

As easy as A-B-E?

**ACETONE-BUTANOL-ETHANOL FERMENTATION:
AN OPTIMISED PROCESS FOR SUSTAINABLE FUELS
AND BIO-BASED PRODUCTS**



BUTANEXT
Next Generation Biobutanol

The ButaNext project optimised the ABE-fermentation process to convert sustainable renewable feedstocks into cost-competitive biobutanol.

Why?

- > The European **bioeconomy** has great potential, see pages 1-3.
- > **Biobutanol** is considered an exciting alternative to first generation biofuels and can be used in the production of a range of bio-based products, see pages 4-5 and 16-17.

How?

- > By optimising the **Acetone-Butanol-Ethanol (ABE) fermentation process**, see pages 4-5.
 - > Sourcing **sustainable feedstock**, see pages 6-7.
 - > Innovative two-stage **biomass pre-treatment** and tailor made **enzyme cocktails** convert sugars at higher yield with lower energy use and costs, see pages 8-9.
 - > High productivity fermentation process combining **novel microbial strains** with ***in situ* product recovery**, see pages 10-11.
 - > **Integrating** the technologies and **upscaling** the process to pilot scale, see pages 12-13.
- > The improved process was **validated** by a range of assessments, see pages 14-15.

A SUSTAINABLE, ADVANCED BIOECONOMY FOR EUROPE

The transition towards a **bioeconomy** is seen as key to combating climate change. Making products and energy from renewable, local bio-resources instead of polluting, finite fossil fuels will help reduce dependence on imported oil, and stimulate jobs and growth in Europe.

But biomass availability is limited. And the use of certain feedstocks, such as arable crops, has raised concerns around their competition with food production and the greenhouse gas impacts of indirect land use change.

Attention is therefore turning to more sustainable feedstocks such as agricultural residues, biological waste and fast growing energy crops, which don't compete with food production or contribute to deforestation.

Transforming these **sustainable feedstocks** into high value products is technically challenging. Research and innovation is being undertaken to develop **improved production processes** that are cheaper, more efficient and environmentally friendly. Europe is leading the way through programmes such as Horizon 2020.¹



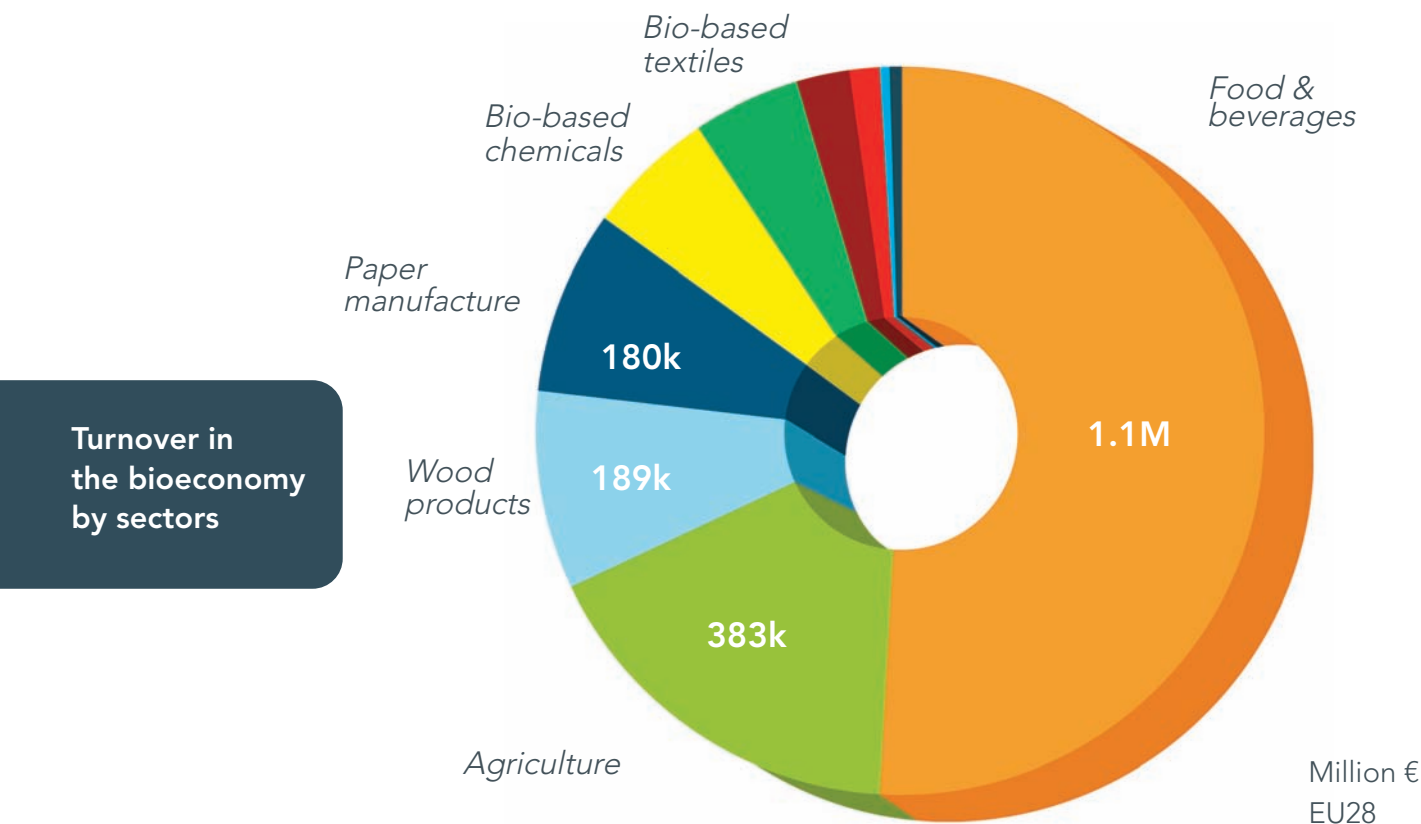
What is the bioeconomy?

