



Phoebus Power



Department for  
Business, Energy  
& Industrial Strategy



**Phoebus Power**  
*Affordable Solutions for a Sustainable World*



## Biohydrogen from Dark and Photo Fermentation

### Final Report

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**Phoebus Power Ltd**



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## List of acronyms

AA – Acetic Acid

AD – Anaerobic Digestion

BA – Butyric Acid

BECCS - Bioenergy Carbon Capture and Storage

BEIS – Department for Business, Energy and Industrial Strategy

CAP – Common Agricultural Practices

CCC – Climate Change Committee

CCS – Carbon Capture and Storage

CFD – Computer Fluid Dynamics

CHN – Carbon, Hydrogen and Nitrogen

CHO – Carbon, Hydrogen and Oxygen

CHP – Combined Heat and Power

COD – Chemical Oxygen Demand

CO<sub>2</sub> – Carbon Dioxide

DEFRA – Department for Environment, Food and Rural Affairs

DF – Dark Fermentation

DNS - Dinitro salicylic acid

ECS – Energy Crops Scheme

FM – Fermentation Media

GHG – Greenhouse Gas Emissions

GC – Gas Chromatography

GM – Growth Media

HCl – Hydrochloric acid

HRT – Hydraulic Retention Time

IRR – Internal Rate of Return

LCA – Life-Cycle Analysis

MFC – Microbial Fuel Cells

MSW – Municipal Solid Waste

Mt – Million tonnes

MTCC – Microbial Type Culture Collection and Gene Bank

NaOH – Sodium hydroxide

NG – Natural Gas

NNFCC – National Non-Food Crops Centre

NPV – Net Present Value

OD – Optical Density

Odt – Oven dried tonnes

OSR – Oilseed Rape

PA – Propionic Acid

PAR – Photosynthetically Active Radiation

PBR – Photo Bioreactor

PF – Photo Fermentation

SRC – Short Rotation Coppice

ROI – Return on Investment

RPM – Rotation Per Minute

TS – Total Solids

UK – United Kingdom

VA – Valeric Acid

VFA-Volatile Fatty Acids

VS – Volatile Solids

WACC – Weighted Average Cost of Capital

## Executive Summary

The feasibility report demonstrated the hydrogen generation from sequential two-stage dark and photo fermentation from various biomass. Among all the biomass Napier grass resulted in the highest yield of biohydrogen followed by willow through a sequential dark and photo fermentation. This H<sub>2</sub> yield is comparable to most of the reported values in literature but is still lower than the theoretical yield. This preliminary study developed a proof-of-concept for biohydrogen production by integrating dark and photo fermentation from various biomass. Three major steps identified that need further optimisation such as pre-treatment approach, use of high H<sub>2</sub> yielding microbes during dark fermentation, in-line gas analysis and process parameters of photo fermentation (pH, VFA, temperature, light intensity), etc.

Other important strategies include genetic engineering of the H<sub>2</sub> producing microbes to enhance the actual yield. This includes overexpression of hydrogen-producing genes (native and heterologous), knockout of competitive pathways, creation of a new productive pathway, and creation of dual systems and genetic mutations. In the long-term, we aim to take this study forward by selecting a few genetically modified strains for high hydrogen production.

The study projects integrative dark and photo fermentation system. The approach involves scale-up from the lab to bench scale leading to demonstration project of 20 tons/day feedstock. The CO<sub>2</sub> from the H<sub>2</sub> generation is captured to produce carbon negative fuels. The study has initiated the upscaling approach by aligning the following strategies:

- Evaluating a range of photobioreactor systems and concluded by designing a photobioreactor system that can be used in two modes: either for the cultivation of phototrophic microorganisms (upright and bubbling) or for hydrogen production or other anaerobic products.
- Modular process and automation of the photobioreactor for batch processing.
- Similarly, a dark fermentative reactor is designed and evaluated based on local weather conditions, feedstock availability and HRT.
- List of local parameters to be considered for setting up the bi-phasic model.
- Two financial models have been developed, based on a throughput of 20 tonnes and 50 tonnes of feedstock per day generating up to 0.35 to 0.875 tonnes/day of hydrogen for assumptions of CAPEX and OPEX.
- Levelised cost of Hydrogen by 2030 for 20 and 50 tons/day is expected to be in the range of £2-£3 /kg.
- Preliminary Life-cycle analysis, environmental and economic benefit-risk analysis have been conducted.
- CO<sub>2</sub> emissions reduction of the biphasic process using microalgal cultivation.

## 1 Introduction

The UK has legally committed to achieving net-zero emissions by 2050. To deliver on this ambition the generation of hydrogen, particularly, to support the decarbonisation of process heat and carbon-intensive ('hard-to-treat') industrial sectors will be crucial. One method to produce hydrogen is via bioenergy using biogenic residues and waste feedstocks which has the potential to generate negative GHG emissions, if combined with CCS. This project is a detailed feasibility study looking at the opportunity for conversion of both organic and lignocellulosic feedstocks into hydrogen. Combustion of hydrogen creates only heat and water as a by-product. Therefore, no carbon emissions are associated with the burning of hydrogen. Biohydrogen has the potential to be cost competitive with Green Hydrogen, which is hydrogen produced via electrolysis with the electricity from renewable energy source like solar or wind. Hydrogen can be stored and dispatched for use as required and any excess production can be either sold to third-parties or utilised onsite for uses other than provision of heat (e.g., to power fuel cell electric farm vehicles).

