

Development of a BIOREFINERY Concept for Integrated Production of Biomedicals, Biochemicals, Feed and Fuels from Selected Plant Materials (BIOREF)



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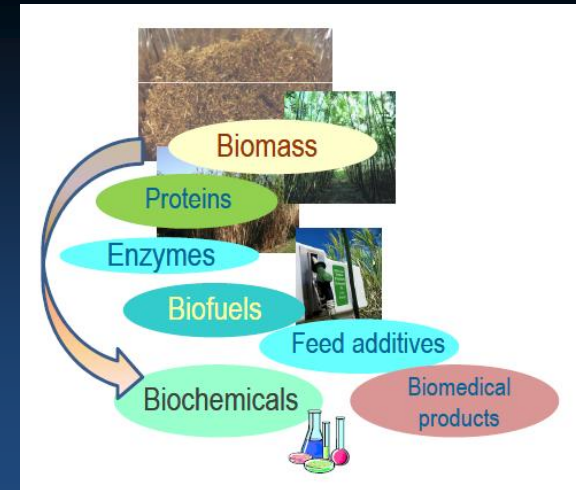
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Background

- Denmark is among the leading countries in the world within the field of lignocellulosic based bioethanol from for instance straw.
- Denmark has a flexible agricultural sector, a strong industrial basis within biotechnology and many small and medium-sized industries specialized in producing machineries and equipment for production facilities.
- To ensure that Denmark and the world will be able to meet the future demands, a large concentrated effort will be necessary involving both universities and industries.
- This program is of major importance for the Danish society and has large economic perspectives for a future industrial sector within the biofuel and biochemical area.

Overview



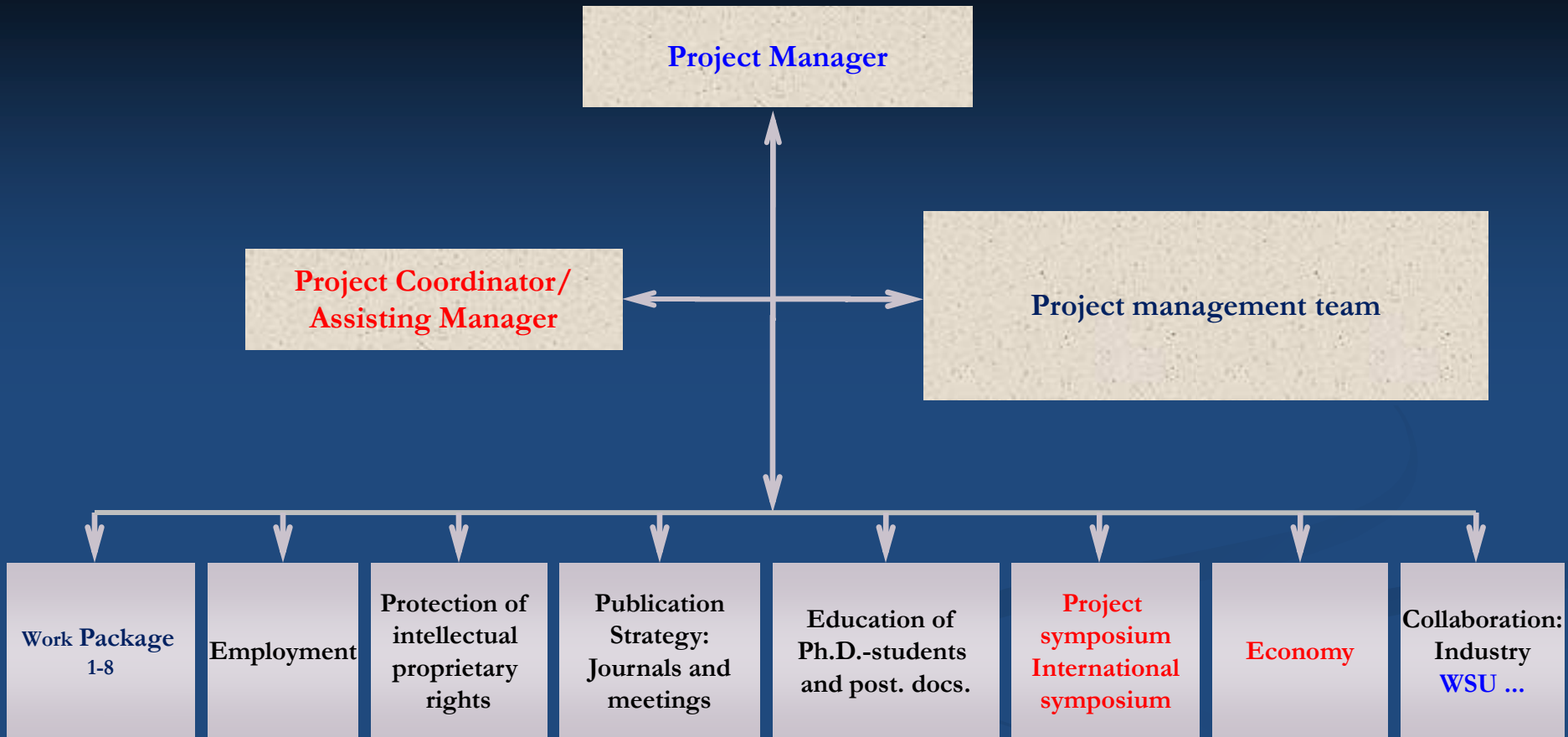
- The aim of the project is to form a biorefinery concept that allows biological production of chemicals, drugs, feed and fuels using residuals from plant materials in well-defined cell-factories.
- The idea is to use residuals that have no or low value for other uses but contain bound energy for biofuels and compounds with broad applications.
- The use of these selected low value plant materials will have an important impact on the environment by decreasing organic waste and CO₂ emissions.
- The production of biofuels and biochemicals will reduce the use of fossil fuel in the transportation sector and chemical industry.