The bioeconomy encompasses the production of renewable biological resources and the conversion of these resources and waste streams into value-added products, such as food, feed, bio-based products and bioenergy.

¹ Horizon 2020 is the biggest EU Research and Innovation programme ever with nearly €80 billion of funding available over 7 years (2014 to 2020) – in addition to the private investment that this money will attract. It promises more breakthroughs, discoveries and world-firsts by taking great ideas from the lab to the market.

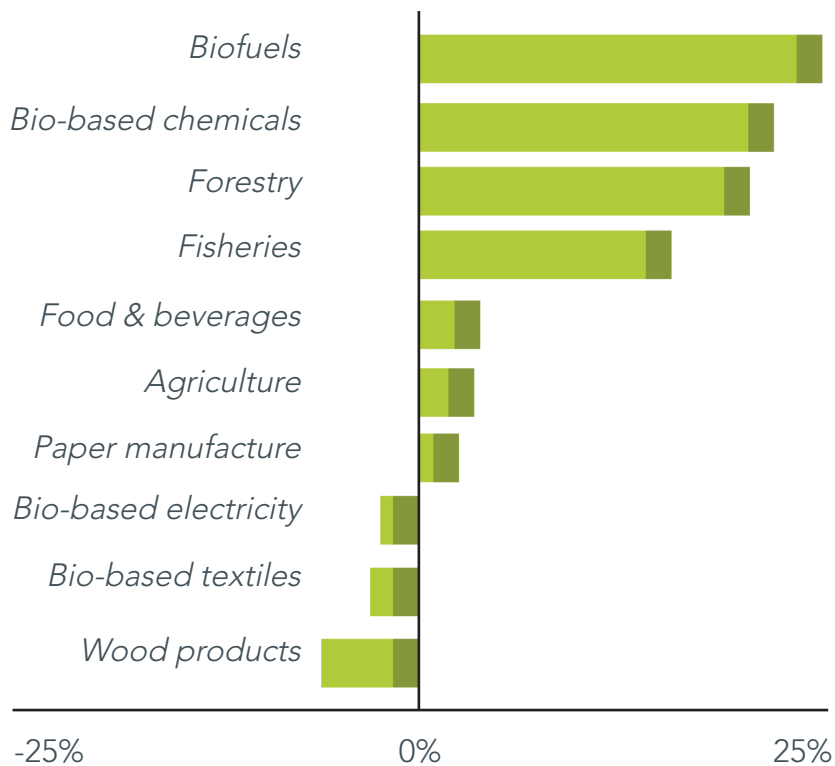


EUROPEAN BIOECONOMY IN NUMBERS



Roughly three quarters of the turnover in the European Bioeconomy is accounted for by the food and beverages sector, and by agriculture and forestry. The other quarter is created by the so-called **bio-based industries** (such as chemicals and plastics, pharmaceuticals, pulp and paper products, forest based industries, textile sector, biofuels and bioenergy). These bio-based industries are responsible for most of the growth experienced by the EU bioeconomy in the last decade.

All data 2014. Source: Eurostat



Turnover growth
in the bioeconomy
by sectors

2008-2014
EU28



18.6 M

NUMBER OF PEOPLE EMPLOYED



€ 2,200

TURNOVER (BILLION €)

🔹 REVIVING AN OLD PROCESS

Fermentation is a conversion method that can be used to turn biomass into valuable products.

Acetone–butanol–ethanol (ABE) fermentation uses bacteria to produce acetone, butanol, and ethanol from carbohydrates such as starch and glucose. The process may be likened to how yeast ferments sugars to produce ethanol for wine, beer, or fuel.

