PROJECT FINAL REPORT

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4.1.1 Executive summary

Bio-QED is a collaborative project financed by the European Union as part of the Seventh Framework Programme in which 14 partners from 7 different countries (Italy, Germany, Belgium, the Netherlands, Croatia, Spain and USA) worked to define innovative and sustainable processes towards the production of biochemicals (1,4-bioBDO and IA) from renewable sources, opening new industrial routes for the production of key building blocks alternative to the traditional petrochemical sources. The project fits perfectly with the EU's Bioeconomy strategy, which is intended to guide the European economic system towards a broader and more sustainable use of resources, reconciling the requirements of agriculture and food safety with the sustainable use of renewable sources for industrial purposes. It is concentrated on the sustainable upscaling of the production of biobased itaconic acid and 1,4-butanediol through industrial biotech processes starting from renewable feedstock.

Bio-QED, officially launched on 1st January 2014 and lasted for 4 years, was broken down into 8 Work Packages (six technical and two non-technical WPs), each targeting partial developments concurring as a whole to demonstrating the technical, economic and environmental sustainability of the production of bio-based 1,4 butanediol and itaconic acid. WP1 - Evaluation of 1st and 2nd generation feedstock - took place during the first 12 months of the project and it has brought to the identification and validation of 1st generation byproducts and innovative 2nd generation sugars that have been successfully used to feed the activities of WP2 and WP4. WP2 and WP3, respectively upstream and downstream processes, have reached a robust microbial fermentation and downstream processes able to support the scale-up in WP4. In particular, a robust variable volume process has been developed for 1,4-bioBDO up to 1m^3, using 1st generation sugars as benchmark. Furthermore using variable volume process and optimising process conditions, lower by-products, higher BDO titer, higher productivity and higher yield have been obtained. Referring the IA upstream process, best results have been achieved with the strain *A. terreus*. Moreover, fermentations with immobilized mycelia of *A. niger* have been tested and analogue/higher results in terms of itaconic acid yields were obtained compared to the fermentation carried out with *A. terreus*. In both IA and 1,4-bioBDO fermentation process, comparable results have been obtained with 2nd generation feedstocks proving that no toxic substances were present. In WP3 – Downstream processes - a cooling crystallization cascade for IA recovery has been successfully tested at bench scale in batch and the protocol to be used as blueprint for the pilot process in WP4 has been defined. On the other hand the Novamont process for 1,4-bioBDO separation based on micro- and nanofiltration has been set up and tested at pilot scale. The first step for 1,4-bioBDO purification, based on primary ion-exchange, allowed to determine the maximum amount of 1,4-bioBDO stream that can be loaded before the saturation of the resin is reached. WP4 was related to 1,4-bioBDO and IA scale-up of the processes up to 20 m^3 and 10m^3, respectively. Purified 1,4-bioBDO and IA have been validated in WP5 for the production of PU, bio-based polyesters for film application and Latex production, showing proprieties comparable to the building blocks from fossil resource. Within WP6 - Sustainability assessment -, a techno-economic assessment (TEE), LCA and LCC analysis have been performed. Certification schemes fitting with the Bio-QED scopes have been identified. Within WP7 (Dissemination and Exploitation), a public and private website section has been constantly updated and the project disseminated in several events, organizing specific stakeholders workshops and open demo events organised at plants premises. IA

Strategic Plan and IP Mapping studies for developments by competitors and emerging technologies outside the Consortium have been developed and constantly update as well.

4.1.2 Summary of the description of project context and objectives

The overall objective of Bio-QED project is to demonstrate how promising research results for two important bio-based chemical building blocks, 1,4-butanediol and itaconic acid, will be guided through the notorious "Innovation Valley of Death" to the level of large scale demonstrations.

Europe is currently investing towards a transition from a fossil-based to a bio-based economy and the European Bioeconomy offers, as described in the EC Strategy Paper and Action plan entitled "Innovating for Sustainable Growth: A Bioeconomy for Europe[1]", a comprehensive answer on key societal challenges, like creating and maintaining jobs, maintaining the competitiveness of the European Process Industries, adaptation to climate change, reducing the dependency on non-renewable feedstocks and "more with less" strategy on renewable biomass used for food, added value chemicals and energy. Moreover, the European Commission reiterated the pivotal role of bio based products and market development in the context of the review of the EU Industrial Policy"[2] Due to its extensive scope and complex character it can be expected that the accomplishment of the desired transition will take at least one to two decades. The starting position for Europe on the biotechnological production of added value chemicals from renewable biomass is still very good, with its leading scientific position on industrial White Biotechnology, the global top positions of the European chemical and agro-food industries and the sophisticated logistic infrastructure, but there is an urgent need to go to the innovation and commercial exploitation phase to prevent that Europe loses its strong starting position to faster acting competing economies.

For this reason an entrepreneurial consortium was built with the joint ambition to generate hard evidence and collect all technical and economic key design parameters needed for investment decisions for the first demonstration of the production of bio-based building blocks 1,4 butanediol and itaconic acid.

The Bio-QED consortium supported the Bioeconomy Strategy by taking a frontrunner position guiding promising research results for these two demonstrations, which have a number of synergetic effects, and proved that:

- **Industrial White Biotechnology** has reached the status that important **bio-based building blocks** can be made on a **scale** and with a level of **robustness and reliability** that it is ready to be **implemented industry,**
- The **costs and efficiency** of industrial biotechnology processes can still be reduced significantly by implementing **novel concepts** like **continuous fermentation** and **highly selective and energy efficient technologies** for separation and purification
- Bio-QED improved the sustainability **profile** of industrial biotechnology processes and promoted the efficient use of resources switching from **1st to 2nd generation feedstock**;
- That the produced building blocks can both be used as **bio-based drop in replacements** as well as for **new applications or products** with **new functionality** or **improved performance**.

