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APPENDIX

Biotechnological Progress in Producing Fuels and Valuable Chemicals and in Bioremediating Environmental Impacts

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ABSTRACT

The 71 poster papers presented at the 16th Symposium on Biotechnology for Fuels and Chemicals provided new information on ethanol production, feedstock pretreatment, enzymatic activities, bioreactor characterization, enzyme and cell immobilization, valuable chemicals production, coal bioconversion, hazardous waste bioremediation, and on miscellaneous subjects. Brief summaries of all presented posters are described here. Further details of many of these are found as complete articles elsewhere in this volume.

INTRODUCTION

The 71 poster papers presented at the 16th Symposium on Biotechnology for Fuels and Chemicals focused on ethanol production, feedstock pretreatment, enzymatic studies, bioreactors and enzyme immobilization, production of chemicals, coal bioconversion, bioremediation of hazardous waste, and on miscellaneous studies (*1*). Presented information is summarized below.

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ETHANOL PRODUCTION

Eight papers showed new data on ethanol production using selected fungal and bacterial strains. Previous work showed that *Saccharomyces cerevisiae* and similar yeasts can successfully ferment six-carbon sugars of filtered polysaccharide hydrolyzates from orange peel to ethanol, but cannot ferment the five-carbon sugars and the galacturonic acid of the hydrolyzates. To overcome this difficulty, ethanol-producing selected bacterial strains are used to produce ethanol from both six- and five-carbon sugars with significant results (*2*). In two other studies, sorghum carbohydrates (cellulose and hemicellulose) fermented to ethanol by a mixed culture of *Fusarium oxysporum* and *S. cerevisiae* selected strains yielded as high as 10.3 and 11.5 g ethanol/100 g of fresh sorghum in batch and fed-batch fermentations, respectively (*3*). Furthermore, separate fermentation of sorghum juice and bagasse indicated that the juice (20.5% sucrose and 2% glucose [w/w]) fermented by a selected strain of *S. cerevisiae* yielded a broth containing 9.2% (w/v) ethanol (5.5 g of ethanol/100 g fresh sorghum), whereas the bagasse (8.7% sucrose, 1% glucose, and 12.3% polysaccharides [w/v]) fermented by a mixed culture of *F. oxysporum* and *S. cerevisiae* yielded a broth containing 4.1% (w/v) ethanol (5.1 g of ethanol/100 g of fresh sorghum) (*4*). Another yeast, *S. uvarum* U4 strain, used in a tower fermenter gave an ethanol production rate of 50 g/L/h. To optimize the process further, cell growth kinetics and the effectiveness factor of the yeast under 10–30% sugar concentrations and 20–45°C temperatures were studied (*5*). Studies on sugar fermentation to ethanol by the LORRE's (Purdue University) yeast 1400, a genetically fused product of *S. diastaticus* and *S. uvarum,* indicated that this yeast is more osmo- and ethanol-tolerant than its parents, although higher glucose concentrations inhibited ethanol production. In order to maintain cell viability and increase ethanol production, fed-batch fermentation was found to be beneficial (*6*).

In a survey of microbes for pentose fermentation, *Zymomonas* and *Lactobacillus* strains were identified as the most promising microorganisms for ethanol production from poplar wood hydrolysate (*7*). Efforts were also made to formulate a minimal medium for *Clostridium thermosaccharolyticum* and *C. thermocellum* to document the effect of various types of nutrient limitation on production of ethanol, acetic acid, lactic acid, and exopolysaccharide in continuous culture, as well as of growth inhibition owing to ethanol and pretreatment byproducts (*8*). It has been suggested that the amount of microbial growth (cell mass) is proportional to the amount of energy (ATP) derived from the metabolism of microbes involved, with a generally accepted value of 10.5 g dry wt/mol ATP (Y_{ATP}). Accordingly, if the molar yields of ATP from glucose and xylose fermentation to ethanol (by a patented *Escherichia coli* strain) are 2.0 and 1.67, the molar growth yield would be 21 and 17.5, respectively, and, consequently, the mass growth yield ($Y_{x/y}$) with respect to glucose and xylose would have the same value, namely 0.117 g dry wt cells/g sugar. In this work, the $Y_{x/y}$

value for glucose and xylose was observed to be 0.116 and 0.71 g/g, respectively; therefore, when xylose is the energy source, either the Y_{ATP} is not a valid biological constant, or the molar yield of ATP is less than the generally assumed value of 1.67 (*9*).

