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## BIOCORE - Final publishable summary report

Michael O'Donohue

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# BIOCORE

## Final publishable summary report

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# 1 Executive Summary

## 1.1 *BIOCORE has produced industrially-relevant outcomes*

### 1.1.1 *Organosolv biomass refining can use multiple feedstocks and procure high quality biomass intermediates (cellulose, C5 sugars and lignin)*

At the end of this work section the level of development of organosolv technology reached a TRL of 7

The deconstruction of non-food lignocellulosic biomass into its major component parts (i.e. biorefining) is a major challenge that has mobilized considerable R&D resources worldwide for several decades. Many processes have focused on the aim of extracting cellulose, since this biomass component is a source of glucose, which can then be used as a feedstock for industrial biotechnology. Nevertheless, biomass is rarely composed of more than 50% w/w cellulose, thus biorefinery technologies also need to extract the other components in a manner that will facilitate their further valorization. This specific challenge is central to the BIOCORE concept that uses organosolv technology to optimize the extraction of the three major components of biomass (i.e. cellulose, hemicelluloses and lignins), and thus add significant value to at least 90% of the biomass feedstock.

A second challenge that is important for the sustainability of biorefining is the ability to use several types of biomass. So far, biorefinery technologies have been rather biomass-specific, for example working best with agricultural residues, such as straws. In biomass constrained regions such as Europe, this is a severe disadvantage and will jeopardize the chances of long-term success of biorefineries. Therefore, another principle central to the BIOCORE concept is the use of multiple feedstocks, including cereal straws, hardwood and wood from short rotation coppice cultures.

#### ***Pilot scale processing of wheat/barley straw with built-in strategies for waste reduction and process integration and optimization***

The BIOCORE partner CIMV S.A. (France) possesses a pilot plant that operates organosolv technology based on the use of a formic/acetic acid solvent system. In BIOCORE, the pilot plant was intensively used and studied in order to optimize several aspects of the process.

Regarding, wheat/barley straw mixtures that constitute the base-case feedstock, the conditioning (chopping) of the biomass for processing has been studied and the waste (fines) associated with this step has been quantified. Moreover, regarding the process itself, working with CIMV, Arkema S.A. has been able to optimize has been able to optimize the hydrogen peroxide formulation, a chemical that is used in the process in order to delignify cellulose pulp and thus remove lignin. In BIOCORE the efficiency of the staged introduction of stabilized hydrogen peroxide was confirmed. This process development procures overall reductions in hydrogen peroxide needs of 5 to 10%, which inevitably leads to process cost reductions and reduces the final lignin content in the cellulose pulp by approximately 8% compared to the base-case process.

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Process design and integration were also used to improve the CIMV organosolv process, studying all of the key process steps and scoping for water and energy savings over the entire process scheme. Accordingly, process design pinpointed three major areas for improvement (i.e. removal of acids from the cellulose pulp, the filtration of the extracted lignin and organosolv acids recovery). Modifications led to significant improvements, with the yields of each of the major components being increased and overall dry matter recovery reaching approximately 100%. Similarly, process modifications aimed at energy savings, reduced power consumption by 13 and 11 MW for hot and cold utilities respectively and scoping for water savings reduced the requirement for freshwater (approximately 19 tonnes per hour and per 19 tonnes of feedstock) by 50%, partly by introducing recycling of process water arising from distillation.

Finally, concerning the primary biomass waste stream generated at the beginning of the process (approximately 5% dry weight of wheat straw feedstock), the use of wheat straw fines as a combustible for thermal processes that can produce heat (electricity) and ashes was studied. Results from preliminary testing indicate that fines from wheat straw are suitable as a combustible in certain circumstances, but further studies will be required to determine how the fly ash can be best used, since the measured chloride levels appear to exclude its use in concrete, but might allow it to be used as a fertilizer.

### ***Extension of the biomass feedstock base***

In addition to the use of wheat straw, the CIMV organosolv process was also tested for its capacity to use rice straw, hardwood and short rotation coppice poplar. Concerning rice straw, the processing of approximately 1 tonne of rice straw shipped from India confirmed that the process could be operated without any special modifications. In the case of deployment of BIOCORE technology in the Punjab state (India), this result implies that the biorefinery could operate using either feedstock and probably accept a mixture of the two. Nevertheless, differences in the composition of these two straws mean that the economics is altered. For example, rice straw will provide less lignin (18% dry weight of rice straw feedstock) and thus the final balance of the product portfolio will be altered.

Beyond straws, studies in the BIOCORE project clearly revealed that the CIMV organosolv process can also use birch wood and wood from short rotation poplar coppicing. However, unlike rice straw, the introduction of these feedstocks requires process modifications. First, hardwoods require drying to achieve the prerequisite maximum allowed humidity level of 15% (w/w) biomass, and then the actual processing of the biomass requires alterations to the solvent system and to the residence time. This latter point implies that a future biorefinery unit should either be equipped with two parallel processing lines or should operate by campaign, processing straws on a seasonal basis, both scenarios being feasible. Looking at the advantages of woody biomass, BIOCORE clearly underlined that Europe possesses surpluses of hardwood and thus there is scope to develop biorefineries along the lines of the BIOCORE concept, particularly in countries such as France, Germany, Italy and Poland. Moreover, from an economic standpoint, hardwoods are interesting due to their relatively high lignin content (approximately 24% weight dry biomass). Assuming that organosolv lignin will sell for €1000 per tonne, economic calculations performed on the basis of a biorefinery capacity of 150,000 tonnes per annum

feedstock indicate that BIOCORE biorefineries using hardwoods are among those that are closest to profitability (i.e. achieve an IRR of 25%).

***Key lessons for the future concerning the CIMV organosolv process***

Overall, the CIMV organosolv process has been found to be well-adapted to the aims of the aims set out in the BIOCORE concept, since the technology provides the means to process several biomass types and successfully extract the biomass components in useful forms for further valorization (see sections below). However, regarding economic and environmental sustainability it is clear that it is almost impossible to evaluate the process without considering the context of whole value chains and process-to-process integration. Nevertheless, it is possible to state that in the CIMV process, the rather precise, ‘clean’ extraction of cellulose, hemicellulose and lignins, comes at a relatively high energy price, thus the technology must be optimal in terms of heat and energy integration and the valuable lignin stream must not be used as combustible material, as is currently the practice in other biorefinery concepts. Moreover, conclusions from process design and integration provide strong arguments to suggest that future BIOCORE biorefinery units should integrate on site the upfront organosolv processing technology with downstream product manufacturing processes. In this way, it will be possible to derive greater benefits from heat and energy integration, using the surplus process heat that is generated by the upfront technology.

***1.1.2 Organosolv cellulose pulp provides a platform for the production bioethanol and itaconic acid***

At the end of this work section itaconic acid and ethanol production reached TRL of 6

The conversion of cellulosic material is currently a major industrial aim, since this will provide fermentable sugars from non-food resources for the industrial biotechnology sector, which is considered to be a key enabling technology and a cornerstone of the bioeconomy. In BIOCORE, cellulose was sourced from cereal straw (notably wheat/barley straw), a feedstock that is widespread around the world (>400 Mt produced worldwide – *source FAOSTAT*) and presents the advantage that, as a by-product of the food chain, its use can vastly improve the GHG performance of the agricultural sector, while providing significant feedstock volumes to the chemical industry.

***Using cellulose as a feedstock for liquid fuels and chemicals***

In BIOCORE the extraction of cellulose from cereal straw has been successfully achieved by CIMV S.A. using organosolv technology, furnishing high quality cellulose that, in the hands of DSM Bio-based Products & Services B.V, proved to be very amenable to subsequent enzyme-mediated hydrolysis, a step required to produce a fermentable glucose hydrolysate. After, using the hydrolysate, DSM Bio-based Products & Services B.V was able to demonstrate the production of two different products by fermentation. The production of 2<sup>nd</sup> generation ethanol opened prospects for its use either as a liquid fuel or as a building block chemical, while the production of itaconic acid opened prospects for its use as a chemical in the coatings and paints sector.

***Itaconic acid market***



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Itaconic acid is an unsaturated C5 dicarboxylic acid that figured among top twelve candidates biobased building blocks identified in the well-known report published by the US DoE (*Eds. Werpy and Petersen, PNNL, 2004*). Itaconic acid can be used as a bio-based monomer in polyesters, either replacing maleic anhydride or be used as a precursor of methyl methacrylate acrylic acid, both of which are currently produced from oil-based benzene (MAN) and acetone (MMA) respectively. Given the scope of substitution, it is foreseeable that itaconic acid will be used to manufacture a range of synthetic resins and fibers, plastics, rubbers (styrene-butadiene rubber) and oil additives that can be applied in various market sectors, such as diapers, feminine pads, detergents, cosmetics, inks, coatings and cleaners. However, despite this potential, the market for itaconic acid has so far remained small (approximately 40,000 tonnes/year - Weastra 2012, published in the framework of FP7 BioConSepT), mainly because of its cost (up to €1600 per tonne - Weastra 2012) and also because it is in competition with other biobased diacids, such as succinic and adipic acid, in certain application areas.

### ***Itaconic acid and BIOCORE***

In BIOCORE, the production of cellulose-based itaconic acid was successful and calculations were made on the basis that the process could be up-scaled to an annual production of 10,000-30,000 tonne/year. Clearly, this production volume represents a large portion of the current market, but Weastra 2012 predicted that the market will be multiplied by 10 in 2020. In this case, one BIOCORE biorefinery unit would produce 5-10% of the world market needs, thus it is likely that market prices will remain mainly unaffected. Therefore, it is expected that cellulose-based itaconic acid made in a BIOCORE biorefinery could sell for approximately €1500 per tonne. Interestingly, in BIOCORE, DSM clearly demonstrated how itaconic acid can be used to make biobased phthalic anhydride-free paint that displayed novel properties, such as better UV stability. Therefore, it is possible to speculate that such an itaconic acid-based could benefit from a green premium since it might generate extra consumer interest. Nevertheless, at this stage it is difficult to precisely evaluate this market bonus, which could inflate the product price by anything from 20 to 100% compared to the price of the reference product (e.g. the paint Uradil AZ516 Z-60).

Regarding the role of itaconic acid in the sustainability of an integrated biorefinery processing lignocellulosic biomass, this product clearly holds the potential to procure game-changing results. In BIOCORE, it was estimated that product portfolios including itaconic acid would be close to economic viability, since estimations of the price support (i.e. subsidies, green premiums or other financial support mechanism) to achieve an IRR of 25% were in the range 11-23%. Significantly, these good results coincide with encouraging environmental indicators, since product portfolios containing itaconic acid also revealed potential to procure, for example, GHG mitigation.

Overall, the manufacture of itaconic acid looks like an appealing option that should be further investigated within the framework of the development of a BIOCORE biorefinery demonstration unit. However, in BIOCORE, the encouraging results obtained for itaconic acid imply that the BIOCORE concept will also prove to be a good technology platform for the production of other organic acids, such as succinic acid. Therefore, the further application of the BIOCORE concept to the general field of organic diacids is recommended.

### ***The current status of bioethanol***

Currently the biobased ethanol sector represents 88 billion litres (2013 figures), with the vast majority being produced from maize and sugarcane and being used as a fuel for cars. Therefore, there is strong political will to move towards cellulose-based ethanol, since this displays the distinct advantage of avoiding direct competition with the food chain.

### ***Pilot scale production of 2<sup>nd</sup> generation bioethanol in BIOCORE***

In BIOCORE, the feasibility of cellulosic bioethanol production from wheat/barley straw was very successfully demonstrated at pilot scale in DSM Bio-based Products & Services B.V., who worked on a cellulose pulp supplied by CIMV S.A. Overall, 1 tonne of wheat/barley straw cellulose was converted into 165 litres of ethanol in a process that successfully demonstrated the different process steps, including the CIMV S.A. organosolv technology and DSM's proprietary thermostable cellulases. The yield of the process after distillation was 70%, which is highly encouraging and suggests that the process is mature enough to be scaled up to demonstration scale (e.g. 1-10 tonnes per hour), especially because the pilot trials pinpointed several aspects that can be readily improved.

Beyond technical feasibility, ethanol production in the context of a BIOCORE biorefinery was also studied from a sustainability point of view, closely examining economic and environmental performance. Overall, these studies did not provide a good economic case for ethanol production, since product portfolios that included cellulose-based ethanol work generated less revenues than those containing alternative products, such as itaconic acid. Nevertheless, the economic data for one scenario in which ethanol production is coupled to xylitol production (the latter from pentose sugars) did appear to be economically-feasible (i.e. achieve an IRR of 25%) if one assumes that the cellulose-based ethanol would receive subsidies at the level that is currently being paid for sucrose- and starch-based ethanol. Moreover, this case displayed environmental advantages, since compared to the reference case it was able to procure GHG savings of approximately 0.4 tCO<sub>2</sub> eq t<sup>-1</sup> biomass. Nevertheless, this positive environmental performance was not systematic for portfolios containing ethanol were not among the best achievers, since in the worst case scenarios (e.g. when both xylose and glucose were converted to ethanol), ethanol production was actually predicted to be a source of positive GHG emissions. However, these data need to be handled with suitable precaution, since they are only relevant within the scope of the study and the boundary conditions that were used. In this respect, it was discovered during the course of the BIOCORE project that the overall economic and environmental performance of the BIOCORE concept is quite scale-sensitive and that industrial units operating at scales higher than the one studied in BIOCORE (i.e. 150,000 tonnes dry biomass per year) are predicted to have better performance. Indeed, this finding was borne out by the simulation performed on a rice-based biorefinery operating at 500,000 tonnes per annum in India. In this case, the biorefinery was predicted to be profitable (revenues >€20 million per annum) and to display GHG savings of approximately 1 tCO<sub>2</sub> eq t<sup>-1</sup> biomass.

### ***Cellulose-based ethanol to PVC: lessons learnt from BIOCORE***

Beyond the use of cellulosic ethanol as a liquid transport fuel, there is also scope for its use as a chemical intermediate, notably for the production of ethylene, which itself is a key bulk chemical that is used in many polymers, especially in polyethylene, polyethylene terephthalate and polyvinylchloride (PVC). In BIOCORE, one aim was to investigate the feasibility of making PVC from cellulose-based ethanol.

The manufacture of PVC from ethanol is a multi-step process that first involves PVC production involves ethanol dehydration, converting ethanol into ethylene, and then ethylene chlorination, producing 1,2-dichloroethane, 1,2-dichloroethane cracking, which produces vinyl-chloride monomer (VCM) and finally VCM polymerization, yielding PVC. During the BIOCORE project, despite some technical setbacks, it was possible to demonstrate that providing that impurities specific to biobased ethanol are accounted for and removed, it is possible to successfully manufacture bio-based PVC. However, the economics and environmental sustainability of the process do not argue in its favour. Indeed, the measurement of environmental performance of biobased PVC clearly highlighted the more generic fact that significant molecular mass losses, coupled to subsequent mass gains, all requiring energy, over the biomass conversion pathway, (e.g. from glucose to ethylene, and ethylene to 1,2-dichloroethane) compromise overall sustainability and thus should be avoided.

### ***1.1.3 Organosolv lignins are promising building blocks for polyurethanes and wood adhesives***

At the end of this work section work on three lignin-based product lines reached TRLs of 5-7

Lignins are major components of lignocellulosic biomass, often representing 20-25% of the total dry weight. From a chemical perspective, lignins are highly complex ramified polyphenolic polymers that have proven to be difficult to extract from biomass in a useful form. For this reason, despite the fact that the paper pulp industry generates several million tonnes per year of lignin, most of these are burnt to generate heat and power. This industrial paradigm is also the dominate one in emerging biorefineries, which are primarily geared towards the isolation of cellulose and heat and power from the combustion of lignins.

In BIOCORE, a totally different approach has been developed, considering lignin as potential source of higher level revenues (compared to cellulose). To achieve this, it has been vital to demonstrate some added-value applications that reveal how the intrinsic quality of organosolv lignins can be exploited.

#### ***Biobased polyol components in polyurethane formulations***

Polyurethanes (PU) are among the most versatile plastic materials known and form the basis of many consumer products, insulating panels, shoes (soles), mattresses, toys, kitchen sponges etc. In general terms PUs are formed by reacting a polyol with a diisocyanate or a polymeric isocyanate in the presence of suitable catalysts and additives. Depending on the exact nature of the components used, the PU can take the form of rigid foam, or that of elastomer-type plastic. In Europe, the demand for PUs amounts to approximately 3 million tonnes (*source PlasticsEurope*), representing approximately 7% of the demand for plastics in Europe.

Increasingly, in a drive towards more sustainable products, major PU producers are commercializing PUs containing biobased polyols, which include vegetable oils. This

is the case for example for BASF's Lupranol Balance 50, a PU which contains castor oil.

### ***Lignin as a polyol for PUs***

In BIOCORE, organosolv lignin was tested as a solid co-polyol for the solvent-free production of castor oil-based PU elastomers and as a liquid co-polyol for rigid PU foams.

In the first example, SYNPO (Czech Republic) devised a pretreatment technology that was used to condition powdered organosolv lignin supplied by CIMV S.A. This pretreatment essentially consisted of mechanical grinding that, by homogenizing and reducing the size of the lignin particles, procured a nanometric lignopolyol that could be added directly to a PU elastomer formulation. When compared to the commercial PU resin, Veropal 3B, it was found that the new PU resin displayed increased tensile strength, toughness, surface hardness and high electrical resistivity. The new PU resin and the method required to make it has been patented (PTC/CZ2013/000111) and the process has been validated in a batch process, producing 25 kg of PU resin. The process is now ready for commercial up-scaling (250 kg batches) and samples have been supplied to several potential clients. In this respect, taking into account the singular electrical properties of the PU resin, SYNPO believes that this product will be well-placed to penetrate the electrical appliances market, a sector that represents approximately 140,000 tonnes per year of PU in the USA alone and that uses flexible PU resins particularly to coat wires and cables.