The ambitions of Bio-QED was realized by a consortium which included:

- strong suppliers of bio-based feedstocks (Cargill – France and Cargill R&D– Belgium),

[1] 1Innovating for Sustainable Growth: A Bioeconomy for Europe, Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions, Brussels, February 13, 2012, 9 pages

[2] European Commission: A Stronger European Industry for Growth and Economic Recovery-Industrial Policy Communication Update COM COM(2012) 582 final

- industrial players leaders in the production of 1,4-bioBDO (Mater-biotech - Italy) and in bio-based biodegradable plastics (Novamont - Italy)
- industrial players that aims to become market leaders in IA and its derivatives (Lubrizol – Spain),
- parties that make semi- or end-products from the building blocks (Lubrizol - Spain, MI-PLAST – Croatia),
- Research & Technology Organizations supporting the transfer of results from lab scale to large scale demonstration (TNO – the Netherlands, Fraunhofer Gesellschaft – Germany)

- specialized SMEs for dissemination and exploitation (Nova Institute – Germany, Patentopolis – the Netherlands),
- one specialized SME in the development of innovative processes for the separation and purification of biobased building blocks (SoliQz)
- a certification body for standardization (RINA – Italy) and
- the involvement of state of the art demonstration facilities (Mater-biotech - Italy, Fraunhofer-CBP – Germany).

The Bio-QED consortium was based on strong industrial leadership on both of the selected products. This ensured that the industrial partners needed for further exploitation and commercialization of the anticipated end-result of Bio-QED were on board. In addition, the present partnership covered the full supply chains for 1,4-bioBDO and IA from the feedstock up to the application of the building blocks in (semi-)products. These applications included a healthy mixture of drop-in replacements with well-developed markets and new applications or products with new functionality or improved performance. Structured in 8WPs, a summary of the main activities and obtained results is reported in the following section.

4.1.3 Description of the main S&T results/foregrounds

Work Package 1 - Evaluation of 1st and 2nd generation feedstocks

The challenges to overcome and the technological advances that this WP intended to realise were aimed at the identification of innovative solutions to use and valorise all the by-products from 1st generation sugars available without competition with the food supply chain as well as supply 2nd generation feedstock. Divided in three tasks, the main partners involved were Cargill, FhG and Novamont.

Task 1.1: Valorization of by-products from 1st generation sugars

One of the aims of the BIO-QED project was to improve the sustainability profile of industrial biotechnology processes and to promote the efficient use of resources through the valorization of by-products from 1^{st} generation sugars for the production processes of the biochemicals 1,4-bioBDO and IA.

As a starting point towards that goal, a non-comprehensive list of 1^{st} generation sugars and potential feedstocks of 1^{st} generation by-products was made by compiling available public and Consortium data around their chemical composition (fermentable sugars, inhibitors, contaminants and their expected impact on key process steps), required (pre)treatment technologies, feedstock availability in the EU, competitive uses of the biomasses, logistics and sustainability considerations, potential cost drivers, among others. Special attention was given to corn and wheat, due to their relevance to the Consortium Partners. Based on such an overview, a selection of 1^{st} generation based feedstock types (benchmark as well as by-products) for further evaluation in the context of the BioQED project by means of toxicity (Task 1.3) and fermentation tests (WP2) was jointly made. Subsequently, Cargill produced and analysed different types of 1^{st} generation (by-)products that were supplied to FhG and Novamont at different volume scales in WP2 and WP4 to further improve the fermentability and hence the valorization of the selected 1^{st} gen by-products

Task 1.2: Preparation of 2nd generation sugars from lignocellulosic feedstock

In this task, FhG used its innovative "lignocellulose biorefinery" pilot plant to prepare 2^{nd} generation sugars from Beech wood and Wheat straw. The pilot plant was a batch process designed to fractionate wood chips by ethanol-water-pulping (Organosolv method). Ethanol-water pulping was carried out discontinuously with batches of 70 kg o.d. debarked beech wood chips. After pulping at temperatures > 100 °C, the cooking liquor was displaced by an ethanol-water solution. Washing and cooling were done two times by displacement with water in order to remove ethanol from the fibres. During the cooking phase, lignin and hemicelluloses were dissolved in the cooking liquor. The hemicellulose present in solution after the filtration of the precipitated lignin was purified and concentrated at the solvent rectification, while the cellulose fraction was disintegrated and washed. After dewatering, an enzymatic hydrolysis of cellulose was carried out in a stirred tank reactor for up to 24 hours. The glucose solution obtained was then filtrated (to remove residue lignin) and finally concentrated for preservation by means of evaporation. The obtained sugars solutions, after toxicity tests, were validated as feedstock into fermentation tests in WP2.

Task 1.3: Toxicity tests and selection of 1st and 2nd generation feedstock

The by-products from 1^{st} generation and the 2^{nd} generation sugars produced respectively by Cargill and FhG in previous tasks, were used for the investigation of the toxicity effect on IA and 1,4-bioBDO on the producing strains.
About the IA production, the more the complex feedstocks the better growth of A. *niger* was registered and, in the case of 2^{nd} generation sugars, the production of IA is higher. In contrast to these results, the usage of the complex feedstocks for cultivation of *Aspergillus terreus* led to an extension of the lag phase, as acetic acid and furfural (HMF) (common by-products during production of 2^{nd} generation sugars from lignocellulosic material) had a significant negative influence to growth and itaconic acid production of *A. terreus*.
Toxicity tests were carried on 1,4-bioBDO-producing strain of *E.coli* using both 1^{st} generation sugars, by-products of sugars production and 2^{nd} generation sugars. 1^{st} generation byproducts led to results comparable to the 1^{st} generation benchmark in terms of 1,4-bioBDO titer and productivity. With 2^{nd} generation sugars, no inhibition of cells growth was observed. Cells grew even faster than the control culture with pure glucose and 1,4-bioBDO production was higher and positively correlated with the amount of 2^{nd} generation sugars supplied. 2^{nd} generation sugars from wheat straw gave the best performance.

