MECHANICAL ENGINEERING | PHYSICS | PRESERVATION OF THE ARCHITECTURAL HERITAGE | SPATIAL PLANNING AND URBAN DEVELOPMENT | STRUCTURAL, SEISMIC AND GEOTECHNICAL ENGINEERING | URBAN PLANNING, DESIGN AND POLICY | AEROSPACE ENGINEERING | ARCHITECTURAL COMPOSITION | ARCHITECTURE, BUILT ENVIRONMENT AND CONSTRUCTION ENGINEERING | ARCHITECTURE, URBAN DESIGN, CONSERVATION OF HOUSING AND LANDSCAPE | ARCHITECTURAL, URBAN AND INTERIOR DESIGN | BIOENGINEERING | DESIGN | ELECTRICAL ENGINEERING | ENERGY AND NUCLEAR SCIENCE AND TECHNOLOGY | ENVIRONMENTAL AND INFRASTRUCTURE ENGINEERING | **INDUSTRIAL CHEMISTRY AND CHEMICAL ENGINEERING** | INFORMATION TECHNOLOGY | INTERIOR ARCHITECTURE AND EXHIBITION DESIGN | MANAGEMENT ENGINEERING | MATERIALS ENGINEERING | MATHEMATICAL MODELS AND METHODS IN ENGINEERING

PhD Yearbook | 2018

DOCTORAL PROGRAM IN INDUSTRIAL CHEMISTRY AND CHEMICAL ENGINEERING

Coordinator:
Prof. Alessio Frassoldati

The Reference Committee
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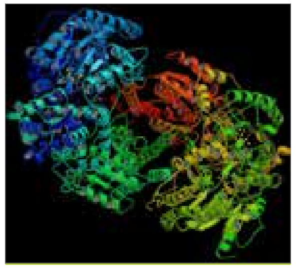
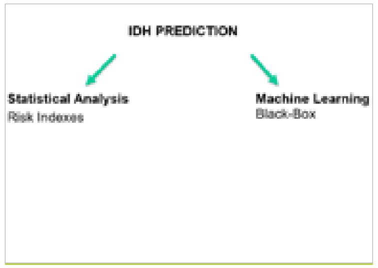
Grants
Pirelli, Captive Systems, Humanitas, EU H2020-MSCA-ITN-2014.

The Doctoral Program in Industrial Chemistry and Chemical Engineering (CII) is designed for students aiming to get a deep expertise on chemical processes and on material processing either as the inherent research or in the process design and development activities. The program is the ideal extension of the Laurea Magistrale degrees in Chemical Engineering, Safety and Prevention Engineering, Materials Science, Material Engineering, Industrial Chemistry and Chemistry, but it is also open to graduated in other scientific disciplines.

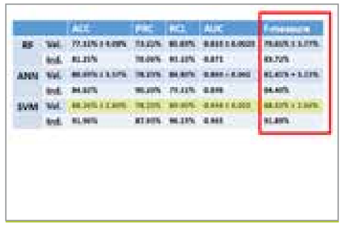


Structure simulation of Nitrogenase enzyme for N_2 conversion to NH_3

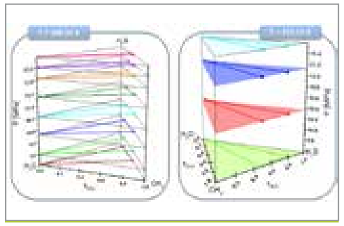


Amyloidal peptide nanohelices in solution as ordered nanomaterials (Nature Commun. 2015, 6:7574, DOI: 10.1038/ncomms8574)

The general topic for the doctoral program in Industrial Chemistry and Chemical Engineering is the application of the chemical and physical knowledge to all the activities related to the synthesis, design, production and transformation of chemical substances and materials (like advanced inorganic, polymers and biomaterials). These studies involve not only the synthesis processes but also the related plants, here analyzed starting from the laboratory tests and the pilot plant experiments up to the industrial size ones, always including the careful evaluation of the related energetic, safety and environmental issues. The final goal is to provide to the PhD student the tools and the skills: (1) to design and manage industrial processes at any size scales; (2) to develop new technical applications and (3) to create and to characterize new products and services. The ***CII*** program covers three years for an overall amounts of 180 credits. The responsibility of the organization and of the contents of the doctoral program is attributed to the Professors Committee (PC). At the beginning of the Doctoral program, a tutor and a thesis advisor is assigned to each student. The tutor has a supervisor function during the whole doctoral program, whereas the thesis advisor is responsible for the thesis work.

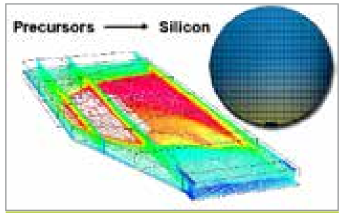


"Biocompatible polymer nanoparticles functionalized with a fluorescent dye for in vivo imaging studies"



VLE and VLLE measurements for the system CH4-H2S-H2O.

The PC defines a specific curriculum for each student, on the basis of the tutor suggestions. The curriculum has to be designed in order to both cover deficiencies in the student scientific formation and to give a high-quality technical and scientific preparation. Since 2001 (XVII cycle) the ***CII*** program graduated more than 100 students, being ~80% now working in industry and the remaining in the university or government research centers. These data demonstrate the great link existing between the performed researches and the industry. In fact, during the years, numerous the research topics were directly supported by industrial companies, like ENEL, LPE Epitaxial Technology, Bracco, Flamma, Mapei, Solvay Specialty Polymers, RSE, Isagro, Tecnimont, Biochemtex, Pirelli.



Simulation of a chemical vapor deposition reaction for silicon films deposition

Hazardous gas dispersion simulation from an industrial accident

CHEMO-ENZYMATIC DEPOLYMERISATION OF LIGNINS

Chiara Allegretti - Supervisor: Prof. Paola D'Arrigo

The experimental work performed in these three years of PhD, locates within the broad field of lignin valorization studies. Lignin represents more than 30 % (by weight) of the total biomass of the earth biosphere and it is one of the three major subcomponents of lignocellulosic biomass.

Although burning lignin still represents a valuable contribution for saving fossil sources, lignin also offers perspectives in terms of higher value-added applications. In fact, after the hydrolysis of lignocellulose polysaccharides, lignin remains as a solid residue, representing one of the major renewable sources for biofuels and fine chemicals (mainly aromatics) production. The main limitation to practical applications for lignin so far is presumably due to the difficult and challenging processing for depolymerisation and valorisation. In this respect, the challenge is to combine chemical and enzymatic methods in order to obtain selective cleavages of the polymeric skeleton. The work of my PhD is inserted in the context of the Valor Plus European Project "Valorisation of biorefinery by-products leading to closed loop systems with improved economic and environmental performance" (FP7 EC-KBBE-CALL-7 grant agreement FP6-KBBE-2013-7-613802 (2013-2017)) that aims to set-up a chemo-enzymatic process for the controlled depolymerisation and transformation of standard lignin feedstocks to value product streams.

The work accomplished in this PhD project is divided into three main sections: Part A - The Lig enzymatic system, Part B - Lignin fractionation, Part C - Lignin depolymerisation.

At first, the Lig enzymatic system (Part A) was studied, which is able to cleave the β-*O*-4 aryl ether linkage, which represents about 50 % of all ethers in various lignins. The cleavage of this type of bond was identified to be part of lignin catabolism in the proteobacterium *Sphingobium* sp. SYK-5. Five enzymes were identified as being involved in this degradation process: LigD/L (Cα-dehydrogenases), LigF/E (β-etherases) and LigG (glutathione lyase). The activity of these recombinant enzymes obtained in our laboratory "The Protein Factory" has been tested on the lignin dimeric model compound 1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol as substrate, proving that this enzymatic system is able to efficiently cleave and process this type of chemical bond in the presence of NAD$^+$ and glutathione. Kinetic studies on the model compound have been performed and the whole process has been optimized.

In parallel to this project, a tool box of enzymes composed of many different classes of enzymes aiming to perform selective transformation of lignin functionalities has been built including commercial enzymes, recombinant enzymes and enzymes obtained through a metagenomics approach. The preliminary unsuccessful results obtained when the unmodified raw material (Protobind™1000 provided by the technical manager of the Valor Plus Project) has been incubated with different enzymes indicated that the enzymatic biotransformations required a more homogeneous starting material.

The development of a pre-treatment strategy became necessary (Part B) to separate the small molecules from the bulky material before carrying out other tests. The first approach has been the dialysis of raw lignin in order to obtain two different fractions, a retentate rich in high molecular weight molecules, and a permeate enriched in small compounds. A further step towards a physical separation in terms of molecular weight has been performed in collaboration with Biobasic Environnement (Saint-Beauzire, France), an industrial partner of the European Project Valor Plus. Two different approaches have been developed. The first method involved a microfiltration followed by two ultrafiltration steps on a starting solution of Protobind™1000 in ethanol/basic water. The process has been set-up with two different sets of membranes (membrane in polyethersulfone with a cut-off of 3 kDa and 1 kDa and membranes in stabilized cellulose with a cut-off of 5 kDa and 2 kDa). The second method consisted of a soxhlet extraction of Protobind™1000 in methyl ethyl ketone (2-butanone, MEK) followed by two ultrafiltration steps using stabilized cellulose based membranes with a cut-off of 5 kDa and 2 kDa. This second method has also been applied to another industrial lignin (Kraft). All the different fractions obtained from these sequential ultra-filtrations had been fully characterized by GPC, GC-MS, ESI-MS, DSC, TGA, GFC and HPLC. Unfortunately, when these more homogeneous fractions (Part C) have been incubated directly with different enzymes, no degradation has been identified. Probably, this was because these polymers were too bulky to be efficiently attacked and modified by the protein activity, and therefore a new approach has been developed based on a two-step cascade process. The rationale behind this approach was that a first oxidation step (which could be chemical or photochemical) was required to partially cleave and oxidize the polymeric skeleton in order to make it more accessible to the enzymatic activity.

Therefore, the optimization of the parameters concerning the chemical degradation approaches were performed, arriving to the conclusion that the best results have been obtained when the samples have been treated with NaBr, NaClO, and TEMPO. However, the chemical approach is always linked to the use of harsh conditions, solvents to extract the products and expensive catalysts. On the other hand the use of a photochemical path is very appealing as it would be a greener alternative since it relies on UV light to catalyze the depolymerization. The photocatalytic step therefore exploits the oxidation capability of UV-irradiated ZnO nanoparticles in order to establish a water based greener oxidative pathway. The photochemical degradation tests provided quite promising results, as the GPC analysis indicated a clear reduction of both Mn (number-average molecular weight) and PDI (polydispersity index). These partially degraded products obtained both from the first photochemical and chemical oxidations have then been tested as substrates for different enzymatic bioconversions.

Different commercial laccases: LAC 3 from *Trametes spec.*, LAC C from *Trametes versicolor* and LAC F from *Funalia trogii* provided by ASA Spezialenzyme partner of the Valor Plus Project have been assayed.

The time course experiments performed on all three enzymes highlighted the best operative conditions for the bioconversions.

The cascade process indeed leads to a clear reduction of the Mn and Mw values, even if the total yields in terms of mass recovery of the two-step process on 1 g scale in each case does not exceed 20 %. Further studies are now in progress for the optimization of the whole process for the application in a pilot plant.

AG2S^{TM} (ACID GAS TO SYNGAS) TECHNOLOGY

Andrea Bassani - Supervisor: Prof. Flavio Manenti

CO_2 is dramatically affecting our life: climate change and consequent social-economic problems are identified as Grand Challenges of current century. Indeed, it is estimated that average overall CO_2 emissions were 35 Gt/y in the last years. Unfortunately, due to its thermodynamic stability and low chemical value, CO_2 presents minor industrial uses. One of its most important consumptions in chemical plants is in urea synthesis. Catalytic dry reforming or hydrogenation of CO_2 represent a viable application for CO_2 consumption; nevertheless, these have not been successfully applied to industrial scale yet, because of considerable energy consumption and need for large amounts of hydrogen. Direct production of methanol from CO_2 is also a potential route, but catalysts and reactors are still in a research phase. However, a new potential way to reuse CO_2 is available: AG2S^{TM} technology. The latter allows to convert CO_2 into syngas by means an oxy-reduction reaction with H_2S which takes place into a regenerative thermal reactor (RTR):

Moreover, AG2S^{TM} technology allows to convert another critical molecule: H_2S. Indeed, H_2S is a poison for industrial and automotive catalysts and a source of corrosion for process equipment. The Claus process is the most widespread large-scale technology adopted for the neutralization of H_2S. Its thermal stage promotes the conversion of H_2S into elemental sulfur and water by a controlled oxidation at high temperatures (900-1500 °C). In this case, H_2S and CO_2 are reacted and a mixture rich in H_2 and CO (known as syngas) is obtained with water and elemental sulfur as harmless by-products. The scope of the Ph.D. activity is to propose and demonstrate the industrial feasibility of this innovative and sustainable process. As first, the involved kinetic mechanism was studied in deep, starting from the simplest mechanisms of H_2S (i.e. H/S system) pyrolysis up H/S/O/C global system. The kinetic was extensive validated with literature and industrial cases and in the well-known conditions of Claus Process. This activity was complemented by the integration of the kinetic mechanisms at the process-scale for studying the industrial feasibility of the process. Indeed, coupling two different software, i.e. Aspen HYSYS and MATLAB®, a complete plant model, able to manage the recycle of unconverted acid gases has been developed (Figure 1).

The core of the process are the RTR where main reaction takes place at temperatures higher than 1000 °C. High temperatures are necessary to (i) activate the reactive system from chemical-thermodynamics standpoint; (ii) quicken kinetics; and (iii) obtain high yields. According to Figure 2, the RTR is composed of two sections, where CO_2/H_2S reaction (regenerative section) and enthalpy recovery (recovering section) are carried-out. Due to high temperatures, the regenerative section corresponds to a refractory lined chamber; the reaction is assisted by a minor injection of oxygen for energy self-sustainability. Enthalpy content of RTR effluents, comprised of products and unreacted feed species, is recovered in a heat exchangers train. This train, a waste heat boiler and a feed/effluents exchanger, is part of the RTR as it contributes to overall yield by quenching of thermal reactions and energy self-sustainability.

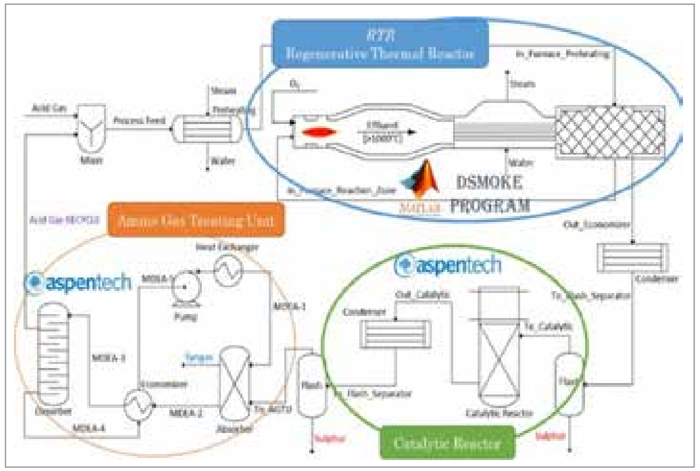


Fig. 1 - PFD of AG2S^{TM} Technology

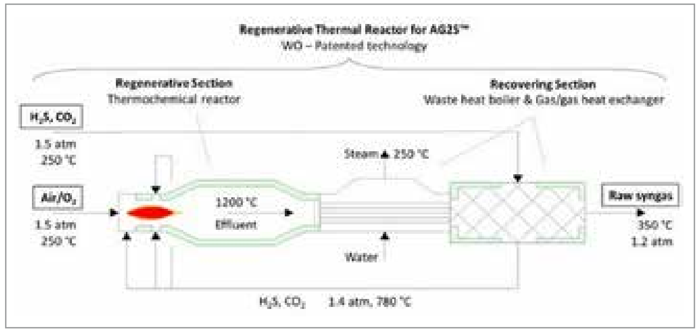


Fig. 2 - Regenerative Thermal Reactor (RTR) configuration

At RTR outlet, effluents are subject to a further cooling in condenser heat exchanger, where by-products (elemental sulfur and water) are removed. Effluents are subsequently processed in catalytic reactors for converting (i) residual SO_2 into elemental sulfur (Claus reaction) and (ii) traces of sulfur-compounds (COS and CS_2) into H_2S and CO_2 (hydrolysis reaction). Finally, the main stream undergoes a separation treatment where unreacted species and H_2/CO mixture are split (chemical washing); resulting streams are recycled to RTR and exported to the next process, respectively. The catalytic reactors are simulated with global reactions (Claus reaction and COS/CS_2 hydrolysis). In order to account for the distribution of sulfur allotropes, the thermodynamic equilibrium is assumed. Moving from the existing plant ENI® Claus plant in Livorno refinery, an evaluation of revamping technical feasibility of AG2S^{TM} technology is analysed. Simulations of the standard and of the revamped Claus plants are compared. The advantages of the revamped process are highlighted, both at technical and environmental levels.
Finally, a preliminary economical assessment is performed to evaluate the Pay Back Time (PBT) of the Tecnimont KT Claus process. By varying the syngas price, a variable PBT of 16-36 months is obtained (excluding the two years of installation without any revenues). This result is acceptable from an industrial point of view. Finally, AG2S^{TM} technology is applied to steam reforming and to CTM (coal to methanol) process. Focusing on the second one, a kinetic based gasifier model (GASDS tool) was validated and implemented in the global process. Moreover, in cooperation with Sotacarbo S.p.A., a gas-solid kinetic scheme, that allows to study the devolatilization of organo-sulfur compound during coal gasification, was developed in order to able to predict H_2S formation. As result for coal to methanol process was found a reduction of 1% of CO_2 emissions in parallel with an extra production of 0.5% of methanol using the same amount of inlet feedstock.

ADDITIVE MANUFACTURING AND METALLIZATION FOR 3D MICROFABRICATION OF FUNCTIONAL DEVICES

Roberto Bernasconi - Supervisor: Prof. Luca Magagnin
Tutor: Prof. Marinella Levi

3D printing is one of the technologies that are having the greatest impact on contemporary world. In fact, the introduction of additive manufacturing techniques is slowly changing the way industrial production is conventionally intended. One of the fields that may be most affected by the introduction of these manufacturing techniques is microfabrication, since a considerable number of modern products contains or will contain microfabricated parts, e.g. sensors, microactuators and many others.

