Final Report

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DEMA Project Executive Summary

The EU funded DEMA (Direct Ethanol from MicroAlgae) project has established an integrated system to produce bioethanol directly from cyanobacteria and extract it as potential ecological source for biofuels that can compete with fossil fuels

The DEMA Consortium has developed, and demonstrated a complete technology for the direct production of bioethanol from microalgae within scalable photobioreactors. The method developed is an advance on the reported literature for a microalgae based biofuel in terms of its ethanol production performance.This biofuel production method has achieved operational -economic and -ecological viability but at the cost of an unviably high capital expenditure.

A comprehensive ecological model has demonstrated, via a detailed Life Cycle Assessment (LCA), that the process is operational on a carbon neutral basis when integrated with non-fungible renewable thermal energy. The catalytic conversion of solar energy, H_2O and CO_2 into ethanol can be undertaken by a metabolically engineered strain of the cyanobacterium, *Synechocystis sp.* PCC 6803 that has overcome the ethanol tolerance limitations reported in the prior state of the art.

The DEMA Process has two main stages. In the first phase, high performing metabolic engineered cyanobacteria directly transform CO_2, H_2O and sunlight into Bioethanol. In the second phase the bioethanol is continuously extracted from the culture media via a membrane technology process. The DEMA process design has shown that this process is technically feasible to generate bioethanol from CO2.

While operational viability is achievable, with the assistance of renewable thermal energy, the energy balance of the core DEMA process is deficient, as the process requires more energy to operate than is contained in the resultant bioethanol. This energy balance in turn means that the core economic performance of the DEMA Process is negative. The cost of the energy inputs are greater than the revenue gained from the bioethanol. In addition, while the economic performance of the DEMA Process can be augmented via the utilisation of renewable energy sources it adds considerably to the capital cost.

An important aspect in the evaluation of the DEMA process has been the comparison of the full Life Cycle Analysis (LCA) to the baseline fossil fuel alternatives. In the case of the core DEMA process the operational LCA performance is problematic since the CO2 footprint generated by the exogenous energy requirements are greater than the CO2 saved via using bioethanol. This operational ecological performance can be improved by integrating the use of renewable energy into the DEMA Process. This improvement in operational LCA does require a larger carbon footprint at the process construction stage however.

The DEMA Process has overcome the technical challenge of producing a biofuel grade ethanol from the low ethanol concentration medium within the Photobioreactor by developing a state of the art low concentration ethanol separation process. The DEMA process was demonstrated at the pilot scale where ethanol was extracted from the ethanol producing culture within the Photobioreactor via highly selective membranes at a location in Lisbon, Portugal. This pilot scale work has helped to achieve more reliable and scalable industrial reference designs.

The DEMA process operational energy performance is enhanced via the use anaerobic digestion of the residual cyanobacteria biomass which enhances both the DEMA energy and ecological balance. This closed loop approach to the utilisation of the metabolically engineered cyanobacteria provides a robust approach to GMO safety. There remains however, a very significant challenge concerning the high capital expenditure required to deliver a DEMA process facility.

The DEMA consortium are continuing to enhance the economic and ecological performance of bioethanol processes by developing and deploying individual process technologies to other biofuel applications. The DEMA project has contributed to the education and development of professionals with expertise in microalgae metabolic engineering, algal microbiology and microalgae cultivation and enhanced culturing systems. By developing this portfolio of knowledge and skills and the development of new biofuel technologies the project has made a valuable addition to Europe's research capacity and bioeconomy.

DEMA Project Context & Objectives:

The aim of the 54 month DEMA Project was to develop, demonstrate and licence a complete economically competitive technology for the direct production of bioethanol from microalgae with low-cost scalable photobioreactors thereby making ecologically produced biofuels more competitive with fossil fuels. The primary rationale for the DEMA project was instead of using the slow growing microalgae as a feedstock for biofuel but to engineer the microalgae to directly produce the bioethanol within the photobioreactor. The DEMA consortium combined novel and innovative microdroplet technology, with sophisticated metabolic engineering approaches, novel ethanol separation technology, and a sound understanding of microalgal cultivation methods to establish an economically, socially, and environmentally positive bioethanol production process.

The DEMA concept is to culture auto-trophically a cyanobacterial strain that is capable of directly producing ethanol at an economically viable energy balance. Strains of engineered microalgae cultivated in closed photo-bioreactors with optimised sun exposure, in a liquid medium supplemented with CO2, nitrogen, phosphate and micro-nutrients offers the means for direct synthesis of bioethanol from sunlight.

Overall Science & Technological objectives

The DEMA project was divided into two main areas of scientific research and technology innovation activity:

1. DEMA Ethanol producing strain development: The main scientific effort was focused on the development of a high performing ethanol producing strain of Synechocystis sp. PCC 6803.
2. DEMA Integrated Process Innovation: The main technology innovation effort was focused on the development of an Integrated DEMA Process that enabled the stable culturing of this ethanol producing strain with Photobioreactors and the efficient extraction of the resultant bioethanol.

DEMA Ethanol producing strain development

The scientific research for the DEMA project was devoted to the enhancement of strain performance, by targeted and non-targeted metabolic engineering, so that it both produced and tolerated higher bioethanol concentration. The experimental lifecycle of the creation, identification and improvement of the optimised cyanobacteria strains was facilitated by a unique high-throughput microfluidics platform. The outputs of the scientific research was governed by the following objectives.

Scientific objectives of the DEMA project:

S01. Maximise the ethanol productivity of cyanobacteria through metabolic engineering;

S02. Increase culture ethanol tolerance in order to obtain higher ethanol concentrations (>1-2%);
S03. Maximize biomass productivity and ethanol production through enhanced culture robustness;
S04. Use the waste biomass as an energy and CO_2 source, optimising the process outputs recovery.

This research was not simply focused on using current methods only in completing the DEMA strain development but also to develop new methods that would accelerate the rate of the new higher performing strain development and evaluation work.

These scientific objectives were transformed into the following activity areas.

Metabolic Engineering of Cyanobacteria
The cyanobacteria Synechocystis sp. PCC 6803 was selected to be the focus of the DEMA project work since there existed a suite of experimental methods that allowed this organism to be modified to produce ethanol.

Microfluidic and Lab Scale Analysis
The microfluidics platform was developed for screening modified cyanobacterial strains, measurement of ethanol production, and optimisation of culture conditions. A range of Lab-scale PBRs were used to evaluate the ethanol producing strains.

Optimisation of Bioethanol-Producing Strain
The proprietary bioethanol-producing cyanobacterial strain was then improved by a combination of enhancing bioethanol production, metabolic network modelling, the knocking out of competing pathways, increasing the ethanol tolerance via directed evolution, and both random and directed mutagenesis strategies.

Culture Stability and Safety
The DEMA project addressed the area of culture robustness and productivity optimisation over a long operating time, based on establishing stable microbial consortia with one or more bacterial partners. Genetic strategies to enhance GMO safety were also developed. The following challenges were identified as having the greatest potential of influencing the success of the Ethanol Producing Strain Development.

Primary DEMA Challenges - Ethanol Producing Strain Development
A central component of the DEMA project was the enhancement of strain performance, by targeted and non-targeted metabolic engineering, so that it both produces and tolerates higher bioethanol concentration. The identification of optimised strains was undertaken by a unique high-throughput microfluidics platform. In addition approaches were developed for enhanced culture robustness in optimised photobioreactors and GMO safety. A list of the identified Primary DEMA Challenges to Ethanol Producing Strain Development and the final status outcome of addressing these challenges are shown next:

1. Product Tolerance was the primary barrier to ethanol production.
   Final Status: Product Tolerance of UL030 has been shown to exceed 2x State of Art and is now not a barrier to ethanol concentration

2. DEMA strain development via high throughput techniques for sorting *Synechocystis* PCC6803 libraries of mutants.

Final Status: High throughput sorting of libraries took place, no strain was identified that outperformed UL030 which was designed using rational techniques.

3. Develop ethanol detection methods for high throughput techniques and use it to select the best bioethanol strains from *Synechocystis* PCC6803 libraries of mutants.
   Final Status: Ethanol detection methods were developed and successfully deployed during RP3.

4. Generate the libraries of mutants of *Synechocystis* PCC6803 via the standard methods used for equivalent work with strain development for other production strains e.g. *Saccharomyces cerevisiae*.
   Final Status: These libraries were developed and sorted using the high throughput microfluidic system.

5. DEMA consortia based robust culture for enhanced pilot PBR performance.
   Final Status: The production of cultures from the pilot PBR were fully characterised however no consortia combination was found that enhanced the ethanol productivity.

6. Role of Rational metabolic engineering techniques for DEMA strain development: These would be used in a modest way to augment the high throughput strain development techniques as a means of gaining a greater knowledge of the relevant metabolic pathways.
   Final Status: The rational metabolic techniques showed the best performance outcomes of all the strain development techniques.

DEMA Integrated Process Innovation

The technology innovation for the DEMA project was devoted to the creation of an integrated process that could be licensed to biofuel producing enterprises. The prime objectives of this work are described as follows:

Technological objectives of the project:

T01. Scale-up low cost photobioreactors and optimise their operation;
T02. Engineer the ethanol separation from the culture media using membrane processes;
T03. Utilization of residual biomass by anaerobic digestion and energy production process;
T04. Establish process integration of all developed and existing technologies
T05. Prove the process viability through scale-up demonstration and a business case.

