OPTISOCHEM

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to bio-ISObutene for bio based CHEMicals"

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Report on delivery of first 2G isobutene samples

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Document Abstract

The first conditioning of IBN from second generation sugars was successfully conducted in GBE's Pilot Plant facility at Pomacle-Bazancourt, using Clariant's Wheat Straw Hydrolysate (WSH) as feedstock.

During the first year of the project, several WSH samples were tested with GBE's IBN producing strain, while the development of new strains adapted to WSH was ongoing. Scale up attempts started in December 2017. GBE's Pilot Plant facility for IBN production includes a 500 liters fermentation unit, and a purification unit allowing IBN purification, condensation and conditioning. Two different WSH batches were tested at the laboratory scale, before scale up in the Pilot Plant. Growth at the laboratory and Pilot scale were very similar, whereas productivity was lower in the Pilot.

A first sample of IBN (kilogram scale) was produced from WSH, and delivered to INEOS for application tests.

Because of the public (PU) nature of this deliverable, details such as WSH composition, strain genotype and process parameters will not be disclosed in this document.

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Abbreviations

GBE: Global Bioenergies

IBN: Isobutene

WSH: Clariant's Wheat Straw Hydrolysate

TPED: Transportable Pressure Equipment Directive

1. INTRODUCTION

GBE develops an isobutene (IBN) production process by fermentation, allowing for the conversion of sugars into gaseous IBN. The fermentation gas is purified and condensed, in order to recover highly concentrated IBN in pressurized cylinders. Clariant has delivered several samples of Wheat Straw Hydrolysate (WSH) to GBE in order to assess the current production process with this feedstock, and to develop new IBN producing microbes, adapted for WSH fermentation.

The adaptation process is ongoing in Evry's research facility based in Evry. The objective is to optimize the simultaneous consumption of the different sugars present in WSH, and to overcome the inhibitory effect of impurities such as hydroxymethylfurfural (HMF) and furfural. Process development is first conducted at the lab scale (1, 10 and 42 liters bioreactors) in order to test and maximize IBN production with the original strain as well as with its WSH-adapted counterparts.

For the production of the first IBN samples from WSH, GBE used its Pilot Plant facility, in Pomacle-Bazancourt, hosted and operated by the BioDemo platform of the *Agro-industrie Recherches et Développement* (ARD) company.

2. Production of bio-IBN in Pomacle

2.1. Strain and protocol development in the Evry laboratory

Strain genotype and detailed process are confidential information. Basically, GBE's strain for IBN production have an IBN metabolic pathway, introduced into a bacterial chassis derived from the E. coli MG1655 bacteria, and modified in order to increase yield. The metabolic pathway can convert the pivotal acetyl-CoA molecule, a central intermediate in metabolism, into IBN, through a cascade of enzymatic reactions performed by a series of recombinant enzymes. These recombinant enzymes include natural enzymes as well as artificial biocatalysts, generated by GBE's enzyme engineering platform, and catalyzing non-naturally occurring reactions.

2.2. The IBN pilot plant

GBE has constructed and commissioned a 500 liter Pilot Plant in 2014. This plant was designed for a production capacity of 10t of IBN per year. The plant includes a fermentation and a purification unit, described in Figure 1.

The fermentation unit comprises an automated and highly instrumented 500L fermenter, and a series of satellites tanks for substrate, basic, and acids solutions. The purification unit comprises a washing column, a drying equipment, a condensation tank and a final product recovery system