FEEDSTOCK PRETREATMENT

Feedstock pretreatment for ethanol and other chemicals bioproduction plays a significant role in enhancement of yield and production rates. At the symposium, seven poster papers dealt directly with feedstock pretreatment. In one study, an electroexplosion reactor was developed to pretreat lignocellulosic matter for better bioconversion. By generating high voltage (15–25 kV) between two electrodes on wet (65–75% humidity) matter in a closed reactor, steam and explosions within the pores of the lignocellulosic matter loosened its structure to a fermentable feedstock (*10*). Xylan was found to be hydrolyzed during the pretreatment of cellulosics with zinc chloride solution, and the xylose yield was >90% (w/w) at 70°C. It was noted that the yield and hydrolysis rate were correlated to temperature and zinc chloride concentration (*11*). Pretreatment of switchgrass by dilute sulfuric acid (0.46–2.42 wt%) at 120–140°C was found to be highly effective in selectively recovering the hemicellulosic sugars from the biomass. In addition, pretreated samples fermented with *Lactobacillus delbrüeckii* to produce lactic acid were evaluated for their enzymatic digestibility (conversion efficiency) and simultaneous saccharification and fermentation (SSF) (*12*).

Preliminary investigations on continuous SSF of milled hybrid popular substrates subjected to various dilute-acid pretreatment conditions focused on the sterility of feedstock, ethanol yield, and glucose and cellobiose levels, as well as on operating conditions and design of the single-stage continuous system used. The levels of glucose and cellobiose in the medium were particularly studied, since the cellulase enzyme complex is extremely sensitive to feedback inhibition by these sugars and, consequently, to SSF ethanol production (*13*). Another research effort indicated that the ruminant digestibility of sugarcane lignocellulosic content was significantly improved after pretreatment with aqueous orthophosphoric acid, which completely depolymerized hemicellulose and enhanced cellulolylsis (*14*).

To evaluate the process economics of producing ethanol from switchgrass, two base-case pretreatment methods were investigated: SO_2-steam explosion, and dilute sulfuric acid pretreatment. Under inherent uncertainties, ethanol production cost was estimated to range between $1.17 and $1.31/gal for the SO_2-steam explosion pretreatment, whereas the cost of a gallon of ethanol using the dilute sulfuric acid pretreatment was projected to be $1.28 (*15*). The technical and economic feasibility and resulting benefits of a biomass chemical pretreatment process that can

concurrently solubilize the hemicellulosic and lignin fractions of biomass and leave a solid residual composed primarily of cellulose were investigated. A technoeconomic assessment of this process alternative on the overall biomass fermentation to ethanol was presented (*16*).

ENZYMATIC STUDIES

Four of the poster papers presented results from studies mainly on *Trichoderma reesei* cellulolytic enzyme activities. A study utilized the strain *T. reesei* RUT-C30 to hydrolyze the cellulosic fraction of municipal solid waste (MSW) to yield glucose units for ethanol production (*17*). Another work found that a selected *T. reesei* mutant was able to produce cellulolytic enzymes on highly viscous liquid media containing up to 12% of lignocellulosic substrate. The substrate obtained from oak bark lignocellulose spent solids (~45–50% lignin and 35–40% cellulose content) and treated by the culture filtrate of the mutant produced 15–20% sugar syrup. The activity of the mutant on this carbon source reached 5–5.5 FPIU/mL (FPIU = filter paper international unit) (*18*). The effects of pentamine ruthenium groups covalently attached to xylanase 2, and cellobiohydrolases I and II produced by *T. reesei*, as well as to a bacterial xylanase were examined on the hydrolysis of various cellulosic and lignocellulosic substrates (*19*). Observations showed that sodium hexachloropalladate is a strong inhibitor of cellobiohydrolase I (CBH I) from *T. reesei*, whereas similar platinum, osmium, iridium, and rhodium complexes had little effect on the enzyme. Furthermore, palladium complexes inhibited avicelase and β-glucanase, suggesting that the inhibition of catalytic activity of celluase is reversible and, possibly, noncompetitive (*20*).