Work Package 2 - Upstream process- fermentation development

In this WP, cost-effective and sustainable processes were established converting the 1^{st} and 2^{nd} generation biomass fractions from WP1 into a broth containing the desired platform chemicals. For this purpose, processes and protocols were developed with the aim to:

- Develop and optimise different robust microbial fermentation processes with 1^{st} and 2^{nd} generation feedstock from WP1 in 50 L scale equipment.
- Enhance time space yield and productivities for the production of the platform chemicals 1,4-bioBDO and IA by batch, fed-batch, (semi)continuous or novel fermentation processes at pilot scale

- Evaluate fermentation processes towards scale-up in WP4

Structured in two tasks, the main partners involved were FhG and Novamont.

Task 2.1: Fermentation with recombinant E.coli for the production of 1,4-bioBDO

In this task, protocols for 1,4-bioBDO production by fermentation process have been developed by Novamont in collaboration with Mater-Biotech, starting from 1^{st} generation feedstocks, up to pilot scale and applicable at demo level. 1,4-bioBDO production with 2^{nd} generation sugars has been successfully obtained at laboratory scale. An alternative novel fermentation process has also been developed based on a variable volume fermentation, in which the substrate is fed not only in the initial medium, but also during the fermentation. In this way, the substrate was kept at the best concentration and the inhibition was avoided by adding the substrate at the same rate at which it was consumed, allowing the feed of larger quantities of substrate. Results pointed out high titer and no residual glucose left in the final medium, avoiding feedstock waste and possible negative impact on the downstream processes. A similar process was successfully applied using 2^{nd} generation. Final titer was about 10% lower than what obtained with 1^{st} generation benchmark, probably due to the lower glucose concentration of the feedstock that causes a dilution of 1,4-bioBDO.

The protocols were developed based on the result of many fermentation tests and of setting up fermentation parameters, feeding strategy, inoculum preparation, pH control. To control the processes, validated analytical methods have been used.

Task 2.2: Fermentation of Aspergillus sp. for the production of IA

A fermentation process for the production of itaconic acid with *Aspergillus terreus* was established by FhG. Therefore an optimized way for the production of spores which can be used at demo scale was developed and overcome the limitation of the traditional spores production . The production of IA with 1^{st} generation feedstock has been successfully demonstrated, while the IA production with 2^{nd} generation feedstocks was validated with the requirement that feedstock needs to be free of acetic acid and furfural for a successful IA production.

Within this task, the production of submers-spores was established and led to a spore production for demo scale. Regarding the feedstock, *A. terreus* could metabolize 1^{st} generation sugars by-products (Cargill feedstock) with faster and earlier IA production up to a scale of 100 L, even though the Itaconic acid production depends on the *A. terreus* strain. The process has also been developed based on a fed-batch strategy for feeding 2^{nd} generation feedstock (beech wood hydrolysate) and the biomass separation has been optimized using a filter press.

Work Package 3 - Downstream process (separation and purification up to 50 l)

In this WP, different separation and purification techniques have been selected for Bio-QED with the aim to:

- Test and implement pre-selected separation techniques to recover 1,4-bioBDO and IA from fermentation broth at a scale up to 50 L.
- Apply 1,4-bioBDO and IA recovery technique as ISPR to improve productivities and feedstock impurities inhibition
- Test and implement techniques to purify 1,4-bioBDO and IA from the stream coming out of the separation step at a scale up to 50 L.

Structured in two tasks, the main partners involved were TNO and Novamont

Task 3.1: Separation

The downstream process designed by Novamont with the support of Mater-Biotech for separation of 1,4-bioBDO from fermentation broth consisted of a filtration step. The purpose of this step was to remove cells, cell debris and higher molecular weight proteins from the 1,4-bioBDO fermentation broth. Upon evaluation of alternative innovative technologies for the recovery of 1,4-bioBDO from

the clarified broth, TNO investigated solvent extraction, followed by eutectic crystallization. Extraction of 1,4-bioBDO from clarified broth with isopropanol (IPA), followed by solvent distillation, was identified as most feasible. In a single stage extraction, 88% of the original 1,4-bioBDO was recovered from broth and after the evaporation an oil of 99% 1,4-bioBDO was produced.
In parallel TNO evaluated and developed technologies for IA recovery from clarified fermentation broth. From the evaluated options, cool (CC) and eutectic freeze crystallization (EFC) were identified as most energy efficient and cost-effective recovery technologies. With both technologies IA crystals were formed upon cooling of the broth. With EFC the broth was cooled down to the eutectic point (< 0°C) at which water was converted into pure ice and therefore theoretical yields above 95% could be achieved. Both technologies were applied at bench scale at the TNO facilities to recover IA from clarified broth that was supplied by FhG. IA crystals produced by means of cool crystallization had purities ranging from 90 to 95%. As a more mature and therefore more robust technology, cool crystallization was selected as recovery technology for the demonstration pilot. A protocol for (semi)continuous cool crystallization at pilot scale was produced and validated at bench scale.

Task 3.2: Purification

Protocol and key design parameters have been designed by Novamont for the purification process to obtain high grade 1,4-bioBDO suitable for further polymerization (99.95% purity) in WP5. After filtration to remove cells and other solids, the process consisted of a combination of additional filtration steps, Ion Exchange process, evaporation and distillation to remove residual carbohydrates, proteins and other high molecular weight impurities, inorganic salts, water, etc
In parallel, TNO applied hydraulic wash column technology (HWC) for purification of 97% 1,4-bioBDO, as produced and supplied by Mater-Biotech, to reach a purity of >99%. HWC is an energy efficient technology that combines continuous solid-liquid separation with efficient counter-current washing with an extremely low wash liquid consumption.
Related to purification of IA, for purification to polymer grade quality, two technologies were evaluated: solvent switch (SS) and additional cool crystallization (CC) technology. Like HWC, SS is an energy efficient technology that combines continuous solid-liquid separation with efficient counter-current washing with an extremely low wash liquid consumption. Both SS and CC were successfully applied at bench scale to produce >99% IA. Cool crystallization was selected for purification at demo scale. A protocol for (semi-)continuous cool crystallization at pilot scale was produced and validated at bench scale.