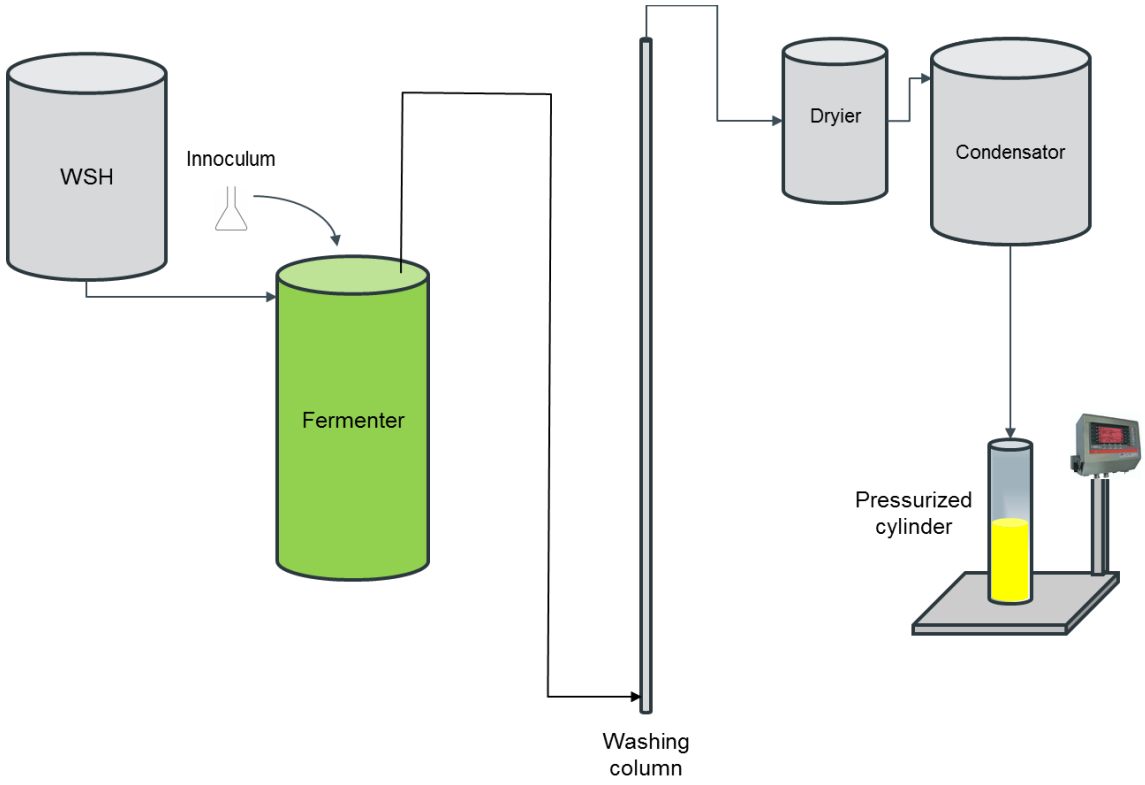

Figure 1. The 500 liters IBN pilot-plant in Pomacle.

2.3. Bold lines of the IBN production process

Pre-culture are prepared in Erlenmeyer flasks, and inoculated into the main fermenter. In parallel, the clean fermenter is prepared with fermentation medium and sterilized, instrumentation (pH, DO, gas analysis) is calibrated, and safety systems are checked.

Upon inoculation of the main fermenter, the growth phase is conducted in a fed-batch mode, in order to reach the desired biomass concentration and physiological state. When the cell density reaches a plateau, the production phase is initiated, and several process parameters are adapted in order to optimize and stabilize IBN production.

IBN is gaseous at the operating temperatures, and exits in the fermentation off-gas, which is sent to the purification unit. There, the off-gas is first washed from the fermentation material that could potentially be carried over. It is then dried and condensed. Condensation occurs at a temperature below the boiling point of IBN, but high enough to avoid the condensation of most other gases.

At the end of the run, the total condensed IBN is recovered in pressurized TPED cylinders allowing for storage and shipping.

2.4. Results at the Pilot scale

A first series of runs at the Pilot scale was scheduled in December 2017. A batch of WSH was provided by Clariant, and tested first at the lab scale in GBE's laboratory facility in Evry, in order to define the preparation, sterilization and fermentation protocols. However, at the pilot scale, several technical issues impacted the production and purification process, and no IBN could be conditioned. However, this first attempt greatly helped in optimizing the operating conditions.

Three new runs at the Pilot scale were scheduled in March 2018. For these runs, a new batch of WSH was provided by Clariant, and first assessed at the laboratory scale. This batch was prepared by a modified procedure, in order to avoid potential concretions upon sterilization. In the 500L fermentation unit, growth and production were comparable with those observed at the laboratory scale (Figure 2A): in spite of a lag phase of a few hours, the final biomass concentration was nearly identical at the laboratory and Pilot scale. At the Pilot scale, a good process reproducibility was observed (Figure 2B). However, productivity was lower than at the laboratory scale (not shown), for unknown reasons.

Gas flow was maintained at a high level during the whole run, resulting in a high dilution of the IBN in the off gas that negatively impacted the production and condensation yield. Nevertheless, a total of 1.2kg of IBN could be collected in cylinders and shipped to Ineos.

