In BIOCORE, VTT (Finland) are investigating the production of xylitol using yeast. To this end, they have developed high-performing yeast strains that convert xylose present in biorefinery hydrolysates into xylitol in a single bioconversion operation. More than 100 g L^{-1} xylitol have been produced at high rate and yield (0.7-0.8 g g^{-1}) from the BIOCORE pentose-rich stream, even though this contains high concentrations of formic acid. Higher rates and titers are expected in less inhibitory hydrolysates, but are dependent on the xylose concentration available. Residual glucose in pentose-rich stream provides energy for the yeast, which also removes other components from the hydrolysate, thus facilitating the downstream processing.

Xylonic acid

Xylonic acid is a five-carbon sugar acid that naturally occurs in some foodstuffs. In industrial foods xylonic acid could be used as a replacement for gluconic acid, thus acting as a latent acid in bakery products, an acidulant in meat products, or as a milk coagulant in cheese curd formation. Xylonic acid has also been shown to be an excellent substitute for gluconic acid in cement formulations, acting as a cement retardant, providing better control of the setting time. Interestingly, the setting time of cement containing xylonic acid is two-fold shorter than that of cement containing gluconic acid, underlining the specific character of xylonic acid. Regarding other applications, xylonic acid also figures in the top 30 building block chemicals from biomass, described by the US National Renewable Energy Laboratory, and has been used as a precursor to synthesize 1,2,4-butanetriol. Moreover, the use of xylonic acid as precursor for the synthesis of polyamides has been published. Nevertheless, despite the potential of xylonic acid, only very limited commercial production appears to exist at this time.

In BIOCORE, scientists in VTT (Finland) have developed a yeast strain for the production of high concentrations of xylonic acid at low pH. More than 140 g L^{-1} have been produced at pH 3, at a rate of ~1 g L^{-1} h^{-1}. Higher yields, titers and rates will no doubt be achieved after further process optimization. This strain also performs well at higher pH, at which even 170 g L^{-1} have been produced. The host strain has an excellent tolerance to most biomass hydrolysate inhibitors.

CONCLUSION

The development of biorefineries using advanced fractionation technologies, such as organosolv, to process lignocellulosic biomass holds the potential to open up new valorization routes for pentose sugars. While significant R&D is still required to develop cost-efficient downstream processing of the pentose-rich syrup produced by CIMV, research performed by BIOCORE researchers is revealing how pure pentose sugars can be used to manufacture useful products.

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SEVENTH FRAMEWORK PROGRAMME

biocore

A biorefinery concept for the transformation of biomass into 2nd generation fuels and polymers

TECHNICAL NOTE ON PENTOSE SUGARS AND THEIR APPLICATIONS

http://www.biocore-europe.org

EDITORIAL NOTE

The European FP7 project BIOCORE focuses on a lignocellulosic biorefinery concept, which includes deriving value from hitherto underused components, such as pentose sugars. To achieve this, BIOCORE partners are devising a range of methods aimed at extracting and converting pentose sugars into useful products. In this technical note, some of BIOCORE's results are related in order to better visualize how pentose-based products will form part of tomorrow's bio-based commercial products.

BIOREFINING AND PENTOSES

Driven by the glucose to ethanol challenge, the extraction and deconstruction of cellulose have been the focus of much research over the last few decades. However, economically-important crops, such as cereals, non-food crops and hardwoods, also contain up to 30% dry weight of hemicelluloses, which are mainly arabinoxylans composed of D-xylose and L-arabinose, or pentose sugars. Current uses for these sugars are limited, partly due to the fact that high purity D-xylose and L-arabinose are not yet produced as commodity chemicals. However, their future use as platform intermediates will be necessary in order to ensure the sustainability and economic viability of lignocellulosic biomass value chains and to avoid excessive use of D-glucose.

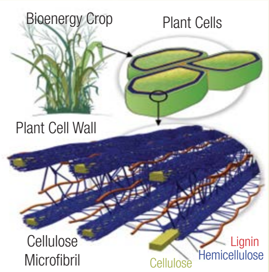

Fig. 1 – Plant cell wall utilization of lignin, hemicellulose and cellulose. © Elsevier - License Number: 3012501219608.

Biorefining in BIOCORE

In BIOCORE, CIMV S.A. (Levallois Perret, France) provides the biomass fractionation technology, which uses a mixture of formic and acetic acid to breakdown the biomass into its component parts, cellulose, lignins and pentose sugars from feedstocks, such as wheat straw, birchwood or poplar. The pentose sugars are obtained in an impure liquid stream that also contains glucose, proteins, phenolic compounds, minerals and acids from the extraction process. It is this biorefinery stream that BIOCORE researchers are using as a raw material to imagine new technologies and products.

Fig. 2 – From left to right: cellulose, powder lignin, wheat straw, C5 syrup (hemicellulose) © CIMV.

PENTOSES TODAY

Pentose sugars are primarily obtained from woody biomass and various crop residues. However, despite their humble origins, the current market value of these two sugars is high (98% pure D-xylose sells for 2000 - 3000 € per ton), mainly because the production of these sugars is costly, and also because xylose is mostly converted, in an integrated way, to xylitol (see below). Arabinose is classified as a rare sugar that is only produced in small amounts, being used as a chemical for specialty applications and as a food ingredient in Japan. Regarding xylose, this sugar can be produced from black liquor, a byproduct of dissolving pulp manufacture. A major European producer is Danisco sweeteners (Lenzing, Austria), a company that sources xylose from black liquor arising from bisulfite pulping of beechwood. In Asia, xylose is made from feedstocks, such as coconut husks. China boasts many xylose/xylitol producing facilities, although recently several of these have closed down due to the application of increasingly tight environmental regulations.