These technological objectives were transformed into the following activity areas.

Photobioreactor Development
The engineering and design optimisation for the photobioreactors (PBRs) was completed. A pilot-scale validation PBR of about 1 m3 was established and used to evaluate the production process configurations with a particular focus on ensuring the reliable production of ethanol within PBR for long time periods in order to maximise the ethanol concentration.

Ethanol Separation

The efficient separation of the media and the product within the PBR required a significant level innovation in dealing with low ethanol concentrations. An ethanol separation process model and evaluation of the alternative methods took place. A high selectivity membrane pervaporation based pilot scale ethanol separation system was developed and tested and benchmarked against the alternatives of distillation, mechanical vapour compression and ethanol stripping methods via in silico models. A suite of reference process designs were be prepared for both large, medium and small scale deployments. The ethanol separation technology is also relevant to other lower concentrations of ethanol applications such as biofuel technologies based on lignocellulosic feedstocks.

DEMA Process and System Integration

The creation of an economically and ecologically competitive biofuel production process required the design efforts and knowledge throughout the entire DEMA project to be integrated into an effective solution. The full DEMA Process was modelled and evaluated from Value Engineering and LCA perspectives. The exploitation of renewable energy such as Anaerobic digestion of the excess biomass is used to supply energy for the ethanol-separation process and improve its energy balance. The information from the pilot scale plant was used to provide the specific detail for the engineering and design necessary for the larger scale reference designs. The modelling of the operational economic (OPEX) and ecological performance provided a foundation for the DEMA process design since it is essential to attain an acceptable energy balance and associated carbon footprint on a day to day basis. A range of reference design configurations were modelled in terms of the capital expenditure (CAPEX) so as to provide guidance on the economic Business Case for firms wishing to invest in a biofuel facility utilising the DEMA process. The modelling of the carbon footprint of DEMA process facility establishment was also very helpful to ensure that the ecological case for the facility was similarly analysed and validated.

The Primary DEMA Challenges - DEMA Integrated Process Innovation and Development are outlined in the following list.

1. DEMA Pilot PBR bioethanol productivity (g/l/d)
   Final Status: Productivity per area approached targets set in the DoW. Productivity in terms of g/l/d was limited by the low biomass concentration which in turn yields a low ethanol concentration. This large capital investment needed for the PBR limits the commercial exploitation potential of the DEMA Process.

2. DEMA Pilot PBR maximal bioethanol concentration titre (g/l)
   Final Status: A concentration of 4.5g/l was achieved however this required special measures within the PBR to achieve this level. This low bioethanol concentration titre in the PBR is a very significant barrier to achieving an economic energy balance. This low ethanol concentration level requires a larger amount of energy to be used in order to extract less energy within the resultant bioethanol.

3. DEMA Pilot PBR rapid setup and longer term sustained culture
   Final Status: The inoculum production proved to be very slow growing due to the partitioning of the carbon for ethanol production.
   The Culture Set-up time Days is the most appropriate performance indicator. This DEMA PBR used a combination of lab scale inoculum producing PBRs and small scale PBRs where the DEMA ethanol producing strain was initially grown. The growth rate of the DEMA ethanol producing strain is much slower than the base wild type strain. This slow growth leads to a longer than culture setup time of 15 days. This is a practical limitation of the DEMA Process PBR module for production deployment.

   The Culture Run Time (Stability) is the performance indicator that was sued to monitor the pilot scale PBRs where the DEMA ethanol producing strain was grown on a daylight basis. The maintenance of a stable culture within the DEMA Process proved to be a considerable challenge with an increased risk of contamination due to the presence of ethanol in the medium. The longest culture duration of 70 days was less than the target of 90 days. The achievement of this stable required considerable expertise and effort on a sustained basis. This limitation of the DEMA Process also represents a significant commercialisation challenge.

4. DEMA Ethanol separation (50 g/l or 10g/l worst case)
   Final Status: A technically feasible DEMA Ethanol Separation process module was proven to operate with an incoming ethanol concentration of 4.5g/l . The associated energy balance is problematic however. This DEMA Ethanol Separation process module can be exploited in other bioethanol applications where the incoming ethanol concentration is better.

5. Deliver an investor ready DEMA process beyond the state of the art and the state of the practice.
   Final Status: Presentation to potential investors was made at the Algae Industry Conference in Nice 2017. The large capital investment in particular will prove a barrier to potential investors.

6. Ethanol Cost €/l has an overarching DEMA objective of €0.40/l
   Final Status: The €0.40/l objective can be achieved at an operational level. A DEMA process configuration has been developed integrates renewable energy as part of the process. If the core DEMA process is considered however without renewable energy then cost of the exogenous energy at commercial rates will be greater than the revenue earned from the bioethanol produced.

DEMA Process – Performance Indicator Portfolio

In order to measure progress towards achieving the Innovation Challenge Outcomes and Scientific Objective fulfilment during the project an appropriate suite of DEMA

performance indicators was developed at the commencement of the project. It was noted early on that it was necessary to have this suite of performance indicators so as to guide and prioritise the work necessary to complete the DEMA Project. This suite of key performance indicators (KPIs) were selected as they were considered to be the best way to summarise the outputs of this complex endeavour. The KPIs were particularly necessary for potential licensees of the technology to evaluate the efficacy of the DEMA process. It was noted the inter-relationships between the KPIs meant that some KPIs were much more dominant influence on the performance outcome for the entire DEMA process than others. It was for this reason that a key theme of the DEMA project was the effort to improve both the ethanol concentration and the ethanol productivity per unit volume within the photobioreactor. The ethanol concentration dominates the operational cost performance of the DEMA process. The ethanol productivity per unit volume within the photobioreactor has a strong influence on the capital cost (CapEx) of the DEMA process.

The list of DEMA Ethanol Separation & Economic Key Performance Indicators (KPI) developed were:

KPI 1 Bioethanol Cost (€/l)

This KPI has been evaluated through the use of process models as well as having the PBR and ethanol separation modules verified via pilot scale prototypes.

The operational cost of €0.40 litre can only be achieved with the extensive use of non-fungible renewable thermal energy. If exogenous or commercial energy sources are used then this operational cost target cannot be achieved. This result causes the DEMA Process to be at TRL level 4 to 5.

KPI 2 Capital Expenditure Performance - (k€ per m3 of ethanol nominal annual capacity)

In all business case scenarios the capital expenditure estimates were computed using basic engineering process models. The low ethanol concentration at all stages requires a very large PBR investment relative to the ethanol output performance. This result means that over a 20 year life time of the process that the capital cost per unit of ethanol produced is an order of magnitude greater than for sugar cane based ethanol production systems. This result causes the DEMA Process to be at TRL level 4 to 5 and represents an important commercialisation barrier.

KPI 3 Operational Expenditure - Fixed - (k€ per m3 per year of ethanol nominal annual capacity)

The annual fixed costs for the DEMA Process was computed using process models using. This cost is dominated by the capital expenditure depreciation and the cost of personnel. The low ethanol productivity and concentration per m3 of PBR has resulted in a very high cost Operational Expenditure – Fixed cost.

KPI 4 Operational Expenditure Performance - Variable - (€/l of ethanol produced)

This KPI has been evaluated through the use of process models as well as having the PBR and ethanol separation modules verified via pilot scale prototypes. The DEMA process can have its Operational Expenditure Performance improved by the use of

non-fungible renewable thermal energy. In the general case the where commercial energy sources of heat and power are used then the DEMA process has a negative added value. The variable cost of energy is greater than the value of the revenue of the bioethanol produced.

KPI 9 Bioethanol Separation Energy Balance Ratio (Lab)
This performance indicator was computed using a laboratory scale ethanol separation process. It showed that the use of electric power to run the membrane systems was energy negative where the electrical energy required was much larger than the energy content of the bioethanol product.

KPI 10 Bioethanol Separation Energy Balance Ratio (Pilot-Boundary)
This performance indicator was computed using a combination of pilot scale ethanol separation process and computation process models. It demonstrated that the use of non-fungible thermal energy can reduced the required electric power to run the ethanol separation system. The total energy consumed still resulted in an energy negative process where the thermal and electrical energy required was larger than the energy content of the bioethanol product.

DEMA Photobioreactor (PBR) Key Performance Indicators (KPI)

KPI 5 Bioethanol per g/M2/day (Photosynthetic- Lab Scale)
This performance indicator was measured from laboratory scale PBRs where the ethanol producing strain was grown on a 24 hour basis. The level achieved of 3 g/m2/day

KPI 6 Bioethanol per g/M2/day (Photosynthetic- Pilot)
This performance indicator was measured with a pilot scale PBRs where the ethanol producing strain was grown on a daylight basis. The level achieved of 1.1 g/m2/day is low which in turn negatively influences the KPI 1 through KPI 4 and KPI 7 – KPI 8 & KPI 11.

KPI 7 Bioethanol Concentration in broth g/L (Lab Scale)
This performance indicator was measured from laboratory scale PBRs where the ethanol producing strain was grown on a 24 hour basis. The achieved level was 4.5 g/l. This level while representing a published state of the art for this process type is still too low to facilitate the commercialisation of the DEMA process.

