ChiBio

Development of an integrated biorefinery for processing chitin rich biowaste to specialty and fine chemicals

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http://www.chibiofp7.fraunhofer.de/

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Publishable Summary (Final Report, December 2014)

Executive summary of the project

The processing of prawns, crabs and shrimps results in the accumulation of large quantities of shell waste. Every year over six million metric tons of these crustacean shells are thrown away, with an estimated several hundred thousand tons of these being within the EU alone. Theoretically the shells could also be valorised. They contain chitin, a biopolymer also occurring in insects and fungi, that consists of nitrogenous sugar molecules (N-acetylglucosamine) strung together in a polymer chain. A use has already been found for a small part of this biogenic resource and in Asia, for example, chitin from crab shells is utilised for the production of chitosan for biomedical applications or as a food additive. Because of the higher proportions of calcium carbonate, the utilisation of European shells is less economical and not very common. Nevertheless, proper disposal of the shell waste – material which poses a contamination risk – is complex and costly owing to EU and country-specific regulations. In the EU-funded ChiBio project, an international team of scientists is working on new processes for developing the shell waste accumulating in the EU as a raw material's source for specialty chemicals. ChiBio is adopting the integrative and sustainable approach of a biorefinery, i.e. the complete utilisation of biogenic raw materials by consecutive material and energetic utilisation process steps, in order to use the biogenic waste material as efficiently and completely as possible.

Summary description of the project context and the main objectives:

The processing of prawns, crabs and shrimps results in the accumulation of large quantities of shell waste. Every year over six million metric tons of these crustacean shells are thrown away, with an estimated several hundred thousand tons of these being within the EU alone. Theoretically the shells could also be valorised. They contain chitin, a biopolymer also occurring in insects and fungi, that consists of nitrogenous sugar molecules (N-acetylglucosamine) strung together in a polymer chain. A use has already been found for a small part of this biogenic resource and in Asia, for example, chitin from crab shells is utilised for the production of chitosan for biomedical applications or as a food additive. Because of the higher proportions of calcium carbonate, the utilisation of European shells is less economical and not very common. Nevertheless, proper disposal of the shell waste – material which poses a contamination risk – is complex and costly owing to EU and country-specific regulations. In the EU-funded ChiBio project, an international team of scientists is working on new processes for developing the shell waste accumulating in the EU as a raw material's source for specialty chemicals. ChiBio is adopting the integrative and sustainable approach of a biorefinery, i.e. the

complete utilisation of biogenic raw materials by consecutive material and energetic utilisation process steps, in order to use the biogenic waste material as efficiently and completely as possible.

Stabilisation of the shells and mobilisation of chitin

In order to prevent the natural rotting process and therefore to avoid any contamination risk, in a first process step methods are being developed to pre-treat and stabilize the waste shells thereby making them storage-stable and transportable. In this step especially the remaining crab meat sticking to the shell is removed. For this so-called mobilisation process, ChiBio is combining chemical with biotechnological (microbiological or enzymatic) methods for a chitin mobilisation as mild as possible. Because biocatalysts are highly specific, biotechnical processes are in many cases more energy- and resource-efficient and thus more environmentally compatible than chemical methods alone. After separating these biomass residues, which consist of proteins and fats, they are directly digested – together with all other biogenic by-products obtained in the overall process – by specific microorganisms to generate biogas as an energy carrier, thus allowing a full usage of the crab shell waste stream.

Enzymatic degradation of chitin

The purified chitin has to be split into its monomeric components (chitin lysate), the nitrogenous sugars N-acetylglucosamine and (after deacetylation, that means cleavage of the acetyl function) glucosamine, respectively. Chemo-catalytic methods used for this degradation step are not particularly sustainable. ChiBio therefore is using specific enzymes or microorganisms as biocatalysts for this process step as well. In contrast to the most abundant sugars like glucose or fructose, both N-acetylglucosamine and glucosamine contain a nitrogen atom, which makes them very interesting for applications in the chemical industry. In nature, chitin is degraded by microorganisms, which use specific enzymes for this task. ChiBio develops biocatalytic degradation methods using these natural chitin-degrading enzymes from fungi and microorganisms (e.g. strains of Trichoderma, Aspergillus and Bacillus) to obtain N-acetylglucosamine and glucosamine as products. For an efficient and economic technical process however, these enzymes have to be optimised to adapt them to the industrial, non-natural conditions and to speed up the chitin degradation which takes a long time in nature.

Functional monomers for polymerisation

N-acetylglucosamine and glucosamine are the monomeric products of the degradation process. Before they can be used as a starting material (or platform chemical) for the catalytical synthesis of new polymers, they have to be equipped with at least two functional groups for linking the single monomers to a polymer chain. ChiBio is pursuing two strategies for this great challenge of obtaining those functional monomers. In the first route, the chitin lysate (containing N-acetylglucosamine and glucosamine) is used as a carbon and energy source for the growth of specialized yeast cells. These cells degrade N-acetylglucosamine and glucosamine and hereby produce functionalized fats/fatty acids and the corresponding aminocarboxylic acids. In a second route, ChiBio develops a multi-enzymatic process to produce functionalized heterocycles from glucosamine. Both routes yield N-containing functional monomers for the polymer industry. N-containing compounds for the production of polyamides and isocyanates are of particular interest for the polymer industry and so far it has not been possible to produce them on the basis of renewable raw materials.