Analysts predict that the xylitol market will undergo significant growth once sufficient quantities of industrial grade, cost competitive xylose (>98% pure) become available. Therefore, the main challenge is to lower the cost of xylose and arabinose extraction and purification.

USES OF D-XYLOSE FOR THE MANUFACTURE OF INDUSTRIAL PRODUCTS

C5 ethanol

Several decades of research have been devoted to the development of microbial strains *S. cerevisiae* that can produce ethanol using D-xylose. The engineering strategies aim to drive D-xylose into the pentose phosphate pathway (PPP) through its conversion into the ketose derivative, D-xylulose. Once phosphorylated, D-xylulose-5-phosphate can enter the central metabolism of *S. cerevisae* via the pentose phosphate pathway. One way to convert D-xylose into D-xylulose is to use a D-xylose isomerase.

In BIOCORE, DSM has further developed the xylose isomerase technology, generating a yeast that is capable of simultaneously converting both C6 and C5 sugars to ethanol. In this manner, ethanol production using hydrolysates of cellulose pulp supplied by CIMV could be boosted by at least 8% when C5 sugars arising from the CIMV process were added to the glucose-rich hydrolysate.

Isopropanol

Other industrial microorganisms also possess the ability to consume pentose sugars. One fine example is *Clostridium acetobutylicu*m, an anaerobic bacterium that for over half a century formed the basis of the industrial ABE (acetone-butanol-ethanol) process, which primarily manufactured acetone, producing butanol and ethanol as co-products. This process was abandoned after 1945, only because of the availability of cheaper acetone made from petroleum resources.

In BIOCORE, INRA-affiliated researchers have been working on the engineering of *C. acetobutylicum* to create a microorganism capable of producing isopropanol, one the World's most widely used industrial solvents and a potential future precursor for the production of propylene (via a dehydration step), a petroleum-derivative that is actually currently used to manufacture isopropanol. In BIOCORE, researchers have engineered a strain that can produce up 21 g L^{-1} of the IBE solvent mixture (*i.e.* replacing acetone by isopropanol) and work is currently underway to achieve the second aim, which is to produce isopropanol as a sole product, disrupting the metabolic steps that lead to the formation of butanol and ethanol.

Furfural

Acid dehydration of D-xylose and L-arabinose leads to the formation of furfural, an industrial chemical that was first produced by the Quaker Oats Co. in Cedar Rapids, Iowa. As a chemical, furfural is used directly as a solvent or as an intermediate for the production of tetrahydrofuran, an important industrial solvent, or furfuryl alcohol, a chemical that is principally used to manufacture resins for bonding foundry sands that compose foundry molds. Currently furfural is made directly from agricultural raw materials in a continuous process that employs sulphuric acid. However, it is expected that advanced biorefining will generate pentose-rich hydrolysates that will constitute new raw materials for this industry. Today, about 90 percent of furfural production capacity is present in just three countries, China, the biggest producer, South Africa and the Dominican Republic, with most furfural being converted into furfuryl alcohol.

In BIOCORE, scientists at DLO are aiming to use furfural to prepare a biobased diisocyanate for the formulation of polyurethanes, which can be used in coating and foam applications. To achieve this, DLO scientists submit the pentose-rich stream from the CIMV process to a thermal conversion process in which the organic acids already present in the pentose syrup act as catalysts for the formation of furfural (Figure 3), thus obviating the need for the addition of mineral acids. So far, results reveal that the pentoses (primarily xylose mono- and oligomers) are effectively converted to furfural, with a yield greater than 50% and a selectivity of 80% , which is better than commercial processes. Having achieved this result, DLO scientists are now studying how to best isolate furfural from the reaction medium.

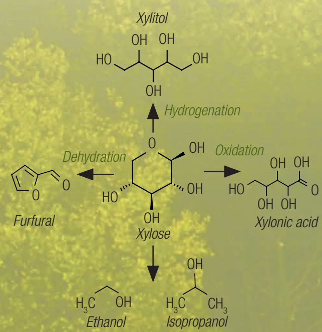

Fig. 3 — Conversion of xylose into commercially-relevant products

Xylitol

Xylitol is a five-carbon sugar alcohol that is best known for its sweetening capacity, which exceeds that of sucrose. As such, it is an excellent artificial sweetener that is now used widely by the confectionary industry, being responsible for the cold, fresh sensation of certain chewing gums for example. Xylitol is also used for caries prevention. However, xylitol is also increasingly used in other industrial sectors, for example in pharmaceutical and cosmetic products such as toothpaste, fluoride tablets and mouthwashes and has been tested as a co-monomer with terephthalatic or sebacic acid for polyester production. The current global demand for xylitol is approximately 160,000 tons per annum, a market that is undergoing a yearly growth rate of about 5%. Most of the current xylitol production is based in China and the world price is situated in the price range €5,000-6,000 per ton. Xylitol is currently manufactured in a 3-step process that involves the catalytic hydrogenation of D-xylose (or xylose-rich hydrolysates) using a Raney nickel catalyst. Advantageously, this catalyst is cheap and displays quite good activity and selectivity, but is nevertheless prone to fast deactivation, due to poisoning, and can result in nickel being present in the xylitol product.