### 1.1 Brief outline of the scope of the project

This project explored the technical and financial feasibility for the conversion of biomass into hydrogen. The project is a Phase 1 project funded through the UK Government's Department for Business, Energy & Industrial Strategy (BEIS). It is a collaboration between Phoebus Power (Lead Partner) and Grassroots Energy (Technology Partner) and Newcastle University as part of the Hydrogen Bioenergy with Carbon Capture and Storage (BECCS) Innovation Phase 1 programme. The outcome of this project is a detailed feasibility study that comprehensively assesses the financial and technical aspects of a biphasic dark fermentation and photo fermentation system (biofermentation) that converts organic and lignocellulosic feedstocks into hydrogen. Four biomass and waste feedstocks have been analysed and characterised: i) Straw (agricultural residues) ii) Short Rotation Coppice (SRC) Willow iii) Napier grass (energy crops) and iv) Hay (green waste).

### 1.2 Project Objectives

1. Explain the feasibility of a unique biphasic system of organic waste by exploring isolated groups of microbes.
2. Explore the feasibility of a biorefinery model with a biphasic anaerobic system as the central unit.
3. Evaluate approaches to use biohydrogen as an energy resource.
4. Feasibility of CO<sub>2</sub> sequestration using an algal pathway.



## 2 Biphasic system assessment and feedstock analysis (Deliverable ID 2)

### 2.1 Characterisation of different feedstocks

#### 2.1.1 Agricultural residues: straw

Agricultural residues come in the form of rice straw, wheat straw, rice husk and corn stover that are primarily left on fields after harvest, deployed as fodder and landfill material or burnt for heat and electricity as well as CHP units. Straw is a naturally abundant biological resource and because it is rich in cellulosic material it makes for an ideal feedstock for biohydrogen production. From a UK perspective, straw is a by-product of cereal crop cultivation with the majority derived from wheat, barley, OSR and to a lesser degree oats. The composition of straw is given in Table 1. The cellulose and hemicellulose content makes extracting fermentable sugars from straw a lot easier in comparison to wood or MSW which are rich in lignin and heterogeneous. Straw is also an enticing feedstock for biohydrogen production due to its biogas yield of 242-324m<sup>3</sup>/t.

**Table 1** Typical composition of agricultural straw residues

%	Wheat	Barley	Oat	OSR
Lignin	15-21	14-19	16-19	18-23
Cellulose	33-40	31-45	31-48	35-40
Hemicellulose	20-25	27-38	23-38	27-31
Ash	3-10	2-7	2-7	3-8

#### 2.1.2 Energy crops

Energy or bioenergy crops tend to be perennial crops that are high yielding. Examples in the UK predominantly come in the form of miscanthus and SRC originating from willow or poplar and are the most widely planted species. Their primary use is for power stations where they are burnt to generate heat and power (electricity) via CHP units. Aside from a minimal amount that is used for animal bedding miscanthus and SRC are dedicated to bioenergy generation making them an appealing feedstock for biohydrogen. Miscanthus has a moderately high biogas yield of 179-218m<sup>3</sup>/t. SRC, specifically, is deemed a feasible bioenergy system when assessing its life-cycle compared to conventional energy production. Akin to straw, they primarily consist of cellulose as well as hemicellulose which again means this type of material is ideal to garner the fermentable sugars needed for biohydrogen production. Their specific composition is stated in Table 2.

**Table 2** Typical composition of energy crops: miscanthus, SRC willow and poplar

%	Miscanthus	Willow	Poplar
Lignin	6-13	25	22-25
Cellulose	31-55	30	35-48
Hemicellulose	25-38	45	19-22



Cellulose	+	61-89	-	-
hemicellulose				
Ash		2-3.9	1-3	1-3

### 2.1.3 Green waste

Green waste, like food waste, is a biodegradable material and is occasionally combined with other organic waste for recycling and composting purposes. It contains garden or amenity land residues which include grass, hedge trimmings and horticultural green waste. For this study, we will be focusing on grass or specifically herbal leys given its significant biogas potential. Herbal leys are composed of a complex seed mixture of grasses, legumes, and herbs. They can be sown on arable and horticultural land, vegetable fields and temporary grassland that can easily fit into arable and mixed farming rotations. What makes grass species appealing especially for biogas and biohydrogen production is its composition: 32-39% is made up of cellulose; 31-43% hemicellulose and 3-6% lignin perfect to create the sugars required for the biofermentation process. Grass has an incredibly high biogas yield of 298-467 m<sup>3</sup>/t.

## 2.2 Availability of feedstocks in the UK

### 2.2.1 Straw

UK production of straw is determined and estimated by applying the straw crop yield for England to the total UK area. Given that production levels in the UK normally range from between 11-12 million tonnes per year, with typical yields equating to 4 tonnes/ha for wheat, 3.9 tonnes/ha for oats and 3.8tonnes/ha for barley (based on an average of straw yields in England since 2014) and knowing around 43% of this will always be used as animal bedding, feed, or soil enhancement as well as 40% sold or exchanged for bedding or feed. The remaining approximately 17% (1.87-2.04 million tonnes per year) can potentially be used to produce biohydrogen.