ABE fermentation became uncompetitive compared to petroleum based production in the second half of the twentieth century, but driven by technological advances and the growing demand for sustainable alternatives to fossil fuels, this century old process is now being rediscovered.

Louis Pasteur first observes butanol as a product of fermentation

1861



Industrial ABE plants built for production of acetone, an important substance for the British war industry during World War I

1916



Green Biologics brings online new commercial ABE plant in the United States

2016

1915

ABE process patented by Chaim Weizmann, renowned biochemist (and later first President of Israel)



1960

Mass production of same three solvents from petroleum begins. ABE fermentation from biomass becomes unprofitable



Source: Green Biologics

THE PRIZE: BIOBUTANOL

The main product of ABE fermentation is butanol: conventionally butanol, acetone and ethanol are produced in a ratio of 6:3:1. Biobutanol is considered an exciting alternative to first generation **biofuels** such as biodiesel and bioethanol. The similarity of butanol to gasoline means that with effective pumping systems it could be implemented immediately within the existing infrastructure.

Biobutanol's energy density is about 20% higher than bioethanol, more similar to that of gasoline. This means a litre of biobutanol will get your car almost as far as a litre of regular gasoline, but with up to 85% less greenhouse gas emissions.

As well as its potential as a transport fuel, biobutanol is also a building block chemical used extensively in the **paints, coatings, adhesives and inks markets**. Given the huge potential on show, funds are quickly rising for biobutanol production.



BUTANEXT: CHEAPER AND MORE EFFICIENT PRODUCTION

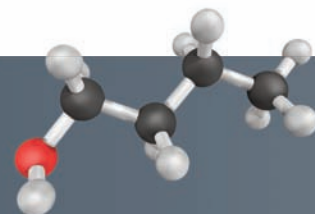
The ABE fermentation process has been improved significantly since the time of Weizmann. However, using it to convert more sustainable woody biomass remains technically challenging. On top of this, each production step is frequently developed separately, leading to difficulties in optimisation and scale up. **In order to overcome this, the ButaNexT project brought together the best experts in Europe to optimise the process at value chain level.**

Together they have developed and demonstrated, at pilot scale, a more cost-competitive, efficient and environmentally friendly process to convert sustainable renewable feedstocks into biobutanol. Their work has been validated by a full environmental, resource, techno-economic and social impact assessment of the entire chain.



Butanol or biobutanol?

Chemically they are the same. The difference is what they are made from – petroleum or biological sources.



1. SUSTAINABLE FEEDSTOCKS

First-generation biofuels are made from the sugars and vegetable oils found in arable crops, which can be easily extracted using conventional technology. In comparison, second-generation biofuels are made from lignocellulosic biomass or woody crops, agricultural residues or waste, which makes it harder to extract the required fuel. The ButaNexT project focused exclusively on the latter, which are considered more sustainable. A feedstock assessment carried out by E4tech considered the availability across Europe of four types of feedstock that could be used for butanol production: **straw**, **Miscanthus**, the **biological fraction of municipal solid waste**, and **woody residues**.

STRAW

The wheat stalks left behind after harvesting are generally used as bedding for livestock or left on fields as a way to enrich the soil. Even when these existing uses of straw are taken into account, a significant straw resource exists that can be sustainably extracted and used for alternative purposes such as biobutanol production.

> Potential EU availability: **58Mt**

MISCANTHUS

Miscanthus (commonly known as Elephant Grass) resembles bamboo, grows over 3 metres tall, and produces a crop every year without the need for replanting. Its rapid growth and high biomass yield make it a promising feedstock for biofuels. Not currently grown widely in Europe, Miscanthus should be planted on unused or underutilised land to mitigate against indirect land use change impacts.