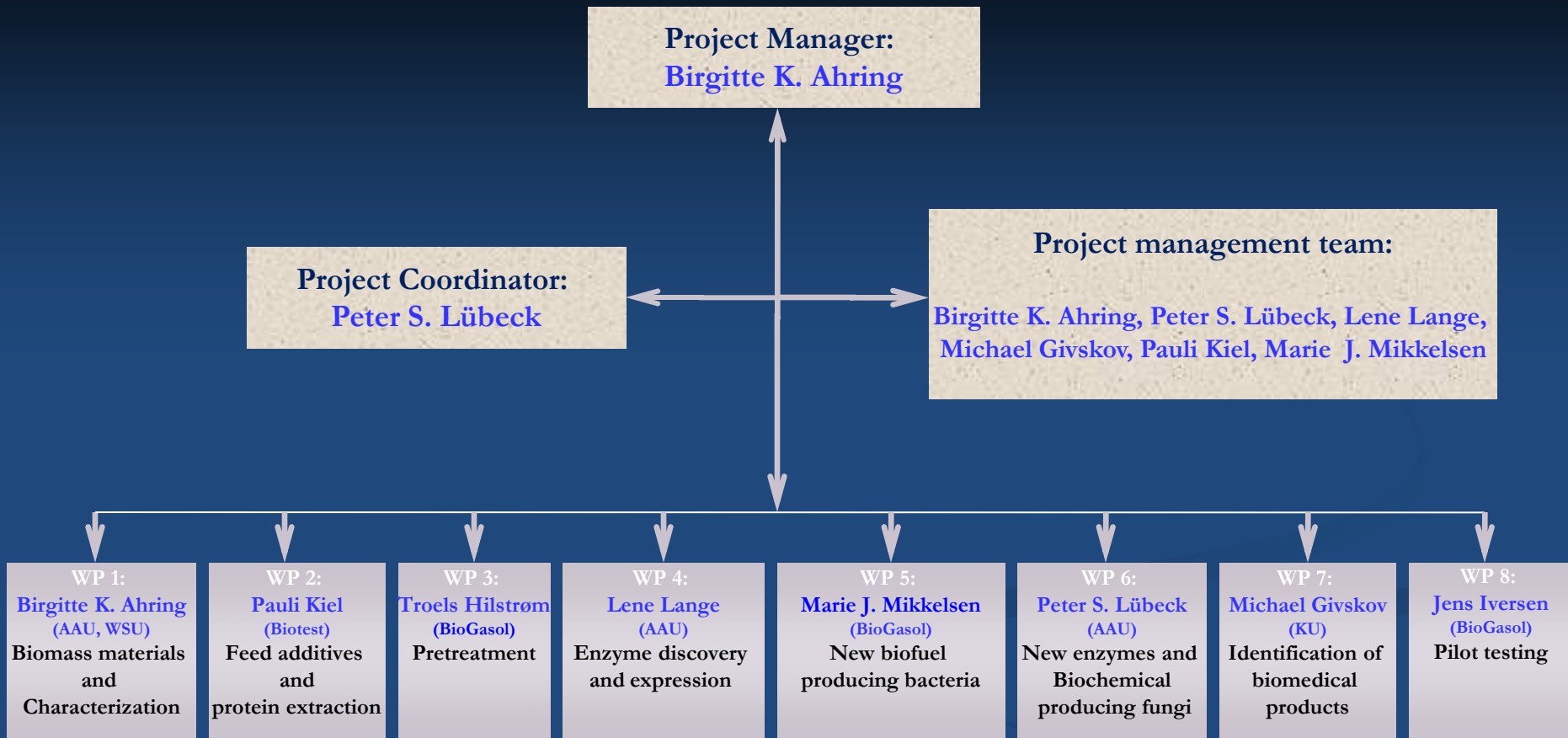
Overview

Important goals:

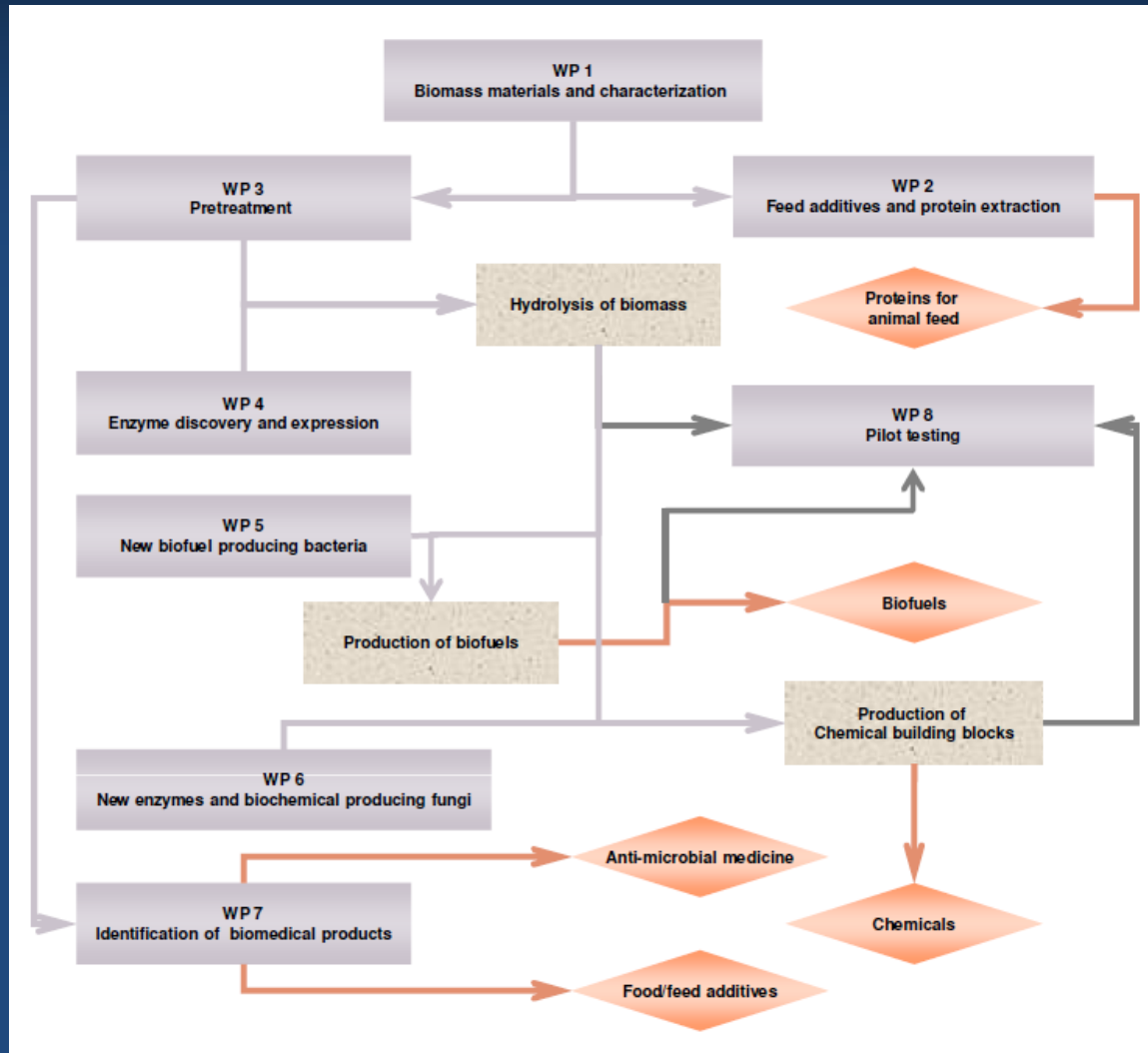
- Discovery of new biocatalysts for production of enzymes, biochemicals and fuels, focusing on thermophilic enzymes and thermophilic biofuel-producers
- Production of high-value compounds of industrial importance.
- Make the basic steps for introducing the biorefinery concept into the Danish research environment.

The ideas possess a significant potential for decreasing the over-all cost of biofuel production.





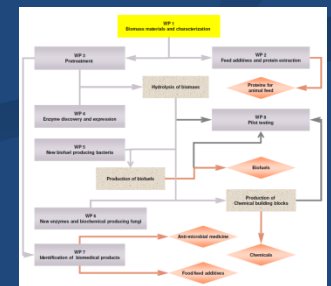
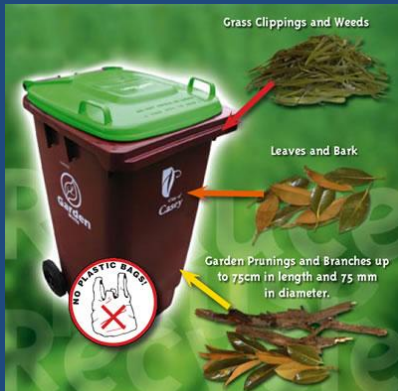
Flow in BIOREF



Biomasses materials and characterization

Aalborg University, Solum A/S

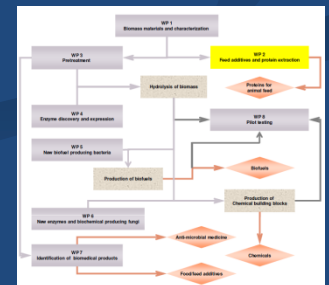
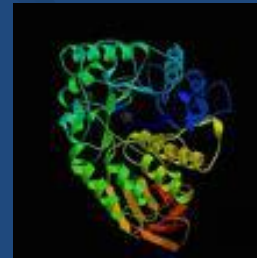
- Biomass materials from crop residues, marginal land and recreational areas (BornBiofuel).
- Biomass materials from garden/park refuse (Solum A/S).
- The garden/park refuse are delivered untreated and after exposure to different degree of composting and anaerobic digestion processes.
- The composition of the biomass are analyzed before and after the different treatments in the biorefinery process.



