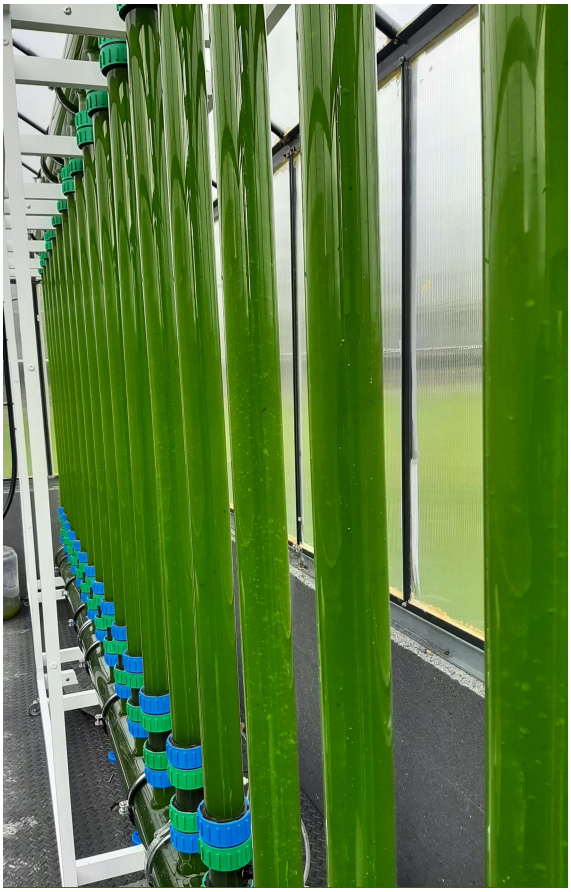

PROJECT MISTY - GRE-303-1

Final Report

28th February 2022

Contents

Executive summary

The MISTY project aimed to increase microalgal biomass by using a microalgae-bacteria consortium grown in brewery wastewaters (effluents). The consortium grew in a photobioreactor which stood inside of a greenhouse, thus making the UK weather conditions less harsh, and using nutrients present in the effluents. In this final report, we present the main results achieved in Phase 1 of MISTY, as well as the commercialization plan developed, aspects related with project management, and we discuss a few aspects pertaining the application for Phase 2 of MISTY.

As summary, during Phase 1 it was possible to demonstrate that by using the proposed innovation:

- It is possible to growth microalgae biomass even during the wintertime in the UK.
- Brewery effluents are excellent media for the cultivation of the microalgae-bacteria consortium since they have the required nutrients.
- 4.2-fold increase in biomass production can be achieved using a microalgal-bacteria consortium, thus proving that the natural symbiotic/mutualistic relationship between microalgae and bacteria boosts the productivity.
- 8.6-fold increase was achieved when the consortium was scaled-up from the laboratory experiments to the photobioreactor in brewery effluent.

1. Technical description of the innovation

Biomass increase is pivotal to regulate the Earth's climate, since it has an essential role in global carbon cycle by removing carbon dioxide from the atmosphere through its growth and by storing it for extended periods of time in soils and plants [1].

Scaling of biomass production poses problems such as the use of land and water, both of which are finite resources, especially if we are talking about arable land and potable water. One way to increase biomass production is to use microalgae. Microalgae have high photosynthetic efficiency, which allows them to grow faster than terrestrial plants [2]. Another advantage is that they can grow on non-arable land and are able to use various water sources (including non-potable water) [3]. Furthermore, microalgae can help to reduce greenhouse gas emissions (GHG; 183 Gtons of CO_2 can produce 100 Gtons of microalgae biomass) [4].

Over the years, countless technoeconomic assessments established that microalgal growth requires much more input in nutrients, energy, water, and labour than plant cultivation [5]. Thus, it is established that the production of competitive microalgae-based production of bioactive compounds such as sustainable/renewable energy supply, needs ground-breaking approaches to overcome these hurdles. In MISTY (Figure 1, Annex 1), we use with three innovative approaches all at once:

1) use of wastewaters as a nutrient source,
2) growth of microalgae together with bacteria (consortium)
3) use mixotrophic cultivation.

Wastewaters are being more used for the growth of microalgae at a lower cost. In MISTY, brewery effluents, which are rich in organic compounds, such as sugars, proteins, phosphates, ammonia and/or nitrate, were used as an alternative medium for the growth of microalgae [6].

Furthermore, the microalgae were grown with bacteria in a consortium, taking advantage of the natural symbiotic/mutualistic relationship between microalgae and bacteria. Consortia between microalgae and bacteria have been described as an effective, sustainable, and commercially viable option. This interaction (Figure 2, Annex 1), based on nutrient exchange between microalgae and bacteria: microalgae produce O_2 for bacteria to use as an electron acceptor and degrade organic matter; in turn, bacteria supply CO_2, fixed nitrogen, vitamin B, phytohormones, and siderophores (high-affinity iron-chelating compounds) to microalgae to support their growth [7]. In fact, this sort of relationship between these two organisms can increase microalgae growth rate by 10-70% and enhance CO_2 fixation [7].

However, the relationship between microalgae and bacteria is not always symbiotic/mutualistic with bacteria being able to produce compounds (metabolites) that can inhibit microalgal growth (and vice-versa) and can also produce algicidal metabolites and, microalgae can produce bactericidal metabolites [7].

When creating the concept of MISTY, it was established that the consortium would grow in two types of "bioreactor systems", one taking advantage of natural sunlight during spring/summer period and the other, autumn/winter, using the organic compounds present in the wastewaters as carbon sources in darkness, which would sit inside of a greenhouse. This "bioreactor systems" wouldn't consist of two separate photobioreactor systems but instead it would be two types of trophic levels that the microalgae species chosen would use depending on the type of nutrients and light they would be receiving, heterotrophic in the autumn/winter and autotrophic in the spring/summer. This was changed in the beginning of the project, and we decided that there would only be one "system" and it would be mixotrophic. Microalgae can be cultivated autotrophically (sunlight and CO_2), heterotrophically (dark conditions utilizing an organic carbon source like glucose as a source of energy and carbon) or mixotrophically (need lower light conditions and consume both organic carbon and CO_2) [8]. However, for some microalgae species, mixotrophic culture always provide higher biomass production than autotrophic and heterotrophic ones [9], acting on both the maximum cell density and growth rate, thus lowering the production costs [10]. Furthermore, when grown under mixotrophic conditions, microalgae tend to accumulate more lipids [10, 11].

Considering the nutritional and environmental requirements, a mixotrophic microalga able to grow well in low light conditions and in lower temperatures, i.e., grow in the UK with minimum energy inputs, we chose to use [redacted]. This microalgal species is one of the most common genera of green microalgae in freshwater environments and it can be found as single-celled individuals but are also capable of forming 2–32 cell coenobia (colonies) although, most usually, it forms a four-celled coenobium that are surrounded by a mucilaginous matrix [12]. The optimum temperature range for the [redacted], which is important since temperature is one of the major environmental factors limiting the microalgae productivity [13]. Another positive aspect of this microalga species is the fact that it is described as having great potential to treat CO_2-rich gaseous effluents [14]. It has also been described that the biomass yield of this microalga is very similar in the 14:10 and 12:12 photoperiods (light: dark) [15], meaning that it can grow when the light period is lower in natural conditions, such as in the UK during wintertime.

Further advantages of MISTY as a way of increasing microalgal biomass, lie in growing the consortium using the effluents of breweries and dairy, which will provide a carbon source for the mixotrophic growth of the microalgae but will also provide other nutrients such as nitrogen and phosphorus, which are vital for microalgal

growth. The idea of using dairy industry effluents was abandoned due to the time scale of the project and, as such, we only focused on brewery effluent.

Water is one of the main ingredients in beer and, traditionally, it takes 3.5 L of water to produce of 1 L of clear beer [16]. Additionally, water is necessary for supporting operations of the brewing process such as cooling, steam raising, packaging, and cleaning. Therefore, the brewing industry is one of the largest industrial users of water, generating vast amounts of polluting effluents [17]. Brewery effluents have high concentrations organic compounds, such as proteins, phosphates, ammonia and/or nitrate, sugars, soluble starch, ethanol, and volatile fatty acids, which lead to high biochemical oxygen demand (BOD) (Table 1, Annex 2) [6]. Although these compounds are extremely biodegradable, most of them must be removed before discharging the effluent into the environment [17]. This removal imposes more energy and costs to the wastewater treatment plant or, in the case of breweries that don't have their own wastewater treatment plant, paying a fee to dispose of the effluents into the municipal sewer for processing at utility wastewater works.

The capability of microalgae to grow in brewery wastewater utilizing their available nutrients is widely reported in literature. The reason why mixotrophic microalgae are used to treat wastewater lies in their capability to use organic and inorganic carbon, as well as organic and inorganic nitrogen and phosphorus in wastewater for their growth [18]. This results in the reduction of the concentration of those substances in the wastewater, therefore promoting bioremediation [19]. Also, by integrating microalgae into wastewater treatment there is the production of O_2 through photosynthesis and this O_2 can then be used by heterotrophic bacteria to biodegrade carbonaceous materials [18, 20]. This natural relationship between microalgae and bacteria is, as mentioned before, the foundation of MISTY, and it is possible to see how the use of wastewater as a growth medium and the consortium are intertwined and have the potential to increase the production of microalgal biomass, as we will report in the section 2 (Innovation results assessment).