> Potential EU availability: **20Mt**

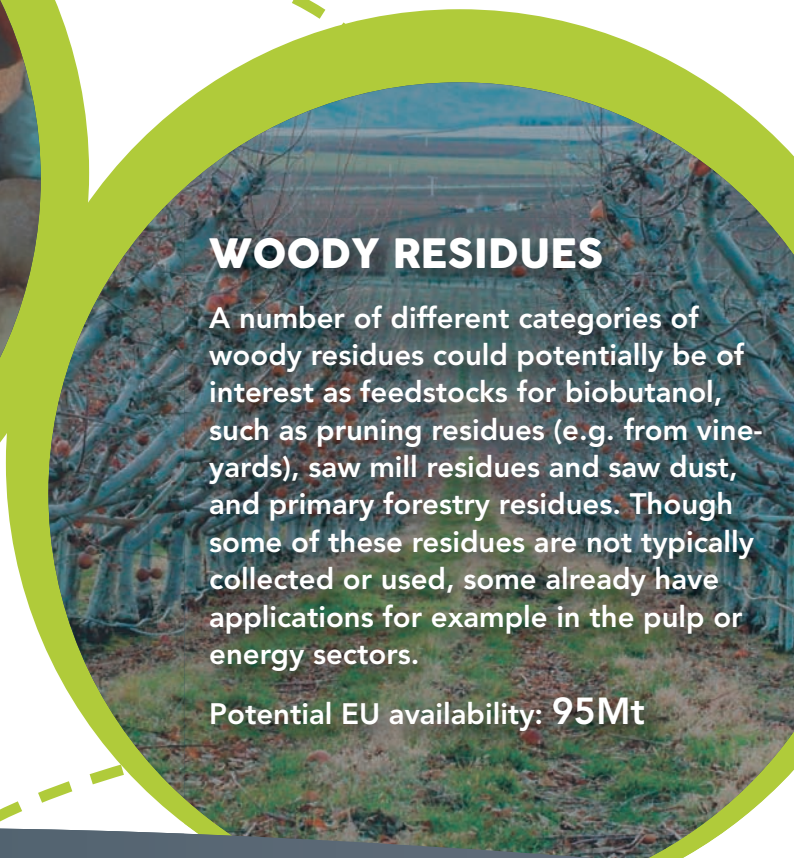
*Source: Bgabrielle
CC BY-SA 3.0*



MUNICIPAL SOLID WASTE

Municipal Solid Waste (MSW) consists of all the everyday items that we use and throw away. This includes organic material such as food waste that could be used as a feedstock for the biobutanol process. In the EU as a whole, around 30 to 40% of MSW is comprised of separately collected biodegradable waste.

Potential EU availability: **150Mt**



WOODY RESIDUES

A number of different categories of woody residues could potentially be of interest as feedstocks for biobutanol, such as pruning residues (e.g. from vineyards), saw mill residues and saw dust, and primary forestry residues. Though some of these residues are not typically collected or used, some already have applications for example in the pulp or energy sectors.

Potential EU availability: **95Mt**



A large amount of sustainable biomass is available in Europe for biobutanol production



400,000 TONNES

APPROXIMATE AMOUNT OF FEEDSTOCK REQUIRED
EACH YEAR FOR A COMMERCIAL BIOBUTANOL PLANT

II. BIOMASS CONVERSION

FLEXIBLE TWO-STAGE PRE-TREATMENT

The pre-treatment of lignocellulosic biomass consists of two stages. The first step involves grinding the irregular shaped feedstock into smaller pieces. Following this a thermochemical treatment is applied. Conventionally the first stage was carried out with a hammer-mill system, which was considered expensive and energy intensive. Within ButaNexT an improved technology has been demonstrated.



Source:
Técnicas
Reunidas

Técnicas Reunidas, together with CENER, have developed a **new two-step pre-treatment process** which is able to convert different lignocellulosic biomass and wastes in such a way that provides **higher yields in subsequent stages**. Crucially, the new milling unit significantly reduces the biomass particle size - to less than half a millimetre. This allows for milder conditions in the subsequent thermochemical treatment, and an improved conversion rate during the hydrolysis stage. It is expected that both capital and operating costs can be reduced – the unit **reduces the energy consumption up to 25%** compared to the conventional technologies studied.

TAILOR MADE ENZYME COCKTAILS

Enzymes are used to break down the cellulose, a polymer of glucose residues, into monomeric glucose molecules. Currently the market is providing general enzymes for any kind of feedstock, not taking into account the specific characteristics of different biomass or pre-treatments and combinations thereof.

Within the ButaNexT project, MetGen has designed and developed tailor-made enzyme solutions for non-food lignocellulosic feedstocks. Enzyme production has already been scaled up to pilot scale (400 litres), and MetGen has the capabilities to increase this to industrial scale (50-150m³) immediately after the end of the project.

After extensive screening of different enzyme combinations and formulations, new enzymatic cocktails for specific feedstocks were identified. The optimised cocktails **increased total sugar yield by 70% to 90%** compared to the initial offerings, **in only half of the hydrolysis time**.

The increased sugar yield with higher hydrolysis efficiency represents a more economically feasible enzyme solution for biorefineries, reducing the cost of the whole process. Importantly, the cocktails were also designed to address possible inhibitors and produce cleaner sugars specifically for the subsequent biobutanol fermentation process.



Source:
MetGen



A range of feedstocks
can be converted to sugars at higher yield,
with lower energy use and costs

III. HIGH PRODUCTIVITY FERMENTATION PROCESS

NOVEL MICROBIAL STRAIN

The acetone-butanol-ethanol (ABE) fermentation process uses clostridial strains of bacteria to produce acetone, butanol and ethanol from sugars. Conventionally, this process produces butanol, acetone and ethanol in a ratio of 6:3:1. Butanol yields are typically low due to solvent toxicity, which inhibits the fermentation, and the individual solvents are expensive to purify using typical downstream processes.