Work Package 4 Scale-up: integration of fermentation and downstream processing

In this WP, the proved and most promising fermentation and purification processes from WP2 and WP3 were scaled-up and optimized at larger scale. Here, 1,4-bioBDO and IA have been produced out of 1st generation feedstock as well as 2nd generation feedstock. The following tasks have been performed:

- Scale up and optimize fermentation processes for 1,4-bioBDO and IA production up to 1 m^3-scale
- Scale up and optimize fermentation processes for 1,4-bioBDO and IA production up to 10 m^3-scale
- Scale up and optimize fermentation processes for 1,4-bioBDO production up to 20 m^3-scale

The main partners involved were Mater-Biotech, Novamont, TNO, FhG and SoliQz.

Task 4.1: Scale-up of 1,4-bioBDO and IA production up to 1 m^3

Specific amount of 1st generation benchmark and by-products (Cargill) and 2nd generation sugars (FhG) were provided to enable upscaling the fermentation processes for *E. coli* and *Aspergillus terreus* in WP4.

In the context of the optimization of (production) technologies for improved performance of the various feedstocks in fermentation, Cargill R&D pursued the patent application WO2017120170A1 regarding the enzyme-assisted fermentation of regular dextrose greens.

The variable volume fermentation process, developed and optimized in WP2 for 1,4-bioBDO production, was initially scaled up in a 500L fermenter, using 1st generation feedstock. The scalability of the process was demonstrated, obtaining 1,4-bioBDO titers comparable (97-98%) to those obtained at 1 L scale, indicating that the variable volume process for 1,4-bioBDO production could be successfully scaled up. The benchmark purification process described in WP3, was used by Novamont at pilot scale in Piana di Monte Verna in order to verify the suitability of different feedstocks for 1,4-bioBDO production. In particular, the use of the selected 1st generation by-products was compared to the benchmark. Although minor differences found in the downstream process, the results indicate that sugar has an impact on the downstream process. 1,4-bioBDO fermentation trials at 1 m^3 scale were successfully carried out, according to the process previously developed and optimized in WP2. The obtained results showed a very promising and robust process, ensuring an almost absent loss of 1,4-bioBDO titer if compared to the lab scale.

The fermentation process with *A. terreus* developed in WP2 was successfully scaled up to 1 m^3 scale. Different batches of IA were produced at different scale (i.e. 100 L, 300 L and 1 m^3 scale) and results were compared. Itaconic acid production was similar up to 72 h cultivation time among the different batches and afterwards, the production was much faster/higher in 300-L-scale and flattened off a bit in 1-m^3-scale compared to the reference in 100-L-scale. At each scale, *A. terreus* grew in pellet morphology, with the size of the pellets that tended to decrease while increasing the scale. More mycelia were also observed at higher scale. Product recovery and purification was carried out through biomass removal, followed by a 2-stage cooling crystallization using a continuous crystallizer from SoliQz and freeze drying of the final crystals.

Task 4.2: Scale-up of 1,4-bioBDO and IA production up to 10 m3

The 1,4-bioBDO process optimized up to 1m^3 by Novamont in Piana di Monte Verna facility has been successfully scaled-up to 10m^3 in Mater-biotech facility located in Bottrighe. To do so, sterility validation of the equipments was successfully performed as first step. 1,4-bioBDO fermentation trials at 10 m^3 scale were successfully carried out, according to the process previously developed and optimized in WP2 and task 4.1.

The obtained results have shown a very promising and robust process with 1st generation sugars, ensuring an almost absent loss of 1,4-bioBDO titer if compared with pilot scale. Indeed, the loss of titer and yield at 10 m^3 demo scale was quantified in just 4,9% and 5,8%, respectively.

Fermentation for IA production was successfully scaled up to 10-m^3 with enhanced titer and yield. A continuous crystallizer from SoliQz for a 2-stage cooling crystallization was implemented at FhG-CBP and allowed a continuous recovery/purification of IA from higher fermentation volumes. Cooling crystallization performed well, with a minor loss in crystal size compared to the 1 m^3 scale demonstration. In total, 31 % itaconic acid was recovered and purified from the fermentation broth.

Task 4.3: Scale-up of 1,4-bioBDO production up to 20m^3

The same approach has been followed to further scale the 1,4-bioBDO production process up to 20m^3. Sterility validation was successfully performed for large scale bioreactors. 1,4-bioBDO fermentation trials at 20 m^3 scale were successfully carried out, according to the process previously developed and optimized in WP2, task 4.1 and task 4.2.

The obtained results have shown a very promising and robust process, ensuring an almost absent loss of 1,4-bioBDO titer if compared with pilot scale trials. Indeed, the loss of titer and yield was quantified on just 3,7% and 7,1%, respectively.

Task 4.4: By-products valorization (water treatment, anaerobic digestion, energy)

To allow an economically sustainable 1,4-bioBDO production, by-products recovery and valorization has been investigate. After a pre-screening of several possibilities, Mater-Biotech focused on a biodigestion system as a method to treat the organic wastes with the maximum recovery value, reducing disposal costs and, at the same time, producing potentially saleable products: biogas (energy) and fertilizer. After a preliminary study in order to define the right system of biodigestion, a single stage biodigestor with sludge recirculation fully integrate with the downstream process (purification) and the waste water treatment (WWT) has been designed and implemented at Mater-Biotech facilities. Feed and operative optimization have been performed, resulting in a good biogas quality (high methane content) and high conversion yield.

