DEINOL

AN INNOVATIVE AND COMPETITIVE SOLUTION
TO CREATE VALUE IN THE 2G-ETHANOL INDUSTRY

OCTOBER 2014

2G BIOFUELS

THE INDUSTRIAL CHALLENGES

Optimizing the yield

- Maximizing the conversion of raw material to sugars
- Maximizing sugar utilization
- Converting simple sugars to ethanol with good productivity

Minimizing the costs

- Restricting the use of commercial enzymes (accounting for 30% of production cost)
- Reducing energy consumption
- Decreasing the number of steps in the process, therefore limiting equipment investment

Having access to a versatile process

- Adapting to different types of industrial raw materials
- Adapting to different pretreatment methods

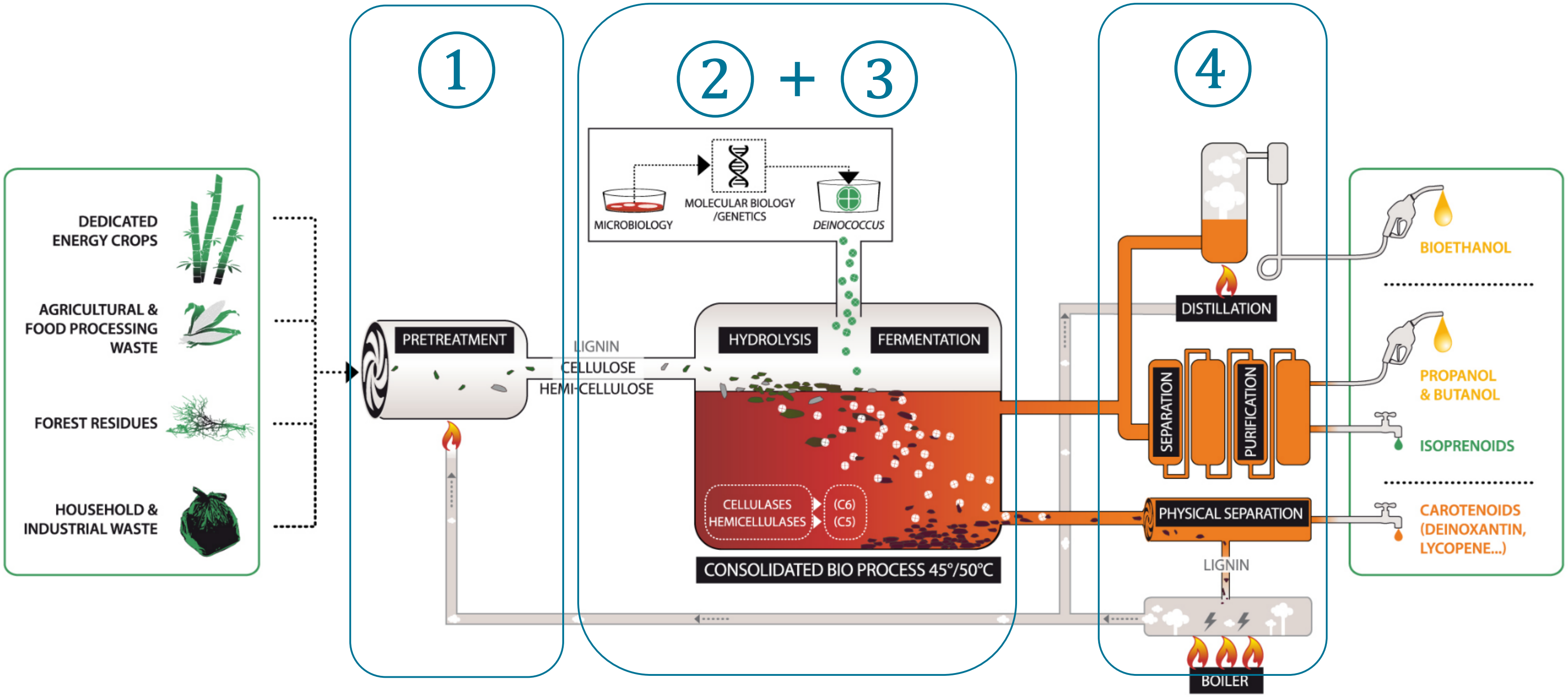
2G BIOFUELS

4 STEPS IN THE BIOMASS-TO-ETHANOL CONVERSION PROCESS

① PRETREATMENT	Chemical, thermal or mechanical separation of the 3 main components of lignocellulosic biomass: • **Cellulose** and **hemicellulose**: complex sugars • **Lignin**: non-fermentable fibres, removed and combusted to produce heat and/or power