Finally, the photobioreactor of MISTY was put inside of a greenhouse as an additional way of increasing biomass production while reducing its costs. Thus, the greenhouse aimed to keep the temperature in the photobioreactor higher than if it stood outside and it also prevented the growth medium to freeze, since the pilot-plant experiments were all done during wintertime, where negative temperatures outside were achieved.

Summing the innovation proposed in the MISTY, we intended to increase microalgal biomass productivity by co-culturing microalgae with bacteria (consortium) mixotrophically using wastewaters from the brewing industry in the UK weather conditions.

2. Innovation results assessment

The assessment of the innovation results was done considering the biomass increase using the growth conditions proposed in MISTY when compared to the traditional microalgal growth. Thus, in the following sub-sections, the main results achieved will be described as well as the corresponding experiments that allowed us to reach extremely positive results in phase 1 of MISTY.

2.1. Microalgae biomass production system design

To increase biomass production, a species of microalgae [redacted] adapted to lower temperatures was used in the experiments.

Three effluent samples were collected from two breweries (Wadworth and Wye Valley Brewery).

Laboratory experiments were performed to identify the optimum growth conditions, how the microalgae grew in the consortium, and to compare the microalgal growth between standard media culture and the effluents. Furthermore, we also identified the species of bacteria present in the three effluents collected and assessed if such species could be used in the consortium. An alternative plan, comprising of a consortium with [redacted] was put in place.

2.1.1. Optimisation of consortium conditions

Before growing [redacted] in brewery effluent and in a consortium in the pilot-plant (200 L photobioreactor), the effluents were analysed, and experiments had to be performed in the laboratory.

Brewery effluent samples (Figure 3, Annex 1) were collected from two different breweries (Wadworth and Wye Valley Brewery): Wadworth (Devizes, UK) and Wye Valley Brewery (Stoke Lacy, UK). Regarding Wadworth brewery, one sample of crude effluent was collected on the 26th of August 2021. For the Wye Valley Brewery two samples were collected, one of crude effluent and the other of final effluent, on the 21st of September 2021.

Chemical and microbiological analysis were performed for the three effluent samples. Chemical analysis results (Table 2, Annex 2) showed the presence of maltose, xylose, and glucose. These can be used by the microalgae as carbon sources, thus proving to have huge potential as a cheaper growth medium for the consortium.

The bacteria present in the effluent samples (Figure 4, Annex 1) were isolated using different media culture (Figure 5, Annex 1); gram staining was done for each isolate (Figure 6, Annex 1), and identified using the API® identification system galleries (Figure 7, Annex 1). We were able to identify a total of 28 different bacterial

species/strains (Table 3, Annex 2) in the three samples collected from Wadworth and Wye Valley breweries.

Most of the bacteria identified on both breweries are found in the environment (mainly in the soil or water) or are part of the microbiota of animals. Most of the bacteria are described as promoting the growth of plants either by nitrogen transfer, phytohormone production, phosphate solubilization, and even biological control by protecting against phytopathogens. However, some of the bacteria identified are nosocomial and pathogenic bacteria that cause opportunistic and nosocomial infections in humans and other warm blood animals. Thus, it was decided that the bacteria should be removed from the effluent before growing the microalgae; and that [redacted] would be used to form the consortium in both the laboratory and pilot-plant experiments.

In laboratory experiments, [redacted] was grown either alone, or in a consortium with [redacted] in two different media (standard media and brewery effluent) for 15 days. Microalgal cells and bacterial colonies were counted at day 0, 1, 4, 6, 8, 11 and 15. Results (Figure 1 and 2, Annex 2) show that:

- Brewery effluent is a good medium for the growth of microalgae.
- When the microalga [redacted] is growing in a consortium, i.e., with the bacterium [redacted], the growth is significantly higher than when growing on its own, with a 5.94-fold increase in the standard media and a 4.19-fold increase for the brewery effluent.

2.1.2. Assessment of microalgal biomass in MISTY (System scale-up)

After performing laboratory experiments that determined that the growth of the microalga [redacted] was successful in brewery effluent; and that it was higher in the consortium, i.e., with the bacteria present, the experiments were scaled-up to the MISTY photobioreactor (PBR).

The photobioreactor (PBR) for the scale-up was the Phyco-Lift, and it was acquired from Varicon Aqua (Worcester, United Kingdom). The PBR (Figure 8a, Annex 1) consists of a single control module with 3x6 tube units assembled on a single frame, with a total volume of 200 L and a working volume of 180 L.

MISTY's PBR stands inside of a greenhouse (Figure 8b, Annex 1) specially built for this project, which has a weather station, both inside and outside, to provide values of temperature and humidity.

Three experiments were done in the PBR during the months of October (2021) to January (2022):

- Experiment 1 – Performed with the aim of acquaintance with the system and creation of Standard Operating Procedures of the PBR. This experiment ran for 15 days.
- Experiment 2 – Performed with the aim of assessing microalgal biomass growth using a model effluent. This experiment ran for 15 days.

- Experiment 3 – Performed with the aim of assessing microalgal biomass growth using brewery effluent from Wadworth and Wye Valley breweries and using a consortium inoculum adapted to the UK environmental conditions. This experiment ran for 11 days.

Successful experiments 2 and 3 in the PBR were assessed both in terms of microalgal growth, i.e., number of microalgal cells, as well as dry weight in the final of the experiment.

At the end of the experiment 2, approximately 50% (80L) of the volume of the PBR was collected to a container (Figure 9a, Annex 1) and was dewatered by centrifuging at 6000 rpm (Figure 9b, Annex 1). 62.4g of the wet microalgal biomass was collected (Figure 9c, Annex 1) and dried (Figure 9d, Annex 1). By the end of this experiment, we achieved a solid dry content of [redacted] of dry microalgal biomass.

The rationale behind leaving 50% of volume in the PBR from experiment 2 to use as inoculum for the experiment 3 in the PBR was to use the semicontinuous growth approach. In the semicontinuous growth, only part of the culture volume is harvested, and the harvested volume is replaced either with fresh brewery effluent. Thus, the culture left in the PBR acts as the inoculum for the experiment 3. This approach allows us to maintain the culture at the optimal biomass concentration and in exponential growth phase at all times thus maximizing productivity [21]. Furthermore, the consortium (microalgae and bacteria) was already adapted to the lower light and temperature conditions. It is known [22] that adaptive laboratory evolution is frequently used to develop beneficial phenotypes in industrial microorganisms, such as microalgae, during long-term selection under specific stress conditions. Thus, this inoculum had the potential to be better than the one grown in laboratory conditions (optimal light and temperature conditions), such as the one used in the experiment 2 in the PBR.

In experiment 3 in the PBR, brewery effluent was cultured with the adapted inoculum left from experiment 2. After dewatering 172L from the PBR, [redacted] of wet biomass was collected and then dried. Results were highly positive, achieving a solid dry content of 20.2 wt.%. Furthermore, compared to laboratory results in brewery effluent, the results from the scale-up experiment 3 in the PBR show an 8.6-fold increase of microalgal biomass growth (Figure 3, Annex 2).

2.2. Environmental benefits and risks

A direct environmental benefit of MISTY is the bioremediation of brewery effluents while increasing microalgal biomass. Presently, depending on the brewery, the effluent is treated on site before discharge to watercourses or it may be discharged to sewerage which then goes to wastewater treatment works. The majority of local breweries have to deal with costs of wastewaters disposal in a safe way, e.g., gate fees and transportation costs, being the most cheap and common solution sending their wastewaters to the drainage after balancing the pH. The biological oxygen

demand (BOD) of these effluents is very high and places demands on processing facilities. The Biochemical Oxygen Demand (BOD) represents the amount of oxygen consumed by bacteria and other microorganisms while they decompose organic matter under aerobic (oxygen is present) conditions at a specified temperature and COD is a measure of the oxygen equivalent of the organic matter in a water sample that is susceptible to oxidation by a strong chemical oxidant. By using brewery effluents, the consortium uses the nutrients present (nitrogen, phosphate, sugars, among others), MISTY would help reduce this demand and render the eventual wastewater of lower impact potentially suitable for discharge following minimal processing. MISTY technology can help reduce the volume of waste effluents while creating value chain with the subsequent environmental benefits, which include the potential for direct water quality improvements.

Experimental work performed in MISTY using a 200 L PBR and a mixture of effluents from Wye Valley and Wadworth breweries showed an rapid decrease for both BOD and COD until day seven. [redacted]. These results suggest that the microalgae, and corresponding consortium have reach between day six and seven the maximum capacity for degrading the organic matter present is the wastewater and most likely it was reached the limit for the growth of the microalgae in mixotrophic conditions too.

Furthermore, as mentioned before, microalgae cultivated mixotrophically can consume both organic carbon and CO_2 [8]. In phase 1 of MISTY, the carbon source for the microalgal growth was the sugars present in the brewery effluent (maltose, xylose and glucose) and also food grade of CO_2 commercially acquired. However, in phase 2 of MISTY we intend to capture the CO_2 from the fermentation process.