### 2.2.2 Short Rotation Coppice

Most SRC has been grown within the ECS when comparing total area of new plantings claimed under the subsidy payment scheme since 2000 (around an average of 2500 ha). NNFCC estimates that the average SRC yield is between 8-17.5 oven-dried tonnes (odt) per hectare per year (factoring in the 2-3 year harvesting period). The Forestry Commission Forest Growth-SRC has modelled an average annual yield of 9 odt per hectare and 10.3 odt per hectare for willow and poplar respectively. Therefore, based on the estimated upper yield annual volume of 33,000 odt the amount of SRC that is accessible per year is 5000 odt. Assuming half of the 5000 oven-dried tonnes is employed for use in small-scale or domestic CHP systems or other end uses, 2500 odt per year could potentially be used in the biphasic fermentation system.

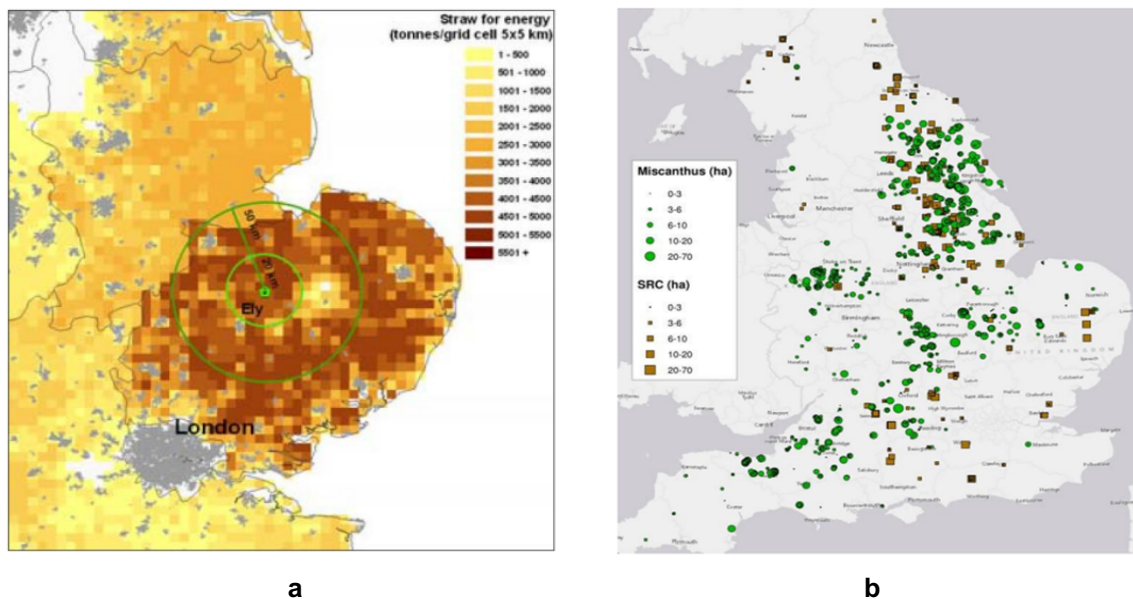
### 2.2.3 Grass and herbal leys

An area of 6.46 million ha of grasslands could be made available to grow herbal leys. Assuming around a tenth of this is accessible for biohydrogen production,

approximately 650,000 thousand tonnes of herbal leys could be used for the biphasic fermentation system producing  $4.72 \times 10^9 \text{ m}^3$  of biogas per year.

### 2.3 Regional mapping of the feedstocks in the UK

The highest amount of unused straw accumulates in Yorkshire, the East Midlands and Eastern England and the density of the available straw in the East of England has been visually represented in Figure 1a. The distribution of miscanthus and SRC are mapped in Figure 1b which is derived from Natural England Data in 2010. Yorkshire and the Humber and the North East are the most prominent regions when it comes to the cultivation of miscanthus and SRC. Other areas of growth are dispersed around the Midlands (East and West) and the South West.



**Figure 1** Maps to depict the availability, density, and distribution of straw in the East of England (a) and miscanthus and SRC throughout England (b)

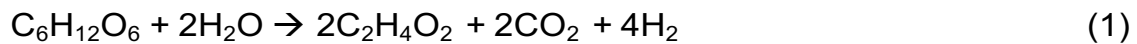
### 2.4 Possible locations for the biphasic fermentation system

Taking into consideration the significant arable and horticultural grassland available for possible herbal ley (grass) production and based on the accessibility of the mapped feedstock above, the four locations where the biofermentation system would thrive are: Yorkshire and the Humber, East of England, East Midlands, The North East. Thus, it can be concluded that the four feedstocks identified have the potential to be processed into hydrogen. The feedstocks are widely available in the UK and in large quantities which is encouraging for developers of hydrogen production technologies to produce hydrogen at scale. Given the limitations of the seasonality of the agricultural feedstocks, based on the sizing of the hydrogen production, storage needs to be planned accordingly for the smooth and continuous generation of clean fuel throughout the year.