3. Conclusions

The first conditioning of IBN from second generation sugars was successfully conducted in GBE's Pilot Plant facility at Pomacle, using Clariant's WSH as feedstock and GBE's IBN producing microbe as catalyst. Different batches of WSH provided by Clariant were assessed at the laboratory scale, before proceeding to the scale up in the 500L fermenter. During scale up, the growth properties observed at the laboratory scale were fully reproduced, but a lower productivity was observed. Further studies will be necessary to understand the reasons for these differences. At the end of the campaign 1.2kg of bio-IBN were recovered in B20 cylinders, and shipped to Ineos, in Köln (Germany).

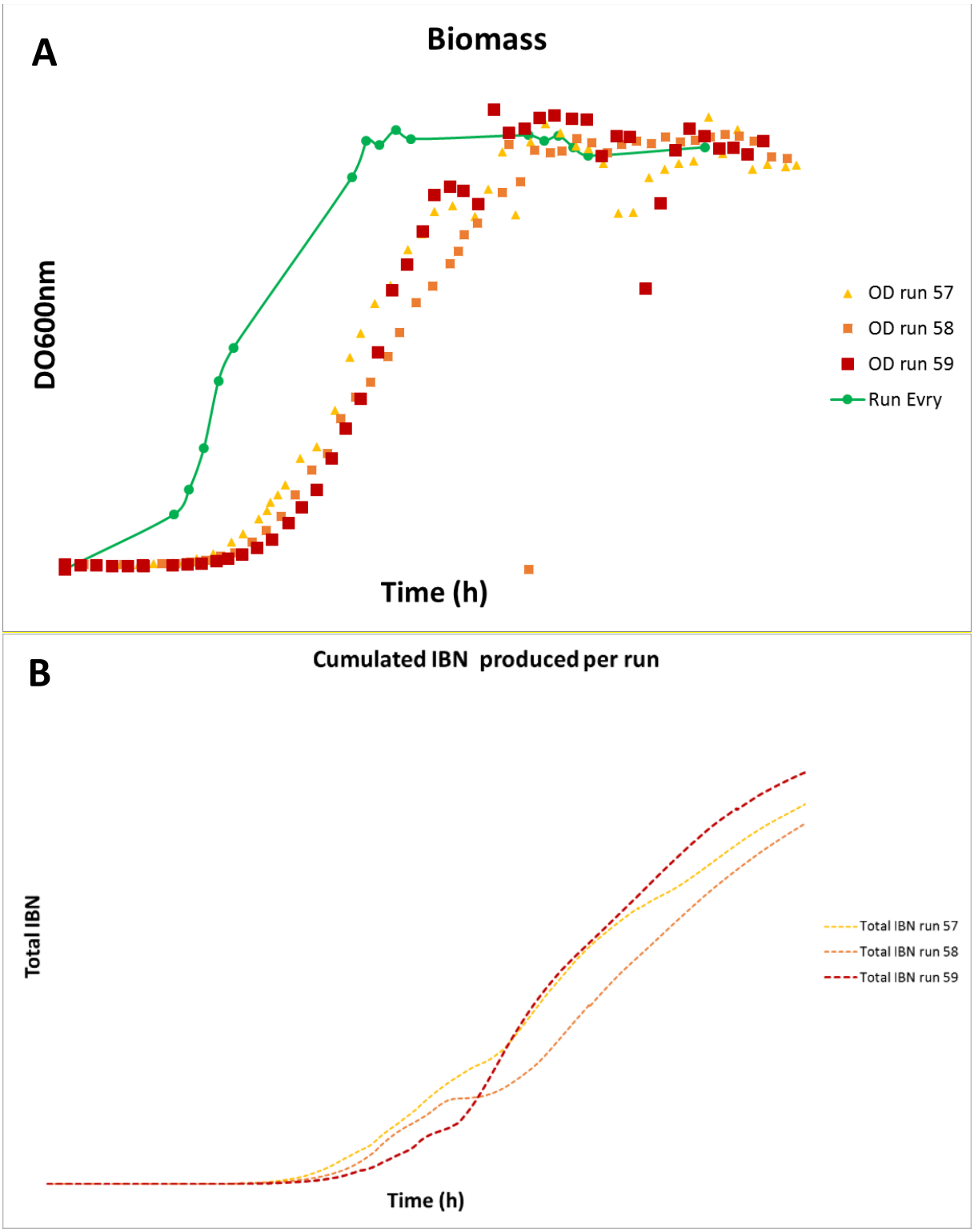

Figure 2. IBN production on WSH at the Pilot and laboratory scale

A. Growth was monitored by optical density at 600 nm (OD600nm). Runs 57, 58 and 59 are runs at the Pilot scale. A representative run at the laboratory scale is represented for comparison. **B.** Overall production over the three runs at the Pilot scale.