0273-2289/94/4546—0935$04.60

APPENDIX

New Research Findings in Biotechnology for Fuels and Chemical Production†

Poster Session Papers

ANTONIOS A. ANTONOPOULOS*,[1] AND KAREL GROHMANN[2]

[1]*Environmental Assessment Division, Argonne National Laboratory, Argonne, IL 60439; and* [2]*US Citrus and Subtropical Research Laboratory, Winter Haven, FL*

The 15th Biotechnology Symposium participants had the opportunity to view 85 poster papers (*1*) presented in an artistic setup as well as to discuss with the authors experimental procedures involved, results obtained, and practical aspects of the research conducted. The papers focused mostly on ethanol bioproduction from biomass material, pretreatment and hydrolysis of cellulosics, enzyme production/characterization, enhanced photoevolution of hydrogen, novel bioreactors, coal solubilization, sulfur bioproduction, generation of high-priced chemicals from biomass, and on bioremediation of hazardous wastes. Several of the papers are included in this volume, and a brief discussion of all of the posters presented at the symposium is the subject of this article.

ETHANOL PRODUCTION

Kluyveromyces marxianus EMS-26 is an organism particularly well adapted to carry out a successful simultaneous saccharification and fermentation (SSF) process (*2*). Despite good thermotolerance, *Kluyveromyces* spp. appeared to have low ethanol tolerance at high temperatures

*Author to whom all correspondence and reprint requests should be addressed.

(3). Some studies have shown that the incorporation of unsaturated fatty acids, sterols, or both into the cellular membrane helps to alleviate ethanol inhibition (4). In this work, the effect of sterol and unsaturated fatty acid addition on the production of ethanol by an SSF process was evaluated (5).

Studies on the physiological characteristics of a genetically engineered *Escherichia coli* B (carrying the PET operon with genes with *Zymomonas*) indicated that, under aerobic conditions:

1. The growth rate was almost seven times faster;
2. The molar growth yield (xylose) was about 4.5 times higher; and
3. The ethanol yield was only 0.29 g/g (57% conversion efficiency), which is similar to that achieved by the parent culture (ATCC 11303) under anaerobic conditions (6).

In an attempt to quantitate the interactive relationship between hexose and pentose metabolism, the relative rates of ethanol production using synthetic hemicellulose hydrolysates (mixtures of glucose, mannose, and xylose) and the recombinant *Escherichia coli* B (ATCC 11303; carrying the plasmid pLOI297 with pyruvate decarboxylase and alcohol dehydrogenase II genes from *Zymomonas mobilis*) were examined (7).

During xylose fermentation by *Pichia stipitis*, it was shown that high aeration levels led to extensive cell mass production and concomitant low product yield. Experiments in which the volume ratio (culture volume to total flask volume) was varied from 0.33 to 0.75 demonstrated that volume ratio > 0.5 created conditions of oxygen limitation, such that near-theoretical ethanol yields could be attained (8).

The production and consumption of sorbitol by *Saccharomyces cerevisiae* ATCC 36859 were studied. It was shown that sorbitol and ethanol were produced in a fructose medium, but only ethanol was generated in a glucose medium. The study indicated that Jerusalem artichokes could be used for the production of sorbitol and ethanol by this strain. The sorbitol, ethanol, and biomass yields were 0.259, 0.160, and 0.07 at the end of the process, respectively (9).

High yields of monomeric sugars were obtained by the hydrolysis of orange peel with pectinase or pectinase supplemented with cellulase enzyme, and relatively rapid rates of enzymatic hydrolysis of all polysaccharides were observed. The major sugars released were glucose, fructose, arabinose, and galactose, as well as galacturonic acid. Preliminary studies of batch fermentations using *Saccharomyces cerevisiae* indicated the presence of yeast inhibitors in whole-peel hydrolyzates (10).

A self-aggregating *Saccharomyces uvarum* strain formed large stable aggregates in a tower fermentor. The ethanol production effectiveness of the aggregates under various sugar concentrations and temperatures was studied (11). Ethanol productivity was 53 g/L/h at a dilution rate of 0.7 h^{-1}, using corn syrup as substrate. Ethanol yield was 96 and 92% of the

theoretical values in corn syrup and sugar-cane black strap molasses, respectively (*12*).

The direct conversion of sweet sorghum carbohydrates to ethanol by a mixed culture of *Fusarium oxysporum* F3, *Saccharomyces cerevisiae* 2541, or *Zymomonas mobilis* CP4 in a fed-batch fermentation system was studied. Optimum ethanol concentrations as high as 75.5–83.6 g/L and yields of 31.2–32.2 and 9.7–10 g ethanol/100 g sorghum carbohydrates and stalks, respectively, were obtained (*13*). The profile of celluloytic enzymes excreted during aerated growth of *Fusarium oxysporum* F3 affected the direct conversion of sweet sorghum polysaccharides to ethanol by a mixed culture of this fungus and *Saccharomyces cerevisiae* 2541. Yields as high as 41.2 and 12.8 g ethanol/100 g sorghum carbohydrates and stalks, respectively, were obtained while the ethanol concentration was 51.7 g/L (*14*).

Preliminary process designs based on laboratory and pilot-scale experiments involving sweet sorghum fermentation to ethanol were evaluated using fixed-capital equipment and production cost estimates. Energy balance analysis showed energy content of the ethanol produced to be greater than all energy requirements for sweet sorghum production, processing, and product recovery (*15*).

New cultivars of Canadian Prairie Spring wheats are being developed with 5% more starch and less protein than durum and bread wheats. Fermentation of high-starch flours yielded substantially higher ethanol concentrations for distillation. Mass and energy balance and ethanol yield were presented (*16*).

To ascertain the ability to ferment L-arabinose to ethanol by microorganisms, a variety of wild-type yeast and filamentous fungi grown in a mixture of arabinose, xylose, and glucose and under oxygen-limited conditions were screened. All of the yeasts and some of the fungi consumed arabinose, although significant fermentation of arabinose to ethanol was not observed for any of the strains tested (*17*).