② HYDROLYSIS	Degradation of complex sugars to fermentable simple sugars, through the action of enzymes: • Sugars with 6 carbons (**C6**), released by the hydrolysis of cellulose: **glucose, galactose, mannose, …** • Sugars with 5 carbons (**C5**) released by the hydrolysis of hemicellulose: **xylose, arabinose, …**
③ ETHANOL FERMENTATION	Biological process in which simple sugars are converted into ethanol, through the action of microorganisms (yeasts or bacteria). Commonly used microorganisms assimilate only part of the total sugars, glucose and successively xylose
④ DISTILLATION	Separation of ethanol from water by heat, to obtain concentrated ethanol

DEINOL 2-IN-1 PROCESS

A REAL VALUE-ADDED TO THE INDUSTRIAL PROCESS



Optimal operating temperature 48°C (vs ~30°C)

- Energy savings
- Savings in enzymes (-30%)
- Contamination prevention

=> Lower operating costs

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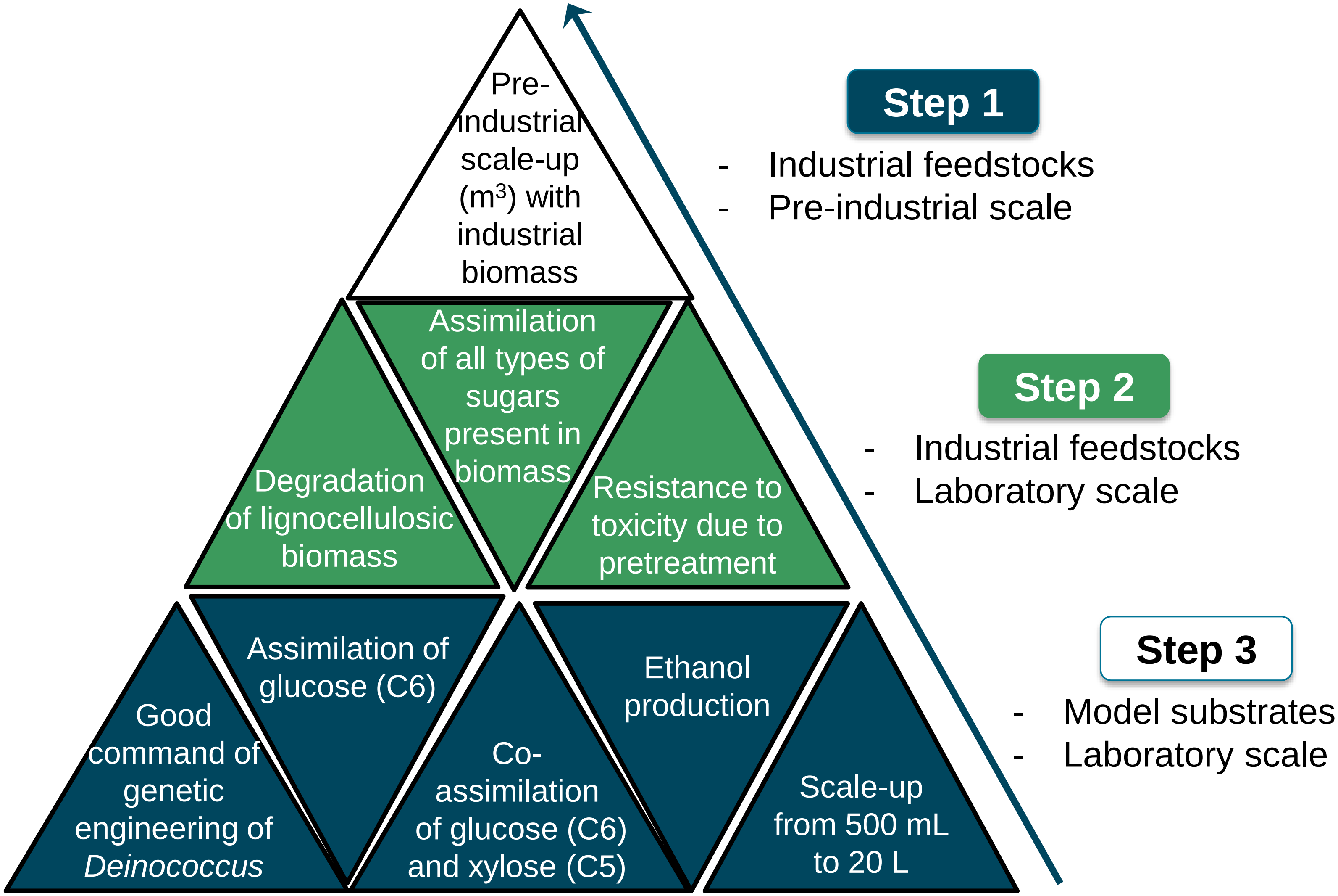
DEINOL PROCESS