Investigation of cross-synergism between CBH I from *T. reesei* and an endoglucanase (CenD) from *Cellulomonas fimi* during hydrolysis of unprinted newspaper indicated that the degree of synergism decreased as the ratio of CBH I to CenD was increased. Addition of an excess amount of β-glucosidase to the hydrolysis reaction further enhanced the degree of synergy (*21*). Several saprophytic fungal strains (isolated from wheat liter) were examined for their cellulolytic ability on various lignocelulosic crop residues. Such residues from jackfruit (*Artocarpus integrifolius*) were found to be the most suitable substrates for cellulase enzyme biosynthesis, whereas maximum cellulase activities were shown by *Penicillium chrysogenum* strains. Different parameters affecting production and activity of cellulase and other hydrolyzing enzymes were also studied (*22*).

The specific activity of an endoglucanase (E1; which displays high specific activities for initial rates of reducing sugar release and thermotolerance) purified from the actinomycetous *Acidothermus cellulolyticus* was studied. Various recombinant vector/host combinations were compared by determining the specific activity of E1 endoglucanase produced from

native genes. Great variations in specific activity were detected. The highest specific activity was achieved by expression of the native gene in *Streptomyces lividans* (23). Specific antibodies were developed for an endoglucanase from *A. cellulolyticus* and an exoglucanase from *Thermomonospora fusca*. Using the antibodies to detect and quantitate these cellulases in complex protein solutions, two distinct enzyme-linked immunosorbent assays (ELISAs) were developed for accurate and reliable quantitation (24). Substrate specificity and thermal and pH optima of stability and activity for two glucanases (EG5 and EG7) and a cellobiohydrolase (CBH3) of *Clostridium thermocellum* F7 isolated from the recombinant strains of *E. coli* hydrolyzing avicel and carboxymethylcellulose were characterized. It was noted that glucose and cellobiose behave as inhibitors of these enzymes, bovine serum albumin stimulates all three enzymes, the enzymes exhibit synergistic action in cellulose hydrolysis, and they are localized in the cellulosome of *C. thermocellum* (25).

Certain metabolic and physical characteristics of the cellulase-producing bacterium NCIB 10462 (*Pseudomonas fluorescens* ssp. or var. *cellulosa*) indicated that this gram-negative cellulolytic bacterium could be a pseudomonad species, but total fatty acid analyses do not support any taxonomic placement at species level. The authors suggest the name *P. cellulosa* for their NCIB 10462 bacterium (26). Immobilized selected mutants of *P. fluorescens* var. *cellulosa*, grown in a continuous fluidized-bed reactor for more than 3 wk, showed high cellulase activity on carboxymethylcellulose and on filter paper (27). Recovered cellulase enzyme from the supernatant in a batch culture of *P. fluorescens* var. *cellulosa* was added to a batch attrition bioreactor in order to study the effects of temperature and pH on waste-paper hydrolysis and enzyme stability and recycle (28).

Ruthenium carbonate reaction with CBH I yielded a protein-bound complex that was modified with imidazole treatment and generated Ru-$(bpy)_2$im His^{2+}. Two such complex molecules were bound to one molecule of CBH I. Light photolysis excited electronically Ru$(bpy)_2$im His^{2+}, a powerful oxidant that could be a very important agent for cellulose and lignocellulose hydrolysis (29). Based on chemical synthesis through biomimetic pathways, horseradish peroxidase (an oxidative enzyme) was encapsulated in water-in-oil microemulsions (reversed micelles) to develop a template for enzymatic polymerization studies. This provided a scheme to elucidate further the enzymatic synthesis of phenolic polymers in lignin (30).

