REPORT TO STAPLEDON MEMORIAL TRUST

VACATION STUDENTSHIP BURSARY

Biorefining of grass to produce high value-added products

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Student : Anaïs Giraudeau Email : anaisgiraudeau@gmail.com

Supervisor: Dr. Sreenivas Rao Ravella Email: rsr@aber.ac.uk

Host Institute: IBERS, Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Gogerddan, Aberystwyth, SY23 3EE

Abstract

The aim of the project is to develop the processes to produce high value-added products from grass. The objective is to learn the Design of experimental (DOE) concepts related to the Taguchi method and techniques of fermentations using grass juice and fibre. The yeasts use the sugar such as in the ray-grass juice to transform ethanol.

Introduction

In the Wales, an area of Mha representing 62% of agricultural land are utilised for growing perennial ryegrass. In IBERS a perennial ryegrass variety has been developed and exploited, AberMagic, for grass biorefining. The biorefining of the grass juice produces ethanol which is a high value-added product. The yeasts ferments the sugar in the grass juice.

The hemicellulose fraction present in the grass fibre be able to use like a raw material to produce high value-added products, such as xylitol and xylooligosaccharides. To increase sugar release and thus economics of an overall process, two types of pre-treatment can be used. Steam explosion followed by enzymatic digestion are used to develop process of xylooligosaccharides and Xylitol from AberMagic grass fibre. To optimize the fermentation process and pre-treatments, the Taguchi method was used.

Materials and methods

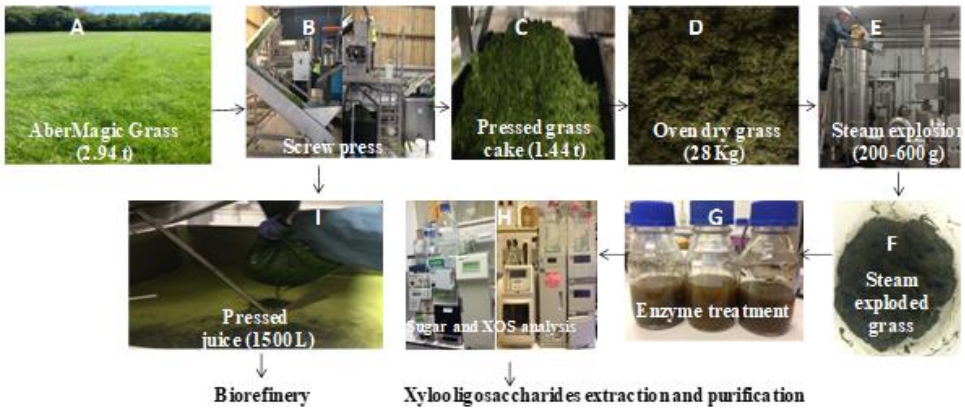

Figure 1: Different steps of the biorefining of grass

Biomass

The raw material for the biorefining of grass is grass juice and fibre. AberMagic, DART and High Sugar Grass were harvested at Aberystwyth University, UK during spring 2009.

To produce grass fibre hydrolysate, ABERMAGIC (H140617) fibre was used. This grass was harvested at Aberystwyth University, UK, in spring 2017 and screw pressed, juice was stored in a freezer and the pressed fibre dried and stored in a barn.

Analytical Methods

Sugars and fermented product analysis

To quantify the monomeric and polymeric concentration of carbohydrates, HPLC technique was used. The different monomeric carbohydrates that were quantified are glucose, xylose, arabinose, fructose ranging from 25 to 3.125 g/ml. For this, Dionex High Performance Anion Exchange Chromatography was used. Samples were centrifuged 1 min at 10000 rpm and the supernatant is removed, diluted with the mobile phase, then filter sterilized through 0.22µm with 25µL of each sample. The samples are injected into the HPLC column.

Figure 2: Collected samples of the YEPX medium for HPLC analysis

Carbohydrate analysis

It is an analytical procedure carbohydrate of the content in the fibre without pre-treatment and in the fibre after the steam explosion were determined. Using 100 mg of freeze dried substrate are added to 1 mL 72% H_2SO_4 and mixed at 30°C for one hour at 200 RPM. Then, was diluted to 4% H_2SO_4 by the addition of 28g of deionized water, autoclaved at 121°C for 60 min, then cooled to room temperature and pH to 6 was adjusted using $CaCO_3$. To quantify sugar monomers, Jasco HPLC equipped was used.

