Development of a generalized platform to produce value-added chemicals from lignocellulose

Biochemical Conversions

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Abstract

The versatility of bacteria promises to solve some of our global challenges, such as production of fuels and chemicals from feedstocks other than petroleum. In the past two decades, considerable research has been conducted on microbial conversion of cheap, renewable biomass into value-added chemicals. However, cellulosic or sugar-based processes have been the main focus of such efforts to develop bio-refineries. Our long-term goal is to develop technologies that exploit the entire biomass, including plants (i.e., cellulose, hemicellulose, and lignin). To this end, we are developing a generalized platform that produces biochemicals by combining upstream thermochemical depolymerization of lignin with downstream biological conversion of phenolic lignin-breakdown intermediates. Specifically, by applying adaptive metabolic evolution, we have improved the ability of Rhodococcus opacus to tolerate and utilize various toxic phenolic compounds as sole carbon sources and to convert them into acetyl-CoA, an important biological precursor of many molecules, including triacylglycerols (TAGs). To identify the genetic causes underlying these phenotypes, we analyzed genomes and transcriptomes of evolved mutants using whole genome and whole transcriptome sequencing. In addition, genetic engineering tools have been developed to enable engineering of pathway and regulatory genes, based on our mechanistic understanding of phenolic tolerance in R. opacus. Our engineered R. opacus strains can be used to produce a diverse set of value-added chemicals and thus represents a prime example of how metabolic engineering combined with systems biology can be applied to provide a generalized chemical production platform.
Quantitative Structure-Activity Relationship (QSAR) Study on Carbonyl Inhibitors in Biochemical Conversion

Biochemical Conversions

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Abstract

Biomass pretreatment generates significant amount of fermentation inhibitors from the degradation of cellulose, hemicellulose, lignin and extractives. Substantial efforts have been made to identify inhibitors in biomass hydrolysates using analytical separation and identification tools including HPLC, GC/MS, LC/MS and NMR. Understanding of the potent inhibitors that have the most pronounced effects on microbial fermentation is yet to be established. Most currently identified inhibitors have a common functional carbonyl group (C=O). Carbonyl compounds are reactive: the positive charge of carbon can initiate a nucleophilic addition between nucleophiles and electrophiles. Consequently, the inhibitory effects of carbonyl compounds are governed by their electrophilic reactivity towards nucleophiles. The objective of this study was to identify the specific molecular descriptors that correlate molecular structure of carbonyls to their inhibitory activity. Quantitative structure-activity relationship (QSAR) modeling was used in assessing the reactivity of carbonyls. Molecular descriptors that were studied in this work included hydrophobicity (Log P), dipole moment, energy of the lowest unoccupied molecular orbital (ELUMO), energy of the highest occupied molecular orbital (EHOMO) and electrophilicity index (ω%). The quantitative information on carbonyl inhibition was evaluated based on the glucose initial consumption rate and the final alcohol yield. We identified that ELUMO is a very good global parameter to correlate the molecular structure of carbonyl compounds to their inhibitory effects. The alcohol dehydrogenase (ADH) activity in response to vanillin inhibition was determined from the batch fermentation results. Effects of acetic acid and benzoic acid on alcoholic fermentation were also studied with and without pH control. Using those data, the correlation between fermentation inhibition and carbonyl molecular descriptors were established.
Investigating production of sophorolipids from hydrolysates derived from different lignocellulosic biomass feedstocks

Biochemical Conversions

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Abstract

Candida (Starmerella) bombicola has been studied on various substrates like glucose, soy, whey concentrate, crude glycerol, oleic acid, and restaurant waste oil for the purpose of producing bio-surfactants like sophorolipids (SLs). These SLs have wide range of applications from cleaning and pharmaceutical products to soil or water remediation. To make the process of producing SLs truly sustainable and renewable, we have been investigating production of SLs from hydrolysates developed from lignocellulosic feedstocks, such as sweet sorghum bagasse (SSB), corn fiber (CF) and corn stover (CS). C. bombicola was grown on the hydrolysates with and without addition of soybean oil or yellow grease. Without addition of oil, a SL concentration of 3.6 and 1.0 g/L was detected from cultures with bagasse and corn fiber hydrolysates, respectively. With the addition of soybean oil at 100 g/L, the yield of sophorolipids from these two hydrolysates in the same order was 84.6 and 15.6 g/L. In addition, we have been evaluating SL yield from the three different hydrolysates on yellow grease. SL production rate, the overall mass balance and the composition of SLs will be elaborated in this presentation.
Computational approaches to improve thermal stability of enzyme via engineered disulfide bridges

Biochemical Conversions

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Abstract

Background: Increasing thermal tolerance of enzymes has direct consequences in increased efficiencies in bioprocessing systems. Cysteine disulfide bonds are known to provide thermal stability of enzymes. In this study we have analyzed the structure and evolution of ~18,000 proteins containing disulfide bridges in an attempt to develop computational approaches to introducing novel disulfide bridges in arbitrary enzymes.