For this reason, exploiting 3D printing for the so-called additive micromanufacturing is one of the most intriguing research topics in modern industrial engineering.

3D printing techniques suitable for microfabrication are however characterized by a common drawback: the materials used for printing are polymers. Since many applications require metallic properties, it is fundamental to individuate a technique to impart such properties to the surface of a 3D printed object.

In the present work, a hybrid approach combining stereolithography 3D printing and wet metallization techniques was developed and applied to the production of functional microdevices. Stereolithography (SLA) was selected as the most suitable 3D printing technique to perform cost effective 3D printing at the submillimetric scale. Wet metallization, both electroless and electrolytic, was selected to impart metallic properties to the printed microdevices.

Wet metallization of SLA printed resins was investigated in the first part of the work. The possibility to obtain good quality metallic coatings using electroless deposition was demonstrated on a set of commercially available and self-formulated photocurable polymers. Pretreatment for metallization was found to have a critical influence on the layers obtained. Different etching methods were tested, but only the alkaline pretreatment proved able to guarantee adhesion between the metal coating from electroless deposition and the resins. In particular, exposure to OH^- containing solution was found to break the ester bond present in most resins due to the presence of acrylate moieties, dissolving thus the photopolymerized material and increasing surface roughness (figure 1).

Optimized alkaline etching parameters were found to be ideal to provide acceptable adhesion and surface quality to electroless plated Cu and NiP layers. Electrolytic deposition was attempted on the surface of 3D printed resins made conductive by a first layer of electroless deposited metal. Many commercially plated metals and alloys were deposited on Cu and NiP base coatings. Both decorative and functional applications were demonstrated.

After optimizing the metallization process and demonstrating its feasibility for the manufacturing of functional devices at the microscale, two realistic application fields were selected: MEMS and microrobotics.

Concerning MEMS, the first 3D-printed and electroless metallized z-axis accelerometer (figure 2) has been designed, fabricated and tested. The tri-dimensionality of the 3D-printing fabrication process has been exploited in the design process of the mechanical structure and the electroless metallization has been employed to allow the capacitive testing of the device. A good agreement between the performances predicted by FEM simulation and the experimental tests has been found, thus showing a very good reliability of the proposed fabrication process.

The work demonstrated that 3D printed and wet metallized accelerometers show comparable or better performance in terms of sensitivity and linearity with respect to current state-of-the-art accelerometers. The marked advantage of the proposed devices is the possibility of full customization and the lack of many design constrains typical of the current MEMS production process.

Regarding the microrobotics applicative field, rolling actuated microrobots for cell deliver and water remediation were produced. Both devices showed full actuability, with a complete control over direction and speed of motion, together with a demonstrated functionality.

Microscaffolds were found to be biocompatible and able to transport cells. They are intended for use in zones of the human body characterized by the presence of cavities presenting suitable dimensions. These may include the gastrointestinal system or the respiratory system.

Photoactive/antimicrobial devices were found to be efficient in photodegrading pollutants and in killing bacteria. Their main application is the control of water quality through the reduction of pollutants concentrations and the containment of bacteria proliferation. They can be used to improve water conditions locally inside closed pipes, canals and water reservoirs by positioning them exactly where their action is needed.

All the devices presented proved to be fully functional, confirming thus the feasibility of the hybrid 3D printing/wet metallization approach for the additive micromanufacturing of functional devices.

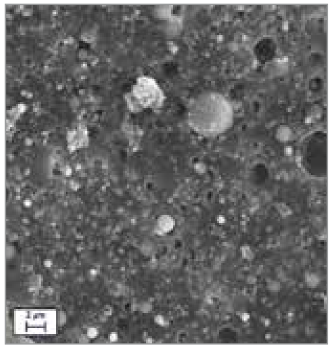


Fig. 1 - DL260 resin surface morphology after alkaline pretreatment

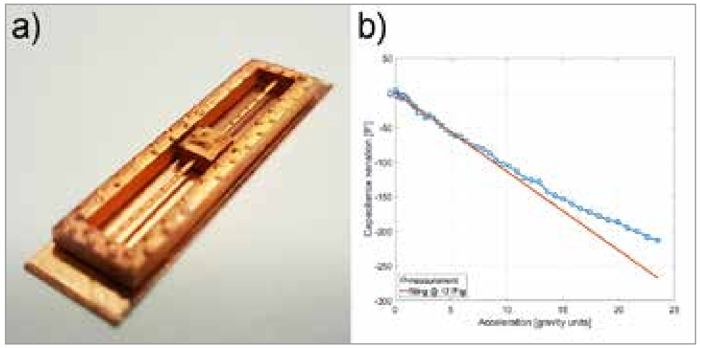


Fig. 2 - 3D-printed and electroless metallized z-axis accelerometer: visual appearance (a) and capacitance variation vs. acceleration (b)

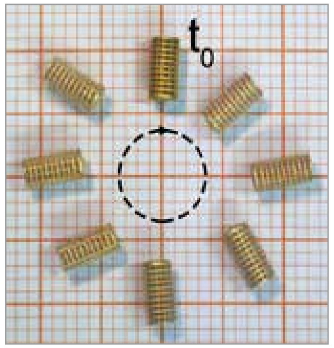


Fig. 3 - Time lapse representation of a circular actuation pattern for a 3D printed and wet metallized scaffold for cell delivery

FUNDAMENTAL INVESTIGATION OF THE TRANSPORT PROPERTIES IN OPEN-CELL FOAMS

Mauro Bracconi - Supervisor: Prof. Enrico Tronconi

Open-cell foams are widely acknowledged as potential enhanced substrates for catalytic reactors, due to a number of promising features. High void fractions, low pressure drops, and the intense gas-to-solid transfer rates make foams attractive for many different catalytic processes in the quest for a more environmentally and energetically sustainable world. Moreover, their totally interconnected solid matrix grants effective heat conduction through the structure, strongly improving the global heat transfer performances of the support. Despite their great potential, however, the application of open-cell foams to industrial catalytic processes is limited by the poor understanding of the related transport phenomena.
The large deviations between the experimental data and the predictions of the available literature correlations for momentum, mass and heat transfer in foams point out the need for a painstaking study of the relevant transport mechanisms.
This thesis aims at the fundamental analysis of the transport properties of open-cell foams by means of Computational Fluid Dynamics (CFD). CFD provides, in fact, a detailed insight into the flow field and in the complex associated transport mechanisms. Moreover, it can be employed for the derivation of lumped parameters and descriptors by means of the hierarchical approach, thus enabling CFD-based engineering correlations. The application of this approach requires new methodologies for the generation of reliable virtual structures, in order to obtain computational domains representative of the complex geometry of foams. Moreover, the interpretation of the simulation data requires adequate models for the foam morphological parameters, like e.g. specific surface area, characteristic lengths.
The focus of the first part of the work is the solution of the main numerical and geometrical problems. In particular, a systematic procedure for the virtual generation of open-cell foams has been developed. Based on a few pieces of readily available geometrical information, i.e. porosity, cell size and strut cross-sectional shape, this methodology provides virtual foam samples (see Figure 1) which totally retain all the geometrical and topological features and properties of real structures. Moreover, the virtual structures accurately reproduce the hydrodynamic behavior of real open-cell foams. In doing so, a fully theoretical geometrical model has been also derived. This model is based on a simplified description of the foam structure by means of an ideal tetrakaidekahedral unit cell and is able to predict the geometrical features of real foams. Moreover, it provides accurate first guesses for the virtual reconstruction procedure.
The solution of those problems granted the instruments required for the accurate investigation of the transport properties and of the underlying mechanism by means of CFD. In particular, the analysis of the gas-to-solid transport properties has been addressed.
The investigation of external mass transfer has been performed in combination with dedicated experimental activity. The effect of the morphological features has been parametrically investigated and the results used to derive lumped parameters. It has been found that the mass transfer coefficients mainly depend on the porosity of the foam. A decrement in the porosity increases the mass transfer properties of the support. A negligible dependence on the strut cross-section shape and on the cell size has been obtained. An engineering correlation able to accurately predict the transport properties has been derived as shown in Figure 2.
The pressure drop has been analyzed by means of an innovative approach. Virtual samples have been manufactured by means of 3D printing. This removes the discrepancies between the real foams and geometrical models and enables a precise control of the geometrical properties of the investigated samples. Numerical simulations on the virtual models and experimental tests on the 3D printed samples are employed jointly to understand the effect of the porosity, cell size and strut shape on the pressure losses. As expected, an increase of both the porosity and the cell size reduces the pressure losses, whereas the pressure drop increases by changing the strut shape from circular to triangular. These results have enabled the derivation of an engineering correlation for pressure drops based on an Ergun-like approach. A detailed comparison of open-cell foams against other conventional catalytic supports has been carried out. Open-cell foams present higher mass transfer rates with respect to honeycomb monoliths at the cost of higher pressure drop. In this perspective, the trade-off between external mass transfer and pressure losses shows that the performances of foams can be comparable or even higher than the ones of monoliths in a limited range of operating conditions.
The global heat transfer performances of foams have been investigated aiming at establishing the impact of the geometrical parameters on their effective thermal conductivity.
In doing so, a parametrical investigation of the effect of the geometrical properties has been performed. The solid fraction has been identified as the controlling parameter along with the solid distribution between nodes and struts. An increase of the solid fraction promotes the heat conduction in the structure, while a reduction of the ratio between node and struts increases the performances at fixed porosity. This result enables the derivation of geometry of open-cell foams that can increase the heat transfer by adopting a constant strut diameter along the axis. These structures cannot be manufactured by conventional techniques, but the virtual model can be easily generated by additive manufacturing techniques such as metal 3D printing.
Finally, I have performed a qualitative analysis of the interactions between multiphase flows and open-cell foams by carrying out numerical simulations aiming at evaluating the static holdup and the liquid spreading through the foam structure.
The numerical results are in line with experimental data present in literature, thus assessing the capability of the CFD simulation in the investigation of these phenomena as well.

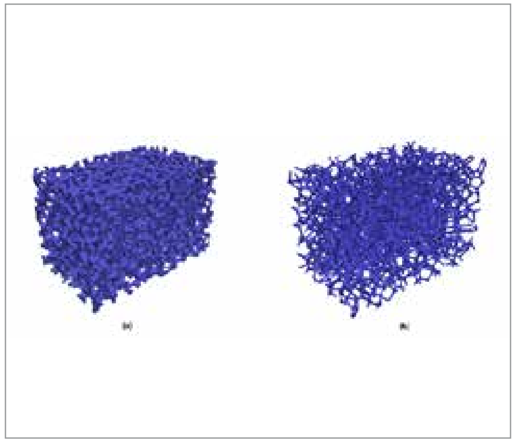


Fig. 1 - Final reconstructed foam samples with circular (a) and triangular (b) struts (c)

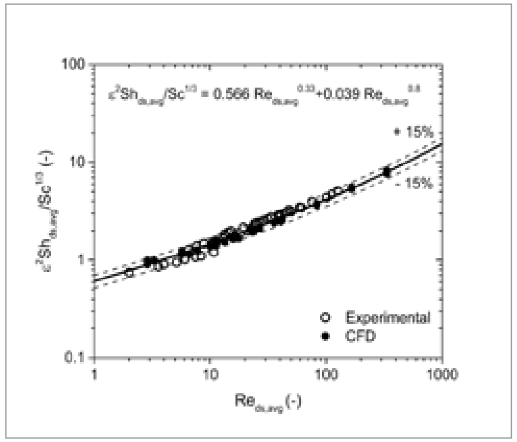


Fig. 2 - Sherwood number as a function of the Reynolds number for experiments (empty symbol) and CFD simulation (full symbol) along with the mass transfer correlation (full line)

THE COMBINATION OF ROP AND RAFT POLYMERIZATION FOR THE SYNTHESIS OF POLYMERIC NANOPARTICLES

Umberto Capasso Palmiero - Supervisor: Prof. Davide Moscatelli

Polymeric nanoparticles (NPs) are colloids in the nanometer size range that find applications in several fields, such as optics, coating, and medicine. In this latter case, they can be used as drug delivery systems for different therapeutics, ranging from lipophilic drugs to oligonucleotides, in order to improve their pharmacological profile, efficacy and to avoid possible side effects in the treatment of several illness, such as cancer. These nano-colloids typically consist of polyesters which are able to degrade into safe and easily removable compounds, such as lactic acid and hydroxycaproic acid. However, NP production is often a complex process that requires mixing devices. In addition, expensive purification steps are necessary to eliminate the unloaded drug and the high amount of organic solvent used in the NP production step. At the end of this process, a lyophilization step is generally adopted to assure a good shelf-life of the final product. All the above-mentioned steps hamper the cost-effective use of a re-formulation of the same therapeutic agent and, in turn, reduce the availability of these treatments among the patient population. The aim of this PhD thesis is to solve this issue and to develop a novel single-step NP production protocol which requires only a syringe and a needle, thereby eliminating the subsequent purification and freeze-drying steps. This advance build on the optimization of the hydrophilic/lipophilic ratio of block-copolymers that are able to directly self-assemble in water. The additional degree of freedom necessary for this optimization was introduced via the synthesis of these materials thorough the combination of the reversible addition-fragmentation chain transfer (RAFT) polymerization, a technique that allows the control over the structure and the molecular weight of the aliphatic polymers, and the ring opening polymerization (ROP), the most diffused process adopted for the production of polyesters. The first part of this work was devoted to the modeling and kinetic study of the RAFT polymerization and copolymerization of water soluble monomers in order to better understand the effects of the reaction parameters on the structure of the final polymer. Subsequently a general model of the NPs formed by a block-copolymer synthesized via RAFT polymerization of a hydrophilic and hydrophobic monomer has been developed. A method that consists in the use of amphiphilic RAFT macromolecules (the so called "RAFT surfmers") has been found to decouple the dependence of the main characteristics of the NP and polymer molecular weight. A good agreement between the prediction of the model and the experimental results has been obtained. As an example of the application of this technology, fluorinated NPs with different surface charges that are useful for optical experiments have been produced. Then the use of RAFT macro-surfmers has been combined with ROP to produce a library of biodegradable amphiphilic block-copolymers able to self-assemble in water via the use of only a syringe in order to avoid all the post-process steps necessary to store them. In fact, the classic polymeric surfactants developed so far require a mixing device to form NPs and to load the drug, a subsequent dialysis to eliminate the large amount of organic solvent, the lyophilization to avoid NP degradation during the storage and finally the reconstitution of the colloidal suspension before injection. Most of these steps are also required in the NPs obtained via emulsion polymerization of biodegradable vinyl monomers, as visible in **Figure 1.1**. The main difference between the two types of NPs resides on the loading step that needs to be carried out after the production of the colloids in the case of the emulsion polymerization. In this work, however, the block-copolymer is optimized to self-assemble in the reduced turbulence conditions of a syringe needle. In this way it can be stored as a dry powder and then dissolved with the therapeutic in a small amount of organic solvent and formulated into NPs just before injection.

As a proof of concept, trabectedin (ET-743), a potent anticancer therapeutic that causes necrosis of the blood vessel at the site of injection, has been loaded on this novel platform with high efficacy. The ability of the NPs to retain the drug inside the core allowed to reduce the "phlebitis" side effect without affecting the trabectedin antitumor activity, potentially leading to a better patience compliance.

The previously optimized block-copolymer has been also used to load paclitaxel, another important anticancer therapeutic. The resulting formulation has been found as effective as the most diffuse commercially available formulation (Taxol®), but without the use of Chremophor EL, a toxic surfactant known to cause several adverse side effects.

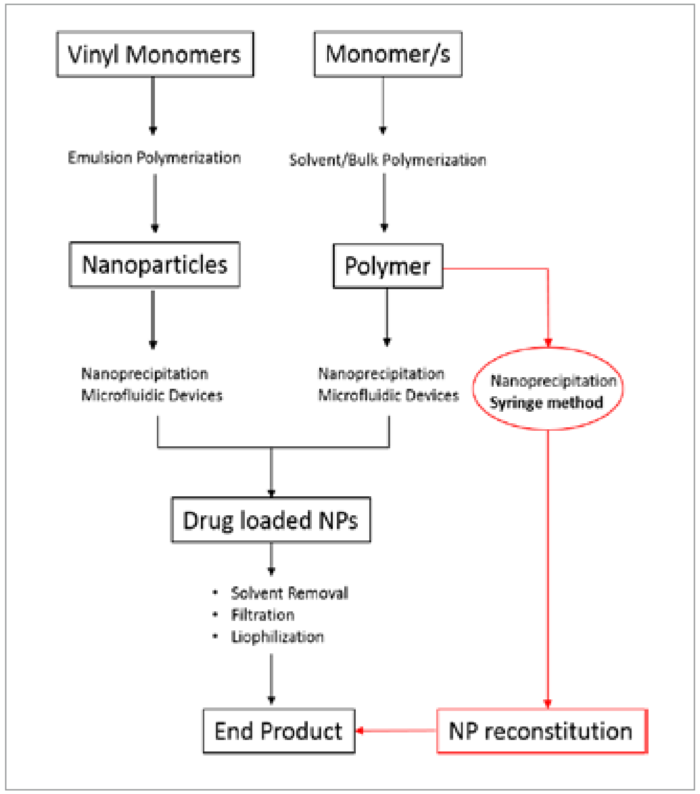


Fig. 1 - Classic pathways for the synthesis of polymer NP-based therapeutics

CATALYTIC PARTIAL OXIDATION OF LIQUID FUELS: EXPERIMENTAL AND MODELING ANALYSIS OF A LAB-SCALE ADIABATIC REFORMER

Andrea Carrera - Tutor: Prof. Carlo Cavallotti
Advisor: Prof. Gianpiero Groppi

The need of efficient processes for energy conversion and abatement of pollutant emissions keeps high the interest towards efficient energy systems, such as fuel cells, and prompts towards the improvement of combustion and exhaust after-treatment systems. Hydrogen is attractive for its possible usage as energy carrier as well for the reduction of pollutant emissions. The superior combustion properties of hydrogen can be exploited in Internal Combustion Engines or Gas Turbines, for the generation of mechanical work, electricity or heat. Syngas, i.e. a mixture of hydrogen and carbon monoxide, when purified from particulate matter and sulfur compounds, may also be fed to Solid Oxide Fuel Cells, for the conversion into electric energy. The development of SOFC-based Auxiliary Power Units has a great interest because of the availability of electric energy when the engine is off. Synthesis gas may also regenerate Lean NO_X Traps during their rich phase. Hydrogen may be either stored or produced on board of vehicles. Though the stationary production of hydrogen is well established, the first solution should face many challenges before becoming a commercial technology. In fact, hydrogen storage is costly, and requires specific infrastructures for its distribution. Hydrogen on-board production represents an interesting alternative, as several technologies allows the conversion of primary fuels into this energy vector. The development of a compact and light device for hydrogen production is necessary when considering mobile applications. Currently, the main commercial hydrogen production processes are steam reforming (SR), partial oxidation (PO, either non-catalytic, or catalytic), auto-thermal reforming (ATR). Steam reforming is a well consolidated technology, which consists in the reaction between hydrocarbons and water, in the presence of a catalyst. Though steam reforming is widely used in the industry, it is an endothermic and energy-demanding process, which requires external heat supply, therefore it is not suited for small-scale applications. In the Partial Oxidation process, oxygen reacts with hydrocarbons to produce carbon dioxide, water, carbon monoxide and hydrogen. This technology works in harsh conditions, hence it is not valid for the small-scale hydrogen production, Catalytic Partial Oxidation requires shorter contact times (t ~ ms) than the non-catalytic process (t ~ s) to achieve equilibrium. The CPO process is globally exothermic and this allows the use of simple adiabatic reactors. In the catalytic Auto-Thermal Reforming, both water and oxygen are reacted with the hydrocarbon feedstock. This process may be thermally neutral or exothermic, according to the reactant composition employed. ATR operates at lower temperatures than CPO, but the former requires slightly higher reactor volumes, as well as water storage and dosing systems.