A SET OF TECHNOLOGICAL BUILDINGS BLOCKS



DEINOL PROGRESS

GOOD COMMAND OF GENOME MODIFICATIONS

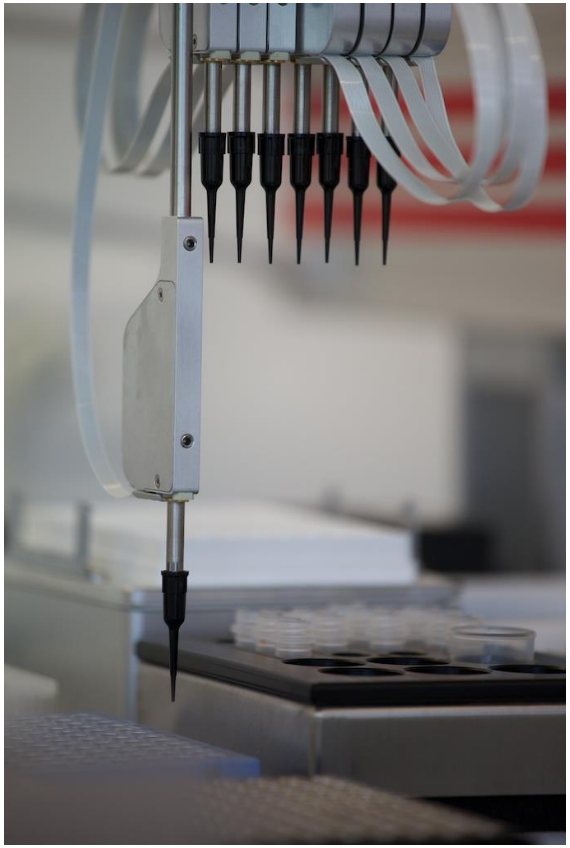
Development of a personalized version of CAD4Bio software which enables DEINOVE to quickly construct customized *Deinococcus* strains

DEINOVE now offers this unique, automated platform to each potential partner and customer so as to be able to produce the ideal *Deinococcus* for each production process.