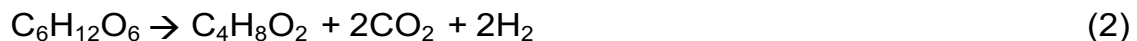
### 3 Biorefinery modelling and state-of-the-art biphasic system design (Deliverable ID 3)

#### 3.1 Stage 1 - Dark fermentation

Dark fermentation (DF) is the most favourable process of biological H<sub>2</sub> production. It is an efficient route of fermentation adopted by all anaerobic bacteria that take place in the dark. A high rate of production and modest reactor designs are the key attributes of dark fermentation which makes it an effective and economical approach. During dark fermentation, anaerobic microbes utilise various carbon sources through metabolic pathways in the absence of oxygen, to produce H<sub>2</sub> gas. Accumulation of volatile fatty acids during dark fermentation lowers the yield of hydrogen, thus restricting the usage of this method for H<sub>2</sub> production. Theoretically, a maximum yield of 4 mol H<sub>2</sub>/mol hexose is produced with acetic acid as the byproduct, as shown in Eq. (1):



Similarly, when anaerobic dark fermentation favours production of butyric acid formation along with H<sub>2</sub>, a maximum yield of 2 mol H<sub>2</sub>/mol glucose (Eq. 2) is produced.



DF is a complex process conducted in an anaerobic environment manifested by a series of enzymatic reactions. The key enzymes that drive this process in diverse groups of bacteria are mostly Fe-only and Fe-Fe hydrogenases. A diverse microbial flora including both strict and facultative anaerobes, are involved in H<sub>2</sub> production by dark fermentation. Bacteria belonging to Clostridium species are strict anaerobes that are efficient producers of H<sub>2</sub> gas as compared to facultative bacteria. These groups of microbes vary in terms of their morphology, H<sub>2</sub> producing pathway, tolerance to oxygen and yield of H<sub>2</sub>.

#### 3.2 Stage 2 - Photofermentation

Photofermentation, as the name suggests, is an organic substrate fermentative conversion with light energy by photosynthetic bacteria. It is an anoxic process where the organic compounds, e.g. acetate, butyrate, and lactate, are degraded and forms H<sub>2</sub> and CO<sub>2</sub> in light. It mainly happens in purple non-sulfur photosynthetic bacteria. The photosynthetic bacterium of species are reported to produce H<sub>2</sub> by photofermentation. These microorganisms degrade wide range of organic substrates from fructose, glucose, and succinate to organic acids like acetic and malic into H<sub>2</sub> by O<sub>2</sub>-sensitive nitrogenase enzyme, which can simultaneously produce H<sub>2</sub> and reduce N<sub>2</sub>. These photosynthetic bacteria cannot split water due to PS-II deficiency, but they can in an anaerobic environment, using organic acids as electron donors. Ferredoxin then transports these electrons to nitrogenase using ATP. In the absence of N<sub>2</sub>, nitrogenase reduces the proton to H<sub>2</sub> using ATP. A simplified reaction for H<sub>2</sub> production by photofermentation can be written as:



Factors like wavelength, light intensity, and illumination pattern influence the H<sub>2</sub> production in these bacteria by photofermentation. Light intensity is directly proportional to H<sub>2</sub> production. An increase in the intensity of light results in photo-inhibition, which reduces O<sub>2</sub> production and enhances H<sub>2</sub> production. Various organic wastes can be used for H<sub>2</sub> production by photo fermentation.

### **3.3 Biphasic system**

The two-stage process involved in biohydrogen production depends on the types of microbes involved. Microbial H<sub>2</sub> production has several challenges over chemical processes and yield, or production rate is one such major hurdle of the process. The integrative approach to generate a single-stage hybrid system or two-stage system for H<sub>2</sub> production has recently gained much attention with the goal to overcome the limitations of single operated processes. Dark fermentation process is characterised by the massive accumulation of organic acids which shows an inhibitory effect on H<sub>2</sub> producing enzymes and the growth of microbes. The acid rich effluents are high in carbon content and can act as a substrate for further energy recovery through a two-stage process.

The dark fermentation effluents generated in the first stage are processed by the second stage that can be used for methanogenesis for methane production or photofermentation for H<sub>2</sub> production, microbial electrolysis cells (MECs) for H<sub>2</sub>, microbial fuel cells (MFCs) for bioelectricity, bioplastic production, and heterotrophic algae cultivation for lipids. These integrated processes are involved in the efficient valorisation of waste effluents for additional energy production or other value-added products. This makes the integrative approach economically feasible and practically applicable to industrial scales. Combining dark and photofermentation sequentially can extract all the energy stored in the substrate. Theoretically, 12 mol of H<sub>2</sub> per mol of glucose can be produced (dark fermentation contributing 4 mol of H<sub>2</sub> and photofermentation contributing 8 mol of H<sub>2</sub> in sequential fermentation).

Thus, combining dark fermentation and photofermentation can yield higher biohydrogen production. The conversion efficiency of heat value in dark fermentation can be enhanced in the two-phase system. However, the theoretical value cannot be achieved because part of the carbohydrate is used for growth and maintenance of the microorganism due to a lower conversion efficiency.

### **3.4 Algal CO<sub>2</sub> sequestration**

The biological route of hydrogen production through either dark fermentation or photofermentation is ametabolic function of microbes that generate various metabolites along with hydrogen as a part of their metabolic activities. Carbon dioxide is a major byproduct of this process which accounts for around 25-33% of the total biogas generated by the anaerobic hydrogen producing bacteria. The generation of such a huge amount of CO<sub>2</sub> along with hydrogen triggers the matter of greenhouse gas emissions, and with an aim to produce clean fuel leading to complete decarbonisation of the process, it is essential to mitigate the CO<sub>2</sub> produced from fermentation. To make the entire process green and sustainable our team has come up with a technology which involves algae as a natural entity that can sequester huge amounts of CO<sub>2</sub> from the atmosphere.