KPI 8 Bioethanol Concentration in broth g/L (Pilot)
This performance indicator was measured with a pilot scale PBRs where the DEMA ethanol producing strain was grown on a daylight basis. The level achieved in pilot scale PBR at the final stage was similar to that achieved in the laboratory. This level required special culturing methods to achieve.

KPI 11 Photosynthetic Energy Efficiency (Sunlight to Ethanol)
This performance indicator was measured within the greenhouse of the pilot scale PBRs where the DEMA ethanol producing strain was grown on a daylight basis. The Photosynthetic Energy Efficiency was less than 2%.

KPI 15 Culture Set-up time Days
This performance indicator was measured from a combination of lab scale inoculum producing PBRs and pilot scale PBRs where the DEMA ethanol producing strain was grown on a daylight basis. The growth rate of the DEMA ethanol producing strain is much slower than the base wild type strain. This slow growth leads to a longer than culture setup time of 15 days.

KPI 16 Culture Run Time (Stability)
This performance indicator was measured from a combination of lab scale inoculum producing PBRs and pilot scale PBRs where the DEMA ethanol producing strain was grown on a daylight basis. The maintenance of a stable culture within the DEMA Process proved to be a considerable challenge with an increased risk of contamination due to the presence of ethanol in the medium. The longest culture duration of 70 days was less than the target of 90 days. The achievement of this stable required considerable expertise and effort on a sustained basis. This limitation of the DEMA Process also represents a significant commercialisation challenge.

DEMA Process Environmental Key Performance Indicators (KPI)
KPI 12 Environmental Performance (g CO2e Emissions per m3 of ethanol nominal annual capacity - Construction)
KPI 13 Environmental Performance - (g CO2e Emissions per l ethanol - Operational)
KPI 14 Environmental Performance - CO2e Savings Vs Petrol (%)

The Performance Indicators KPI 12 – KPI 14 were computed using process modelling software with inputs coming from the basic engineering designs of all the DEMA Process modules. In the best case scenario of where a large proportion of non-fungible thermal renewable energy is used the operational environmental performance is still deficient compared to first and second generation ethanol production techniques. In this scenario the KPI 12 Environmental Performance (g CO2e Emissions per m3 of ethanol nominal annual capacity - Construction) becomes the most salient KPI. The critical issue is the environmental footprint per unit of ethanol producing capacity is large which in turn creates a significant environmental challenge.

In the scenario where exogenous commercial energy sources of heat and power are used then the environmental performance of the DEMA process is deficient compared to conventional fossil fuel. This is a key barrier to commercialisation of the DEMA process.

The DEMA consortium believe that these KPIs are of benefit in any biofuel production benchmarking activity. The DEMA process was benchmarked against first

and second generation ethanol production techniques and also biodiesel production processes during the project.

In terms of the Project Objectives i.e. ethanol producing strain development and developing a unique Integrated Process Innovation: the results of the work towards these objectives surpass the state of the art and provide clear pathways for future development.

Table 1 DEMA KPI Portfolio Target Vs Outcome

KPI 1	**Bioethanol Cost (€/l)**			
	Target			**0.4**
	Outcome at end of Project			0.4
KPI 2	**Capital Expenditure Performance - (k€ per m3 of ethanol nominal**			
	Target			**21**
	Outcome at end of Project			>40
KPI 3	**Operational Expenditure - Fixed - (k€ per m3 per year of ethanol**			
	Target			**0.1**
	Outcome at end of Project			0.2
KPI 4	**Operational Expenditure Performance - Variable - (€/l of ethanol**			
	Target			**0.3**
	Outcome at end of Project			0.4
KPI 5	**Bioethanol per g/M2/day (Photosynthetic- Lab Scale)**			
	Target			**12**
	Outcome at end of Project			3
KPI 6	**Bioethanol per g/M2/day (Photosynthetic- Pilot)**			
	Target			**9.3**
	Outcome at end of Project			1.1
KPI 7	**Bioethanol Concentration in broth g/L (Lab Scale)**			
	Target			**50**
	Outcome at end of Project			4.5
KPI 8	**Bioethanol Concentration in broth g/L (Pilot)**			
	Target			**50**
	Outcome at end of Project			4.5
KPI 9	**Bioethanol Separation Energy Balance Ratio (Lab)**			
	Target			**3**
	Outcome at end of Project			0.4
KPI 10	**Bioethanol Separation Energy Balance Ratio (Pilot-Boundary)**			
	Target			**4**
	Outcome at end of Project			4
KPI 11	**Photosynehetic Energy Efficiency (Sunlight to Ethanol)**			
	Target			**13**
	Outcome at end of Project			<2
KPI 12	**Environmental Performance (g CO2e Emissions per m3 of ethanol**			
	Target			1.85t
	Outcome at end of Project			0.35t
KPI 13	**Environmental Performance - (g CO2e Emissions per MJ ethanol -**			
	Target			**7**
	Outcome at end of Project			5
KPI 14	**Environmental Performance - CO2e Savings Vs Petrol (%)**			
	Target			**95**
	Outcome at end of Project			>90
KPI 15	**Culture Set-up time Days**			
	Target			**0.5**
	Outcome at end of Project			15
KPI 16	**Culture Run Time (Stability)**			
	Target			**90**
	Outcome at end of Project			70

Potential Impact

When the DEMA Consortium was originally formed a key motivation from all partners was to collaborate in creating an environmentally favourable biofuel that also was economically sustainable. At the completion stage of the DEMA Project it is opportune examine the project outcomes in terms of the impact that they will have on the relevant stakeholders. The impact categories that will be considered are as follows:

a) Impact on the Environmental Sustainability – Global benefits
b) Impact on EU Policy Priorities
c) Impact via Commercial Exploitation
d) Impact Barriers and Sensitivity Analysis

Impact on the Environmental Sustainability – Global benefits

The increasing levels of greenhouse gases in the atmosphere have been created as a side effect from global economic activity. These supply chains have generated externalities in the form of Greenhouse Gases (GHG) in the form of CO2, CH4 and NOx being released.
The DEMA Project has delivered a technology portfolio that captures CO2 prior to atmospheric release and uses it as a biofuel feedstock to produce bioethanol. The DEMA Process is one of only a few value chains that does not add to the stock of (GHGs) in the environment. The direct production of bioethanol from microalgae within low-cost scalable photobioreactors has added to the portfolio of available biofuel technologies that can be used to meet the increasing global demand for ecologically viable biofuels that do not compete with food production resources. The ecological engineering of the DEMA process has shown via Life Cycle Assessments (LCA) models and energy balance that it is feasible to use microalgae to produce bioethanol for a reduced carbon footprint than the cost of its biodiesel based microalgae equivalents.

Given the nature of the environment then any improvement in a biofuel carbon footprint will generate global benefits.

This DEMA CO2 feedstock approach also minimises the unintended consequences of many first generation and second biofuel technologies which can be described as follows:

1. The DEMA Process does not compete with food supply directly for feed stocks, such as maize, wheat or sugar beet.
2. The DEMA Process does not compete with food supply indirectly by using valuable arable land and soils and scarce fertiliser nutrient resources.
3. The DEMA Process is not limited by the supply of finite biomass resources (eg. straw or corn stover), which is not available throughout the year. While the supply of lignocellulosic feedstock does not compete directly with food there can be indirect competition due to the requirement for arable land. The process to transform the lignocellulosic feedstock from straw to bioethanol is complex and tends only to be economic at very large scales of operation. This in turn is problematic for transport costs of the raw material from a wide area.
4. In the biofuels sector in particular the first generation technologies have had some unintended consequences with biodiesel from palm tree plantations causing change of

land use environmental problems. Over the past twenty years an area the size of Ireland has been transformed from tropical forest into a palm oil monoculture.

Impact on EU Policy Priorities

Economic competitiveness and energy security
Since the EU is not energy self-sufficient and relies on continuous access to fossil fuel reserves from third countries which transfers considerable economic resources that could be used instead to promote economic added value within the EU. The DEMA process enables local EU production of biofuels while also reducing the carbon footprint.

Environmental Policy Options
The most important potential contribution of the DEMA Technology from an EU policy perspective is that it can provides a biofuel technology meets personal mobility demand with a carbon neutral capability. In the case of global warming situation there is a very urgent need to stop increasing the atmospheric CO2 level then the DEMA technology is available to meet this need without the negative consequences for world food production. Carbon negative technologies such as the DEMA process make become a valuable policy option if the experience of climate change in future years creates a more immediate imperative to reduce CO2 in the atmosphere.

The European Technology and Innovation Platform Bioenergy (ETIP Bioenergy) Initiative of the SET-Plan
When the DEMA project concept was being developed it was strongly informed by the SET Plan envisioning a Strategy for competitive, sustainable and secure energy for the EU. The SET Plan focused on the Role of the Technology Platforms that which would boost research, demonstrate innovation and accelerate development and deployment of technology. The DEMA Project was fully aligned with the bioenergy value chain #7: Bioenergy carriers produced by micro-organisms (algae, bacteria) from CO_2 and sunlight.

The DEMA project contributes to meeting the 2020 targets for biofuels from biomass since it helps to reach the key performance indicators (KPI´s) for second generation biofuels developed by the European Industrial Bioenergy Initiative (EIBI team) for ethanol and higher alcohols from lignocellulosic biomass by biological processes. The EIBI was one of the industrial initiatives under the SET Plan that aim to prioritise and facilitate 'first-of-a-kind' demonstration of innovative 'clean energy' technologies in Europe.