In the second example, IWC (Latvia) used lignin from CIMV S.A. as a starting point to make a liquid lignin-based polyol. The new polyol and the method required to make it have been patented (Latvian patent LV 14722A, 20/09/2013). This was achieved through an oxyalkylation process, which furnished the organosolv lignin in a liquefied, highly functionalized form, which was suitable for incorporation into a rigid PU foam formulation. Interestingly, when compared with a standard rigid PU, the lignopolyol-based foam (containing approximately up 11.2% w/w lignin) displayed higher UV and dimensional stability, and better flame resistance, properties that are highly desirable in insulating panels used for construction purposes. Moreover, this type of formulation could be handled using standard, commercial-scale, high pressure spraying equipment. Therefore, it is possible to postulate that this process is quite mature and that commercialization could become possible in the short-term, providing that the higher viscosity of the lignopolyol-based PU formulation can be correctly dealt with at commercial scale. In the case of commercialization, it is noteworthy that the rigid PU foam market is a significant one, representing over 300,000 tonnes per year and involving 88,000 jobs across Europe (*source: Isopa*).

### ***PF resins in the wood panel industry***

Resins made from phenol and formaldehyde (PF resins) are the major adhesives used to bind wood, creating products such as panels, molded products, lumber and timber products. In the area of wood panels, PF resins are used to manufacture a wide variety of types, including plywood, particleboard, medium density fiberboards etc., which in some cases will be specifically designed to resist outdoor use conditions. In 2011, it was estimated that the global market PF resins was growing and will reach approximately 16 million tonnes by 2016, with a third of this being produced in Asia, although growth of annual sales was also predicted for Europe.



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Regarding plywood, Europe (+Russia) produces over 4 million cubic meters per year. However, this only represents about 50% of the local market, which means that a considerable part of the plywood used in Europe is imported (*source: European Federation of the Plywood Industry*).

### ***Novel lignin-based PF resins for plywood***

In BIOCORE, CHIMAR (Greece) has investigated how organosolv lignin can be used to directly substitute phenol in the preparation of PF resins for the manufacture of plywood panels. Briefly, CHIMAR developed a process that allows the direct incorporation of lignin (supplied by CIMV in a milled, washed and dried form) into PF resins. Several substitution levels were tested, but for pilot scale manufacture of plywood panels a PF resin in which 50% of the phenol component was replaced by lignin was prepared. The resulting plywood panels (3, 5 and 9 layers) displayed technical properties (shear strength and wood failure after pre-treatments described in the EN314.1 & EN314.2 standards) that were perfectly comparable with commercial ones, meaning that it is feasible to commercialize lignin-based plywood panels for interior or exterior use that comply with current EU standards (EN314.1 and 2).

Bearing in mind that the current market price of phenol is situated somewhere in the range of €1200-1500 per tonne, CHIMAR's result is a significant one for the viability of the BIOCORE concept, since the implication is that this application could consume over 1 million tonnes of organosolv lignin. Assuming that a single BIOCORE biorefinery operating at 150,000 tonne (biomass) per year scale will produce approximately 24,000 tonnes of lignin, a 1.2 million-tonne market represents the capacity of 40-50 biorefinery units (if one supposes that lignin can fully penetrate the PF resin market sector).

These BIOCORE examples show that lignin-based products that valorize lignin are valuable features of higher performance product portfolios

Overall, the economic and environmental analyses performed in BIOCORE clearly confirmed that the non-energetic valorization of lignin is crucial to the BIOCORE concept. Moreover, in environmental analyses the credits acquired by lignin-based materials were often essential to tip the balance towards environmental benefits, notably in terms of GHG savings. In the case of PF resins and PU elastomers this is particularly true because the extracted organosolv lignins require relatively little processing to allow their use in finalized products.

### ***1.1.4 Concluding comments on the socioeconomic relevance of BIOCORE results***

The pilot scale studies performed in BIOCORE have taken a number of technologies forward to TRL 5-6, revealing the advantages, limits and future hurdles for these. Taken together, the results reveal that organosolv biorefining can play a part in the future bioeconomy, probably as a raw material supplier for the chemical industry rather than a crucial element in the energy sector. Moreover, both environmental and economic analyses suggest that organosolv biorefineries (BIOCORE concept) will need to be conceived as tightly integrated industrial units that combine on-site the production of biomass intermediates and their conversion into a range of biobased products that can be supplied to secondary transformer industries. The future

operation of such a complex industrial scheme will require further study and optimization and will necessitate the construction of one or more demonstration plants (TRL 7-8) that can actually furnish biobased products to potential markets. Apart from the financial barrier itself, among the probable hurdles that will have to be surmounted figure the question of biomass supply in an increasingly biomass-constrained Europe and the ramp up of the price-competitive production of pentose sugars and products thereof.

The current state of play suggests that the CIMV organosolv process is mature enough for demonstration, thus assuming that sufficient financing is available (probably €20-25 million), such a unit could become operational in Europe sometime in 2016. Making the further assumption that the demonstration is successful, a full scale unit (>220,000 tonnes biomass per year) could come online in 2019-20.

Concerning some of the potential products that have been investigated in BIOCORE, it is noteworthy that among these figure several plastics and resins. In this respect, it is important to note that the production capacity of the European plastics industry has steadily lost influence at the world level over the last 50 years, producing almost 50% of world requirements in 1980 and less than 20% in 2010. Therefore, the introduction of innovative biobased plastics and resins is likely to be a welcome boost to this sector, since these are likely to provide extra cutting edge in a highly competitive sector and in turn increase sales and revenues.

## ***1.2 BIOCORE has significantly contributed to the development of biorefinery-enabling technologies***

### ***1.2.1 Engineered microbial strains for the conversion of pentose sugars into added-value products***

This R&D work on the biotechnological production of xylitol and xylonic acid reached a TRL of 3-4 and 4 respectively. Xylitol is now ready for transfer to TRL 5-6

The 20<sup>th</sup> century conception of biorefining was very much centred on glucose. However, the use of lignocellulosic biomass as a raw material demands that economically- and environmentally-sustainable conversion routes for pentose sugars be developed, since these are significant components of most common lignocellulosic biomass resources. In BIOCORE, VTT (Finland) has focused on the development of high-performance microbial strains that are able to produce valuable chemicals such as xylitol or xylonic acid, using an industrial pentose hydrolysate as the feedstock.

#### ***What has been achieved?***

In BIOCORE the challenge to develop microbial strains that will convert pentose hydrolysates into xylitol or xylonic acid was two-fold. First, it was necessary to move beyond state-of-the-art in terms of intrinsic parameters, such as productivity and yield, and second it was also necessary to develop robust strains able to function in adverse conditions, notably in the presence of organic acids present in the sugar feedstock.

#### ***Xylitol-producing strains***

VTT carried out strain engineering work to improve xylitol productivities with various yeasts, including *Saccharomyces cerevisiae* and *Pichia kudriavzevii*. Several different xylose reductase enzymes were tested (that convert D-xylose to xylitol) and the enzyme expression levels were increased. Excellent production strains were obtained that, when growing on pure xylose, can produce over 160 g.L<sup>-1</sup> xylitol, with production rates of over 2 g.L<sup>-1</sup>.h<sup>-1</sup> and yields well over 0.9 g xylitol from 1 g xylose. Regarding the robustness of these strains, partially deacidified pentose syrup could be used as a 70% feed (supplemented with 30% pure xylose). In this case, xylitol titers of over 100 g.L<sup>-1</sup>, production rates of 0.82 g.L<sup>-1</sup>.h<sup>-1</sup> and yields of 0.89 g.g<sup>-1</sup> were obtained. Small scale experiments indicated that if the pentose syrup is further purified using the most advanced technology that was achieved in BIOCORE, then it could be used directly for xylitol production.

#### *Xylonic acid-producing strains*

VTT also did extensive strain engineering work to produce xylonic acid (XA) from xylose. Several xylose dehydrogenase enzymes (that convert D-xylose to D-xylonic acid) with different cofactor specificities were tested in various host species. The yeast *P.kudriavzevii* turned out to be the best host of the several species studied. On pure xylose, the XA titers were over 160 g.L<sup>-1</sup>, production rates 1.4 g.L<sup>-1</sup>.h<sup>-1</sup> and yields of XA over 0.9 g.g<sup>-1</sup> xylose provided. Remarkably, the productivities were similar at low pH, pH 3, which is important for the economy of downstream purification. Preliminary trials were carried out with the industrial pentose syrup fed at 50% (w/v), with 50% (w/v) pure xylose. In this case, xylonic acid titers were 40 g.L<sup>-1</sup>, overall production rates 0.43 g.L<sup>-1</sup>.h<sup>-1</sup> and the yield was 0.83 g.g<sup>-1</sup> (XA/xylose). Higher productivity can be obtained using alternative hydrolysates.

#### **Conclusions**

Overall, this R&D work shows that production of xylitol and xylonic acid with yeast is industrially feasible and that high productivities can be obtained. Assuming that suitable pentose hydrolysates will become available in the near future, the strains developed in this work can be used for pilot scale trials that will allow the study of downstream product purification issues.

#### **1.2.2 Engineered microbial strains for the production of an isopropanol-containing solvent mixture**

This R&D work on the biotechnological production of a solvent mixture containing isopropanol, butanol and ethanol acid reached a TRL of 2 and is now ready for proof of concept and subsequent transfer to TRL 4-5

The ABE process developed by Chaim Weizmann in the early 1900's employs anaerobic bacteria from the genus *Clostridium* that can produce a mixture of acetone, butanol and ethanol using sugar-based carbon sources as feedstock. This process was operated late into the 1980's, primarily to produce acetone, before being abandoned due to strong competition from the petrochemical sector.

#### ***What was targeted and what has been achieved?***

Now that the prospect of competitive biobased processes for the production of chemicals is once again becoming a serious industrial option, the goal of scientists affiliated to INRA (France) was to engineer the bacterium *Clostridium acetobutylicum*

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to produce isopropanol. First a strain deleted in butyric acid production was obtained, and thereafter several parallel strain engineering strategies were performed to further improve isopropanol production. Optimal natural enzyme, or engineered variants, were tested for two key enzymes, an NADH-dependent hydrogenase enzyme for redox cofactor equilibration and CoA transferase for improvement of acetate utilization for isopropanol conversion. However, within the timeline of BIOCORE, it was not possible to obtain a strain that produces isopropanol as the sole product. However, a very good *C. acetobutylicum* strain was created that produces a mixture of isopropanol, butanol and ethanol (IBE) at overall titers of 21 g.L<sup>-1</sup> (including isopropanol 5 g.L<sup>-1</sup>), productivity of 0.8 g.L<sup>-1</sup>.h<sup>-1</sup> and a yield of 0.34 g IBE /g of glucose utilized. Similar productivities were also obtained on pure xylose and on xylan (lower rates and yields on xylan). The IBE mixture is suitable as a biofuel, or as a source of the individual chemicals. Such high performance IBE production in batch cultures is a novel result, since this had not yet been demonstrated in *Clostridium*.

In complementary work, DLO (The Netherlands) carried out experiments to investigate the *in situ* recovery of IBE mixtures produced by the wild type strain, *Clostridium beijerinckii* NRRL B593. Gas stripping and absorbent materials (activated carbon or zeolite) were studied in detail. In best combinations 70-85% of products could be recovered. Removal of products through gas stripping and/or absorption during fermentation by *Clostridium* increased IBE productivity by at least 50%. Modelling suggests that the combination of the recombinant strain produced by INRA with the *in situ* recovery methods developed DLO would increase the IBE productivity 8-fold in comparison with the wild type strain cultured in normal conditions.

### *Concluding remarks*

Several companies have been looking at the opportunity to launch the biobased production of butanol or isobutanol. These include British Petroleum, Gevo (USA) and, more recently, Green Biologics Ltd., a UK-based company that is relaunching the ABE process. Moreover, several companies, including Gevo and Genomatica (USA) are pursuing the development of isopropanol-producing strains, which is unsurprising for a chemical whose worldwide consumption is 2.5-3.2 million tonnes. Therefore, the results generated in BIOCORE are highly pertinent, but have to be considered in the light of strong international competition. Therefore, future developments will need to account for this, identifying routes to highly original IP.

### **1.2.3 A sugar-derived biobased plasticizer outperforms the standard phthalate plasticizers in PVC for its flexibility properties**

This R&D work on the development of a new non-phthalate plasticizer reached a TRL of 3. More R&D is required to reach TRL 4 and beyond.

Plasticizers are important additives that are used to increase the flexibility of plastics. They work by embedding themselves between the chains of polymers, spacing them apart (increasing the "free volume"), and thus significantly lowering the plastic's glass transition temperature and increasing softness or flexibility. Approximately 6 million tonnes of plasticizers are used worldwide and >80% of these are phthalate-based molecules (*source ECPI*), with most being used in PVC. Until recently, low molecular weight phthalates such as di-2-ethylhexyl phthalate (DEHP) were widespread, but health concerns about these has led to a progress move towards higher molecular



phthalates, the current European market now strongly favoring the latter (87% of the market, *source ECPI*).

*A non-phthalate plasticizer for PVC*

In BIOCORE, researchers from DLO (The Netherlands) have devised a synthesis route for the preparation of a sugar-based plasticizer that has been designated DEH-bio. The plasticizer has been used for incorporation into PVC blends which has allowed the production of flexible sheets, without any associated difficulties. Compared to reference sheets prepared using DEHP-containing PVC, or PVC containing di-isononyl cyclohexanoate (DINCH), DEH-bio PVC, prepared using approximately 38% (w/w) DEH bio, was tangibly more flexible, rather like sheets prepared using and PVC containing another non-phthalate plasticizer, di-2-ethylhexyl adipate (DEHA). However, the heat stability of the DEH bio PVC was poorer than the DEHP PVC, though heat stability was improved by doubling the amount of stabilizer.

When the biobased plasticizer was used in so-called plastisol PVC, formulated to constitute the wear layer of flooring, an increase in yellowing (at least +50%), which was obvious even just after coating, despite the fact that the formulation had not been submitted to oven-level heat. Therefore, although the biobased plasticizer displays considerable promise, there is still a number of issues to address before the product can be considered for commercial applications.

**1.2.4 New process design methodologies that address the specific challenges of advanced biorefineries**

This R&D work constitutes a support activity that will provide vital accompaniment to future projects in their transition through TRL 5-9

Advanced biorefineries are complex industrial systems that unlike the oil and gas industry are characterized by very large feedstock variability and relatively uncharacterized processes (from an engineering perspective). Therefore, advanced biorefineries pose specific challenges to the process engineer, which call for the development of new tools and methodologies.

***What has been achieved?***

*Smart methods to identify the most promising process routes and product portfolios*

A biorefinery can potentially produce a very large amount of products, starting from the three major biomass intermediates, cellulose, pentose sugars and lignin. Therefore, an early strategic challenge in the development of a R&D program is to determine which are the most promising process pathways and product portfolios. In the course of BIOCORE, chemical engineers from NTUA (Greece) pioneered a strategy that addresses this conundrum. Working on 70 different product pathways, presenting overlapping chemistries, processing branches, intermediates and products, they formulated models and conducted searches, taking into account isolated and integrated pathways. Mass and energy balances were compounded from BIOCORE simulations, and conceptual cost models were developed using LHV-based correlations. Using current market and economic indicators, the approach deployed optimization to screen for the best-performing product portfolios. Additional

studies addressed the impact of process and economic parameters on the final selections.

Having eliminated in the preliminary analysis what appeared to be the most unprofitable biorefinery pathways, process integration and process-to-process integration was further extended and elaborated, focusing on a smaller number of identify best-performing product portfolios. To achieve this, the chemical pathways were translated into complete flowsheets, which were then integrated, creating new models that capitalized on both literature data and on expert knowledge, thus offering a convenient background to analyze, cost and scale-up the different variants of the biorefinery concept. Moreover, process integration was extended to all of the process operations, setting targets for energy and water efficiencies and generating Grand Composite Curves, the results providing energy and water footprints.

#### *Investigating the relative benefits of different biorefinery deployment scenarios*

The oil and gas industry is built around the concept of refineries that convert raw material, such as oil into more useful intermediates that are either distributed to off-site to user industries, or are delivered to co-localized facilities. A typical example of co-localization in Europe is the highly industrialized port of Rotterdam, which is characterized by a cluster of oil terminals, oil refiners and various chemical industry players. In the case of advanced biorefineries, no industrial models exist yet, because these will emerge with the development of specific biorefinery concepts. In BIOCORE, in order to investigate the pros and cons of centralized versus distributed production, and thus the co-location of the different biorefinery processes, a Total Site Analysis (TSA) was conducted. This TSA constitutes the first ever study of this kind performed on a biorefinery. Focusing on some of the more mature processes that were studied in BIOCORE, the TSA produced results that indicate that savings from process-to-process integration may range from 14 to 85%, and that savings increase as the number of products increase, with diminishing benefits still being detected as portfolios extend beyond 4 to 5 products. The range of savings indicates that the exact choice of product portfolio will have a significant impact on the sustainability of the biorefinery, whereas the magnitude of the savings supports scenarios with co-located production.

#### **1.2.5 New methods to analyze and integrate the various impacts of biorefineries**

This R&D work constitutes a support activity that will provide vital accompaniment to future projects in their transition through TRL 5-9

For many years now, well-developed international standards have been applied to environmental life cycle assessments, even if the refinement of these standards is an ongoing process. However, to fully understand the impacts of biorefineries, taking a comprehensive view of sustainability, it is necessary to consider a wider range of factors including economics, and social and environmental issues, the latter being differentiated between local and global/regional effects, and be able to weight their relative importance. Ultimately, all of this is vital to correctly inform public opinion and provide decision-makers with the necessary elements required to implement policy or strategy.

## **New tools to probe sustainability impacts**

In BIOCORE, a group of researchers from IFEU, Nova Institut, IUS (Germany), SOLAGRO (France), TERI (India), Imperial College London (UK) have worked on the development of a new methods that are designed to capture information linked to the different facets of biorefinery sustainability. To achieve this, a common set of system boundaries were adopted in order to probe the major sustainability issues (i.e. environment, society and economy).

Regarding the environmental impacts of BIOCORE biorefining, these were quantified using screening life cycle assessment (LCA) and supplemented by elements borrowed from environmental impact assessment (EIA). However, unlike standard EIA studies, which are site- and project-specific, the assessment of local environmental impacts was performed at a generic level, and took a life cycle perspective and was product-specific. For convenience, the modified method was designated life cycle environmental impact assessment or LC-EIA. In terms of progress, compared to the current LCA methodology, a supplemental LC-EIA enlarges the scope of a LCA study, providing adequate coverage of local environmental impacts.