Recent studies of the relationship between key SSF parameters/conditions and ethanol yield were presented. The effects of fermentation conditions, such as pH, temperature, inoculum size/age, agitation, growth medium, biomass concentration, and cellulase enzyme levels were evaluated in shake flasks and stirred-tank bioreactors (*18*).

A study was conducted to quantify the chemical and energy input and output of the ethanol from biomass fuel cycle in comparison with reformulated gasoline from crude oil. Feedstock production and transport, as well as fuel production, transport, and end use were analyzed. The air emissions evaluated were carbon monoxide, sulfur dioxide, nitrogen oxides, volatile organic compounds, and carbon dioxide (*19*).

Focusing on the reduction of carbon dioxide released to the atmosphere, an analysis of fuel utilization, energy flow, and carbon dioxide balance was provided for fossil fuels used. On the other hand, the benefits of biofuels utilization were emphasized. Biofuels technologies require little

if any fossil fuel inputs; carbon is recycled through their use, reducing substantially the net amount of carbon dioxide released to the atmosphere (*20*).

The US Department of Energy and the National Renewable Energy Laboratory are installing a Process Development Unit (PDU) to facilitate cooperative development of biomass-to-ethanol conversion technologies by the private industry, academia, and government. The unit is expected to begin operation in early 1994 (*21*).

FEEDSTOCK AND ENZYME STUDIES

Energy crops, industrial food residuals, and food crops were examined with respect to adoption as feedstocks by the chemical industry. Prices of raw materials, pretreatment needs, storage requirements, potential fermentable sugar yields, opportunities for waste minimization, level of technology base, crop requirements, and land usage were evaluated for each candidate feedstock (*22*).

An improved prototype apparatus and process for separating cellulosic materials from municipal solid waste was fabricated. The recovered recyclables are of high quality, and the cellulosic product is more suitable for combustion, composting, or as a feedstock for biological or chemical conversion to fuels and chemicals (*23*).

It was proven that step changes of reaction temperature during percolation of acid-catalyzed selective hydrolysis of hemicellulose from cellulosic biomass is advantageous especially in improving the sugar yield. The most significant improvement was seen with application of two-stage reverse-flow reactor arrangement with temperature change (*24*).

The power requirements and size distribution for milling of several biomass feedstocks were determined on a variety of pilot-scale size-reduction equipment. The milling (reduction to small particles) suspended mass- and heat-transfer limitations during the enzymatic hydrolysis of cellulosics (*25*).

The kinetics of the adsorption of catalytically inactivated *Trichoderma reesei* cellobiohydrolase I (CBH I) and resulting effects on the structure of crystalline cellulose (cotton) fibers were studied. The catalytic activity of CBH I was abolished by treatment with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC). Incubation of modified CBH I with cotton linters had no effect on their appearance (*26*).

To follow the adsorption and absorption of the individual components of *Trichoderma reesei* cellulase complex during cellulose hydrolysis, a chromatofocusing column (Mono-P) in a fast-protein liquid chromatography (FPLC) system was used to identify these components (*27*). A study was made on the capability of *Trichoderma reesei* (RUT-C30) to saccharify the cellulose fractions of the municipal solid waste (MSW) stream. Batch culture of RUT-C30 showed a significantly higher enzyme activity when

grown on MSW compared to microcrystalline celullose (Avicel). Additionally, a significant reduction in the total solids of the biodegradable fraction of the MSW was observed (*28*).

Mixtures of bacterial and fungal endoglucanases and exoglucanases used at very low total cellulase loadings were evaluated. Endoglucanases utilized included E1 from *Acidothermus cellulolyticus*, EG1 from *Trichoderma reesei*, and E_3 from *Thermomonospora fusca*. It was found that the degree of synergistic effect: (1) for the E1/CBH I and E_5/CBH I mixtures, peaked sharply at the low end of the range of endo/exo ratios, and (2) varied not only with the enzyme ratios, but also with the extent of substrate conversion during digestion (*29*). Acetate, succinate, citrate, phthalate, and phosphate buffers at different concentrations and in various combinations were tested to develop a buffered medium for an economic production of cellulases (*30*).

Endoglucanase 5 (EG5; isolated from the strain *E. coli* TG1 harboring recombinant plasmid pCU108, which contains *ce15* gene of *Clostridium thermocellum*) cleaves carboxymethyl cellulose (CMC), amorphous cellulose, xylan, lichenan, and Avicel. It was found that:

1. Cellobiose in concentrations up to 200 μm/mL did not inhibit the hydrolysis of CMC by EG5;
2. Calcium chloride stimulated Avicel hydrolysis by EG5;
3. EG5 together with *C. thermocellum* endoglucanase 7 (EG7) and cellobiohydrolase 3 (CBH3), also produced in *E. coli*, acted synergistically in cleaving Avicel;
4. The synergism extent depended on the order of addition of enzymes to Avicel; and
5. The yield of reducing sugars under simultaneous action of enzymes was two times higher than when they acted consecutively (*31*).

Discrete cellulase activities were examined utilizing both preparative native polyacrylamide gel electrophoresis (PAGE) and preparative isoelectric focusing. These endoglucanases were fingerprinted by obtaining sodium dodecyl sulfate (SDS)-PAGE-derived molecular weights and isoelectric pHs (*32*).

The susceptibility of a cellulosic substrate to enzymatic hydrolysis depends on the ability of cellulase to bind to cellulose. In lignocellulosic substrates, hemicellulose and lignin not only form a physical barrier restricting access to cellulose, but may also adsorb cellulases. CBCcex is the cellulose-binding domain from an exoglucanase/xylanase produced by *Cellulomonas fimi*. Since CBDcex binds to substrates without hydrolyzing them, it was used as a probe to investigate the binding capacity of various cellulosic substrates. When commercial cellulose substrates were used, initial results showed that the maximal initial hydrolysis rate obtained by cellulases from *Trichoderma reesei* could be correlated with maximal binding of CBDcex (*33*).

Agricultural wastes were used as the substrate for culturing *Aspergillus* sp. G-393. The optimal growth conditions for the production by the strain of cellulase was pH 3 at 60°C, and for xylanase was pH 5 at the same temperature (*34*). Cellulose hydrolysis was carried out in a gel state in a zinc chloride solution. The hydrolysis rate at various temperatures and its products and yields were studied. The methods for recycling zinc chloride were also investigated (*35*).