Work Package 5 Application development and end of use

The bio-based building blocks produced and purified in previous WPs have been supplied to WP5 with the aim to:

- Transform 1,4-bioBDO, and IA into intermediary derivatives;
- Further process intermediates into end-user / value added applications;
- Characterize properties and evaluate the performance of these applications;
- Provide feedback/guidance for the developments in other WPs;

Structured into five tasks, the main partners involved were Lubrizol, Novamont, TNO, VLCI, Itaconix and Miplast.

Task 5.1: Synthesis of polyurethane from 1,4-bio BDO

In this task, 1,4-bioBDO was evaluated by Lubrizol as a raw-material in the production of thermoplastic polyurethanes, and specifically as a glycol in the production of regular polyester (Butanediol Polyadipate) and also as a chain-extender. The performance of polyurethanes obtained from 1,4-bioBDO has been also compared to the polyurethanes available on the market and made of petro-BDO.

Evaluations have been carried out on different 1,4-bioBDO batches obtained both at pilot and demo scale. Results suggested that 1,4-bioBDO had the same performance of petro-BDO regarding the production of thermoplastic polyurethanes. Consequently, 1,4-bioBDO can be used as a glycol in the production of regular polyester (butanediol polyadipate) and also as a chain-extender. The global properties of the thermoplastic polyurethanes produced using 1,4-BDO of both sources are very similar. A special focus was dedicated to the colour of polyurethane, which improved according to the increasing production scale. However, it was verified that all samples produced with 1,4-bioBDO are in line with market specifications for the production of thermoplastic polyurethanes.

Finally, an analysis of 1,4-bioBDO to predict the colour of the polyurethane was developed by Lubrizol in order to provide useful feedback to Mater-Biotech regarding the stage of the 1,4-bioBDO production process that is more sensitive to develop colour in the subsequent polyurethane.

Task 5.2: Preparation of acrylic copolymers from IA

The objective of this task was the evaluation of Itaconic acid in the production of acrylic copolymers. Itaconic acid has been used by Lubrizol into 5 selected co-polymers for application into paper coating (wallpaper), textile coating (blinds), paper coating (filtration), paper coating (tea bags) and wet lamination adhesives.

IA samples obtained both at pilot and demo scale and with different purity levels were tested and acrylic copolymers targeting the specific applications have been synthesized and characterized. According to the results, the purity of itaconic acid affected the polymerization mainly in physical-chemical characteristics and in the filtration of the emulsion, which is an important step at pilot and

industrial scale. Influence of the IA purity has been also registered in the performance, especially for water uptake and Dry or Wet Strength (MD).
Evidences collected at lab scale for the different co-polymers have been validated at pilot scale and results from performance tests regarding the use of IA samples obtained in the Bio-QED project suggested the feasibility of the application in the production of acrylic copolymers for the targeted applications.

Task 5.3: Latex production from IA (through esterification and emulsion polymerization of alkyl itaconates)

Three different and parallel activities have been carried out within this task.

1. Synthesis of latex via di-alkyl itaconate

The production of di-alkyl itaconate (DnBI) from itaconic acid and a combination of alcohols has been demonstrated successfully at pilot scale by Itaconix. The yield of the synthesis of dibutyl monomers was increased from 73% for the first batch to ~92% for later batches. The purity and reactivity of the monomers obtained were very similar to reference batches obtained at smaller scale as confirmed by HPLC characterization, and also by emulsion polymerization, where monomers remained stable and reactive for polymerization for more than a year.
The emulsion polymerization process was optimized in order to overcome the slow polymerization rate of itaconate diesters by working with slow feed and low reaction temperatures. The slower monomer feeding rate increased the conversion and also the shelf stability significantly, and led to a solid content of 46 % and particle size at 177 nm.

2. Synthesis of self-healing polymers through emulsion polymerization

In parallel, polymerization of n-butyl methacrylate and itaconic anhydride (that can be produced from the IA) was established by TNO towards the production of a self-emulsifying polymer after treatment with ammonia, with the most important process variable being the ratio between the monomers. Monomer ratios nBMA/IA varying from 5 to 9 led to almost complete conversion.
Two parallel approaches were followed in the development of itaconic acid based self-healing polymers: one based on itaconic anhydride and the other based on diethyl itaconate as co-monomer in the polymer formulation. The self-healing properties of both polymers would result from their possibility to cross-link through thermo-reversible Diels-Alder additions. Although itaconic anhydride based formulation did not perform as expected due to undesired side reactions, most likely resulting from the presence of the strong acid moiety of itaconic anhydride itself, the diethyl itaconate based formulations resulted in thermo-reversible crosslinked polymer networks with improved solvent resistance and mechanical properties. At room temperature, the coatings crafted from these polymers behaved as thermoset polymer networks, while at higher temperatures the coatings behaved as thermoplastics, which demonstrated their self-healing capabilities.

3. Synthesis of latex for wall paint formulation

Different batches of IA were tested by VLCI in a guide formulation of a vinylacetate:butylacrylate 70:30 emulsion polymer, compared to the standard available on the market and to acrylic acid. The guide formulation was found to be suitable in order to distinguish differences in the IA batches, by evaluation polymerizations and their films, therefore this was used as a quality control process. With the best IA batches, application in a wall paint was evaluated too. The polymers and paints were prepared via the High Throughput system to achieve consistent results, and to be more precise than manual. The used guide formulation was further optimized by applying HLD-NAC, which is a formulation science to efficiently select (bio-based) surfactants and develop emulsion with better and lower amount of surfactants. In this case, it led to the selection of a glucoside based surfactant which could be used in a lower amount than the standard guide formulation provided by the supplier. For the results of all the polymerizations, it was found that the quality of IA improved during the project,

resulting in good batches at the end of the project. Both the demo plants (1 m^3 and 10 m^3) have produced IA suitable for emulsion polymerization, of high quality and without any impurities affecting the synthesis.
The best latexes produced were successfully tested in a wall paint formulation, and compared with a commercial binder to determine the real-world potential of the inclusion of bio-based materials in the market. The performed tests led to very positive results, matching or exceeding the properties of the commercial binder in the formulation.