Pre-treatment

The pre-treatment of grass fibre was performed using steam explosion to release monomeric sugars like xylose and arabinose from hemicellulose fraction, further for the yeast to bio-convert it to xylitol or ethanol. For the pre-treatment, 1% of phosphoric acid was used to imbibe the grass fibre before steam explosion. The samples were incubated at 50°C for 1 hour and the excess liquid of grass fibre was separated then steam exploded.

Isolation of yeasts

Five samples of rotten wood were collected in the forest of Gogerddan, at Aberystwyth, in Wales. Yeasts were isolated using Rose Bengal agar medium with chloramphenicol and later identified as *Kluyveromyces dobzhanskii* and *Scheffersomyces sp.*,. Also, *Metschnikowia chrysoperlae* (M2) which was isolated in July 2017 was used for grass juice fermentations.

Results:

Identification of yeasts

Based on genomic DNA (D1/D2 domain of LSU rRNA gene) analysis yeasts were identified as follows

Strain ID	Putative Genus Species	Query Coverage	Identity
W4	*Kluyveromyces dobzhanskii*	98	98
W7	*Kluyveromyces dobzhanskii*	97	97
W9	*Scheffersomyces sp.*	94	98
M2	*Metschnikowia aff. chrysoperlae*	87	99
S1	*Metschnikowia aff. chrysoperlae*	88	99

Fermentation of glucose and xylose using novel yeasts

Fermentation using yeasts strains W4, W7, W9 and A were carried out to produce xylitol from xylose and S1, M2 were carried out to produce ethanol from glucose. W9 and A were selected to ferment the grass fibre hydrolysate. M2 was selected to ferment the grass juice to produce ethanol. Moreover, the fermentation of the juice and grass fibre hydrolysate was a success and the A strain was able to produce xylitol from grass fibre hydrolysate with in 48 hours.

Economic and market potential

IBERS scientists are classed seven in rank in the research areas of agricultural, veterinary and food sciences in UK. Scientists at IBERS have successfully commercialised agricultural rye grasses for many years and recently, at the breeding centre, they have developed a high sugar rich (30-40 % sugar) grass that can be potentially converted into bio-based products. IBERS research's goal is to help farmers in developing pasture management systems, which can allow different grasses to be produced for their desired levels of livestock whilst having the option of producing a surplus for biobased products. Moreover, grassland could be multi-functional for both biobased products and livestock production.

IBERS is home to the National Plant Phenomics Centre and the BEACON centre of excellence for biorefining; a £ 20 million partnership between Aberystwyth, Bangor and Swansea universities.

BEACON is a winner of European Union RegioStar award in 2014. The BEACON centre developed at IBERS has been using this developed high sugar grass for biorefining. BEACON team also works with up to 140 businesses located in Wales.

Within 2030, the total production of grass will represent between 20 and 111 Mt in Europe. The article 'Future biogas : Animal manure, straw and grass potentials for a sustainable European biogas production' allows to evaluate the amount of grass available in Europe by this time. The aim of the study is to know if there is enough grass available to be used for the Biorefining in industrial scale.

Experience gained during this project and impact

This internship in the BEACON with IBERS biorefining research team has improved my knowledge of the biotechnology field and learn more about the biorefining processes. The objectives of this project were fulfilled successfully in due course. During this internship, I have acquired good laboratory practice, industrial biotechnology competencies at pilot scale. Also, this experience was a good opportunity to introduce myself to the biorefining concept and its economy. The internship made me learn a lot on how to manage a project and how to organize in order to finish it in time which is a crucial quality in research.

The BEACON+ is an award-winning project, which has assisted more than 150 businesses and undertook collaborative R&D projects with 50 businesses. The research that BEACON+ has undertaken with businesses is helping to foster the transition to a low carbon society, presenting significant environmental and business impacts.

Acknowledgement

I would like to have a special thanks to Stapledon Memorial Trust which made this 2018 summer internship at the IBERS at Aberystwyth a reality. I would like to thank the Dr Sreenivas Rao Ravella, who welcomed me three months in his laboratory this summer. I was glad to be part of his team during this internship. I also would like to thank David Thomas and Steven Bourne who helped me a lot during this internship in our different tasks. Finally, a special thank the whole Beacon team for their hospitality.

Anaïs Giraudeau