Intensive Mass-Transfer Reactor (IMTR), a bioreactor that transforms the energy of electromagnetic field into kinetic energy of ferromagnetic particles, was used to provide an intensive agitation of microcrystalline cellulose reaction mixtures. The effects of various factors on the efficiency of enzymatic hydrolysis of microcrystalline cellulose were studied. A significant improvement of the hydrolytic efficiency was observed owing to the enhancement of mobility of cellulolytic enzymes and cellulose susceptibility to cellulases (*36*).

Samples of 15 species of hard and soft woods were collected from saw mills located in the Appalachian region, pretreated, and subjected to SSF with *Saccharomyces cerevisiae* D_5A. It was observed that the glucan content of each wood tested was relatively invariant, whereas hemicellulose sugar and lignin contents were unique to each wood (*37*). It was found that the gastric juice from *Megalobulimus paranaguensis* was able in vitro to hydrolyze xyloglucans completely as well as release protoplasts from leaves of Parana pine, oats, cotton, and cassava (*38*).

HYDROGEN PHOTOEVOLUTION

During photosynthetic water splitting (photoevolution of H_2 and O_2), the carbon dioxide concentration of *Chlamydomonas reinhardtii* was regulated as a means of partitioning photosynthetic reductant between the Calvin cycle and the hydrogenase/hydrogen-evolving pathway. It was found that control of atmospheric CO_2 concentration could be used to regulate the branching ratio between the hydrogen evolution/hydrogenase pathway and CO_2-assimilating Calvin-Benson cycle pathway (*39*). Simultaneous photoproduction of H_2 and O_2 by water splitting was demonstrated in platinized thylakoids using hexachloroplatinate ($PtCl_6$). In addition, hexachloroosmiate $(O_sCl_6)^{-2}$, and hexachloroiridates $(IrCl_6)^{-2}$ and $(IrCl_6)^{-3}$ were also tested (*40*).

A novel process was analyzed to photoconvert biomass waste efficiently into natural biological plastics. Thermally gasified biomass releases carbon monoxide and hydrogen (among other compounds), which can be photoassimilated by isolated unique strains of photosynthetic bacteria into new cell mass. Under unbalanced culture conditions, a large fraction

of the new cell mass was found as granules of poly-3-hydroxybutyrate (PHB), a high-molecular-weight thermoplastic similar to polysterene or polypropylene. When a green alga was cocultured with the photosynthetic bacteria in light/dark cycles, a copolymer comprised of 70% 3-hydroxybutyrate and 30% 3-hdyroxyvalerate (PHB-V) was produced within the bacteria (*41*).

Strains of photosynthetic bacteria produced H_2 from organic acids, which appeared to photoassimilate the organic acids into endogenous sugars that were subsequently converted into fermentation products, including H_2 and CO_2, which were rapidly used by methanogenic bacteria. Repetitive light/dark cycles depleted organic acid pools and enhanced methane production. In mixed culture with methanogenic enrichments, photosynthetic bacteria were capable of facilitating the nearly complete conversion of propionic, butyric, or acetic acids into methane (*42*).

A stable biophotolysis system with an alternating light/dark cycle using green alga and photosynthetic bacterium was studied. Into a dark anaerobic fermentation reactor the alga rapidly degraded intracellular starch, and the photosynthetic bacterium produced hydrogen from the organic compounds released by green alga. The CR-8 mutant of *Rhodopseudomonas* sp. strain W1S (a marine photosynthetic bacterium) was isolated and tested (*43*).

BIOREACTORS—ENZYME IMMOBILIZATION

Aqueous biphasic extraction can be used to separate particles (including whole and broken cells, vesicles, and ribosomes) from each other or from soluble materials. In order to understand this phenomenon, various factors related to the thermodynamics and fluid dynamics of particle partitioning were studied using BSA-coated latex spheres as model particles (*44*).

Beads of copolymerized methacrylamide and *N,N'*-methylenebisacrylamide were prepared by means of the W/O-type suspension polymerization. The polymer was activated by various reagents and tested as a support for immobilizing invertase (*45*).

In order to account for spatial differences in the colloidal/solid pectin substrate, the batch reaction with pectinase was modeled with a two-zone model. Imperfect mixing was, thus, able to consider early time concentrations of pectin in the zone into which it was initially added (*46*).

In a fluidized-bed reactor, the saccharification of amylase liquefied starch solution was carried out with glucoamylase immobilized in controlled-pore silica. The obtained data showed that the fluidized-bed model led to higher conversion efficiency than the fixed bed (*47*).

Neural network techniques were employed to develop model schemes for the optimization of fed-batch bioreactors for treatment of toxic chemicals. Using this model, an approach was developed both to optimize feeding profiles and control schemes. This technique was illustrated using methanol and thiodiglycol as the toxic compounds for treatment (*48*).

PRODUCTION OF FUELS AND CHEMICALS

Because of their high-viscosity, vegetable oils are not suitable for use in modern diesel engines. Transesterification of the oils with short-chain alcohols to corresponding fatty esters can eliminate the high-viscosity problem and accommodate their use as diesel fuels. A transesterification method was applied to crude safflower seed oil using methanol. A significant improvement was observed in viscosity and other physical properties with the ester product compared to the parent oil (*49*). An evaluation of using safflower seed oil as a diesel fuel substitute indicated that the methyl ester of the oil had similar engine performance characteristics as grade No. 2-D diesel fuel. Furthermore, lower CO, EC, and particulates emissions were achieved when methyl ester was used (*50*).

The growth of *Clostridium ljungdahlii* and production of acetic acid were examined for carbon monoxide, and for hydrogen with carbon dioxide (components of synthesis gas) in mineral medium. The thermochemistry of the fermentation reactions, the acetogenic pathway, the nutritional requirements of the organism, and implications for process design were considered (*51*).

To enhance enzymatic hydrolysis of inulin, caproyl-, or cholesteryl moities from the respective chlorides were chemically transferred to native inulin and the resulting derivatives were assayed as inulinase inducers using selected *Kluyveromyces* yeast strains. Supplemented caproylated inulin proved to be a superior inulinase inducer compared to cholesteryl-inulin (*52*).