Task 5.4: Polymerization by polycondensation to synthesize polyesters and co-polyesters
The present task aimed to produce aliphatic-aromatic co-polyesters based on renewable 1,4-bioBDO obtained by fermentation processes produced within the Bio-QED framework.
Initially activities performed at laboratory scale were focused on demonstrating that the properties of the co-polyester obtained using 1,4-bioBDO perfectly matched the properties of the co-polyester obtained using the fossil-based 1,4-BDO.
Different process conditions were tested, such as reaction time, temperatures, catalysts, stirring profile and filling level. Activities also involved the synthesis of aliphatic-aromatic co-polyester based on at least three different carboxylic acid (typically two aliphatic acids and one aromatic acid). No differences were remarked on both process and quality by partial substitution of one aliphatic dicarboxylic acid with another.
Moreover, the feasibility to incorporate IA as branching agent during the synthesis of polyesters has been demonstrated. The best results were achieved incorporating the sample obtained with additional Cool Crystallization, with a concentration up to 0.5% molar with respect to the sum of dicarboxylic acids.
Finally, this task was completed performing synthesis at demo scale level using 1,4-bioBDO obtained at demo scale by Mater-Biotech. The co-polyester obtained at demo scale was used to produce starch-based compounds suitable for carrier bags and mulching film that were validated into final products in task 5.5.

Task 5.5: Film applications development
The main purpose of this task was to optimize the process conditions towards the obtaining of four final applications, all in line with UNI EN13432, respectively two types of carrier bags and mulch films for short and long crop cultivation (biodegradable in soil). Activities has initially involved the transformation of 1,4-bioBDO thermoplastic compound through blown extrusion process and different compounds were evaluated. Equipment (blown extrusion and welding lines) was adjusted during project implementation in order to achieve high properties of the films. During such tests, a significant differences between traditional fossil based materials (which are nowadays mostly used) and this new generation of biopolymer blends were noticed, especially in terms of thermal properties and density. Results suggested that the materials transformed into final applications during the processing stage showed high processing possibilities and the potential of achieving optimal processing parameters for targeted applications. Indeed, in the different section of the process, such as for single screw extrusion process BUR, DDR, frost line, bubble height and for welding process, optimal sealing temperatures and sealing time were achieved.
The characterization of the obtained carrier bags and mulch films led significant results, especially in terms of the most important mechanical properties, i.e. tensile properties and propagation tear resistance. The obtained samples were also characterized in terms of biodegradability and compostability according to EN 13432, leading also in this case to very promising results.

Work Package 6 Sustainability assessment
While technological aspects were evaluated in WP1, 2, 3, 4 and 5, cross-cutting activities related to the sustainability (both economic and environmental) have been addressed in WP6. Economic aspects

covered the analyses of cost competitiveness, CAPEX and OPEX of the new processes compared to common technologies, as well as the market potential for new products. Environmental aspects were analysed using well established LCA approaches following ISO 14040 and ISO 14044. These different aspects were integrated and updated with results of the other WPs to provide an inbuilt sustainability, market and reality checks for the whole project.
Structured into four tasks, the main partners involved were Novamont, Nova-Institut and Rina.

Task 6.1: Market studies

The market study on IA and 1,4-BDO has been carried out starting from the current existing literature and integrated with interviews to relevant stakeholders. The main activities involved the data collection on prices and production capacity for 1,4-BDO and IA, the analysis of the past and current markets of the targeted building blocks, interviews with stakeholders of chemical industry to acquire more information of IA market development, the analysis of the potential technical substitution for 1,4 BDO and IA, in order to estimate their theoretical future market volumes, and the data collection on feedstock availabilities (in terms of volumes, prices etc.) for producing 1st and 2nd generation sugars.
From the conduced studies, bio-based building blocks represent a highly promising innovation for two main reasons:

1. The use of renewable and potentially more sustainable raw materials (biomass instead of crude oil)
2. The possibility to develop an efficient and synergic system (i.e. biorefinery) based on principle of circular economy able to revitalize chemical sector with a strong environmental, social and economic perspective.

1,4-BDO and IA are well known compounds globally produced for decades. 1,4-BDO is currently mainly produced from petroleum-based sources. Nonetheless, within a unique moment of interest and demand for sustainable chemicals driven by consumer attitudes and volatile petroleum markets, the production of 1,4-BDO from renewable feedstock is today being regarded as an interesting alternative also in the light that the global 1,4-BDO market size was valued at USD 6.19 billion in 2015 and is expected to grow at an estimated CAGR of 7.7% from 2016 to 2025. IA represents a niche product among the different bio-based building blocks, although it has a high technical substitution potential. IA is only produced via fermentation and currently its global yearly production is in the range of 42 ktonnes, most of it manufactured in China.

Task 6.2: Techno-economical assessment

Within this task, a techno-economic assessment of the organosolv process based on wheat straw and beech wood as well as of the conversion of glucose into IA and 1,4-bioBDO has been carried out. This assessment was conducted by collecting process data from the Bio-QED partners and applying a calculation model developed by Nova-Institute fitted for a situation with limited data.
For the different processes, annual profits and losses as well as the Internal Rate of Return (IRR) as a prominent indicator of profitability have been calculated. Positive results were showed for the organosolv process using lignocellulosic biomass for the recovery of glucose as a chemical feedstock together with the recovery and valorisation of the lignin (a co-product of the process), which can be used to produce the energy for sustaining the process.
The economic viability of the studied processes for the conversion of glucose into 1,4-bioBDO an IA showed positive results as well.
Moreover, the whole economic and environmental sustainability of the production of bio-based building blocks from alternative sources of sugars could be further enhanced in the future when processes will be fully upscaled and industrialized and all the process steps will be integrated following a zero waste biorefinery approach, where also by- and co-products will be recovered and valorized for the production of bioenergy and additional added value biochemicals.

Bio-QED project outcomes represent a valuable contribution towards biorefineries development which represent the bridge between agriculture and bio-economy.