Using the combination of LCA and LC-EIA, it was shown that biorefineries based on the BIOCORE concept could provoke a variety of impacts, ranging from significant environmental benefits to distinctly detrimental impacts. Among the drivers of these impacts are key factors, such as the choice of product portfolio, the mode of implementation and external influences. Depending on the exact nature of these factors, in some cases environmentally-advantageous impacts can occur simultaneously with negative impacts, with no obvious pattern being detected, while in others, detrimental environmental impacts emerge across all of the environmental impact categories. Nevertheless, in many cases the careful analysis of the different impacts has revealed a significant number of opportunities for mitigation and overall environmental performance optimization.

To study social impacts of biorefining, several methods were developed and tested. These include social impact assessment (SIA), and social life cycle assessment (sLCA), which together provided the means to investigate large number of social issues and themes. Overall, these methods revealed BIOCORE biorefineries could be the source of new jobs and rural development. However, it was also apparent that competition for biomass is a threat and that part of the solution to dealing with this is tight collaboration with local stakeholders, in particular farmers/forest owners. In India, social studies revealed other threats, including the currently insufficient development of infrastructures in rural areas and a lack of qualified workforce.

Finally, regarding economics, extensive investigations were performed. Among these was a study of the so-called green premium, which is the additional price that a market actor is willing to pay for the enhanced performance and/or intangible benefits that might be associated with a bio-based product. To perform the study, extensive surveys were done on 35 cases of bio-based chemicals, polymers and plastics. These revealed that green premiums do exist, especially for new bio-based products that are sold within the European market, and that these can be in the range 10 to 300% depending on the product, although 20-40% would be most typical. Moreover, by studying the potential economic performance of BIOCORE value-chains, it was

demonstrated that green premiums could play a major role in achieving economic viability.

### **Towards an integrated view of sustainability**

The controversy surrounding the question of the net benefits of bioenergy compared to bio-based materials is derived from the fact that there is increasing awareness that the replacement of fossil resources by biomass is not sustainable *per se*. Therefore, to better address this debate, BIOCORE applied a multi-criteria sustainability assessment of the overall concept, comparing products from the BIOCORE biorefinery to conventional reference products, and BIOCORE processes with competing biomass-based systems, which will compete for both biomass and land. Finally, all sustainability aspects were integrated into an overall sustainability assessment, using multi-dimensional comparison metrics. For the final interpretation of the results, rather than using mathematical means (e.g. attribution of scores to impacts using weighting factors or a weighting algorithm) to provide a series of consolidated scores, it was decided to provide transparent analysis of the results, discussing the pros, cons and conflicts of all of the options under study. To prepare this discussion a method for structured comparison was developed and decision-making options were presented using multi-criteria analysis.

The key outcomes of the integrated assessment show that the complexity of the BIOCORE system, characterized by multiple process pathways and products, gives rise to a multitude of results when comparison with a conventional system is attempted. Specifically, the extent to which any given product portfolio can be considered sustainable depends very much on the nature of the products, the technology employed, the scale of the industrial operation and the biomass feedstock type. Hence, no general conclusion can be drawn for the BIOCORE concept as a whole, but only for its individual implementations. Nevertheless, some general comments are possible:

- (i) when using lignocellulosic biomass, it appears vital to use all of the biomass components, converting them into value-added products. This observation confirms one of the fundamental suppositions that underpinned the BIOCORE concept.
- (ii) the choice of product is very important, since products that preserve the molecular mass and chemical functions of the biomass intermediates display the best environmental and economic performances
- (iii) lignin should not be used for energy purposes.
- (iv) in the case of the BIOCORE concept, close-to-optimum technical implementation is paramount, since under the most favorable conditions, environmental advantages and economic viability increase substantially. Moreover, process integration is crucial since the CIMV process yields considerable amounts of residual heat which is available to downstream processes. This means that biomass fractionation and downstream processes should take place at the same location.

However, the success of biorefineries is not just about resolving technical challenges. The SWOT and biomass competition analyses revealed that a major challenge is how to establish a supply of sustainable biomass in a near future characterized by the multiplication of biomass uses. To meet this challenge, a number of issues need to be addressed including the relations with local stakeholders, the development of

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appropriate infrastructures (e.g. transport routes, storage facilities etc.) and efficient policy / legal frameworks that will regulate biomass uses.

When the BIOCORE system was compared with other biomass-based systems, the issue of biomass competition becomes even more pertinent and crucial. However, putting this aside, and assuming that technical issues can be resolved in a satisfactory manner, the integrated analysis reveals that BIOCORE biorefineries hold the potential to outperform (from an environmental standpoint) any first generation biofuel biorefinery and even biomass-fired CHP plants. Therefore, with regard to the controversy mentioned above, the conclusions of the assessment plead in favor of new policy that would establish a level playing field for bio-based products, imposing harmonized performance criteria that should apply to all biomass uses.

### Recommendations for policy makers

1. Introduce active measures to manage increasing biomass and land use competition
2. Incorporate the notion of biorefineries and the wide needs of the bioeconomy in regional planning policies
3. Introduce mandatory sustainability criteria for all biomass, perhaps including animal feed
4. Create a level-playing field for bioenergy, biofuels and bio-based products
5. Revise the current RED policy framework, because currently it leads to misallocation of biomass and undesired effects (iRUC=indirect residue use change)
6. Provide financial assistance for the construction of advanced biorefineries (more attractive than pay-back via multiple counting schemes)
7. Consider the application of the Equator principles (World Bank) for high investment biorefinery ventures

### 1.3 Intellectual property indicators at the end of BIOCORE

Five patent applications have been filed in the framework of BIOCORE:

Partner	Patent subject	Title	Date of filling	Status
ECN <sup>1</sup>	The Pyrolysis of Lignin	The Pyrolysis of Lignin	June 14, 2011	PCT filed
SYNPO	New solvent-free preparation method of unmodified CIMV biolignin that can be used as a macromonomer in polyurethanes.	The way for preparation of polyurethane materials containing lignin, polyurethane material prepared this way	September 21st, 2012 Waiting for approval of Czech government	Waiting for approval of Czech government PCT filed
ECN	Ethanol-organosolv process modification	Process for the treatment of lignocellulosic biomass	February 15 <sup>th</sup> , 2013	PCT filed



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IWC	Polyurethane foams obtained from lignin	Method for production of heat insulating materials	June 20 <sup>th</sup> , 2013	Latvian application European patent application
ARKEMA	H <sub>2</sub> O <sub>2</sub> compositions and conditions to use them	Compositions of Hydrogen peroxide for delignification of biomass and their uses	January 10 <sup>th</sup> , 2014	French application

<sup>1</sup> This is not a strictly a BIOCORE patent, but background ECN knowledge that has been brought into the BIOCORE project. Results, based on this knowledge within BIOCORE enable the improvement of this patent which is called LIBRA. This patent is in preparation and will be filed in 2014.

And four are currently in preparation:

Partner	Patent subject	Status
DLO	Lignin fractionation	In preparation
DLO	Biobased plasticizers	In preparation
ECN	A lignin biorefinery approach which uses a dedicated pyrolysis technology Working title: LIBRA	In preparation
VTT	Xylitol production	Analyzing an assessment of the patentability of the results

### 1.4 Summary all pathways in terms of the TRL state of readiness

Type of result	Result description	Next steps	Current TRL
<b>Microbial strain construction</b>	Strain for xylitol production	Scale-up to pilot (m <sup>3</sup> )	4
	Pentose-acting, glycosynthetic enzymes	Further R&D required and basic economic analysis should be performed. Proof of concept is needed.	2
	Strain(s) for glucaric acid production	Optimization needed (culture conditions and strains - alternative host system) Eventually, transfer to pilot scale	2
	Strain(s) for isopropanol production	Optimization needed (strains and DSP) Scale-up to pilot	3
	Strain(s) for ethylene production	Overhaul of strategy. Possibly synthetic biology	1
	Strain(s) for xylonate production	Optimization needed (culture conditions and strains). Scale-up to pilot (m <sup>3</sup> ).	3-4
<b>Full or partial biotech and chemical processes</b>	Organosolv (CIMV) pretreatment of cereal straws and woody biomass	Scale up to commercial demonstration	5-6
	Organosolv (ECN) pretreatment of woody biomass	Integrated pilot scale testing, including initial removal of extractives	4-5
	Process for itaconic acid production	Commercial production	6
	Process for the production of 2 <sup>nd</sup> generation ethanol	Demonstration scale production	6
	Synthesis of alkylpolypentosides	Further R&D required	1-2
	Lignin pyrolysis	Further R&D. Identify partnerships for further development and scale-up	5
	3-component PU elastomer formulation using lignin	Attract market take-up for commercialization	7
	Process to make rigid PU foams using lignopolyols	Establish industrial partnership and scale up to demonstration	6

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		(need to secure sufficient lignin supply)	
	Process to manufacture plywood panels with lignin-based adhesive	Establish industrial partnership and scale up to commercial manufacture  (need to secure sufficient lignin supply)	7
	One-pot conversion of cellulose into lower polyols	For sorbitol/isosorbide, no prospect for further development (required purification comprises the economic viability).	3
		For ethylene-glycol, necessary to establish feasibility at labscale (proof of concept)	1-2
	Synthesis of furfuryl diisocyanates/carbamates	More R&D work needed	1-2
	Furfural production from hemicellulose-rich stream	The post-production isolation of furfural needs to be established. Once achieved, scale-up to pilot	2
	Preparation of a sugar-derived biobased plasticizer	Properties need to be improved. R&D to pilot to be performed	3
	Preparation of biobased PVC from 2 <sup>nd</sup> generation ethanol	Up-scaling to demonstration if economic viability can be established for the whole value chain.	5
	Synthesis of xylonic/gluconic acid-based hydrogels	Pursue R&D in order to achieve proof of concept	1

## 2 Summary description of project context and objectives

Europe has recently witnessed the inauguration of a biorefinery in Crescentino, Italy. This biorefinery constitutes a major step forward for Europe, since this is the first industrial size 2<sup>nd</sup> generation biorefinery currently in operation in Europe. Nevertheless, for the time being, using two lignocellulosic feedstocks (Arundo donax and wheat straw), the Crescentino facility will only produce ethanol (40 kt per year), heat and power as major products, all being low value, high volume energy products.

In BIOCORE, the overarching aim was to develop an even more advanced concept than the one that is being operated in Crescentino, since the ambition was to develop



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a multifeedstock, multiproduct biorefinery that is designed to make full and optimal use of the incoming biomass resources, thus generating maximum profit, while limiting the environmental footprint.

Currently, both developed and developing economies are increasingly facing the multiple challenges associated with a growing world population, increased consumption of energy and climate changes that can be directly attributed to Man's excessive reliance on fossil resources. In response to these challenges, Europe has set ambitious targets to reduce GHG emissions and oil dependency. To achieve these goals, while limiting the impact of non-food biomass production on the food production industry, the European Commission has supported the development of advanced technologies, which will use lignocellulosic biomass from various sources to produce both energy vectors, such as fuels, and higher value products, such as chemicals and materials. Within this context, the European Commission furnished a major effort in 2008, launching an 80 M€ project call for advanced biorefinery projects. The FP7 project BIOCORE is one of the projects that was selected for financing at the end of the selection process.

The BIOCORE consortium was composed of 25 partners coming from 14 countries, including India. The project benefitted from strong European Commission support, with the EC contributing approximately 14 M€ to the overall 20 M€ budget. The project began on the 1<sup>st</sup> March 2010 and was finalized on the 28<sup>th</sup> February 2014.

The major ambition of BIOCORE being to develop an advanced biorefinery concept, a key element of the project was the choice of pretreatment technology, which is the upfront process that allows biomass to be cracked and fractionated into its major components. In this respect, and accounting for the fact that a second ambition of BIOCORE was to maximize the use of the incoming biomass, and thus all of the biomass components, the choice of the pretreatment was vital in order to ensure that components, such as lignin and pentose sugars would be isolated in a way that favors their conversion into commercially-valuable products. With this criterion in mind and the ambition to use multiple biomass feeds, such as woody biomass and agricultural coproducts (e.g. straws), the choice of pretreatment was narrowed down to organosolv technologies. Organosolv technologies use organic solvents to dissolve the lignin component of biomass, thus providing a solid, delignified cellulose pulp. Europe boasts several pilot-scale organosolv facilities. These include the Chempolis biorefinery park (Oulu, Finland), the lignocellulose biorefinery, Fraunhofer Center for Chemical-Biotechnological Processes (Leuna Germany) and the CIMV S.A. pilot plant (Pomacle, France). In BIOCORE, CIMV provided its pilot scale facilities, which allowed the project partners to gain access to kg scale batches of cellulose, lignin and hemicellulose-rich syrup and provided CIMV, a SME, with a rich R&D network to develop its technology. This choice of biomass pretreatment technology provided BIOCORE with the possibility to process several types of herbaceous biomass and woody biomass from broadleaved tree species.

Consistent with the ambition of BIOCORE, the project was also designed to derive maximum value from the biomass feedstock, mainly by targeting the production of biobased chemicals and polymer building blocks. This product choice was motivated by the fact that like oil, biomass can furnish both carbon and energy, making it unique among the renewable solutions. Moreover, targeting polymer building blocks is a strategic choice, since the biobased polymers is an emerging market that meets the

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long-term goals of European industry, who are looking for ways to reduce dependence on petrochemical feedstocks, and the shorter term expectations of consumers, who are looking for value and/or new products, while choosing environmentally-acceptable options. Moreover, the development of innovative, biobased plastics in Europe will be a considerable boost to the European plastics industry, which has lost a considerable part of its market over recent decades.

Intrinsic to the ambition of producing chemicals and polymers from biomass, in BIOCORE it was considered vital to provide focus, not only on the conversion of glucose into valuable products, but also lignin and hemicellulose. Therefore in order to reach this goal, considerable effort was focused on the use of lignins to produce added-value products, thus demonstrating how the exquisite chemical complexity of lignins can be exploited rather than destroyed (i.e. the usual fate of lignin when used as a combustible), and on the development of biotechnological processes for the use of xylose.

Finally, in order to clearly establish how biorefining can be operated in a sustainable manner and thus contribute to Europe's dual drive towards GHG reductions and renewed industrial growth, BIOCORE set out to develop and implement an extensive sustainability assessment action. This vital part of the project was designed to carefully examine many aspects of sustainability, including environmental impacts (local and global), economic performance and social acceptability and thus provide new insight into some of the key issues that are currently fuelling public debate. These include fundamental questions concerning competition between food and non-food value, chains, the actual sustainability of biorefining as an alternative industrial pathway and the relative sustainability of different biomass uses. By addressing these issues, it was hoped to contribute to the emergence of a clearer policy roadmap and a more stable framework for financial investors.

### **3 Description of the main S&T results/foregrounds**

#### **3.1 WP1: Biomass production**

To reveal how biorefineries can be implemented within local contexts, WP1 was designed to perform detailed case studies that were intended to provide information on local supply chains, logistics and local social and environmental impacts. As a first step in the planning of these case studies, BIOCORE researchers assessed the availability of certain types of biomass feedstocks both in Europe (hardwood, wheat straw, SRC poplar, maize straw, and Miscanthus) and in India (rice and wheat straw), by agglomerating data from a variety of reliable sources. Overall, this preliminary analysis of biomass availability provided the basis for the selection of several regions that then became the subject of more detailed research and enquiry. Accordingly, in the BIOCORE project, the following regions were targeted for the implementation of the case study approach:

- Beauce (France)
- Nordrhein-Westfalen, Rheinland-Pfalz, Saarland and Hessen federal states (Germany)
- Zala, Somogy, Barany and Tolna counties (Hungary)

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- Faridkot (India) and
- Sangrur (India)

### **3.1.1 Biomass availability**

According to our findings, the amount of potentially extractable, surplus wheat straw in Europe represents approximately 35 Mt DM. This considerable, but nevertheless quite limited, reserve of available biomass can be completed by another 15 Mt of maize straw. Likewise, surplus hardwood in European countries amounts to between 2.5 and 5.5 Mt, particularly in countries such as France, Germany, Italy, Poland, and Romania. In India, the northern states of Punjab and Haryana are well-endowed with excess biomass, especially rice straw that is currently an underused resource that is the source of environmental pollution due to in-field burning. An annual paddy-wheat rotation occupies at least 60% of total cultivated land in Punjab and Haryana, with these crops providing approximately 145 Mt of surplus dry biomass per year.

In WP1, two timeframes (2015 and 2025) were fixed for the case studies. Likewise, regarding biomass availability, our data revealed that there will be a significant increase in competition for biomass. This conclusion was reached, assuming that biomass production will decrease (notably crop residues) and that competitive uses will increase (for both crop residues and stem wood). Other constraints are also foreseen, such as climate change, environmental regulations and the greater implementation of low input farming systems (LIFS) and organic farming. Although LIFS and organic farming can procure a number of beneficial effects (e.g. less irrigation and fertilization, lower or no use of pesticides, and lowered fuel consumption), in our study we assumed that these will also generally lead to decreased crop yields.

### **3.1.2 Stakeholder views**

Within the scope of the case studies, stakeholders from each region were questioned in order to measure their level of acceptance of the BIOCORE concept. This enquiry revealed that a majority of stakeholders support the biorefinery concept and consider that it constitutes a promising solution for the substitution of fossil-based products. Overall, stakeholders considered biorefinery as a high-tech development that would potentially bring significant benefits to regional development, notably in terms of skilled job creation, resource-efficiency and sustainable development. Similarly, stakeholders were aware of the fact that biomass can be used for several purposes and considered advanced biorefining as a good way to get more out of biomass than just liquid fuel.

In the French case study region a wheat/barley straw-based biorefinery was modelled. In discussions with stakeholders (farmers, advisers, cooperatives), it transpired that there were concerns about the quantities of straw export that would be required by a biorefinery. Stakeholders believe that the export of surplus straw could ultimately harm soil organic carbon levels and thus jeopardize grain yield, even though agronomic models demonstrate the feasibility of increasing straw export in the region. Furthermore, it was apparent that it would be difficult to convince farmers to sell surplus straw within the framework of long term contracts (5-10 years). This is primarily because local farmers' attention is focused on grain production.