Within ButaNexT, Green Biologics has developed an **improved clostridial strain specifically for use with lignocellulosic feedstocks**. Experimental evolution techniques were used to naturally select for robust fermentation microbes under a variety of growth conditions.



Dr Holly Smith, Head of Fermentation, Green Biologics

> What improvements have Green Biologics made to the fermentation process?

Green Biologics has developed strains with increased tolerance to butanol; we have shown that when these strains were grown in the presence of higher solvent concentrations they maintained a **25% higher rate of sugar usage** than the original strain. We have also developed strains with increased tolerance to feedstock inhibitors and shown that these strains have up to **40% higher rate of solvent production** than the original strain in lab scale tests.

> What impact will this have on the overall ButaNexT process?

We can achieve higher rates of solvent production and improved sugar conversion yields than have been demonstrated with the conventional ABE process. We have also shown that it is possible to use second generation feedstocks with these microbes. In addition, these improved characteristics lead to **lower energy costs** and **reduction in waste water** generated leading to **savings in the total plant and operational costs** of the ButaNexT process.

IN SITU PRODUCT RECOVERY

Butanol productivities can be even further increased when the inhibitory solvents are removed from the fermentation broth while they are produced. The Flemish Institute for Technological Research (VITO) has extensive experience in coupling membrane technology – in particular organophilic pervaporation – to the fermentation to achieve such an *in situ* product recovery (ISPR). This hybrid fermentation concept not only alleviates product inhibition but also leads to partial product purification and enrichment, **thus improving water balances and reducing energy consumption** in further downstream processing.

VITO thoroughly evaluated and optimised its ISPR concept with the newly developed strains from Green Biologics, with the overall objective of **maximising butanol productivity** from pre-treated lignocellulosic feedstocks. It was found that continuous operating conditions have the edge over (fed)-batch conditions in terms of labour intensity, water consumption, solvent enrichment, and volumetric productivity. While glucose was always completely consumed, a trade-off between xylose utilisation and productivity was shown. Moreover, alternative membranes were tested and found to achieve fluxes which were twice as high as observed in earlier work while no membrane fouling was observed. This innovative approach to further reduce energy consumption was patented.