Feed additives and protein extraction

Biotest ApS

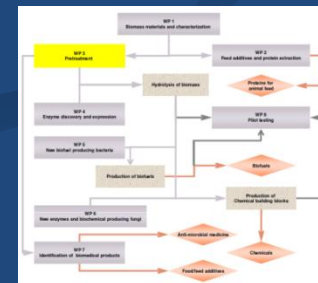
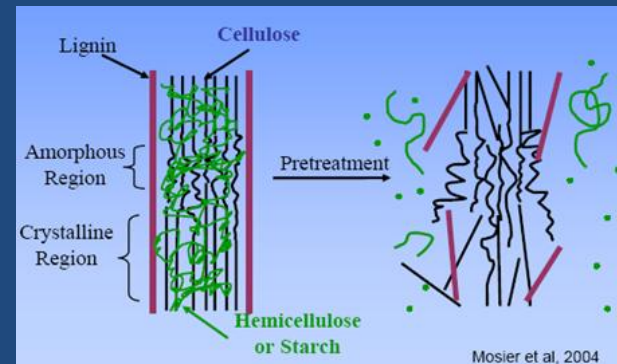
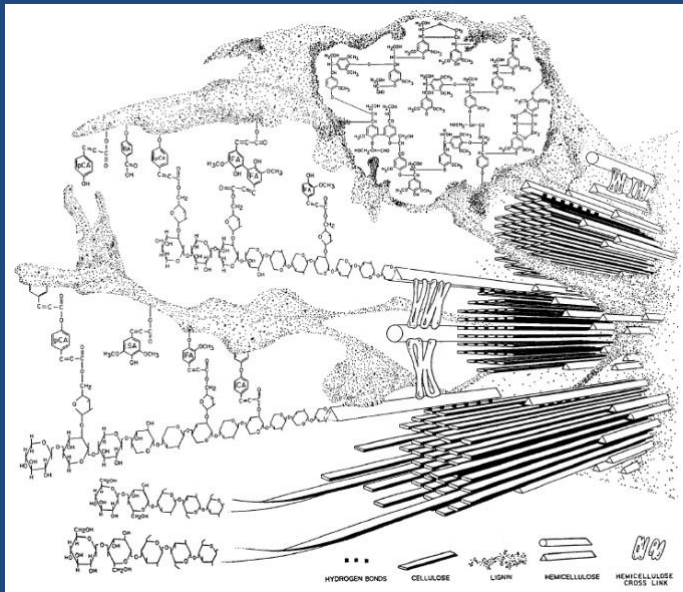
- Isolation of native, non-denatured proteins from freshly harvested plant material (**Lucerne**) before the conversion of the residue to biofuels and other products.
- Removing compounds (*e.g.* ammonia) that can harm a variety of downstream processes.
- The protocols developed depends on the final use of the proteins.
 - Main purpose is developing of protocols for isolation of proteins for feed use, but further work with proteins for food and medical applications is a future aspect to consider.



Pretreatment

BioGasol ApS

- Pretreating the biomass material using patented wet-explosion method in order to break the lignocellulotic shield.
- The pretreatment is optimized for the different biomasses and processes in the biorefinery individually and for the process as one.



Enzyme discovery and expression

Aalborg University/Mette Lübeck

Introduction of new efficient hydrolytic enzymes facilitates optimization of the biomass conversion – two strategies are used for enzyme discovery:

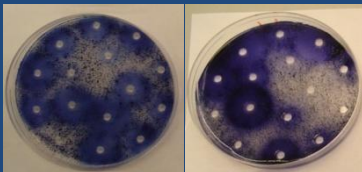
- Thermophilic/mesophilic filamentous fungi from culture collections and from new isolations from relevant habitats, e.g. composts, are grown on the biomass materials.
- The resulting enzyme blends are fingerprinted for cell wall degrading activities and the most interesting strains selected.
- Genes encoding for novel and interesting cellulases will be selected and cloned from cDNA libraries or genomic libraries.
- The selected genes will be expressed in a fungal expression system currently under development.
- The enzymes will be further characterized by testing on the pretreated biomass and the most interesting will be tested under up-scaled process conditions in a Pilot plant.