BIOREACTORS-ENZYME IMMOBILIZATION

The effects of total solids content of sludge, digester fill level, agitator configuration, and mixing speed were evaluated for a laboratory-scale high-solids digester design. Visualized trends in horsepower requirements

were shown in a matrix approach focusing on the establishment of optimum parameters for minimizing mixing energy requirements (*31*). To elucidate the dynamic response of changes in the dilution rate of an activated sludge in an aerated continuous stirred-tank reactor, comparisons were made and evaluated among the dilution rates, cell levels, and substrate concentrations (*32*). Studies on the effect of temperature and impurities contaminations on the stability of immobilized amyloglucosidase in a fluidized-bed reactor fermenting 30% w/v liquefied cassava starch solution indicated that to obtain high half-lives in the immobilized enzyme process, in addition to maintaining optimum temperature levels, the substrate must also be purified and free of contaminations (*33*).

The effects of agitation and aeration on production of gluconic acid by *A. niger* from a glucose medium in a 20-L stirred-tank fermenter were investigated. It was noted that changes in the oxygen-transfer characteristics and gluconic acid production are correlated to the evolution of the broth rheology over the course of the fermentation (*34*). The rheology of *A. niger* broths was characterized using the impeller viscometer approach (*35*). A new continuous adsorption-desorption reactor (CADR) for bioconversion of lignocellulosics is proposed. It is based on the adsorbed enzyme redistribution phenomenon and consists of two chambers, each being modeled as a number of independent cells, where the content of both chambers is stirred and moved from input to output around a membrane (*36*). A discussion of the advantages of immobilized strict anaerobes in bioreactors and the methodology of enhancement of continuous anaerobic processes was presented (*37*).

A model describing the anaerobic degradation of 2-ethylhexanoic acid (2-EHA; persistent and inhibitory to bioprocesses waste) was developed and applied to simulate substrate utilization, product formation, and bacterial cell growth. The model considered the grouping of anaerobic bacteria according to their metabolic activities, description of the reactions and mixed-culture interactions, and the effect of unionized 2-EHA acid on inhibition of anaerobiosis (*38*). Furthermore, a 15-L anaerobic fixed-film reactor was evaluated for treating a trade effluent containing inhibitory concentrations of 2-EHA and neopentanoic acid. Methane gas was produced from the process (*39*).

PRODUCTION OF CHEMICALS

In considering bioconversion of potato-processing residuals to valuable chemicals, it was estimated that total US potato-processing waste could generate from 44 million to 1.55 billion lb of glycerol/yr, representing a 2.9–103% displacement of current glycerol production. Furthermore, total US waste could generate 1.41 million to 28.2 million lb of surfactin and

880,000 to 17.6 million lb lichenysin/yr (*40*). Glycerol, an important feedstock for chemical and plastic industries, can be produced from xylose fermentation by a selected strain of *Rhizopus javanicus*. Manipulation of xylose concentration, nitrogen source and level content of the medium, aeration rate, pH, and inoculum size can improve glycerol production rate and yield (*41*).

Investigation of acetic acid fermentation by *Clostridium thermoaceticum* for the production of the deicers calcium magnesium acetate and potassium acetate indicated that acetate production in batch fermentation is very low. The productivity was improved by achieving high cell density in a membrane bioreactor, although acetate concentration decreased. To overcome this limitation, two-stage CSTR and fed-batch processes were employed. Therefore, for the same acetate concentration in the product, both fed-batch and two-stage CSTRs had higher productivity than was seen in a single-stage CSTR (*42*). Lactic-acid-producing bacteria were evaluated for their ability to tolerate the acidity produced during bioconversion of carbohydrate to lactic acid. Lactic acid concentration and pH limited the continuous conversion of carbohydrate to acid. At pH 5, where most lactic cultures cease to produce acid, 2% lactic acid was extremely inhibitory to *Lactobacillus pentosus*. Consequently, buffering of the substrate is essential to maximize conversion of carbohydrate to lactic acid (*43*).