## 4 Technical feasibility of hydrogen-oriented microbes and dark fermentation

### 4.1 Sourcing of the biomass feedstocks

Barley straw, hay, wheat straw, and willow samples were sourced by the Newcastle University team in the UK from Cockle Park Farm in Morpeth. The collected biomass was dried in oven followed by size reduction to get a particle size of 1-2 mm by grinding as demonstrated in Figure 2. The Grassroots Energy team in India procured Napier grass that was prepared and chopped before pretreatment.



**Figure 2** The samples of feedstocks oven dried and ground in the Newcastle University laboratory sourced from Cockle Park Farm, Morpeth

### 4.2 Characterisation of the biomass feedstocks

Elemental composition is one of the most important properties for biomass utilisation. The experimental determination of carbon, hydrogen, and nitrogen (CHN) elements in biomass is conducted by using a CHN analyser. The compositional and elemental analysis (CHN) of all the biomass before and after pretreatment revealed degradation of cellulose content upto 20%.

### 4.3 Pretreatment of biomass using thermochemical and biological methods (Deliverable ID 4.1)

#### 4.3.1 Thermochemical pretreatment

Acid pretreatment is a promising process for the deconstruction of recalcitrant lignocellulosic biomass, capable of producing high yields of hemicellulosic sugars in the hydrolysate and enhancing enzymatic yields of glucose in the solid fraction. This study will use thermochemical pretreatment to treat the biomass.

#### 4.3.2 Biological pretreatment and hydrolysis of Napier grass

Preliminary experiments were set up for microbial pretreatment of Napier grass feedstock. The initial experiments were carried out with chopped Napier grass using a mixed hydrolytic microbial consortium. Samples were collected at various intervals and stored for analysis of total reducing sugars and COD.

#### 4.3.3 Total carbohydrate analysis

The samples obtained after pretreatment of each biomass were tested for total carbohydrates using the phenol-sulfuric acid method. The phenol-sulfuric acid technique is a fast and easy method for determining the amount of carbohydrates

present in an experiment sample. It can detect virtually all kinds of carbohydrates including di-, mono-, and even oligo and polysaccharides. In this process, the concentrated sulfuric acid can break down all polysaccharides and disaccharides and oligosaccharides into monosaccharides. These pentose sugars (5-carbon substances) are later dehydrated to furfural and the hexoses (6-carbon substances) to the hydroxymethyl furfural. The compounds react with phenol, resulting in an orange-gold hue. The amount of total carbohydrate present in the sample solution was calculated using the standard graph. The standard graph was prepared using glucose. Hay and willow resulted in total carbohydrates in the range of 3-6 g/L whereas napier grass resulted in less total carbohydrate compared to other biomass.

#### **4.3.4 COD analysis**

Samples of feedstock were added to the COD vials with proper dilution. The COD analyser was set to 150°C. Once the temperature was attained, the COD vials containing the samples were placed in the analyser and incubated for 2 hours. After two hours the vials were removed from the analyser and allowed to cool to room temperature. The absorbance was read using a spectrophotometer at the appropriate wavelength. The COD measured after pretreatment for all biomass was in the range of 3000-7000 mg/L.

#### **4.3.5 Volatile fatty acid analysis**

VFA analysis was carried out using GC. The samples collected after dark fermentation were tested for VFA composition at Newcastle University. A comparison of the feedstock samples collected before and after fermentation based on total carbohydrates, COD and VFA was analysed. The COD values after and before fermentation remained almost the same. Gas samples were collected and stored for the GC analysis. VFA analysis revealed that DF effluent is rich in acetic acid and formic acid. Butyric acid, isobutyric acid, isovaleric acid, propionic acid, valeric acid was not detected. Higher concentrations of acetic acid in the DF effluent are indicative of a high hydrogen yield. However, GC analysis of the headspace gas was carried out to prove this observation.

#### **4.4 Inoculum for dark fermentation (Deliverable ID 4.1)**

Pure culture of gram-negative bacteria was used by Newcastle University as the microbial inoculum for hydrogen production in this study. Preliminary studies were initiated to determine the growth curve of this sps. on lactose and glucose. The dark fermentation consortia prepared by the Grassroots Energy team was optimised for its growth in different media compositions named growth media 1 (GM1), growth media 2 (GM2), and growth media 3 (GM3). The growth profile of the consortia was monitored in all three media at OD 600 nm. It was observed that the highest growth of the consortia was obtained in GM2 which was used further for inoculum preparation.

## **4.5 Technical feasibility for H<sub>2</sub> production by Dark fermentation (stage 1) (Deliverable ID 4.2)**

### **4.5.1 Preliminary experiments on lab-scale biohydrogen production**

Like growth media, dark fermentation media was optimised to achieve maximum hydrogen production by the Grassroots Energy team. Preliminary experiments were set up at lab scale by monitoring the total gas volume produced in each media selected for fermentation with the defined DF consortia. Four types of media named as fermentation media 1 (FM1), fermentation media 2 (FM2), fermentation media 3 (FM3), and fermentation media 5 (FM5) were used in these experiments and it was observed that maximum gas volume was obtained in tube 3 which contained bacterial consortia grown in GM2 and fermentation media FM2. These experiments are monitored purely based on the total gas volume produced during the fermentation process at lab-scale. Further GC analysis is required to confirm the content of hydrogen in the total gas obtained.

