



# Sustainable Energy Authority of Ireland

National Energy Research,  
Development & Demonstration  
Funding Programme

## FINAL REPORT TEMPLATE

### SECTION 1: PROJECT DETAILS – FOR PUBLICATION

<b>Project Title</b>	A novel technology to maximise biofuel efficiency
<b>Lead Grantee (Organisation)</b>	Nektr Technologies Ltd
<b>Lead Grantee (Name)</b>	Monika Ehrensberger
<b>Final Report Prepared By</b>	Monika Ehrensberger
<b>Report Submission Date</b>	2/10/2023

	<b>Name</b>	<b>Organisation</b>
<b>Project Partner(s)</b>	Cormac Murphy	UCD
<b>Collaborators</b>		

#### Project Summary (max 500 words)

*Bioethanol is a potentially sustainable alternative to fossil fuels; however, challenges such as substrate shortages, low ethanol yield and high production costs all contribute to its relatively high price. It was recently discovered that by exploiting the hormetic effect of compounds such as H<sub>2</sub>O<sub>2</sub>, the reproductive ability and ethanol tolerance of biofuel producing organisms can be increased. Hormesis is a biological phenomenon characterised by beneficial results from exposure to low doses of a chemical that would be toxic at higher doses.*

*A3IS is a novel compound created by Nektr Technologies and its key characteristic is the slow and sustained release of low levels of H<sub>2</sub>O<sub>2</sub> in a controlled manner. All components of A3IS are Generally Recognised As Safe (GRAS), thus it could be added to the fermentation medium without process changes.*

*The aim of this project was to investigate the potential of A3IS to improve ethanol production in fermentation of *Saccharomyces cerevisiae* and other yeasts. The addition of A3IS to growing batch cultures of *S. cerevisiae* resulted in a longer lag phase, but pre-exposure of the inoculum to A3IS resolved this, demonstrating classical hormetic behaviour. Exposing the yeasts to A3IS improved tolerance to 25 % (v/v) ethanol in comparison to cultures that were not exposed, with up to 3-fold more cells surviving in the A3IS-treated cultures. Most interestingly, ethanol production also improved by approx. 12.5 % after exposure to A3IS. As A3IS is a biological*

*system, timing is crucial and various testing parameters have to be brought in line to produce repeatable levels of ethanol in each experiment.  
In conclusion, results obtained by this project indicate that A3IS has the potential to increase ethanol output and further research is warranted.*

**Keywords (min 3 and max 10)**

Hormesis, A3IS, Ethanol, Biofuel, Yeast, Fermentation

**NB – Both Section 1 and Section 2 of this Final Report will be made publicly available in a Final Technical Report uploaded online to the [National Energy Research Database](#).**

*In the following Section, please provide a clear overview of your project, including details of the key findings, outcomes and recommendations. The section headings below are provided as a guide, please update or add to these as best suits your project.*

*By submitting this project report to SEAI, you confirm you are happy for Section 1 and Section 2 of this report to be made publicly available. If you wish to request edits to this section in advance of publication, please contact SEAI at [EnergyResearch@seai.ie](mailto:EnergyResearch@seai.ie).*

## SECTION 2: FINAL TECHNICAL REPORT – FOR PUBLICATION

(max 10 pages)

### 2.1 Executive Summary

*Bioethanol is a potentially sustainable alternative to fossil fuels; however, challenges such as substrate shortages, low ethanol yield and high production costs all contribute to its relatively high price. It was recently discovered that by exploiting the hormetic effect of compounds such as H<sub>2</sub>O<sub>2</sub>, the reproductive ability and ethanol tolerance of biofuel producing organisms can be increased. Hormesis is a biological phenomenon characterised by beneficial results from exposure to low doses of a chemical that would be toxic at higher doses.*

*A3IS is a novel compound created by Nektr Technologies and its key characteristic is the slow and sustained release of low levels of H<sub>2</sub>O<sub>2</sub> in a controlled manner. All components of A3IS are Generally Recognised As Safe (GRAS). The aim of this project was to investigate the potential of A3IS to improve ethanol production in fermentation of *Saccharomyces cerevisiae* and other yeasts.*

*The key findings were in increased ethanol resistance in *S. cerevisiae* and other biofuel producing microbes after exposure to A3IS which follows the principles of the classical hormetic effect. An 12.5% increase in ethanol production was also achieved when an in-house brewing yeast was treated with A3IS. The results of this pilot project indicate that A3IS is a potential biofuel production enhancer and further research is warranted.*