At the commencement of the DEMA project the Strategic Energy Technology (SET) Plan of the European plan had a bioethanol target price before taxes equal or lower than 0,50 €/litre. DEMA project has reached the operational target of 0,40 €/litre with the use of renewable energy resources. Additionally, the KPI for GHG savings for bioethanol compared to petrol shall be at least 60% to be long-term sustainable according to the current EU Biofuels policy. This GHG target requires that not more

than 33.5 g CO_2/MJ of GHG should be emitted for all value chain. Given that the DEMA process can exploit renewable sources of energy the amount of CO2 required for each litre of bioethanol can be even lower.
The DEMA Process technology has been modelled in terms of energy savings and careful life cycle analysis would be carried out at all stages of the technology development. It is our understanding that a maximum of 10 g CO_2/MJ should be attained for the whole value chain in DEMA. In some cases where renewable technology is used the DEMA Project can achieve a carbon neutral performance.
The use of microalgae as biomass grown under closed and tightly controlled reactors does not raise problems concerning land use changes (either direct or indirect) since microalgae facilities only requires set aside and marginal lands. In short, DEMA project adds to the technology portfolio that can contribute towards main goal of SET Plans for biofuels, which is to deploy demonstrate plants in the selected Bioenergy value chain of production of bioenergy carriers by microorganisms from CO_2 and sunlight, before 2020.

Connecting economic and environmental gains – the Circular Economy

The DEMA Process can be also seen as initial version of the technology contributes to the roadmap of the SPIRE cPPP and to the focus area "Connecting economic and environmental gains – the Circular Economy". In this case the CO2 is captured and reused before being released into the atmosphere.

The DEMA ethanol separation reference designs for both small and large scale facilities can be applied to biofuel technologies based upon lignocellulosic feedstocks. In many cases these biofuel technologies are constrained by lower ethanol concentrations which negatively impact their economic performance. In the cases the DEMA ethanol separation would improve their economic performance to a level when there are more competitive since it would reduce the operational energy cost of this key process step.

Responsible Research and Innovation (RRI)
The DEMA Process is also an example of the Responsible Research and Innovation (RRI) means societal actors (researchers, citizens, policy makers, businesses, third sector organisations, etc.) working together during the whole research and innovation process to align the processes and outcomes with the values, needs and expectations of society. In the case of the DEMA Project the practical imperatives of the transport market. In the DEMA project execution the RRI values were implemented as a package by enabling easier access to scientific results via open access publications. The DEMA Project also supported the gender dimension and ethics within its research and innovation activities.

Impact via Commercial Exploitation

The DEMA Consortium has developed, demonstrated and completed a well-received investor pitch of a complete DEMA Process design. Enterprises that wish to produce bioethanol with the most ecological and operationally competitive technology

attended an end-of-project exploitation workshop which has led to follow-on discussions on aspects of the DEMA process.

Exploitation of Results

In working towards the set of performance challenges towards the scientific and technical objectives the DEMA consortium have generated significant contributions to the theory as well as the practice of biofuel production. The primary achievement of the DEMA project is that it has advanced the opportunity to exploit a unique strength of microalgae: the ability to produce bioethanol using energy from photosynthesis. The DEMA project has closed key gaps in the direct ethanol production from microalgae scientific and technical knowledge domains and has advanced the technology readiness level from TRL level 2-3 to TRL level 4-5. The results from the DEMA project have better elucidated the commercialisation pathway for this innovative solution to EU biofuel needs. The US firm Algenol has previously reported that they were close to commercialisation however they have recently paused their work on the production of biofuels in order to focus on other sustainable products such as natural colorants, proteins and personal care ingredients.

In comparing the performance of the DEMA process to the other methods of producing bioethanol via first or second generation methods it is important to note the following benefits:

The value engineering of the DEMA process has yielded a system design that is less complex and has superior operational economic performance than the alternative microalgae biodiesel based biofuel production technologies. The most important difference between DEMA process and the biodiesel based microalgae technology alternatives is that in the DEMA case the microalgae is utilised to produce biofuel for seventy days rather than simply growing the microalgae and then immediately converting it to a biofuel. In this way this catalytic conversion of solar energy, H_2O and CO_2 into ethanol using the slow growing microalgae as a biocatalyst is a superior approach.

The DEMA Process can be exploited in any area where there is a source of CO_2, reliable sunlight (high insolation) and unused land resources. In many cases where there are good solar insolation conditions there is also considerable arid or non-agricultural land available. In order to gain maximal market traction for the demand for the biofuel produced with the DEMA process it will be essential that the environmental credentials of the resulting bioethanol be able to command a premium in the marketplace by demonstrating the extremely high level of environmental performance of the DEMA biofuel production lifecycle.

The initial expectation that the DEMA process would be deployed in smaller scale plants. It was initially expected that the DEMA Project results will be deployed in two main ways:

1. Large scale facilities where the CO_2 and waste heat are available where the daily ethanol production volume is 400 m^3/day

2. Medium scale facilities where the CO2 and waste heat are available where the daily ethanol production volume is 40 m3/day
3. Ethanol Farmers where the daily production volume is 1m3/day.

The DEMA Process value engineering work has shown that biofuel facilities have strong economy of scale effects making it economically challenging to deploy these smaller scale plants. The effect of scale can also been seen in first generation biofuel facilities with typical plant sugar cane ethanol plants typically producing 500 to 2000 m3 per day.

The considerable experience and expertise of the DEMA consortium has devised a range of business scenarios to show how the DEMA process could be exploited.

Technology Commercialisation Phase

Two business case scenarios approaches has been considered:

- One is an "Energy only scenario" in which all ethanol produced is used for energy generation as a biofuel while the biomass in its totality is used for anaerobic digestion for production of biogas. The biogas is afterwards used in a co-generation plant to generate electricity, heat and carbon dioxide that can be fed back to the DEMA PBR.

- The second approach is the "Biorefinery scenario" which is similar to the "Energy only scenario" with the exception that the biomass before going to anaerobic digestion is used to extract Zeaxanthin and other high value pigments. The remaining biomass after extraction is then used for anaerobic digestion.

In addition to these two approaches the impact of the GMO legislation environment with regards to the conditions for their utilisation will be studied. Some legislation are very strict and require total confinement of the plant (e.g Europe) while others may allow less strict rules. The study of the DEMA strain fitness in the wild at the location of implantation has shown that there GMO will not be viable outside of the PBR. This GMO safety Standard Operating Procedure developed by DEMA will ensure that the local legal requirements demand a total confinement of the GMO plant the impact of the infrastructure needed to comply will be fulfilled.

Although the outputs from the DEMA project are of great scientific and technical quality and significance, the technology still has a range of challenges that limit the commercial exploitation of technology. In order to demonstrate the DEMA technology at commercial scale, it is necessary to bring the technology to TRL 9. There are a number of performance areas that the DEMA technology that if improved will have a positive strong influence on the exploitation opportunity. The main technical activities of the technology commercialisation phase will be to "debug" the DEMA pilot level prototype, build a commercial grade demonstrator and perform long term measurements of the impact of this prototype on plant level performance. Reliability testing of the technology would also be of additional value. Documentation beyond the basic engineering level will also be required to define further the manufacturing standards, installation procedures and further refinement

in the operating procedures. The outputs of this post pilot scale activity would further verify the performance benchmarks of the DEMA technology against conventional first generation ethanol and other microalgae based biofuel technologies.

Impact Barriers and Sensitivity Analysis

DEMA Technology – Impact Sensitivity Analysis

There are a number of developments in allied technologies that can strongly enhance the commercialisation potential of the DEMA technology portfolio. The following areas are the most important:

1. Capital Cost per unit of Ethanol Production capacity
   The capital cost of greater than >40k€ per M3 of ethanol production capacity is an important limitation of the DEMA process. This high capital cost is driven by the low concentration of ethanol being produced at all stages of the process. This high capital cost means that it is more economic to invest in to biofuel production techniques at this time. This issue reduces DEMA Process TRL to 4-5 with a considerable technical development required.

2. Operational Expenditure Performance - Variable
   The DEMA process can have its Operational Expenditure Performance improved by the use of non-fungible renewable thermal energy. In the general case where commercial energy sources of heat and power are used then the DEMA process has a negative added value. The variable cost of energy is greater than the value of the revenue of the bioethanol produced. This issue reduces DEMA Process to TRL 4-5. This TRL requires a considerable technical development required the process economically to commercialisation.

3. Photobioreactor materials economic and ecological cost
   Reductions in the carbon foot print and cost of photobioreactor raw materials especially the glass tubing. These component materials are significant for the capital investment required for the DEMA Process. The energy and associated carbon footprint for these materials are also significant.

4. Photobioreactor temperature control
   The DEMA Process Unilayer PBR design relies on the application of water for temperature control. In cases where the PBR temperature may exceed 30C then the water requirement can be substantial. The control of temperatures within PBRs without water can be accomplished however it will add to the DEMA process Capital Expenditure (CapEx) requirements.