MISTY aimed at reducing the relatively standard practice of throwing waste liquids into UK's rivers and seas, therefore reducing the environmental impact of breweries while helping to increase their reputation in the set-up of green technologies to create value and technology from waste. By doing so and helping the biggest alcoholic beverage industry in the UK to go greener, MISTY will bring the UK a little closer to the Green Revolution.

Concerning risks, there would be a potential risk related with spillage of water from the photobioreactor. However, the content of the PBR would mainly be microalgae and bacteria that had already degraded the organic and inorganic compounds present in the effluent. Furthermore, this aqueous content is described to have biostimulant properties due to the release of phytohormones by the microalgae when they are growing and their benefits include better rooting, higher crop and fruit yields, drought and salinity tolerance, photosynthetic activity and pathogen resistance [23, 24]. Additionally, this water also contains [redacted], which is one of the major plant growth-promoting rhizobacteria, being already used in biofertilizers (e.g., Xtreme Gardening Azos) and well-recognized bacterium with the ability to fix

atmospheric nitrogen, capable of synthesizing phytohormones (mainly indole-3-acetic acid) and, increasing plants tolerance of abiotic and biotic stresses [25, 26].

3. Agronomic productivity plan

MISTY technology will boost productivity of microalgal biomass in the UK, by using a consortium-based culture under mixotrophic conditions.

Deployment of this technology in the UK will involve: (a) establishment of algal growth technologies at wastewater sites (initially, breweries), with on-site harvesting and dewatering capability; (b) consolidation of biomass at one or more central facilities where hydrothermal liquefaction processes will be used to produce sustainable transport fuels.

3.1 Stakeholder research and commercialisation

We have conducted supply chain research with a limited panel of breweries as candidates for a Phase 2 demonstration site. On Phase 2, further engagement will include wider engagement with the sector.

We identified downstream stakeholders and supply chain, in the intended application, which comprise users of sustainable transport fuels, especially in the marine and Agro-industrial sectors. Green Fuels has excellent links in this sector.

3.1.1. Business Opportunity

The MISTY project aims to develop a new technology that will be commercialised by exploiting the company's industry connections in the Animal and Aquaculture Feed industry. The developed solution will have significant impacts on biomass supply chain in the UK. Currently 30 per cent of the world algal production is used for animal feed.

Aquaculture

Algae have a great potential for use in sustainable aquaculture as they are not only a source of proteins, lipids, carbohydrates, essential minerals, and vitamins and have other nutritional qualities, but they are phototrophic organisms so produce that can use these directly from sunlight as energy source. Algae can fix carbon dioxide (CO_2) because they need it for their metabolism. In fact, producing 100 tons of algal biomass also fixes roughly 183 tons of CO_2 which, under the current period of climate change, has obvious implications in this period of climate change is highly desirable.

Animal Feed

The world's population is growing fast—and with it, our demand for not only meat but also animal feed. Soy is a common protein supplement in animal feed, but growing soybeans requires fresh water, fertilizer, and vast swaths of land. Protein-rich microalgae need less of these resources. The results show that microalgae have the potential to be a more sustainable alternative to soy in animal feed, the researchers say.

The researchers also compared the amino acid profiles of S. obliquus and soy. Farmers often must supplement soy-containing cattle feed with methionine, but since the microalgae contained twice as much methionine as soy, they may not need to do that with microalgae-containing cattle feed.

According to an industry-led UK Roundtable on Sustainable Soya convened by the UK Government in 2018, The UK annually imports 3.2 million tonnes Soya bean equivalents, with 68% or 2.2 million tonnes being meal of which 97% is used in animal feed.

New technologies

This project will develop the new technology that utilise wastewaters from breweries to grow microalgae in consortium with bacteria. The product that will be commercially exploited in this project is algae biomass.

This new technology will develop Intellectual Property (IP) and therefore new revenue streams for Green Fuels Research Ltd. The MISTY project will use the growth of microalgae in mixotrophic conditions in a consortium with bacteria using waste effluents. The biomass product will be used to produce commercially viable products, mainly in aquaculture and animal feed, with other potential markets biofuels, and biofertilizers.

IP opportunities

The MISTY project will develop new technologies that will exploit wastewaters from the breweries. The IP from these new technologies will be exploited to generate revenue streams for breweries and Green Fuels Research Ltd.

The technologies listed in the following or some components of these will be developed within the MISTY project, and their commercial potential will be exploited:

- Optimization of microalgae growth in wastewaters
- Optimization of microalgae and bacteria consortium in wastewaters
- Biorefinery combining two systems for increased biomass production

3.1.2. Benefits of MISTY generated biomass

The brewery industry in the UK generates over £8 billion of revenue each year. However, the breweries produce significant levels of liquid waste. The MISTY project will make substantial contributions to this sector by sustainably handling wastewaters and creating new industry and jobs around breweries. This will beneficially impact the UK economy and green targets.

The project will use locally available wastewaters to provide biomass, which will create local industry and jobs as well as decrease disposal of these waters into drainage and local rivers.

The MISTY project will provide new sources of biomass. This will lead to a reduction in farm emissions and clean water usage and will promote inclusive green economic

growth via biorefinery based on a mixotrophic system where microalgae grow in a consortium with bacteria.

Economic benefits

- New revenue streams created within the brewery industry
- New economic links between GFR, the brewery industry and biomass receivers
- Research and development opportunities in the UK
- A reduction in costs of wastewaters treatment
- Socio-economic impact on the lives of local workers
- Replacement Animal & Aquaculture feed

Environmental benefits

- Maximising wastewater utilisation and a reduction in the amount of waste generated by the breweries
- Reduced environmental impact of the brewery industry
- Growing microalgae for animal feed would not only remove planet-warming CO_2 from industrial emissions but also watershed-polluting nitrate from wastewater, which is also consumed by microalgae.
- Positively impacting arable land and clean water usage, while reducing the use of soy imports.

3.1.3. The Market Attractiveness

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

Commercialisation

GFR will provide the necessary model for breweries and dairies to exploit their wastewater by removing all or a significant portion of the cost of the discharge fee.

The photo bioreactors will be purchased by the brewery or dairy either outright or with asset finance over an agreed term. The purchase can partly be offset with capital allowances. Until 31 March 2023, companies investing in new qualifying new plant assets will benefit from a 130% first year capital allowance.

[redacted]

With Green Fuels Research experience in the biofuels industry and with global feedstock and commodity prices rising there is a viable option to produce bio-oil (via HTL) from the microalgae biomass which can be further processed into a variety of sustainable, renewable transport fuels including road and marine use.

3.2 Engineering design and production cost analysis

Engineering of the photobioreactor (PBR) system plays an important role in design optimization when it comes to maximize the production of microalgal biomass. The vertical transparent tubes PBR has been identified as significantly promising in regard to this purpose. The phase 1 of MISTY was able to show the improvements and huge potential of yield of biomass in this specific PBR closed system.

Accordingly, production costs can be considerably optimized by the yield maximization of the production process, as it can mitigate the high initial capital cost granting economic feasibility and future commercialization plans. Other important factors to consider in cost optimization are energy efficiency, and long-term process running, which are also connected to the design and engineering of the system. Furthermore, two cost efficient features come from the bioremediation of the brewery's effluent. The microalgae feed requirements can be sourced at a very low cost thanks to their ability to absorb the nutrients of the wastewater effluent. This

also represents a saving in the treatment of wastewaters as it has been reported that microalgal wastewater treatment is cheaper than treatment processes currently adopted.

Phase 2 of MISTY will begin with FEED for a demonstration facility. This will inform techno-economic analysis of capex, opex and ultimate biomass cost. The architectural design concept for the demonstration airlift photobioreactor to be installed on site at the new Wadworth Brewery can be seen in Figure 1, Annex 4.

4 Project plan assessment

The project was managed through a series of tools to assure meeting deadlines, while keeping the quality of the work at the best standards.

The first tool used was the project plan (Figure 1, Annex 3), which was reviewed as the project progressed. Any revisions were consulted and communicated with the MO. Tasks were grouped into WPs and milestones were established.

4.1 Timelines for deliverables

Project plan deliverables were identified from the project plan, costs for them were calculated, and were grouped into payment milestones with their respective due dates. Examples of the tables used can be seen below (Table 1 and Table 2, Annex 3).

Aside from the payment milestones, deliverables were grouped into project milestones in the project plan. Key project milestones were:

- M1.1. Consortium Biomass productivity demonstration (WP1)
    - D1.1 Report on consortium optimised conditions
    - D1.2 Report on scale-up and bacterial load on biomass
- M2.1. Identify potential stakeholders (WP2)
    - D2.1 Commercialisation & exploitation plan
    - D2.2 Report on design, cost, & environmental analyses
- Project completion
    - D3.1 Quarterly report
    - D3.2 Final report

4.2 Project management

The project management was carried out through a modified Prince2 process. The team involved a diverse group of professionals from GFR.

The relevant GFR team members (one-page CV can be found in Final Report Annex 3) consist of:

- **Dr Inês Baptista**, Research Scientist, holds a PhD in Biology with a Microbiology specialization from the Universidade de Aveiro (Portugal). Dr Baptista was the Delivery Team Leader in Phase 1 of MISTY.
- **Chris Clutterbuck**, Laboratory Technician; holds a MSc in Biotechnology and BSc in Biochemistry from Nottingham Trent University. Mr. Clutterbuck's role during Phase 1 of MISTY was in commissioning the PBR and conducting the scale-up experiments; from loading the PBR, to the harvesting of the biomass.
- **Ailin Donati**, Business Analyst, holds a Bachelor of Science in International Business and Economics from the University of Otto-von-Guericke

(Germany). Miss Donati's contribution in Phase 1 of MISTY was related with environmental and cost analysis.