Prior to the production of new bio-based polymers from the functional monomers, these have to be purified; ChiBio develops strategies for this task and uses the purified molecules for polymerisation trials. Subsequently, the polymers are characterized for their application in the industry. All steps in the whole project are additionally evaluated by Life Cycle assessment studies. Therefore the goal of ChiBio is not only to use renewable waste streams as raw material for new polymers but additionally developing a sustainable and economically viable process for this challenge.

Description of the main S & T results/foregrounds

We have developed more sustainable methods for the pretreatment of crustacean shell wastes and the mobilisation of chitin. This includes a moderate chemical precipitation step to remove the lipid and protein fraction, and the identification of 2 novel strains for the fermentative mobilisation of chitin. Moreover, the composition of the shell wastes was monitored on a monthly base to show seasonal and typal variations. An emphasize of these works was put on the pretreatment of European shell wastes (e.g. brown crab). The separated lipd and protein fraction was intensively studied as substrate for anaerobic digestion to biogas and gave good results (293 – 400 mL/g TVO) being in the range of common organic waste materials.

Next to that ChiBio made available a complete set of enzymes & enzymatic cocktails from existing and novel strains for the depolymerisation of chitin (and chitosan) to either gain hydrolysates or the corresponding monomeric sugar units such as N-Acetylglucosamine and glucosamine. This includes the proposed upscaling and demonstration activities where the most relevant enzymes where made available at the multi gram level in >200 L fermentations after feasible expression hosts were identified.

Thus, we have been able to degrade various forms of pretreated chitin with efficiencies that are promising, considering the fact that we only had 3 years for all the optimization work. Specifically when considering that the development of an efficient depolymersiation process for cellulose took 30 years and more. Several promising oleaginous yeast strains growing on glucosamine or shell hydrolysate have also been identified. These are capable of producing ca. 35 -50 % lipids / dry cell weight whereas most of the lipids produced are unsaturated fatty acids of chain length C16-18.
We also developed technically feasible purification protocols for sugar monomers, lipid congeners and N-containing heterocyclic monomers. The purified monomers i.e. the di carbonic acids were successfully used as copolymers in polyamides (1-10 % w/w) giving promising results for the lipids from the yeast oils. The results for the di carbonic acid based on the N-containing heterocylce were less promising as it seemed to make the material brittle. The full platform of polymer characterisation was performed for 2 selected prototypes that were produced in kg-amounts.

A full set of process relevant data was collected from all partners working on ChiBios process chain and used to set up a realistic LCA model. Our process engineers worked out the full process chain of the full ChiBio biorefinery including costing.

Description of the potential impact (including socio-economic impact and the wider societal implications of the project so far) and the main dissemination activities and the exploitation of results

It is an overall goal of ChiBio to improve the sustainability of the biomass processing industry and to increase the competitiveness of the European biotechnology industry. ChiBio will convert an industrial biowaste stream (Crustacean shells, e.g. crab shells) that accumulates in significant amounts in numerous European countries (mainly in Ireland, Norway, UK, the Netherlands, Germany, Denmark, France, Iceland etc.) as well as worldwide (e.g. in Asia and Africa) posing a severe risk to the environment into valuable bio-based plastics.

The aim of ChiBio was not simply to replace fossil oil as starting material, but to provide bio-based products with specific innovative properties that have advantages over other products, e.g. that are less-toxic to soil and water. Elemental parts

of the process chain including the pretreatment of the shell waste and conversion into biobased bifunctional monomers will employ and advance the full range of known biotechnological and genetic methodologies.

Following the proposed cascading approach, all separated by-products (proteins, lipids etc.) will be used as feed for biogas-production (as alternative energy source). Worldwide discards of shellfish wastes exceed 20 million tons per annum; if simply disposed in landfills, the chitin-rich wastes are considered hazardous due to their high perishability, becoming rapidly colonized by pathogens and spoilage organisms, causing environmental and public health concerns. The EU and other countries have responded to these issues by setting a specific maximum limit for biodegradable municipal wastes that may be disposed in landfills and at sea. In practice, in maritime European countries such as Ireland where local fisheries are an important source of local wealth and employment, this means an additional monetary burden for local industry for the European shell fish industry especially for the SMEs. Since most of this industry is situated in economically deprived rural areas with high unemployment rates, this additional financial burden can jeopardize the future of the European shellfish processing industry.

The isolation of chitin from shellfish waste is an established industrial process that consists of three chemical steps utilising large quantities of harmful components. The chemical removal of CaCO3 by mineral acids releases considerable amounts of CO2 and leads to the accumulation of salts as a precipitant, which are problematic in downstream processing operations and have a negative environmental impact. The removal of CaCO3 and protein fractions is usually carried out at high temperatures ~100-300°C, which is not energy efficient. Therefore, the aim of ChiBio is to selectively solubilise chitin and protein factions, while at the same time optimizing the existing biotechnological degradation methods.

Moreover, the subsequent depolymerisation step on the industrial level is also based on more or less harsh chemical processes and represents serious market hurdles relative to regulatory requirements and consumer perception and results in negative eco-efficiencies. Clearly, an enzymatic conversion of chitin/chitosan directly to its monomeric units N-Acetylglucosamine and glucosamine is the most economical and environmental sound strategy and will be assessed in-depth by the ChiBio consortium.

Finally, the target compounds of the ChiBio process chain are bifunctional monomers (e.g. N-containing heterocycles or lipid congeners) that will be specifically tailored to the European polymer industry needs and act as “drop-in” synthons that can easily be integrated into existing high value polyamide production processes of our industrial partners without any further chemical modification steps.

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