5. CO2 Pricing Initiatives
   While there has been recent efforts to price GHG externalities of many economic activities in the form of Carbon Credits however the current market prices do not appear to provide EU and global value chains with a compelling motivation to

adapt to more sustainable operating methods. An additional challenge that has emerged since the DEMA project commenced has been the change in the pricing of CO2 emissions. There has been a significant reduction of cap and trade and similar type carbon credit pricing initiatives. This has meant that the revenue opportunities to reward the plant owners of DEMA type facilities for the capture and reuse of CO2 is not available to the extent that was expected at the beginning of the DEMA project. The current price of €8 for CO2 European Emission Allowances is much less than half the level it was at the beginning of the DEMA project. A higher carbon credit price would better incentivise the deployment of DEMA ecologically responsible facilities.

6. CO2 direct air capture
   At the moment the DEMA process is limited to large CO2 point sources where it is captured from flue gas. This limits the number of potential deployment locations for the DEMA process. The case of direct air capture for the DEMA process is made easier since it does not need high purity CO2. The initial work as part of the DEMA process has shown that the use of Metal Organic Framework (MOF) materials works well for CO2 extraction from flue gas. This technology would enable the DEMA process to be located as part of vast biofuel production facility in available large scale arid areas with good solar insolation.

7. Metabolic Engineering with switchable components
   In the development of the DEMA process there was a considerable effort invested to enhance the proportion CO2 that was used to produce ethanol within the DEMA strain. When the strain is producing ethanol within PBR this approach is appropriate. It does create a challenge for the production of the Ethanol producing strain inoculum production phase. The growth rate of the inoculum is limited since it is devoting a lot of energy to also producing ethanol. There is a need for a better methods to turn on and off the ethanol production elements of the pathway. In this way when during inoculum production its growth rate of biomass / biocatalyst is maximised. When the resultant inoculum is then transferred to the ethanol producing photobioreactor then it the ethanol production capabilities of the strain should be switched on so that the majority of the energy is devoted to producing ethanol. This approach would reduce the capital expenditure necessary for inoculum production. Riboswitch was developed within the DEMA project however it reduced the ethanol production levels. The DEMA process would benefit considerably from a technology that enables an effective separation of the biomass/biocatalyst production and ethanol production.

8. Fossil Fuel Pricing
   One of the competitive issues that have emerged since the beginning of the DEMA project has been the reduction in fossil fuel prices. In the time period prior to the beginning of the DEMA project oil prices had reached close to US$150 /b whereas prices now are a third of this. There has been significant Fossil Price changes since the DEMA Project commencement. When the DEMA project was conceived oil prices had been on an increasing trend with the expectation that

competing biofuel technologies would soon be able to compete solely on price. This was based upon the long term trend showing a longer term fossil fuel price increase trend. This experience of fossil fuel prices created an expectation that biofuels were an imperative for economic reasons. The reversal of this trend where the price benchmarks has fallen from US$150 pbl to close to US$50 pbl has had a negative influence even on investment patterns in both first generation bioethanol facilities worldwide as well as on advanced biofuel technologies.

9. Competition from First Generation Bioethanol
   In the case of first generation bioethanol technologies the DEMA technology the most important benchmark process is the sugar cane ethanol system. This process has achieved the lowest economic cost per litre and indeed it is a key part of the existing automotive fuel industry particularly in Brazil where over a 30 year period the technology has been evolved into a mature status. The sugar cane system is also among the better performing bioethanol production systems from an ecological perspective since it uses the residual bagasse to provide process energy. In the case of EU based first generation systems such as sugar beet, wheat or corn based systems the ecological performance is limited by the use of exogenous fossil fuel based energy sources. The main limitations of this first generation systems is that it diverts resources from food to fuel with significant problematic food price consequences for low income populations. It is also limited where these systems can be applied where there is a need for land and a set of specific climatic conditions.

10. Fossil Fuel Pricing and First Generation Bioethanol
    An additional issue for DEMA ethanol competing in the EU biofuel market is that much of the bioethanol within the EU is produced from wheat and sugar beet crops. In these cases exogenous energy sources such as natural gas and electric power are used to provide process energy. When the cost of natural gas falls it actually improves the economic competitiveness of these first generation bioethanol *vis a vis* second or third generation biofuel technologies.

Overall Impact of DEMA Project

The overall impact of the DEMA project can be summarised as proving the technical feasibility of producing ethanol directly from microalgae. The most ecological DEMA Process configuration requires a high capital investment however in order to provide drop in transport fuel.

DEMA Project Results

This section presents the main results which were achieved throughout the DEMA project

Direct Ethanol from Microalgae (DEMA) Process

1. The DEMA Consortium has delivered an integrated process that is capable of producing bioethanol for less than €0.40/L operational cost using captured CO2 as a feedstock. This has been achieved by improving the volumetric productivity and ethanol tolerance, thereby enabling cost-effective transformation of solar energy, CO2 and H2O into a ready-to-use biofuel. This work has maximised the ethanol productivity of cyanobacteria through metabolic engineering; This DEMA Process configuration relies on the use of renewable energy. The core-DEMA process has a negative energy and economic balance if exogenous energy (heat and power) at commercial prices are used.

DEMA Ethanol Producing strain

2. In WP3 of the project, the catalytic conversion of solar energy, H2O and CO2 into ethanol has been carried out by a metabolically engineered strain of the cyanobacterium, Synechocystis sp. PCC 6803 that has overcome the ethanol tolerance limitations reported in the prior state of the art. This direct approach to the generation of bioethanol within the photobioreactor enables the economic and energy efficient production of biofuel at a reduced operational expenditure compared to other microalgae biofuel production techniques. This work has enhanced culture ethanol tolerance in order to obtain higher ethanol productivity and concentration.

Culture Stability and Safety

3. In WP7 and WP4 of the project, The DEMA Process ethanol producing culture that can be sustained for over 70 days was developed. This culture has been developed as a closed loop system that keeps the metabolically engineered cyanobacteria residual material contained within the DEMA process by using it as a feedstock to the anaerobic digestion module. This approach ensures the containment of any GMO materials within the DEMA system boundary. A comprehensive microalgae GMO Management Standard operating procedure has been developed as a secure methodology. This methodology has been evaluated within the DEMA pilot photobioreactor and it has shown that the metabolically engineered cyanobacteria do not survive outside of the culture conditions prevalent within the DEMA culture. In order to exploit the DEMA results it was noted that an extremely reliable GMO risk minimisation strategy was necessary to reduce the risk to a very remote level. In addition to the behaviour in the laboratory of single strains, it is essential to assess the potential invasiveness and persistence of ethanol-producing strains in natural communities. This was carried out by co-culturing the GMO with environmental samples under controlled conditions at A4F. This three-phase experiment was carried out under ideal laboratory conditions and then transferred to the GMO glasshouse facility at A4F. In parallel a method was developed for quantification of UL030 growing in these environmental populations, namely measurements of ethanol productivity, microscopic observation, and a PCR product check of the two genetic cassettes carried by the engineered strain. This work has maximised the biomass productivity and ethanol production through enhanced culture robustness

Constructs and process to increase the carbon dioxide uptake

4. In WP3 of the project, constructs and process were created to increase the carbon dioxide uptake of which can be applied to all phototropic species. The increased CO2

uptake enhanced cyanobacteria growth rates. Cloning of a series of carbon transporters to increase the flux of carbon into the cell. Enhancement of CO2 uptake has increased available CO2 for fixation and stabilised the loss of carbon that occurs as the flux from pyruvate to ethanol occurs.

Constructs and process to optimise ethanol production

5. In WP3 of the project, constructs and process to optimise ethanol production were developed via a library of pathway designs was created, in which the expression of each gene in the operon was varied using a series of differentially strength RBS elements. Genes included pyruvate decarboxylase (e.g. Zymomonas mobilis PDC), acetaldehyde reductase (e.g. slr1192 or E.coli yjgB), pyruvate kinase and/or bifunctional fructose-1,6-bisphosphatase/sedoheptulose-1,7-bisphosphatase. (BiBP)

Photobioreactor Process for clean cultivation of engineered cyanobacteria

6. In WP5 of the project, a process for clean cultivation of engineered cyanobacteria for high accumulation of EtOH in the culture medium and continuous extraction was developed. A new operational strategy for enhanced EtoH concentration as an alternative to further microalgae genetic improvement was developed by A4F.

DEMA Photobioreactor - Unilayer Horizontal Tublar Photobioreactor.

7. In WP5 of the project, a DEMA Photobioreactor - Unilayer Horizontal Tublar Photobioreactor has a superior surface area/volume ratio has been developed. Due to the GMO nature of strains in DEMA project, the open systems – Raceways (RW) and Circulating Raceways (CRW) – cannot be used as productions systems for the DEMA process, however they can be very effective systems to capture photons since the photobioreactor area is projected in a horizontal surface. On the other hand, vertical PBRs – tubular or flat-panels – presented a photosynthetic area that is dependent on the position of the sun. The use of this type of reactors requires an analysis of the solar path to determine the total surface exposed to sunlight for a given moment and location. Tools for such analysis have been developed and optimized by A4F for years now. During this period improvements were introduced to the existing flat-panel PBR, namely the coupling of glass tubes in an horizontal plane in order to improve the surface to volume (S/V) ratio of the photobioreactor and therefore the potential to increase of ethanol concentration in the culture.

Ethanol Separation Process.