### **3.1.3 Environmental impacts**

As mentioned earlier, within the scope of WP1, BIOCORE researchers assessed the probable local environmental of biorefining within the context of the different case study regions. Overall, some negative environmental impacts were identified in the European case studies. These include the intensification of forest harvesting, reductions in soil organic matter and nutrients (due to straw or wood export), and an increase of anthropic pressures on the environment. Nevertheless, it is expected that these negative effects could be, to some extent, minimized through the implementation of preventive actions, such as leaving forest residues on the ground, targeting only areas where biomass density is high, increasing the return rate of crop residues (>70% is recommended for cereal straws in the European case studies), thus maintaining SOM and nutrient levels. Other mitigation actions could involve improvement of biomass logistics, design and use of good forestry management practices, which would guarantee the integrity and/or improvement of forest resources, lowered use of forest stem wood (use alternative raw materials, reduce the demand of hardwood for thermal uses, or increase wood availability), introduction of reduced or no tillage methods, reduction of pressure on straw (use of alternative raw materials in cereal area, such as chaff (fine cut straw), or reduced straw needs for livestock).

In the Indian case studies the environmental impacts were positive. This is because, although leaving rice residues on the field would be beneficial for soil fertility and soil organic matter content (and thus crop yields), this option is hardly ever implemented. Indeed, in the two-crop wheat/rice rotation, after rice harvesting farmers only have approximately one month to clear fields before wheat planting. Therefore, to expedite this process farmers generally choose to burn rice residues in the field. This in-field residue burning is a source of both environmental and health problems, and is increasingly condemned by Indian government authorities. In this context, it is easy to appreciate that biorefining, which would create considerable financial incentives for the collection of rice crop residues, will significantly reduce these highly negative impacts.

### **3.1.4 Local biomass procurement costs**

For each case study, a primary feedstock was defined (e.g. rice straw in Indian case studies). Therefore, for each case study, the procurement costs of the primary feedstock were calculated up to farm gate/roadside. Furthermore, additional costs up to the factory gate (mainly transport, logistics costs, storing and drying, if applicable) were calculated and the supply chains were optimized based on this economic assessment.

A large variation of feedstock prices was found between the case study regions. According to the results, wood pellets in Germany are the most expensive feedstock (average €165 per tonne dry matter). Rice straw in India is the most economic feedstock (€29 per tonne dry matter) followed by Indian wheat and Hungarian wheat, barley and maize. However, including transportation and storage costs changes the economic order and Hungarian and German forest biomass, Hungarian poplar, German wood chips and French miscanthus become competitive.

Concerning the transport, processing and storage components of biomass procurement costs, it was generally ascertained that the use of transport modes,

such as railways can favourably influence overall costs. For example in the French and Hungarian studies, it was predicted that the smart integration of railways and trucks could produce up to 10% cost reductions. However, the use of railways is rendered difficult when the biomass production zones are scattered across a territory. In the German study this reduction was already intrinsic to the basic scenario, since the region under study is well-equipped with rail and fluvial infrastructures. Also, in the German study the proportional weight of transport in the overall procurement cost was lower, because the transport of woody biomass, especially dense derivatives such as pellets, is much more cost efficient than the transport of baled straw for example. Finally, in India, the cost of transport was found to be very much dependant on the availability of modern baling technologies that provide the means to make higher density bales. The complete implementation of baling in the two study regions was predicted to reduce the proportional weight of transport in the overall biomass procurement cost from 13 to 7% (Faridkot) and 25 to 21% (Sangrur) respectively.

### **3.1.5 Optimal supply chain**

The definition of supply chains was found to be very case specific. According to the way in which the BIOCORE case studies were designed, the French region was perceived as one that is highly reliant on a single biomass feedstock, while the Hungarian region could benefit from high availability of a variety of feedstocks, and displays good potential for the growth of dedicated biomass crops. In Germany, the feedstock (hardwood) is expensive, but transportation costs are low. Finally in India the establishment of an industrial use for rice straw presents many advantages, including an opportunity to contribute to the energy demand in the study region.

One of the critical features of any project that relies on the use of biomass is the security of the biomass supply chain. Therefore, a biorefinery can only become financially viable if it is associated with a feedstock provision plan that secures biomass supply for at least a 10-year period. The exact choice of a site also depends on other factors such as the presence of an appropriately skilled labour force, the quality of local transportation infrastructures and the proximity to end-user industries. Regarding infrastructures, the case studies performed in India illustrate a key requirement for the implementation of biorefineries, which is adequate storage capacity.

Other critical factors include the adoption of adequate technology. This will largely depend on the product portfolio that is targeted. One of the major strengths of the two organosolv technologies that have been tested and operated in BIOCORE resides in the fact that several different feedstocks types can be used.

## **3.2 WP2: Biomass fractionation and enzymatic and physico-chemical fraction refinement**

Using the Organosolv processes, in particular the one operated at pilot scale by CIMV S.A., as the cornerstone for biorefining, the principle aim of WP2 was to (i) extend the range of biomass feedstock that can be used with this fractionation technology and (ii) to develop all of the refining technologies required to produce high quality lignin, glucose and pentose syrups with optimal specifications for downstream conversion and product manufacture in WP3 and WP4. Additionally, WP2 set out to investigate the direct valorization of certain primary fractions, mainly via qualitative



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analysis and techno-economic application R&D, and to assess the potential use of process residues for the generation of heat and power for the biorefinery or for export in the form of alternative products (e.g. building materials or fertilizers). The work within WP2 was conducted in five tasks:

Task 2.1: Mechanical biomass pre-treatment (Comminution) and feeding

Task 2.2: Optimization of organosolv technology for multiple feedstocks

Task 2.3: Application-specific refinement of hemicellulose-rich fraction

Task 2.4: Cellulose refinement and techno-economic application

Task 2.5: CHP generation from organic residues and use of minerals

### **3.2.1 Mechanical biomass pre-treatment (Comminution) and feeding**

During the early phase of BIOCORE, several partners including CIMV S.A., successfully completed the development and selection of industrial scale comminution technologies, providing optimum acid diffusion for all BIOCORE feedstocks. For cereal straws, such as wheat and rice straw, the use of biomass particles presenting a size of 5-15 cm was validated at pilot scale. For hardwoods, the work performed in BIOCORE revealed that the optimum particle size is smaller (<1 cm). Accordingly, a prospective study performed within the scope of this work (described in deliverable 2.1) identified “micro-chipping” as a potentially useful technology for subsequent industrialization of the CIMV process. Micro-chips are smaller (<10 mm) than standard wood chips (<20 mm) and can be used directly in the CIMV biorefinery without further size reduction thereby improving the overall energy balance significantly.

### **3.2.2 Adapting organosolv technologies for alternative biomass processing**

At the outset of BIOCORE, the two organosolv technologies developed by CIMV S.A. and ECN respectively were essentially optimized for wheat straw. Therefore, a task in BIOCORE was to identify the correct operating conditions for other feedstock, such as hardwoods, including birch and short rotation poplar. In fact this was achieved in both cases by introducing changes into the operating conditions. For the CIMV process this involves altering the ratio of the solvent components (i.e. acetic and formic acid) and prolonging the residence time. In this phase, both organosolv processes were also tested on softwood. In the case of the CIMV process, trials were globally unsuccessful, although it was established that the presence of up to 10% (w/w) softwood in a hardwood batch does not prevent successful pulping. Regarding ECN technology (ethanol-water organosolv process), this proved to be more tolerant to softwood, since it was possible to generate a pulp from softwood. Nevertheless, this pulp proved to be rather unsuitable for subsequent processing by cellulases, and thus the production of fermentable glucose syrups. Overall, results indicate that to use softwood as a feedstock for the technologies tested in BIOCORE it will be necessary to introduce a process step before organosolv pulping that can remove the resins that prevent the correct penetration of the solvent systems. In this respect, WP2 work performed on the ECN technology provided a new development, which involves the introduction of a new process step, prior to the organosolv pulping. This process step removes proteins, waxes and other extractives from feedstock, notably straws, and thus provides a higher purity cellulose pulp and a lignin fraction whose

structure more closely resembles that of native lignin. A patent application has been filed for this new process route.

ECN has successfully adapted the benchmark ethanol/water organosolv technology and determined optimum process conditions for wheat straw, rice straw, poplar and birch.

### **3.2.3 Characterization and refinement of the hemicellulose-rich fraction**

Within the framework of BIOCORE, a considerable effort was made to characterize the rather complex hemicellulose-rich fraction (HRS) that is obtained as primary intermediates from the CIMV organosolv processes. This work was led by KULeuven who provided their considerable know-how in sugar analytics, and was facilitated by the collaboration of several partners. This combined effort provided the basis for quite robust analytics of a fraction (the CIMV-derived HRS) that proved to be particularly difficult to characterize. Overall, the fraction's dry matter was found to contain 60% (w/w) sugars, of which approximately half was xylose. The other components were xylo-oligosaccharides, glucose and a substantial amount of acetylated and/or glucuronic acid-substituted sugars.

Following the intensive effort required to characterize the CIMV-derived HRS, a mass balance was also done on the CIMV organosolv pulping process, performed at lab scale. This revealed that the recovery of the major biomass components was reasonable to good, although significant sugar (10-15%) and protein (23%) losses were observed. Concomitantly, lignin recovery exceeded 100%, which led to the assumption that some lignin-protein adducts had been formed. The final purity of cellulose and lignin were found to be 60 and 78% respectively, which was lower than that obtained at pilot scale (89 and 92% respectively). The results of the mass balance analysis have been prepared as a publication that was published in 2014 in the journal *Bioresource Technology*.

Regarding the refinement of the CIMV-derived HRS obtained at pilot scale, the main aim was to remove residual organic acids that are present in significant amounts after organosolv pulping. This is because organic acids, such as acetic and formic are known to be fermentation inhibitors, and indeed this was confirmed in collaboration with WP3 at early stage of the project. Nevertheless, beyond the idea that it was necessary to remove organic acids, refinement proved to be a rather complex task, because of the absence of a precise compositional analysis during the initial phase of the project (see above), and afterwards, due the discovery of components, such as acetylated and/or glucuronic acid-substituted sugars.

To refine the HRS, two stages were envisaged and developed, the first being acids removal, and the second being enzymatic hydrolysis (to obtain a syrup composed essentially of monomeric xylose). To tackle acids removal, several partners tried different technologies, including electrodialysis, steam stripping and ion exchange chromatography. Similarly, working with various, more or less purified, samples of the HRS, several partners (notably DSM), tested hemicellulolytic enzymes on the HRS. In both cases, progress proved laborious, because it was difficult to reduce acid concentrations to those necessary for fermentation and, when used at reasonable loading levels, most enzymes appeared incapable of completely depolymerizing the sugar mixture. Nevertheless, regarding enzyme hydrolysis, DSM did manage obtain some good results using a newly developed hemicellulase (HC) cocktail, which

yielded up to 90% xylose in monomeric form from a de-acidified sample of the HRS, working at 10% and 30% dry matter content respectively. Moreover, in the course of their studies, DSM identified a beta-xylosidase that was useful for the hydrolysis of the xylo-oligomers contained within the CIMV HRS.

Finally, at a quite late stage in the project, CIMV developed a new refinement process based on the use of separation-purification chromatography (SPC), which proved to be effective, because it was able to reduce the residual acid and ash contents in the HRS down to 1% (w/v) and 5% (w/v) respectively, while furnishing a syrup of which almost 30% dry weight was xylose (40% dry weight) and increased free glucose content from 3-8% (dry weight). Pursuing its work on SPC, at the end of the BIOCORE project, CIMV attempted a first pilot scale trial of its newly-established refinement scheme for the hemicellulose-rich stream. However, unfortunately, due to handling errors the pilot trial was unsuccessful no xylose-enriched syrups could be made available for fermentation trials.

In another part of the refinement task, KULeuven first studied the potential of the HRS as a source of prebiotic compounds (i.e. xylo-oligosaccharides or XOS) and then pursued work on model XOS in order to accumulate data that will be useful for this application when suitably-sized XOS can be obtained in pure form, either from the CIMV or ECN processes.

Similarly, in anticipation of advances in the purification of CIMV HRS, INRA developed work on enzyme immobilization with a view to developing a fixed bed bioreactor. To achieve this, beyond state of the art work was performed in order to evolve the fitness of a xylosidase for immobilization. Several approaches were attempted and promising enzyme variants that display an improved capacity for immobilization were isolated and characterized.

### **3.2.4 Cellulose refinement and processing**

A key aim of WP2 was to deliver pilot scale batches of cellulose pulp for further processing into glucose syrup and then for fermentation into target chemicals. Accordingly, in one example, CIMV delivered a sufficient quantity of pulp to DSM in order to allow the production of 275 kg of glucose, which was then into converted into 165 L of bioethanol using yeast fermentation. To achieve this, DSM deployed its state-of-the-art thermostable cellulose cocktail for the liquefaction step, achieving good to excellent (75-90%) glucose yields. Best hydrolysis yields were achieved by DSM after enzyme mixture optimization, which provided the optimized cellulase cocktail, designated C-mix opt.

Regarding pilot scale hydrolysis and fermentation, no scale-up issues were encountered by DSM and the overall yield in pilot trials was  $0.38 \text{ g.g}^{-1}$  glucose, which 78% of the time was higher than the best results achieved at lab-scale ( $0.35 \text{ g.g}^{-1}$ ).

### **3.2.5 Application testing for process by-products and wastes**

The major process residues of the BIOCORE concept are fines from straw or wood milling and fermentation residues. Of course,  $\text{CO}_2$  is also a by-product of fermentation, but this was not considered in the work performed. Representative samples of each of the process residues were analyzed and the optimum energy conversion routes were identified. Regarding the fines, results of tests performed by



ECN using a Bubbling Fluidized Bed (BFB) Thermal Converter (5kWth) revealed that the combustion of wheat straw fines was stable and no agglomeration was observed up to 900°C, unlike rice straw, which did cause bed agglomeration. In either case, flue gas cleaning was required to remove HCl, SO<sub>x</sub> and NO<sub>x</sub>. Using a Labscale Combustion Simulation (LCS) system, ECN also assessed the thermal conversion of fines via Low Temperature (LT) gasification, Pulverized Fuel Burner (PFB) and co-firing in coal fired power plants (cases are relevant for the EC and India). Results showed that wheat and rice straw fines are difficult fuels to use on a stand-alone basis (mainly due to high alkaline content) unless remediation measures are taken (e.g. addition of potassium binder and/or co-firing with coal or another biomass). For LT gasification, results revealed that the fines are more suited to gas production (for electricity generation) and that the use of a MgO (dolomite) bed would be recommendable to prevent agglomeration. Finally, analysis of the combustion residues (fly ashes) revealed that these did not comply with EU criteria for use in concrete (EN-450-1), but did comply with the criteria of several EU member states for use as fertilizer. Unlike the European context, the current regulatory situation in India, would allow the generation of electricity from fines using simple open-top gasification units, which could replace diesel generators in rural areas.

Analysis of the raw effluent and its liquid fraction, arising from cellulose hydrolysis and fermentation, revealed that these have high (80 and 70 g.L<sup>-1</sup> respectively) Chemical Oxygen Demand (COD) values and that the solids content (Total Suspended Solids (TSS) of the raw effluent was around 12 g.L<sup>-1</sup>. The raw effluent and its liquid fraction were readily digested in an anaerobic digester, with an average BMP value of 24 m<sup>3</sup> methane per m<sup>3</sup> of raw effluent and 22 m<sup>3</sup> methane per m<sup>3</sup> liquid fraction (measured at 30°C). Both inocula gave comparable final results. No acute inhibition of the methanogenic organisms was observed, but the fast acidification was identified as a risk. This latter feature needs to be taken into account in the design of both buffer tanks and anaerobic reactors. From the conducted batch tests no conclusions can be drawn about possible inhibition as a result of long-term exposure to the substrates.

Finally, the fermentation residue resulting from enzymatic hydrolysis and fermentation of the cellulose pulp was positively evaluated for application as animal feed additive.

### **3.2.6 Benchmarking CIMV acetic/formic acid organosolv biomass treatment against ECN's ethanol/water technology**

The main upfront biomass processing technology employed in BIOCORE is that operated by CIMV, this technology relies on the use of an organic to dissolve lignin and generate a cellulose pulp. If compared to more conventional biomass pretreatment methods, such as steam explosion, it is obvious that organosolv technologies are a quite different concept, which makes extensive benchmarking quite difficult to achieve. For this reason, in BIOCORE the CIMV process was benchmarked against a well-studied, alternative organosolv technology (ECN technology), which relies on the use of an ethanol/water solvent system, used with, or without, catalytic amounts of inorganic acid.

From a conceptual point of view, the ECN and CIMV technologies proved to be highly similar, because both processes can convert cereal straws or hardwood into a

cellulose pulp and an isolated, modified lignin fraction. Additionally, both processes remove hemicelluloses, which are obtained in a third fraction. However, closer comparison of the two technologies revealed a number of differences. Although the cellulose pulps arising from the two processes appeared to be of similar quality, being readily amenable to enzyme hydrolysis and fermentation, the hemicellulose fractions were very different. In the CIMV process, hemicelluloses were only partially depolymerized and were partly acetylated, while in the ECN process a large proportion of the pentose sugars were converted into furfural. Likewise, the lignin fractions were quite different, since those obtained in the CIMV process displayed higher average molecular weight and a higher level of carbohydrate impurities. One consequence of the observed differences in lignins from the two processes was the amenability to pyrolysis (i.e. the CIMV does not melt so easily and so has a lesser tendency to clog up the system) and the level of lignin incorporation in PF resins (higher phenol substitution levels could be reached using CIMV lignins). It should be noted that a water-recycle has not been taken into account in the calculations of the ethanol / water process.

In terms of CAPEX, preliminary estimates revealed that the two processes might have very similar investment economics (€149 million for CIMV, and €131 million for ECN), although the OPEX might be significantly higher (more than 40%) in the case of the ethanol/water process. In this respect, one notable difference between the two processes is the requirement for process water, which is much higher in the case of the ECN process.