The CPO process is globally exothermic and may be carried out in adiabatic reactors, loaded with a structured catalyst, which offer a better trade-off between pressure drops and transfer properties with respect to random packings. Rh catalysts offer several advantages, compared to transition metal and Pt catalysts, such as high activity and selectivity to syngas, low tendency to form coke and good thermal stability.

In this work, the CPO of iso-octane and n-octane has been investigated on Rh based catalysts, supported over a 400 CPSI cordierite honeycomb monolith in a lab-scale auto-thermal reformer, equipped with the spatially resolved sampling technique. A one dimensional, single-channel, heterogeneous, adiabatic reactor model have been used to verify the reaction mechanism and to better understand the interplay between chemical kinetics and transport phenomena.

Both iso-octane and n-octane exhibit an indirect-consecutive reaction scheme, with oxidation of the fuel to CO_2 and H_2O and reforming of the excess fuel with H_2O with production of syngas, which results in the presence of an Oxy-Reforming zone next to the catalyst inlet and a Reforming zone downstream, and leads to the onset of a hot spot of temperature.

The impact of mass transfer on the CPO of octane isomers is important, and affects significantly the thermal management of the reactor. In fact, due to the decrease of molecular diffusivity with increasing size of the hydrocarbon molecule, and thus to the decrease of steam reforming efficiency, the hot spot temperature increases when passing from methane to octanes, at constant carbon and oxygen flow rates, as detailed in Figure 1. Temperature profiles along the reactor axial coordinate are similar between the two octane isomers, as reported in Figure 2. High temperatures in the gas phase (T>700°C) activate gas phase cracking reactions, with the production of olefins and light alkanes, which can condensate, forming soot or coke, or can further react on the catalyst surface, producing syngas. While the heterogeneous reactivity of the two isomers, n-octane and iso-octane is similar, the two octanes undergo different gas phase decomposition pathways, which lead to different distributions of olefins and light alkanes: iso-octane is more selective towards iso-butylene and propylene, while n-octane mainly produces ethylene.

Temperature Programmed Oxidation revealed that different forms of coke are deposited onto the catalytic monolith. Coke deposition mainly occurs in the reforming zone and is more pronounced in the case of n-octane. Deposition of carbon species is thermodynamically favored by N_2 dilution, which was adopted in the experiments to prevent catalyst overheating. However, reactant dilution can be practically implemented in combination with Internal Combustion Engines using the Exhaust Gas Recirculation. Thermodynamic calculations show that, thanks to the H_2O content of the diluting gas, this is effective in the reduction of the operating temperature of the reactor, while keeping the process in a thermodynamically safe zone with respect to coke deposition. Still, coke deposition is an important issue, especially when using alumina as support. The introduction of a more basic support with higher surface area than α-alumina, such as $MgAl_2O_4$ spinel, contributes to enhance the rate of steam reforming, with the twofold benefit of lowering the hot spot temperature and hindering the deposition of carbon species on the catalyst; both aspects contribute to preserve the catalyst stability under octanes CPO conditions. TPO analysis confirms that the alumina support, more acidic, is subjected to higher coke deposition than magnesium aluminate. The co-feed of aromatics and naphthenic hydrocarbons and sulphur poisoning should be addressed in more detail in the future.

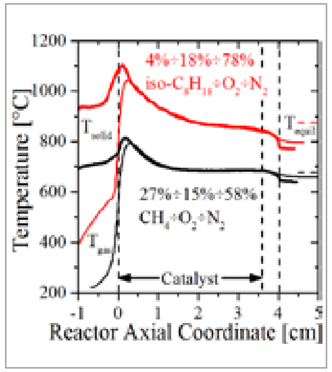


Fig. 1 - Comparison among experimental temperature profiles in the CPO of methane and iso-octane; methane CPO: CH_4=27.3%, C/O=0.9, F=10Nl/min, T_{IN}=25°C; iso-octane CPO: iso-C_8H_{18}=4%, C/O=0.9, F=10Nl/min, T_{IN}=85°C.

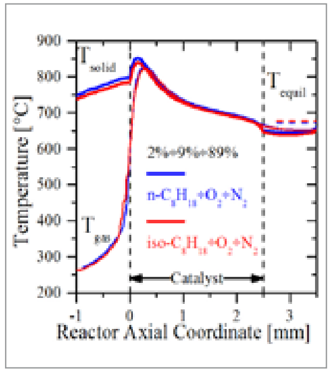


Fig. 2 - Comparison among experimental temperature profiles in the CPO of iso-octane and n-octane; x-C_8H_{18}=2%, C/O=0.9, F=10Nl/min, T_{IN}=85°C.

EVALUATION AND DEVELOPMENT OF BIOMASS CONVERSION PROCESSES FOR THE CHEMICAL AND BIOCHEMICAL VALORIZATION OF LIGNIN

Chiara Francesca Carrozza - Supervisor: Prof. Citterio Attilio

Tutor: Prof. Brenna Maria Elisabetta

Through the integration of green chemistry into biorefineries and the use of low environmental impact technologies, future sustainable production chains of biofuels and high value chemicals from biomass could quickly forecast. Despite the fact that a massive amount of lignin is obtained from the biorefineries, the use of lignin in real industrial processes is far to be obtained. Hence, approaches to develop lignin conversion into high-value co-products are progressively investigated in the attempt to improve the economics of the biorefineries.

In this sense, the PhD project deals with the valorization of biorefinery lignin as by-product from second-generation bioethanol production involving either a biochemical or chemical treatments. The starting materials were obtained from different stages of the cellulosic ethanol plants powered by Proesa™ technology and operated in Crescentino (Italy).

More specifically, the research activity was divided in four main Chapters:

Chapter 1: Introduction

In order to face the challenges of becoming independent of crude oil and switching to a more sustainable and carbon neutral society, biomass has emerged as most prospective raw material in the next future. The basic knowledge about plant origin materials, with specific attention to lignin component will be introduced in this chapter. In this sense, a deep understanding of biomass as renewable feedstock will be provided. Then, the sustainable use of lignin, as the other major constituent in biomass and its potential as starting material for the production of chemicals due to its aromatic structure, will be investigated. Several approaches either focus on the direct utilization of lignin with suitable modification to find application in low value products including dispersant, binders, emulsifiers and resins, or target the depolymerization of lignin macromolecule into high value aromatic fine chemicals will be presented. Finally, a general overview of Crescentino plant and its technology will be reported in order to understand where the raw materials used among all experiments come from.

Chapter 2: Lignin isolation and characterization

Starting from protocol procedures, optimization of extraction and purification treatments combining mechanical treatment (ball milling) and an intensive washing step will be performed. All the materials were fully characterized by means of different spectroscopic techniques and their properties compared with the raw material. It was found that soxhlet extraction as washing procedure will lead to a material containing 96% of lignin.

Meanwhile, in collaboration with the University of Pavia, the effect of the growth of two fungal species, *Trichoderma asperellum* EVT4 and *Pleurotus ostreatus*, which respectively are able to degrade polysaccharides or lignin aiming the biodegradation of the material, was tested. It was found that *Trichoderma asperellum* EVT4 is able to purify the material lowering the content of sugar (reducing sugars or glucose) in a strong way but leaves a complicated tri-dimensional structure.

Comparison between chemical and biochemical purification have been done and it can be assumed that both procedure can purify lignin rich residue with a good degree of lignin purity but with different structural properties.

Finally, in collaboration with Lawrence Berkeley National Laboratory, a complete biorefinery process (pretreatment, hydrolysis and fermentation) in lab scale using ionic liquid as solvent to degrade and solubilized two different feedstocks, switchgrass and poplar, was performed. Compared with the traditional pretreatments, IL pretreatment is a relatively new approach and has several parameters that need more investigation. The main drawbacks in ILs pretreatment consist in the extensive washing procedure of the pretreated biomass to remove residual amounts of ILs that may inhibit downstream saccharification and fermentation. To overcome this issue, recently a new wash-free approach was developed. It is based on a one-pot system where IL pretreatment, saccharification and fermentation followed by direct extraction of sugar and recovery of lignin will take place simultaneously. The one-pot process performed gaves us good result in term of ethanol yield since the whole hydrolysed glucose was converted. Hence, it was demonstrated that IL pretreatment improves the “delignification” process and significantly increases the enzymatic hydrolysis efficiency. Physical and chemical alterations in lignin structure were investigated with the aim to understand its reactivity during the whole IL treatment.

Chapter 3 and Chapter 4: Catalytic oxidation of lignin and lignin depolymerization by hydrogenolysis.

Since lignin is the richest source of renewable aromatic compounds on earth, its conversion into well-defined aromatic chemicals was deeply investigated by either oxidative and reductive approaches in mild conditions of temperature and pressure.

As it is known in literature, the recalcitrance of lignin and its ability to recondensate represent a challenge that inhibit an efficient depolymerization.

In the first case, two different catalytic conditions were tested on the purified material by using $CuSO_4$ or Co(salen) as catalysts. With copper sulphate the three main aldehydes (vanillin, siringaldehyde and p-hydroxy aldehyde) were produced at pressure equal to 3 bar with an overall conversion of about 5% (coherent with literature results). Instead by using Co complex, p-benzoquinones were formed (mainly methoxy benzoquinone and dimethoxy benzoquinone) with a total yield of 7.2% at 3.5 bar.

On the other hand, the purified materials were depolymerized by catalytic hydrogenolysis in alcoholic media with a combination of palladium on carbon and/or nickel(II) acetate as catalyst. To increase the efficiency of the reaction, *in-situ* preparation of the nickel-based catalyst was carried out by adsorption of metal salt on the material. In 1,2-propanendiol as solvent at 210 °C, up to 80% of the material was solubilized with an overall yield of reduced monolignol derivatives of about 18%. Depolymerized lignin fragments (both the liquid residue and the solid residue recovered after precipitation with water) were detected by MALDI-ToF analysis with a molecular weight between 400-1400 Da.

To this end, catalytic depolymerization reaction through hydrogenolysis was performed on the one-pot ionic liquid residues. Different conditions of temperature and pressure were tested: at 200 °C the alcoholic solvent (1,2-propanendiol) acts as source of hydrogen, while at 90 °C and 140 °C the reaction was performed adding 20 bar of H_2. Solid residues, from either switchgrass or poplar, reach a maximum value of about 35% but the distribution of product is opposite: about 60% of S unit in switchgrass residue and about 60% of G unit in poplar residue.

TOWARDS ENGINEERING OF SOLID-STATE SUPRAMOLECULAR ROTORS VIA HALOGEN BONDING

Luca Catalano - Supervisor: Prof. Pierangelo Metrangolo

My PhD research activity had a twofold objective:
a) Studying halogen-bonding interactions (XB) at a fundamental level by focusing on new tools for the identification and characterization of this non-covalent interaction;
b) Engineering crystalline supramolecular rotors based on halogen bonding and studying the effect of the strength of XB on the dynamics of these systems.

Far-IR spectroscopy is seldom employed to prove the occurrence of halogen bonding. This is essentially due to low frequencies (< 400 cm-1) and weak intensities of the vibrations involving halogens. However thanks to new standard instrumentations the detection limit of vibrational spectroscopy is moving to lower frequencies allowing the exploration of the Far-IR region. To proof this concept, we built a prototypical series of supramolecular adducts based on halogen bonding and then we characterized them with X-ray diffraction, DSC and IR spectroscopy. We coupled the experimental IR analysis with calculations in order to correctly assign the different vibrations to the peaks of the IR spectra. The adducts were formed between strong halogen bond donors (iodopentafluorobenzene and bromopentafluorobenzene) and strong Lewis bases (pyridyl moieties and TMEDA).

All new species were obtained via cocrystallization from standard solvents and their crystal structures were either confirmed (if already known) or solved through XRD analysis. One species was liquid at room temperature, thus, to obtain its crystal structure, we successfully employed the *in situ* cryo-crystallization technique. Far-IR analysis showed red-shifting and intensity increase of the main vibrations involving the halogen atoms. These effects are diagnostic of the occurrence and the relative strength of the XB.

Solid-state nuclear magnetic resonance (SSNMR) spectroscopy is a versatile characterization technique that can provide a plethora of information complementary to single crystal X-ray diffraction analysis. In order to apply this powerful technique to the study of XB, we designed an experimental and computational investigation of the relationship between the geometry of XB and the SSNMR chemical shifts of the non-quadrupolar nuclei either directly involved in the interaction (^{15}N) or covalently bonded to the halogen atom (^{13}C).

To reach this goal we prepared two series of X-bonded cocrystals based upon two different dipyridyl modules, and several halobenzenes and diiodoalkanes, as XB-donors. All cocrystals were fully characterized via IR spectroscopy, thermal analysis, single crystal and powder XRD.

We then investigated our systems *via* SSNMR. For the first time, the change in the ^{15}N SSNMR chemical shifts upon XB formation is shown to experimentally correlate with the normalized distance parameter of the XB. The same overall trend is confirmed by density functional theory (DFT) calculations of the chemical shifts. ^{13}C NQS experiments show a positive, linear correlation between the chemical shifts and the C-I elongation, which is an indirect probe of the strength of the XB. These correlations can be of general utility to estimate the geometry of the XB.

Amphydynamic crystals are materials built to possess rapidly moving components in the solid state. Nowadays there is a growing interest in this class of compounds for the development of new functional materials and molecular machines.

The aim of our research is to take advantage of crystal engineering principles to assemble stators and rotators into cocrystals shown highly efficient molecular dynamics. This strategy has few advantages such as its intrinsic flexibility and hence the trivial access to a vast number of different supramolecular rotors. Firstly, we were able to synthesized crystalline supramolecular rotors self-assembled by XB of diazabicylo[2.2.2]octane (dabco), a well known C_3-symmetric cylindrically shaped rotator, and a set of five fluorine-substituted iodobenzenes, acting as strong halogen bond donors, that take the role of the stators. We characterized the adducts via single crystal and powders XRD, IR spectroscopy and melting point measurements to confirm their formation. Then we used variable temperature ^{1}H T_1 spin-lattice relaxation measurements to characterize the dynamics of the rotors. All structures display ultrafast Brownian rotation around the XB with activation energies ranging from 2.4 to 4.9 kcal/mol,. In all cases the activation energies are lower than the one of pure dabco (8.3 kcal/mol). Within the cocrystals, the rotators are partially isolated from their neighbouring rotator molecules by XB donors, which work both as stators and bearings. The pre-exponential factors of the dynamics are in the range 1–9 x 10^{12} s^{-1}. These results are comparable to those found in rotors with covalent rotational axis, suggesting that XB is robust enough to work as an efficient axle of rotation. Lineshape analysis of quadrupolar echo ^{2}H NMR measurements in selected samples indicated rotational trajectories consistent with both 3-fold and 6-fold symmetric potential of the rotator.

The second step of the supramolecular rotors project was to understand how the strength of XB, working as axle of rotation, influences the dynamics of the rotors. To do so, we successfully realized two new halogen-bonded supramolecular rotors with isomorphic crystal structures at 100 K and room temperature. The only difference lies in the halogen atom involved in the XB (X = Br, I). Solvent assisted mechanochemical crystallization allowed us to prepare pure cocrystals. The increasing dynamic disorder of dabco from low to high temperature observed by single crystal X-ray diffraction and quadrupolar-echo ^{2}H NMR led us to conclude that the rotation of dabco in both cocrystals can be described by a six-fold rotation potential surface with three low energy minima and three minima of higher energy that, by this reason, at low temperatures are less populated. ^{1}H T_1 relaxometry experiments allowed us to calculate the activation parameters for the rotation of dabco in both cocrystals. We observed good correlation between the low crystal packing, C_k = 0.66 at 100 K, and the small rotational barriers of dabco, 1.15 and 0.71 kcal/mol, respectively, with the later being the lowest reported in the field of molecular rotors. The two cocrystals showed pre-exponential factors of almost the same magnitude (τ_0^{-1}), 1.3 x 10^{12} s^{-2} and 1.3 x 10^{12} s^{-2} respectively.

Using isomorphous cocrystals, as demonstrated by X-ray diffraction and IR data, we were able to sort out the origin of enthalpy and entropy of activation. Accordingly, by fitting ^{1}H T_1 data to the Arrhenius, Eyring and Kubo-Tomita equations, it was found negative entropies of activation of the same magnitude ($\Delta S^{\neq}$ = -3.0 cal/mol K) supporting the similarity of the normal modes and lattice phonons. On the other hand, the enthalpy of activation for the rotation of dabco in co-crystal involving iodine ($\Delta H^{\neq}$ = 0.95 kcal/mol) was shown to be almost twice as that of the one involving bromine ($\Delta H^{\neq}$ = 0.54 kcal/mol) that correlates well with the subtle crystal structure differences, namely a more hindered environment in the dabco cavity in the iodine-based cocrystal.

In conclusion relevant results were obtained in both the research strands of my PhD career.