- **Dr Przemysław Ociepa**, Senior Research Scientist, has a PhD in plant biology obtained from the University of Southampton in collaboration with universities in France and Norway. Dr Ociepa was part of the original team of MISTY and, started the work with the microalgae but had to step back due to illness.
- **Dr Lucía Benavente**, Project Manager, holds a PhD in Process and Environmental Engineering from the Université de Toulouse (France). Dr. Benavente has ample experience in research and project management, having participated in several projects funded by different UK agencies. Dr. Benavente was involved in Phase 1 of MISTY as the Project Manager.
- **Dr Sérgio Lima**, Research Manager of GFR, with a PhD in Chemistry and expertise's in chemical catalysed processes for biofuels production. Dr. Lima was the Project Leader in Phase 1 of MISTY.
- **Jason Askey-Wood**, UK Managing Director, founded Uptown Biodiesel in 2007 becoming London's leading Biodiesel manufacturer. Mr Askey-Wood's role in Phase 1 of MISTY was in the exploitation and business analysis.
- **Dr Paul Hilditch**, Director and Chief Strategy Officer is a Cambridge graduate with a PhD in photosynthetic biochemistry.
- **James Hygate**, Director and Green Fuels founder, has over 18 years' experience in the biofuels industry, including with the world's first biojet fuel demonstration in 2007.

4.3 Risks and risk management

Risks were assessed at the beginning of the project and updated when triggered and/or when new risks were identified (Table 3, Annex 3). They were divided into technical, commercialisation, environmental, and Covid-19 risks. Among the technical risks, the major ones identified were related to the effect of temperature during winter dropping below the required level, existence of competition/ parasitism by bacteria towards microalgae, and the presence of algicidal microorganisms.

During the period, one new risk was identified and triggered; algae-bacteria consortium in wastewater at risk due to contamination by bacteria from wastewater external source. Therefore, mitigation actions were put in place, such as dilution methods, filtration, UV-C light and/or antibiotics, in order to minimise or eradicate the bacterial contamination prior to the addition of the desired bacterial cultures. As a backup plan, an uncontaminated microalgal inoculum was also grown. The actions were effective into mitigating the risks, and the advancement in the project was not compromised.

4.4 Quality assurance

Internal quality assurance was set through meeting project goals and budget, while meeting high experimental standards.

The right people were identified for the right tasks.

Access to documentation describing tasks, plans, and budgets were available.

Communication channels were set and clarified for the team.

4.5 Project controls and governance

Oversight of the project was done by the Directors of Green Fuels, via monthly internal project meetings and financial reporting.

Regular internal meetings were held throughout the duration of the project. Monthly meetings were held with the external MO to communicate on the developments of the project and general updates, while setting goals and reminders for deliverable due dates.

5 Project plan for Phase 2

[redacted] in the Phase 2 supplying the wastewater and agreeing in principle to the installation of the demonstration photobioreactor plant at their location. This facility will use the effluent from the brewery and capture CO_2 from the brewing process that would otherwise be lost to atmosphere (Annex 4, Figure 1).

MISTY2 has a significant potential to be used in many different ways such as the sustainable production renewable fuels. We intend to use the microalgae biomass in MISTY2 in another research project (own-funded project and totally independent of MISTY2) [redacted]. We will explore (in our own-funded work outside this project) the upgrading of the microalgal-based bio-oil for the production of blendstock for fossil marine fuel, decarbonizing the UK shipping industry.

5.1 Timelines

The Phase 2 of MISTY would be anticipated to start on 1 June 2022 and finish on 7 March 2025. A Gantt chart (Annex 4, Figure 2) was provided in the proposal.

Phase 2 of MISTY would be comprised of 3 work packages (WPs):

[redacted]

[redacted]

- [redacted]

5.2 Project management

We will use an agile project management approach, aimed to reduce delays due to dependencies, although modified to accommodate BEIS' requirement for stage gates. The project will have an assigned Project Manager on Green Fuels' staff. Financial reporting will be the responsibility of the Chief Financial Officer.

5.3 Risks and risk management

Risks for Phase 2 of MISTY and the mitigation to each of those risks were identified in the proposal submitted. The risk register identifies technical, environmental, regulatory, financial, commercialisation and COVID19 (Health) related risks.

The main risks from the technical side include the effect and mitigation actions regarding extremes of temperatures (to be mitigated in the plant design), and contamination of the system.

Environmental risks include the possibility of leakage from the system (releasing microflora to the environment).

Regulatory risks include the establishment of stricter regulations for the wastewater parameters, while commercialisation risks involve the stakeholder engagement and treatment plant costs being too high, hence the risk of not raising enough commercial interest.

Main Covid19 risks identified are the transmission of the virus in the workplace and external visitors.

5.4 Quality assurance

Internal quality assurance is expected to be conducted in a similar manner to Phase 1 and adapting it as necessary to the new challenges.

- Establishment of meeting project goals and budget, while meeting high experimental standards.
- The right people to be identified for the right tasks.
- Access to documentation describing tasks, plans, and budgets to be made available.
- Communication channels to be set and clarified for the team involved.

5.5 Project controls and governance

Oversight of the project will be via the Directors of Green Fuels, via monthly internal project meetings and financial reporting.

Regular internal meetings to be held throughout the duration of the project. Monthly meetings to be held with the external MO to communicate on the developments of the project and general updates, while setting goals and reminders for deliverable due dates.

5.6 Reporting plans

Reporting to BEIS will be via monthly monitoring meetings and quarterly reports, and other requirements set by BEIS.

6 Conclusions

The innovative approach used in MISTY, i.e. the cultivation of microalgal biomass using wastewaters as a nutrient source, growth of microalgae together with bacteria (consortium) and use mixotrophic cultivation can be effective in producing microalgal biomass in the UK, even during the wintertime. In particular, a 4.2-fold increase of microalgal biomass growth can be achieved using a microalgal-bacteria consortium, i.e. natural symbiotic/mutualistic relationship between microalgae and bacteria. In addition, a 8.6-fold increase of microalgal biomass growth was achieved when the consortium was scaled-up from the laboratory experiments to the photobioreactor in brewery effluent, showing that once adapted to weather conditions, e.g. low irradiance and low temperatures, the biomass productivity can be increase using the brewery effluents instead of fresh waters and suppling expensive nutrients, such us phosphorus sources, sugars or vitamins.

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Annex 1

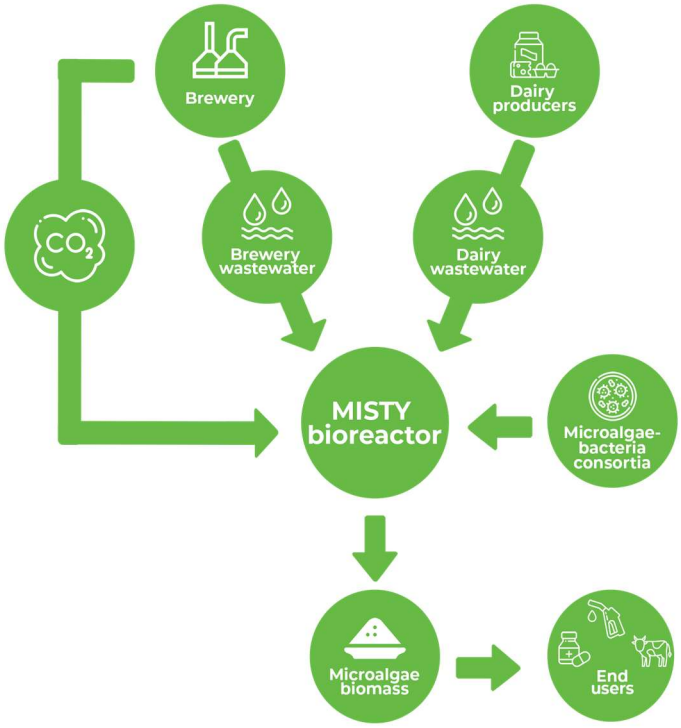

Figure 1 - MISTY concept for Phase 1.