Approach: If a cysteine is not involved in a disulfide bridge it tends to be a highly reactive and most likely an important and conserved functional residue in the protein. As a corollary, the deletion of one of the cysteines in a disulfide bridge is frequently deleterious to the protein. Statistical analysis of disulfide bridges in >10,000 proteins reveals that that approximately 40% of cysteines in a disulfide bond simultaneously disappear in related structures. In addition to the statistical distributions of the various bonds of the disulfide bridge and their context within the protein structure (i.e. the backbone angles, B factor, secondary structure and structural domains in the protein) provide us with valuable insights into the nature of disulfide bonds and allow the development of algorithms to predict how proteins may be modified to improve structural stability.

Results: The characterization of the structure and evolution of disulfide bonds is encoded as a computational system that predicts the location and feasibility of novel disulfide bonds in an arbitrary PDB structure. In the presentation we will show preliminary results that our software can predict disulfide bridges in known situations, and may constitute a rapid computational screening tool for enzyme / protein engineering.
Methane Potential of Mixed Food Waste and Algal Biomass using an Optimized Anaerobic Digestion Procedure

Biochemical Conversions

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Abstract

The anaerobic digestion process is complex and requires a carefully controlled set of conditions to be successful. To optimize the anaerobic digestion process and thus maximize the methane potential, several digester variables were optimized, including digester temperature, inoculum to substrate ratio, pH buffering capacity, anaerobic environment, and inoculum quality. Once the process was optimized, a test matrix was developed to compare the methane potentials of several feedstocks. The feedstocks tested included single substrate food wastes, mixed food wastes, and algal biomass. The algal biomass required pretreatment to disrupt the cell wall prior to digestion. The effectiveness of sonication, microwave radiation and thermal pretreatments were compared. A set of twelve 2 liter anaerobic digesters was used to determine the effect of various digester conditions and determine optimal digester conditions. Select digesters were outfitted with pH electrodes to monitor system pH and ensure inhibition did not occur due to acidic environments. Upon achieving optimal control, single substrate feedstocks with known chemical compositions were tested for their methane potential in a set of fifteen 500 mL digesters to minimize the time needed for complete digestion. The experimental potential was then compared to the predicted value based on the chemical composition. After verifying the reliability of the system, several mixed food wastes were digested to determine their respective methane potentials. Finally, various types of algal biomass were digested to determine their methane potentials. Algal biomass is difficult to digest because of its thick protective cell wall and pretreatment was required to disrupt the cell wall and allow the biomass to be digested. Several pretreatments were compared using both the methane potential and the ratio of soluble to total COD. The ratio of soluble to total COD was used to estimate the fraction of biomass available for digestion with a ratio of 1 suggesting all of the biomass was available for digestion. Elemental analysis was also performed using a CHNS-O analyzer. The results of the elemental analysis was used to estimate the theoretical maximum amount of methane that can be generated from the anaerobic digestion of the biomass. The experimental methane potential was compared to the theoretical value to determine the percentage of digestion.
USING SELECTIVE BIO-CATALYTIC ACTIVITIES AS A NEW ROUTE FOR CONVERSION OF WASTE COAL TO METHANE

Biochemical Conversions

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Abstract

Mining and processing coal generates massive quantities of coal waste, resulting in serious environmental problems that are difficult to assess as well as financial liabilities associated with its storage and maintenance. This study aimed at exploring a new route to convert coal waste to methane either in situ or ex situ via selective bio-catalytic activities. For this purpose, we elucidated the microbial structure of the formation water collected from a coal mine. Understanding the presence and distribution of bacteria and archa species in the coal mine can assist future efforts for enhancing methane production in situ. In addition, we have studied the population distribution of an adapted consortium that are dedicated for use in converting coal to methane ex situ. This consortium comprises aerotolerant bacteria and archaea can be used for producing methane from mined out coal or coal waste in bioreactors. Besides results from next generating sequencing of the original community and the adapted consortium, we have evaluated methane yield for both in a laboratory environment with and without adding a nutrient solution. Furthermore, to evaluate effect of bioconversion on coal, the residual coal was studied on its adsorption and desorption behavior. Results regarding surface area and porosity of the residual coal will be presented.
Modeling mass and heat transfer in baled lignocellulosic feedstock during solid-state aerobic fungi pretreatment and anaerobic bacteria fermentation

Biochemical Conversions

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Abstract

Heat and mass transfer during solid-state fermentation was modeled via differential equations which were solved by finite elemental analysis. Bale preparation, where temperature and moisture content were adjusted to the optimum level by flushing warm or cold water, was modeled based on 1-D heat diffusion involving conductive and convective heat transfer. The approximate preparation time was predicted to be 16 to 20 hours for the 667 m3 feedstock bunker. Solid-state fermentation was modeled based on microbial (white rot fungi or Clostridia) growth kinetics and heat diffusion. Moisture loss was significant in aerobic fungi fermentation due to constant aeration to ensure a viable oxygen concentration. By comparison, Clostridia anaerobic fermentation showed less moisture loss and less temperature increase. Also anaerobic fermentation appeared to have a homogenous microbial growth, and temperature and moisture change along the height of the bunker. White rot fungi, on the other hand, showed variations on microbial growth, and heat and mass transfer among different layers of the bunker.