### **4.5.2 Process parameters for biohydrogen production**

Various process parameters for dark fermentative hydrogen production such as initial pH, total volatile fatty acids (VFA), COD and alkalinity. were recorded at the zeroth hour of fermentation by the Grassroots Energy team. The initial pH for all the fermentation media was in the range of 6-7. These variations in pH are due to the composition of the media and the change in initial pH monitored over time. The change in total VFA and COD during the fermentation process was monitored and the composition of VFA was dominated by acetic acid, propionic acid, butyric acid, and valeric acid.

### **4.5.3 Dark fermentation experiments**

The biomass hydrolysate obtained after pretreatment of Napier grass using microbial consortia H was diluted before fermentation. The production of total gas was measured among which second resulted in a higher volume of gas in 4 days. A control experiment of DF was set using glucose as the substrate. The gas collected showed a flame after burning which qualitatively confirmed the presence of hydrogen in the gas.

At Newcastle University, initial experiments were set up with pretreated hydrolysate samples of barley, wheat, hay, and willow biomass. The experiments were conducted in small serum bottles. Raw and pretreated biomass was used. For standard, the same concentration of glucose and lactose were used.

After sealing the vials with rubber septum stoppers. Two needles were inserted in the rubber septum, one for the inlet of nitrogen and the other for the outlet of gas. Purging was done for 3-5 minutes. All experiments were conducted at 200 RPM and 35°C in the dark. After every 24 hours, gas samples were collected. The initial detection of hydrogen was confirmed by GC-MS in fermentation experiments with lactose as the carbon source. The biohydrogen yield was calculated based on the % area of hydrogen peak in GC and total gas volume obtained with DF of each biomass.



## **5 Photofermentation and technical feasibility of a photobioreactor (stage 2) for hydrogen production**

### **5.1 Photofermentation (stage 2)**

Grassroots Energy team sourced the photosynthetic bacterial cultures from the culture banks. Photofermentation experiments were conducted using the lab-scale 500ml assembly by the Grassroots Energy team and Newcastle University.

### **5.2 Technical feasibility for H<sub>2</sub> production by photofermentation (stage 2) (Deliverable ID 5.1)**

There are many configurations and designs of photobioreactors suitable for microalgae cultivation. However, the number of previously explored designs for photobioreactors used for hydrogen production is much more limited. The main purpose here was to construct and determine a technically feasible photobioreactor to be used for both microalgae cultivation and hydrogen production.

#### **5.2.1 Risks of the PBRs / hydrogen generation process**

1. Hydrogen gas collection and storage, presence of inhibitory compounds like furfural in lignocellulosic hydrolysate which may lower the yield of H<sub>2</sub>.
2. Maintenance of anaerobic environment in both stages of dark and photofermentation.
3. Oxygen sensitivity microbes.
4. Requirement of an external light source for photofermentation.

### **5.3 Evaluation, Design and Optimisation of the photobioreactor (stage 2) for H<sub>2</sub> (Deliverable ID 5.2)**

A range of photobioreactor systems have been used by different research groups for lab-scale hydrogen production experiments, and a few attempts have been made to upscale the hydrogen production process. Even though a photobioreactor system for hydrogen production does require special construction properties only very few attempts have been made to design photobioreactors specifically for the purpose of hydrogen production. We have designed a photobioreactor system that can be used in two modes: either for the cultivation of phototrophic microorganisms or to produce hydrogen or other anaerobic products.

Special emphasis has been taken to avoid any hydrogen leakages. The photobioreactor system will be assembled with a custom-built control system that can log and control temperature, pH, and optical density and additionally log the amount of produced gas and dissolved oxygen concentration. The same PBR can be used in series under natural light conditions.

### **5.4 Fabrication of the photobioreactor (stage 2) for hydrogen production (Deliverable ID 5.3)**

The reactors for photofermentation (stage 2) have been designed by the Grassroots Energy team.

## 6 Challenges and learnings during the experimental phase

During the pre-treatment studies, we have come across various challenges such as:

- Yield of reducing sugars and total carbohydrates.
- Microbial hydrolysis resistance to the recalcitrant nature of the biomass composition.

The learnings that have been uncovered during the experimental phase:

1. Longer time of incubation during pretreatment
2. Standardisation of the protocol using standard sugars.
3. Analytical parameters like COD, total carbohydrates using standard procedures
4. Follow standard procedures for sampling of gas samples to reduce error

These learnings will be considered to further improve the experimental approach and results. The biohydrogen yields determined from the gas samples from each feedstock for dark, photo fermentation, and the overall integrated biphasic system calculated are elucidated. Overall mass balances depicting a 20 tonne/day biphasic system using the DF and PF data collected from both Newcastle University and Grassroots Energy labs have been captured in this study.