### **3.3 WP3: White biotechnologies for product manufacture**

The objectives of WP3 were to establish biotechnology-based product pipelines for known technologies (e.g. cellulose to ethanol production), and to develop other ground breaking technologies to make bulk (e.g. organic acids, xylitol, isopropanol) and specialty (alkylpolyglycosides) chemicals. Production of bioethanol, xylitol, itaconic acid and xylic acid were candidates for piloting, whereas the aim for glucaric acid, isopropanol, ethylene and alkylpolypentosides was to demonstrate the feasibility of their biotechnical production. Xylitol, xylic acid and the surfactant alkylpolypentoside all relied on the C5 syrup, xylose and xylo-oligosaccharides as raw materials while other products can be produced from the C6 fraction. A special emphasis also was to optimise the process conditions for the use of these fractions. As elaborated in WP1, the use of the C5 fraction was more challenging and required extra efforts in strain and process optimisation. The work in WP3 was conducted in the following Tasks:

- Task 3.1: Production of bioethanol and xylitol using sugars derived from lignocellulose
- Task 3.2: Bioproduction of organic acids from glucose and pentoses
- Task 3.3: Bioproduction of ethylene and isopropanol, a propylene precursor
- Task 3.4: Synthesis of alkylpolypentosides

#### **3.3.1 Optimization of bioethanol and itaconic acid production towards piloting**

DSM used their proprietary C6 and C5-fermenting *Saccharomyces* yeast strains to address the suitability of the CIMV cellulose pulp (C6) and hemicellulose syrup (C5)

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fractions for bioethanol production. The cellulose pulp fraction was highly amenable to enzyme hydrolysis and the high initial cellulose content of the C6 pulp provided the opportunity to produce ethanol at levels at almost 10 % (v/v) in fermentation. Of all cellulose available, about 90% was converted into glucose during the enzymatic hydrolysis, and the amount of ethanol produced represented 93% of the theoretical maximum amount. The main challenge when handling the cellulose pulp was linked to mixing, in order to introduce the cellulolytic enzymes. At lab scale, this could be overcome by mixing the C6 pulp with partially deacidified C5 liquor. Interestingly, this was an effective strategy, because not only did it fluidify the mixture, but it provided the means to achieve higher ethanol yields (519 L.t<sup>-1</sup> cellulose dry mass, on 17% DM), compared to those achieved on cellulose alone (477 L.t<sup>-1</sup> cellulose dry mass, on 12.5% DM). In contrast, using C5 syrup alone only yielded 162 L.tonne<sup>-1</sup> xylose dry mass (on 15% DM). During BIOCORE, DSM also selected and tested strains of *Aspergillus*, a filamentous fungus, for the production of itaconic acid (IA), which is a useful platform chemical and a polymer precursor. Various engineered and mutant strains of *Aspergillus* were screened and the best ones selected for production trials. Experiments were carried out to test optimal pH, media composition and oxygenation level for production. A key target was to address the suitability of the CIMV cellulose pulp as raw material. As shown earlier for ethanol, CIMV cellulose pulp was well-suited to enzymatic hydrolysis and fermentation. In contrast, the C5 syrup was not, since it had a strong inhibitory effect on the *Aspergillus* strains. Working with glucose derived from cellulose pulp, at lab scale DSM was able to produce 49.2 g L<sup>-1</sup> culture of IA over 107 h. The final yield of IA was 0.32 g.g<sup>-1</sup> glucose (theoretically available in the cellulose pulp), or 0.44 g.g<sup>-1</sup> in the fermentation broth and the volumetric productivity 0.41 g L<sup>-1</sup> h<sup>-1</sup>. Importantly, fermentation could be carried out at pH 2.4, which is beneficial for purification. IA was purified from fermentation broths with yields of 75-88% and crystal purity above 99%. Overall, these results were sufficiently encouraging for DSM to take the process forward to pilot scale trials.

In summary, the results showed that the cellulose pulp from the CIMV process is an excellent raw material for the production of glucose syrup that can be used in biotechnological processes. In BIOCORE, the usefulness of the CIMV cellulose was demonstrated through ethanol and IA production.

### **3.3.2 Engineering of yeasts for xylitol and xylonate production on the C5 syrup fraction**

One key aim in BIOCORE was to establish added-value uses for the C5 syrup (HRS) that is obtained from the CIMV process. To this end, the production of xylitol and xylonic acid by fermentation processes was explored. It was found that in particular the high formic acid concentration of the HRS imposes limits on yeast performance. In addition, around half of the xylose contained within the HRS is 'bound' in oligomeric or polymeric form and thus requires a hydrolysis step to render it available for fermentation. However, if efficient depolymerization of the 'bound' xylose was possible, it would mechanically raise the overall xylose concentration, allowing a xylose concentration of ~200 g.L<sup>-1</sup> to be achieved, which is the minimum concentration needed in order to attain industrial feasibility.

In BIOCORE, VTT worked extensively on various HRS obtained from refinement work performed in WP2, optimizing fermentation whenever possible. Additionally, to overcome yeast inhibition by formic acid, further yeast engineering was performed.

Using these yeast, it was possible to tolerate up to  $\sim 40 \text{ g.L}^{-1}$  of formic acid in the HRS,.

#### *Xylitol-producing strains*

VTT carried out strain engineering work to improve xylitol productivities, working on various yeast, including *Saccharomyces cerevisiae* and *Pichia kudriavzevii*. Several different xylose reductases were tested (that convert D-xylose to xylitol) and the enzyme expression levels were increased. Excellent production strains were obtained that, when growing on pure xylose, can produce over  $160 \text{ g.L}^{-1}$  xylitol, with production rates of over  $2 \text{ g.L}^{-1}\text{h}^{-1}$  and yields well over  $0.9 \text{ g xylitol from 1 g xylose}$ . Regarding the robustness of these strains, partially deacidified pentose syrup could be used as a 70% feed (supplemented with 30% pure xylose). In this case, xylitol titers of over  $100 \text{ g.L}^{-1}$ , production rates of  $0.82 \text{ g.L}^{-1}\text{h}^{-1}$  and yields of  $0.89 \text{ g g}^{-1}$  were obtained. Small scale experiments indicated that if the pentose syrup is further purified using the most advanced technology that was achieved in BIOCORE, then it could be used directly for xylitol production.

#### *Xylonic acid-producing strains*

VTT also did extensive strain engineering work to produce xylonic acid (XA) from xylose. Several xylose dehydrogenase enzymes (that convert D-xylose to D-xylonic acid) with different cofactor specificities were tested in various host strains. The yeast *P.kudriavzevii* turned out to be the best host of the several species studied. On pure xylose, the XA titers were over  $160 \text{ g.L}^{-1}$ , production rates  $1.4 \text{ g.L}^{-1}\text{h}^{-1}$  and yields of XA over  $0.9 \text{ g.g}^{-1}$  xylose provided. Remarkably, the productivities were similar at pH 3, which is important for the economy of downstream purification. Preliminary trials were carried out with the industrial pentose syrup fed at 50% (w/v), with 50% (w/v) pure xylose. In this case, xylonic acid titers were  $40 \text{ g.L}^{-1}$ , overall production rates  $0.43 \text{ g.L}^{-1}\text{h}^{-1}$  and the yield was  $0.83 \text{ g XA g}^{-1}$  xylose.

The work showed that production of xylitol and xylonic acid with yeast is industrially feasible and high productivities can be obtained. Nevertheless, at the end of BIOCORE the use of the CIMV C5 syrup in fermentation still posed problem, even though some very encouraging preliminary results were obtained with a final small batch of refined syrup. Unfortunately, these results could not be confirmed at larger scale, due to the late failure of a pilot scale refinement of the CIMV C5 syrup (reported elsewhere in this report).

### **3.3.3 Strain engineering to demonstrate the feasibility of biotechnical production of isopropanol, glucaric acid and ethylene**

Isopropanol and ethylene are platform chemicals and precursors of polymers, such as polypropylene and polyethylene, respectively. Glucaric acid is also a potential platform chemical that can be obtained from biomass hydrolysates and used as a polyester precursor.

At the outset of BIOCORE, the production of each of the aforementioned molecules was at a very preliminary stage (TRL1-2), so the aim was to move forward, supplying lab-scale proof of concepts for each. To achieve this, extensive microbial strain engineering was performed.

#### *Isopropanol production*



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The goal of scientists affiliated to INRA (France) was to engineer the bacterium *Clostridium acetobutylicum* to produce isopropanol. First a strain deleted in butyric acid production was obtained, and thereafter several parallel strain engineering strategies were performed to further improve isopropanol production. This involved the introduction of two key enzymes, NADH-dependent hydrogenase enzyme for redox cofactor equilibration and CoA transferase for improvement of acetate utilization for isopropanol conversion. For this, genes encoding either best performing naturally-occurring enzymes or engineered enzymes were tested. However, within the timeline of BIOCORE, it was not possible to obtain a strain that produces isopropanol as the sole product. Nevertheless, a very good *C. acetobutylicum* strain was created that produces a mixture of isopropanol, butanol and ethanol (IBE) at overall titers of 21 g.L<sup>-1</sup> (including isopropanol 5 g.L<sup>-1</sup>), productivity of 0.8 g.L<sup>-1</sup>h<sup>-1</sup> and a yield of 0.34 g IBE per g of glucose utilized. Similar productivities were also obtained on pure xylose and on xylan (lower rates and yields on xylan). The IBE mixture is suitable as a biofuel, or as a source of the individual chemicals. Such high performance IBE production in batch cultures is a novel result, since this had not yet been demonstrated in *Clostridium*.

In complementary work, DLO (The Netherlands) carried out experiments to investigate the *in situ* recovery of IBE mixtures produced by the wild type strain, *Clostridium beijerinckii* NRRL B593. Gas stripping and absorbent materials (activated carbon or zeolite) were studied in detail. In best combinations 70-85% of products could be recovered. Removal of products through gas stripping and/or absorption during fermentation by *Clostridium* increased IBE productivity by at least 50%. Modelling suggests that the combination of the recombinant strain produced by INRA with the *in situ* recovery methods developed DLO would increase the IBE productivity 8-fold in comparison with the wild type strain cultured in normal conditions.

### *Glucaric acid production*

VTT worked on the production of glucarate and constructed a 4-step pathway from glucose to glucarate in a modified strain of *S. cerevisiae*. The viability of the strain is impaired, but when glucose was fed at low concentrations and nitrogen provision was limited, yields of 0.3 g of glucarate per g glucose were obtained. At the end of BIOCORE, production rates remained low in this *S. cerevisiae* host, with titers of 1.3-1.5 g.L<sup>-1</sup> being achieved. However, the results demonstrate that engineered yeast can produce glucarate, although significant efforts are still needed to develop an industrially-viable biotechnical production process.

### *Ethylene production*

Chalmers engineered the yeast *S. cerevisiae* to produce ethylene by expressing the plant ethylene forming enzyme, EFE. One aim in BIOCORE was to better understand EFE function, in order to increase the intracellular availability of 2-oxoglutarate and concomitantly decrease the amount of arginine, which is an alternative EFE substrate that does not provide ethylene.

During the course of work in BIOCORE, genetic modifications and culture optimization did provide some improvements in ethylene production. However, attempts to engineer EFE for efficient ethylene formation proved difficult. Moreover, metabolic modelling clearly demonstrated that oxygen availability will always be a

limiting factor, since oxygen is not only a substrate for EFE, but is also required for the respiration of the NADH generated in ethylene formation.

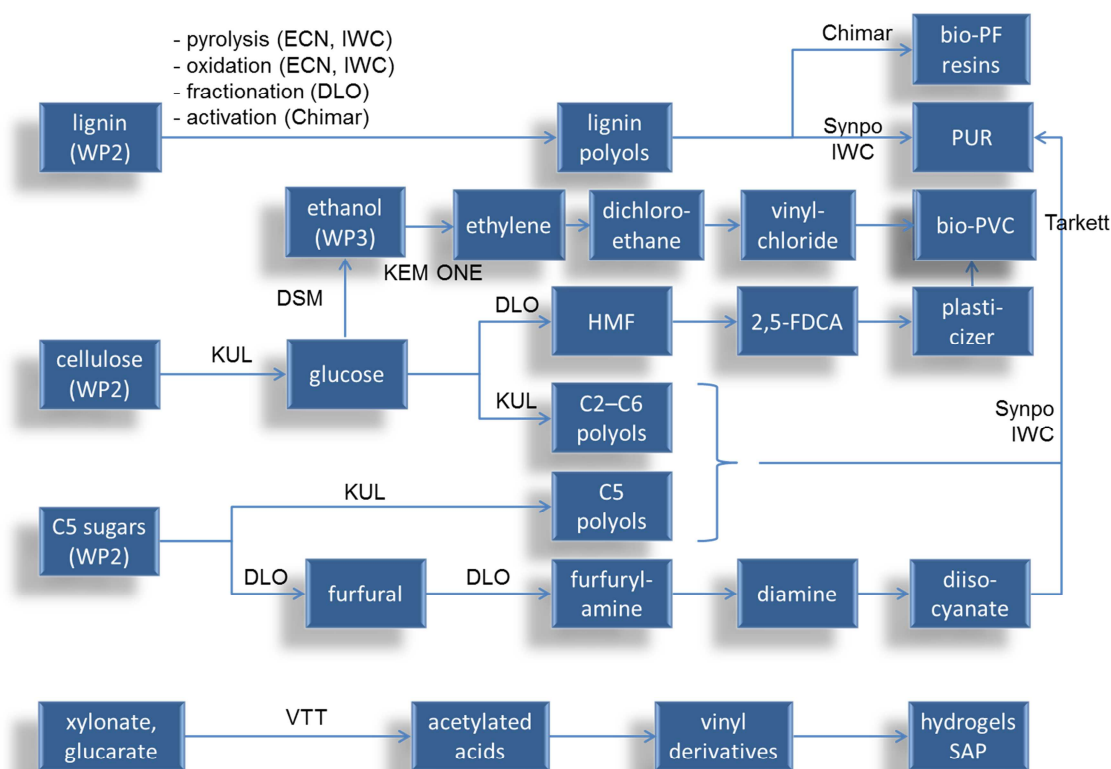
### **3.3.4 3.3.4 Enzymatic conversion of pento-oligosaccharides to alkyl polypentosides**

Alkyl polypentosides (APP) can be used as surfactants and can be formed through *in vitro* enzymatic transglycosylation reactions. INRA analyzed APP production using xylo-oligosaccharides (XOS) and different alcohol donors and demonstrated the feasibility of using a fungal xylanase in this transglycosylation reaction. The XOS contained within the CIMV C5-rich syrup were found to be rather short (low DP) and unsuitable substrates for xylanases. Consequently, the focus turned to beta-xylosidases that accept smaller xylo-oligosaccharides. The C5 syrup was successfully used for transglycosylation with beta-xylosidase, but the syrup must first be purified/detoxified using anionic exchange chromatography. In other work, a suitable immobilization method for the enzyme was found, which enables a repeated reaction process. In addition, INRA performed significant work on enzyme engineering, which was aimed at increasing the transglycosylation capability of the *Trichoderma* xylanase II enzyme, shift its activity way from hydrolysis and towards transglycosylation. This work was successful and an enzyme variant was obtained which showed an approximately 35-fold reduction in hydrolysis, with the hydrolysis/transglycosylation ratio decreasing from 481 to 14.

## **3.4 WP4: Chemical and thermochemical transformations**

The principle goal of WP4 was to investigate different chemistry-based options for the conversion biomass intermediates (lignin, cellulose and hemicelluloses), as supplied by WP2, into useful compounds, polymers and, in some cases, finalized products. The different routes that were investigated are shown in the figure below.

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### 3.4.1 Conversion organosolv lignins into useful building blocks

Lignin depolymerization work, either via oxidation or using pyrolysis, revealed that lower molecular weight lignins and phenolic fractions could be obtained. Using catalytic oxidation, ECN achieved up to 50% depolymerization, with oligo-phenols (average Mw = 1500 Da) forming the principle product. These contained an increased content of carboxy-phenyl groups, implying that a possible application could be the substitution of terephthalic acid. Similarly, catalytic fluidized bed pyrolysis technology was optimised by ECN and used to continuously convert Biolignin™ (i.e. CIMV's lignin) into a phenolic bio-oil (40 - 50 wt%) and biochar (20 - 40 wt%). A techno-economic analysis of the bio-oil, which contained up to 10 % (w/w) monomers, revealed that the most profitable valorization route would be to isolate the monomeric phenols and use the char fraction as carbon black, carbon fiber and activated carbon. However, the use of the bio-oil as a bitumen additive and the char as carbon fiber could also be profitable.

The chemical modification of lignin using a variety of methods (polyoxymetalates, or POM-mediated oxidation, oxypropylation, phenolation and glyoxylation) was investigated and clear improvements in lignin functionalities for target applications, such as bio-resins and bio-PUR, were obtained. Similarly, physical/mechanical modifications of raw Biolignin™ provided some beneficial effects with respect to functionality. Specifically, washing and particle size reduction and homogenization provided the means to directly introduce Biolignin™ into Phenol-Formaldehyde (PF) wood resins (up to 70% phenol substitution) and flexible polyurethane (PU) formulations.

Fractionation of Biolignin™ using environmentally-friendly solvents and a packed column (kg scale) allowed DLO to isolate several macromonomer lignin fractions that could be used for PU applications.

### **3.4.2 Transformation routes for cellulose and hemicelluloses**

An early success in BIOCORE was the successful lab-scale one-pot catalytic conversion of cellulose pulp into isosorbide, using commercial tungstosilicic acid (SiW) and Ru/C. Interestingly, KU Leuven showed that the CIMV-derived cellulose pulp was a particularly good feedstock for this process. In a similar manner, cellulose pulp was also converted into ethylene glycol, with yields up to 47 wt%, using a bifunctional 2%Ni-30%W<sub>2</sub>C/AC-973 catalyst.

Other work involving C<sub>6</sub> sugars included the preparation of sorbitol ester and FAME-based surfactants. To achieve this, SYNPO employed enzyme technology to catalyze trans-esterification reactions.

Finally, regarding hemicelluloses, DLO demonstrated how isocyanate-free polyurethanes and polyurea can be accessed using a pentose-derived dicarbamate, and how the presence of formic and acetic acid in the CIMV HRS can be beneficial for the conversion of xylose into furfural in good yield, above 50%, and at high selectivity, over 80%. A higher furfural selectivity was found in a bi-phasic system, but isolation of the product was not performed.