Task 6.3: Environmental sustainability assessment/evaluation

The environmental sustainability assessment aimed at analyzing the environmental profile of new two bio-based products, i.e. 1,4-bioBDO and IA produced from 1st and 2nd generation sugars through the investigation of the relevant aspects of environmental performance from a life cycle perspective. Moreover, such an assessment has foreseen a comparison of the environmental performance of bio-based products with a benchmark (fossil-based 1,4-BDO and acrylic acid – no fossil based IA is produced and acrylic acid is considered the fossil-based counterpart) and has provided recommendations to the project partners in order to improve the environmental profile of the innovative products. Finally, a sensitivity analysis for the most important key parameters was performed to increase the reliability of the research outcomes.

The LCA study was carried out from "Cradle to gate". Two scenarios base were taken into account:

- Scenario A: 1,4-bioBDO and IA produced from 1st generation sugars;
- Scenario B: 1,4-bioBDO and IA produced from 2nd generation sugars in an integrated process (i.e. lignin recovery)

Overall the most relevant aspects for both 1,4-bioBDO and IA's life cycle are those ascribable to the use of energy (heat and electricity) requested by the organosolv and the fermentation processes. Energy audit and energy efficiency projects would be therefore recommended. Regarding the biomass production, it was interesting to notice that wheat straw and beech wood impacts were about 1/5 of those observed for maize, potato and wheat.

Task 6.4: Products certifications

The activities carried out focused on the possibility of release guidelines for sustainability of the value chain. Such guidelines have been developed based on the analysis and selection of the main standards related to the Bio-QED project. The standards have been selected considering all three pillars of sustainability (i.e. environmental, social and economic aspects) to provide horizontal sustainability criteria applicable to the bio-based part of all bio-based products, excluding food, feed and energy. Starting from these results, the most significant sustainability criteria for Bio-QED supply chains have been defined, and finally guideline/reference practice have been elaborated to provide all information to satisfy the sustainability requirements and best practices.

In parallel, the activity has been also enriched by a comparative analysis of circularity of two different end products, i.e. a biodegradable mulch film made of polyesters from 1,4-bioBDO and a traditional non- biodegradable mulch film made of fossil-based polyethylene.

The achieved results have highlighted that bio-based mulch films is characterized by a significantly higher Material Circularity Index compared to the traditional PE film, mainly due to a higher content in biodegradable materials. Such an index remains higher even compared to the one obtained from partially recycled PE film, where the amount of recycled material in the initial feedstock enabled to increase the circularity of traditional polyethylene

Work Package 7: Dissemination and exploitation

During the project life, Bio-QED employed a broad set of dissemination and exploitation activities with the aim to:

- Promote Bio-QED and its results as widely and as effectively as possible to all relevant stakeholders
- Ensure widespread dissemination of the results to the stakeholders, the broad public and the scientific community;
- Ensure proper and effective handling of Intellectual Property

- Implement a toolbox-supported IP Strategy in order to maximize the exploitation of project results for individual and combined partners inside and outside the consortium

Structured into three tasks, all partners were involved in this WP.

Task 7.1: Dissemination

The overall objectives of the task were to promote Bio-QED and its results as widely and as effectively as possible to all relevant stakeholders and to ensure a widespread dissemination of the results to stakeholders, including the general interested public society, industry, scientific community and policy makers. The dissemination part was integrated in the Bio-QED framework and linked to all technical processes, building a frame together with the management and the sustainability assessment within the project. The planned activities have been carried out successfully and without major difficulties. Such activities have included the preparation of a project identity with color selection and coding, logos, presentation sides and other materials, the set-up and frequent updates of a website for the project at http://www.bio-qed.eu (will stay online for 5 years after the project) with an internal and an external area (in the latter case, an internal communication area within the website has been set up with download area for official documents, deliverables, milestones, logos and other materials), and the preparation and updates of a leaflet for the project (both printed version and pdf download at the website). Moreover, in order to increase the disseminate the obtained results to all stakeholders, two workshop have been organized: the first one in Brussels on June 2015 as side event to the final BIO-TIC conference, organized by EuropaBio with BIO-TIC partners (Nova-Institute and TNO included); and the second one in Cologne on May 2016 as side event to the 10th International Conference on Bio-based Materials, organised by Nova-Institute.

Finally, in order to further increase the dissemination activities, different Open Demo Events have been organized (one in Leuna at Fraunhofer CPB facilities and five in Bottrighe at Mater-Biotech facilities), involving more than 700 stakeholders among politicians, scientists, students, etc.

Task 7.2: Standardization

The following task has focused on two main topics

- Strategy and proposal for measures to be undertaken towards standardization.
- Digestate's characterization for agricultural's use: test and sampling activity

Regarding the first topic, a guideline based on a specific checklist has been developed to provide a set of requirements selected by reference standards. The reference standards covered different aspects of the identified macro-areas, i.e. *upstream, core, applications* and *end-of-life*.

The guidelines aimed at providing all information to satisfy the sustainability requirements and best practices and could be considered as the tool to outline an internal verification of the production cycle of the polymer obtained by the production process based on 1,4-bioBDO or IA, in order to evaluate the sustainability aspects.

Regarding the digestate's characterization for agricultural use, the activity was focused on types, technical and regulatory context. In order to define testing methods, the PAS 110:2014 "Specification for whole digestate, separated liquor and separated fibre derived from the anaerobic digestion of source-segregated biodegradable materials" has been considered as reference.

Different digestate's sample with statistical significance (5 months for samplings; 27 samples collected) have been characterized through chemical and microbiological tests. All the obtained data led to conclude that results are in compliance with the considered limits and, consequently, digestate might be valorize in agriculture, especially regarding the production of ammonium sulphate.

Task 7.3: IPR management and exploitation

The objectives of this task were to provide a strategy toolkit to prepare and deploy strategic plan regarding two value chains 1,4-bioBDO and IA; and to support Partners with their IP operations for implementing the plans. The work performed has been structure in different steps.

