SUNLIBB

Sustainable Liquid Biofuels from Biomass Biorefining

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ENERGY

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Deliverable 6.5

"Demonstration of ethanol production from lignin from the breakdown of C4 biomass"

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Workpackage: **6**
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SUNLIBB deliverables

Del No: 6.5	Deliverable Name: Demonstration of ethanol production from lignin from the breakdown of C4 biomass			
WP: 6	**Lead partner:** 11	**Dissemination level:** PU	**Delivery date (proj. month):** 38	**Actual delivery date:** 36

1. Objective

The objective of this deliverable was to demonstrate the potential of generating ethanol from the breakdown of C4 biomass.

2. Description of work

Fermentation experiments were carried out (Mahendra Raut) using Miscanthus hydrolysate sent to the University of Sheffield by partner 1 (University of York) and commercially obtained plant materials. Data from these experiments will be fed forward into Work Package 7 for simulation purposes.

Lignocellulosic biomass is a potential source of energy, but its recalcitrant nature is a major roadblock. The degradation step is the basic hurdle and needs to be optimised. The innate capacity of native microbes cannot be underestimated and throughout the SUNLIBB project and from the literature, it is clear that a single microbial system may not be best solution. For wider commercial use under difficult conditions in the field there are problems with recombinant strain development. As shown in SUNLIBB and elsewhere, both chemical and biological treatments for lignocellulose degradation are promising. It is becoming clearer that in addition to traditional strains such as *Saccharomyces cerevisiae* etc, anaerobes are raising increased interest among researchers.

In addition to our yeast work and against this background, we decided to examine the utility of anaerobes at degrading C4 biomass. We chose ***Fibrobacter succinogenes S85*** and ***Clostridium acetobutylicum ATCC824*** for the following reasons:

Fibrobacter succinogenes S85

- Efficient cellulose degrader
- Symbiotically lives in rumen
- Degrades cellulose to glucose and cellodextrin
- Provides carbon substrates to host organism and other microbes

Clostridium acetobutylicum ATCC824

- Efficient solvent producer
- Utilises variety of sugar components
- Produces acetone, ethanol, butanol and H_2

We had two major experimental programmes along these lines, as follows:

EXPERIMENT 1 - Chemical degradation & fermentation: (a) To investigate the ability of *C. acetobutylicum* to utilise sugar components from biomass hydrolysate, (b) To investigate the influence of different chemical treatments on biofuel generation. In EXPT-1 we used Chemical degradation and fermentation: (1) H_2O, (2) 0.2N H_2SO_4, (3) 0.2N NaOH.

Fig.1 Miscanthus hydrolysate

EXPERIMENT 2 - Biological degradation & fermentation: (a) To develop a co-culture system for lignocellulosic biofuel production, (b) Using innate capacity of microbes to develop microbial consortia for simultaneous saccharification and fermentation. IN EXPT2 we used biological degradation and fermentation: (1) Acid swollen (AS) cellulose (0.5%), (2) Microcrystalline cellulose (MC), (3) Raw biomass (Miscanthus).

The following methods were used, as summarised in Fig.2.

Growth on either substrate type was done and products/metabolites primarily analysed with GC-MS.

3. Results

We were able to successfully grow *C. acetobutylicum* on a range of relevant feedstocks. Furthermore, we were able to produce H_2, butanol and ethanol, as can be seen in Fig.3.

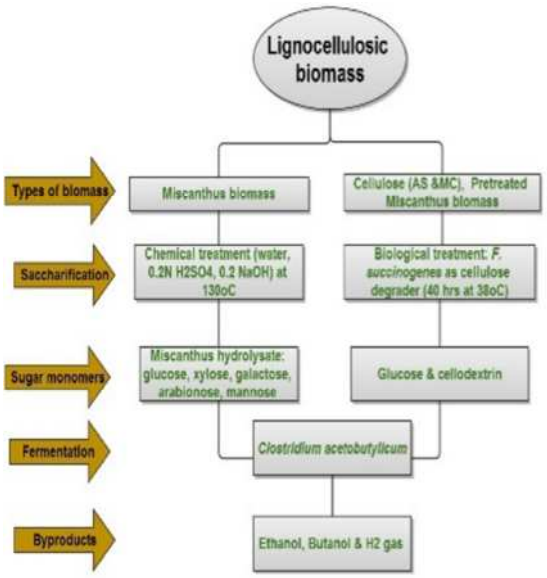

Fig.2 Experimental outline flow diagram

It can be seen that there is a distribution of performance across the three biofuels. Ethanol yields are higher than butanol.