Enzymatic or acid hydrolysis of inulin may produce difructo-furanose anhydride (DFA III). Among the several anhydrofructobiose isomers, DFA III provoked disturbance of free D-fructose metabolism. The known DFA-producer *Arthrobacter ureafaciens* was comparatively studied with two *Dahlia* tuber inulin rotters (*53*).

Canola meal is a byproduct of oil production from canola seeds, which is rich in protein and contains 3–7% phytic acid (myoinositol hexaphosphoric acid). Phytic acid has to be removed from canola meal prior to its utilization as a feedstuff. *Aspergillus carbonarius* was used in a solid-state fermentation process to study the production of phytase enzymes and the phytic acid reduction in canola meal (*54*). Furthermore, canola contains about ten times phenolic compounds (mainly esters of sinapic acid, like sinapine, contributing to the bitter flavor and dark color of the

meal) found in soybean. An enzyme was used that reduced up to 95% the phenolic compounds of the canola meal (*55*).

Seed xyloglucan and lipophilic components from seeds, leaves, bark, and roots of *Annona* sp. ("fruta-do-conde") and *Rollina* sp. ("biriba") were submitted to organosolv isolation procedures to isolate tumorgenesis blockade compounds. Preliminary tests explored brine shrimp lethality and inhibition of *Agrobacterium tumefaciens* development on potato disks (*56*).

The permeation of plasma membrane as well as the pH-dependent accumulation of acetic acid and acidification of the *Zymomonas* cytoplasm were examined (*57*). The production of lactic acid from pure substrates, such as cellulose, glucose, and xylose, was investigated. Various *Lactobacillus* strains and fermentation conditions were examined for the efficient conversion of glucose and xylose. Experiments on SSF of cellulose to lactic acid were also carried out using exogenously added cellulase enzyme and the microorganism (*58*). Basic anion-exchange resins with imidazole, pyridine, and amine functional groups were evaluated as absorbents to recover lactic acid from fermentation broth of *Lactobacillus delbrueckii* (*59*).

A continuous biparticle fluidized-bed reactor was developed for the simultaneous fermentation and purification of lactic acid, which did not require addition of salts for pH control and product separation as the inhibitory factor. The system was demonstrated in a biphasic fluidized bed of immobilized *Lactobacillus delbreuckii* (*60*). Eight *Lactobacillus* cultures were evaluated for their ability to produce lactic acid (which can be manufactured into degradable polylactic acid plastics) from simple sugars from acid-hydrolyzed municipal solid wastes (MSW). Effective treatments to remove microbial inhibitors from MSW were developed. The lactic acid yield and productivity were 65 mg/mL and 1.3–1.6 g/L-h, respectively. The repetitive regeneration of the loaded resin (polyvinyl pyridine) was investigated (*61*).

The acetone-butanol production by simultaneous saccharification and extractive fermentation (SSEF) was investigated. The SSEF-employing cellulase enzymes and *Clostridium acetobutylicum* have shown that both glucan and xylan fractions of pretreated aspen wood are concurrently converted into acetone and butanol (*62*). *Pseudomonas fluorescens* strain BTP9 was used as a biocatalyst to produce vanillic acid from vanillin (*63*).

COAL AND SULFUR STUDIES

An investigation has been made into the ability of several cultures to liquefy lignite samples that had been pretreated with very dilute-acid solutions at elevated temperatures and pressures. A *Candida* sp. strain ML-13 and an unidentified bacterial strain LSC have each shown an ability to liquefy samples of lignite significantly (*64*).

A study was undertaken to test selected anaerobic microbial consortia for their ability to degrade and produce methane from bituminous coals. At least three consortia have shown the ability to produce methane from hard coals without the presence of yeast extract in the culture medium (*65*).

A model was developed for an accurate hydrodynamic characterization of the fluidized-bed bioreactor for coal biosolubilization. The bioreactor design and its scale-up were also addressed (*66*).

Liquid fluidized beds of small coal particles have been investigated for the biocatalytic solubilization/liquefaction of coal at near-ambient temperatures. This work has undertaken the direct visualization of coal particles (20–150 μm) in operating fludized beds using fluorescent microscopy. The fluorescence technique has been developed and fully validated (*67*).

Work demonstrated that the sulfate-reducing bacterium *Desulfatomaculum orientis* can be grown in batch cultures on H_2 as an energy source, CO_2 as a carbon source, and SO_2 as a terminal electron acceptor. Complete reduction of SO_2 to H_2S was observed within 1–2 s of gas–liquid contact time. The stoichiometry of SO_2 reduction with H_2 as the electron donor has been determined, and batch reactors have been optimized with respect to percent conversion of H_2 into gas feed via gas recycle (*68*).

The anaerobic photosynthetic bacterium *Chlorobium thiosulfatophilum* may be used for bioconversion of H_2S to elemental sulfur or sulfate. A bench-scale study using a CSTR with a sulfur-settling separator was carried out to determine the optimum process conditions for maximizing H_2S conversion, cell growth, and elemental sulfur production, and for minimizing sulfate production (*69*). Another work presented kinetic information on the rate of growth of *C. thiosulfatophilum*, as well as the rate of uptake of both H_2S and CO_2. Modeling results indicated that growth is dependent on light intensity according to a Monod-type relationship (*70*). *Thiobacillus denitrificans* has been proposed for the removal of H_2S from gases and sulfides from sulfide-laden (sour) water to give sulfur. However, this technology requires an economical means of growing and harvesting the bacterial biomass. It was shown that *T. denitrificans* may be flocculated by coculture with floc-forming heterotrophs to produce sulfide-active floc with excellent settling properties. The economics and practical aspects of this technology were presented (*71*).

BIOREMEDIATION OF HAZARDOUS WASTE

In a columnar reactor, the selected microbes were able to remove hydrocarbons (pentane and isobutane) from air. Bioreactor concepts that provide for interaction with gaseous substrates are being investigated (*72*).