### **3.4.3 Polymer formulation studies.**

As mentioned above, Biolignin™, Glyoxalated Biolignin™ and ECN organosolv lignins were used by CHIMAR to substitute phenol in PF resins. In the best case, washed and milled Biolignin™ could be directly incorporated into PF resins, substituting up to 70% of phenol. The resultant resins were suitable for plywood manufacture. Plywood boards made with Biolignin™-based resins met current European standards, and also met the severe CARB II rule limits for formaldehyde emission. These excellent results motivated the choice of this process for further scaling for pilot testing. Similarly, finely milled Biolignin™ was also directly incorporated into a flexible polyurethane elastomer formulation. The addition of Biolignin™ led to improvements in several properties, including surface hardness and electrical resistance. The process was patented by SYNPO.

Regarding chemically-modified lignin, the oxypropylation of Biolignin™ led to the successful preparation of liquid polyols. IWC used these in PU formulations and showed that they displayed high reactivity toward traditional isocyanates, no doubt thanks to the increased content of hydroxyl functions. Overall, work by IWC and Synpo demonstrated that liquid polyols, containing up to 30% of Biolignin™, could be used to formulate both polyurethane foams and films.

In a global effort to provide innovation in the PVC sector, KEM ONE and DLO worked on different aspects of a strategy aimed at producing bio-based components for PVC, with KEM ONE adapting the ethanol to vinyl chloride monomer process to the use of 2G ethanol (reported in WP6). Regarding DLO, they developed an improved di(2-ethylhexyl) ester of tetrahydro-2,5-furandicarboxylic acid (DEH-bio), starting with a C<sub>6</sub> sugar. The resulting compound proved to be an excellent PVC plasticizer, compared to the commercial counterpart, although its thermal stability remains an



issue. In the final stage of the work, DEH-bio was incorporated into a PVC formulation, which was prepared as a flooring component. PVC sheets were successfully produced, but a clear yellowing problem was identified, which is linked to the presence of DEH-bio. Therefore, this issue will require further investigation.

Finally, concerning the functionalization of xylonic acid (a target compound in WP3), VTT on the synthesis of a polymer building block. One route, incorporating gluconate/xylonate into a polymer backbone via an amide linkage, provided the basis for a successful polymerization trial aimed at producing a hydrogel product.

### **3.5 WP5 Process design**

In BIOCORE, WP5 fulfilled the important task of producing conceptual design(s) for a full-scale biorefinery. The different tasks of this WP were thus focused on the implementation of state-of-the-art technologies in process synthesis, process integration, modelling, flowsheeting, and optimization to produce advanced process designs in which feedstocks were varied and product portfolios, rather than single products, were taken into account. At the end of the project, WP5 work delivered a systematic layered approach for the development of integrated biorefineries that is generic and amenable to high-throughput analysis. The different layers involve synthesis technology to tackle the combinatorial challenges of the design, process integration to establish efficiency targets (raw materials, energy, and water) and flowsheeting to deliver detailed blueprints and models. Accordingly, WP5 tasks covered flowsheeting (WP5.1), synthesis (WP5.2), integration (WP5.3), concept-based optimization (WP5.4), and technology deployment (WP5.5).

#### **3.5.1 Modelling the core organosolv process**

At the outset of the design and optimization process, it was necessary to build basic models to describe the core process that is operated by CIMV S.A (France), establishing mass and energy balances, sizing units, and calculating the composition of intermediate bulk streams (i.e. cellulose, C5 stream and lignin). The process flowsheets that were generated accounted for six different sections (extraction, de-acidification, evaporation, lignin precipitation, C5-evaporation, distillation) and involved unit operations for reaction, separation, heat transfer, and several mass transfer steps. The models were validated within the actual process, whereas optimization studies fine-tuned design and operating parameters (e.g. location of feed trays, temperature and pressure changes). Interactions with experimental groups proved vital due to a general lack of data (in stream properties, thermodynamics, VLE/LLE, and reaction engineering) and a parallel need to produce models of non-conventional processes. To capture data, an Excel-based template was developed, which was used to formalize data communication and exchange with all BIOCORE partners, especially those unfamiliar with the specific needs of process designers. The results and detailed flowsheets are included in deliverable D5.1. Once models for the basic process had been established, process integration was studied, scoping for energy and water savings. Likewise, **Pinch Analysis** identified quite impressive scope for energy reduction (up to 70%), pinpointing specific sections and processes for modification. However, conventional **Water Pinch** was not readily applicable. Therefore, within the framework of BIOCORE, a new set of methods, tailored to the specific problems and challenges of a biorefinery, were developed.

These combined Water Pinch with mathematical programming. The new methodology was applied to set targets and designs for water re-use (45%), water-recycle (55%) and water regeneration-recycle (60%). Savings, integrated designs and a summary of process modifications are described in deliverable D5.2.

### ***3.5.2 Selecting best-performing product portfolios***

In the BIOCORE concept, the idea is to use biomass as raw material for the simultaneous manufacture of several products using the specific chemical potential of each of the bulk intermediates. Therefore, one of the most exciting features of the process design work was the selection of processing pathways and product portfolios (task 5.2). To achieve this, WP5 developed a pioneering strategy, using an ambitious and holistic approach that used superstructures to systematize the search using optimization and synthesis technology. The approach formulated models that embraced approximately 70 different pathways with overlapping chemistries, processing branches, intermediates and products, and the search accounted for both single and integrated pathways. Mass and energy balances were compounded from BIOCORE simulations, whereas conceptual cost models were developed using LHV-based correlations. Using current market and economic parameters, the approach deployed optimization to screen for the best-performing product portfolios. Additional studies addressed the impact of process and economic parameters (e.g. conversion to products, market demand and prices, business biases on particular products) on the final selections. Overall, this work accomplished an important milestone, providing a selection of new processes for integration, and provided a key achievement, supplying a new methodology that offers the capability to link experimental accomplishments with a synthesis stage that selects chemistries and products for an industrial plant.

### ***3.5.3 Synthesis models flowsheeting of pilot processes***

In the latter stages of WP5, work was subsequently diverted from a sequential case-by-case approach towards a systematic and simultaneous methodology. Beyond its holistic nature, the methodology developed is generic, systematic and readily amenable to further extensions. Meanwhile, WP5 remained an active hub of communication channeling information to researchers working on integrated sustainability analyses and pilot trialing respectively. In parallel, WP5 produced a revised version of the flowsheet of the core CIMV process, which incorporates improved models for solids and waste by-products, cost models for benchmark processes (ECN), cost models for the CIMV process and a range of process flowsheets to support the pilot studies performed in WP6. The details of much of this work can be found in deliverables D5.3 (economic data and the optimization models) and D5.4 (synthesis models). the synthesis representation, the development of mass and energy balances and the costing approach. In the course of this work, WP5 was challenged by minor delays, which were linked to the challenges of communication with the experimental groups, who were often uncomfortable about communicating what they perceived to be preliminary data on (among other things) conversion efficiencies, energy requirements, and descriptions of process byproducts.

### **3.5.4 Process integration and process-to-process integration**

Process integration and process-to-process integration extended and elaborated the findings of synthesis, which had already provided the means to eliminate unprofitable pathways and identify best-performing product portfolios.

First, the chemical pathways were translated into complete flowsheets. In relation to the processes that were submitted for pilot testing in BIOCORE (described by 8 basic flowsheets and 3 additional variants), 23 new flowsheets were integrated in order to account for the selected pathways. The latter included 14 processes for cellulose, 7 processes for C5 sugars and 2 new processes for lignin. The new models capitalized on both literature-derived and BIOCORE expert knowledge, offering a convenient background to analyse, cost and scale-up the different variants of the CIMV-based biorefinery concept. Next, process integration was extended from the CIMV core process to address all of the process operations in the BIOCORE pilot trials, setting targets for energy and water efficiencies. In the form of **Grand Composite Curves**, the results provided energy and water footprints, which were necessary in subsequent studies of **Total Site Analysis**. In parallel, work was performed to update the CIMV process, following the decision by the CIMV team to act upon a recommendation made in D5.1 concerning the better integration of evaporation and distillation units. This led to the retrofitting of these process units and thus changes to the CIMV process, notably in terms of safety, since lower pressure is employed in distillation, and target achievement, since a multi-effect distillation is attained, with integrated sections both below and above the Pinch. All of these modifications were reported in deliverable D5.5, which also provides detailed descriptions of all flowsheeting models produced after synthesis.

### **3.5.5 Total site analysis**

The penultimate stage of the process design work focused on Total Site Analysis (TSA) and process-to-process integration, a study that was designed to investigate the relative benefits (e.g. assess trade-offs between centralized vs. distributed production) of the colocation of the different biorefinery processes. So far, these methods have mainly been used to study petrochemical installations and so our study essentially represents the first TSA study ever performed on biorefineries. Unlike the case of oil and gas installations, in which all processes are fixed, biorefinery processes represent degrees of freedom. When applied to the BIOCORE processes that had been submitted to pilot scale studies, TSA produced results that indicate that savings from process-to-process integration may range from 14 to 85%, and that savings increase as the number of products increase, with diminishing benefits still being detected as portfolios extend beyond 4 to 5 products. The range of savings indicates that the exact choice of product portfolio will have a significant impact on the sustainability of the biorefinery, whereas the magnitude of the savings supports scenarios with co-located production. The new screening methodology and the results thereof are summarized in deliverable D5.6.

### **3.5.6 Integrating new value chain features**

In the final stage of the process design work, the biorefinery was integrated with the supply chains, assessing the impact of uncertainties, such as market, price and technology changes. In parallel, WP5 augmented the designs with waste treatment

sections, finalizing flowsheets and calculating detailed costs in each case. The flowsheets that describe wheat straw processing were extended to process rice, poplar and birch wood. In the case of hardwood, it is noteworthy that additional energy was required for drying. However, process integration asserted that minor process modifications will be enough to render the additional energy (e.g. offering free drying with better designs). Similar conclusions have been drawn for single-feedstock and multi-feedstock processes. An additional round of studies evaluated the impact of process uncertainties. No major changes were reported for changes below 20% of the nominal value; minor modifications appeared within 20-40%; new solutions emerged as the level of uncertainties increased further. Waste management has been addressed from a holistic perspective that simultaneously reviewed all the waste streams of the plant. WP5 applied a superstructure approach to assess alternative waste treatment technologies (e.g. anaerobic digestion, rotating biological contractors, incineration etc.) and incentives for cogeneration and energy production. Detailed results of the concept-based analysis are presented in deliverable D5.7. A final version of the integrated biorefinery (including flowsheets, detailed costs models using APEA: the Aspen Plus Economic Analyzer, stream compositions, process variants with different feedstocks, and energy analysis) are included in deliverable D5.8.

### **3.6 WP6: Pilot and industrial pilot scale demonstration**

WP6 relied on the extensive industrial knowledge and existing pilot facilities of several partners to perform pilot trials. In this way, a pan European, virtual pilot was used for the demonstration tasks. The scale of each pilot operation was chosen either to enable the proper upscaling of the process parameters, or to provide sufficient material for application work.

#### **3.6.1 Piloting the cellulose to products processes**

Cellulose pulp produced by CIMV S.A. from 1 tonne of wheat straw proved to be readily hydrolysable using DSM knowhow and enzymes, and the yeast *Saccharomyces cerevisiae*. In the pilot trial, 275 kg of glucose was fermented in a 4 m<sup>3</sup> vessel, producing 165 L of bioethanol, which represents 74% of the theoretical yield. The pilot trial provided a clear demonstration of the amenability of the cellulose pulp to cellulase action and the suitability of the resulting hydrolysate for fermentation.

In a second cellulose-based pilot trial, 290 kg of cellulose pulp was hydrolyzed, producing a glucose syrup that could be used a feedstock for fermentation using a strain of the filamentous fungus *Aspergillus terreus*, thus allowing the production of itaconic acid. Like for ethanol production, the pilot trial was very successful, surpassing the performance of laboratory scale trials and revealing that the cellulose pulp obtained from the CIMV organosolv process is also a suitable raw material for *A. terreus*.

Regarding the further transformation of the 2G ethanol and itaconic acid into potentially marketable products, the former was used to investigate whether it could be used to produce PVC, while itaconic acid was used to formulate alkyd coatings. Interestingly, for ethanol it appeared that this could be readily dehydrated, procuring ethylene, using standard industrial protocols and no specific purification steps.

Nevertheless, the subsequent step in the process (*i.e.* the production of dichloroethane) failed due to the presence of minor impurities in the biobased ethylene, although it was rapidly established that these can be eliminated using standard techniques, such as molecular sieves, thus allowing the production of PVC. Regarding itaconic acid, approximately 9 kg of pure, crystalline product was produced and used to prepare alkyd coatings, one formulated using phthalate anhydride (70% biobased resin) and another using rosin (100% biobased, phthalate-free). Both coatings were formulated in sufficient quantities to perform application testing. This revealed that the itaconic-based displayed gloss and hardness comparable to reference paints and even increased opacity and outdoor durability. Moreover, it is important to underline the fact that one of the resins was phthalate-free, a point that could confer this product with market attractiveness.

Finally, the chemical conversion of cellulosic glucose into sorbitol via classical hydrogenation was also tested at pilot scale using standard catalysts and refining techniques. Unfortunately, in this case protein impurities in the glucose syrup inhibited the activity of the hydrogenation catalyst and thus reduced the process performance to a suboptimal level and rendered it impracticable in an economically-viable framework.

### **3.6.2 Processing of the hemicellulose stream**

Work performed in BIOCORE clearly revealed that, in terms of product manufacture, the hemicellulose-rich stream from the CIMV process is the hardest to use as a feedstock. This is because this fraction is chemically complex and thus, unlike the lignin and cellulose fractions, requires further refining for most of the target product routes. Until a quite late stage of the project it was anticipated that a small scale pilot run (5 L fermentation) could be performed to produce xylitol using the engineered yeast strains created by VTT. However, despite the fact that CIMV did succeed in preparing 10 kg of chromatographically-purified hemicellulose syrup, a handling error during the preparation procedure resulted in accidental microbial-mediated sugar degradation and thus sample destruction. Therefore, no pilot scale fermentation trials have been performed. Nevertheless, the most recent status of the work on the hemicellulose fraction has supplied encouraging results, indicating that with some further effort it will be possible to pilot fermentations that are fed with refined hemicellulose syrups from the CIMV process.

### **3.6.3 Production of lignin-based products**

Within the scope of WP6, three different pilot trials were implemented using lignins from the CIMV process (*i.e.* Biolignin™). Two of these used Biolignin™ in almost direct manner, relying on the intrinsic properties of the organosolv lignins. However, a third trial involved further chemical modification of the Biolignin™ in order to produce a liquefied derivative.

The first of the pilot trials concerned the production of a flexible PU elastomer, by adding lignin into a biobased commercial formulation. To prepare the Biolignin™ for pilot trialing at 25 kg scale, 6 kg of Biolignin™ was washed and ground to reduce particle size before being added into the PU formulation. When compared to the reference elastomer, the Biolignin™-filled elastomer displayed quite modified properties, notably vastly increased elongation and tensile strength, and slightly



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increased surface harness. Also, remarkably the electrical resistance of the resulting elastomer was increased. As mentioned earlier, considering the attractive properties of the new lignin-based PU elastomer, the formulation has been patented (number PTC/CZ2013/000111) and scale up for commercial production (= 250 kg batch size) is feasible. Target applications for this elastomer are electrical appliances and customer prospection is underway.

For the second pilot trial, 75 kg of resin was produced with washed and ground Biolignin™. The lignin was used as a substitute for phenol in thermo-curing phenol-formaldehyde resin at the level of 50%. The resin was used for the preparation of plywood panels composed of 3, 5 and 9 layers. The technical properties of the panels were determined and evaluated according to the relative European standards EN314-1:2004 and EN314-2:1993. It was found that the Biolignin™-based panels resulting from the pilot trial meet the requirements of the above standards and are perfectly comparable with commercial ones. Such plywood panels are suitable for interior or exterior use. Moreover, as before it was noted, the lignin-based plywood panels displayed extremely low emissions of formaldehyde, meaning that these panels can meet the CARB II rule.

The final pilot trial involving lignin concerned the preparation of rigid polyurethane (PU) foams, scaling up to 30 kg scale the previously established procedure, which involves the oxypropylation of Biolignin™ and thus the preparation a liquid, Biolignin™-based polyol component. As for the lab scale trials, the resulting PU foams, which contain 7.5 % (w/w) Biolignin™ or 25% (w/w) liquid Biolignin™-based polyol, displayed a certain number of advantages when compared with a standard rigid PU foam. These include higher UV and dimensional stability, and better flame resistance. Nevertheless, the pilot trial also revealed that the Biolignin™-based polyol containing more than 30% (w/w) of Biolignin™ leads to a PU foam formulation that displays higher viscosity, a point that could create problems in a manufacturing process.

### **3.7 WP7: Integrated assessment of overall sustainability**

An overarching priority for BIOCORE was to investigate the sustainability of the BIOCORE biorefinery concept in a fully integrated way, considering environmental, economic and social aspects along the value chain, the goal being to identify the most sustainable biorefinery options. To achieve this, it was necessary to devise new methodologies, since integrated sustainability assessments do not yet possess an internationally-standardised methodological framework. Nevertheless, recent methodological developments (e.g. life cycle sustainability assessment, LCSA) were used as a source of inspiration and a set of existing state-of-the-art analytic tools was implemented. The list includes environmental life cycle assessment (LCA), elements of environmental impact assessment (EIA) and social impact assessment (SIA) / social life cycle assessment (sLCA), economic and market analysis, as well as an analysis of other sustainability-related aspects, such as policy and regulatory issues and biomass competition issues. The major advancement of this comprehensive and streamlined approach is that it (i) covers all major aspects of sustainability and (ii) ensures that the individual assessments are based on exactly the same system boundaries.

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In a first step, these vital aspects of sustainability were addressed individually. The results of these individual analyses were subsequently subjected to a multi-criteria evaluation.