The effect of dissolved oxygen on citric acid yields and productivities was evaluated in batch, fed-batch, and cell-recycle fermentation systems using *Candida lipolytica* Y 1095. In the batch fermenter, citric acid yields were found to be virtually independent of dissolved oxygen concentration during the initial production phase at a biomass concentration of 3% w/v. However, at a biomass concentration of 5% w/v, citric acid yields increased with an increase in dissolved oxygen during the initial production phase. The maximum observed volumetric productivity in the batch, fed-batch, and cell-recycle fermenters was 1.14, 1.29, and 1.32 g citric acid L^{-1}/h, respectively (*44*). In synthesis-gas fermentations into alcohols and acids, the interphase mass transfer is the rate-limiting step owing to the very low aqueous solubility of carbon monoxide and hydrogen gases (synthesis gas components), and therefore, the use of microbubbles (surfactant-stabilized bubbles) was investigated (*45*). The soft parts of the columnar cactus *Cereus peruvianus* were found to be a novel source for homo-(α-1,4-linked)-galacturonan, and the viscosity and gelling properties of *Cereus* pectate proved superior to those observed for a commercial pectin (*46*). A process (steam classification) has been developed for the treatment of MSW to recover cellulosics as a feedstock for biological or chemical conversion to fuel, fertilizer, and/or fermentable sugars. The recovered material was found by composting to yield a suitable compost, by anaerobic digestion to generate good amounts of methane, and by enzymatic hydrolysis to yield glucose (*47*).

COAL STUDIES

Bituminous coal subjected to successive solvent extraction with anthracene oil and liquid paraffin was pyrolyzed. This solvent extraction/refining was found to free coal from its undesirable volatilizable components, and made it a cleaner and higher calorific fuel (*48*). A direct visualization of coal particles in an operating fluidized bed (biocatalytic solubilization/liquefaction of coal) was conducted using fluorescence light, which provided quantitative information on particle-size distribution and the effects of dispersion and solubilization reactor kinetics (*49*).

An investigation was made into the ability of several microbial cultures to liquefy lignite samples pretreated with dilute nitric acid solutions at elevated temperatures and pressures. The *Candida* sp. ML-13 strain, along with other lignolytic fungi, demonstrated an ability to liquefy lignite samples significantly from Louisiana deposits (*50*). Biogasification of Texas lignite was studied using a proprietary anaerobic consortium, Mic-1, isolated from the hindguts of soil-eating termites (*Zootermopsis* sp.). To understand the mechanism of coal biogasification, the attachment of the Mic-1 consortium to the coal particles was studied. It was found that the process can be at least 24-fold economical by supplementing the currently used yeast extract and trypsoy broth mixture with Sheftone™ as a nutrient amendment that enhanced the attachment of the Mic-1 consortium to the coal particles. High biomethanation rates were observed in the presence of 10 m*M* citrate or 0.5% methanol (*51*).

BIOREMEDIATION OF HAZARDOUS WASTE

Alginate-immobilized and free cells of *Pseudomonas putida* in an 800-mL air-uplift-type fluidized-bed batch reactor were used to study the substrate- and product-dependent kinetics of bioremediation of the toxic environmental pollutants sodium cyanide and acetonitrile (*52*). A microbial culture that can degrade the dilute gaseous hydrocarbons (*n*-pentane and isobutane) from effluent airstreams was enriched and sustained in a liquid-continuous packed column for over 28 mo using the degradation products as the sole carbon and energy sources. Shake-flask kinetic tests indicated that the process is mass-transfer-limited because of the low solubility of the alkanes (*53*).