Soil column studies evaluated effects of treatments on petroleum hydrocarbon (PHC) remediation in nutrient-deficient, alkaline soils of Kwajalein Atoll (west-central Pacific Ocean). Bioaugmentation with indigenous PHC degraders did not enhance bioremediation. Although the

potential for bioremediation was demonstrated by air, water, and nutrient amendments, bioremediation time could be lengthy (*73*).

The fixation by photosynthetic microalgae of CO_2 emitted from power-plant boilers was investigated. Algal growth rate under different sunlight conditions was evaluated (*74*).

The mechanism of uranium binding by the strain *Pseudomonas aeruginosa* CSU was examined. Resting cells were shown to remove uranium from dilute aqueous solutions of $UO_2(NO_3)_2$. Metal loadings were enhanced by pretreatment of the cells with polar organic solvents. Uranium binding was significantly inhibited by ferric iron [Fe(III)]. Within 24 h, all bound uranium was located intracellularly, as amorphous material. Although the cells were exposed to hexavalent uranium [U(VI)], bound material existed in the tetravalent state [U(IV)] (*75*). Complexation and consequent mobilization of radionuclides by EDTA can be a significant problem at treated sites, and consequently, *in situ* degradation of EDTA is an attractive option for remediation. The most well-characterized organism that can degrade EDTA is *Agrobacterium radiobacter*. The effects of various media compositions were examined. It was found that with Fe-EDTA as a carbon source, EDTA degradation resulted in a rise in pH and ammonia concentration. Addition of glycerol to the media resulted in suppression of Fe-EDTA degradation, and in a decrease in pH and NH_4 in the media (*76*).

Several species of algae, cyanobacteria, and bacteria were screened for their ability to biosorb ionic nickel from a nickel-containing waste. Cyanobacteria demonstrated a greater capacity for nickel biosorption than any of the other organisms tested. Biosorption was greater, and the results more consistent, when using heat-killed cells than with live cells (*77*).

It was found that hen eggshell membrane (ESM) protein eliminated various heavy-metal ions from their dilute aqueous solutions in high yield and in short contact time. Metal ions complex formation with ESM tested were of common metals (Na, K, Cu, Ni, Zn, Fe, and others), toxic metals (Hg, Cd, Pb), actinides and lanthanides (U, La, Nd, Pr, Gd, Ce, Sm, Eu), and precious elements (Au, Pd, Pt, Ag) (*78*).

Porphyrin-catalyzed reduction of nitroaromatics to corresponding amino-substituted products was demonstrated in both batch and continuous reactors. Intermediate and final products of porphyrin-catalyzed reduction of mono-, di-, and trinitrotoluenes were identified. Of several reductants tested, dithiothreitol, sodium dithionite, and sodium sulfide were seen to give significant reduction of nitrobodies. Cobalt (III) and iron (III) ions were also compared as the chelated metal ion in the reduction of 2-nitrotoluene. If the nitroaromatics are components of munition wastes, porphyrin pretreatment can render the wastes safer to handle and more amenable to biotreatment (*79*).

The growth of bacteria in samples from the deep subsurface at western arid sites after addition of water and nutrients to the solid material was studied (*80*). A method was developed to examine the ability of deep sub-

surface bacteria to assimilate recalcitrant natural organic matter (humic/fulvic material) and their interactions with inorganic nutrients. Microbial growth was measured by increases in optical density. Isolates tested to date have shown variable capabilities for utilizing organic matter (*81*).

MISCELLANEOUS STUDIES

A variety of *Phanerochaete chrysosporium* mutants have been isolated through UV and γ-ray mutagenesis. Some of the mutants appears to produce the key lignolytic enzymes (peroxidases) under high-nitrogen growth conditions. The mutant 76UV produced 272 U of lignin peroxidase enzyme activity/L under high-nitrogen growth conditions in 6 d. The parent strain does not produce lignin peroxidase at all under these conditions (*82*).

Optimized conditions for acetylesterase enzyme production using *Aspergillus niger* ATCC 10864 in 14 L fermentation jars were determined to be 33°C, 1.5 vvm aeration, and 300 rpm agitation without pH control. Stability, kinetic, and other characteristics of the acetylesterase were determined. The mol wt of the enzyme was 35,000 dalton. The N-terminal amino acid sequence, the glycosylation composition, isoelectric point, and SDS molecular weight were also determined (*83*).

A mathematical model has been developed for the key reactions taking place in the process of cheese ripening. The intracellular peptidases released through cell lysis during ripening was found to have the most profound effect on cheese flavor (*84*). In another work, the kinetics of cheese whey fermentation using the yeast *Kluyveromyces fragilis* for single-cell protein production and pollution prevention were studied. The fermentation process was successful in reducing the COD, total solids, and ammonium nitrogen by 50, 53, and 90%, respectively. A yield of 0.78 g cell/g lactose was achieved (*85*).

Use of conductivity change in the medium for the on-line monitoring of fresh weights was investigated in cultures of carrot hairy-roots (*86*).

The effect of solid concentration on the growth of heterogenous microbial populations in swine manure was studied under batch and continuous aerobic conditions. Dehydrogenase activity was used as a measure of active biomass in the manure. High concentration of manure had an inhibitory effect on the microbial growth under both batch and continuous conditions (*87*).

The metabolism of suspected compounds (volatile fatty acids, phenols, indols, benzoic acid, and their derivatives) to be involved in odor releases from manure slurries was studied, and their metabolic pathways when amino acids and other precursors were added to slurries were followed. It was found that the chemical and microbiological composition of slurries dictates the odor releases (*88*).

New wood substitute composites from wood, newspaper, and agricultural biomass combined with compounds, such as polypropylene and Epoline E-43, utilize delignification technology via Xylan, Inc.'s patented process, which is based on steam explosion of biomass. The advantages and environmental benefits of this new product utilization were presented (*89*).

CONCLUDING REMARKS

The symposium poster paper presentation emphasized the current trends of biotechnological research focusing on:

1. Ethanol, methane, and hydrogen production from biomass;
2. Enhancement of bioprocesses by key-parameter optimization, development of novel bioreactors, and feedstock pretreatment;
3. Elucidation of feedstock composition, and characterization of involved enzymes and enzymatic mechanisms;
4. Clarification of hydrogen photoevolution and factors implicated;
5. Enhancement of coal solubilization and bioconversion to methane fuel;
6. Development and characterization of novel bioremediation processes for elimination/mitigation of hazardous waste impacts; and
7. Bioproduction of new high-valued chemicals.