## 7 Environmental and Economic feasibility of H<sub>2</sub> applications (stages 1 and 2) at distributed scale

### 7.1 Design assumptions for the biofermentation plant (Deliverable ID: 6.1)

The dark fermentation (stage 1) digester design assumptions and photobioreactor assumptions (stage 2) are depicted in this study.

### 7.2 The location and local factors to design the digesters for the respective feedstocks (Deliverable ID: 6.2)

The location and local factors in Newcastle-upon-Tyne and Cockle Park Farm in Morpeth, Northumberland was considered. The average monthly climate in Newcastle-upon-Tyne, the air temperature, atmospheric pressure, relative humidity, and net radiation readings at the Cockle Park Farm site from November 2021 to October 2022 was considered in this study. The support infrastructure in the North East is vast and has a well-connected 'Energy Gateway' including three major ports, enterprise zone sites and two pioneering research and development centres, the National Centre for Energy Systems Integration (CESI) and the Integrated Transport, Electricity, and Gas Research Laboratory (InTEGRel) both spearheaded by Newcastle University.

The North East hydrogen network is excellent with two projects already demonstrating the use of hydrogen in domestic applications, the H21 and HyDeploy projects. The East Coast Hydrogen mega-infrastructure programme is ongoing in the region thus, making it an ideal area to construct the biphasic system.

### 7.3 The local and international ecosystem for non-core components and equipment (Deliverable ID: 6.3)

The preliminary list of vendors to procure the equipment, components, and non-core equipment required for the biofermentation plant as well as the local ecosystem in the North East and Newcastle to identify potential partners who will help source the necessary equipment have been identified.

### 7.4 Financial model scenarios for CAPEX and OPEX (Deliverable ID: 6.4)

Table 3 and 5 depict two financial models one scenario based on a theoretical throughput of 20 tonnes of feedstock per day generating up to 115 tonnes/year of hydrogen (up to 0.35 tonnes per day or 17.5kg per tonne of feedstock per day) and the second scenario predicated on a capacity of 50 tonnes of feedstock per day producing up to 1188 tonnes/year of hydrogen (up to 3.6 tonnes per day or 72kg per tonne of feedstock per day).

These assumptions are subject to ideal criteria being met in terms of optimised feedstock selection, weather conditions, equipment, and scale-up analysis over the next two years as the technology is developed and validated. The CAPEX and OPEX for the scale have been calculated over the lifecycle.

#### 7.4.1 Scenario 1 – 20 tonnes of feedstock per day in 24 months and by 2030

20 tons / day feedstock input		
	Scenario 1: 24 months	Scenario 2: 2030
Agricultural Waste and Biomass (tons/day)	20	20
Days of operations	330	330
H2 (Kg/ton feedstock/day) generated	17.5	54.3
H2 (tons/day) generated	0.35	1.08
H2 (tons/year) generated	115	358.4
Capital Cost (CAPEX)	£ 4,450,000	£ 3,115,000
Operational Cost (OPEX)	£ 836,250	£ 585, 375
Total cost	£ 5,286,250	£ 3,255,375
Discount rate	5%	5%
NPV (10 years) Total Costs	£ 11,553, 273	£ 7,663,481
NPV (10 years) H2 Production	£ 1,884, 331	£ 2,723,223
<b>H2 Cost / Kg</b>	<b>£ 6.13</b>	<b>£ 2.81</b>

Table 3 – Scenario 1 financial model

## 7.4.2 Scenario 2 – 50 tonnes of feedstock per day in 24 months and by 2030

50 tons / day feedstock input		
	Scenario 1: 24 months	Scenario 2: 2030
Agricultural Waste and Biomass (tons/day)	50	50
Days of operations	330	330
H2 (Kg/ton/day) generated	17.5	54.3
H2 (tons/day) generated	0.875	2.715
Capital Cost (CAPEX)	£ 6,555,000	£ 4,588,500
Operational Cost (OPEX)	£ 1,985,550	£ 1,389,885
Total cost	£ 8,540,550	£ 5,978, 385
Discount rate	5%	5%
NPV (10 years) Total Costs	£ 23,611, 647	£ 16, 528, 153
NPV (10 years) H2 Production	£ 4,710,829	£ 6,808, 057
<b>H2 Cost / Kg</b>	<b>£ 5.01</b>	<b>£ 2.43</b>

Table 5 – Scenario 2 financial model

## 7.5 Preliminary Environmental and Economic Benefit-Risk Analysis and Carbon Life-Cycle Analysis (Deliverable ID: 6.5)

### 7.5.1 Preliminary Environmental and Economic Benefit-Risk Analysis

A detailed Benefit-Risk value tree has been developed to capture the environmental and economic benefits biohydrogen production will bring to the future UK energy mix and its associated risks.