- We demonstrated that Far-IR spectroscopy and SSNMR are powerful diagnostic tools for the detection XB and for the characterization of its strength and geometry.
- We synthesized and fully characterized the first examples of XB-based amphidynamic cocrystals. Furthermore we were able to sort out the origin of enthalpy and entropy of activation of the rotators dynamics. The discovery of these new crystalline multicomponent systems thus opens up new avenues in the development of new smart materials and molecular machines.

A STRUCTURAL APPROACH TOWARDS CELL ADHESION MODULATION AND PROTEIN ENGINEERING

Andrea Dalle Vedove - Supervisor: Dr. Emilio Parisini

Protein X-ray crystallography is a very powerful tool that provides, at the molecular level, the high quality and high resolution structural information that is necessary for structure-function correlation analysis, protein engineering, structure based drug design and molecular dynamics (MD) simulations.

In this thesis, this technique has been extensively used for the investigation of the structure, properties and possible application of the extracellular portion of some selected cell-adhesion proteins of the type I classical cadherin family and of a deglycating enzyme from the large Fructosyl Amino Oxidase family, called Amadoriase I.

Cadherins are calcium-dependent trans-membrane proteins that comprise three clearly discernible regions: intracellular, trans-membrane and extracellular. The extracellular portion is formed by a variable number of so-called extracellular cadherin (EC) repeat domains, each formed by approximately 110 amino acidic residues and rigidified by the presence of Ca^{2+} ions between them.

They are of critical importance for cell-cell adhesion, a fundamental process that results in cellular organization and tissue differentiation, thus allowing the formation of tissues and organs and ultimately the development of complex multicellular organisms.

Other than their adhesive function, cadherins also perform a cell-cell signaling function. It is well known that variations in cadherin natural expression level and changes in their ability to form either homophilic dimers or bind to selected protein substrates correlate with the onset and the progression of diseases such as cancer, asthma and chronic inflammation states.

Although the cadherin main functions (adhesion and signaling) have been quite extensively investigated over the last two decades, the mechanism by which such tasks are performed still needs to be fully elucidated. Over time, a combination of biophysical techniques (mainly X-ray diffraction, NMR and SAXS) have provided a clear, albeit still incomplete, picture of the adhesion mechanism, leaving the complete trajectory that leads, very dynamically, from the monomer to the dimeric adhesive state and back still partially elusive. The implications of such lack of complete structural characterization are important: to date, no clear molecular bases for cadherin homo-selectivity and for their energy activation profiles have been unambiguously identified.

In this thesis work, I have studied selected cadherin family members at the molecular level by single crystal X-ray crystallography. Overall, my goal was to contribute to the characterization of their adhesion mechanism. Furthermore, in the context of this thesis, I combined this structural analysis with biophysical, computational and functional studies aimed at the development or the identification of molecules that are capable of modulating cadherin homophilic adhesion. Finally, starting from the structural information, I have engineered a cadherin family member with the goal of producing functionalized biomaterials. In fact, due to the important role of the protein in tissue sorting and formation, such cadherin-functionalized hybrid materials may be employed as scaffolds in tissue engineering and tissue regeneration or be used for the development of cell-sorting or sensing lab-on-chip platforms.

The second project that I focused on in my thesis is the X-ray crystal structure of the deglycating enzyme Amadoriase I. A member of the Fructosyl Amino Oxidase family, this enzyme is capable of hydrolyzing the bond between the amine and the sugar moiety of a glycated amino acid.

From a biomedical point of view, the study of this enzyme is very important since protein glycation reactions occur spontaneously in the body over time due to the sugar present in blood. As a result of this spontaneous glycation reaction (usually referred to as the Maillard reaction) proteins are progressively glycated and, especially those with long half-life, tend to become heavily crosslinked over time. These non specific modifications negatively affect the function of the proteins and may eventually lead to the development of diseases such as Alzheimer's disease, arteriosclerosis, nephropathy and retinopathy, with a higher incidence in elderly people and in people with abnormally high blood sugar levels.

Moreover, deglycating enzymes are also utilized for the measurement of the concentration of the glycated form of hemoglobin (HbA1c) in the blood. In fact, due to the relatively long life-time of hemoglobin (90-120 days) and therefore its tendency to be glycated over time, this is a very good indicator of the concentration of blood glucose over a period of 2-3 months. Unfortunately, however, due to the fact that the available enzymes only work on glycated amino acids, this process is not immediate since HbA1c must be proteolytically cleaved before the enzymatic detection of selected glycated amino acids can actually take place.

During my thesis, I contributed to the structural characterization of of the apo form and the substrate-bound form of Amadoriase I from *Aspergillus fumigatus*. Moreover, I have been actively involved in the first attempts to engineer this enzyme in order to enhance its natural substrate recognition capabilities. All these studies have been conducted using a combination of molecular biology, protein chemistry, X-ray crystallography and molecular dynamics techniques. The ultimate goal with this project is to engineer the Amadoriase I enzyme in order to enlarge its catalytic cavity and allow its catalytic activity on large substrates such as polypeptides or even whole glycated proteins. This would, for instance, potentially improve the current procedures employed for the measurement of HbA1c in diabetic patients, possibly leading to new, efficient and low cost diagnostic tools for diabetes monitoring. Other potential applications of such molecular technology include the use of such chimeric enzyme to slow or even reverse collagen rigidification in aging tissues as well as in the food industry, to limit protein glycation in aliments that need thermal treatment (e.g. UHT milk).

CATALYTIC CO_2 VALORIZATION PRODUCING FUELS AND CHEMICALS

Leonardo Falbo - Supervisor: Prof. Luca Lietti

The increase of the atmospheric carbon dioxide concentration is blamed as one of the main causes of the climate change (global warming). For this reason, during the last 20 years several studies have been carried out to intensify the technologies aiming at its reduction. The decrease of CO_2 emissions through the increase of energetic efficiency of the existing fossil fuel based industrial processes and the utilization of renewable energy sources are obviously options of choice. However, strategies able to accomplish a net reduction of the CO_2 concentration in the atmosphere are also being considered. Among these, the CO_2 Capture and Utilization technologies, consisting in the chemical conversion of CO_2 into valuable products, are reaching great industrial and environmental appeal. In this regard, the purpose of this research work is to gain more insights in the intensification of sustainable CO_2 utilization catalytic processes producing synthetic fuels (particularly liquid hydrocarbons and synthetic natural gas) and chemicals (particularly “lower olefins” in the C_2-C_4 range).

The production of liquid hydrocarbons through the one-pot catalytic CO_2 hydrogenation is explored on typical iron and cobalt based Fischer-Tropsch catalysts. Co-based catalysts lead principally to the synthesis of light saturated hydrocarbons, whereas Fe-based catalysts produce more heavy hydrocarbons.

On both Co- and Fe-based catalysts, CO and CO_2 hydrogenation processes follow a common reaction pathway, with CO acting as intermediate in the case of CO_2 conversion. The different selectivity of the two processes is mainly ascribed to different H/C ratio on the catalyst surface. During the CO_2 hydrogenation, a high H/C surface ratio is attained due to the slow CO_2 adsorption, if compared with respect to CO adsorption. This behavior favors the hydrogenation reactions of the adsorbed surface intermediates, hence decreasing the chain growth probability and increasing the olefin hydrogenations. Furthermore, on iron-based catalysts, the process yield is also controlled by the equilibrium between the chain growth sites (iron carbides) and the hydrogenation sites (iron oxides and reduced iron centers). The former sites are not stable in the absence of high CO partial pressures and they can evolve with the time on stream into the latter sites, which are active in the secondary reactions of olefins. The relative abundance of these two types of sites affects conversion and selectivity of the catalysts. Although the process selectivity during CO and CO_2 hydrogenation is not dramatically different by using Fe-based catalysts, these catalytic systems need promoters, both structural and electronic, in order to boost process yields. In this regard, the promotion with potassium and zinc, more than with manganese, increases the catalyst activity and selectivity to high molecular weight hydrocarbons, by changing the basicity of the catalyst surface.

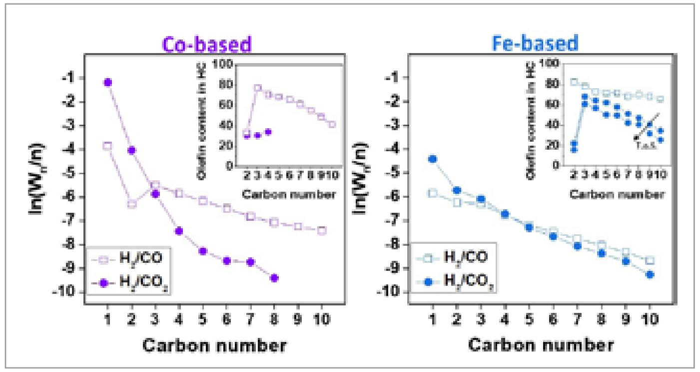


Fig. 1 - ASF plot and olefin content in hydrocarbons for Co-based (220°C, 20 bar_g. H_2/CO_x=2.5 v/v) and Fe-based catalyst (220°C, 30 bar_g, H_2/CO_x=1 v/v) during CO and CO_2 hydrogenation.

The production of synthetic natural gas is here investigated on low-loading Ru-based catalysts supported on alumina. The catalyst is appropriate to carry out the Sabatier reaction (CO_2 methanation) under process conditions relevant for the *Power-to-Gas* application, with an almost complete selectivity to methane. The catalyst performance over a wide range of process conditions, previously unexplored, is described by a developed kinetic rate equation.

Furthermore, the reactivity of Ru-based catalysts in the hydrogenation of CO/CO_2 gas mixtures is investigated to assess the possibility to carry out the CO_2 methanation even in the presence of CO-containing feed streams. When CO is present in the gas feed, Ru-based catalysts show deactivation at low process temperature. The extent of activity loss increases by increasing CO/CO_2 ratio in the inlet stream and by decreasing the catalyst bed temperature, so by working in condition where the CO adsorption is enhanced. Furthermore, the role of CO in CO_2 methanation is also investigated by looking at the catalyst surface through dedicated infrared spectroscopic experiments. By cofeeding CO at low temperature, the surface CO coverage is high and it increases with time on stream. In this way, the methanation becomes kinetically inhibited due to the lack of available hydrogen on the metal sites. Additionally, in the presence of CO, some adsorbed species, identified as carboxylates, are formed on the catalyst surface, blocking the CO_2 adsorption when process conditions are switched back to pure CO_2 methanation. In this regard, high temperatures are needed to avoid a gradual catalyst deactivation due to the strong adsorption of CO on the Ru sites and to the presence of surface blocking adsorbed species.

Eventually, the hydrogenation of CO_2 to lower olefins is investigated by using a bulk K-promoted Fe-based catalyst with an iron spinel structure and high surface area. The catalyst preparation method and the thermal treatments have dramatic effects on the morphological properties and on the crystal structure of the final catalyst. The obtained material showed very good performance in the reaction by operating at mild process conditions.

In conclusion, Fe- and Ru-based catalysts can be successfully used to obtain high added-value products from CO_2 hydrogenation, even in the presence of H_2 deficient feed. The results obtained in this work pave the way for the development of more efficient catalytic processes for CO_2 hydrogenation, which associated with a supply of renewable H_2 and process intensification can mitigate the dramatic effects of elevated CO_2 concentration in the atmosphere.

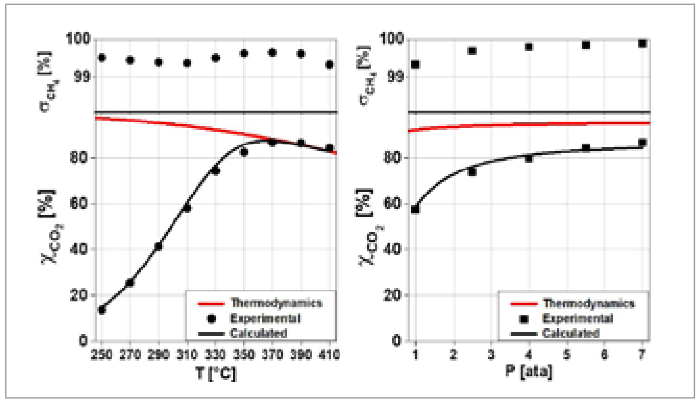


Fig. 2 - Effect of temperature and pressure on CO_2 conversion and CH_4 selectivity. Experimental CO_2 conversion data are compared to model predictions. (Central process conditions: 310°C, 1 ata, H_2/CO_2=4 v/v, 10 mol.% of N_2, 5 L(STP)/h/g_{cat}

COMBINED ADVANCED STRUCTURAL TOOLS FOR CHARACTERIZATION OF POLYSACCHARIDIC POLYMERS

Monica Ferro - Advisor: Prof. Andrea Mele

The work object of this doctoral thesis been focused on the study of different polysaccharidic materials with different characteristics. Linear and branched polysaccharides of both natural and synthetic origin have been studied in solution, in the solid or in the gel state. The work of my PhD can be divided into two parts: the first part (A) regarded the study of cellulose and cellulose derivatives; the second part (B) was focused on the diffusion study of small molecules loaded in different hydrogels composed by cross-linked polysaccharides (Agar-carbomer) or nanoporous cyclodextrin-based cross-linked materials (cyclodextrin nanosponges). Part (A). Cellulose is the most important natural polymer: it is mainly used in the production of paper, but also for the production of relevant industrial derivative such as cellophane and rayon. In the last decades cellulose has also been studied for the production of biofuels as non-fossil fuel source. From the analytical point of view, cellulose was studied by different academic and industrial groups using different solid state techniques such as: Powder X Ray Diffraction (PXRD), Fourier Transform Infra-Red Spectroscopy (FT-IR), solid state NMR spectroscopy, Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). In my work, I mainly used powder X-ray diffraction (PXRD) to study cellulosic materials.

The objective was to develop innovative, easy to apply and accurate analytical methods for cellulose characterization and to monitor cellulose reactivity.

I developed an accurate deconvolution method based on PXRD peaks and I applied it on industrial cellulose samples.

Moreover, I applied multivariate statistical analysis to PXRD data in order to evaluate and predict the reactivity of cellulose. Within the study of cellulose and its derivatives, I studied carboxymethylcellulose (CMC). CMC is a linear, anionic polyether cellulose derivative, generally water soluble and with high molecular weight. The industrial synthesis of CMC is based on a slurry process and involves two steps: mercerization and etherification. The Degree of Substitution (DS) represents the average number of carboxymethyl groups present in each glucosidic unit. The typical DS range of commercial CMC is between 0.6 and 1.25. Several methods to provide DS of CMC have been previously reported in the literature. Most of them are based on chromatography, FT-IR spectroscopy or Scanning Electron Microscopy. The most commonly used method is based on the HPLC analysis of the hydrolysis products of CMCs with strong acids. In addition to this chromatographic method, different promising NMR methods for DS determination have been previously described. Some of these (both ^{1}H and ^{13}C) are also based on the analysis of hydrolyzed CMC. Others are non-destructive methods. The latter approaches are based on quantitative ^{13}C NMR in solution (inverse GATED acquisition mode). DS value is obtained by peak integration. Severe limitations of these methods arise from the spectral overlaps generated by the mixture of regioisomers. Furthermore, the application is restricted only to CMC solutions with low viscosity in order to avoid line broadening.

A novel, non-destructive method based on ^{13}C HR-MAS NMR spectroscopy is proposed in this work. The method herein reported couples a wide applicability to CMCs with different purification degree, different viscosity and different origin (cotton linters or wood) with a non-destructive procedure. Thus the results of the analysis give more reliable picture of the DS of the CMC in its original state.

Part (B). In the last decades hydrogels gained increasing interest as drug delivery scaffolds and permeation membranes due to their ability to control solute transport. The diffusion in polymers or in porous materials was previously studied by different groups using either NMR in solution or solid state techniques. However, a direct characterization of the active pharmaceutical ingredient (API) in the molecular environment of the drug delivery formulation (i.e. in the hydrogel) was missing in the literature.

The innovation of the work herein discussed is in the direct observation of the dynamic behaviour of small molecules confined in hydrogel matrixes.

The study was carried out by using a High Resolution Magic Angle Spinning (HR-MAS) NMR probe. HR-MAS NMR allows to study the molecular environment and the transport properties of the drugs entrapped in the hydrogels. To the best of my knowledge, this type of study is unprecedented in the literature. Diffusion experiments were performed on Ethosuximide and Ibuprofen, taken as model drugs, loaded in hydrogels obtained from Agar-Carbomer (AC) and cyclodextrins nanosponges (CDNS). The signal intensity decays were collected using different observation time values (td) in the milliseconds range by means of the pulsed field gradient spin-echo (PFGSE) method. The experiments were repeated at several concentrations of the drug molecules dissolved in hydrogels having different mesh size. The signal decay provided the mean square displacement (MSD) values for each drug concentration.

The MSD along the z reference axis <z2> is proportional to the observation time elevated to a power factor α. The α values define the normal Fickian random motion (α = 1), or anomalous, non-Fickian (α < 1, subdiffusive regime, α > 1 superdiffusive regime).

NOVEL COBALT-BASED CATALYSTS FOR THE INTENSIFICATION OF THE FISCHER-TROPSCH SYNTHESIS

Laura Fratalocchi - Supervisor: Prof. Luca Lietti

The development of the optimal cobalt based FT catalyst, with improved activity and selectivity to desired products, has been the goal of this PhD thesis work. This goal has been pursued by a combination of experimental activities at different scales, starting from the preparation of the catalysts, to their characterization and eventually the assessment of their catalytic performances in the FTS.

In the first part of this thesis work the development of a novel preparation procedure for an eggshell-type Co/γ-Al_2O_3 catalyst is reported. The main characteristics of the obtained material are a diameter smaller than which is the most common value in the scientific literature, and a diffusive length ensuring the absence of mass transfer limitations. Interestingly, the prepared eggshell catalyst shows great catalytic performances if compared with those of a powdered catalyst (obtained by grinding and sieving the eggshell catalyst) working in a kinetically controlled regime. This result has been explained by considering the onset of weak mass transport limitations in the case of the eggshell catalyst, which boost both the CO conversion kinetics and the catalyst hydrogenating ability. These results show that engineered eggshell catalysts with an optimal thickness of the active shell represent an optimal solution for applications purpose. In fact, it is possible to achieve high C_{15+} yields per mass of cobalt in the reactor, while limiting the ΔP in compact packed-bed reactors for the FTS.