### **3.7.1 Environmental assessment**

Like any other product, biorefinery products will generate environmental impacts at different spatial levels (i.e. global, regional and local levels). The environmental impacts of BIOCORE products were quantified by means of screening life cycle assessment (LCA) and supplemented by elements borrowed from environmental impact assessment (EIA). However, unlike standard EIA studies, which are site- and project-specific, the assessment of local environmental impacts was performed at a generic level, and took a life cycle perspective and was product-specific. For convenience, the modified method was designated life cycle environmental impact assessment or LC-EIA. In terms of progress, compared to the current LCA methodology, a supplemental LC-EIA enlarges the scope of a LCAs study, providing adequate coverage of local environmental impacts.

Using the combination of LCA and LC-EIA, it was possible to reveal that biorefineries based on the BIOCORE concept will cause a wide spectrum of potential impacts, ranging from significant environmental benefits to distinctly detrimental impacts. Among the drivers of these impacts are key factors, such as the choice of product portfolio, the mode of implementation and external influences. Depending on the exact nature of these factors, in some cases environmentally-advantageous impacts can occur simultaneously with negative impacts, with no obvious pattern being detected, while in others, detrimental environmental impacts emerge across all of the environmental impact categories. Nevertheless, in many cases the careful analysis of the different impacts has revealed a significant number of opportunities for mitigation and overall environmental performance optimization.

Concerning biomass supply, many of the value chain variants were shown to be characterized by quite low environmental impacts, especially with regard to local impacts on soil, water quality, fauna and flora. In this respect, the BIOCORE biorefinery concept is well-placed in comparison to conventional production practices for bioenergy uses and biofuels, although the precise choice of the most environmentally-sound feedstock is very dependent on the prevailing local conditions.

The factor that was revealed to be the most determining for environmental impacts was the choice of product portfolio. In this respect, a combination of xylitol production (hemicellulose stream), itaconic acid-based superabsorbers or polyester resins (cellulose) and bio-based polyurethanes (Biolignin™) performed best. In contrast, when ethanol was introduced into the portfolio additional environmental burdens were generated, irrespective of the nature of the lignin and hemicellulose-based products. This observation leads to a more general conclusion that suggests that strong reductions of molecular mass from biomass intermediates to products (e.g. glucose to ethanol or ethylene) should be avoided and that whenever possible the intrinsic chemical functions and features of the biomass intermediates should be exploited. This is exemplified by the good environmental performance of certain products, such as lignin-based resins or itaconic acid-based polyesters.

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The preservation and exploitation of the chemical attributes of the biomass intermediates is particularly crucial in the BIOCORE concept, because the energetic cost to manufacture products is high when compared to other biorefinery processes, which furnish less refined biomass fractions and do not necessarily employ biotechnological processes. The high energy demand is specifically caused by catalyst recycling for the organosolv pretreatment (formic and acetic acid) and the recovery of water soluble products from aqueous culture broths, which is often required when using biotechnological processes. To mitigate these negative effects, it is clear that further R&D should focus on the improvement of these two unit operations, seeking to discover alternative recycling and recovery methods, or ways to circumvent the need for such steps.

Beyond the results mentioned above, it is clear that a number of mitigation strategies can be implemented to reduce the environmental burdens of biorefining. However, to identify these with precision, it will be necessary to account for the specific interdependencies of local factors, this only being possible when actual industrial biorefineries are planned. Moreover, whenever a biorefinery is planned, the results obtained in BIOCORE underline the need for a precise appraisal of biomass availability, taking into account potential alternative uses of the biomass.

Regarding biomass availability and competition between uses, results obtained in BIOCORE indicate that in certain circumstances of rigorous optimization BIOCORE biorefineries could outperform biomass-based cogeneration (combined heat and power) units, which constitute the most environmentally-friendly use of biomass today. In the current context of increasing competition between uses of both biomass and land, it is clear that adequate policy instruments will be required to reduce environmental pressure, while securing the necessary stable framework for the growth of the biorefinery sector. To achieve this, it is proposed that land allocation planning should be implemented at national or European level and that this should be associated with binding measures to ensure that a common set of land and cultivation-related sustainability criteria are respected, irrespective of the final product (i.e. identical regulations are applied to food/feed, bio-based materials, chemicals, fuels and energy carriers). A first step towards this goal would be the freezing or gradual phasing out of product-specific measures, such as biofuel quota, in favour of support schemes that reward environmental benefits and sustainability whatever the product.

In summary, in terms of environmental sustainability, work in BIOCORE has shown that the biorefinery concept does hold the potential to deliver environmental benefits and that these could, in specific circumstances, be greater than those procured by current biomass-based processes that produce energy.

### **3.7.2 Economic assessment and market analysis**

The assessment of the economic sustainability of any industrial venture must necessarily provide answers to a series of interrelated questions related to the overall performance of the industrial process and the business model. In the case of biorefineries, these questions are further complicated by the current immaturity of the biorefinery sector, by market phenomena, such as green premiums, which constitute added market value accorded by consumers to some bio-based products, and by the

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fact that it is expected that BIOCORE biorefineries will co-manufacture several products that will occupy markets of very different volumes and revenues.

Regarding the immaturity of the biorefinery sector, this implies that CAPEX calculations are not straightforward, since process designs are relatively preliminary (i.e. advanced biorefinery designs are not yet industrially or financially proven), unlike those in the petrochemical sector. Moreover, the relative immaturity of the biorefinery sector increases the likelihood that first of a kind biorefineries will be unprofitable and will thus require subsidy schemes to support them. In BIOCORE, to circumvent the CAPEX calculation problem a shortcut method that correlates fixed capital investment to the total rated power of the process equipment was applied. Using this method, which was validated using some data describing known bio-based processes (e.g. a starch refinery), it was possible to reveal that BIOCORE processes (150 kt per annum capacity) will require a CAPEX in the range 120 to 160 M€, depending on the exact product portfolio and according to certain assumptions about integrated energy demand. Regarding OPEX, this is more variable lying between 80 and 150 M€ depending on the assumptions made. Overall, taking into account the total financial investment, the economic analysis revealed that very few of the product portfolios, judged under the most favourable conditions, would generate profitable scenarios and none can achieve a 25% internal rate of return. However, scoping for green premium opportunities revealed that some of the BIOCORE biorefinery products could benefit from extra added value (e.g. 10-20% for bio based intermediates and plastics), which will favour profitability. Moreover, results indicate that some of the BIOCORE product portfolios could become profitable either when supported by moderate subsidies (i.e. 20% output price support), lower than those currently accorded to biodiesel (on average about 45% in the EU) and bioethanol about (on average 45 and 60% respectively in the EU) or when CAPEX reductions are received (i.e. through public assistance schemes).

Finally, a study of the impact of scale clearly underlined the fact that BIOCORE biorefineries can be profitable at higher scale. For example, it was calculated that when operating with rice straw in India, a 150 kt plant would make losses of 10 M€, whereas a 500 kt plant would make profits of 40 M€.

Overall, the economic assessment provided clear indications that a biorefinery producing chemicals will be more profitable than an ethanol biorefinery and that certain products could benefit from significant green premiums. Moreover, when using an expensive technology such as organosolv, the study underlined the importance of converting lignin into added-value products rather than energy. Finally, the economic analysis provided compelling arguments in favour of a new subsidy policy for biobased products, which would provide subsidies for bio-based chemicals.

### **3.7.3 Social and policy assessment**

The third pillar of sustainability concerns social issues, thus in the framework of BIOCORE the assessment of these focuses on the impacts that biorefining will have on human society, in terms of poverty and the distribution of wealth, access to health care, education and housing. However, since it is expected that advanced concepts such as biorefining should do more than ensuring the bare minimum, other aspects such as employment, and equal opportunities, particularly regarding gender and political equality, need to be included. To achieve the aim of exploring, a variety of

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methods to measure social sustainability have been developed and applied, using regional-based case studies to contextualize the work and thus define boundaries.

For the European scenarios, two methods were developed (social impact assessment, or SIA, and social life cycle assessment, sLCA) and implemented, thus assessing a large number of social issues and themes. Overall, in terms of job creation and rural development, the advanced biorefinery concept proposed in BIOCORE holds potential to have positive impacts, without generating significant risks, for example, for gender equity or health. Nevertheless, the results did underline risks due to competition for biomass. In Germany for example, stakeholders expressed concern about the reduction in the availability of wood for domestic use in heating and in Hungary concerns were expressed regarding competition with an existing bioethanol production facility. However, the extent to which these concerns and others might affect a biomass producer's willingness to sell biomass to an advanced biorefinery was not ascertained and thus this would need to be achieved in the case of an actual industrial project.

In the state of Punjab in India, social impacts assessed using a multicriteria analysis, based on an analytic hierarchy procedure, revealed similar results to those obtained in Europe states, with the BIOCORE biorefinery concept being seen as a vector for the creation of new employment opportunities. Additionally, the implementation of biorefineries was considered by some stakeholders to be positive in terms of improving health, environmental and food security, although this was not a view shared by all. However, according to many stakeholders, factors that might not support the case of advanced biorefining in Punjab, include the current levels of research and development, and rural development within the state. Regarding the latter, concerns are linked to anticipated needs of biorefineries in terms of better infrastructures, including road systems and biomass storage facilities. These concerns were confirmed by agro-industrial operators already active in Punjab.

Finally, it is important to note that any future plans to build an advanced biorefinery according to the BIOCORE concept will require a thorough social impact assessment, taking into account of the whole value chain. Moreover, since the economic assessment indicates that such an industrial project will involve substantial financial investment, it will be advisable to apply the Equator Principles, which are internationally-recognized standards in terms of social and environmental impact assessment designed to assist in risk decision-making.

### **3.7.4 SWOT and biomass competition analyses**

The SWOT and biomass competition analyses provided useful insight into success and failure factors for BIOCORE biorefineries. This insight should be of use for many stakeholders, including policymakers, R&D strategic planners, companies and biomass producers (farmers etc).

Overall, the SWOT revealed that the BIOCORE biorefinery concept is well-perceived thought to hold high potential for rural development and resource security. Nevertheless, boundary conditions have to be designed carefully to ensure sustainability. Sustainable biomass supply is crucial for the success of biorefineries. The analysis of biomass availability and biomass competition came to the conclusion that there are currently unused biomass feedstocks available, both in Europe and in India. But, a lack of infrastructure and markets hinders the successful mobilisation of



available biomass potentials. A large share of the available (future) resources and land is situated in Eastern Europe, while most of the current or planned biorefinery sites in Europe are located in Western Europe, close to good infrastructures and existing chemical industries. Therefore, such geographic challenges might need to be faced in order to reconcile biomass supply and demand. To meet the European bioenergy targets, it is widely held that biomass imports from outside the EU will be required in future. In this respect, the local (i.e. in the EU) implementation of sustainability criteria reduces the amount of biomass actually available, compared to total theoretical biomass availability, and thus drives the requirement for biomass imports. The risk here is that the negative impacts of biomass production might be shifted to areas outside the EU, unless similar strict sustainability criteria were applied to the imported biomass. Overall, to secure sustainable biomass supply, it appears necessary to develop both reliable sustainability criteria and biomass allocation plans, which will ensure full biomass traceability and coordination between different biomass value chains respectively. Finally, the SWOT analysis revealed that it is vital to involve all stakeholders from the outset of an industrial biorefinery project in order to achieve acceptance and support. The findings highlight the importance of site and case-specific assessments, taking into account environmental, technological, economic and social aspects.

### **3.7.5 Integrated sustainability assessment**

The controversy surrounding the question of the net benefits of bioenergy compared to bio-based materials is derived from the fact that there is increasing awareness that the replacement of fossil resources by biomass is not sustainable *per se*. Therefore, to better address this debate, BIOCORE applied a multi-criteria sustainability assessment of the overall concept, comparing products from the BIOCORE biorefinery to conventional reference products, and BIOCORE processes with competing biomass-based systems, which will compete for both biomass and land. Finally, all sustainability aspects were integrated into an overall sustainability assessment, using multi-dimensional comparison metrics. For the final interpretation of the results, rather than using mathematical means (e.g. attribution of scores to impacts using weighting factors or a weighting algorithm) to provide a series of consolidated scores, it was decided to provide transparent analysis of the results, discussing the pros, cons and conflicts of all of the options under study. To prepare this discussion a method for structured comparison was developed and decision-making options were presented using multi-criteria analysis.

The key outcomes of the integrated assessment show that the complexity of the BIOCORE system, characterized by multiple process pathways and products, gives rise to a multitude of results when comparison with a conventional system is attempted. Specifically, the extent to which any given product portfolio can be considered sustainable depends very much on the nature of the products, the technology employed, the scale of the industrial operation and the biomass feedstock type. Hence, no general conclusion can be drawn for the BIOCORE concept as a whole, but only for its individual implementations. Nevertheless, some general comments are possible:

(i) when using lignocellulosic biomass, it appears vital to use all of the biomass components, converting them into value-added products. This observation confirms one of the fundamental suppositions that underpinned the BIOCORE concept.

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(ii) the choice of product is very important, since products that preserve the molecular mass and chemical functions of the biomass intermediates display the best environmental and economic performances

(iii) lignin should not be used for energy purposes.

(iv) in the case of the BIOCORE concept, close-to-optimum technical implementation is paramount, since under the most favorable conditions, environmental advantages and economic viability increase substantially. Moreover, process integration is crucial since the CIMV process yields considerable amounts of residual heat which is available to downstream processes. This means that biomass fractionation and downstream processes should take place at the same location.

However, the success of biorefineries is not just about resolving technical challenges. The SWOT and biomass competition analyses revealed that a major challenge is how to establish a supply of sustainable biomass in a near future characterized by the multiplication of biomass uses. To meet this challenge, a number of issues need to be addressed including the relations with local stakeholders, the development of appropriate infrastructures (e.g. transport routes, storage facilities etc.) and efficient policy / legal frameworks that will regulate biomass uses.

When the BIOCORE system was compared with other biomass-based systems, the issue of biomass competition becomes even more pertinent and crucial. However, putting this aside, and assuming that technical issues can be resolved in a satisfactory manner, the integrated analysis reveals that BIOCORE biorefineries hold the potential to outperform (from an environmental standpoint) any first generation biofuel biorefinery and even biomass-fired CHP plants. Therefore, with regard to the controversy mentioned above, the conclusions of the assessment plead in favor of new policy that would establish a level playing field for bio-based products, imposing harmonized performance criteria that should apply to all biomass uses.

### **Recommendations for policy makers**

1. Introduce active measures to manage increasing biomass and land use competition
2. Incorporate the notion of biorefineries and the wide needs of the bioeconomy in regional planning policies
3. Introduce mandatory sustainability criteria for all biomass, perhaps including animal feed
4. Create a level-playing field for bioenergy, biofuels and bio-based products
5. Revise the current RED policy framework, because currently it leads to misallocation of biomass and undesired effects (iRUC=indirect residue use change)
6. Provide financial assistance for the construction of advanced biorefineries (more attractive than pay-back via multiple counting schemes)
7. Consider the application of the Equator principles (World Bank) for high investment biorefinery ventures

## 4 Potential impact, the main dissemination activities and exploitation of results

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

### 4.1 Biomass production

The work performed in WP1, using a case study approach, has provided considerable insight into how BIOCORE biorefineries could perform in specific contexts. This work has provided several essential elements of a generic methodology that can be used to investigate the suitability of any given region with regard to the deployment of advanced biorefineries. In this respect, the methodology constitutes a useful contribution that will hopefully be adopted in the future as a fully-fledged decision-making tool to examine advanced biorefinery business cases.

Regarding the conclusions of the work performed in WP1, several impacts are anticipated. First, the findings of work on biomass availability support previous findings concerning both Europe and India. For Europe, the data produced in BIOCORE clearly show that there is some potential to develop biomass-based industrial activities. Nevertheless, the data also reveal that biomass availability is subject to many conflicting factors that will need to be carefully accounted for in future biorefinery projects, and in strategic planning at member state and EU level. Regarding India, the data from BIOCORE reveal a considerable biomass potential and support the widely held view that rice straw burning can and must be phased out in favour of sustainable industrial activities that hold the potential to create jobs and new wealth in rural India.

Leading on from biomass availability, the case studies clearly revealed the advantages of feedstock-flexible biorefineries, especially in the more biomass-constrained European context. This information should impact on future developments and serve to orientate EU support for biorefinery projects.

Concerning territorial planning, the results from the BIOCORE case studies serve to underline the importance of consistent, coordinated territorial development, and in this respect converge with the notion of regional smart specialization that is currently propounded by the European Commission. Specifically, the case studies underlined the importance of well-planned transport networks and the creation of storage facilities for advanced biorefineries. Moreover, directly or indirectly, the case studies further support the notion that Europe needs consistent policy, both with regard to biomass use, providing a level playing field for all biobased products, and with regard to land use, providing a more integrated view of land use (including urbanization and alternative farming methods that affect yield).

Finally, the case studies also provided interesting feedback from stakeholders and thus provided information that will be useful for future biorefinery planning. In particular, the need for early consultation and implication of all stakeholders, including the local populations and the biomass producers was made apparent.

To correctly disseminate the useful information produced by WP1, and thus to amplify the different potential impacts of the conclusions of the study, a 27-page summary document has been prepared for wide dissemination. This non-technical report has been disseminated to a wide stakeholder community including entrepreneurs and policymakers and was presented at a major event, organized in Brussels on the 11<sup>th</sup> February 2014. It is also downloadable at the following address:

#### ***4.2 Biomass fractionation and enzymatic and physico-chemical fraction refinement***

Activities in WP2 have provided a series of results and developments that we anticipate will have impacts either in the short term, or in the mid/longterm.

Regarding immediate impacts, a major part of the work performed in WP2 has already impacted on the business development of the upfront technology provider, CIMV S.A. As a SME, CIMV S.A. has greatly benefitted from the R&D activities that characterized work WP2 and has allowed this company to move its technology through TRLs 5 and 6, making it ready for immediate industrial demonstration (TRL 7-8). In particular, work on biomass pre-processing (i.e. size reduction) has provided CIMV with the opportunity to extend its knowledge of biomass handling to woody biomass, notably short rotation coppice poplar and to rice straw, which is not so readily available to a European-based company such as CIMV. Moreover, regarding the actual CIMV organosolv process, WP2 activities have provided the framework to extend the process and demonstrate the feedstock flexibility of the technology. This is a vital point, because feedstock flexibility will be an extremely strong argument in CIMV's business case, and should help to persuade investors that CIMV will be able to secure a stable supply of biomass on a long-term basis (10-20 years).