=> Productivity

=> Quality

=> Traceability



DEINOL PROGRESS

Step 1

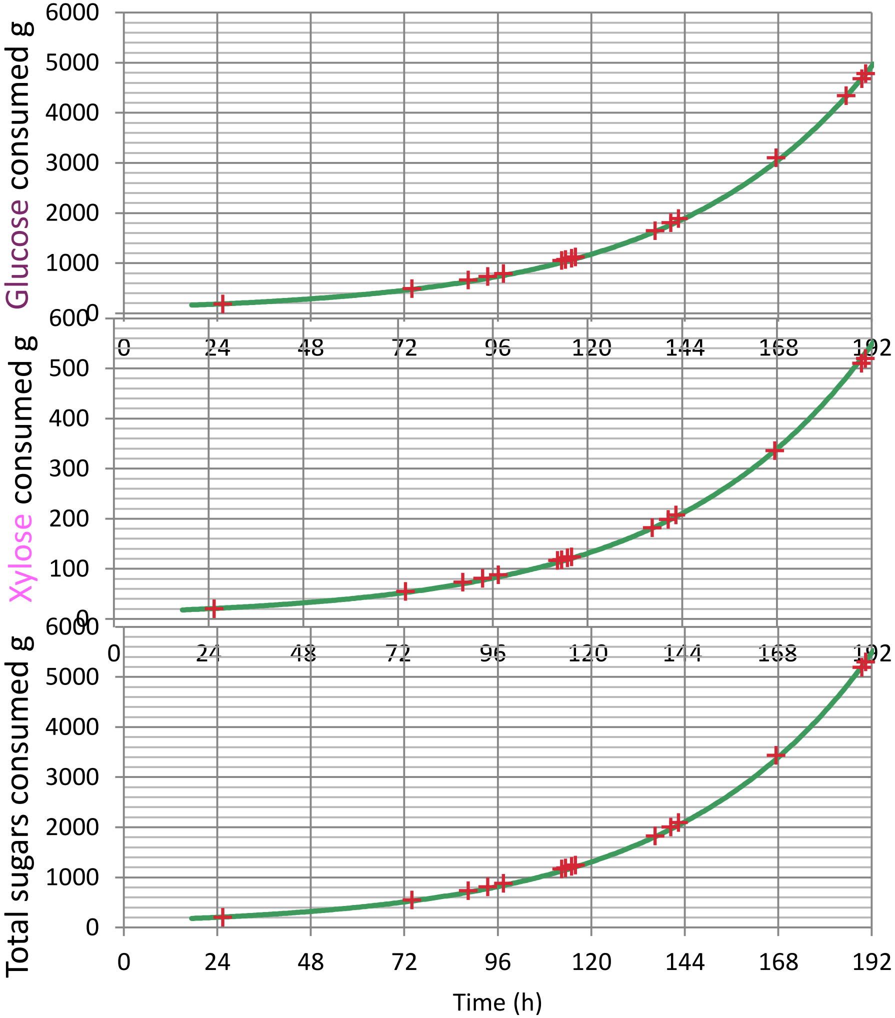
PARALLEL ASSIMILATION OF C6 AND C5

Yeasts preferentially metabolize the glucose (C6) and only after the glucose has been exhausted do the yeasts switch to the xylose (C5) (phenomenon of “diauxie”).

Deinococcus bacteria metabolize all of the sugars simultaneously.

⇒ **Better productivity**

⇒ **Better yield**



DEINOL PROGRESS

ETHANOL PRODUCTION

Step 1

9 % ethanol:

Unmatched performance in the bacterial fermentation

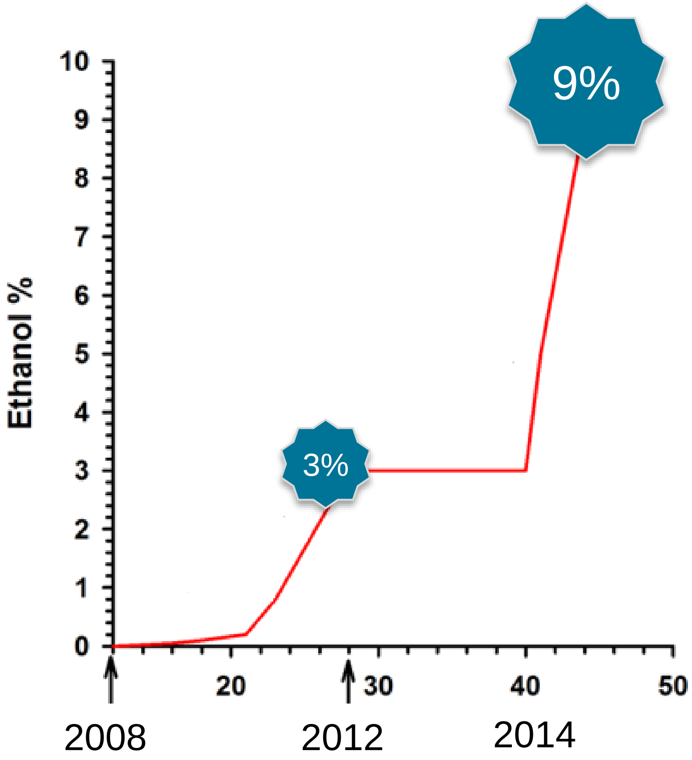
High-level productivity: 2 g / L / h

Significant conversion yield:

0,43 g ethanol / g sugar (theoretical: 0,51)

Conditions of the test:

- **20 L Fermenter**
- **From glucose (model substrate)**
- **Basic culture medium**



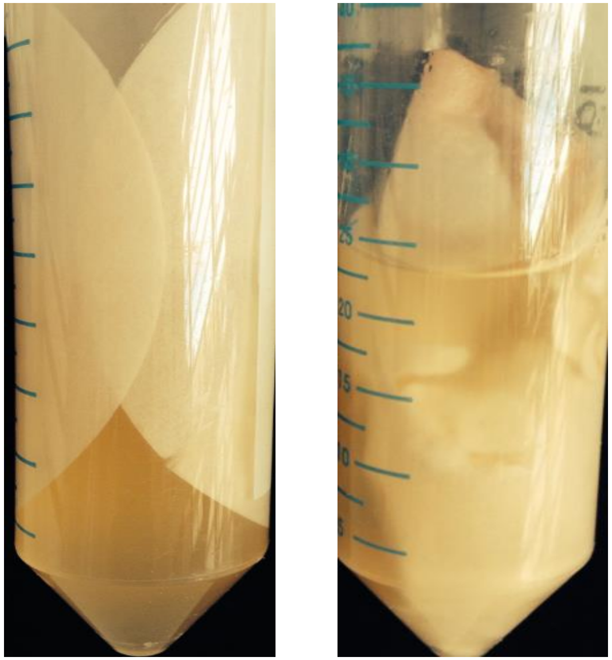
Step 2

DEGRADATION OF LIGNOCELLULOSIC BIOMASS

Current processes use commercial enzymes to break the cellulose and hemicellulose down into simple sugars.
=> 20-30% of total operating costs

The *Deinococcus* are able to naturally produce some of these enzymes, and by doing so requires fewer commercial enzymes.

=> Cost savings



Paper: lignocellulosic-based material
On the left side: no bacterium in the test tube
On the right side: action of Deinococcus, paper is digested

DEINOL PROGRESS

ASSIMILATION OF ALL SUGARS PRESENT IN BIOMASS

Step 2

Assimilating a wide range of sugars is critical to optimize the ethanol/biomass ratio.

In the current processes, several C5 sugars (e.g. arabinose) are not converted into ethanol => Yield loss

Deinococcus bacteria are able to assimilate all major biomass sugars.

=> Increased yield

	Sugars assimilated by *Deinococcus*	
C6	Glucose	✓
C6	Galactose	✓
C6	Mannose	✓
C6	Fructose	✓
C5	Xylose	✓
C5	Arabinose	✓

DEINOL PROGRESS

Step 2

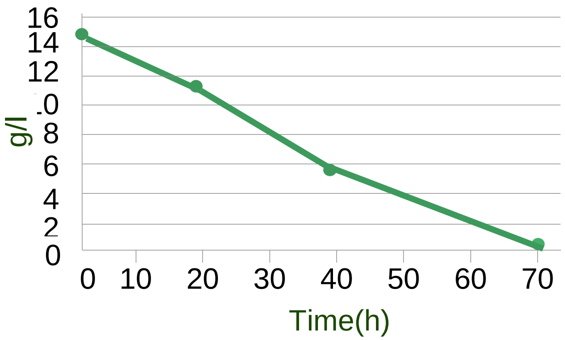
ASSIMILATION OF OLIGOMERS

An oligomer consists of a few monomer units (e.g. few simple sugars). Dimers, trimmers and tetramers are, for instance, oligomers composed of two, three and four monomers, respectively.