The second part of this thesis work focuses in developing a highly active Co/γ-Al_2O_3 catalyst as uniformly impregnated with a pellet size of 300 μm in diameter. In compact FT reactors, such diameter represents a compromise to keep under control mass transfer and pressure drop issues. In this regard, two strategies have been adopted in this thesis work to increase the number of cobalt metal surface sites available for the FTS. The decrease of the cobalt metal particle size through the improvement of the preparation methods commonly applied at the industrial scale (in view of the necessity not to complicate the catalyst synthesis) and the enhancement of the catalyst reducibility with the addition of a small amount of noble metals (in view of the catalyst cost).

Concerning the first approach, a highly active Co-based catalyst has been synthetized by diluting the Co-nitrates impregnating solution with an organic compound (i.e. diethylen glycol, DEG). It has been found that thanks to the occurrence of combustion phenomena between Co nitrates, which act as oxidizers, and DEG, which act as fuel, the decomposition from Co nitrates to Co oxides species during calcination becomes exothermic and fast. This generates highly dispersed Co oxides particles, which, however, are difficult to reduce by a standard reduction treatment in H_2 at 400°C. Despite the lower reducibility, when compared with a catalyst with the same formulation but prepared without using DEG, the catalyst prepared with DEG shows very interesting catalytic performances, thanks to the fact that the small Co^0 particles are intrinsically very active. In line with the decrease of the Co^0 crystallite size, the catalyst prepared with DEG shows slightly higher hydrogenating activity. Nevertheless, the increase of the CO conversion overcompensates the decrease of the C_{5+} selectivity, thus resulting in a raised C_{5+} yield.

Concerning the second approach, the possibility to increase the number of active Co^0 centers through the addition of small amount of platinum in the catalyst formulation has been investigated. In particular, the effect of 0.1wt.% of Pt has been studied on the properties and catalytic performances of a catalyst prepared with (small crystallites) and without (big crystallites) DEG, in the Co-impregnating solution. The Pt-promoted catalysts have been prepared by varying the impregnation order of Pt and Co: (i) Pt after Co (sequential deposition order, SDO) and (ii) Pt before Co (reverse sequential deposition order, RSDO). Regardless of the initial Co_3O_4 crystallites size, all the Pt-promoted catalysts show a strong enhancement of the catalyst reducibility and Co^0 dispersion with respect to the corresponding unpromoted catalysts. This indicates the ability of Pt in favoring the reduction of cobalt oxides species, even if present in small quantity. Moreover, it has been found that the catalytic activity trend reflects that of the Co^0 dispersion with the catalysts prepared without DEG, thus justifying why the RSDO catalyst is more active than SDO, which in turn is more active than the unpromoted sample. Furthermore, the selectivity to the main FTS products is almost unvaried with respect to the unpromoted sample, thanks to the small amount of Pt used. On the contrary, the promising results obtained with the Pt-promoted catalysts prepared with DEG in terms of catalyst characterization (good reducibility and increase of the number of Co^0 sites) do not reflect the catalyst activity, which results (in the bad case) almost halved with respect to the unpromoted catalyst. This result has been explained with the fact that the intrinsic activity of the Co^0 sites (turnover frequency, TOF) of the Pt-promoted catalysts is significantly decreased. This has been attributed to the fact that these catalysts have a Co^0 particle size distribution shifted to lower values than that of the unpromoted sample, thus entering in the TOF-size range where the FTS has been reported to be structure sensitive.

In the final part of this thesis work, the effect of water on the catalytic performances of Co-based catalyst is investigated. This is of interest in view of the fact that commercial FTS practices require that Co-based catalysts withstand long-term use at high CO conversion, and hence at high water concentration levels. The obtained data indicate that water addition leads to a remarkable catalyst deactivation already at low water concentration. This phenomenon is a rather slow process, whose rate depends on the feed concentration of water and whose extent depends on the duration of water co-feeding. The presence of water in the feed also affects the process selectivity, and both reversible and irreversible effects have been observed. In particular, the exposure of the catalyst to water results in an irreversible increase of the olefin to paraffin ratio (mostly due to the increased olefin selectivity), of the 1-olefins in the alkenes pool (due to the decreased double bond shift activity) and of the CO_2 selectivity (due to increased WGS activity). These effects have been explained considering that the active sites evolve during the water co-feeding in those cobalt oxides species which are formed as a result of the oxidation of a fraction of the metallic cobalt sites initially present on the catalyst. Reversible effects, which mostly consist in the increased chain growth probability and CO_2 selectivity and in the decreased CH_4 and alcohol selectivities, have been explained by assuming that the water inhibits the hydrogenation reactions and acts as reactant in the WGS reaction.

Giancarlo Gentile - Relatore: Tiziano Faravelli

The thermochemical conversion of biomass is a promising technology to produce fungible substitutes for petroleum-derived fuels and chemicals. This is a topic of increasing interest within the scientific community in light of the recent concerns regarding the energy availability and the sustainability of the industrial processes. Thermochemical conversion routes provide remarkable advantages in the exploitation of biomass because of the possibility to handle wide range of feedstock and address the production of different type of fuels and chemicals. However, current technologies still face significant industrial challenges, mainly in term of efficiency and operation scale-up, which severely hinder the worldwide industrialization of these technologies. In this framework, spending modeling efforts in the direction of a clearer and deeper understanding of biomass potential is crucial towards the massive exploitation of this renewable energy source.

The aim of this thesis is to develop a new comprehensive and predictive particle based model able to provide a better insight into the fundamental chemical/physical phenomena occurring during the biomass conversion. The proposed problem is quite complex as it requires several levels of analysis and consideration, as highlighted in Figure 1. Moreover, the strong interactions between chemical kinetics and heat/mass transfer phenomena make the mathematical model demanding from a computational point of view.

The work presented in this thesis steps from the definition of the methodological basis. Firstly, a model for the biomass characterization was presented. It is able to predict the biochemical composition of a generic biomass sample starting from its elemental analysis. The second step regards the definition of a comprehensive kinetic mechanism for the biomass pyrolysis and devolatilization. A multistep kinetic scheme with lumped first-order reactions was employed in this work. The peculiarity of this model, respect the state-of-the-art in the literature, is a detailed characterization of the pyrolysis products, including a wide set of permanent gas and tar species. This kinetic mechanism was eventually coupled with the heat/mass transfer limitations in the developed particle-based model. The resulted code, named bioSMOKE, was implemented within the OpenFOAM® framework in order to simulate the thermal degradation of anisotropy biomass particles of arbitrary shapes. The mathematical model requires the solution of the Navier-Stokes equations, applied to porous media. One of the original aspects of this work is represented by the development of a comprehensive approach to model the spatial-dependent particle shrinking during the thermal decomposition. It is based on the evaluation of a shrinking wave front which progressively evolves during the transient and allows for handling arbitrary multi-dimensional particle deformations due to anisotropy and/or non-uniform boundary conditions. In spite of the outstanding importance of this topic, only few models are available in the literature, usually based on empirical correlations or fitting parameters. The code was validated against comparisons with experimental data of particle pyrolysis in different range of operative conditions. Three different geometries were analyzed (namely, sphere, cylinder and slab), to show the reliability and the capability of bioSMOKE in handling particle shrinking. Despite of the unavoidable uncertainties of such a system, the model turns out to be in reasonable agreement with the experimental data. The model successfully predicted the temperature history of the particle, the mass/volume loss, as well as the evolution of the major species released during the pyrolysis.

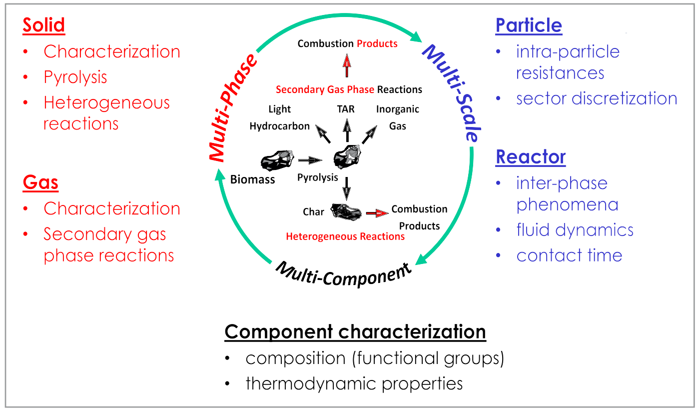


Fig. 1 - Biomass thermal conversion: a multicomponent, multi-phase and multi-scale

The fundamental analysis at the particle scale is an essential step towards the rational modeling of complex multiphase reactors (i.e. fluidized/fixed bed gasifier). In this regard, one the critical issues is the coupling between the particle-scale phenomena and the reactor-scale transport. The existing state-of-the-art methods crudely consider the particle degradation because of the complexity of the mathematical formulation and the huge computational costs required for tracking individual particles. Global kinetics are typically defined which cannot capture the key dynamics of particle conversion. To this end, a reduced-order model (ROM) for biomass particle degradation was developed aiming to describe the release rate of primary pyrolysis products by means of a simpler and less demanding methodology.

DESIGN OF LIPOSOMAL AND POLYMERIC NANOCARRIERS CAPABLE OF CROSSING BIOLOGICAL BARRIERS

Xiaoyi Huang - Supervisor: Prof. Francesco Cellesi

This PhD thesis work focused on the design of new liposomal and polymeric nanocarriers capable of crossing specific biological barriers. Depending on the application, the physicochemical characteristics of the nanocarriers were tuned in order to maximize barrier penetration and drug delivery at the specific site.

Firstly, new targeted therapies directed to treat glomerular diseases in kidneys were developed, based on the use of new protein drug-loaded, engineered liposomes.

These lipid-based nanocarriers were designed to target the glomerular endothelium and release the drug in close proximity to the glomerular filtration barrier. Once released, the drug should present a hydrodynamic diameter small enough to penetrate the glomerular barrier by diffusion and reach podocytes, i.e. glomerular cells which are known to play a central role in kidney diseases. In order to identify the key parameters which may influence drug encapsulation and release, different lipid formulations and payloads were investigated.

Four thermosensitive liposomal formulations were selected: DPPC/DSPE-PEG2000 95/5 (mol/mol) (TSL1), DPPC/DSPC/DSPE-PEG2000 80/15/5 (mol/mol) (TSL2), DPPC/DSPC/Chol/DSPE-PEG2000 50/25/15/3 (mol/mol) (TTSL) and DPPC/P-lyso-PC/DSPE-PEG2000 90/10/4 (mol/mol) (LTSL).

Water soluble molecules, including a low molecular weight molecule (Carboxyfluorescein (CF)), a hydrophilic linear polymer (dextran), a custom-made PEGylated star polymer and a protein (albumin) were used as model drug molecules for encapsulation and release. Results showed that all liposomal formulations displayed higher loading efficiency for carboxyfluorescein (CF) mainly due to its low molecular weight. The presence of hydrophobic domains in albumin enhanced the interaction between this protein and the hydrophobic lipid bilayer to some extent, thus leading to higher encapsulation efficiency compared to that of dextran and PEGylated star polymer. LTSL not only showed the highest encapsulation efficiency for albumin compared to other liposomal formulations, but it was also able to release CF and albumin in the mild-hyperthermia temperature range (40-42°C). The release of albumin was much more limited than that of CF due to slower diffusion and potential interaction with the lipid bilayer through hydrophobic domains. LTSL was further selected for encapsulation and release of therapeutic proteins, such as lysozyme and the brain-derived neurotrophic factor (BDNF), which has been recognized as a potential therapeutic agent for kidney diseases. *In vitro* release tests confirmed that LTSL was capable of retaining lysozyme and BDNF at the physiological temperature while releasing them under mild hyperthermia conditions. Moreover, BDNF maintained structural stability and bioactivity towards damaged podocytes during the encapsulation process, as confirmed by SDS-PAGE analysis and immunofluorescent tests. The BDNF-loaded LTSL showed good therapeutic effects towards podocytes in standard 2D cell cultures (**Fig. 1**) as well as in a 3D podocyte–endothelial co-culture system, which was designed to mimic the glomerular filtration barrier *in vitro*. Therefore, BDNF-loaded LTSL nanotherapeutic may present a powerful new strategy for effective treatment of kidney glomerular diseases.

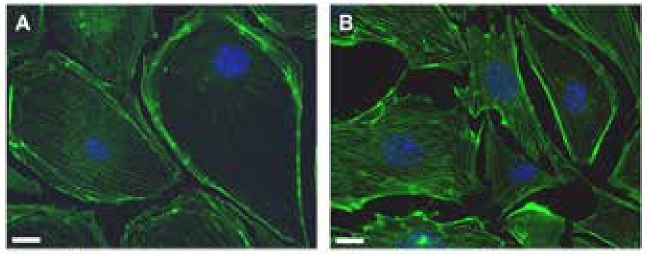


Fig. 1. Immunofluorescence studies of podocytes. The cytoskeleton of podocytes was stained by phalloidin (green) and the nuclei were stained by DAPI (blue). Scale bar: 20 μm. Damaged podocytes (A). Repaired podocytes after incubated with BDNF-loaded LTSL (B).

In order to enhance the targeting properties of liposomes towards glomerular endothelial cells, specific peptides, such as cRGD, Ac-IELLQARC-NH$_2$ (IEL), Ac-HITSLLSC-NH$_2$ (HIT) and Ac-CLPVASCK-NH$_2$ (CLP), were selected as ligands and conjugated to liposomes. Cellular uptake experiments showed that cRGD-conjugated liposomes exhibited extremely high uptake by mouse microvascular endothelial cells (EOMA) and glomerular endothelial cells isolated from rat glomeruli. cRGD conjugated liposome uptake was likely due to the overexpression of αvβ3 integrin receptors on the cell surface. IEL conjugated liposomes also showed substantial interaction with EOMA due to the expression of E-selectin (a receptor for IEL) on the tumour vascular endothelial cells. HIT and CLP showed enhanced selectivity towards rat glomerular endothelial cells (**Fig. 2**). *In vivo* biodistribution of liposomes in mice revealed that the conjugation of cRGD to liposomes enhanced accumulation of these nanocarriers in the liver, spleen and kidney. Therefore, the specificity of peptidic ligands towards kidney should be enhanced to improve the targeting delivery to the glomeruli; HIT and CLP could be potential candidates in achieving this goal.

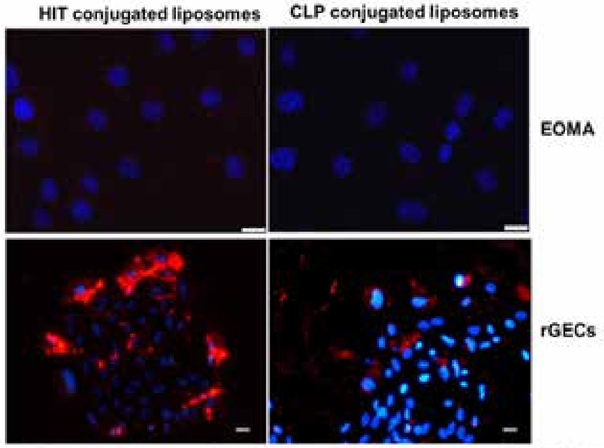


Fig. 2. Fluorescent images of mouse microvascular endothelial cells (EOMA) (top panel) and rat glomerular endothelial cells (rGECs) (down panel) after being incubated with HIT conjugated liposomes (0.5 mg/mL) (left panel) and CLP conjugated liposomes (0.5 mg/mL) (right panel) for 24 h. Peptides conjugated liposomes were labeled by rhodamine (red) and the nuclei were stained by DAPI (blue). Scale bar: 20 μm.

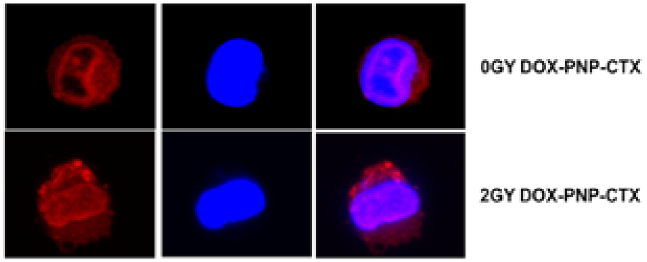


Fig. 3. CLSM images of glioblastoma multiform (GBM) cells after 4 h incubation with CTX conjugated DOX-loaded PNP (DOX-PNP-CTX) either treated with 2 GY radiation dosage or not. For each panel, images from left to right show DOX fluorescence in cells (red), cell nuclei stained by DAPI (blue), and overlays of two images.

Secondly, a glioblastoma multiform-targeted therapeutic approach based on the use of PLGA-PEG polymeric nanoparticles (PNPs) loaded with the anti-tumor drug doxorubicin (DOX), was developed. DOX was efficiently loaded into PNPs by nanoprecipitation, and DOX loaded PNPs were further functionalized with chlorotoxin (CTX), which is known to selectively bind to glioma cells with high affinity. The engineered targeted PNPs offer the potential for delivering therapies directly to invasive brain cancer cells, thus improving the desired therapeutic effects while minimising unwanted toxicity. Preliminary *in vitro* cellular uptake and cytotoxicity studies suggested that further modification of the polymer structure may be necessary to control the release rate of DOX and improve cell targeting. Moreover, a combination strategy based on radiation therapy and CTX modified PNPs may have a great potential in enhancing blood brain barrier penetration, and strengthening the anti-tumor efficacy of chemotherapeutics towards glioblastoma multiform diseases (**Fig. 3**).

Keywords: Thermosensitive liposomes; Polymeric nanocarriers; Biological barrier; Kidney diseases; Glomerular filtration barrier; Glioblastoma multiform; Blood brain barrier; Targeted delivery

HYDROGEN AND HALOGEN BONDING TOWARDS MOLECULAR RECOGNITION AND SEPARATION

Vijith Kumar - Supervisor: Prof. Giuseppe Resnati

Molecular self-assembly has been well-acknowledged as one of the efficient bottom-up approaches to synthesize various materials with controllable architectures and useful properties. It has clearly been proven that the formation of the well-ordered structures of self-assembled architectures can be driven by a single interaction or by the synergistic action of multiple interactions. In this thesis metal coordination, hydrogen bond (HB) and halogen bond (XB) have been used for the design and synthesis of self-assembled systems tailored to topological studies, separation processes and obtainment of supramolecular functional materials.

The first part of the thesis describes how metal coordination enables twenty four bis-pyridyl ligands (L), functionalized with an iodotetrafluorobenzene moieties, and twelve Pd(II) ions (M) to undergo, in solutions, a quantitative self-assembly process and to form discrete nanocages of general formula $M_{12}L_{24}$. Artificial self-assembled cages can often encapsulate guest molecules and promote unusual reactivity; the XB donor groups decorating the inside face of the obtained nanosized capsule will be used to control the nature of the guest molecule(s) encapsulated in the cage and to direct the reactivity of these molecule(s).