Mixed cultures of *Thiobacillus denitrificans* (a chemoautotroph) and mixed floc-forming heterotrophs successfully treated samples of water coproduced with petroleum, which may contain sulfides and organic constituents that prevent its surface discharge. Complete removal of benzene, toluene, phenol, acetate, sulfides, and aquatic toxicity was achieved (*54*). A sulfide-tolerant strain of *T. denitrificans* (strain F) was isolated by enrichment and was used in a successful field test for the

treatment of sour water coproduced with petroleum at an Amoco Production Co. site in Wyoming. Samples of this sour water were used to produce an enrichment culture for sulfide oxidizers which showed to be strictly aerobic and to grow on sulfide as an energy source with complete oxidation of sulfite to sulfate (*55*).

Tetrachloroethylene has been shown to be reductively dechlorinated by dithiothreitol and titanium citrate in a reaction catalyzed by vitamin B_{12} (cyanocobalamine) at pH 9.0 and 45°C. Conversion of 50–60% to 1,2-trichloroethylene and 1,2-dichloroethylene was observed (*56*). A complete removal of nitric oxide (NO; 0.5%) from a feed gas sparged into autotrophic and heterotrophic denitrifying bacterial cultures was observed. In addition, the reduction of NO to nitrous oxide (N_2O) by dithiothreitol in a reaction catalyzed by cobalt-centered porphyrins and cyanocobalamine was investigated. Reaction rates were twofold faster for the corrin than Co-centered porphyrins, and 2 mol NO were lost/mol N_2O produced (*57*). An evaluation of the reported polychlorinated biphenyl (PCB) bioremediation activities and an assessment of the addition of known PCB-degrading microorganisms to a research site as it relates to biotransformation effectiveness and survival of added bacterial and fungal strains were presented (*58*).

Experiments using denitrifying bacterial consortia focused on determination of microbial attachment and detachment rates in porous media. The ultimate purpose of this work was to integrate the generated data into an *in situ* bioremediation model that will predict the effects of stimulating a denitrifying bacterial consortium to degrade carbon tetrachloride in both aquifer and vadose zones (*59*). To predict the effectiveness of an *in situ* biotransformation of hazardous materials strategy requires an understanding of both kinetics of the dominant metabolic processes and of the process rates that affect the accumulation of microorganisms on soil surfaces. Data obtained from an aerobic differential packed-bed reactor using glucose as the electron donor were used to determine kinetic parameters for attachment and detachment expressions, and further to develop and apply mathematical descriptions of microbial accumulation in porous media (*60*). An overview of the commonly used reactors to degrade contaminants during site restoration, as well as of the key operating parameters and designs involved was presented (*61*).

MISCELLANEOUS STUDIES

Using sewage sludge as a source of water for acid solution preparation, laboratory hydrolysis of newsprint was conducted to determine liquid-to-solid ratios, retention times, and temperatures necessary for optimum sugar yields (*62*). An oxidative model system using horseradish peroxidase (HRP)/hydrogen peroxide as the enzyme/substrate complex and

p-cresol as the secondary donor substrate was developed to investigate the reaction and interfacial transport phenomena of biphasic liquid–liquid systems. It focused on the effect of HRP on the continuous extraction of *p*-cresol from toluene into an aqueous phase (*63*). A new method was developed to activate cellulose (wood) photocatalytically by high-pressure (20,000 psi) insertion of specific photosensitizers into it. Such photosensitizers are quantum dot colloids, such as ZnO, and photoredox active ions, such as ferric ions. On irradiation of cellulose with near-UV light, a simultaneous photoevolution of molecular oxygen and volatile hydrocarbons was observed (*64*).