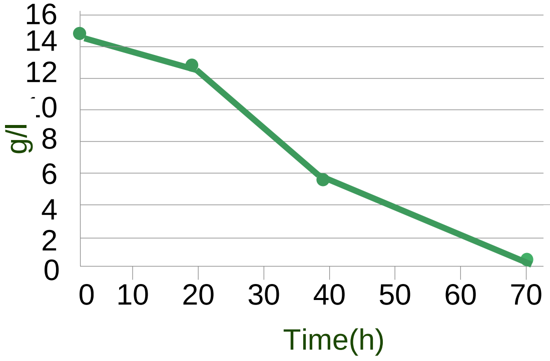
Deinococcus has the unique ability to assimilate oligomers.

=> Enzymes use reduction
=> Increased yield

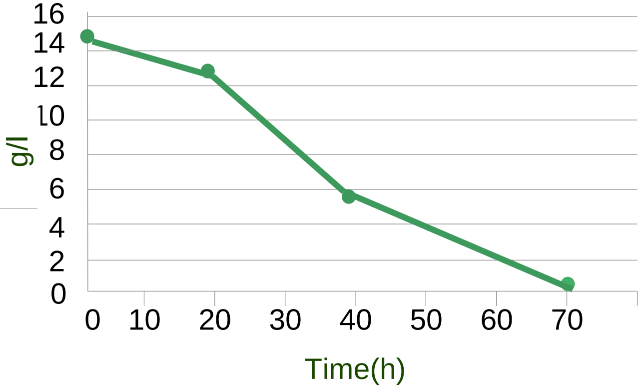
CELLOBIOSE:
2 glucose molecules



CELLOTRIOSE:
3 glucose molecules



CELLOTETRAOSE:
4 glucose molecules



Step 2

RESISTANCE TO INHIBITORS FROM LIGNOCELLULOSIC FEEDSTOCKS

Various lignocellulose pretreatments release inhibitors, toxic molecules to the microorganism responsible for the fermentation => slow down the process

Optimized *Deinococcus* can withstand higher levels of toxic molecules

=> Increased yield and productivity

IC_{50} (g/L)	5-HMF	Furfural	Aldehyde
E. coli	26	3	5
S. cerevisiae	18	1	3
D. Radiodurans R1	93	20	14
D. Geothermalis MX6-1E	104	42	12

ONGOING STEPS

Step 2

ADAPTATION TO DIFFERENT TYPES OF RAW MATERIALS & PRETREATMENT

DEINOL technology has been initially developed from model substrates:

Simple substrates: glucose, xylose, …

Switch to industrial substrates has started and is already showing signs of success:

mbi Derisking and scale-up of bio-based technologies

Various sources of industrial substrates (from several industrial partners): agricultural residues, forest residues, household waste…

Various types of pretreatment methods: acid, alkaline, thermal…

Step 3

NEXT STEPS

PRE-INDUSTRIAL SCALE-UP

1-2 L
Fermenter

X 10

20 L
Fermenter

X 10

200-300 L
Fermenter

X 10

3-5 m^3
Fermenter

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NEXT STEPS

INDUSTRIALIZATION WITH OUR PARTNERS

ABENGOA BIOENERGIA
The Global Ethanol Company

A leading global bioethanol producer: 3 billion liters a year

In Europe, United States and Brazil

A commercial scale biomass-to-2Gethanol biorefinery facility

suez environnement

Collects the waste produced by nearly 52 million people

Operates in all five continents

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