In the second part of the thesis, HB is employed in selective recognition and effective separation of industrially important dicarboxylic acids from either their mixtures through solid or solution phase processes. The size-matching of the interacting partners plays a major role in allowing for selective self-assembly and ensuing separation process. Specifically, we have demonstrated that bis-(trimethylammonium) alkane diiodides, a well-known class of porous organic salts, can reversibly encapsulate dicarboxylic acids through intermolecular hydrogen bonding between the host I^- anions and the guest carboxylic OH group. The process is highly effective for separating in pure form of dicarboxylic acid chain that forms an $I^-\cdots HOOC–(CH_2/CF_2)_n–COOH\cdots I^-$ superanion matches in length to the chosen dication. The size-matching controlled formation of cocrystals allows for the selective solubility variation of dicarboxylic acids and provides a new direction for selective recognition and separation.

The strategy is reminiscent of the effective exchange of α,ω-diiodoperfluoroalkanes via the formation of halogen bonded $I^-\cdots I(CF_2)_nI\cdots I^-$ adducts where cation/supramolecular anion size matching plays a key role.

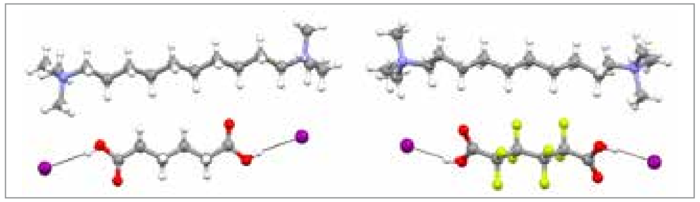


Fig. 1 - Crystal structure of supramolecular size matching complexes: Top: decamethonium iodide adipic acid complex; Bottom: decamethonium iodide octafluoro adipic acid complex; Colour codes: grey, carbon; blue, nitrogen; white, hydrogen; red, oxygen; green, fluorine; magenta, iodine.

In the third part of the thesis XB is used to form a great variety of supramolecular architectures. Specifically, naked halide anions have been used to form several halogen bonded networks with different and fascinating topologies. A library of supramolecular anionic networks showing Borromean interpenetration has been prepared by self-assembly of crypt-222, several metal or ammonium halides, and five bis-homologous α,ω–diiodoperfluoroalkanes. Halogen bonding has driven the formation of these anionic networks. Borromean entanglement has been obtained starting from all the four used cations, all the three used anions, but only two of the five used diiodoperfluoroalkanes.

As the change of the diiodoperfluoroalkane, the cation, or the anion has a different relative effect on the metrics and bondings of the self-assembled systems, it can be generalized that bonding, namely energetic, features play here a less influential role than metric features in determining the topology of the prepared tetra-component cocrystals. This conclusion may hold true for other multi-component systems and may function as a general heuristic principle when pursuing the preparation of multi-component systems having the same topology but different composition.

In addition to this, the role of $I\cdots I^-$ XB is used to assess the proton localization in the product that hydrogen iodide forms with crypt-111, a proton sponge with unique and useful protonation kinetics.

Finally the synthesis and characterization of new trimeric complexes obtained upon XB driven self-assembly of 1,4-diiodotetrafluorobenzene or α,ω–diiodoperfluoroalkanes, acting as XB-donors, with an alkoxystilbazole derivative functionalized with a methacrylate terminal group , acting as XB-acceptor has examined in detail. Despite the fact that the starting materials are not mesomorphic in nature, the obtained halogen-bonded complexes exhibit monotropic LC behaviour with smectic A phases possibly resulting from segregation between fluorocarbon and hydrocarbon chains. The obtained supramolecular mesogens possess reactive groups suitable for incorporation into liquid crystalline elastomeric actuators.

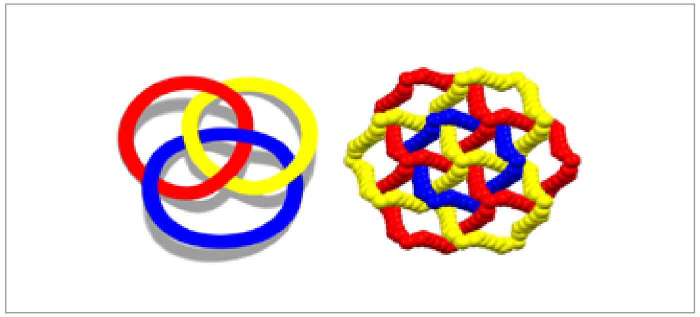
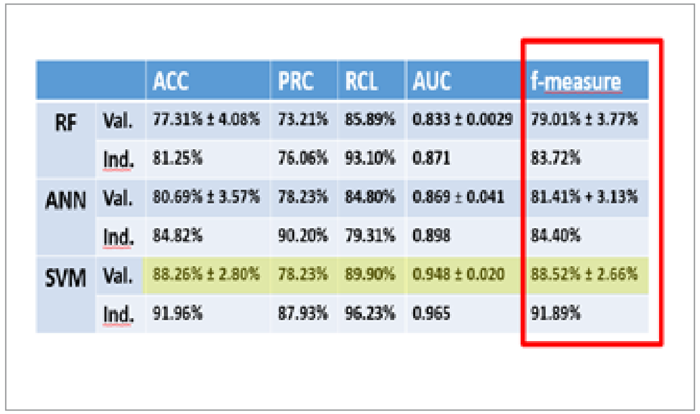


Fig. 2 - Left: Schematic view of discrete Borromean ring. Right: Partial view (Mercury 3.8, space-filling) of the three honeycomb nets present in the cocrystal crypt-222 potassium iodide diiodoperfluorooctane. Red, yellow, and blue colours differentiate the three translationally related nets showing Borromean entanglement. Supercations K+Ì1 are omitted for clarity.



		ACC	PRC	RCL	AUC	f-measure
RF	Val.	77.31% ± 4.08%	73.21%	85.89%	0.833 ± 0.0029	79.01% ± 3.77%
	Ind.	81.25%	76.06%	93.10%	0.871	83.72%
ANN	Val.	80.69% ± 3.57%	78.23%	84.80%	0.869 ± 0.041	81.41% + 3.13%
	Ind.	84.82%	90.20%	79.31%	0.898	84.40%
SVM	Val.	88.26% ± 2.80%	78.23%	89.90%	0.948 ± 0.020	88.52% ± 2.66%
	Ind.	91.96%	87.93%	96.23%	0.965	91.89%

Fig. 3 - Crystal packing of complexes alkoxystilbazole diiodotetrafluorobenzene (top) and alkoxystilbazole diiodooctafluorobutane (bottom) showing a clear segregation between hydrocarbon and perfluorocarbon modules in complex. Hydrogen atoms have been omitted for the sake of clarity. Color codes: grey, carbon; blue, nitrogen; magenta, iodine; red, oxygen; yellow, fluorine.

RECOVERY OF SUGARS FROM LIGNOCELLULOSIC HYDROLYSATES BY CONTINUOUS ION EXCLUSION CHROMATOGRAPHY FOR THE PRODUCTION OF GREEN CHEMICALS

Gabriele Lodi - Supervisor: Prof. Laura Annamaria Pellegrini

Tutor: Prof. Davide Moscatelli

Today, the depletion of fossil resources, the increasing greenhouse gas emissions and the resulting climate change, joined with a growing world population, make imperative to find processes able to produce fuels and chemicals from alternative, renewable resources. Lignocellulosic biomasses, e.g. forestry wastes, wheat straw, corn stover and sugarcane bagasse, can be used to obtain monomeric sugars, which are valuable raw materials and can be subsequently converted into fuels or chemicals through chemical or biochemical processes.

Biochemical conversion of biomasses involves pretreatment and hydrolysis processes for deconstructing the recalcitrant lignocellulosic matrix and for hydrolyzing the polysaccharides (namely, cellulose and hemicellulose) to monosaccharides like glucose, xylose, arabinose, galactose and mannose. These monosaccharides are then fermented or catalytically converted into the desired products. Biochemical processes are often referred to as “sugar-platform” conversions: the production of monomeric sugars from lignocellulosic biomass is thus the key to a sustainable, renewable chemical industry. However, pretreatments of lignocelluloses release not only the desired pentose and hexose sugars, but also various compounds that can inhibit the fermentation microorganism or poison the conversion catalyst. This can deeply reduce the product yield and process productivity, severely limiting the usefulness of the derived sugars. In order to enhance the efficiency of sugars conversion, it is necessary to remove these inhibitors from the hydrolysate before the fermentation.

The recovery of sugars from hydrolysates and the removal of by-products can be successfully accomplished using chromatography. The separation is based on a chromatographic technique known as Ion Exclusion. In this technique, strong electrolytes are separated from nonelectrolytes and weak electrolytes using a strong ion-exchange resin as a stationary phase. The strong electrolytes are excluded from the resin due to electrostatic repulsion with the fixed groups, while the nonelectrolytes and weak electrolytes are partitioned between the mobile phase and the stagnant liquid inside the particles. No actual ion exchange takes place during the separation and hence no chemical regeneration of the resin is required.

The Italian company Biochemtex developed the GREG™ process, in which monosaccharides are converted into ethylene and propylene glycols. The process is based on traditional catalysts and a straightforward purification of the sugars is thus essential in order to increase the catalyst lifetime. The process involves an innovative pretreatment, where the hydrolysate is neutralized to pH 6 before the chromatographic separation making all the electrolytes in solution completely dissociated and therefore excluded from the resin pores. The electrolytes elute unseparated as a first group in the chromatogram followed by the sugars, allowing a baseline binary separation that is performed in continuous in an I-SMB process.

In this work, the recovery of lignocellulosic sugars obtained with this innovative pretreatment has been studied.

Experimental investigations on model systems, aimed to understand the mechanisms involved in the separation, have been carried out at different scales. The column dynamics has been analyzed using equilibrium and pulse elution data measured in batch columns. A systematic investigation of the adsorption behavior of the main components found in the hydrolysate has been performed and the effects of the main electrolytes on resin shrinking and sugars adsorption have been studied. Afterwards, a quantitative description of the experimental observations was provided.

On this basis, a dynamic column model for IEC has been developed and used to simulate pulse tests of real hydrolysates. The exclusion of ions from the resin pores has been explained in terms of Donnan Theory, which describes the partition of charged species between two phases separated by a semipermeable membrane. The equilibrium relationships for this model have been derived and implemented in the material balances. These were written taking into account changes in interparticle and intraparticle porosity due to resin shrinking.

A model for the I-SMB process was developed coupling the single-column dynamic model to the appropriate material balances at the nodes. The model was then used to predict experimental data of continuous separations, obtained with the I-SMB unit of the GREG™ pilot plant. The results were in good agreement with the experimental data, correctly representing the main features of this separation. The simulation tool can be used for the design and optimization of the operating conditions of the I-SMB separation process.

ODOUR EMISSIONS FROM NON-ACTIVE AREA SOURCES

Federico Lucernoni - Relatore: Prof. Ing. Laura M. T. Capelli

During the PhD, the focus was on the problem of odour sampling on non-active area sources. The final goal was to provide and answer to the question "Which is the best way to asses odour emissions from non-active area sources?". It is necessary to clarify that this is a complex open issue. In the first part of the research it was necessary to understand the sources that were the object of investigation. A first result was understanding that the previous distinction between passive and active area sources was too simplistic and that it is necessary to distinguish the non-active sources in at least three categories, as among these categories there are significant differences that can't be neglected if the aim is defining a method to sample and assess the odour emissions capable of producing reliable data. Once this important first result was achieved, the focus shifted on the description and characterization of the three identified kinds of non-active area sources: semi-passive sources, liquid passive sources, solid passive sources. This led to divide the main problem in three different sub-problems, and the initial question into three distinct questions, each requiring a different procedure in order to obtain an answer (see the scheme in Fig. 1).

Fig. 1 - Scheme of the subdivision of the problem.

Semi-passive sources, are defined like this as they have a proper endogenous emission flux, even if it is typically low; such sources are typically landfill surfaces. For these sources, the main results obtained were the definition of the sampling hood design and the definition of the sampling method, both validated via a detailed on-site study as well as a thorough CFD analysis (see Fig. 2).

The purely passive sources, instead, are characterized by absence of said endogenous emission flux and emission is due to volatilization phenomena. Liquid surfaces are typically represented by waste water treatment tanks. For these sources it was defined a reliable sampling system, and another important achievement was understanding how important intra-phase transport phenomena are for a precise description of the phenomena leading to the emission. For solid sources, typically represented by contaminated soils, it became evident that they are characterized by a high complexity and that in this case more than in any other, transport phenomena and the chemical-physical characteristics of the source itself are crucial for a proper approach to the problem. Another important result was understanding that the methods usually adopted, normally assimilating liquid sources to the solid ones, neglecting the transport phenomena and the chemical-physical aspects, are not proper. This difference from before and after this research project is a big change and it is fundamental. In conclusion, one of the most important results of the work, is the distinction between the

different types of non-active area sources, as it became evident how not all are the same and they can't be treated all in one single fashion, also how it is necessary to differentiate them and how each kind needs to be treated distinctly. Then, another important achievement was obtained, keeping a more engineering approach to the problem, that is the understanding of the crucial role - in the case of passive sources - played by the transport phenomena and the characteristic chemical-physical parameters of the source itself.

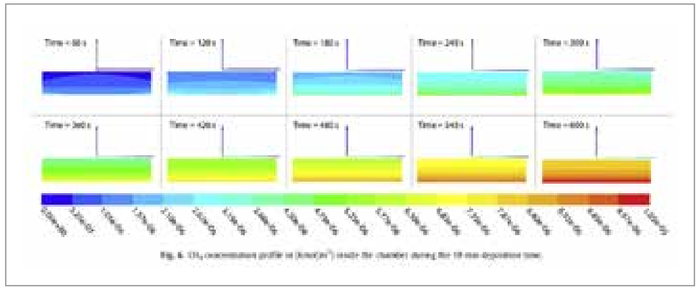


Fig. 2 - CFD analysis of the behavior of the sampling device adopted for semi-passive area sources, exploiting the software Fluent®. It is shown here the methane concentration profile in [kmol/m3] inside the chamber during the 10 min static deposition time, for one of the scenarios inspected during the study.

FUNCTIONALIZED HYDROGELS FOR DRUG AND CELL DELIVERY IN CENTRAL NERVOUS SYSTEM

Emanuele Mauri - Supervisor: Prof. Filippo Rossi

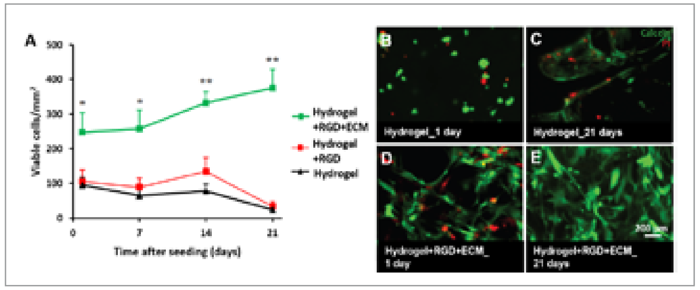


Fig. 1 - In vitro hMSCs viability and morphology. A: cell viability profiles; B-C: hMSCs in unmodified hydrogel; D-E: hMSCs in hydrogel with RGD and ECM.

Central nervous system (CNS) injuries represent one of the most critical scenarios for human health, society and economy. Nowadays, the lesions to brain or spinal cord are still looking for efficient and sustainable solutions. In particular, in spinal cord injury, the neurodegeneration is related to the influx of immune cells and the scar formation, which generates permanent interruption of information transmission to and from the CNS. Researchers are moving towards therapeutic approaches based on the use of biomaterials to reduce the post-traumatic degeneration. These tools involve the use of anti-inflammatory agents and cells to counteract the adverse effects and to rebuild cellular interconnections. Recent literature is focused on *in situ* treatments applying three-dimensional hydrogel scaffolds, due to their properties: the ability to retain water mimicking living tissues, their high biocompatibility, the possibility to allow controlled release rates and control the cell fate.

In this work, functionalized hydrogel systems, composed by synthetic and natural polymers, are synthetized as hospitable cell delivery and as carriers for controlled drug release. The functionalization strategies are post-polymerization modifications grafting chemical groups that are not present in the polymer structures.

Hydrogels are obtained as bulk systems and nanostructures (nanogels). The first ones are developed as appealing cell niches linking RGD tripeptide to the scaffold through click chemistry and introducing the extracellular matrix (ECM) deposition: the *in vitro* and *in vivo* studies with human mesenchymal stem cells (hMSCs) have showed a significant improvement in cell viability and density (Fig. 1), promoting the activation of anti-inflammatory and curative actions in the target site.

About the drug delivery guidelines, the use of pH-sensitive hydrazone and ester linkers between drugs and polymer gives the opportunity to synthetize tunable tools with different release kinetics, to promote a sustained and prolonged drug release. At different pH, with hydrazone and ester functionalizations, drug release has occurred as double Fickian diffusion regime (Fig. 2): the transition and duration of the two regimes depends on the nature of the functionalization and probably correspond to ester/hydrazone lability at the interface hydrogel/external environment and ester/hydrazone bonds present in the inner core.

Moreover, the versatility of functionalization techniques allows the design of a polymeric scaffold as injectable tool for magnetic resonance imaging (MRI). In this case, the hydrogel with paramagnetic properties is obtained through azide-alkyne cycloaddition between polyethylene glycol and TEMPO.

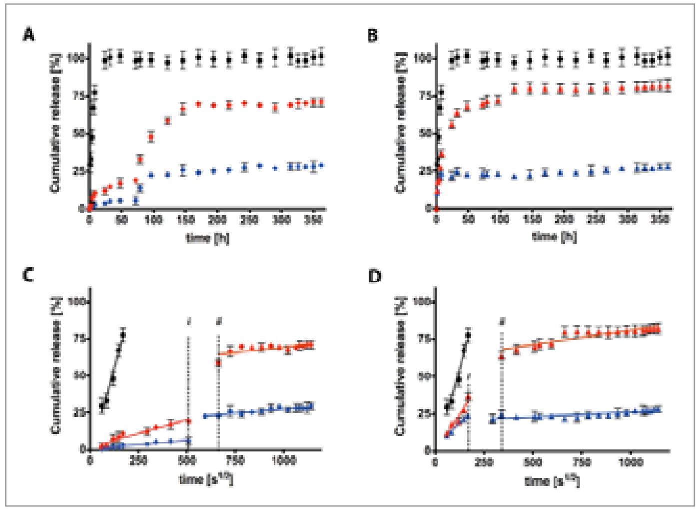


Fig. 2 - A-C: In vitro release profiles and slope representative of Fickian diffusion coefficient from unmodified hydrogel (•) and ester-hydrogel at pH 7.4 (♦, blue) and at pH 8.5 (♦, red). B-D: In vitro release profiles and slope representative of Fickian diffusion coefficient from unmodified hydrogel (•) and hydrazone-hydrogel at pH 7.4 (▲, blue) and at pH 8.5(▲, red).