Enzyme discovery and expression

Aalborg University/Lene Lange

- Composite samples from thermophilic/mesophilic habitats, will be used as basis for construction of ecological cDNA libraries.
 - cDNA libraries will be expressed in *Saccharomyces* or *Pichia* for full length genes of successfully secreted proteins.
- Cellulases with the most interesting activities and temperature stability will be selected for further over-expression in *Pichia pastoris*.



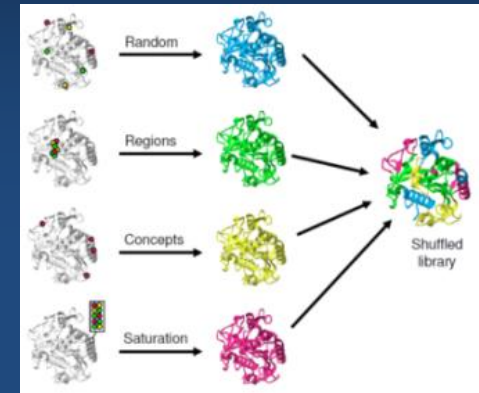
Enzyme discovery and expression

Aalborg University

The enzymes can be modified in such a way that they perform better for specific purposes:

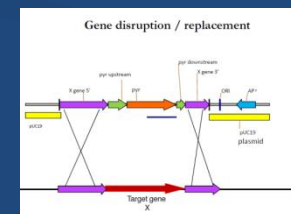
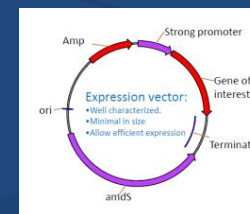
■ Improving enzymes:

- Improving enzyme activity or stability.
- Fine-tune specificity of enzymes.
- Create novel specificities and activities in enzymes.
- Change substrate specificity of enzymes.
- Change stereospecificity of enzymes.
- Create thermostability.



■ Improving microorganisms:

- Optimize existing pathways.
- Create new pathways for synthesis of novel compounds.
- Delete unwanted site effects as proteases and secondary metabolites.



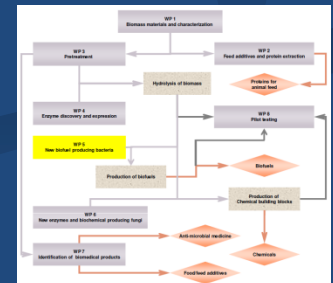
New biofuel producing bacteria

Aalborg University, BioGasol ApS

- Development of thermophilic bacteria that produce liquid biofuels from carbohydrates obtained from biomass hydrolysis by genetic modification .
- Modification of the thermophilic bacterium *Caldicellulosiruptor*, which is naturally able to degrade cellulose to enhanced alcohol production (improve process economics).
- Improvement of xylanolytic thermophilic bacteria by genetic modification for improved alcohol production.
- The residue after alcohol production will be tested for its methane and hydrogen potential.



