetic field as well as the influence of pH, temperature, ionic strength, and particle size were investigated (*31*). Work also focused on microbial immobilization on fibers in a fluidized-bed reactor (*32*). Models to predict the coal particle segregation and/or mixing effects on the performance of an advanced fluidized-bed bioreactor for coal liquefaction were presented (*33*).

The successful removal of the inhibitory product, acetic acid, by adsorption in a biparticle fluidized-bed bioreactor employing immobilized cells of the *Acetobacter pasteurianus* or the anaerobe *Clostridium termoaceticum* was presented (*34*). Acid-hydrolyzed MSW digested by *Clostridium thermoaceticum* yielded low concentrations of acetic acid, whereas yields from glucose and D-xylose were 94 and 84%, respectively (*35*).

Strains of methanol-utilizing bacteria were tested for their growth and production of polyhydroxybutyrate (a biodegradable plastic) from methanol (*36*). Incorporation of wood, wood pulp, or wood fibers into graft copolymers made thermoplastic composites (*37*). Cultures of *Bacillus licheniformis* were studied for the production of an insoluble polysaccharide soil plugging agent with encouraging results (*38*). A novel continuous production and in high yields of catechol from phenol by using a strain of *Bacillus stearothermophilus* was presented (*39*). Safflower seed oil was used as a 10%-to-90% additive to diesel oil, and engine tests were performed with promising results (*40*).

Research presented the purification, characterization, and properties of acylated aminopolyols that were produced by enzymatic coupling of pelargonic acid (fatty acid) and the aminated polyols: 1-amino-1-deoxysorbitol and *N*-methyl-1-amino-1-deoxysorbitol (*41*). The production and characterization of proteinases and dipeptidases from *Streptococcus lactis* spp. *cremoris* strains (used as starters in the manufacture of ripened cheeses) were monitored during the cell growth and lytic phases, as well as the effect of temperature on enzyme activity was investigated (*42*). Studies with β-galactosidase immobilized by covalent bonding to sepharose spheres indicated that the galactose of fresh skimmed milk and fresh whey was hydrolyzed close to 100%, thus preparing lactose-free milk and whey (*43*).

An investigation of the effects of CO_2 concentration and light intensity on the rates of photosynthetic hydrogen and oxygen production from algae was conducted with the purpose to optimize the rates of light-driven hydrogen production via the photosynthetic water-splitting reaction (*44*). Researchers developed a computer code simulating the synthesis of a given product in a photobiological reactor subject to typical insolation over a 12-h period. The code results can be regarded as ideal in the sense that only light intensity appears as a limiting factor for the photosynthetic

reactors (*45*). Transmission electron microscopy was used to study chloroplast-prepared platinum and osmium catalysts, which impacts new photobiocatalytic properties to photosynthetic membranes (*46*). A new method for the determination of time-dependent photosynthetic quotients (O_2 evolved/CO_2 assimilated) in spinach leaves and green alga *Scenedesmus* under anaerobic conditions was reported (*47*). Results of a study focusing on the mass transfer and kinetic parameters in a CSTR in which the photosynthetic bacterium *Rhodospirillum rubrum* was evaluated for its ability to carry out the water gas shift reaction to produce hydrogen from carbon monoxide and water, were presented (*48*).

The cyanobacterial alga *Scytonema* sp. was reported to possess antibiotic (toward *Bacillus subtilis*) and mitogenic activities. Results of investigating the optimization of antibiotic production were also presented (*49*). The coccolithophoric alga *Pleurochrysis carterae* was used to produce ultrafine coccolith particles of calcite ($CaCO_3$) and organic matter (sugars, proteins, and lipids). Enzymes and antibodies were successfully immobilized on the coccoliths (*50*).

Studies demonstrated that the heterotrophic bacterium *Paracoccus denitrificans* may utilize nitric oxide (NO) as a terminal electron acceptor when grown anaerobically on succinate or heat and alkali pretreated sewage sludge as carbon and energy sources, and, therefore, this denitrifying bacterium can be used to convert NOx to elemental nitrogen for disposal (*51*). In a heat and alkali pretreated sewage sludge *Desulfotomaculum orientis* was grown. It was reported that this sulfate-reducing bacterium can be grown on H_2 as an energy source, CO_2 as a carbon source, and SO_2 as a terminal electron acceptor, which conducted a complete reduction of SO_2 to H_2S (*52*).

Studies with a single-pass packed-bed bioreactor (packed with sand containing microbes from a contaminated site) focused on the biodegradation of chlorinated aliphatic hydrocarbons (trichloroethylene, tetrachloroethylene, and other chlorinated organics). Greater than 99% degradation of trichloroethylene was observed in a residence time from 1.9 to 3.2 days (*53*). Studies with *Phanerochaete chrysosporium* indicated that the fungus degraded extensively the organophosphorous insecticides terbufos, chlorpyrifos, and fonofos tested (*54*). It was reported that live or heat-killed cells of *Micrococcus luteus* instantaneously adsorbed strontium. This strontium binding is perhaps mediated by an ion-exchange phenomenon with some specificity for divalent cations (*55*). Four fungal (*Saccharomyces cerevisiae, Rhizopus arrhizus, R. oligosporus,* and *Aspergillus niger*) and three bacterial (*Streptomyces longwoodensis, Pseudomonas aeruginosa,* and *Citrobacter freundii*) strains were tested for uranium biosorbent ability in comparison with two commercial biosorbents. It was concluded that the microorganisms tested are superior to generic metal biosorbents (*56*). Predictive simulations were presented that were used to develop effective *in situ*

remediation strategies to clean groundwater contaminated with carbon tetrachloride, nitrate, and other organic and inorganic contaminants (*57*).

It should be pointed out that an outline of the goals, technical directions, and strategies of the DOE's Biological and Chemical Technologies Research Program was presented within the context of the National Energy Strategy (*58*).

In closing, the poster paper presentation emphasized the current trends of biotechnological activities focusing on:

1. Ethanol, methane, and hydrogen production from biomass;
2. Enhancement of bioprocesses by optimization of key parameters, development of novel bioreactors, and feedstock pretreatments;
3. Elucidation of feedstock composition, and characterization of involved enzymes and enzymatic mechanisms;
4. Clarification of photosynthetic pathways and factors implicated;
5. Development and characterization of novel bioremediation processes for elimination/mitigation of hazardous waste impacts; and
6. Bioproduction of new high-value chemicals.

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