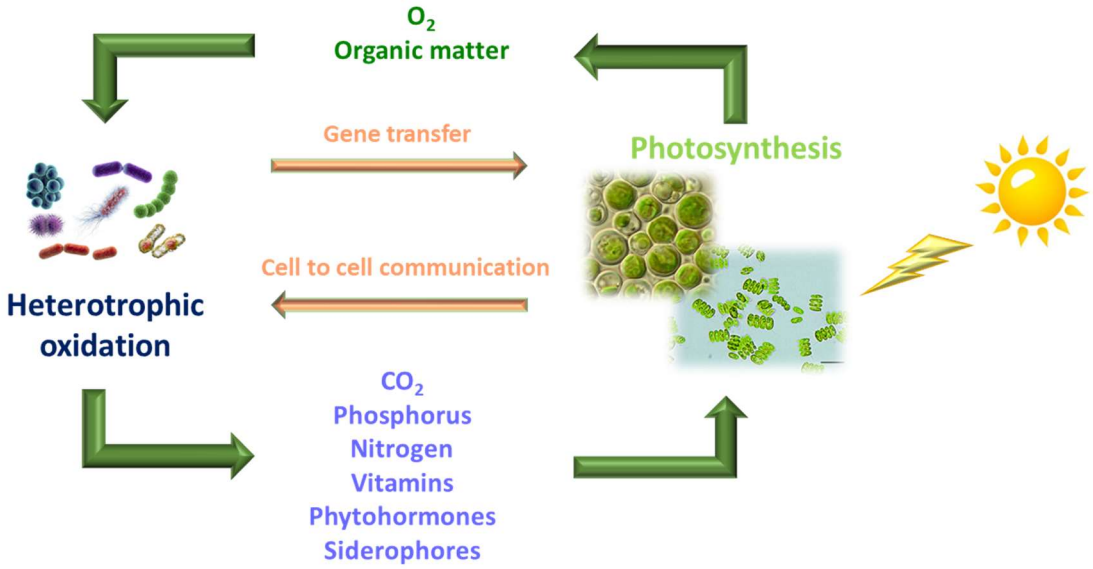

Figure 2 - A general structure of the interaction modes between microalgae and bacteria.

Figure 3 - Samples collected from Wadworth Brewery and Wye Valley Brewery.

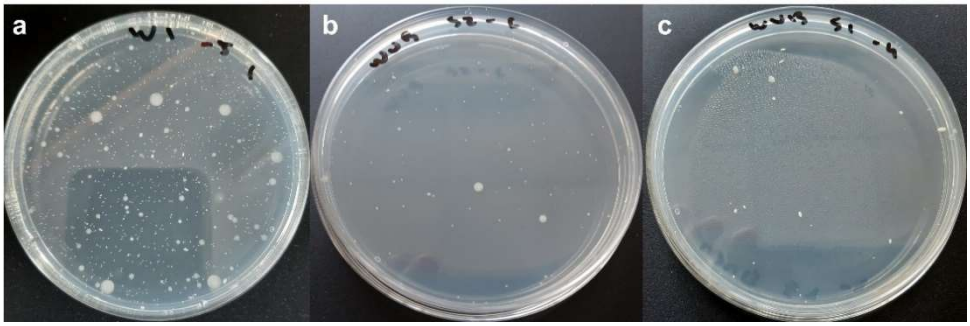

Figure 4 - Cultures of the samples collected from the different effluents: a) crude effluent from Wadworth Brewery, b) crude effluent from Wye Valley Brewery and c) final effluent from Wye Valley Brewery.

Figure 5 – Growth of three different isolates in the different media culture and at different temperatures. From this picture it is possible to observe that the morphology from the isolate a has a different morphology (rhizoid) than the isolates b and c (circular). We can also observe the absence of growth in some of the media or different sugar fermentations depending on the media. We can also observe for example, that isolate c is a fastidious bacterium.

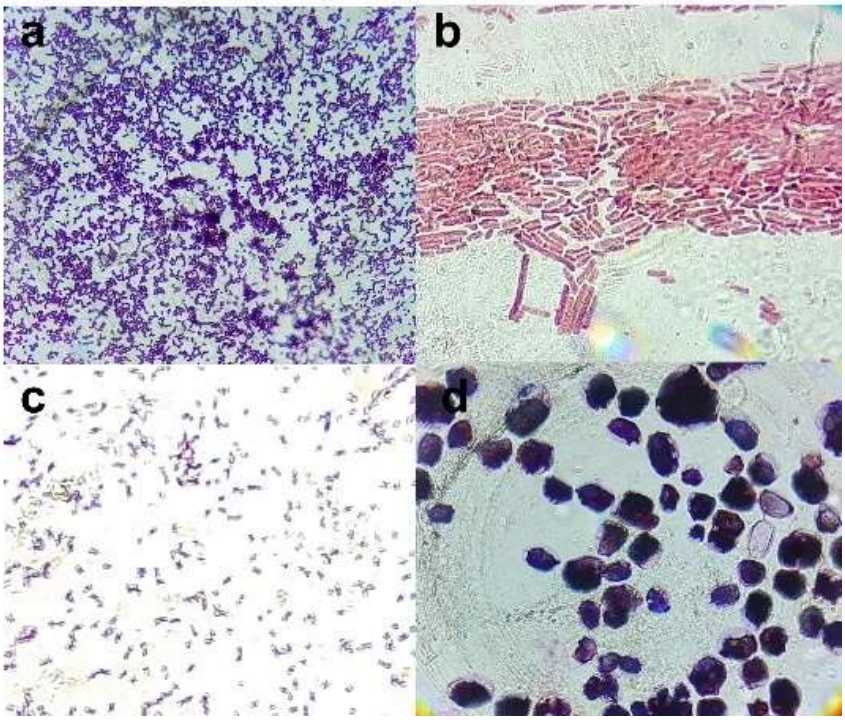

Figure 6 - Gram staining of four isolates from effluent samples from both breweries. In this figure it is possible to observe gram positive coccobacilli (a), gram negative bacilli (b), gram positive bacilli with central endospores (c) and yeasts (d).

Figure 7 - API® identification galleries for six of the from the brewery effluents: a) *Burkholderia cepacia*; b) *Klebsiella pneumoniae ssp pneumoniae* 1; c) *Escherichia coli*; d) *Proteus vulgaris*; e) *Micrococcus* spp.; and f) *Staphylococcus lentus*.

Figure 8 - MISTY system: a) Varicon Aqua Phyco-Lift photobioreactor standing inside of a greenhouse; b) The greenhouse in wintertime with an outside temperature of 0.94 °C and an inside temperature of 9.31 °C.

Figure 9 - End of second experiment including: a) collection of the microalgal biomass in a vessel before centrifugation; b) centrifuge with microalgal biomass being collected; c) wet microalgal biomass; d) dried microalgal biomass.

Annex 2

Table 1 - General characteristics of brewery wastewater (Retrieved from Simate et al., 2011).

Parameter	Value
pH	3-12
Temperature (°C)	18-40
TKN ($mg.L^{-1}$)	25-80
PO_4^{3-} ($mg.L^{-1}$)	10-50
COD ($mg.L^{-1}$)	2000-6000
BOD ($mg.L^{-1}$)	1200-3600
TSS ($mg.L^{-1}$)	2901-3000
TSD ($mg.L^{-1}$)	2020-5940
VFA ($mg.L^{-1}$)	1000-2500

TKN, total Kjeldahl nitrogen; COD, chemical oxygen demand; BOD, biochemical oxygen demand; TSS, total suspended solids; TDS, total dissolved solids; VFA, volatile fatty acids.

Table 2 - Chemical analysis from the effluent samples from Wadworth and Wye Valley breweries.

Parameter	Crude effluent (Wadworth)	Crude effluent (Wye Valley)	Final effluent (Wye Valley)
[redacted]			

TSS, Total suspended solids.

Table 3 - Bacterial species/strains found in the effluent samples from Wye Valley and Wadworth breweries.

Bacterial species/strains	
Staphylococcus lentus[a]	*Pseudomonas oryzihabitans*[a]
Alcaligenes faecalis[a]	*Proteus vulgaris*[a]
Pseudomonas fluorescens[a]	*Proteus penneri*[a]
Comamonas testosteroni[a]	*Bifidobacterium spp 2*[a]

Micrococcus spp.[a]	*Pseudomonas luteola*[a]
[redacted]	

[b] Found both in Wye Valley and Wadworth effluent samples
[c] Found only in Wadworth effluent sample

