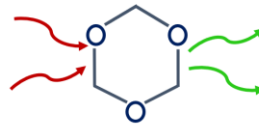


BIOLOGICAL CONVERSION OF TRIOXANE INTO BIOPRODUCTS

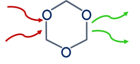
FeedstocksUnited



Jan Wery

janwery@feedstocksuntited.com

The MB6.0 Fall meeting of the
Microbial Biotechnology section of the KNVM
November 4th, 2019

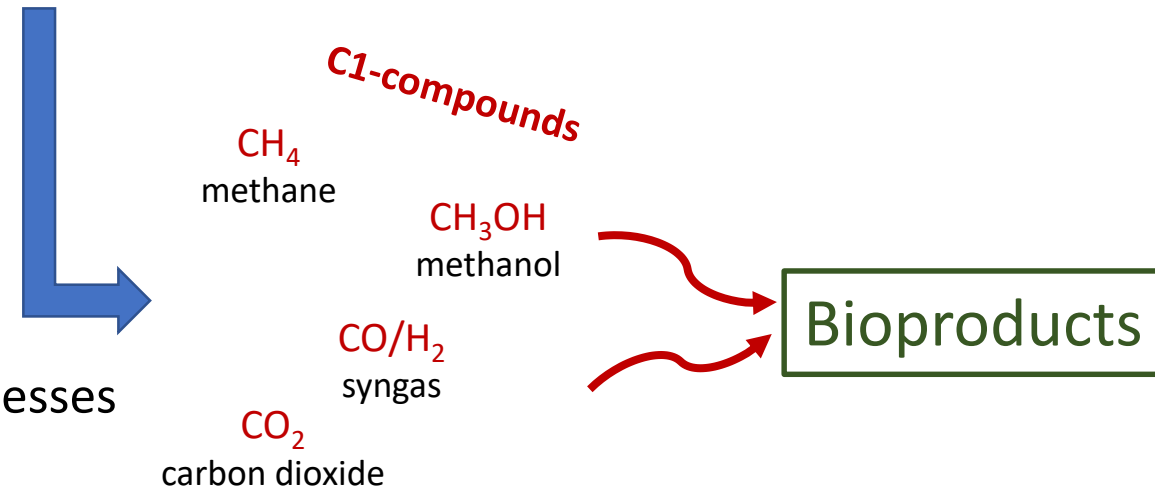


Why one-carbon compounds as feedstock for bioproduction?

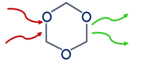
- In many cases cheaper than sugar

natural gas, off-gases, biogas, biomass, wastes

- Abundantly available
- Decoupling production from agriculture, no land-use
- Possibility to integrate with renewable electrification processes
- Great CO₂-reduction potential