ACKNOWLEDGMENT

This work supported by the US Department of Energy, Assistant Secretary for Energy Efficiency and Renewable Energy, under contract W-31-109-Eng-38.

REFERENCES

1. Fifteenth Symposium on Biotechnology for Fuels and Chemicals: Program and Abstracts, Colorado Springs, Colorado, May 10–14, 1993, National Renewable Energy Laboratory.
2. Ballesteros, I. et al. (1981), *Appl. Biochem. Biotech.* **28/29,** 307–315.
3. Hughes, D. B., Tudroszen, N. J., and Moye, C. J. (1984), *Biotechnol. Lett.* **6(1),** 1–13.
4. Thomas, S. D., Hossak, J. A., and Rose, A. H. (1978), *Arch. Microbiol.* **117,** 239–245.

5. Ballesteros, I., Oliva, J. M., Carrasco, J. E., and Ballesteros, M. (1993), *Effect of Media Supplementation on Ethanol Production by the Simultaneous Saccharification and Fermentation Process.* [20]*
6. Lawford, H. G., and Rousseau, J. D. (1993), *Effect of Oxygen on Ethanol Production by a Recombinant Ethanologenic* E. coli *B (pLOI297).* [39]
7. Lawford, H. G., and Rousseau, J. D. (1993), *Relative Rates of Ethanol Production by an Ethanologenic* E. coli *B. (pLOI297) Using Mixtures of Glucose, Mannose, and Xylose.* [40]
8. Boynton, B. L. and McMillan, J. D. (1993), *High Yield Shake Flask Fermentation of Xylose to Ethanol.* [57]
9. Duan, Z. D., Turcotte, G., and Duvnjak, Z. (1993), *Conversion of Inulin from Jerusalem Artichokes to Sorbitol and Ethanol by Saccharomyces cerevisiae.* [24]
10. Grohmann, K. and Baldwin, E. A. (1993), *Bioconversion of Carbohydrates in Orange Peel to Ethanol.* [31]
11. Chen, C. S., Wang, S. I., Chen, L. G., and Chen, L. F. (1993), *Temperature Dependent Kinetics of Ethanol Fermentation Using a Self-Aggregating Saccharomyces uvarum.* [58]
12. Chen, C. S., Gong, C. S., and Chen, L. F. (1993), *Ethanol Fermentation in a Tower Fermentor Using Self-Aggregating Saccharomyces uvarum.* [61]
13. Lezinou, V., Christakopoulos, P., Kekos, D., and Macris, B. J. (1993), *Fed Batch Fermentation of Sweet Sorghum Carbohydrates to Ethanol by a Mixed Culture.* [69]
14. Lezinou, V., Christakopoulos, P., Kekos, D., and Macris, B. J. (1993), *On the Mechanism of Direct Bioconversion of Sweet Sorghum Carbohydrates to Ethanol: Effect of Cellulolytic Enzymes.* [70]
15. Linden, J. C., Henk, L. L., Murphy, V. G., Lange, K. D., and Tengerdy, R. P. (1993), *Silage Processing of Forage Biomass to Alcohol Fuels.* [85]
16. Sosulski, K. and Sosulski, F. (1993), *Wheat as a Feedstock for Fuel Ethanol.* [15]
17. McMillan, J. D. and Boynton, B. L. (1993), *Arabinose Utilization by Xylose-Fermenting Yeast and Fungi.* [72]
18. Smith, T. K. and Philippidis, G. P. (1993), *Ethanol Production from Lignocellulosic Biomass: Evaluation of SSF Conditions and Parameters.* [78]
19. Bull, S. R., Riley, C. J., Tyson, K. S., and Costello, R. (1993), *Biofuels Full Fuel Cycle Analysis.* [97]
20. Wyman, C. E. (1993), *Reduction of Carbon Dioxide Accumulation by Use of Alternative Fuels from Biomass.* [105]
21. Schell, D., Duff, B., and Bergeron, P. (1993), *Design and Installation of the NREL Biomass-to-Ethanol Process Development Unit.* [2]
22. Polman, J. K. (1993), *Analysis of Renewable Feedstocks for the Production of Commodity Chemicals.* [86]
23. Eley, M. H. (1993), *An Improved Prototype Apparatus and Process for Separating Cellulosic Materials from Municipal Solid Waste.* [4]
24. Kim, B. J., Lee, Y. Y., and Torget, R. (1993), *Simulation of a Modified Percolation Process in Dilute-Acid Hydrolysis of Biphasic Hemicellulose.* [11]
25. Schell, D. and Harwood, C. (1993), *Milling of Lignocellulosic Biomass: Results of Pilot Scale Testing.* [14]
26. Donner T. R., Woodward, J., and Affholter, K. (1993), *The Role of the Cellulose-Binding Domain of* Trichoderma reesei *Cellobiohydrolase I in Cellulose Hydrolysis.* [23]

* Number in brackets indicates the poster's number in Ref. 1.