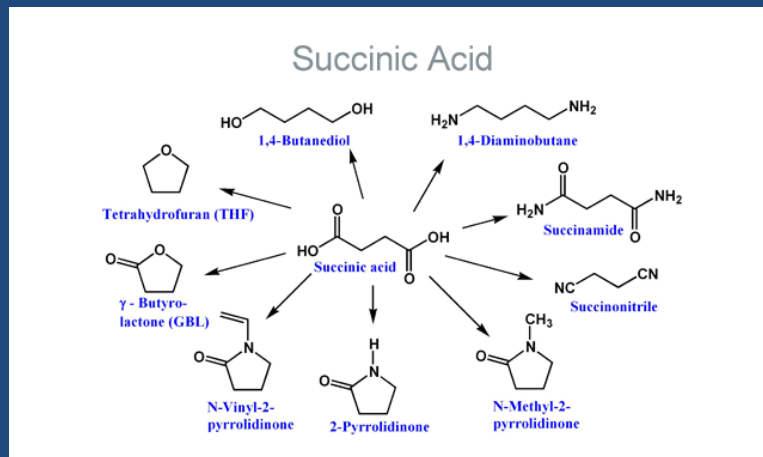
Jonathan M Gitlin, 2008



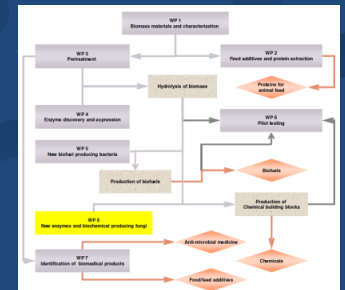
New biochemical producing fungi

Aalborg University/Peter Lübeck

- Biochemicals can substitute petroleum-derived chemicals
 - E.g. Maleic anhydride can be substituted by succinic acid produced by microorganisms.
- Fungi are tested for their ability to produce succinic acid and other acids when cultivated on pre-treated biomass material.
- A few strains that produce succinic acid most efficiently or strains that are identified as good organic acid producers are selected for genetic engineering.
- Based on knowledge from metabolic modelling studies of the production of succinic acid, pathway engineering of relevant genes will be carried out.



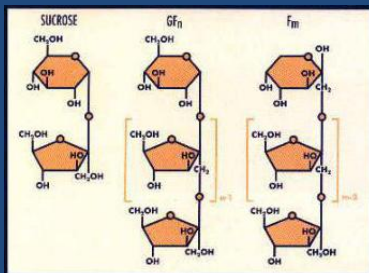
Adapted from U.S. Department of Energy Top Ten Chemicals from Biomass Report



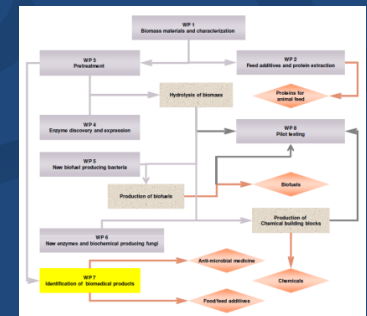
Identification of biomedical products

University of Copenhagen

- The degradation of biomass can lead to a range of products useful in the food, feed and medico industry.
- From the lignin fraction, potential blockers of bacterial cell to cell signalling (Quorum Sensing (QS)) which can prevent bacterial infections, are identified.
- In addition, sugars that can be used as prebiotics with anti-microbial activities will be identified.
- Test of the efficiency of these novel anti-microbial measures on infective animal models.



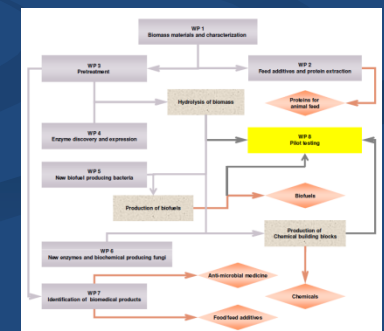
Prebiotics, James Collier, 2004



Pilot testing BioGasol ApS

Testing the biorefinery in pilot plant scale:

- Selected cellulolytic enzymes identified and expressed in selected production strains will be tested on the biomasses un-treated and pretreated.
- Developed biofuel production bacterial strains will be tested on the hydrolyzed biomass.
- Further testing in the pilot plant will depend on the results obtained in the other work packages.



Participants

Universities:

Aalborg University:

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Professor Lene Lange

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Ph.D.-student

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Manager Pauli Kiel

Assistent Margrethe Kiel

Solum:

Chief Technology Officer Morten Kristensen

Assistent