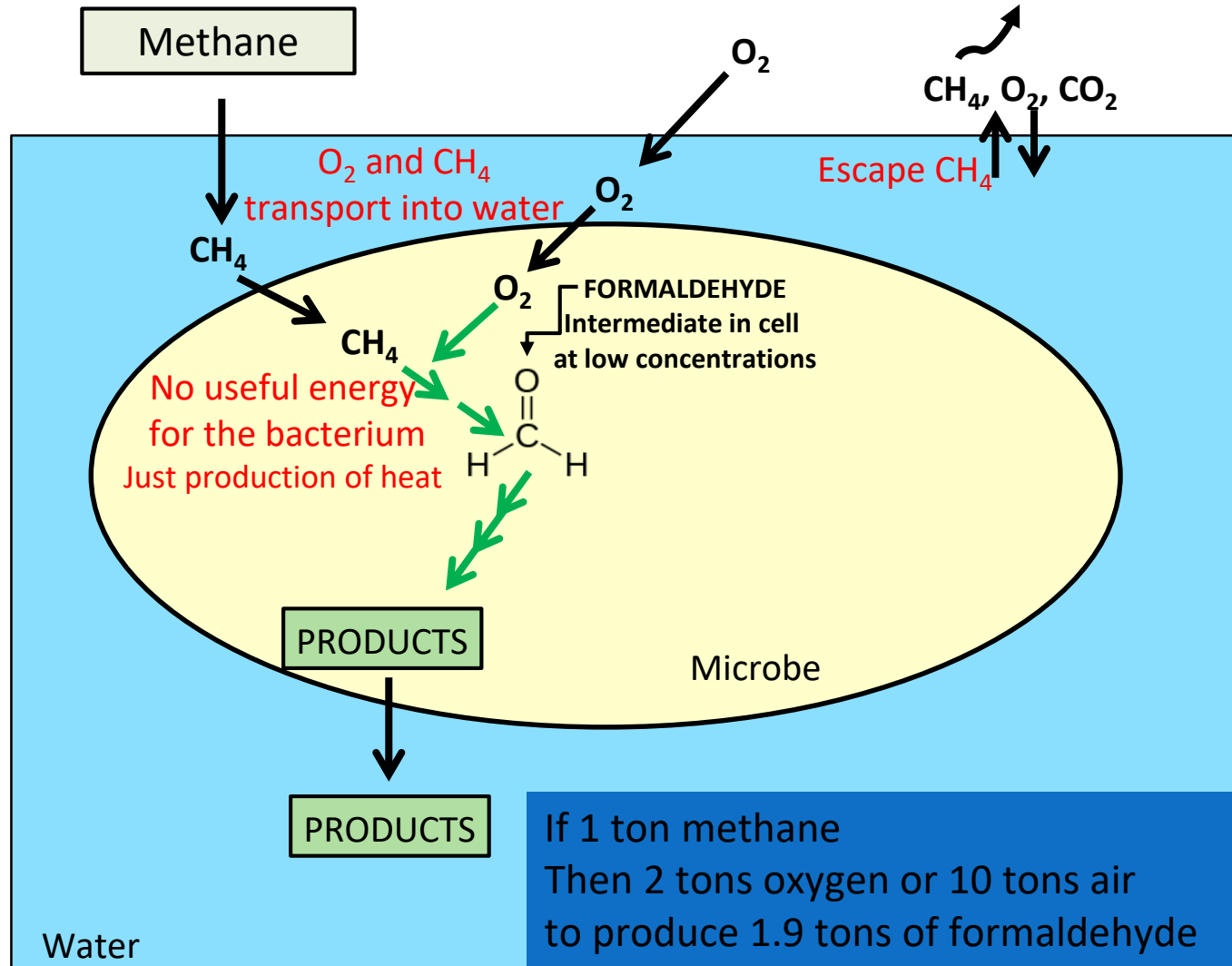
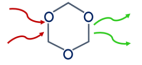


Selection of bioproducts considered on the basis of C1-compounds



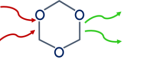
| Bioproduct | C1-substrate | Reference |
|---------------------------------------|---------------------|---|
| Single Cell Protein (SCP) | Methane | Feedkind.com (Calysta/BP/Cargill), Unibio.dk |
| Lactic acid/PLA | Methane | Calysta/Natureworksllc.com |
| Butanediols, Isobutanol, etc. | Methane | MBP Titan (Formerly Intrexon) |
| Ethanol and others | Syngas | lanzatech.com |
| Polyhydroxyalkanoates (PHA) | Methane | Strong <i>et al.</i> 2016 |
| Methionine and other amino acids | H2 and CO2 | trelystech.com |
| Lysine and other amino acids | Methanol | Brautaset <i>et al.</i> 2007 |
| Amino acids, dicarboxylic acids, etc. | Methanol | Pfeifenschneider <i>et al.</i> 2017 |
| Single Cell Protein (Pruteen) | Methanol | Imperial Chemical Industries |

Direct fermentation of C1-compounds is challenging!