8. In WP6 of the project, an ethanol separation process has been developed that is able to separate ethanol from concentrations as low as 4.5 g/l. This represents a significant advance on the state of the art for low ethanol concentration. It has been accomplished via high selectivity membranes for small scale ethanol production facilities 1m3/day and using heat driven compression multi effect ethanol stripping for large scale applications of 40 m3/day or 400 m3/day type ethanol production facilities.

High Selectivity Membranes for Ethanol Separation.

9. In WP6 of the project, higher Selectivity Membranes for Ethanol Separation membrane-driven processes have been developed. The improvement of the organophilic PDMS membrane has been essential to achieving ethanol separation at low concentrations. The membrane selectivity was increased from 6-7 to 13-14 and evaluated at the pilot scale with ethanol containing broth from the pilot scale photobioreactor.

Ceramic HybSci Membranes for fuel grade ethanol separation.

10. Fuel grade bioethanol for E85 type applications are required to have concentration levels of greater than 99.5%. This concentration level requires a dehydration processes to remove the water from the azeotropic ethanol/water mixture. In the DEMA Ethanol Separation process the DEMA project partner Pervatech developed a Ceramic HybSci Membrane which was shown to work with input concentrations of between 80-85%. This approach enables a lower energy footprint than the conventional approach of distillation to a 95.5% azeotrope level followed by dehydration via molecular sieves.

DEMA Process Development and Deployment Modelling

11. In WP6 and WP7 of the project, a suite of DEMA process models were developed that support the design, development and integration of the component technologies. The DEMA process models have been developed for two main activities. The first is the initial technology development within the DEMA project and the second is as part of the DEMA Process deployment. The DEMA Process Development Models were focused on predicting the energy and mass balance for the main modules of the DEMA process and provided key guidance in the evolution of the component technology designs. The modelling of the DEMA process Capital Expenditure from initial feasibility analysis, basic engineering, project management, construction and facility commissioning has been completed. The information from the pilot scale plant evaluations was used to provide the specific detail for the engineering and design necessary for the differing scale reference designs. In the case of the initial establishment of the DEMA Process the ethanol volumetric productivity has a dominant influence on the DEMA capital expenditure on the Photobioreactor facility.

    The DEMA Process Deployment Models support the following stages of a deployment project lifecycle:

    i) Parametric Model Design Ethanol Separation Process
    ii) Parametric DEMA Process Piping & Instrumentation Diagram Generation
    iii) DEMA Process – Integrated Control System
    iv) Parametric DEMA Process- Equipment Sizing and Construction Lifecycle Management
    v) LCA Modelling for Operational Ecological Evaluation
    vi) DEMA Process Operational Expenditure (OpEx) modelling.
    vii) DEMA Process Capital Expenditure and techno-economic viability Modelling

Sustainability assessment of the DEMA Process

The LCA model of the DEMA Process provided the inputs to the Sustainability assessment of the DEMA Process in when benchmarking its performance against other biofuel technologies. A full Life Cycle Analysis has been completed. The functional unit chosen for this study is 1 MJ of produced bioethanol, as the DEMA process is seen as a biorefinery. In terms of system boundaries was selected for this study the approach from cradle-to-gate. For this approach a number of differing scenarios were modelled.

The environmental sustainability assessment of the DEMA process includes the comparison to a status quo fuel production system which fossil diesel or petrol transport products. The baseline fossil fuel production system can be substituted for by the DEMA approach. This is a well know process and relies on increasing the proportion of ethanol in the petrol. In practice the maximal amount for inclusion is 85% with remaining 15% being petrol. This biofuel type also exploits the existing fuel infrastructure which is a sunk investment from an economic and ecological perspective. The economic and ecological performance of fossil fuel systems is driven by the very large economies of scale of conventional fuel production compared to the DEMA process value chain.

DEMA Process Technology assessment

In gaining an understanding of the status of a technology it has proven helpful to apply the Technology Readiness Level (TRL) approach to the DEMA Process. In the case of the DEMA process it was planned to achieve a TRL 8 where the DEMA Process would be provide evidence that the DEMA Process is proven in an operational environment and competitive in biofuel production in the case of key enabling technologies. In the case of the DEMA Process scenario outlined where renewable energy is used the delivered TRL in in the range TRL 4-TRL 5. The pilot scale evaluation of the DEMA process shows that TRL 4-5 has been well achieved as operational technology. If the core DEMA Process is evaluated then while the TRL level is unchanged the economic performance is problematic.

The primary work to be completed to get to TRL 9 for the DEMA process is to increase the ethanol concentration throughout the system by tenfold. This is a very considerable outstanding challenge. This development of the DEMA Process mainly in the area of improving the capital expenditure (CapEx) and the environmental impact of the DEMA process initial establishment.

The technology assessment of the concepts developed for the DEMA process show that due to the actual GHG emissions (biochemical only) and the primary energy demand of the DEMA Process (current TRL) have improved from their prior state but are not at (TRL 9). The main areas for improvement in the GHG emissions are the electricity demand especially needed for PBR operation and the ethanol separation stage. The energy provided by the anaerobic digestion of the biomass mitigates this issue. While the actual demonstrated state of technology is feasible on an operational basis. The fixed costs for personnel, capital depreciation and for exogenous energy does not achieve a positive economic position in terms of return on capital.

Due to actual demonstrated state of technology being at pilot level (TRL 4-5) due to the very high capital expenditure requirements. Further technology development is needed in order to achieve a TRL 9. A comparison using the guide values of the modelled full scale commercial and integrated DEMA Process was made to analyse the gap between the current TRL and the guiding values for TRL 9. An assessment of the actual state of technology, based on the developed concepts, in comparison to TRL 9 is provided.

WP2 MICROFLUIDICS & LAB-SCALE ANALYSIS

The main purpose of WP2 was to develop and use a microfluidic platform to improve the efficacy of the DEMA cyanobacterial experimental effort. This microdroplet platform was used to reduce the time and cost of evaluating a larger variety of strains when completing research on ethanol producing microalgae for biofuel applications. In particular this microdroplet capability was used to complete many small scale evaluations of differing microalgae versions in parallel which greatly accelerated the knowledge development process and reduced the cost per individual experiment.
This analytical infrastructure was used to support the scientific and technological objectives of entire DEMA project through all phases of development.

This work package fulfilled its original objectives by achieving the following outcomes:

i) The development of a robust microdroplets platform for high throughput analyses of cyanobacteria, both for strain selection and also to rapidly establish optimum growth conditions and other environmental parameters relating to ethanol production.
ii) The Verification and further optimization of cyanobacterial cultivation in lab scale photobioreactors (PBR), acted as an intermediate link between the microfluidic based microdroplet platform and pilot-scale photobioreactor cultivation within WP5.
iii) The microfluidic platform collaborates extensively with WP4 by exchanging information on how best to optimised strains bioethanol-producing strains Synechocystis PCC 6803, and assessing the behaviour of consortia for culture robustness and productivity.
iv) The microdroplets platform is available to make an important contribution to photobiological biotechnology on a broad level, both within and well beyond the DEMA project domain.

Key findings and conclusions:
This work package has contributed to the experimental technologies and methods available to progress microalgae research. In particular the development of the microfluidic platform has the potential to reduce the experimental effort and cost for evaluating a larger variety of strains when completing research on microalgae for biofuel applications. In particular the capability to complete many small scale evaluations of differing microalgae versions in parallel can greatly accelerate the knowledge development process and reduce the cost per individual experiment.

In particular the following results have been achieved.
1. New microfluidic devices were designed and fabricated for incubating cyanobacteria in microdroplets.
2. An automatic cells counting program was developed to calculate the number of cyanobacteria in microdroplets.
3. A novel and robust enzymatic assay was created to measure ethanol secretion in microdroplets.

4. A platform to screen cyanobacterial cells. A novel high throughput sorting instrument was built to screen a library of cyanobacterial variants.
5. A set of new software was developed to improve the speed, reliability, precision, and automation of the microdroplet sorting process.
6. A microdroplet encapsulation system to co-culture cyanobacteria with other species in isolated microdroplets was developed.
7. A device for holding microdroplets in arrays.
8. Two-channel fluorescence laser system which facilitates a high-throughput, indirect method to explore relationships between two organisms encapsulated in a microdroplet.
9. The microdroplets platform is an important contribution to the domain of photobiological biotechnology on a broad level, both within and well beyond the DEMA project.

WP3 Optimisation of Bioethanol-Producing Strain

The main purpose of WP3 was to use rational metabolic engineering methods to generate ethanol producing strains of the cyanobacterium Synechocystis sp. PCC 6803, with an ethanol productivity that exceeds the current state of the art by a factor of 5 or more. Prior to the commencement of the DEMA project there had been some prior publications and one start-up firm focused on this domain. The DEMA project has delivered over seventy ethanol producing strains of results Synechocystis sp. PCC 6803. The best DEMA Process strain produced was UL030 and it has been shown to have achieved the highest level of ethanol titer reported in the literature.

The performance of UL030 was benchmarked with Prokaryotic ethanol-producing strains have previously reported and it was shown that they have enhanced several-fold by several rational strategies with the DEMA project.

The following four main factors that were individually addressed and combined for complementary and synergistic enhancement:

(i) Pathway performance – Total catalytic activity, metabolic channelling and co-factor availability

(ii) Optimisation of host metabolism - Substrate-availability (carbon, electrons)

(iii) Ethanol-tolerance – Although current production levels are well below estimated tolerance-limits, these have only been tested by exogenous addition

Key findings and conclusions:

The work package has generated over seventy new ethanol producing versions of the cyanobacteria Synechocystis sp. PCC 6803. The following are the most salient results from this work package.

