INDIVIDUAL FELLOWSHIPS

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AquaCat, *June 2012 – May 2014*
***GA-No.* 301723 (FP7-MC-IIF)**
http://www.igb.fraunhofer.de/de/kompetenzen/biocat/aquacat.html

Publishable Summary, July 2014

"AquaCat" was an IIF project (Grant no. 301723), funded by the European Union FP7 in accordance with the strategic research agenda in the area of "biocatalytic process design". The project started June, 2012 and ended May, 2014. Experiments were performed in Germany (Straubing) at Fraunhofer IGB, Project Group BioCat for 20 months and in France at ICEEL, Laboratory of Macromolecular Chemical Physics in Nancy for 4 months. AquaCat wanted to bring sustainable solutions to the production of two economically important commercial products; polylactone nanoparticles (Fig. 1 a & b) and sucrose fatty acid esters. Both find extensive application in the field of biomedicals, food&feed, cosmetics and pharmaceuticals.

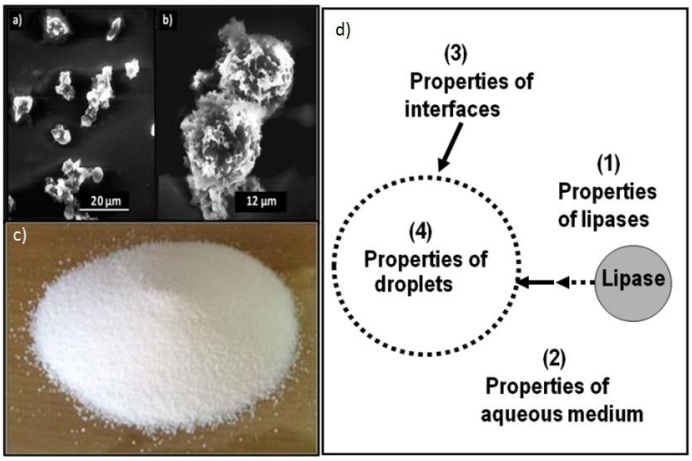

Fig.1:

a) & b) SEM-pictures of polylactone nanoparticles

c) purified sucrose ester and

d) Model of reaction mechanism for ROP of polylactone nanoparticles.

AquaCat wanted to circumvent major problems in the current manufacturing processes involving the use of toxic organometallic catalysts, hazardous organic solvents, high energy consumption and the laborious multi-step work-up of nanoparticles by working in aqueous dispersions. Therefore we applied lipases (a class of enzymes, i.e. biocatalysts) to a tailored microenvironment, designed the reaction medium making lipase function as synthetic catalyst of confined organic substrates and identified the relevant parameters for industrial scale up of these processes. The general objective was to develop sustainable industrial processes to synthesize polylactones derived from ε–caprolactones of sufficiently high molar mass (> 10.000 g mol^{-1}) in water for application as drug carriers and sucrose fatty acid esters having 1 to 3 fatty acid ester substitutions as bio-surfactants, respectively. This general objective can be translated into 2 more specific objectives:

1) Engineering of the aqueous biphasic system for the synthesis of poly(lactone)

The basic knowledge on the lipase-catalyzed ring-opening polymerization (ROP) of lactones in water was not available before starting with AquaCat. Consequently, we had to start with a systematic study on the four keys parameters influencing the lipase-catalyzed ring-opening polymerization in aqueous medium; (1) properties of lipases, (2) properties of aqueous medium (3) qualities of lactone/water interface, and (4) properties of droplets (Fig.1 d)). ω-pentadecalactone (ω-PDL), was chosen as model substrate thanks to the ease of emulsion preparation with controlled properties. Using lipase PS (*Burkhodoria cepacia* lipase), the influence of interfacial properties, influence of kinetic parameters as well as the influence of percent lipase PS adsorption capacity at ω-PDL/water interface on the molar mass of poly (ω-PDL) was elucidated. The obtained results allowed the development of a model describing how the lipase PS catalyzed the ROP of ω-PDL in biphasic system. On the

basis of this model we were able for the first time to synthesize oligo (ω-PDL) with a molar mass of 3557 g mol^{-1} in water.