27. Yu, A. H. C. and Saddler, J. N. (1993), *Compositional Changes of Adsorbed Cellulases During Cellulose Hydrolysis.* [47]
28. Maxey, A., Huckabay, H., Chen, X.-J., and Elmore, B. B. (1993), *Cellulase Activity of* Trichoderma reesei *(Rut-C30) on Municipal Solid Waste.* [89]
29. Baker, J. O., Adney, W. S., Nieves, R. A., Thomas, S. R., Wilson, D. B., and Himmel, M. E. (1993), *Synergism Studies of Bacterial and Fungal Cellulases at Low Total Cellulase Loadings.* [17]
30. Kadam, K. L. and Keutzer, W. J. (1993), *Development of a Buffered Medium for Cellulase Fermentation Studies with* T. reesei *Rut-C30.* [35]
31. Kalyuzhnyi, S. V., Mosolova, T. P., Varfolomeyev, S. D., and Velikodvorskaya, G. A. (1993), *Synergism in Cleaving Avicel by* Clostridium thermocellum *Cellulases Produced in* Escherichia coli. [37]
32. Rivard, C. J., Nieves, R. A., Nagle, N. J., and Himmel, M. E. (1993), *Evaluation of Discrete Cellulase Enzyme Activities from Anaerobic Digester Sludge Fed a Municipal Solid Waste Feedstock.* [46]
33. Lee, D., Wong, K. K. Y., Yu, A. H. C., Saddler, J. N., Warren, A., Killburn, D., and Gilkes, N. (1993), *Use of a Cellulose-Binding Domain to Measure the Accessibility of Various Cellulosic Substrates to Cellulases.* [48]
34. Wang, S. I., Chen, L. G., Chen, C. S., and Chen, L. F. (1993), *Cellulase and Xylanase Production by* Aspergillus sp. *G-393.* [60]
35. Cao, N. J., Xu, Q., Gong, C. S., and Chen, L. F. (1993), *Cellulose Hydrolysis Using Zinc Chloride as a Solvent and Catalyst.* [59]
36. Gusakov, A. V., Sinitsyn, A. P., Davydkin, I. Yu., and Davydkin, V.Yu. (1993), *Effect of Various Factors of the Efficiency of Enzymatic Cellulose Hydrolysis in the Intensive Mass Transfer Reactor.* [64]
37. Vinzant, T. B., Ponfick, L. R., Nagle, N. J., Ehrman, T. I., Magill, K. M., Reynolds, J. B., and Himmel, M. E. (1993), *SSF Comparison of Selected Woods from Southern Saw Mills.* [83]
38. Fontana, J. D., Gebara, M., and Lyra, I. N. (1993), *α-Xylo-β-glucan Monomerization and Plant Protoplast Release Using the Hydrolase Complex from the Gastric Juice of the Land Snail Megalobulimus.* [no. 28]
39. Greenbaum, E., Blankinship, S. L., and Cinco, R. M. (1993), *New Methods of Improving Hydrogen Yields by Photosynthetic Water Splitting.* [30]
40. Lee, J. W., Tevault, G. V., Blankinship, S. L., Collins, R. T., and Greenbaum, E. (1993), In situ *Photoprecipitation of Metallocatalysts for Molecular Hydrogen Production with Photosynthetic Water Splitting in Thylakoids.* [41]
41. Maness, P.-C. and Weaver, P. F. (1993), *Photobiological Conversion of Synthesis of Gas into Biodegradable Plastics.* [45]
42. Weaver, P. F. and Maness, P.-C. (1993), *Photoenhanced Anaerobic Digestion of Organic Acids into Methane.* [53]
43. Yagi, K., Maeda, I., Idehara, K., Miura, Y., Akano, T., Fukatu, K., Ikuta, Y., and Nakamura, H. K. (1993), *Removal of Inhibition by Ammonium Ion in Nitrogenase-dependent H_2 Evolution of Marine Photosynthetic Bacterium,* Rhodopseudomonas *sp. Strain W1S.* [54]
44. Hawker, D. T. L., Davis, R. H., Todd, P., and Johansson, G. (1993), *Aqueous Biphasic Systems for Biological Particle Partitioning.* [65]
45. Huang, C.-T. and Lee, W.-C. (1993), *Immobilization of Invertase onto a Copolymer of Methacrylamide and N,N'-Methylenebis(Acrylamide) and Characterization.* [68]

46. Tseng, W., Prokop, A., and Tanner, R. D. (1993), *A Two Zone Mixing Model for Describing the Kinetics of a Batch Pectin-Pectinase Reaction Process.* [80]
47. Zanin, G. M., Kambara, L. M., Calsavara, L. P. V., and de Moraes, F. F. (1993), *Performance of a Fluidized Bed Reactor with Immobilized Enzyme.* [84]
48. Chen, Q. and Weigand, W. A. (1993), *Modeling and Optimization of Fed-Batch Bioreactors for Treatment of Toxic Chemicals Using Neural Networks.* [104]
49. Isigigur, A., Karaosmanoglu, F., and Aksoy, H. A. (1993), *Methyl Ester from Safflower Seed Oil of Turkish Origin as a Biofuel for Diesel Engines.* [10]
50. Isigigur, A., Karaosmanoglu, F., and Aksoy, H. A. (1993), *Performance and Emission Characteristics of a Diesel Engine Operating on a Safflower Seed Oil Methyl Ester.* [9]
51. Phillips, J. R., Clausen, E. C., and Gaddy, J. L. (1993), *Synthesis Gas as Substrate for Biological Fuels and Chemicals Production.* [13]
52. Fontana, J. D., Baron, M., and Franco, V. C. (1993), *Microorganism Inulinase Secretion Using Chemically Modified Inulins.* [18]
53. Fontana, J. D., Astolfi Fo., S., Kaiss, J., Hauly, M. C. O., Franco, V. C., Rogelin, R., and Baron, M. (1993), *PCR Protocol and Inulin Catabolism-Based Differentiation of Inulinolytic Soil Bacteria.* [19]
54. Al-Asheh, S. and Duvnjak, Z. (1993), *Production of Phytase and Reduction of Phytic Acid Content in Canola Meal by* Aspergillus carbonarius *During a Solid State Fermentation Process.* [25]
55. Lacki, K. and Duvnjak, Z. (1993), *Upgrading of Canola Meal by Reducing Its Sinapic Acid Esters Content.* [26]
56. Fontana, J. D., Baron, M., Deschamps, F. C., Guimaraes, M. F., Zanette, F., Cervi, A. C., and Almeida, E. R. A. (1993), *Screening of Acetogenin-Like Compounds in Brazilian Flora:* Annona *and* Rollinia *spp.* [27]
57. Lawford, H. G. and Rousseau, J. D. (1993), *The pH-Dependent Energetic Uncoupling of* Zymomonas *by Acetic Acid.* [38]
58. Padukone, N., Schmidt, S., and Goodman, B. (1993), *Production of Lactic Acid from Lignocellulosic Biomass.* [73]
59. Evangelista, R. L. and Nikolov, Z. L. (1993), *Recovery of Lactic Acid by Adsorption: Resin Evaluation.* [12]
60. Kaufman, E. N., Cooper, S. P., and Davison, B. H. (1993), *Development of a Biparticle Fluidized Bed Bioreactor for the Continuous and Simultaneous Fermentation and Purification of Lactic Acid.* [66]
61. McCaskey, T. A., Zhou, S., and Strickland, R. C. (1993), *Bioconversion of MSW to Lactic Acid by* Lactobacillus *species.* [71]
62. Shah, M. M. and Lee, Y. Y. (1993), *Acetone-Butanol Production from Biomass by Simultaneous Saccharification and Extractive Fermentation.* [75]
63. Bare, G., Dulaunois, V., Rikir, R., and Thonart, P. H. (1993), *Bioconversion of Vanillin to Vanillic Acid in One- and Two-Phase Systems.* [81]
64. Huckabay, H., Rao, A., Maxey, A., and Elmore, B. B. (1993), *Microbial Liquefaction of Lignite Pretreated with Dilute Acid at Elevated Temperature and Pressure.* [5]
65. Johnson, E. R., Klasson, K. T., Basu, R., Volkwein, J. C., Clausen, E. C., and Gaddy, J. L. (1993), *Microbial Conversion of High Rank Coals to Methane.* [34]