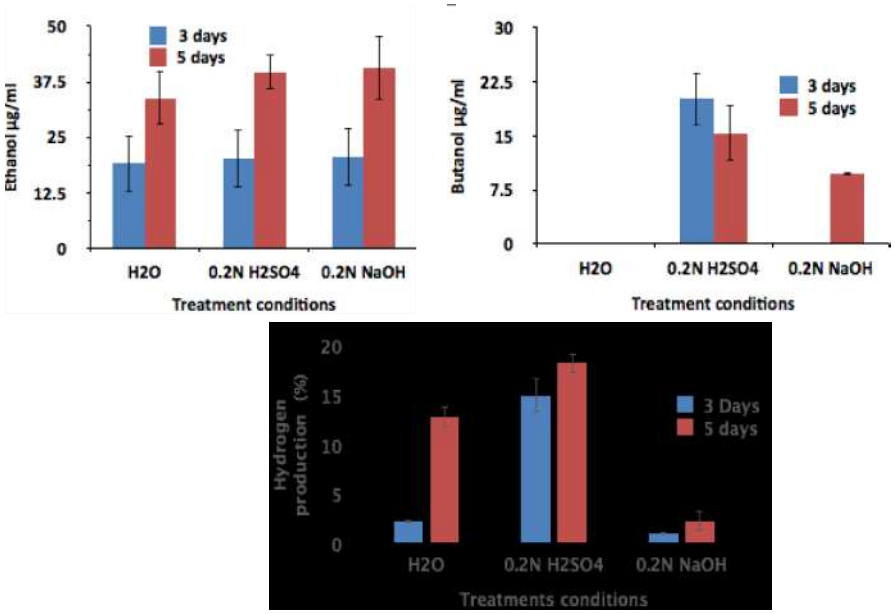

Fig.3 Biofuel generation from Miscanthus biomass hydrolysate and other treatments (as per Exp. 1) using *Clostridium acetobutylicum* ATCC824

Furthermore, we also worked with co-cultures of *Fibrobacter succinogenes* S85 and *Clostridium acetobutylicum* ATCC824. This required media optimisation as the growth media of the two strains are quite different and a compromise was sought (Fig.4). 40FS/60CA media was the best compromise.