In vivo results have showed the traceability of MRI hydrogel signal at least 1 month, without any adverse reactions.

On the other hand, the design of nanogels is aimed to monitor and interfere in the activation of inner-cell inflammatory response: in particular, this work has investigated the modulation of nanogel cellular internalization and their use as drug carrier within the cytosol. The development of different polymer-based coating layers has led to the modulation of cell uptake, laying the milestone for the advance of nanogels capable of selective cell interactions: a promising approach to introduce targeting curative therapy, avoiding side-effects. The combination of orthogonal chemistry and physical properties has offered the opportunity to synthetize nanogels for drug delivery releasing their therapeutic cargo within the cellular environment. It is proposed an innovative nanogel where the drug is grafted to the nanostructure through a disulfide bond: after the cellular uptake, the drug is released without any external stimuli or inflammatory conditions, but exploiting the cellular components cysteine and glutathione able to disrupt the disulfide bond and make available the bioactive element previously loaded. As alternative, the modifications in the experimental conditions of nanogel synthesis allows tuning the amount of drug loaded and released from the nanosystems, showing their potential use as drug delivery without constraints related to the presence of drug functional groups, otherwise necessary to form a chemical bond with a polymeric network.

In summary, this work, through the interdisciplinary approach involving chemistry, engineering and biology, aims at developing smart drug and cell delivery systems, with the idea to improve their healing effects in the central nervous system disorders.

The combinatorial delivery of drugs and cells through tailored biomaterials is a promising and innovative approach that may represent a breakthrough in the treatments, with the potential for rapid translation to patients and contribute to the advancement in the design and construction of biomedical devices.

DEVELOPMENT OF KINETIC MECHANISMS FOR THE COMBUSTION OF RENEWABLE FUELS

Matteo Pelucchi - Supervisor: Prof. Tiziano Faravelli

Pursuing a sustainable energy scenario for transportation requires the blending of fuels from renewable sources (alcohols, methylesters etc.) into hydrocarbon fuels from fossil sources (gasoline, diesels, jet fuels etc.). In fact, while effective alternatives to combustion exist for electricity production (nuclear, hydroelectricity, solar, wind etc.), the high energy density required for road, sea and air transport endorses biofuels as the only viable and realistic option. Moreover, from an environmental perspective, undeniable and dramatic climate change phenomena impose to act with long term sustainable solutions for reducing greenhouse gases, NO_x and soot emissions. If on one side the production of biofuels from biomasses satisfies the requirement of a net *zero-*CO_2 balance, new fuels and new engines technology have been investigated to improve fuel economy and reduce pollutant emissions. The correct characterization of a fuel or fuel mixture reactivity and the evaluation of its compatibility with existing engine infrastructures in terms of pollution and efficiency is, for a major part, a chemical kinetics problem.

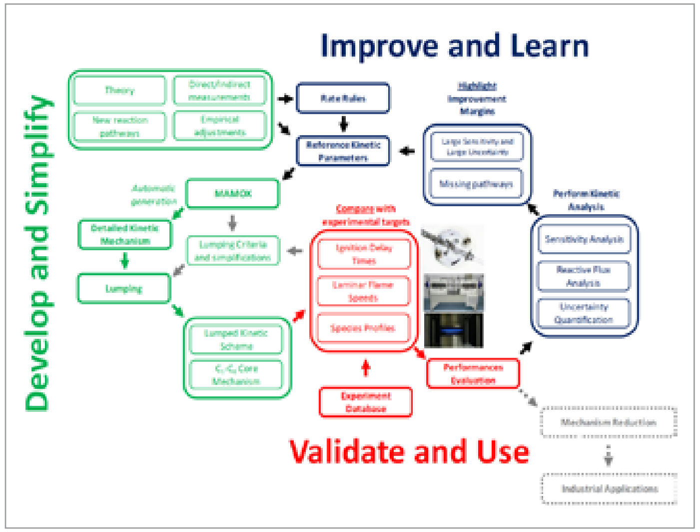


Fig. 1 - Development and validation procedure of the chemical kinetic mechanisms for pyrolysis and combustion of hydrocarbon fuels.

Combustion kinetic modelling, which is the topic of *this thesis*, has been driving fuels and engines development for the last ~25 years. Figure 1 schematically shows the development and validation procedure of a kinetic mechanism according to which this thesis has been developed.

While most of the focus in the first decade has been devoted to the understanding of alkanes chemistry, the need of better representing commercial fuels by means of surrogate mixtures, extended the interest to aromatics and lastly to oxygenated fuels from renewable sources. *This thesis* extended the knowledge of the chemistry involved in alkanes (e.g. n-heptane), aromatics (e.g. toluene) and oxygenated (e.g. alcohols, aldehydes, ketones etc.) fuels combustion, by means of an effective coupling of theory and experiments, within the CRECK group approach to combustion kinetics developed in the last 30 years of research activity at Politecnico di Milano. The left panel of Figure 3 shows a comparison between experimental measurements of the reaction OH+toluene and results from theoretical calculation presented in this thesis. Also the impact of the updated rate constants on ignition delay times is reported in the right panel.

The model obtained coupling the different revised portions, accurately reproduces recent experimental measurements of surrogate mixtures representative of real gasoline fuels. Ignition delay time measurements are compared with model predictions in Figure 3 for mixtures of butanol/heptane (left panel) and n-heptane/iso-octane/toluene/butanol (right panel).

Beside the definition of key model parameters and standard kinetic mechanisms validation procedures, other important challenges of modern kinetic modelling have been a topic of research. The necessity of automatically assessing the validity of increasing complexity kinetic mechanisms has been tackled, providing an innovative and effective method, of application also to mechanism reduction and optimization, and to experimental design.

Inputs for future refinement of the presented models have been provided throughout the discussion, highlighting for example the need of a hierarchical revision starting from the core C_0-C_4 portion of the POLIMI mechanism or a better assessment of other key channels, whose parameters still carry high degrees of uncertainty.

Perspectives concerning the possibility and potentials of effectively and extensively exploiting theoretical kinetics, and the necessity of fully automatized the iterative process of kinetic mechanisms development are also discussed, providing directions for future research efforts.

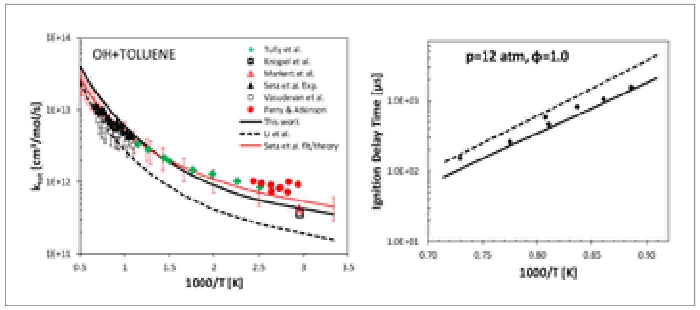


Fig. 2 - Left panel: comparison of the total rate constant of OH+toluene with other theoretical studies and with experimental measurements from the literature. Right panel: Impact of the update rate constant on toluene/air ignition delay time predictions (solid line). Dashed line: POLIMI mechanism with different rate constants for OH+toluene

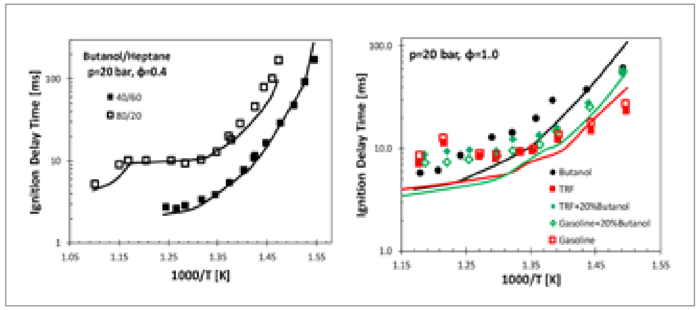


Fig. 3 - Left panel: Ignition delay times for n-butanol/heptane blends at p=20 bar and φ=0.4. Right panel: Ignition delays for TRF on blending with 20% n-butanol, stoichiometric TRF and n-butanol mixtures at p = 20 bar in Rapid Compression Machine. Symbols: experimental data, lines: POLIMI mechanism.

HALOGEN BONDING AS A NEW SUPRAMOLECULAR TOOL TO CONTROL PROTEIN AND PEPTIDE SELF-ASSEMBLY

Andrea Pizzi - Supervisor: Prof. Pierangelo Metrangolo

Although the abundance of halogens in the environment is not remotely comparable to that of other elements, a remarkable number (more than 5000) of naturally occurring halogenated compounds has been discovered to date. Halogenation is a minimal structural modification; however, it can induce deep changes in the molecular properties because of the different chemical features characterizing halogen atoms. Among these features, their tendency to act as electrophilic species is by far the most studied. The strong, specific and directional non-covalent interaction resulting from this electrophilic behavior is halogen bonding (XB). Although halogens are among the most electronegative elements, they can act as electrophilic species because of their anisotropic distribution of the electron density around their nucleus. When halogen atoms form covalent bonds, the new chemical environment affects the halogen electron density, which is no more equally distributed in all directions. The electrostatic potential of the halogen atom becomes anisotropic, showing distinct regions around the nucleus with different chemical behavior. The external region located along the extension of the covalent bond results in lower electron density. Differently, the region perpendicular to the covalent bond shows increased electron density. The resulting shape of the halogen atom is an ellipsoid having the shorter radius (named polar flattening) along the covalent bond direction. According to this electrostatic configuration, nucleophiles form linear interactions (160°-180°) respect to the C−X bond, while electrophiles give rise to lateral contacts (90°-120°) against the bond axis. The strength of halogen bond can be tuned upon changing the nature of the halogen atom and its covalently bound residues. Increasing halogen atom polarizability (decreasing electronegativity) and raising electron-withdrawing propensity of its covalently bound moiety result in a stronger XB. Consequently, halogen bond strength increases following the trend I > Br > Cl >> F. In general, strong – hence short – interactions are more directional than long ones, thus even XB directionality follows the same trend depending on halogen atom polarizability, with iodine that is the more prone to be involved in halogen bonding. Because of its peculiar properties of strength and directionality, halogen bonding has become one of the most exploited interactions in supramolecular chemistry and crystal engineering. However, extensive studies focusing on XB relevance in biomolecular systems started quite recently. Since it is known that halogen atoms can improve biochemical properties like membrane permeability and half-life, their introduction in potential drug candidates is a common procedure of pharmaceutical optimization. Although the importance of halogens in biomolecular systems is longer established, a molecular interpretation correlating the interaction pattern of halogen atoms with the chemical properties of biomolecules is unusual. A recent survey on the Protein Data Bank (PDB) nicely rationalizes XB in biological systems, showing the more frequently occurring halogen bond acceptors. Considering the 567 halogen bonds resulting from this survey, 430 involve halogen atoms and protein residues. All of the twenty amino acids form C−X•••Y halogen bonds, with leucine as favorite residue. XBs involving amino acid main chains are the majority (64.6%) respect to the ones formed with side chains (35.4%). Considering C−X•••π halogen bonds involving aromatic amino acids, nearly half (46.4%) are C−Cl•••Tyr contacts while 22.3% are C−Cl•••Phe interactions. This thesis highlights the role of XB in the self-assembly of biomolecules containing halogenated phenylalanine (Phe) residues. The first part of the thesis describes halogenation as a tool to improve the self-assembly properties of a phenylalanine-based organic gelator: N-Fmoc-Phe. The hydrogel rigidity of mono halogenated derivatives of N-Fmoc-Phe (Fmoc-4-X-Phe, where X = F, Cl, Br, I) has been related for the first time with halogen atom polarizability, i.e. the propensity to act as halogen bond donor. In particular, iodinated and brominated derivatives (containing the most polarizable substituents) were found to form the strongest gels. This trend is opposite to the one reported in previous studies, where hydrogel strength increases with halogen electronegativity. Kinetic studies confirm that iodinated and brominated compounds have the highest fibrillation propensity, indicating a greater efficiency to assemble into high ordered and compact materials. Gel strength and self-assembly efficiency are due to the contribution of halogen bonding in driving the aggregation process, as demonstrated by the crystal structures of Fmoc-4-I-Phe and Fmoc-4-Br-Phe, showing halogen bond between the halogen atom and the electron density of Fmoc aromatic moiety. The second part of the thesis shows the impact of halogenation on the self-assembly behavior of a more complex system. The peptide KLVFF, core sequence of the amyloid beta (Aβ) protein, was modified at the para position of the phenylalanine residues to obtain seven different halogenated derivatives. It was found that some of the halogenated peptides showed an increased rate of fibrillation compared to the wild-type sequence. In general, iodinated derivatives self-assembled into more stable nanostructures, as confirmed by rheological experiments showing the mechanical properties of the peptide hydrogels directly connected with halogen substituent polarizability. Similar results were obtained with another amyloidogenic sequence (DFNKF) although there was a lack of structural evidence about the role of halogen atoms in the self-assembly of this segment. In this thesis, the crystal structure of some of the halogenated KLVFF peptides is the first, direct demonstration that halogen interaction pattern nicely correlates with the aggregation properties of the peptides. The crystal structure of the di iodinated peptide KLVF(I)F(I), showing the peculiar cross-β spine further stabilized by halogen bonding between iodine and peptide carbonyl oxygens, is consistent with the strong self-assembly efficiency of iodinated derivatives. Brominated and chlorinated derivatives, forming less compact aggregates, do not show the contribution of halogen bonding in their respective crystal structures. In addition to the dependence on halogen atom polarizability, the self-assembly of the halogenated KLVFF peptides resulted to be strongly affected by the number and position of halogen atoms in the amino acidic sequence. A single point mutation like halogenation was able to induce a deep change in the self-assembly of the peptide. Indeed, TEM images of each halogenated derivative showed an impressive variety of nanostructures. These results enriches the structural landscape for peptide self-assembly, giving access to a new supramolecular tool to control the morphology of peptide-based materials. Considering the biological context, these results may also shed light on the impact of halogenation in vivo, where oxidative stress mechanisms lead spontaneously to halogenated biomolecules. Finally, halogens were used as "heavy atoms" to aid the phase determination in X-ray diffraction experiments. The KLVFF peptide was modified by placing iodine and bromine substituents on the para position of the terminal phenylalanine ring. KLVFF(I) and KLVFF(Br) were successfully crystallized and their structure was determined with excellent resolution, by using a conventional X-ray source. Since these crystal structures do not show the halogen substituents involved in any kind of specific interaction, they can be considered a reliable model of the wild-type sequence, which structure has never been solved. These crystal structures confirm the overall features of the amyloid cross-β spine, showing in detail the key non-covalent interactions driving the self-assembly of the peptide. For this reason, the crystal structures of these KLVFF derivatives may contribute to enlighten the amyloidogenic behavior of this deeply studied core sequence.

DEVELOPMENT OF HIERARCHICAL METHODOLOGY FOR THE ANALYSIS OF NOVEL CATALYTIC REACTORS: AN APPLICATION TO MICRO PACKED BED REACTORS

Stefano Rebughini - Supervisor: Prof. Matteo Maestri

The first-principles reactor engineering is becoming a very promising tool for the analysis of catalytic reactors. It relies on a fundamental description of all the phenomena occurring in the reactor, where each scale is represented by means of its own governing equations. In heterogeneous catalysis, this approach allows for the accurate description of the interplay between chemistry and transport processes, which is of primary importance in the understanding of the macroscopic observed functionality of the catalyst.
Despite its attractive potential, the first-principles reactor engineering is still hampered by numerical issues and computational costs, even for simple reactor geometries. Therefore, it requires the development of specific tools and methodologies to enable its application to advanced reactor design.
The main aims of this Ph.D. are to develop a numerical framework for multi-scale simulations of catalytic reactors and to propose a hierarchical methodology for the design and the analysis of unconventional and novel reactor configurations. In fact, despite its attractive potential, the first principles multi-scale modeling of complex reactor geometries is still impractical. Indeed, it is mainly limited by numerical issues/limitations related to high number of variables and the high computational cost. Therefore, the first aim of this Ph.D. is the development a framework, which can be used for the first principles multi-scale modeling of catalytic reactors. In particular, a new numerical multi-scale framework able to represent catalytic reactor with different geometries has been developed. This new framework enables the concomitant description of transport phenomena in the gas phase and in the catalyst. It has been applied to analyze the effect of the channel cross-section in the catalytic partial oxidation of methane in a honeycomb reactor. In particular, this framework allows a detail description of the transport phenomena between the gas and the catalyst, which has been used to investigate the effect of the geometry on the reactor performance. This analysis shows that the channel shape strongly affects the reactor behavior in terms of temperature and composition profile. In fact, due to the different diffusion length of the two different cross-sections, a different amount of reactants reach the catalytic wall, affecting the reactor performance.
One of the main limitations of this framework is the high computational cost. Thus a numerical method to reduce the computational effort when the surface chemistry is described by means of microkinetic models has been developed. This methodology enables the use of complex homogeneous kinetic schemes coupled with micro-kinetic heterogeneous schemes, which are applied to investigate the adequacy of lumped parameters in describing the heterogeneous and homogeneous chemistries interaction in the catalytic partial oxidation of hydrocarbons fuel in monolith reactors. This analysis shows that when the effect of the homogeneous chemistry is weak the lumped parameters implemented in the 1D model are adequate to describe the reactor behavior. On the contrary, when the effect of the homogeneous chemistry is enhanced by increasing the pressure, the lumped parameters are not accurate enough to correctly describe the interaction between the surface and the gas chemistries.
The previous analyses clearly show the deeper understanding of the reactor behavior which can be obtained by using this multi-scale numerical framework. However, the high computational cost of multi-scale simulations limits their application for routinely reaction design of complex reactor geometries (e.g. packed bed, foams), which is still based on classical chemical reaction engineering models. This type of modeling adopts lumped parameters, usually experimentally derived, to represent the main phenomena occurring inside the reactor. Therefore, the second goal of this Ph.D. is to develop a methodology to enable the application of the multi-scale framework to the routinely reactor design.
In this regard, the hierarchical approach has been selected as an interesting approach to meet this goal. In fact, it can be adopted to enable a first principles multi-scale design of the novel reactors with an affordable computational cost. In essence, as shown in Figure 1, it consists in using the first principles multi-scale model to analyze a selected and limited number of operating conditions for the novel reactor geometry. Then, the results of these simulations are used to derive lumped parameters. Consequently, the implementation of these parameters in simplified models enables a fundamental description of the phenomena in the reactors with a reasonable computational cost. The potentialities of this methodology are presented by analysis the behavior of micro packed bed reactors in high exothermic processes. The hierarchical modeling is applied to investigate the capabilities of micro packed bed reactors in dealing with high exothermic processes. In particular, the hierarchical approach can be also used to determine the overall heat transfer coefficient between the micro-packing and the wall of the single channel of the honeycomb matrix. Figure 2 illustrates the comparison between the overall heat transfer coefficient estimated with CFD simulations and the literature correlations.