The lignolytic fungus *Phanerochaete chrysosporium* was found to decolorize methylene blue, dextran blue, remazol blue, and coomassie blue, whereas the lignolytic *Pleurotus sajor-caju,* the cellulolytic fungi *Chaetomium cellulolyticum* and *Trichoderma reesei,* and mutants of actinomycete *Streptomyces lividans* did not decolorize the tested dyes. The authors conclude that this decoloration of dyes is correlated to lignolytic activity (*65*). Based on the flavodoxin pathway in *Azotobacter vinelandii,* the investigator of this work is proposing a revision of the biological nitrogen-fixation concept. It was noted that the NADPH:flavodoxin oxidoreductase remained about 100-fold active during either aerobic or anaerobic purification, and its kinetic properties suggested a fundamental role in nitrogen fixation. Furthermore, nitrogenase stimulated the formation of flavodoxin hydroquinone by NADPH:flavodoxin oxidoreductase. Analyses suggested a mechanism of flavodixin reduction (hydroquinone formation) (*66*). Research based on extensive chemical analysis and greenhouse studies with corn indicated that the residues from anaerobically digested MSW are valuable amendments to supply nutrients and enhance water and nutrient-binding capacities to the soil (*67*).

The effect of sodium chloride concentrations on *Spirulina maxima,* an algal cyanobacterium, was examined. It was found that the alga was stressed since the NaCl was higher than 0.1*M*. As a response to stress (higher NaCl concentrations), the total carbohydrate and oxygen production increased, whereas the growth rate and total chlorophyll yield decreased, a response similar to many algae (*68*). Temperature effects on hydrogen and oxygen production in two *Chlamydomonas* strains, CCMP1619 (isolated from an ice-covered lake in Antarctica) and wild-type 137c, were studied. Temperature had a significant effect on the rates of hydrogenase induction and on the "dark" and photodependent hydrogen productions. After hydrogenase induction, simultaneous photoproduction of hydrogen and oxygen by photosynthetic water splitting at 4°C was observed for over 50 h in both strains. A novel photobiological reactor has been developed to study hydrogen production by microalgal water splitting (*69*). To achieve the maximum foreign protein expression for a fed-batch process, a sequential quadratic programming (SQP) algorith with a structured model that

described cell growth and product formation for recombinant *E. coli* was employed. It was found that optimized time profiles of feed substrate concentration and the feed flow rate can increase foreign protein productivity by more than 50% (*70*).

Twenty different *Bothrops* sp. snakes in Brazil were screened for hemorrhagins and L-amino acid oxidase (LAO). Among them, the venom of *B. cotiara* contained highly hemorrhagic and LAO fractions. Since in several human pathological conditions (i.e., congenital porphyrias, saturnism, tyrosinemia, and AIDS-provoked porphyrias) a reliable quantitation of δ-aminolevulinic acid (δ-ALA) is required, the use of LAO from the venom of *Bothrops* snakes can be very useful in removing free L-amino acids from human plasma and thus improving δ-ALA quantitation (*71*).

A high-performance liquid chromatography (HPLC) method was developed to separate and quantify organic acids, which utilizes Polypore H (Applied Biosystems, San Jose, CA) and PRP-X300 (Hamilton, Reno, NV) HPLC columns in series. This method allowed the separation of various organic acids, and it successfully quantified succinic acid in glucose-based culture designed for osmotolerant microorganisms, lactic acid in medium designed for sulfate-reducing bacteria, and acetic acid in medium designed for osmotolerant nitrate reducers (*72*).

CONCLUDING REMARKS

Research efforts presented at the poster session of the symposium projected the current fronts of biotechnological work in the areas of fuels and chemicals bioproduction and bioremediation of environmental impacts caused by various chemical releases. Presented results were mostly referred to: production of ethanol, methane, and other chemicals from biomass via microbial processes; characterization of enzymes involved and optimization of their activities for maximum productivity; feedstock pretreatment; development, evaluation, and optimization of novel bioreactors; enhancement of bioprocesses by key-parameter optimization; enhancement of coal liquefaction and bioconversion to methane fuel; economic and engineering analysis of bioprocesses involved; and development and evaluation of novel remediation bioprocesses for mitigation and elimination of hazardous waste impacts.

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