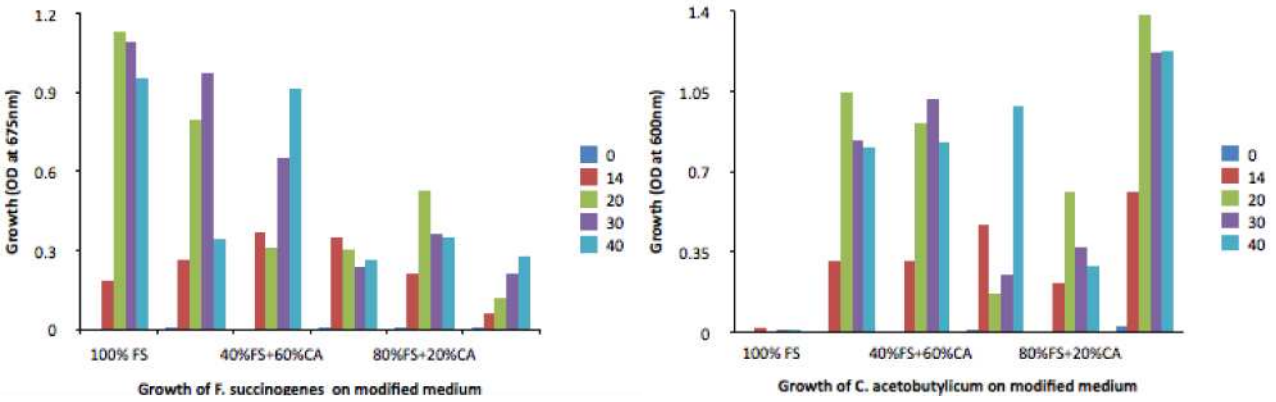

Fig. 4 Media optimisations

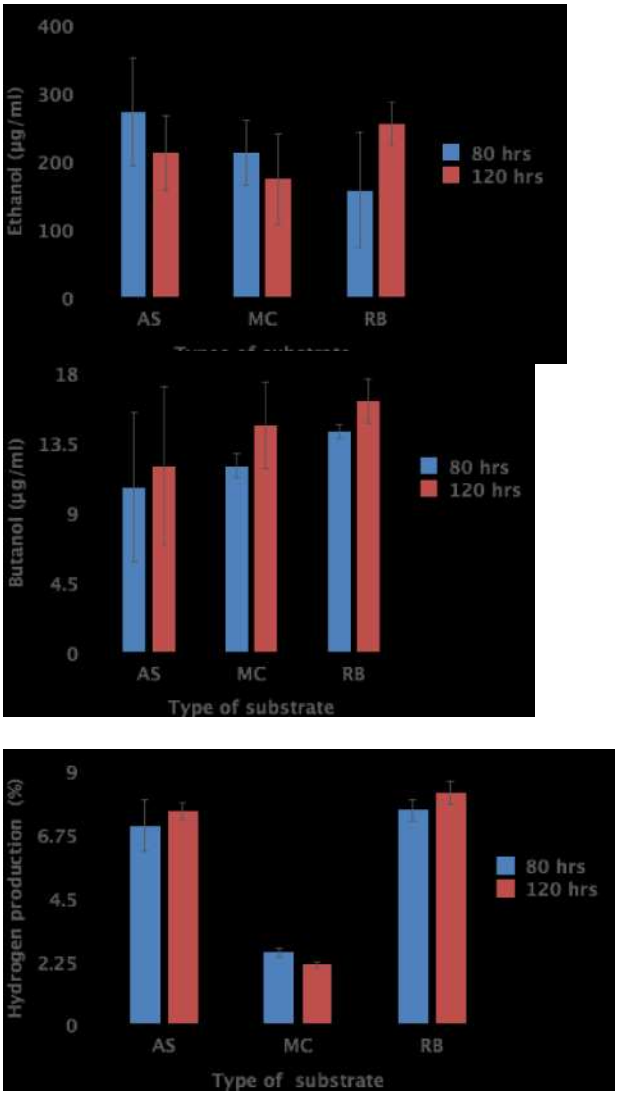

Fig.5 Biofuel generation performance of mixed consortium

As can be seen in Fig.5, considerably higher ethanol is made than in the single species (Fig.1).

4. Conclusions

Clostridium acetobutylicum can be a good candidate to utilise sugar components from biomass hydrolysate to produce a range of biofuels. Microbial consortia for biofuel production using *F. succinogenes* and *C. acetobutylicum* can provide major breakthrough in CBP development.

5. Future Work

We are spending time optimising conditions for higher yields. We are also planning multiplex – omics studies to examine mechanisms. We will attempt other mixes of feedstocks.

6. References

We are writing up three journal papers based on this work, as we have proteomics data as well. This was updated at the SUNLIBB/CeProBIO dissemination meeting in Ghent, September 2014.

The following clarification is in response to a query by the EC Reviewer about the breakdown of lignin during fermentation.
The experiments reported in Deliverable 6.5 suggest that removal of lignin from pretreated biomass could increase biofuel production.
Further experiments were carried out to investigate the ability of *Clostridium acetobutylicum* to utilise lignin.
It was shown that *C. acetobutylicum* could grow on a lignin-based medium, albeit very slowly (Fig.6). This growth led to a reduction of soluble lignin in the medium, especially under anaerobic conditions (Fig.7). Longer-term growth experiments are planned to investigate this further.
In the presence of lignin, metabolite production and metabolic processes were significantly altered. For example, 579 proteins were identified with differential expression by iTRAQ-mediated quantitative shotgun proteomics analysis.
Lignin degradation analysis and lignin by-products analysis will be carried out, and samples have already been sent to VIB (Ghent) to facilitate this.