1. Genes encoding the ethanol-synthesis pathway have been codon optimized for improvement of the protein expression level. Screening of several PDC / ADH enzymes have been carried out to optimize kinetics towards higher flux to ethanol, double cassettes , one inserted into the phb gene to generate a Δphb deletion knocking out a competing pathway have been generated and overexpressed carbon transporters have been generated (bicA and sbtA expressed with the light activated promoter pSBA2).

2. Trials to maximise PDC and ADH expression via the utilisation of native high copy number host plasmids, use of strong promoters and markerless strains.

3. Competing pathways in central carbon metabolism and storage-product accumulation were modified. In addition, overexpression of key enzymes to enhance pyruvate concentrations and photosynthesis rates were carried out in order to redirect the metabolic flux towards the ethanol pathway.

4. Increasing carbon uptake by PCC 6803 cells has been targeted via overexpression of carbon concentrating pathways. Constructs and process to increase the carbon dioxide uptake of which can be applied to all phototropic species.

5. Examination of the separate contributions of the two enzymes [PDC/ADH] of the ethanol-forming pathway was carried out to titrate the optimum amount of each enzyme. Intermediate production formation was quantified [Acetaldehyde yield] to determine bottleneck in productivity.

6. Optimization of the expression of genes involved in the ethanol production - PDC and ADH by the use of different ribosome binding sites in a combination with two different promoters (PA1lacO1 and Ptrc)

7. PDC & ADH co-localization with SH3 tags has been carried out in order to enable intermediate channelling from one enzyme to another to improve reaction rates.

8. A strategy to utilise a high copy number native plasmid pCA2.4 have been utilised and proof of concept using expression of the YFP protein have been carried out demonstrating some 120 fold increase in protein expression.

9. Constructs and Process to create UL004. UL004 was constructed to generate an ethanol cassette. It contains the Zymomonas pdc and native Synechocystis adh.

10. Constructs and Process to create UL030. UL030 is an advance on UL004 in that it contains two functioning cassettes. This strain in addition to the construct outlined in UL004 contains another cassette which contains another cassette with the Zymomonas mobilis pdc fused to the Synechocystis adh.

11. Optimisation via Metabolic control analysis (MCA).
12. Introduction of Malic Enzyme for additional carbon supply and modulation of ADPH/NADH supply.

WP4 CULTURE STABILITY AND SAFETY

The main goal in WP4 was to develop novel methods to enhance culture robustness and optimise productivity over a long operating time. A known issue with microalgae cultures is that they can become unstable due to contamination and become unproductive. The longer the culture duration the higher the risk of the culture failing. Given that an ethanol producing culture has a higher risk of contamination due to having a readily available carbon source then it is essential this risk is mitigated.

Genetic strategies to enhance GMO safety have also been developed.

The outcome of WP4, development of biotechnological strategies to enhance production robustness and safety, is expected to make a strong contribution to the development of large-scale cyanobacteria biotechnology on a broad level, not just this DEMA project. WP4 collaborates in particular with WP2 (analytical infrastructure) and WP3 (strain improvement) by provision of analytical insight and exchange of strains.

Key findings and conclusions:
The work package focus on the imperative of creating a knowledgebase that has enabled the transformation of the use of ethanol producing strains from a low experimental TRL level to a point where the DEMA process has the reliability and performance dependability of other fermentation processes.

The most important results from this workpackage are as follows:

1. Novel methods to enhance culture robustness

WP4 has developed several functional novel methods to enhance culture robustness, with a focus on co-culture systems (Vit B12, NH(x)), but also alternative targeted (antibiotic - against prokaryotes, antifungal - against fungus) and non-targeted (probiotic) strategies. In order to evaluate culture robustness under relevant conditions (for the targeted industrial process) comprehensive efforts have been spent to understand the nature of the components of the A4F bioreactor ethanol production system and what impact additional biotic factors have on ethanol production.

2. Optimize productivity of a long operation time

A series of strategic experiments at a pilot-plant bioreactor level at A4F coupled with analytical support from all WP4 partners has contributed to increase the quantity of ethanol produced and length of time that production can occur. Through these efforts, A4F managed to increase the production time, in pilot scale and at significant ethanol levels, up to 26 days and in the Lab scale to at least 60 days.

3. Genetic strategies to enhance GMO safety

The introduction of an ethanol pathway into Synechocystis results in lower fitness relative to the wild-type. Field experiments demonstrated that all measurable traces of the genetically modified strain was lost over time, most likely as a consequence of the reduced fitness of the engineered strain. A comprehensive SOP for detecting and monitoring genetically engineered cyanobacteria was developed and documented.

Lessons learned on Culture stability throughout the DEMA project

- The composition of algal cultures are complex and change dynamically. Countering this may require more generalised and broader acting solutions as the "removal" of one species is likely to be followed by the uprising of another.
- The combination of multiple organisms in biotechnology is largely unchartered territory in the field of algae and leads to increased complexity and slower progress, but represents an untapped potential area for further development. Several multi-species "systems" were developed in DEMA and shown to be functional. Although only limited insight into the consequences of switching from mono- to poly-cultures were obtained, all the ingredients are now in place to evaluate this question using relevant materials and exploit this novel "technology" further.
- Genetic instability also influences large-scale production. Although not surprising, has become an important future research opportunity to further understand and solve this general challenge in order for the algae industry to commercially utilise genetically optimised strains under long-term conditions
- Genetic contamination of the environment may be less of a problem than anticipated, given the lack of persistence of engineered genetic elements in the absence of concrete solutions to minimize its spread.

WP5 PHOTOBIOREACTOR DESIGN & DEVELOPMENT

WP Objectives

This purpose of this workpackage was to provide the global engineering support for the transfer of the ethanol producing strain from the laboratory scale test and verification environment to the pilot scale photobioreactor (PBR). This pilot scale generated more industrially relevant information on for culture growth rates of the modified bioethanol-producing Synechocystis strain. The optimisation of these growth rates provide important design information on requirements the PBR construction and operation, and the masterplan for the full scale reference DEMA Photobioreator Plants.

The main objectives are:

(i) Engineering and design optimisation of photobioreactors for the Pilot plant covering production in photobioreactors, ethanol production optimisation, microalgae filtration, ethanol extraction and downstream processing of the biomass. The activity on this work package interacted closely with WP7 DEMA Process and System Integration to ensure that all available knowledge was gathered from all of the relevant partner activities. This work in turn was used in the design efforts for the entire DEMA Process. The DEMA Process testing within the pilot photobioreator provided the key technical information for biomass production, ethanol concentration enhancement, ethanol harvesting and purification are applied and integrated in the entire DEMA process (including small plants, pilot and larger scale DEMA plants).

(ii) The Pilot Plant has the objectives to test at smaller (than commercial) level the selected strains and the whole production process configuration in order to provide the specific details and operating data for the engineering and design of the small scale plant and specific information on productivity and operational parameters with the selected strains. In particular, most relevant objectives are: define the configuration of photobioreactors to be built and used, and perform a validation of full scale industrial production and of the techno-economic viability studies.

Key findings and conclusions:

The work package integrates all of the knowledge on the ethanol producing strains from the laboratory and transforms it into an ethanol production process with a stable culture within an actual Photobioreactor. Any limitations on the properties of the DEMA ethanol producing strain emerged within this work package. In conjunction with WP4 the knowledgebase was created that enable transformation from the use of ethanol producing from a low experimental TRL level to a point where the DEMA process has the reliability and performance dependability of other fermentation processes.

DEMA Photobioreactor Development Methodology

The Engineering and design optimisation of photobioreactors for the Pilot plant was focused on the following five areas:

a. Inoculum preparation and pilot scale production in photobioreactors,
b. Ethanol production,
c. Microalgae filtration,
d. Ethanol separation
e. Downstream processing of the biomass via anaerobic digestion.

The knowledge outcomes from the photobioreactor Pilot Plant DEMA culture evaluation of the selected ethanol producing strains was used to define the entire DEMA photobioreator

production process configuration. The main knowledge outcomes from this work were as follows:

i) Provided the specific details and operating data for the engineering and design of the small scale, medium scale and large scale DEMA photobioreactor facilities.
ii) Generated specific information on productivity and operational parameters with the selected strains
iii) Generated the define the configuration of photobioreactors to be built
iv) Completed a validation to verify the key design parameters to complete the of full scale industrial production
v) Provide the information that information techno-economic viability studies completed within work package WP7.

2. DEMA Photobioreactor - Unilayer Horizontal Tublar Photobioreactor

Due to the GMO nature of strains in DEMA project, the open systems – RW and CRW – cannot be used as productions systems, however they can be very effective systems to capture photons since the photobioreactor area is projected in a horizontal surface. On the other hand, vertical PBRs – tubular or flat-panels – presented a photosynthetic area that is dependent on the position of the sun. The use of this type of reactors requires an analysis of the solar path to determine the total surface exposed to sunlight for a given moment and location. Tools for such analysis have been developed and optimized by A4F for years now.
During this period improvements were introduced to the existing flat-panel PBR, namely the coupling of glass tubes in an horizontal plane in order to improve the surface to volume (S/V) ratio of the photobioreactor and therefore potentiate the increase of ethanol concentration in the culture. This improvement gave rise to a new type of reactor configuration developed by A4F denominated Unilayer Horizontal Tubular PBR (UHT-PBR). The performance of a multilayer horizontal tubular PBR (MHT-PBR) was compared to that of a unilayer horizontal tubular PBR (UHT-PBR) at the A4F pilot scale plant in Lisbon. For this test, the microalgae strain Synechocystis sp. UL030 was used in both systems and both were operated in similar conditions and with the same media.