All the collected data were periodically updated and at the end of the project, the following achievements were reached where IPR management is concerned:

- Full analysis for BDO and IA value chains, including identification and assessment of relevant IP from third parties, and identification and assessment Partners' Current IP.
- Strengthening current innovation position. A Patent was also claimed.

Identification of routes for further exploitation especially for Foreground. More details are reported in section B

4.1.4 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

Bio-QED supported the realisation of two innovative platforms dedicated to the production of the biobased chemical building blocks 1,4-bioBDO and IA from renewable resources through the use of innovative fermentation processes, enabling a breakthrough in costs by making the processes for 1,4-bioBDO and IA production (and especially the fermentation) continuous and by using highly selective and novel technologies for separation and purification. Moreover Bio-QED has demonstrated at relevant scale the sustainability of the biobased production of 1,4-bioBDO e IA with respect to environmental and economic criteria, validating their application into biobased materials and products. These results surely open up new perspectives not only for the consortium partners, but also for the European chemical sector and all its related downstream industries, the majority of them being SMEs. This opportunity, together with the opportunity of applying an integrated chain, would definitely result in clear added benefits for economy, society and the environment.

Thanks to the efforts of industries and public and private research centres, Bio-QED set for itself the ambitious goal of rethinking of the development of the Biotech and chemical industry – presently undergoing a wide-reaching contraction due to the financial and economic crises – by integrating biotechnology, chemistry, agriculture and the environment. In fact, Bio-QED platforms could develop innovative bio-based processes, leveraging new investments with the impact of possibly regenerating the use of existing facilities that were abandoned in the last years due to economic crisis or delocalization. Production with low environmental impact, using renewable resources, such as those obtained in Bio-QED, can be suitably exploited to revitalize (Bio)chemistry in an environmental framework in the countries that will host the demonstration platforms and then offering good practice for future replication of the initiative in other countries.

Last but not least, Bio-QED project aimed at contributing to the main EU policies through a strong alignment with Horizon 2020, the Bioeconomy Strategy and the Strategy on Key Enabling Technologies. This has been achieved thanks to the strong systemic integration of the processes and the market-driven approach guiding the innovation efforts performed. In this way, industrial biotechnology becomes the key pillar on which new processes for the production of added value products can be developed with an efficient use of resources and with strong environmental and social benefits. An example is represented by Mater-Biotech, which was established with the aim to realise the first industrial plant in Europe producing 1,4-bioBDO directly from renewable feedstocks. Bio-QED enforced the potential of Mater-Biotech facility by further reducing production costs, improving the sustainability profile of industrial biotechnology processes and promoting the efficient use of resources.

To maximize the project impacts, several dissemination activities have been performed during the four years of the project, focusing on disseminating the project’s results and achieved objectives to the academic and research communities, industrial networks and the general public.

A midterm stakeholder workshop has been held in Brussels on June 24th, 2015 in connection with the Bio-TIC conference to target highly relevant stakeholders. A second stakeholder workshop has been held in Cologne, Germany on May 10th, 2017, organized in conjunction with the 10th International Conference on Bio-based Materials. Both high-level events have been focused on bio-based building blocks and platform chemicals. After a short overview of the project and the market for the Bio-QED platform chemicals 1,4-bioBDO and IA, the workshop has been focused on the final applications of these value chains.

At the final phase of the project, different demo sites events have been organized in order to show the main demo facilities involved in the demonstration of the two value chains (IA and 1,4-bioBDO):

- IA demo event has been organized by Fraunhofer IGB/CBP in collaboration with the Association of German Engineers (VDI). The event took place at Leuna's facility on 12th April 2017 and addressed students and representatives of industry and research. The workshop was completed by a visit tour of the facilities of the Fraunhofer Center for Chemical-Biotechnological Processes CBP and an open discussion of current topics within research and development of bioeconomy.
- 1,4-bioBDO event has been organized by Mater-Biotech. It has been structured in several open days in order to cover different actors along the value chain (institutions, students, citizens, NGOs) and increase the awareness on the project. During the last year of the project, five open days have been organized (one with Legambiente, one with WWF Italy, one for students from Delft University of Technology, one for citizens and institutions and 1 for the Cré – Composting Association of Ireland) with more than 700 people involved.

4.1.5 Contact information

For additional information concerning the Bio-QED project please visit the website or contact:

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Project website
www.bio-qed.eu

Project logo

Project Consortium

No	Name	Country	Project entry month	Project exit month
1	NOVAMONT SPA	Italy	1	48
2	LUBRIZOL ADVANCED MATERIALS MANUFACTURING SPAIN SL	Spain	1	48
4	MATER-BIOTECH SPA	Italy	1	48
5	CARGILL HAUBOURDIN SAS	France	1	22
6	MI-PLAST DOO ZA PROIZVODNJU TRGOVINU I PRUZANJE USLUGA - MI-PLAST LLC MANUFACTURING, TRADING AND SERVICES MIPLAST	Croatia	1	48
7	NEDERLANDSE ORGANISATIE VOOR TOEGEPAST NATUURWETENSCHAPPELIJK ONDERZOEK - TNO	Netherlands	1	48
8	FRAUNHOFER-GESELLSCHAFT ZUR FOERDERUNG DER ANGEWANDTEN FORSCHUNG E.V	Germany	1	48
9	PATENTOPOLIS BV	Netherlands	1	48
10	RINA SERVICES SPA	Italy	1	48
11	NOVA-INSTITUT FUR POLITISCHE UND OKOLOGISCHE INNOVATION GMBH	Germany	1	48
12	VAN LOON CHEMICAL INNOVATIONS BV	Netherlands	17	48
13	ITACONIX CORPORATION	United States	19	48
14	SOLIQZ BV	Netherlands	25	48
15	CARGILL R&D CENTRE EUROPE BVBA	Belgium	23	48