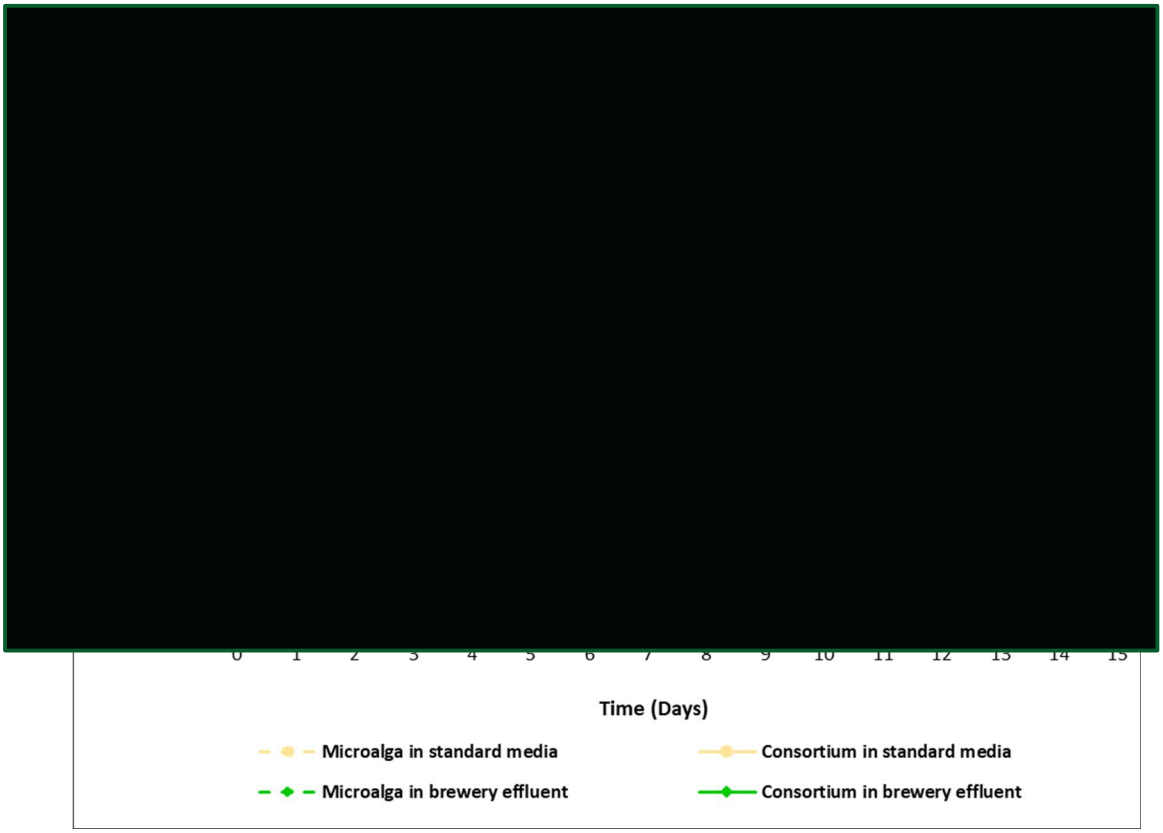

Figure 3 - Microalgal viability over the course of 15 days in laboratory experiments: circle markers stand for microalgal growth in standard media with dotted yellow line standing for growth without bacteria and full yellow line standing for growth in the consortium (i.e., with bacterium); diamond markers stand for microalgal growth in brewery effluent with dotted green line standing for growth without bacteria and full green line standing for growth in the consortium (i.e., with bacterium).

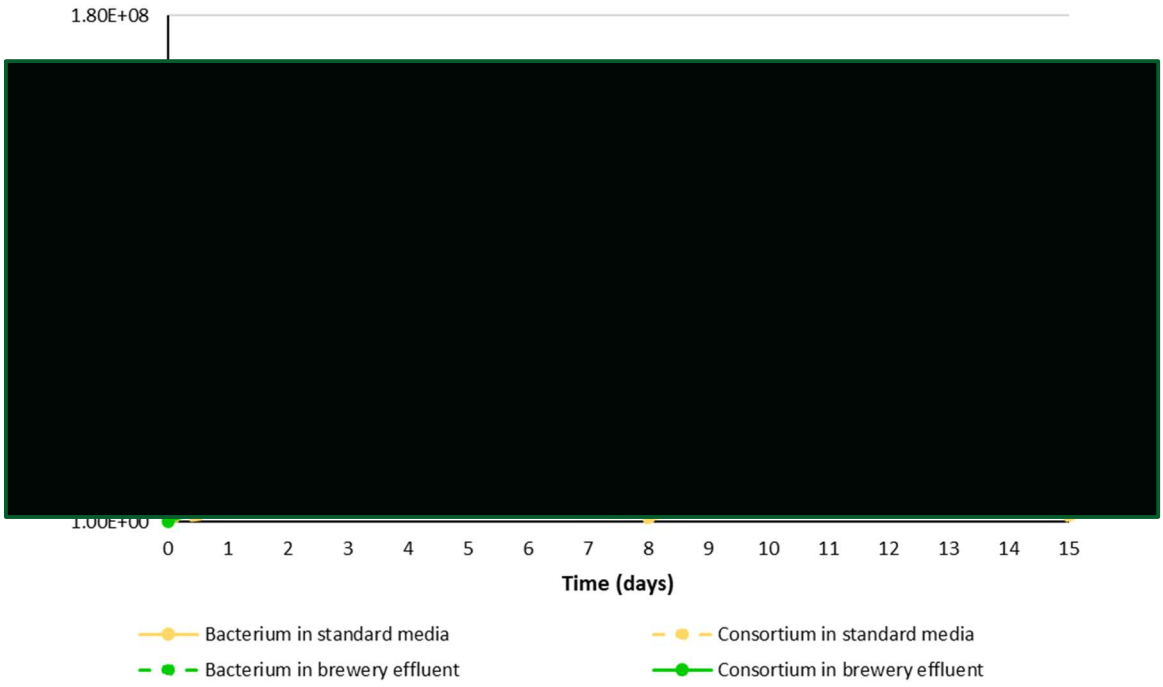

Figure 4 - Bacterial viability over the course of 15 days in laboratory experiments: circle markers stand for bacterial growth in standard media with dotted yellow line standing for growth without microalgae and full yellow line standing for growth in the consortium (i.e., with microalgae); diamond markers stand for bacterial growth in brewery effluent with dotted green line standing for growth without microalgae and full green line standing for growth in the consortium (i.e., with microalgae).

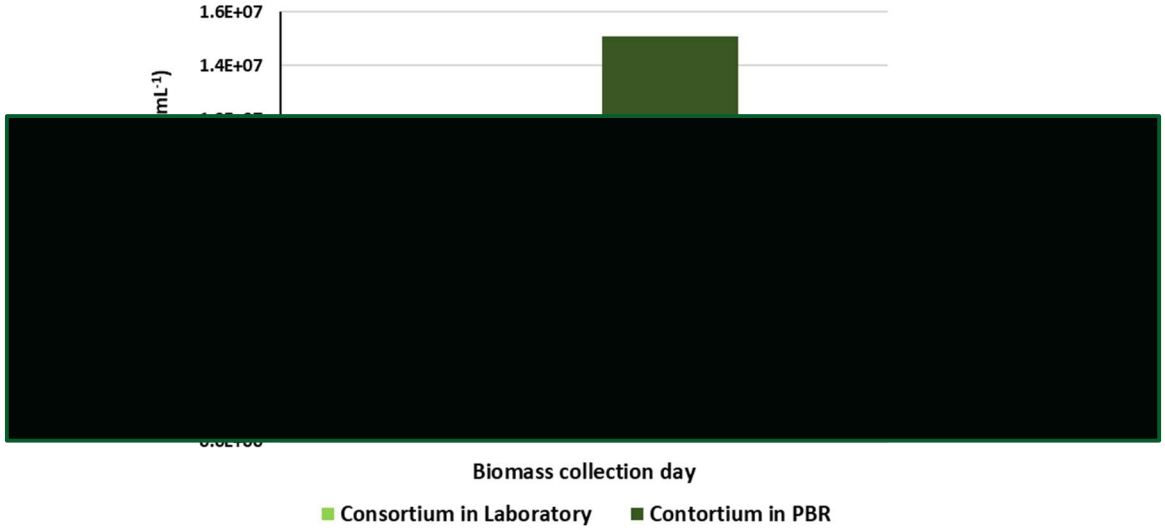

Figure 5 - Plot showing the difference on microalgal growth between laboratory experiments and the experiment 3 in the photobioreactor on the day that the biomass was collected (Day 11).

Annex 3

Project Management

ID	Task Name	Duration	Start	Finish
1	**WP1 Microalgae biomass production system design**	**151 days**	**Sun 01/08/21**	**Mon 28/02/22**
2	T1.1 Optimisation of consortium conditions	4 mons	Sun 01/08/21	Thu 18/11/21
3	T1.2 System scale-up	3.5 mons	Mon 02/08/21	Fri 05/11/21
4	T1.3 Assessment of bacterial load in biomass	4 mons	Mon 02/08/21	Fri 19/11/21
5	D1.1 Report on consortium optimised conditions	20 days	Fri 29/10/21	Thu 25/11/21
6	D1.2. Report on scale-up and bacterial load on biomass	41 days	Mon 03/01/22	Mon 28/02/22
7	M1.1 Consortium biomass productivity demonstration	0 days	Mon 28/02/22	Mon 28/02/22
8	**WP2 Agronomic productivity plan**	**151 days**	**Sun 01/08/21**	**Mon 28/02/22**
9	T2.1 Stakeholder research and commercialisation	2 mons	Sun 01/08/21	Thu 23/09/21
10	T2.2 Engineering design and production cost analysis	4 mons	Fri 03/09/21	Thu 23/12/21
11	D2.1 Commercialisation & exploitation plan	25 days	Fri 24/09/21	Thu 28/10/21
12	D2.2 Report on design, cost, & environmental analyses	26 days	Mon 24/01/22	Mon 28/02/22
13	M2.1 Identify potential stakeholders	0 days	Mon 28/02/22	Mon 28/02/22
14	**WP0 Project management**	**151 days**	**Mon 02/08/21**	**Mon 28/02/22**
15	T0.1 Kick-off meeting	1 day	Mon 02/08/21	Mon 02/08/21
16	T0.2 Quarterly report writing	21 days	Fri 01/10/21	Fri 29/10/21
17	T0.3 Final report writing	21 days	Mon 03/01/22	Mon 31/01/22
18	T0.4 Activities to protect IP	131 days	Mon 02/08/21	Mon 31/01/22
19	T0.5 Activities to develop customer relationships	131 days	Mon 02/08/21	Mon 31/01/22
20	D0.1 Quarterly report	21 days	Fri 01/10/21	Fri 29/10/21
21	D0.2 Final report	21 days	Mon 31/01/22	Mon 28/02/22
22	M0.1 Project completion	1 day	Mon 28/02/22	Mon 28/02/22

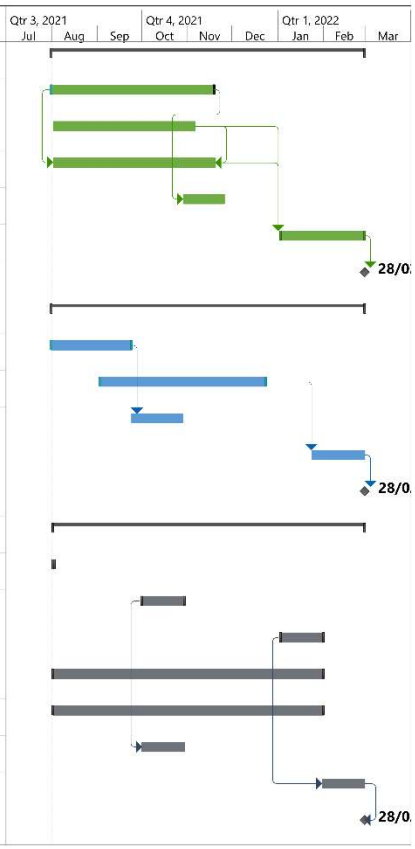

Figure 6. Project plan for MISTY

Table 4 - Payment milestone schedule.