Regarding CIMV's process, WP2 has also provided the framework for collaboration between Arkema and CIMV, which was focused on the H<sub>2</sub>O<sub>2</sub>-bleaching step. The successful outcome of this work has provided the means to reduce process costs (a direct benefit for CIMV) and to provide a competitive product and customer-beneficial knowhow (a direct benefit for Arkema as a provider of industrial grade H<sub>2</sub>O<sub>2</sub>).

Apart from focus on CIMV's organosolv technology, WP2 has also provided the framework for progress on ECN's organosolv technology. In particular, BIOCORE has provide the necessary resources and impetus to extend the feedstock base of the water/ethanol process, and even to assess softwood pulping. Significantly, work in WP2 has allowed ECN to develop a pre-extraction process for which a patent application has been filed. This development could provide ECN with the necessary cutting-edge to develop their process further to pilot scale, or alternatively offer ECN the possibility to contribute to the development of similar processes that are currently under study in Europe (e.g. the lignocellulosics biorefinery plant in Fraunhofer Center for Chemical-Biotechnological Processes CBP, Leuna).

Another area in which WP2 is likely to have significant impact concerns the work performed on the HRS arising from the CIMV process. Work in WP2 involved extensive analytics, which amongst other things, led to a complete mass balance study of the CIMV that has been published in the journal *Bioresource Technology*

(Snelders et al. 2014. doi: 10.1016/j.biortech.2014.01.069. Biorefining of wheat straw using an acetic and formic acid based organosolv fractionation process). Furthermore, the work provided a much more comprehensive chemical compositional analysis of the HRS, revealing information that will be vital for the further processing of this major biorefinery stream. Similarly, the intensive effort that was focused on the refining of the HRS allowed CIMV to make considerable progress, developing a process to hydrolyse and purify the HRS that will no doubt be a determining factor for the future development of the whole CIMV process.

In WP2, DSM in collaboration with CIMV also laid the technical foundations for successful pilot trials on cellulose pulp that were performed in WP6. The impact of these is discussed in WP6.

Finally, WP2 also provided some key results concerning the use of process waste streams to make commercial products. The development of fully sustainable advanced biorefineries depends on the development of smart processes that minimize or even eliminate waste streams *per se*. Therefore, the demonstration of how two major process waste streams arising from the CIMV and ECN processes can be dealt with should impact on the future development of closed-loop biorefineries.

### **4.3 White biotechnologies for product manufacture**

Work performed in WP3 has provided a series of results that demonstrate that biotechnology can provide efficient and sustainable production processes for bulk and higher-added value products. The most promising products in terms of microbial productivity were bioethanol, itaconic acid, xylitol and xylonic acid. For each of these products, microbial conversions provided industrially relevant titers, yields and production rates and the work procured microbial strains that are now ready for further process optimization (TRL 5-7) towards industrial production.

Concerning the bioconversion of the glucose syrup obtained from cellulose, the highly encouraging results obtained in WP3 allowed pilot scale trials (TRL4-5) to be performed in WP6 (discussed) below. Unfortunately, this was not the case for the hemicellulose or C5-rich stream that is obtained from the CIMV organosolv process, because the purity of this fraction was insufficient. Nevertheless, in terms of biotechnology, the work performed in BIOCORE by VTT provided compelling proof that once suitable C5 hydrolysates become available at an industrial scale it will be possible to efficiently produce xylitol and xylonic acid using different yeasts species, such as *Pichia sp.* Moreover, results obtained in BIOCORE clearly indicate that the yeast strains studied are amenable to engineering, in particular to increase their tolerance to feedstock impurities, thus it is foreseeable in the near future that when combined with further efforts to purify the CIMV C5 hydrolysate it will be possible to ferment this feedstock and produce xylitol or xylonic acid.

Although slightly less mature at the end of BIOCORE than the examples mentioned above, work performed on the engineering of *Clostridium acetobutylicum* reached TRL3, providing a strain that can produce in a rather efficient way a mixture of isopropanol, butanol, ethanol (IBE). This is an interesting result, because C.



*acetobutylicum* forms the basis of the ABE (acetone-butanol-ethanol) process, which was operated at industrial scale for several decades. Therefore, assuming that productivity could be further improved, possibly by using the *in situ* recovery methods developed during BIOCORE to ensure continuous removal of the fermentation products, it is foreseeable that this strain could be used in a revamped industrial process. In this respect it is noteworthy that the British company, Green Biologics Ltd. is currently driving the industrial revival of the ABE process and is opening production facilities in China and the USA.

Finally, other work on the production of glucaric acid by fermentation, and alkyl polypentosides by enzymatic processes made less progress during the BIOCORE project, although some encouraging results were obtained, especially regarding glucaric acid. Nevertheless, in the short/medium term more R&D is likely to bring these processes to a more mature state (TRL 3), thus allowing their proper evaluation.

In summary, BIOCORE has supplied some biotechnology processes that reach TRL 4 and others that have reached 6, and data that open pathways towards further technology development for many of the processes that were selected for study. In particular, it is noteworthy that some of the biotechnology processes that have been developed only require suitable feedstocks (i.e. sufficiently pure C5 hydrolysates) in order to be further tested and developed.

#### **4.4 Chemical and thermochemical transformations**

Work performed in WP4 has provided a number of potentially high impact innovations whose levels of development have reached TRL 5 and that could provide the basis for commercial products.

It is widely recognized that, compared to most other biomass pretreatment technologies, organosolv technologies furnish lignins of good technical quality that display high potential for commercial use. In WP4, several concrete demonstrations of this potential were provided, notably showing how relatively simple, low intensity lignin refining processes can be used to transform lignins produced by a biorefinery unit into chemical reagents that can effectively substitute for phenol in phenol-formaldehyde (PF) resins and polyurethane (PU) coating formulations. In the case of PF resins, these provide the basis for the manufacture of environmentally-friendly wood panels that display 70% less fossil phenol content and emit formaldehyde at values meeting the CARB II rule limits. Similarly, the superior properties of the lignin-based PU formulation, which has been patented, provides the basis for the development of a new coating that might be well-adapted for use in electrical appliances. Together these two innovations provide strong evidence that the use of organosolv technology can provide commercially-valuable lignins that will penetrate into million tonne-sized markets and thus contradict the well-known adage “you can make everything with lignins, except money”. Other encouraging demonstrations of how lignin can be converted into valuable chemicals were provided using chemical or thermochemical processes that procured, for example, liquid polyols that can be incorporated into PU rigid foams, thus providing the possibility to make more environmentally-sustainable building (insulation) materials, and bio-oils and biochar, which can be used a valuable source of biobased aromatics, and in materials and fertilizer respectively. To appropriately promote all of the lignin-based innovations,

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BIOCORE partners have used a variety of strategies, including patenting, the production of demo-products, publications. Additionally, a technical note on lignins and their applications was written, professionally-edited and disseminated to a large group of industrial stakeholders. This note has also been distributed at several events.

Regarding WP4 work on biomass-derived carbohydrates, much of the work is also expected to have impact at different levels. The direct catalytic conversion of cellulose pulp into polyols and heterocyclic diols constitutes an elegant demonstration of how biomass can be made into useful products and underlined the advantages obtained when using CIMV organosolv pulp. This, now published, work has provided a follow-up breakthrough, which demonstrates how cellulose pulp can be directly converted into alkanes. This innovation is currently undergoing appraisal to determine patentability. Other innovations concern the production of a pentose-based substitute for the fossil-based isocyanates, which are used in the production of PU resins, and sugar-based plasticizer for PVC. The associated markets are worth billions of euros, so the economic potential for replacement compounds is high. Moreover, in the case of phthalates, some conventional compounds such as Di-2-ethylhexyl phthalate are currently being phased out under REACH regulations. Therefore, the development of new, potentially less toxic, phthalates is timely.

Finally, early work on the production of furfural from CIMV's HRS has produced promising results at TRL 3 that should give rise to follow-up pilot scale trials (TRL 5 and 6).

### ***4.5 Designing and modelling biorefinery processes***

The work performed in WP5 fulfilled critical support and exploration roles, providing formalized process descriptions and flowsheets, as well as pioneering systems approaches to account for the complexity of integrated biorefineries. Regarding the latter, the approach managed to combine state-of-the-art methods into a concrete framework that will be applicable to generic problems. Using this approach, it has been possible to identify profitable manufacturing pathways that offer significant savings in energy and water, and to further explore process-to-process integration, producing a significant number of flowsheets.

An early impact of the process design work was the revision of the CIMV process. This revision has already provided CIMV S.A. with a technically-better process that increases the overall attractiveness of the company's technology.

In terms of the methods developed, systematization offers the ability to perform high-throughput analyses and is easily extendable to include new chemistries and engineering developments. Therefore, it is expected that this methodology will not only be useful for the further development of the BIOCORE concept, but also applicable to other advanced biorefinery concepts. Indeed, this is already the case for an algo-biorefinery which is now being studied using the design methods developed in BIOCORE.

As outlined previously, Total site analysis work in WP5 has revealed the considerable advantages in terms of energy savings that can be gained from the co-location of the

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biomass refining process and the product manufacture processes. This result is particularly important, and is likely to have considerable impact on the further development of advanced biorefineries, such as the one described in the BIOCORE concept, because it strongly argues in favour of certain types of business development, including joint ventures with complementary technology providers.

As well as producing a series of design and decision making tools, WP5 has produced a substantial amount of detailed flowsheets (about 80-90 processes) that will be useful for future research and training. One way to use this data could be to create an open access database that would be designed to motivate further studies in the area. Examples of future studies include the development of shortcut models for process costing (CAPEX and OPEX) or for performing fast LCA. As remarked in the economic analysis performed in BIOCORE, relatively accurate, short-cut costing models, adapted to the specific case of biorefineries, are currently lacking.

Finally, regarding education, the material produced in WP5 has already been used within the framework of the bio refinery schools initiated by the BIOCORE consortium and two other EU programmes. Similarly, this material will continue to be used to support lectures in future biorefinery schools (e.g. the upcoming school organized by Climate KIC in Budapest). Moreover, it is noteworthy that so far 12 diploma dissertations making use of material produced in WP5 have been submitted. Finally, work in BIOCORE has clearly pinpointed some of the limitations of currently available design methods when applied to biorefinery design. To address this issue Marie Curie ITN network has been set up. The role of this network is to deliver next generation PSE tools dedicated to integrated biorefineries. The ITN network will train new engineers and biorefinery practitioners using the BIOCORE cases as reference problems and case studies.

### ***4.6 Pilot and industrial pilot scale demonstration***

In WP6 several of the more promising technologies that were developed in BIOCORE were tested at pilot scale. In each case, the pilot work involved the use of the biomass refining technology, operated by CIMV at its pilot facility, coupled to a conversion technology. In most cases the work that was performed reached a TRL of 5/6 and provided precious information on the manufacturing processes and on the characteristics of potential products. In this respect, work performed in WP6 was pioneering and will have strong impact on how the technologies developed in BIOCORE will be further pursued.

Work on the production of products from cellulose was highly successful and constitutes milestones in the development of 2<sup>nd</sup> generation technologies. Notably, this work provided DSM with the opportunity to further validate its thermostable cellulase mixture and its proprietary yeast technology, both of which are important elements in DSM's 2<sup>nd</sup> generation ethanol development program, which is now reaching fruition with the anticipated startup in 2014 of the POET-DSM Advanced Biofuels LLC plant in Emmetsburg, Iowa (USA). Moreover, the successful completion of the pilot trials constituted precious operational experience for the pilot facilities of the recently created BE-Basic Foundation, which is an international public-private partnership that develops sustainable industrial biobased solutions. Finally, these pilot trials provided CIMV S.A. with a first opportunity to demonstrate the power and near-industrial readiness of its technology.

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Regarding the conversion of chemical intermediates (ethanol and itaconic acid) into final products of the cellulose-based pilot trials, this provided valuable insight into future commercial options. Specifically, the attempted conversion of the 2<sup>nd</sup> generation ethanol into PVC allowed KEM ONE to better understand the pitfalls of this process and to take a decision about whether to pursue R&D in this area. Likewise, the production of an alkyd coating using itaconic acid allowed DSM to explore the potential of this molecule in a concrete market application. In this respect, it is important to note that alkyd paints represent a 450,000-tonne market in Europe. Although the use of alkyd paints in Europe is tending to diminish because of environmental concerns linked to the use of non-aqueous solvents, alkyd coatings remain preferred paint options for certain applications, since they display high glossiness and hardness. Therefore, the development of increasingly biobased alkyd paints (they already contain vegetable oils) is one way to reduce the environmental impact of the final market product. In this respect, it is also noteworthy that the use of itaconic acid to prepare the alkyd paints constitutes an alternative to the use of phthalic anhydrides, which are known to cause a range of common respiratory afflictions, such as asthma, bronchitis and rhinitis. Finally, with a current market price of approximately €1600 per tonne and a predicted world market of 400 kt in 2020, itaconic acid is an attractive biorefinery product. Therefore, the convincing demonstration in BIOCORE of its production is an extremely positive argument for the development of advanced biorefining.

The highly successful nature of the pilot scale trials performed using lignin and its derivatives is also likely to have significant impact on the future development of advanced biorefineries. Indeed, in BIOCORE it has been possible to supply almost unique insight into how lignin can be used for purposes other than heat and power production. The production of plywood panels using lignin-based wood adhesive is powerful argument, since this represents a potentially large market for Biolignin<sup>TM</sup> (approximately 8 million m<sup>3</sup> of panels in Europe alone), consuming several million tonnes of petrochemical-based phenol per year (>10 million tonnes). Assuming that lignin can replace 50% of phenol in wood adhesives, one can deduce that this application could absorb perhaps 1 Mt Biolignin<sup>TM</sup> worldwide, which is approximately equivalent to the combined capacity of 40-50 advanced BIOCORE biorefineries, each converting 150 kt per year of dry biomass into products. If this estimation proves to be accurate, it would provide a strong basis on which to build a bankable business case, especially because the results obtained in BIOCORE also clearly indicated that CIMV's Biolignins<sup>TM</sup> can also be used to make polyurethane elastomer coatings and PU rigid foams. Although according to BIOCORE's partner SYNPO, the market volume for the former would be quite small, consuming only a few thousand tonnes of lignin on an annual basis, the PU foam market is larger at approximately 4 Mt and could thus consume over 300 kt of Biolignin<sup>TM</sup> (assuming that the lignin-based liquid polyol represents approximately 25% of the foam formulation), which represents the capacity of a further 4 industrial biorefineries.

Overall, the outcomes of the pilot studies performed in WP6 provide a solid foundation for a business case that, in the medium term, could lead to the launch of a profitable biorefinery industry based on the BIOCORE concept and CIMV biomass pretreatment technology. Although, the cellulose-based applications are common to many 2<sup>nd</sup> generation biorefinery concepts, so far there is no evidence that alternative biomass pretreatment technologies, such as steam explosion, can easily provide



lignins suitable for the PF resin and PU foams sectors. In this respect, it is possible that the BIOCORE concept could thrive in a competitive 2<sup>nd</sup> generation market.

#### **4.7 Integrated assessment of overall sustainability**

Very importantly, the overall sustainability assessment revealed that a BIOCORE biorefinery could deliver a wide range of solutions, some of which display high potential for sustainability, while others display very poor potential. This analysis also underlined the fact that sustainability in the BIOCORE concept will depend strongly on the choice of product portfolio, on the conditions of implementation and on a certain number of external factors. Significantly for future debate and decision making, the integrated assessment clearly identified several biorefinery configurations that would be likely to deliver more benefits (measured according to the multiple sustainability criteria) than competing conventional product value chains and competing biomass use options. Remarkably, the LCA and the economic assessment resulted in very similar trends.

The implications of the results generated in WP7 are as wide-ranging as the results themselves. However, some findings will be very valuable in order to clarify some of the most controversial issues surrounding biorefining. One of these concerns the use of biomass for bioenergy purposes. Although it has been often assumed that bioenergy is intrinsically sustainable because it uses renewable biomass as its feedstock, this is now contested and challenged by the idea that biomass should be used for other more sustainable purposes. In BIOCORE, the multi-criteria sustainability assessment of the overall concept and the final integration of the results provides insight into this question and some guidelines on how to go forward.

First, in comparison with conventional products and systems (most often fossil-based ones), although no general conclusions can be drawn, it is clear that when produced in an optimal way, some products (e.g. chemicals such as xylitol, or polymer based on the use of itaconic acid or lignins) can deliver environmental advantages and economic viability, while being beneficial for society. Beyond this, a key lesson that can be drawn from BIOCORE results is that, at least in the framework of the BIOCORE concept, the production of molecules that imply significant reductions in molar mass during the conversion of the biomass intermediates into products should be avoided, since these will provide less favorable results in terms of sustainability. In other terms, it is highly advisable to preserve the chemical structures and functions of the biomass intermediates.

Second, comparing the BIOCORE system with other biomass-based systems, it is apparent that certain BIOCORE implementation scenarios could outcompete both 1<sup>st</sup> generation biofuel biorefineries and even biomass-fired heat and power facilities. This information is useful, because it implies that indeed when appropriate conditions are met, the production of bio-based chemicals that can be used to manufacture polymers and materials will provide more sustainable uses for biomass than energy production, irrespective of whether this is energy for transport or stationary applications.

Overall considering the findings of the integrated sustainability assessment performed in BIOCORE, which includes the observation that biomass availability is likely to become ever more constrained in Europe over the next decade, the work



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performed in WP7 provides a certain number of conclusions that give rise to guidelines that can be useful for future policy and strategic planning.

### **Recommendations for policy makers**

1. Introduce active measures to manage increasing biomass and land use competition
2. Incorporate the notion of biorefineries and the wide needs of the bioeconomy in regional planning policies
3. Introduce mandatory sustainability criteria for all biomass, perhaps including animal feed
4. Create a level-playing field for bioenergy, biofuels and bio-based products
5. Revise the current RED policy framework, because currently it leads to misallocation of biomass and undesired effects (iRUC=indirect residue use change)
6. Provide financial assistance for the construction of advanced biorefineries (more attractive than pay-back via multiple counting schemes)
7. Consider the application of the Equator principles (World Bank) for high investment biorefinery ventures

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