### 7.5.2 Carbon Life-Cycle Analyses

Two life-cycle models have been developed based on a throughput of 20 tonnes per day (life-cycle model one, Table 5 and 50 tonnes per day (life-cycle model two, Table 6). Assuming natural gas as the alternative power and heat source and that the biphasic system is a CO<sub>2</sub> neutral technology. Transport emissions are assumed as 1kg of CO<sub>2</sub> per day and the biomass feedstock sources (energy crops and agricultural residues) are carbon neutral taking into account the direct and indirect land use emissions (carbon sequestration). The life-cycle models have been developed with entire carbon emissions associated with the biofermentation plants. The methodologies used to calculate the life-cycle and carbon emissions (LCA):

1. Scope 2 Power Consumption = (CO<sub>2</sub> emissions from electricity (UK) x Annual consumption)/1000
2. Scope 3 Transport = (Transport emissions x uptime)/1000
3. Scope 3 Natural gas = -1 x mass of H<sub>2</sub> per day (tonnes) x mass natural gas/Mass H<sub>2</sub> ratio x CO<sub>2</sub> emission for natural gas combustion x uptime



**Table 5** The Life-Cycle model and carbon emissions of the 20 tonnes per day biphasic system from end-to-end using natural gas as the comparison and alternative heat and power source

LCA		CO <sub>2</sub> emissions (tonnes/year)	Removed (tonnes/year)	Tonnes of CO <sub>2</sub> avoided annually
Scope 1	Fermentation Process	0	0	
Scope 2	Power Consumption	442.70		
Scope 3	Transport	0.30		
	Natural Gas		-2268.75	
Total		443	-2268.75	<b>-1825.75</b>

**Table 6** The Life-Cycle model and carbon emissions of the 50 tonnes per day biphasic system from end-to-end using natural gas as the comparison and alternative heat and power source

LCA		CO <sub>2</sub> emissions (tonnes/year)	Removed (tonnes/year)	Tonnes of CO <sub>2</sub> avoided annually
Scope 1	Fermentation Process	0	0	
Scope 2	Power Consumption	894.72		
Scope 3	Transport	0.33		
	Natural Gas		-8167.50	
Total		895.05	-8167.50	<b>-7272.45</b>

## 8 Conclusion and lessons learned

In conclusion, the findings and results discovered during the Biohydrogen from Dark and Photofermentation phase 1 Hydrogen BECCS project can be summarised as:

- Energy Crops (SRC Willow and Napier grass) are promising feedstocks for biohydrogen production.
- Both pure strains and mixed consortia can be used as an inoculum for DF.
- Both chemical and microbiological pretreatment methods including hydrolysis produced similar results and hydrogen yields.

The key metrics identified during the project have been displayed below in Table 7. These have been measured against the current best-in-class along with the projected targets foreseen over the next 24 months and from 2030 onwards.



**Table 7** Key metrics measured during the project against the current best-in-class along with the projected targets over the next 24 months and from 2030 onwards [28, 29]

Key Metric		Best-in-Class	Solution Today	24-month target	Long-term target (2030 onwards)
<b>Dark fermentation</b>					
Yield (kg H <sub>2</sub> / tonne of biomass)	Napier	7.5 <sup>[28, 29]</sup>	1.2 <sup>#</sup>	3.7	7.5
	Willow	--	0.3 <sup>#</sup>		
	Glucose	22.1 <sup>[28, 29]</sup>	3.4 <sup>#</sup>	11.1	22.1
<b>Photo fermentation</b>					
Yield (kg H <sub>2</sub> / tonne of biomass)	Napier	--	0.22 <sup>#</sup>	6.4	32.2
	Glucose	32.2 <sup>[28, 29]</sup>	--		
Substrate conversion efficiency (%)	Dark fermentation	50	10	25	50
	Photofermentation	35	10	20	35

*#This study*

## 9. Market / competitor appraisal, commercialisation plan for Hydrogen BECCS Innovation programme: phase 2

### Competition Appraisal:

The other technologies for our solution include:

- Biomethane to Steam methane reformer (SMR). This can be done at a certain scale and is an energy intensive process.
- Stand-alone Dark or Photo fermentation being offered which limits the hydrogen potential.
- Biomass Pyrolysis to Hydrogen which is energy intensive process.

### Market Appraisal:

Biohydrogen has the potential to have the lowest LCOH by 2025 ~£5/kg. The UK produces over 100 million tonnes of biomass, annually, suitable for biohydrogen production equivalent to a potential of 1.5 million tonnes of low-cost biohydrogen per year or 59TWh of energy annually. There are over 600 AD plants in the UK that can provide huge potential for retrofitting to produce hydrogen. The hydrogen can be produced in addition to the biogas being generated, giving an incentive for the developers to adopt the technology.

### Commercialisation plan:

Phoebus Power has access to land across the UK, especially in areas of good biomass availability. This gives a reliable source of feedstock availability throughout the year as well as visibility to investors and end-users. Phoebus Power has access to commercial and industrial customers for hydrogen offtake for electricity generation and thermal applications.

The Dissemination Activities have been planned to be taken up by Phoebus Power, Grassroots Energy and Newcastle University till Q2 2023.

The technology partner, Grassroots Energy has been working on different design options to reduce the capital and operating costs to drive the cost of generation. A detailed engineering design proposed for a biofermentation phase 2 has been developed. The Bill of Materials for the dark and photo fermentation equipment to procure for the installation and construction of the 20 tonnes/day biphasic system have been identified.

The residue from the operations can be enriched to produce organic fertilisers replacing chemical agricultural inputs. The market linkages for the distribution of organic fertilisers have been developed to leverage the produce. The team has access to Investments Funds and IPPs who have expressed considerable interest in scale up of this innovative process once it is ready for commercialisation.

**Exploitation Planning:**

Multiple activities are planned to leverage the results from Phase 1.

**IP Protection:**

IP being created from the technology development is explored for patents.