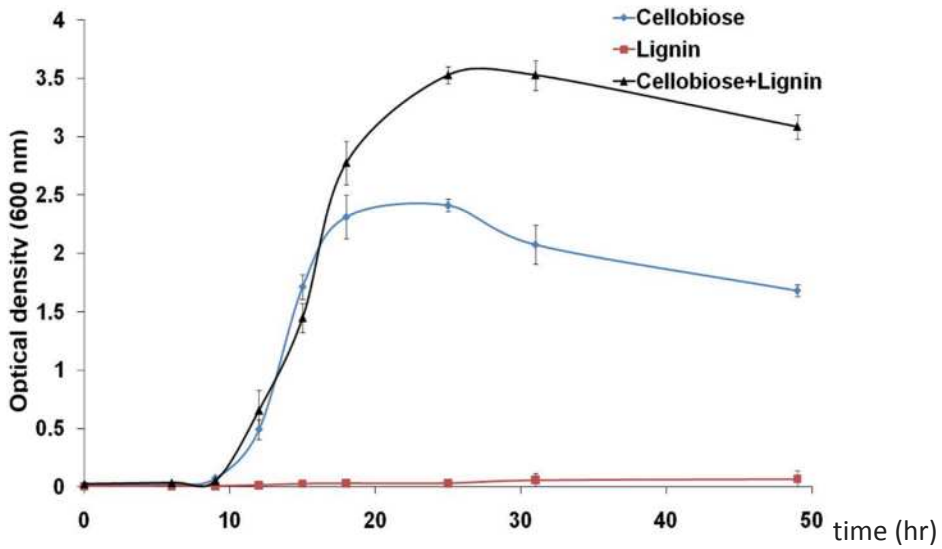

Fig.6 Growth pattern of *C. acetobutylicum*.

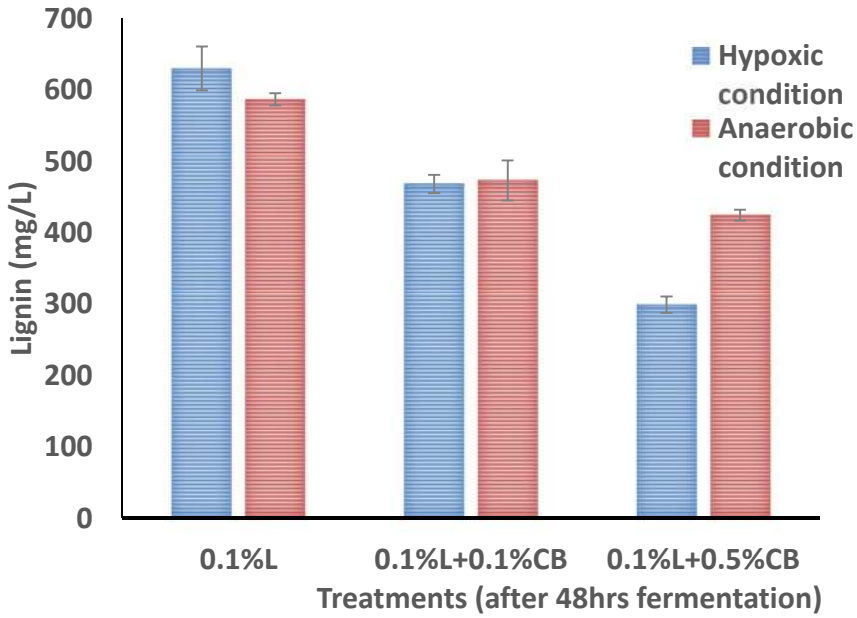

Fig.7 Changes in soluble Lignin concentration after fermentation by *C. acetobutylicum* (Comparing to control =1000mg/l). L = lignin, CB = cellobiose.