The research was then moved to the synthesis of more relevant polylactones such as polycaprolactones. The direct application of the model established for ω-PDL was problematic due to the hydrophilicity of the ε-CL/water interface onto which the necessary adsorption of most lipases is more difficult. Only lipases without interfacial activity such as CALB (lipase B from *Candida antarctica*) catalyzed the polymerisation of ε-CL in aqueous dispersion. Moreover, immobilization of CALB enabled a significant increase of the number-average degree of polymerization of ε-CL oligomers (up to 38) as compared to dissolved CALB (8 at the maximum). The understanding on the synthetic activity of lipases in water allowed the synthesis of oligolactones of molar mass about 4000 g mol^{-1} in water. According to the model, the limit of this technology to achieve the molar mass of 10.000 g mol^{-1} comes from the low interfacial activity of lipases. Reaching this goal was not possible with commercially available lipases and future works should try to increase lipases' activity by genetic engineering. Significant results generated from lipase-catalyzed ROP of lactones in aqueous dispersions were published in 2 peer-reviewed journals [DOI: 10.1016/j.molcatb.2012.11.008 and DOI: 10.1016/j.colsurfb.2013.09.011].

2) Design of a lipase-catalyzed process for the synthesis of sugar esters as a proof of concept

Sucrose and fatty acids as well as fatty acid esters were chosen as starting materials for lipase-catalyzed esterification processes (Figure 2). At first, efforts were spent on the preparation of biphasic media containing sucrose and the fatty acid enclosed in the same droplets, dispersed in aqueous phase. This was not successful due to very high polarity of sucrose. Therefore, the knowledge obtained from the synthesis of polylactones could not be directly be applied to the synthesis of sucrose esters in biphasic medium. To achieve AquaCats objective the synthesis of sucrose ester a new concept was established which consisted in increasing the reactivity of sucrose, the selectivity of lipases toward sucrose, the reduction of water activity as well as by substitution of water with another inert solvents with enhanced properties in solubilizing sucrose and esters. The combination of these strategies allowed us to set-up a feasible process for the selective enzymatic production of sucrose esters. The process we developed is considered to be industrially relevant due to its high possible substrate loads (100 g L^{-1} to 400 g L^{-1}), due to complete conversion in short time and under mild reaction and due to the selectivity towards the degree of substitution varying from 1 to 3. Moreover, a simple downstream process was set up to purify the obtained sucrose esters. This process was easily scaled up to achieve the synthesis of gram amounts of different sucrose fatty acid esters.

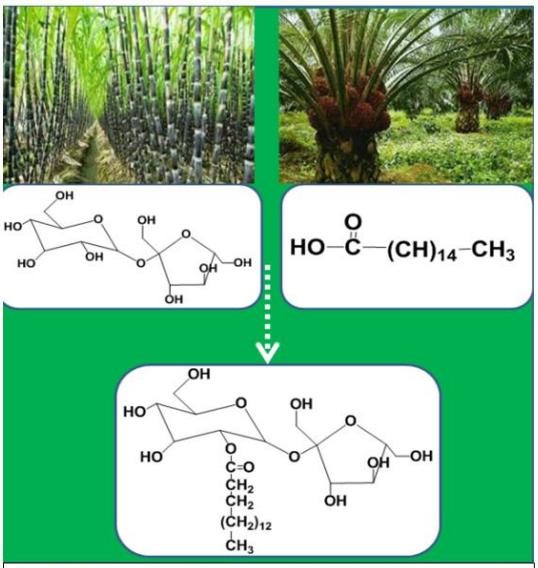

Fig.2: Sucrose and palmitic acid as substrates for the enzymatic synthesis of sucrose esters.

AquaCat relates to a process for producing novel nanoparticles and sugar esters which are mid- to high-value compounds with a high application potential in the European Chemical, Food and Pharmaceutical Industry. In accordance with the strategic research agenda in the area of "Biocatalytic process design", the novel principle of lipase-catalyzed synthesis in aqueous biphasic systems bring technological solutions to the conventional process involving the conversion of oleochemicals and carbohydrates substrates in general. The publication and application of AquaCat principle is thus beneficial, not only for the scientific society working in the domain of green chemistry, but also for the industries and policy maker involving in the sustainable chemistry.

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