In a MHT-PBR, the photosynthetic area, defined as the projected shadow of the photosynthetic zone of the PBR, varies during the year and during the day, resulting in different productivities. The variation on the photosynthetic area leads to the self-shading of the PBR in the winter or the losing of radiation to the ground in the summer. In a UHT-PBR, the photosynthetic area does not vary during the year and during the day and there is no significant shading between the tubes. The following Figure 3.1 and Figure 3.2 describe both PBR characteristics and performances.

WP6 ETHANOL SEPARATION

In order to produce a bioethanol from the DEMA process it is essential step is to extract the bioethanol from within the broth/medium contained within the PBR. The DEMA process has overcome the challenge of extracting the ethanol from the broth within the PBR at low concentrations. The technology necessary to effectively transform the output ethanol broth from the growth of the bioethanol-producing cyanobacteria grown in the pilot-scale Photobioreactors into fuel grade ethanol. In the DEMA Process the higher the ethanol concentration, the better efficacy of the Ethanol Separation Process. The initial expectation at the beginning of the DEMA process was for an higher ethanol starting concentration in the range of 10-20 g/l. The ethanol concentration within the PBR for the highest performing strain was 4-5 g/l which posed a significant additional challenge for this work package.

This work package has successfully meet this challenge and produced a technically functional ethanol separation technology.

The work package produced the following prime outcomes:

i) Designed, built and evaluated a skid mounted pilot plant ethanol separation process that produces ready-for-use bioethanol.
ii) Prepared a reference design and a comprehensive model for 1000 l/day capacity ethanol separation process for a distributed scale process.
iii) Prepared a reference design and a comprehensive model for 40,000 l/day capacity ethanol separation process for a large scale integrated process applications.
iv) Prepared a reference design and a comprehensive model for 400,000 l/day capacity ethanol separation process for very large scale integrated process applications.

The original WP6 Ethanol Separation performance objectives aimed at developing an ethanol separation process to achieve an energy balance of greater than 80% with an incremental cost of less than €0.20 per litre of bioethanol. These performance outcomes can only be achieved with the integration of renewal energy sources within the DEMA Process. If exogenous power and heat sources are used within the ethanol separation process the then these performance goals are very difficult to achieve given the low ethanol concentration in the broth.

Key findings and conclusions:

1. Limited availability off low ethanol concentration separation solutions

The initial expectation is at the beginning of the DEMA project was that the topic of ethanol separation was a well solved problem. While this is true for high concentration situations however in the case of low ethanol concentration fermentations this was not to be the case in practice. It was found from a detailed review of the literature and the current state of the practice of ethanol separation within EU as well as the sugar ethanol systems prevalent in Brazil that effective ethanol extraction from dilute aqueous solutions is not well developed.

2. The current state of practice distillation based approach to ethanol separation for first generation biofuel production is very difficult to adapt to low ethanol concentration applications. This means that the when completing a reference plant design then the ethanol separation system development represents a significant technical challenge.

3. The integration of renewable energy within the DEMA Process enables the ethanol separation process incremental cost to less €0.20 per litre of bioethanol. The capital investment in the supporting equipment however is substantial.

4. In comparing the business case for ethanol separation between first generation biofuels and the DEMA process it is important to note the source of the process energy. In the case of sugar cane ethanol production system the process energy is sourced from the residual bagasse biomass. In the EU context most of ethanol is produced from sugar beet, wheat or maize with the process energy being provided by fossil fuel sources. These sources of bioethanol create additional demand for fossil fuel energy and displace a substantial portion of the ecological benefit associated with using a biofuel.

WP7 DEMA Process and System Integration

The purpose of the work package WP7 DEMA Process and System Integration was to integrate all of the component technologies into a technical feasible and economically viable DEMA Process that was ready to deployment by biofuel facility investors. The creation of an economically and ecologically competitive biofuel production process required the design efforts and knowledge throughout the entire DEMA project to be integrated into an effective solution. The work package demonstrated the capability of the optimised process (cultivation and ethanol separation and biomass processing) at pilot scale under representative industrial conditions, based on the technology and methodologies developed in the previous WPs. The prime effort of this work package was to provide the linkage between the results of the individual work packages and demonstrate the efficacy of the DEMA Process and the pilot plant scale.

The main DEMA process integration activities can be broken down as follows:
i) Enhance the DEMA Process energy balance by valorising the excess microalgae biomass via anaerobic digestion and use the resultant biogas to provide renewable energy to the ethanol separation process.
ii). Developed an effective Integration between the bioethanol production system developed within work package WP5 Photobioreactor design and development and work package WP6 Ethanol Separation.
iii) Developed an integrated process control system for DEMA process operation was developed that can accelerate the deployment of the entire DEMA Process.
iv) Development of a suite of modelling tools to support the full project lifecycle of a DEMA process deployment.

Key findings and conclusions:

1. The three reference designs for small, medium and large scale DEMA process facilities show that the DEMA Process is economically viable on an operational basis.
2. The DEMA Process Value Engineering has been applied firstly to the operational / variable economic performance of the DEMA process. This portion of the Value Engineering effort has confirmed the strong operational economic (OpEx) consequence of the ethanol concentration at all stages of the process since it affects the ethanol separation energy requirements.
3. The modelling of the DEMA process Capital Expenditure from initial feasibility analysis, basic engineering, project management, construction and facility commissioning has been completed. The information from the pilot scale plant evaluations was used to provide the specific detail for the engineering and design necessary for the differing scale reference designs. In the case of the initial establishment of the DEMA Process the ethanol volumetric productivity has a dominant influence on the DEMA capital expenditure.
4. DEMA Process Integration for Heat and renewable energy.
   In order to drive the DEMA ethanol separation process it is necessary to provide a significant portion of heat. If this heat has to be provided from exogenous fossil fuel sources then the DEMA process will not be not be economic on an operational basis. It is also noted that the carbon footprint of these exogenous energy sources would also make DEMA process problematic from an ecological perspective.

5. DEMA Process Integration CO2 Capture

The capture of CO2 from external flue gas is also augmented by capturing the internally generated CO2 from the Anaerobic Digester process. In the case of the DEMA process the CO2 capture utilises Metal Organic Framework (MOF) materials via thermal swing process. While this MOF based CO2 capture process does not produce pure CO2 it is of value in the DEMA process case since typical CO2 concentrations are less than 5%.

6. The full DEMA Process was modelled and evaluated from a Value Engineering perspective. The modelling of the operational economic (OPEX) and ecological performance has provided a foundation for business case development of the DEMA process design since it is essential to attain competitive operational performance levels.

The most important finding from WP7 are as follows:

DEMA Process - Capital Cost per unit of Ethanol Production capacity

The capital cost of greater than >40k€ per M3 of ethanol production capacity is an important limitation of the DEMA process. This high capital cost is driven by the low concentration of ethanol being produced at all stages of the process. This high capital cost means that it is more economic to invest in to biofuel production techniques at this time. This issue reduces DEMA Process TRL to 4-5 with a considerable technical development required.

DEMA Process - Operational Expenditure Performance - Variable

The DEMA process can have its Operational Expenditure Performance improved by the use of non-fungible renewable thermal energy. In the general case where commercial energy sources of heat and power are used then the DEMA process has a negative added value. The variable cost of energy is greater than the value of the revenue of the bioethanol produced. This issue reduces DEMA Process to TRL 4-5. This TRL requires a considerable technical development required the process economically to commercialisation.

WP8 Business Plan, Exploitation and Dissemination

The purpose of the work package WP8 Business Plan, Exploitation and Dissemination was to plan the exploitation and dissemination of the DEMA scientific and technical results. The primary effort was to complete a market analysis. This understanding of the market enabled the development of the DEMA Process business model. This was work was supported by IP and knowledge management activities where the assemblage of the multi-technologies was combined into an IP package and made available for further exploitation. A DEMA Process business plan and associated technology exploitation plan were prepared. An investor pitch was completed which also included the dissemination of the DEMA project outcomes.

DEMA Process - Business Scenario – Small, Medium and Large Scale Plants

The DEMA project large scale application case study for the reference plan designs are best deployed in locations with the following attributes:

1. A substantial renewable CO2 point source of over 1,400,000 kg/day . (A fossil fuel point source can also be used however the LCA is obviously much improved if the net CO2 in the atmosphere is not increased when the bioethanol is used.)
2. Solar insolation of over 300 days per year. The Reunion Island case study location has reliable solar insolation for over 340 days per year. This is superior to Southern EU locations where the reliability of microalgae producing sunshine is lower.
3. Consistent ambient temperature of over 25 C so as to avoid reduced bioethanol productivity due to low temperature. Ambient temperatures of over 30 C also limit the bioethanol productivity.
4. Availability of low cost non arable land which does not displace food production or incur change of land use environmental costs.
5. Scale of deployment site > 700 ha