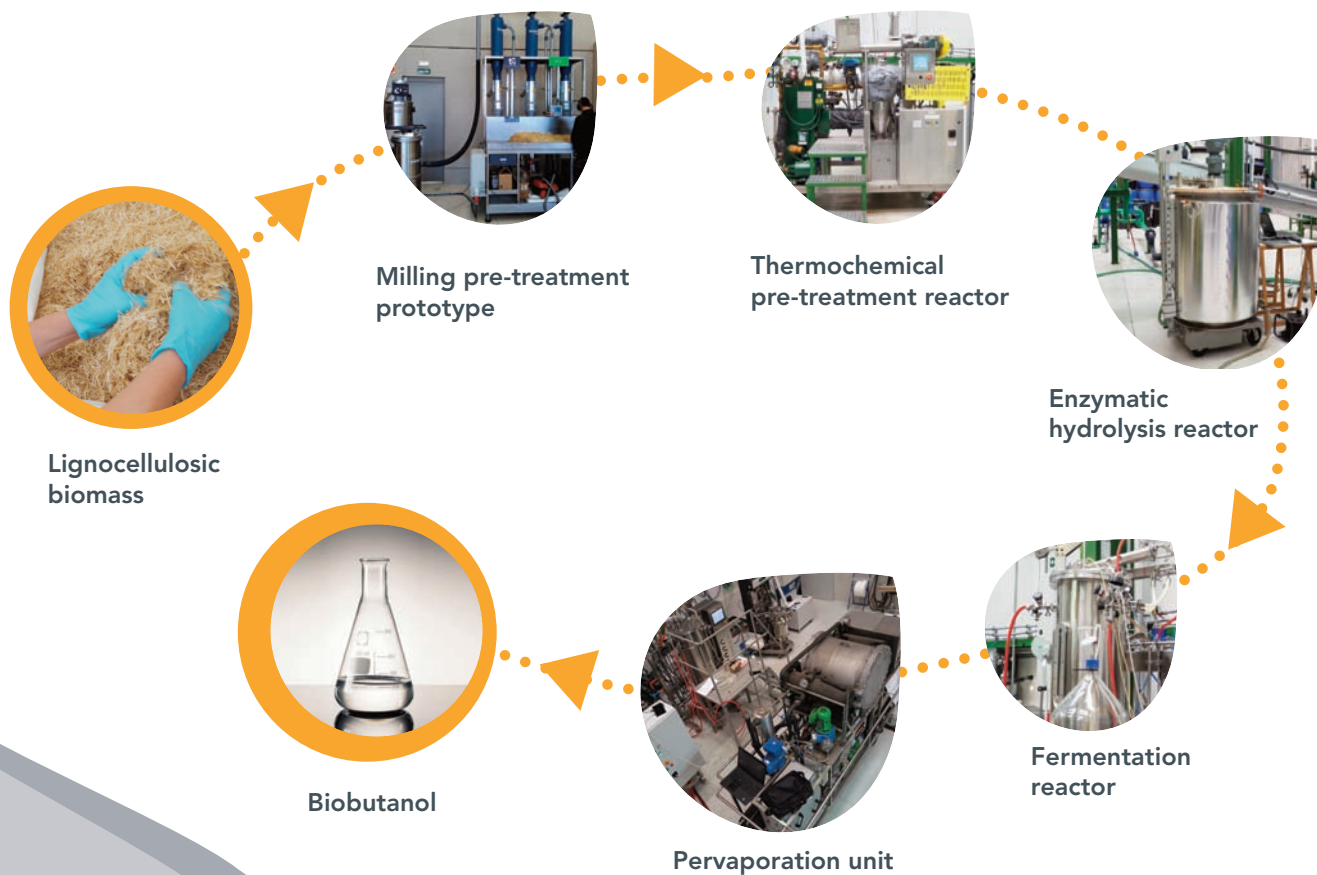
Source:
VITO

! Biobutanol yields and productivity have been improved while using less water and energy

IV. INTEGRATION AND UPSCALING

Having separately optimised all the individual stages of biobutanol production at lab scale, the next step was to test the whole process at a larger scale, in one single location. A pilot plant was installed at the Second Generation Biofuels Centre operated by CENER - Spain's National Renewable Energy Centre - in Aoiz, Navarra.

The pilot unit integrates all the steps of the ButaNext process from biomass handling through to biobutanol recovery



Inés del Campo, R&D Senior Engineer, CENER



> **What are the main challenges for integration and upscaling?**

The main challenge is that **scaling-up is non-linear**; you cannot take a chemical process from the lab and drop it into a pilot plant simply by increasing the chemical dosages and equipment proportionately. Important factors such as reaction kinetics, fluid dynamics and thermodynamics will all be different in larger sized equipment.

Furthermore, in the case of processes where microorganisms or enzymes are involved, it is important to guarantee **stable conditions** like temperature and pH during the process as well as a sterile environment to avoid contaminations.

> **How are you able to test this at CENER?**

At CENER we have technical staff with extensive knowledge and experience in the upscaling of biochemical processes. We know for upscaling it is important to carry out **exhaustive monitoring**, registering parameters and taking periodic samples. In the case of ABE fermentation it is important to monitor factors such as: microorganism growth; sugar concentration; inhibitory compounds coming from the hydrolysate; solvents concentration; and organic acids that can indicate the presence of contamination in the reactor.

> **What activities have you carried out until now, and what are the next steps?**

At first we prepared the layout of the pilot plant. A Tecnicas Reunidas technician then visited CENER to carry out the mechanical pre-treatment of the biomass to be used for the assays.

The following step was the preparation of the biomass hydrolysate. CENER technicians carried out the thermochemical pre-treatment of the feedstock and produced more than 500 kg (in batches) of pre-treated slurry for the assays. After pre-treatment this slurry has been sequentially subjected to enzymatic hydrolysis using enzymes supplied by MetGen. The hydrolysate produced has been filtered to avoid the presence of particles that can alter the operation of the pervaporation unit. The **achieved sugar yield has been around 80% of the initial feedstock**.

At the moment we are finishing the fermentation runs using the filtrated sugar hydrolysate. We are using the microorganism transferred by Green Biologics, and the operation is going smoothly with **high sugar consumption rates**. We are trying to optimise the solvent productivity and achieve the maximum possible sugar conversion.

💧 VALIDATING THE PROCESS

An assessment of the environmental, economic and social impact of producing biobutanol through the process developed in the ButaNexT project was carried out by E4tech.

ENVIRONMENTAL IMPACT

To gauge the environmental impact of the ButaNexT process, a Greenhouse Gas (GHG) Assessment of butanol produced in the pilot plant, and in a theoretical commercial-scale plant was carried out. The butanol was assessed using a Renewable Energy Directive (RED)-compliant methodology, with data from the ButaNexT pilot plant, from process modelling carried out by the ButaNexT partners, and from the scientific literature. The biggest contributors to the overall GHG emissions are the enzymes and the provision of heat and power to the plant. Depending on the type of feedstocks used, feedstock GHG emissions can also contribute substantially to the overall GHG emissions of the butanol. Therefore, in order to reduce butanol's GHG emissions, focus should be placed on **reducing the amount or GHG intensity of enzymes used**, sourcing **feedstock with low GHG emissions**, and **reducing the heat and power demand** of the process.