66. Asif, M., Peterson, J. N., and Scott, T. C. (1993), *Hydrodynamic Modeling of a Liquid Fluidized Bed Bioreactor for Coal Biosolubilization.* [55]
67. Kaufman, E. N. and Scott, T. C. (1993), *Study of Coal Particle Segregation in a Liquid Fluidized Bed:* In Situ *Visualization Using Fluorescence Microscopy.* [67]
68. Lee, C.-M. and Sublette, K. L. (1993), *Autotrophic Growth of* Desulfatomaculum orientis *with Reduction of Sulfur Dioxide.* [50]
69. Basu, R., Ramakrishnan, S., Clausen, E. C., and Gaddy, J. L. (1993), *Conversion of Hydrogen Sulfide to Elemental Sulfur by* Chlorobium thiosulfatophilum *in a CSTR with a Sulfur Settling Separator.* [56]
70. Basu, R., Klasson, K. T., Clausen, E. C., and Gaddy, J. L. (1993), *Removal of Carbonyl Sulfide and Hydrogen Sulfide from Synthesis Gas by* Chlorobium thiosulfatophilum. [87]
71. Hasan, S., Rajganesh, B., and Sublette, K. L. (1993), *Large-Scale Production of* Thiobacillus denitrificans *Biomass to Support Pilot and Field Tests of Microbial Sulfide Oxidations.* [101]
72. Davison, B. H. and Thompson, J. E. (1993), *The Removal of Volatile Organics in a Gas-Phase Bioreactor.* [88]
73. Phelps, T. J., Siegrist, R. L., Korte, N. E., Pickering, D. A., Strong-Gunderson, J. M., Palumbo, A. V., Walker, J. F., Morrissey, C. M., and Mackowski, R. (1993), *Bioremediation of Petroleum Hydrocarbons in Soil Column Lysimeters from Kwajalein Island.* [95]
74. Ikuta, Y., Hukuda, Y., Hamasaki, A., Shioji, N., Makita, T., Hirayama, K., Matsuzaki, H., and Tukamoto, T. (1993), *Carbon Dioxide Fixation by Microalgal Photosynthesis Using Actual Flue Gas Discharged from a Boiler.* [90]
75. Munroe, N. D. H., Bonner, J. D., Williams, R., Pattison, K. F., Norman, J. M., and Faison, B. D. (1993), *Binding of Dissolved Uranium by* Pseudomonas aeruginosa *CSU.* [92]
76. Palumbo, A. V., Lee, S. Y., and Boerman, P. (1993), *The Effect of Media Composition on EDTA Degradation by* Agrobacterium radiobacter. [93]
77. Reeves, M. and Corder, L. (1993), *Microbial Biosorption of Nickel in Complex Aqueous Wastes.* [96]
78. Suyama, K., Fukazawa, Y., and Umetsu, Y. (1993), *A New Biomaterial to Eliminate Heavy Metal Ions from Their Dilute Waste Solutions.* [102]
79. Cho, J.-G. and Sublette, K. L. (1993), *Porphyrin-Catalyzed Reduction of Nitroaromatics for the Treatment of Munitions Wastes.* [100]
80. Palumbo, A. V., Parker, A., McCarthy, J. F., Phelps, T. J., and Strong-Gunderson, J. (1993), *Potential for Microbial Growth in Arid Subsurface Sediments.* [94]
81. Strong-Gunderson, J. M., Palumbo, A. V., McCarthy, J., Scarborough, S., and Mehlhorn, T. (1993), *Differential Bioavailability of Deep Subsurface Natural Organic Matter to Microbial Isolates: Implications to* In Situ *Bioremediation.* [99]
82. Kakar, S. N. (1993), *Phanerochaete Mutants with Enhanced Lignolytic Activity.* [36]
83. Linden, J. C., Samara, M., Thomas, E., Joy, M., Decker, S., Pecs, M., Adney, W., and Himmel, M. (1993), *Purification and Characterization of an Acetylesterase from* Aspergillus niger. [42]
84. Kim, J., Starzak, M., Preckshot, G. W., and Bajpai, R. K. (1993), *Critical*

Reactions in Ripening of Cheeses: A Kinetic Analysis. [1]
85. Ghaly, A. E. (1993), *Cheese Whey Fermentation for Single Cell Protein Production and Pollution Potential Reduction.* [63]
86. Ko, H.-H., Park, D.-H., Hwang, B., and Tanner, R. D. (1993), *On-Line Monitoring of Fresh-Weights in Carrot Hairy-Root Cultures by Conductometry.* [74]
87. Ghaly, A. E. (1993), *Dehydrogenase Activity Measurement in Aerobic Waste Treatment Systems.* [29]
88. Antoine, P., Godefroid, J., Anselme, P., De Poorter, M.-P., Vande Woestyne, M., Rolleghem, P., Verstraete, W., and Thonart, P. (1993), *A New Way of Investigation for the Control of Slurry Odours.* [103]
89. Tyson, G. J. and Jelle, L. L. (1993), *Advanced, New Wood Substitute Composites from Agricultural Biomass.* [106]