[redacted]

Table 5 - Project deliverables description and cost.

Project Deliverables Table						
WP ID	Deliverable	Deliverable Name	Description (inc. outputs)	£ Cost (exc VAT)	£ Cost (inc VAT)	Baseline Due Date
1	1.1	Report on consortium optimised conditions	The deliverable will include a written report on the findings for this WP on the optimised consortium conditions.	£69,152	£82,982	28/10/2021
1	1.2	Report on scale-up and bacterial load on biomass	D1.2 will describe the scale-up experiment results and the findings on bacterial load on biomass	£50,302	£60,363	28/02/2022
2	2.1	Commercialisation & exploitation plan	D2.1. will contain the findings in T2.1. which consist on the industries that will benefit from MISTY and general commercial and stakeholder environment.	£29,832	£35,799	28/10/2021
2	2.2	Report on design, cost, & environmental analyses	Following the results from T2.2, this deliverable will summarise the engineering design and production cost for a future implementation of the project, as well as an initial asessment on implementation sites.	£29,832	£35,799	28/02/2022
3	3.1	Quarterly report	Quarterly report which describes the development status of the projects in the active WPs.	£7,088	£8,506	29/10/2021
3	3.2	Final report	Final report which summarises the overall findings of MISTY with respect to the work performed in all WPs.	£7,088	£8,506	28/02/2022

Table 6 - Risk assessment.

Risk (Identify and describe all key project risks, including: financial, technology, supply chain, regulatory, etc)	**Overall risk rating:** (Probability x Impact) High, Medium or Low	**Mitigation actions** (Describe the actions taken or planned responses to reduce the impact and/or probability of the risk)	**Residual risk rating, after mitigation applied:** (Probability x Impact) High, Medium or Low
TECHNICAL RISKS			
Consortium of *Chlorella*, *Scenedesmus*, and bacteria is not efficient	Medium	Algae will be grown in a separate consortium (*Chlorella*+bacteria; *Scenedesmus*+bacteria) or each algae grown by themselves.	Low
Algae-bacteria consortium in wastewater at risk due to contamination by bacteria from wastewater-external source.	Medium	Algae will be treated with dilution methods, filtration, UV-C light and/or antibiotics to minimise or eradicate the bacterial contamination prior to the addition of the desired bacterial cultures.	Low
There is not enough irradiance during the testing period to sustain microalgae growth	Medium	The artificial lighting powered by a wind turbine will be attached in the greenhouse.	Low
The temperatures during the winter period drop below the required level	High	The mixotrophic bioreactor will have heating system	Medium
Excessive temperatures in the summer causing overheating of the bioreactor	Medium	Use an evaporative cooling system where the remediated water will be recycled and cooled.	Low
The wastewater does not contain enough carbon sources for the heterotrophic bioreactor	Medium	Supplement the wastewater with crude glycerol derived from biodiesel production.	Low
The wastewater is not suitable for the consortium growth due to contaminants (such as heavy metals or antibiotics)	Medium	The anaerobically treated wastewater will be used as an alternative	Low
Existence of competition/parasitism by bacteria towards microalgae.	High	Control the bacterial growth through dilution and filtration.	Medium
Presence of algicidal microorganisms.	High	Filtration of the wastewater and inoculation with pure cultures of bacteria.	Medium
Contaminants get into system	Medium	The bioreactor and transfer line from mixotrophic to heterotrophic systems will be sealed	Low
Insufficient access to feedstock	Low	Engagements with the local brewery and dairy producers will be established to ensure feedstock for the research.	Low
Scale-up more challenging than expected	Medium	Scale-up options have been developed, literature research and experimentation are expected to mitigate any challenges on the scale-up of the system.	Low
COMMERCIALISATION RISKS			
Problems with engaging the stakeholders	Low	Dissemination of technology through existing connections	Low
ENVIRONMENTAL RISKS			
System leaks and microflora released to the environment	Low	Sealed system; Close monitoring for early identification	Low
COVID-19 RISKS			
Transmission of the virus in the workplace	Medium	Government recommendations in place, NHS rapid tests in place	Low
External visitors	Medium		Low
Staff not available	Low	Effective resource management	Low
Funding cuts	Low		Low
Delayed payments	Low	Efficient communication system with funding organisation to be promoted, to establish deadlines and requirements for transfer of payments.	Low

Team CVs

Dr Inês Baptista has 15 years or experience in microbiology and her main research interests are environmental and food microbiology, and in bacterial metabolic pathways.

Over her career, Dr Baptista was involved in several projects concerning heterotrophic bacterioplankton biomass production in estuaries and in the ocean to evaluate the impacts of global warming in aquatic environments, from which she published 6 peer-reviewed scientific papers and 1 book chapter on the subject in international scientific journals as co-author. Working in this field for a great part of her career, Dr Baptista became passionate on the topic and acquired important knowledge concerning the relations between bacterial and other aquatic organisms, such as microalgae, which the MISTY project stands upon.

At GFR, where Dr. Baptista has worked since 2019, she began as being involved in using bacterial fermentations for the production of biofuels from wastes (vWa) and did the assessment and application of biosafety protocols for a new drop-in marine fuel (SALMO). She was also involved in Phase 1 of MISTY, since its conception to being responsible for the microbiological side of the project (identification of bacteria present in the brewery effluents and selection of the bacterial species to use in the consortium) and taking over the microalgal growth after changes had been made to the MISTY team.

Education: Licentiate in Biology (2007) by Universidade de Aveiro, Portugal. MSc in Microbiology (2008) by Universidade de Aveiro, Portugal. PhD in Biology with specialization in Microbiology (2018), University of Aveiro-Portugal.

Publications: Co-author of 13 peer-reviewed scientific papers and 1 book chapter.

Dr Lucía Benavente holds a PhD in Process and Environmental Engineering from the Université de Toulouse (France), a Msc. In Membrane Engineering (Erasmus Mundus Programme), and a Food Engineering degree (UdelaR, Uruguay).

Throughout her international development, Dr. Benavente has gained experience and knowledge in separation processes, bioengineering, microbiology, and materials engineering, while taking part and/or managing multi-disciplinary international teams. Her interests lie in the design and application of circular, zero-waste solutions that valorise waste streams, and that aim at the decentralization of resources which could empower local communities.

Dr. Benavente is a native Spanish speaker and is fluent in English and French, with intermediate knowledge of German. She has one journal publication and has participated in several international conferences as a presenter or as part of the organization committee. She has volunteered at animal shelters (France), and at community outreach events for English learners (Mexico). She regularly engages in plogging (jogging + picking up litter) activities.

As part of the GFR team, Dr. Benavente has participated in a total of 8 funded projects and several other project application processes. She was the main researcher on the REFLOW project and has been managing follow-up projects at GFR, such as SALMO and Distributed scale sustainable fuels in Brazil (FCO – Prosperity Fund). Dr. Benavente is part of the MISTY – Phase 1 team as Project Manager.

Chris Clutterbuck is a Nottingham Trent University graduate with a BSc in Biochemistry, as well as an MSc in Bio-technology. He has worked as a civil engineer for PC Moleing Services Ltd, before joining Green Fuels Research as a Laboratory Technician in March 2018. During his time at Green Fuels, he has worked on several research projects such as REFLOW, SALMO, and MISTY. His work also includes analysis of oil and biodiesel samples from customers, such as water content, free fatty acid content, and fatty acid methyl ester analysis. He was also involved with the construction of biodiesel processing equipment that has been sent to Brazil, Oman and the Shetland Islands.

Dr Przemyslaw Ociepa, Senior Research Scientist, has a PhD in plant biology obtained from the University of Southampton in collaboration with universities in France and Norway. His involvement in biology students' community was recognised with the Good Citizenship award. He has gained his experience in plant developmental biology, cell cultures, and plant biochemistry working in the world-leading plant biology institutions in Cambridge (Sainsbury Laboratory) and London (Imperial College). While working at GFR, Dr Ociepa was involved in the DryGro Energy Crops and REFLOW projects, where he was responsible for growing and maintaining duckweed and microalgae cultures, respectively. Currently he works on vWa project assessing sugarcane wastes for biofuels production. Dr Ociepa was part of the original team of MISTY and, started the work with the microalgae but stepped back due to illness.