This study allows the selection of the most adequate literature correlations for describing the transport phenomena in micro packed bed reactors. Then, this correlation is implemented in a steady-state, pseudo-continuous 2D heterogeneous reactor model to analyze the selective oxidation of o-xylene to phthalic anhydride in this novel reactor configuration. This study shows that the micro packed bed reactor is characterized by higher heat transfer properties than a packed bed reactor. In fact, the micro packed bed reactor has a quasi-isothermal behavior.
On one side, this work shows that micro packed bed reactors are a valid alternative to the classical multi tubular packed bed reactor for selectivity oxidation. On the other side, it clearly shows the potentiality of the hierarchical analysis and how it can be employed for the efficient and fundamental analysis and design of novel reactor technologies.

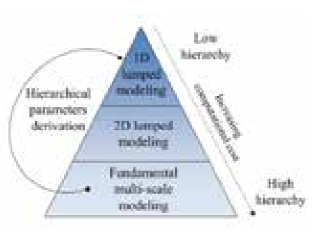


Fig. 1 - Schematic description of the hierarchical modeling applied to novel reactor configurations

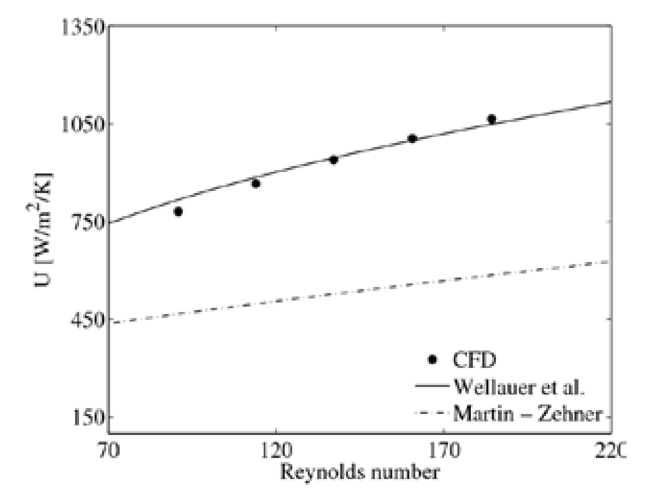


Fig. 2 - Overall heat transfer coefficient as a function of the Reynolds number

PROCESS SYSTEMS ENGINEERING FOR EARLY-STAGE PROCESS DEVELOPMENT: THE CASE OF SUSTAINABLE BIO-DERIVED ADIPIC ACID

Alessandro Rosengart - Supervisor: prof. Attilio Citterio

The awareness of the negative impacts of human activity against environment and public health has pushed western governments to support long-term programs aimed at mitigating pollution and reducing resource consumption. In this spirit, both industry and academia are searching for new solutions towards a “green” manufacturing practice, and the concept of “biorefinery” is taking place, as a renewable counterpart of the ill-famed oil industry. Biorefineries are supposed to produce entire classes of chemicals and fuels just as a real refinery, with the great difference that the carbon source is no more fossil, but follows the natural cycle of CO_2, which is captured from atmosphere and fixed into living organisms (plants, algae, bacteria). My Doctoral Thesis deals with the feasibility evaluation of a drop-in biorefining application for the production of sustainable adipic acid from biomass (2^{nd} generation technology), defining the full-scale process flowsheet, assessing the environmental and economic performances, and identifying the current challenges that R&D should address before industrialization. Some of these challenges have been tackled in this work applying several Process Systems Engineering computational tools, in particular dealing with problems of predictive models development, uncertainty propagation study and parameter regression from experimental data.

The current adipic acid production covers a market of 3.7 million tons per year (with a 4.1% of yearly growth) and, in spite of 70 years of technological maturity, the traditional benzene-based processes still raises serious safety and environmental concerns.

For these reasons, both private and public research institutions have pursued alternative bio (and chemical) routes for adipic acid; still none of these processes has reached industrialization yet, also due to the oil-price fall in 2014. This event evidenced the main weakness of drop-in biorefineries: the need to compete in costs with a well-established and optimized technology. A novel approach to process development is therefore required for the case of bulk bio-derived chemical with low added value. In particular, conceptual design acquires particular importance from the early stage of process development, to produce reliable cost estimates and projections, and to define a strategy for R&D.

The first activity carried out for this Doctoral study was the collection and systematisation of the extensive and interdisciplinary available knowledge on adipic acid, identifying the current alternative processing routes and assessing their actual sustainability with objective green metrics. In the specific, a two step biological-chemical process was considered worth of more detailed investigation for its good yields and sustainability potential. This process consists in a first fermentation to produce an unsaturated intermediate, muconic acid, starting from either glucose (from cellulose) or benzoic acid (from lignin). Muconic acid is then catalytically hydrogenated to adipic acid.

A computer aided process synthesis-and-design methodology was therefore applied for the case study of adipic acid from muconic acid, to evaluate systematically the highest number of process alternatives to produce the best flowsheet concept at the state of the art. The method is implemented in a tool belonging to “ICAS software”, developed at the collaborating institution DTU (Denmark Technical University, Copenhagen). This tool, given a number of alternative feedstocks, technologies (intended as unit operations/process conditions), and products, allows building a superstructure, which is translated into a Mixed Integer (Non) Linear Programming optimization problem solved in GAMS®. The solution is a processing route that maximizes the objective function (economic potential) providing also the material and energy balances. The plant feasibility is then evaluated for different market scenarios, the process bottlenecks are identified and more detailed green metrics are calculated (e.g. water consumption, energy consumption, CO_2 equivalent). Thus, the process flowsheet concept is achieved avoiding the simplified approaches of order-of-magnitude estimates or the “analogy principle”. Also, the used methodology guarantees in general higher flexibility than a detailed process simulator, as each unit operation is defined by few user-defined parameters, that allow giving some cost/performance estimates even in presence of preliminary, lab-scale data. In case of missing information, some assumptions can be made, which become the object of future research, if proven determinant in the process economics (SMART objectives definition).

The problem of how scarce information at early stage of research can affect the trustworthiness of a cost function was addressed in detail for a unit operation which relies much on experimental data: bacterial cross flow microfiltration. In facts, using literature data in analogy with existing plants can be extremely deceptive, as the design and the operating conditions (transmembrane pressure, crossflow velocity, membrane regeneration) are calibrated on the specific properties of a particular microorganism. A grey-box model was developed on the structure of a Darcy additive resistance equation, to represent the dynamic behaviour of the progressive bacterial fouling of a filtration membrane. The model, a system of algebraic and differential equations, is general and flexible, able to represent different strains in virtue of its adaptive parameters. The parameters “carry” the uncertainties deriving from the experimental error or from the actual representation limits of the model. Further uncertainties are introduced when using the model in a predictive way, i.e. extending the parameters validity to “similar” systems, as performed for the case of the strains for muconic acid production, whose filterability properties have not been measured yet. The uncertainty propagation was therefore studied applying Possibility theory and the Fuzzy Logic of Zadeh. This uncertainty analysis allows identifying the most likely range of filtration performances of an industrial membrane system, which, in association with a cost function, provides an indication of the error of cost estimates and the risk in the absence of specific R&D.

The final conversion step for the production of green adipic acid, i.e. the catalytic hydrogenation of muconic acid, lacked as well of the sufficient data to perform reliable estimates on the reaction scale up. In this case, an experimental campaign was started, in collaboration with the laboratories of Industrial Chemistry of Università degli Studi di Milano. The purpose was to identify the optimal reaction conditions (low pressure, low temperature, and catalyst recyclability) and identify the hydrogenation mechanism, to develop the first kinetic model for the system. Several models with LHHW structure were used to interpret the experimental values, considering the species adsorption-desorption equilibria of the involved species. A dual-step hydrogenation mechanism was demonstrated, with hydrogen dissociation on the metal (Pt/C 5%). Muconic acid (in its *trans,trans* form) is first hydrogenated to hexenedioic acid (present in its two *cis* and *trans* isomers, in equilibrium) which is then converted to adipic. The model parameter regression was performed with the C++ library BzzMath, characterized by robust minimization algorithms, to tackle the computational challenges related to the use of models with strong collinearity. The models were thus re-parametrized and progressively simplified, obtaining a good representation of the experimental data and providing the first reference values of the species activation energies.

MULTICOMPONENT AND CASCADE REACTIONS FOR THE PREPARATION OF HETEROCYCLIC MOLECULES OF BIOLOGICAL AND CHEMICAL INTEREST

Arianna Rossetti - Supervisor: Prof. Alessandro Sacchetti

In the last century, the strong demand for new chemical compounds drove the development of novel synthetic strategies for the easy and fast generation of molecules libraries. In particular, in the field of *Diversity Oriented Synthesis* (DOS), convergent synthetic methods, such as the *Multicomponent Reactions* (MCRs), have emerged as powerful tools for this target.

In this context, my PhD research project has focused on the synthesis of new chemical scaffolds through different multicomponent and cascade reactions. The approaches investigated have led to the production of different classes of molecules with either biological or chemical interests. The final aim was the creation of libraries of compounds with high degree of diversity and possible useful future applications in the fields of drug discovery, catalysis or new material sciences.
In details, the synthetic procedures for the applied reactions are well established, nevertheless each type of MCR has been optimised in its conditions, by testing different temperatures, solvents and combination of equivalents before the creation of the library of desired compounds.
Concerning the molecules with a potential pharmaceutical interest, the syntheses were assisted and integrated by *in silico* evaluation of the targets.
Finally, for selected compounds, preliminary biological tests towards cytotoxicity and antimicrobial activities have been performed. This is the case of the products from the Gewald reaction, developed as promising antitumoral and antimicrobial agents, or the macrocylic scaffolds from the Ugi reaction, designed to be PPI inhibitors.

After a brief overview of the MCRs world (**chapter 1**), the thesis is organized dividing the libraries of heterocycles synthesized according to the corresponding multicomponent or cascade reaction applied.
In detail, the first reaction explored (**chapter 2**) was an isocyanide-based cascade used for a new and facile synthesis of *N*-substituted-benzimidazoles.
Then the Ugi reaction, a multicomponent approach for peptidomimetics synthesis, was investigated both on 3,4-dihydro-isoquinolines as reverse turn mimics (**chapter 3a**) and on macrocycles as PPI inhibitors (**chapter 3b**).
A series of Gewald MCR afforded highly functionalized 2-aminothiophenes in the design of thiophene[3,2-d]pyrimidine scaffolds and derivatives as potential PPI targeting peptidomimetics (**chapter 4a**) and in the synthesis of novel tetrahydrothieno[2,3-c]pyridine derivatives as new antimicrobial agents (**chapter 4b**).
Last, Mannich reactions were used for the creation of highly functionalized bispidinones, whose applications vary from material science, as in the case of our new MOFs scaffolds synthesis (**chapter 5a**), to the catalysis world in enantioselective Henry reactions (**chapter 5b**).

ENANTIOSELECTIVE SYNTHESIS OF CHIRAL PHARMACEUTICAL INTERMEDIATES BY WHOLE CELL MICROORGANISMS AND ENGINEERED ISOLATED ENZYMES

Sara Santangelo - Supervisor: Prof. Maria Elisabetta Brenna

Tutor: Prof. Carlo Punta

The role of biocatalyzed reactions is becoming more and more influential in the toolbox of available reactions for the synthesis of enantiopure compounds, also on industrial scale.

In the scenario of the possible options to design the synthetic pathway of chiral molecules, enzymatic biotransformations started to be evaluated more frequently, also considering the distinctive features of this methodology. Enzyme catalyzed reactions are highly chemo, regio and stereo-selective and therefore of great interest for fine chemical synthesis, both for economic and environmental reasons.

Enzymes catalysed reactions can be performed in different conditions: the natural expressing microorganism can be considered as source of enzyme and it can be used directly as biocatalyst. To improve overexpression and to reduce side reactions, a more convenient host can be identified. The enzymes can work in a more known and controlled overexpression system, leading to better results. Using engineered procedures, the possibility to purify the enzymes becomes available. In this way, they can be used for biotransformations also as purified enzymes. All these options have been considered and investigated.

The work during these past three years has explored the effectiveness of combining chemical and biochemical procedures for the synthesis of chiral building blocks in high enantiomeric purity. Specifically, this has involved three different topics:

- Stereoselective reduction of C=C and C=O double bonds;
- Desymmetrisation of achiral compounds by biocatalysed oxidative biotransformation
- Combination of multiple enzymes in cascade reactions

A complete investigation of the steric and electronic effects of substituents on the course of ER-mediated reductions of alkenes has been performed, considering the substitutions on aromatic rings and on alkyl chains. The conversion yields and the enantioselectivity of the reactions were then evaluated.

At the same time, the effect of different electron withdrawing groups has been investigated: in order to perform the enzymatically catalysed reduction of the molecules, the of C=C double bond has to be efficiently activated under the electronic point of view. The role of the nitrile (CN) and the nitro (NO_2) functions as EWG, either alone or in combination with other functional moieties have been considered. The enantioselective reduction of alpha-methylenic nitrile derivatives catalysed by ene-reductases affords the corresponding (R)-2-arylpropanenitriles with high conversion values. The reaction is investigated either in aqueous medium (with an organic cosolvent or by loading the substrate unto hydrophobic resins), and in a biphasic ionic liquid / water system). Compounds bearing either alkyl chains of increasing length at the carbon atom in position β to the nitro group or different substituents on the aromatic ring are prepared and submitted to bioreduction, in order to define the synthetic potential of this enantioselective reaction in the preparation of chiral fine chemicals. A complete screening has been performed, analysing all the factors influencing the reactions. The synthetic versatility of the nitrile and nitro functions were further investigated with the transformation of reduced compounds in the corresponding chiral amides, acids and amines.

When the wildtype enzyme wasn't able to perform the required biotransformations, also the possibility of use an enzyme library, a whole set of variants, was considered. Protein engineering procedures were employed to obtain a single mutation in a position with a critical role for the catalytic function of the protein. The effects of this mutation on enantioselectivity were deepen in relationship with the substrates of interest.

The study of the desymmetrisation of achiral 1,3-diols by biocatalysed oxidation has been conducted by exploring the scarcely known world of Acetic Acid Bacteria. The ability of AAB to oxidize several substrates has long been known and is still attracting attention. The oxidation of alcohols by AAB is an old and established microbial method for obtaining production of vinegar and various carboxylic acids, sometimes with remarkable chemo and enantioselectivity. The enzymatic oxidation of primary alcohol is attractive because it can be carried out under mild conditions that are also suited for labile products. Two different enzymes are involved in two different steps for the oxidation to obtain carboxylic acid in physiologic pathways and conditions: The first reaction is catalyzed by alcohol dehydrogenase (ADH) and the second by aldehyde dehydrogenase (ALDH). The procedure, carried out in aqueous medium under mild conditions of pH, temperature and pressure, contributes to enlarge the portfolio of enzymatic oxidations available to organic chemists for the development of sustainable manufacturing processes.

The optimization of enzymatic cascade procedures has involved coupling more than one enzyme into an efficient pathway for the biosynthesis of compounds of commercial interest. The most odorous stereoisomers of the chiral commercial fragrance Muguesia® are prepared by a very effective linear biocatalysed cascade reaction, in which a suitable unsaturated ketone is submitted to the sequential action of two enzymes, an ene-reductase and an alcohol dehydrogenase, which are added together to the same reaction vessel with the cofactor regeneration system.

Two stereogenic centres in 1,2 relative position are thus created under high stereochemical control by a two-step one-pot enzymatic procedure.

These procedures represent a further demonstration of the synthetic potential of enzyme-mediated reactions: the high chemo and stereoselectivity that enzymes can achieve are key requisites for the optimization processes, which are now extensively investigated for the synthesis of valuable compounds.

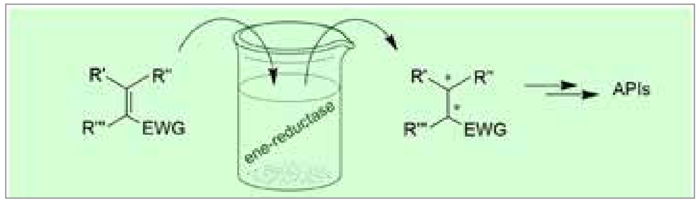


Fig. 1

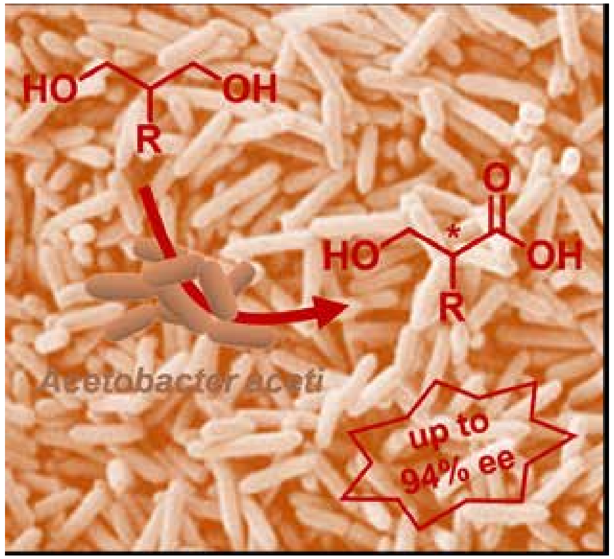


Fig. 2