SOCIAL IMPACT

The social impact assessment carried out used the National Renewable Energy Laboratory's (NREL) open-source Jobs and Economic Development Impact (JEDI) model to assess the likely impact of a commercial-scale butanol plant. The results indicate that there are **substantial job opportunities**, particularly in the agriculture sector, from lignocellulosic biobutanol production.



Source:
CENER

TECHNO-ECONOMIC ASSESSMENT

A techno-economic assessment was made of a commercial-scale biobutanol production process, incorporating the technologies and improvements made over the course of the ButaNexT project. Cash-flow modelling was used to estimate the financial viability of a plant. The largest contribution to the levelised cost of biobutanol comes from the plant capital cost, and the cost of feedstock and enzymes. The cost of electricity could also have a large impact on the levelised cost, depending on how heat and power is provided to the plant.

A sensitivity analysis was carried out on a range of parameters, including enzyme costs, heat and power demand scenarios, feedstock costs and the possibility of energy demand reductions. The scenarios indicate that there is potential for the technology to be **economically viable**. This is likely to depend on **capital and other cost reductions being achieved**, which should be the focus of future analysis.



Source:
CENER



MARKET APPLICATIONS

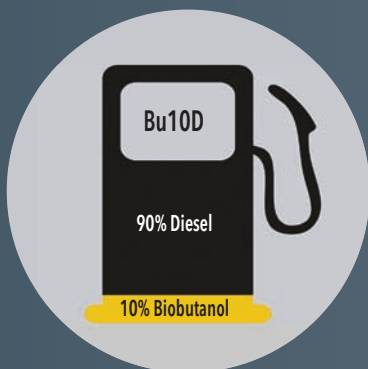
ADVANCED FUEL

Biobutanol is increasingly considered as a superior transport fuel compared to bioethanol, in part due to its higher energy content. Currently however, no vehicles are approved by the manufacturer to run on 100% biobutanol. Blends - a mixture of biobutanol with traditional fuels – can work with current technologies (both spark-ignition and diesel engines) while paving the way for future integration.

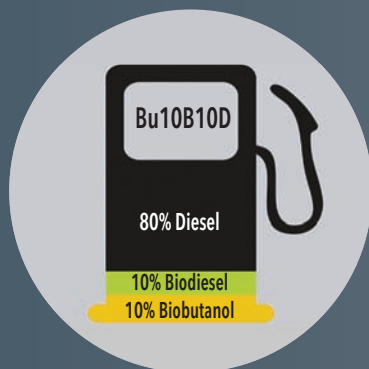
Fuel blends

Within the ButaNexT project, researchers at the University of Castilla-La Mancha (UCLM) investigated different blends incorporating biobutanol. They found that, in general, biobutanol as a blend component does not reduce engine efficiency and is beneficial for reducing particulate emissions (up to a maximum reduction of 60% in mass and 20% in particle number). This is a positive result in the context of the public revelations about diesel emissions, and increasing concerns about air quality. Bu10D (10% biobutanol, 90% diesel) and Bu10B10D (10% biobutanol, 10% biodiesel and 80% diesel) were identified as the most promising blends to substitute 100% diesel fuel in conventional diesel engines.

Furthermore, studies derived from soot analysis conclude that biobutanol-diesel blends reduce frequency of the particulate filter regeneration, leading to an increase in the durability of the particulate filters and to a reduction in the fuel consumption. UCLM found that blends with butanol content above 13% could lead to start problems at cold ambient conditions.



Source:
UCLM



BIO-BASED PRODUCTS

Biobutanol has many applications in chemicals markets, since it is used as a key raw material in the production of paints, coatings, plasticisers and adhesives. It can be used as a drop-in replacement for petroleum based butanol in almost all applications. Its ability to be used as an additive has resulted in increasing demand from the pharmaceutical industry. The uptake of ButaNexT biobutanol by those industries could help them to be more sustainable in terms of their raw material use, and allow them to position themselves within the green 'bio-based' market segment.



Global bio-butanol market

€ 6.4 billion 2014 > € 14.4 billion 2022 (expected)

ADVANCED PROCESSES

In fact most of the innovations and developments achieved in the ButaNexT project will have a broader impact than for biobutanol as a fuel alone. They can also be used for transforming lignocellulosic biomass into other biofuels and biobased chemicals in general:

- New clostridial strains for fermentation of sugars from cellulosic feedstocks, Green Biologics
- High productivity fermentation process for butanol, Green Biologics
- Two-step pre-treatment process for optimal fermentable output, Tecnicas Reunidas and CENER
- New enzyme cocktail for pre-treatment hydrolysis, MetGen
- *In-situ* product recovery (ISPR) capability for bio-processes, VITO
- Biological process scale-up and design, and pilot production service, CENER
- Know-how for optimisation of diesel engines for butanol biodiesel, UCLM



Biobutanol can improve fuel performance and emissions as a blend component, and has wider applications in the formulation of bio-based products.