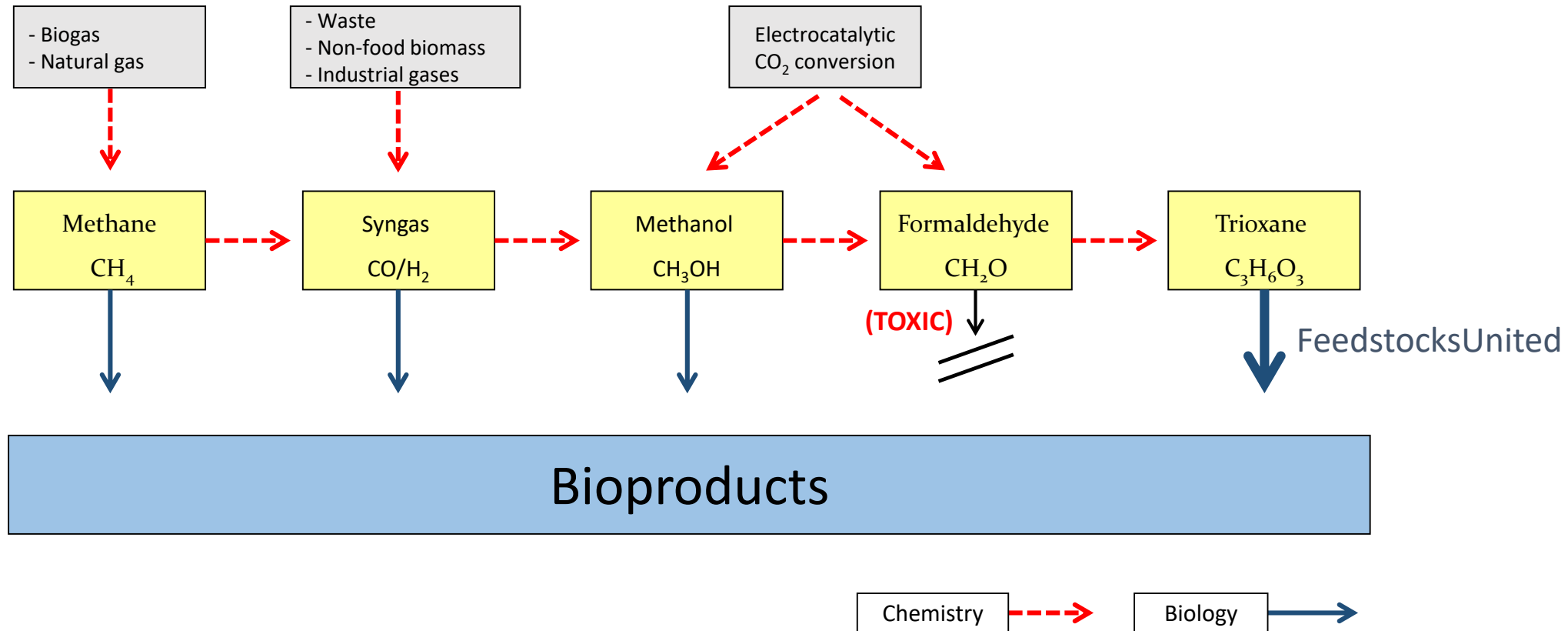


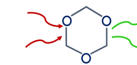
Key issues

- Oxygen supply and cooling
 - Limited volumetric productivity due to limitations in substrate and oxygen transfer
 - Excess unwanted biomass and by-product formation
 - Safety (explosion limits, handling)
- ➔ No commercial large scale process implemented after 40 years of work on the topic