Ailin Donati is a Salt Lake CC, Utah (USA) Associated degree graduate that continued her degree studies at the University of Otto-Von-Guericke, Magdeburg (Germany) earning a Bachelor of Science degree in International Business and Economics. She has always been an environmental activist and she joined Green Fuels as a Business Analyst in 2020. Since then, she worked with market research, data analysis, Power BI, and LCAs providing environmental impacts and business insights to the company, and participating in several projects aimed at environmental sustainability like India Aris GF, Brazil FCO – Prosperity Fund, SALMO, PoWGEN and MISTY.

Dr Sérgio Lima is a chemist with more than 17 years of academic and industrial experience in R&D in research areas such as: Catalysis, Material Science (catalyst design, synthesis and characterisation), Analytical Chemistry, Chemical & Reaction Engineering (down- and upstream upgrading biomass), Process Development and Scaling-Up for a variety of chemical operations.

In particular:

-Development and optimization of heterogeneous chemical catalyzed processes for the conversion of renewable (waste) feedstocks (e.g. lignocellulosic biomass, oleochemicals, CO_2, pyrolytic bio-oils, etc) to sustainable commodity, platform chemicals and advance biofuels, i.e. drop-in biofuels. Process design, process development & scaling-up: from bench-top to commercial demo-plants.

-Design, synthesis and characterization of new advanced heterogeneous (inorganic) catalysts (bulk and metal supported) with improved textural properties (e.g. micro/mesoporous materials: zeolites, aluminosilicates, composite materials, delaminated layered zeolites, mixed oxides, etc.) and particles sizes (from nano to micro) using several synthesis methods (e.g. sol-gel, supramolecular assembling, surface chemical modification procedures).

-Design, construction and commissioning of high-pressure continuous-flow reactors.

-Development, optimisation and validation of analytical methodologies chemicals compounds identification and quantification. Planning and interpretation of experimental data.

-Research Management.

Current position at Green Fuels Research Ltd is focussed on process engineering, research management, design and development of catalytic routes for (waste) biomass conversion to advance transport biofuels, e.g. Sustainable Aviation Fuel (SAF), Renewable Marine Fuel (RMF), from lab scale to demonstration plants.

Previous work experience was related with chemical catalyzed processes development for furanic aldehydes upgrading to platform chemicals for polymer industry, from catalyst design to product optimization, scale-up, and engineering support of processes to convert carbohydrate biomass to chemicals (Chemical Engineering Department, Imperial College London, UK, 2015-2018). CO_2 valorisation to specialty organic chemicals for a petrochemical industry in a variety of processing situations (Tarragona, Spain, 2012-2014). Process development, process engineering and research scientist for lignocellulosic biomass valorisation to furanic aldehydes (University of Aveiro, Portugal, 2005-2012). Design, construction and operation of hazardous chemical continuous and batch processing facilities. Operation of state-of-the-art testing facilities and interpretation of experimental results.

Education: B.E. (Chemistry) 1999, University of Aveiro-Portugal, Ph.D. (Chemistry) 2005, University of Aveiro-Portugal.

Publications: Co-author of 42 peer-reviewed scientific papers, 2 book chapters and 6 scientific papers in proceedings books

Jason Askey-Wood founded Uptown Biodiesel in 2007 becoming London's leading Biodiesel manufacturer. Jason developed the supply chain from feedstock to fuel, building relationships with significant, 'blue-chip' offtakers, at a time where biofuels usage was in its infancy. ABP Food Groups' renewables division, Olleco acquired Uptown in January 2018, where Jason has spent the last 3 years working with their Biofuel and 3 Anaerobic Digestion Plants as Head of Sales and Origination, sourcing

UCO, food waste and category 3 animal by products which are recycled into Biodiesel, Biogas and Fertiliser showcasing the Circular Economy in action and helping the fight against climate change.

Dr Paul Hilditch has an MA in Natural Sciences from the University of Cambridge and a PhD in Biochemistry conducted at the Welsh Plant Breeding Station (now part of the University of Wales), Aberystwyth. He has experience in academic and industrial research (protein and membrane biochemistry, plant physiology, immunology, electrochemistry, microbiology), software development, technology transfer and IP management, science communication (as owner and Managing Director of communications agency Watermeadow Medical) and research team leadership. He is a Director and CSO of Green Fuels Research, coordinating a team of reseachers engaged on in-house, government and EU funded research projects, and a Director of Aris Green Fuels Pvt Ltd and of Green Fuels Ventures Ltd, producing renewable fuels in Brazil, India and Oman.

James Hygate has over 19 years' experience in the biofuels industry, developing industry leading technologies and novel processes for Green Fuels Ltd, the company he founded. Mr Hygate has been developing renewable aviation fuel for the past 13 years and was involved with the first biojet fuel supply in 2007. He is the Royal Warrant holder for Green Fuels and in 2015 was awarded the New Energy and Cleantech Entrepreneur of the Year.

GFR has a diverse team of professionals that includes expertise in algal research, microbiology, downstream processing, biotechnology, waste processing, business and market research, project management, and biofuel technology.

Annex 4

Figure 7 - Architectural design concept for the demonstration airlift photobioreactor to be installed on site at the new Wadworth Brewery.

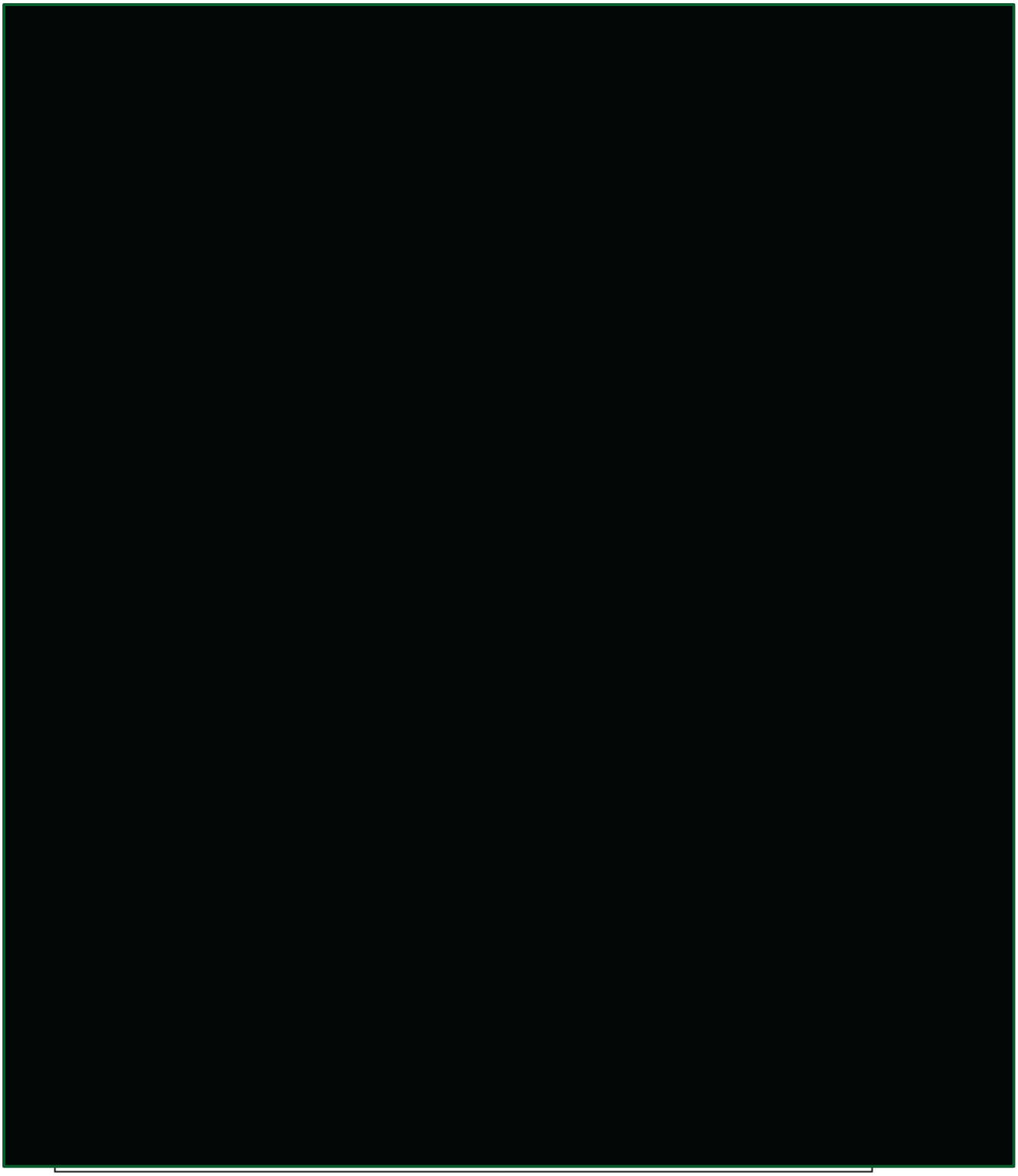

Figure 1 - Gantt chart proposed