BUTANEXT PUBLICATIONS

- > **Lignocellulosic Biomass: Supply and characterisation**, Alvaro Pallares, Clemente García (Técnicas Reunidas), ButaNexT project deliverable, 2015
- > **Performance data with VITO fed-batch & continuous ISPR system on pure and cellulosic sugar: Butanol productivities > 0.75g/L/h**, Wouter Van Hecke, Heleen De Wever (VITO), ButaNexT project deliverable, 2016
- > **Performance Data on Cellulosic Sugar with GBL BEST system: Butanol productivities > 0.75g/L/h**, Aleksandra Mrzyglocka, Charlotte Durant, Abdul Saqib, Kimberley Baker, Barnabas Owoh (Green Biologics), ButaNexT project deliverable, 2016
- > **Vehicle performances and emissions under ambient and cold temperatures when using butanol blends**, Fuels and Engines Group, University of Castilla-La Mancha, ButaNexT project deliverable, 2016
- > **Availability of different potential feedstocks across Europe**, Claire Chudziak, Lizzie German, Ausilio Bauen, Lucy Nattrass (E4tech), ButaNexT project deliverable, 2015
- > **Preliminary Energy Balance and GHG Assessments of ButaNexT process**, Lizzie German, Richard Taylor, Ausilio Bauen (E4tech), ButaNexT project deliverable, 2016
- > **Modeling viscosity of butanol and ethanol blends with diesel and biodiesel fuels**, Magín Lapuerta, José Rodríguez-Fernández, David Fernández-Rodríguez, Rayda Patiño-Camino, Fuel, Vol. 199, 2017, pp. 332-338
- > **Strategies to Introduce n-Butanol in Gasoline Blends**, Magín Lapuerta, Rosario Ballesteros and Javier Barba, Sustainability 2017, 9(4), 589
- > **Biobutanol production from C5/C6 carbohydrates integrated with pervaporation: experimental results and conceptual plant design**, Wouter Van Hecke, Pieter Vandezande, Marjorie Dubreuil, Maarten Uyttebroek, Herman Beckers, Heleen De Wever, Journal of Industrial Microbiology & Biotechnology, 2016, Vol. 43(1), pp 25-36

- > **Autoignition of blends of n-butanol and ethanol with diesel or biodiesel fuels in a constant-volume combustion chamber**, Magín Lapuerta, Juan José Hernández, David Fernández-Rodríguez, Alexis Cova-Bonillo, Energy, 2017, Vol 118, pp 613-621
- > **Modelling of evaporative losses in n-alcohol/diesel fuel blends**, Juan P. Hernández, Magín Lapuerta, Reyes García-Contreras, John R. Agudelo, Applied Thermal Engineering, Vol 102, 2016, pp 302-310
- > **High-flux POMS organophilic pervaporation for ABE recovery applied in fed-batch and continuous set-ups**, Van Hecke, W., De Wever, H. Journal of Membrane Science, Vol 540, 2017, pp 321-332
- > **Paving the way for a next generation biobutanol (ButaNexT)**, ButaNexT consortium, 2017

All these publications can be found on the ButaNexT website: www.butanext.eu/en/publications

ASSOCIATED PROJECTS



WASTE2FUELS aims to develop next generation biofuel technologies capable of converting agrofood waste (AFW) streams into high quality biobutanol.

www.waste2fuels.eu



ADVANCEFUEL will generate new knowledge, tools, standards and recommendations for overcoming barriers to the commercialisation of renewable transport fuels.

www.advancefuel.eu

BUTANEXT PROJECT

The present booklet was produced in the framework of the **ButaNexT** project (www.butanext.eu), which received funding from the European Union Horizon 2020 Research and Innovation Programme to develop highly efficient production processes and convert sustainable feedstocks for the next generation of biobutanol. This will contribute to overcoming the current challenges and limitations exhibited by the first generation of biofuels.

The ButaNexT consortium is a multi-disciplinary team comprised of SMEs, a large company and research centres from Belgium, Finland, Spain and the United Kingdom. The team aspires to optimise each stage of the biobutanol production value chain: biomass pre-treatment, fermentation, downstream processing and blending.

Between 2015 and 2018 the project partners succeeded in developing and demonstrating, at pilot scale, a more cost-competitive, efficient and environmentally friendly process to convert sustainable renewable feedstocks into biobutanol. Their work was validated by a full environmental, resource, techno-economic and social impact assessment of the entire chain.



CONSORTIUM



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Graphic design: Fastlane (www.takethefastlane.eu)

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The ButaNexT project has received funding from the European Union Horizon 2020 Research and innovation Programme under grant agreement n° 640462



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