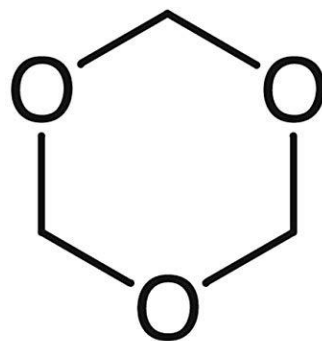


FeedstocksUnited proposes to use trioxane rather than other one-carbon compounds

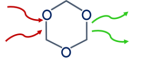




TRIOXANE

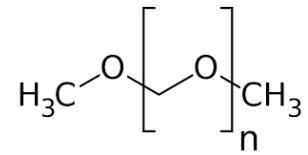


Cyclic acetal



Trioxane production from formaldehyde

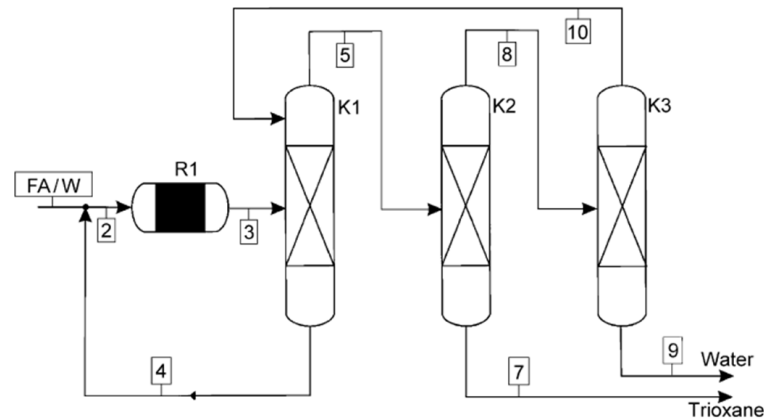
Trioxane is produced as a monomeric compound in the manufacture of polyoxymethylene (POM)



Production of POM is ~500,000 tons/year



T. Grützner et al. / Chemical Engineering Science 62 (2007) 5613–5620

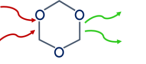


Development of a new industrial process for trioxane production

Thomas Grützner^{a,1}, Hans Hasse^{a,*}, Neven Lang^b, Markus Siegert^b, Eckhard Ströfer^b

^aInstitute of Thermodynamics and Thermal Process Engineering, University of Stuttgart, D-70567 Stuttgart, Germany
^bChemicals Research and Engineering – Process Development GCE/C, BASF AG D-67056 Ludwigshafen, Germany

Prof. Hans Hasse, now the director of the
 Laboratory of Engineering Thermodynamics,
 Technical University of Kaiserslautern, Germany

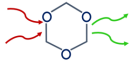


Trioxane production for fermentation purposes

Trioxane for POM production has to be ultra-pure and free of water

Trioxane for fermentations can be less pure, and water obviously is no issue

Hence, the cost price of trioxane production at fermentation specs will be substantially lower (target = sugar price range)



Trioxane as a fermentation feedstock

Physical properties:

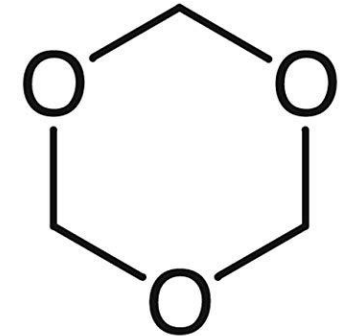
- Good solubility in water of up to 800 g/l (depending on temperature)
- Stable in water
- Volatility no issue

Bioprocess advantages:

- Not toxic to microbes
- Limited problems in combatting contaminations
- Clean stream which is very beneficial in downstream processing of bioproducts

Biological aspects:

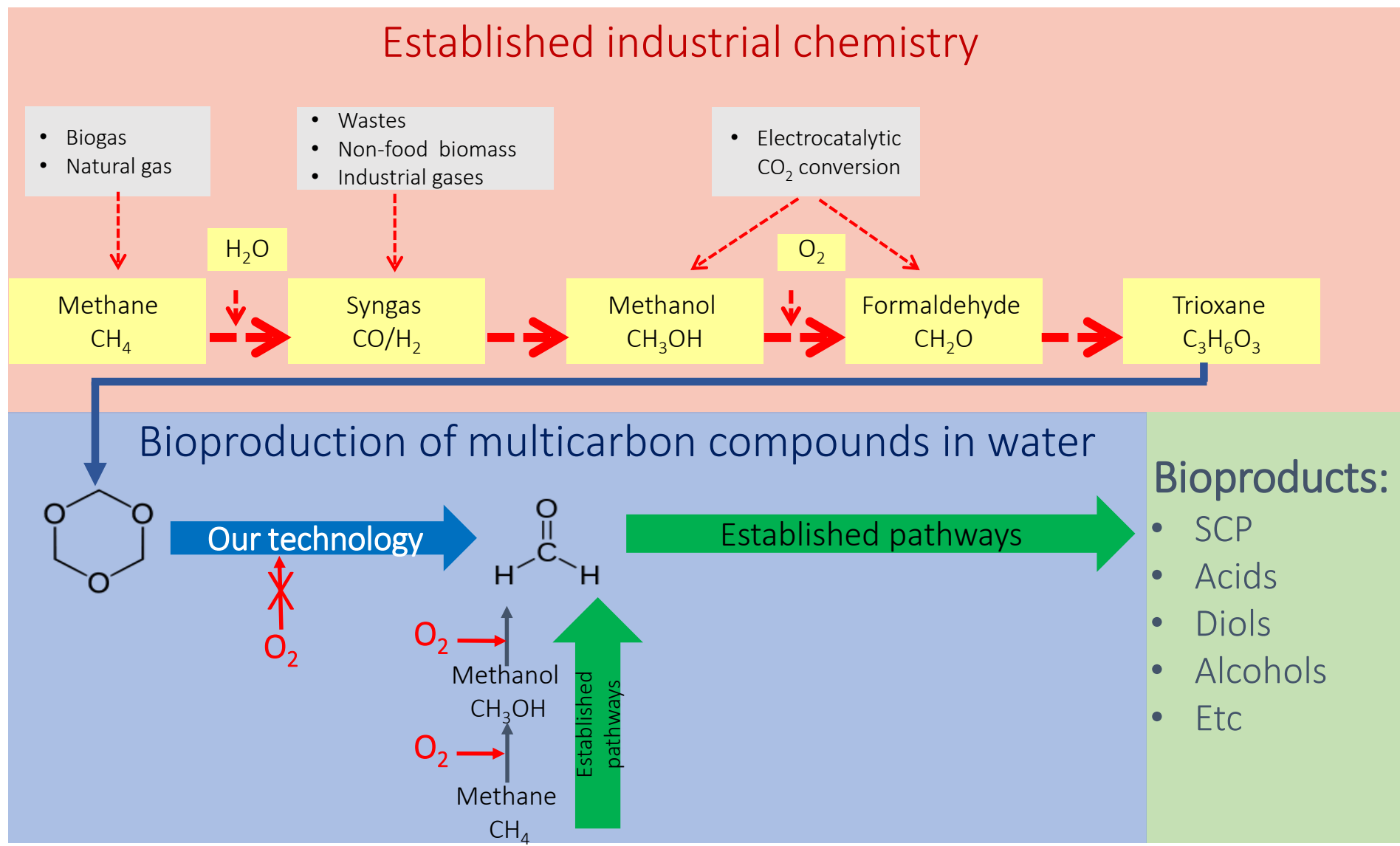
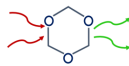
- Oxygen-independent enzymatic degradation (**our technology**)
- The oxidation/reduction levels of trioxane and sugars are the same

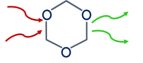


Cyclic acetal

Trioxane could be used like a sugar in fermentations

The FeedstocksUnited concept connects industrial chemistry and biology





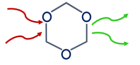
Technology: status and next steps

Status

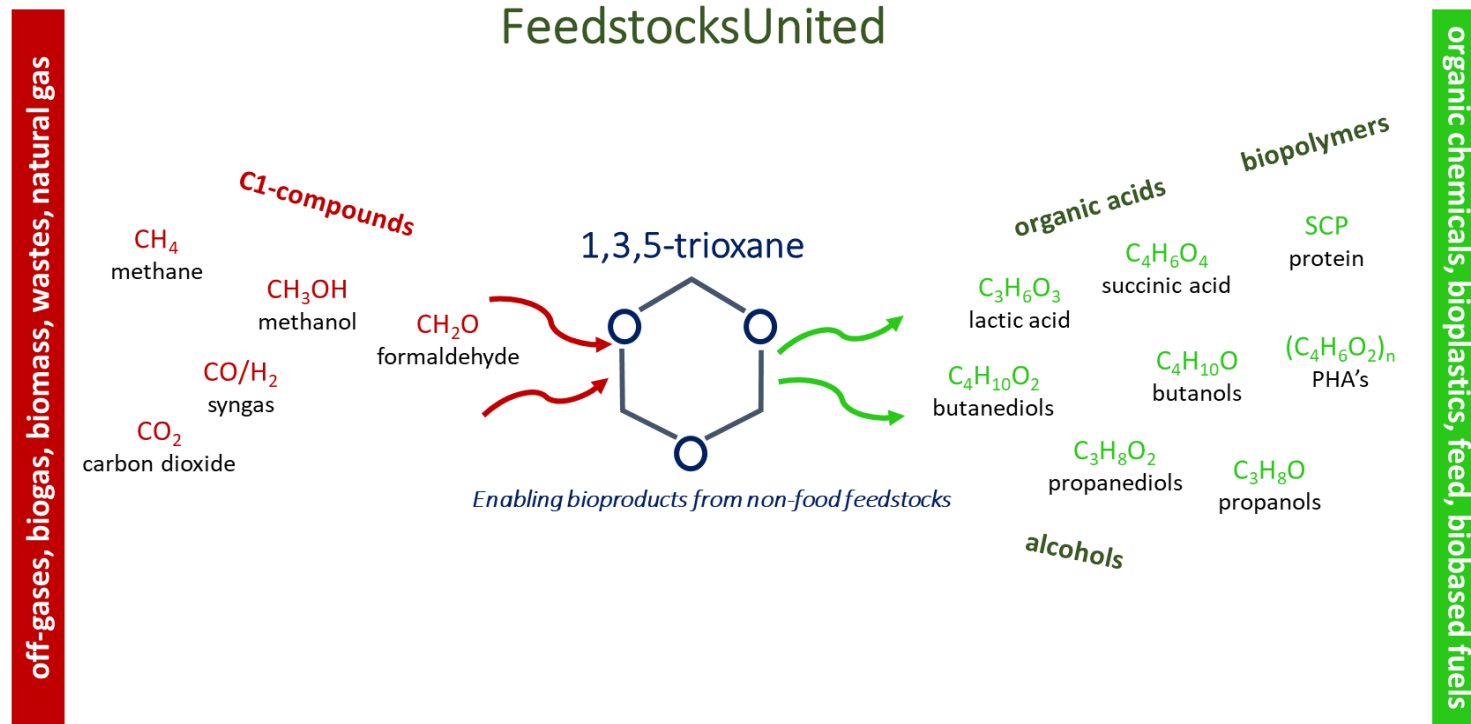
- Bacterial anoxic and oxic degradation of trioxane at very low growth rates demonstrated
- The genome of one strain has been sequenced.
- Genes encoding putative trioxane degrading activity have been identified
- A patent application has been filed with claims covering genes, organisms and processes.

Next steps

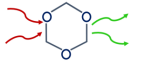
- Generate a proof-of-concept: produce desired product from trioxane by expression in a suitable host
- Strengthen patent position
- Demonstrate potential of the technology
- Assess the economic and sustainability potential in detail



Opportunities for the FeedstocksUnited concept



- **Shorter term:** cheap natural gas funneled into trioxane via established bulk chemistry
- **Longer term:** waste streams (e.g. CO₂ and biomass) funneled into trioxane via biomass gasification and electrocatalytic CO₂-conversion



In conclusion

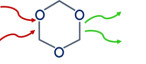
The FeedstockUnited concept enables utilization of cheap methane and derived C1-molecules by:

- Funneling these C1-molecules into trioxane via established chemical routes
- Rendering suitable microbial production hosts proprietary enzymatic activities that enable them to use trioxane “like sugar”

Advantages of trioxane over other C1-compounds in fermentations

- ✓ Less oxygen demand during fermentation
- ✓ Less heat generation and thus less cooling requirements
- ✓ Higher volumetric productivity
- ✓ Lower risk of microbial contaminations
- ✓ Lower toxicity and less safety issues
- ✓ Higher density, more efficient handling

Management FeedstocksUnited



Founders:



Jan de Bont
Managing Director



Jan Wery
R&D Director



Bart Swinkels
IP Director

Advisors:



Ger Bemer
Business



Hans Hasse
Chemical Process Engineering