### 2.2 Introduction to Project

*Due to the current global challenges of providing energy security while ensuring environmental safety, the demand for an alternative and eco-friendly energy source is growing substantially [1]. The burning of fossil fuels contributes to greenhouse gas emissions as well as global warming causing rise in sea levels, climate change, loss of biodiversity and urban pollution [1-3].*

*Biofuel produced from various renewable sources is regarded as one of the most promising alternatives to fossil fuels. Many countries, such as USA, Brazil, China, Canada and several EU member-states have already invested interests in biofuel programs as the commitment to reduce the dependence on fossil fuels continues [1]. Bioethanol is a promising energy source which reduces greenhouse gas emissions by 19-48% (corn-based) and up to 100% (cellulosic feedstock) when compared to common gasoline while enhancing its performance when blended [4]. However, due to a shortage in substrate availability, low fermentation yield and relatively high production costs, there has been a considerable debate about the sustainability of biofuels [5-7].*

*It was recently discovered that hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) plays a crucial role in the induction of hormesis, a biological phenomenon by which a beneficial effect results from exposure to low doses of an agent that is toxic when given at higher doses [8]. The induction of mild stress can improve an organism's biological function and result in the development of resistance to either higher doses of the same stressor or other disturbing factors [9, 10]. The phenomenon has been observed in various organisms: from bacteria to humans. Current as well as early research suggests that exposure to low doses of H<sub>2</sub>O<sub>2</sub> induces cell growth in yeast cultures as well as reduced ethanol sensitivity [10-12]. In a study by Semchyshyn et al. [11], the tested *Saccharomyces cerevisiae* yeast strains grown in glucose and fructose medium demonstrated the peak hormetic response at 25mmol/L and 50mmol/L H<sub>2</sub>O<sub>2</sub>, respectively. At the hormetic concentrations, the yeast strains showed 130%-155% of the initial reproductive ability. A study carried out in 2014 [10] set the hormetic zone for several strains of *S. cerevisiae* at a hydrogen peroxide concentration of < 0.4mmol. The authors also showed that yeast*

exposed to hormetic doses of  $H_2O_2$  subsequently developed an increased resistance to ethanol exposure. Colony growths of 134% and 118% was observed when yeast was pre-incubation with 0.25mmol hydrogen peroxide and then challenged with 15% and 20% ethanol [10].

The proposed research project sets out to investigate an innovative solution to increase the market competitiveness, sustainability, and efficiency of biofuel production. A unique, patented technology (A3IS) owned by Nektr Technologies and developed in the Atlantic Technological University will be explored. The technology's key characteristic is the sustained and slow release of hydrogen peroxide at safe and controlled levels which is a distinct advantage over highly unstable pure hydrogen peroxide. All components of the novel technology are on the Generally Recognised as Safe (GRAS) Index with minimal environmental impact. During initial trials, the hormetic effect induced by A3IS was recorded and published in the patents protecting this technology.

Nektr Technologies hypothesises that the addition of A3IS to the fermentation process will increase biofuel production and raise its sustainability and efficacy while reducing the production cost. The continuous slow release of hydrogen peroxide will induce the hormetic effect, increasing fermentation microorganisms' reproductive ability while developing a higher resistance against ethanol.

## 2.3 Project Objectives

1. Screen and quantify the hormetic effect on biofuel producing microorganisms for example *Saccharomyces cerevisiae*.
2. Establish if the hormetic dose increases the resistance against ethanol.
3. Establish if the hormetic dose increases biofuel production.
4. Investigate enzymatic activity of the A3IS hormetic effect in yeast

## 2.4 Summary of Key Findings/Outcomes

This was the first trials assessing the potential of A3IS as a biofuel production enhancer. Previous research indicated the ability of  $H_2O_2$  to induce an hormetic effect on biofuel producing microbes increasing reproductive ability and ethanol tolerance. However, the impact of those improvements on biofuel production was never assessed before. The project yielded positive results indicating the potential of the novel technology as a fermentation enhancer. However further research is needed to optimise the process and investigate the inclusion of A3IS at industrial scale manufacture.

Address each innovation in a bullet point below. Add as many bullet points as you need:

- *Innovation 1: Positive effect of A3IS on ethanol tolerance in S. cerevisiae*  
*S. cerevisiae* cultures were treated with different concentrations of A3IS and then exposed to 25 % ethanol for 1 hour, the surviving cells were counted. Significant improvement of surviving cell counts of 33-185% were noted depending on the A3IS concentration used.
- *Innovation 2: Increased ethanol production*  
 The production of ethanol in an in-house brewing yeast strain was established in YPD cultures that were supplemented with 20 % glucose and grown for 5 days. Trials recorded a 12.5 % improvement in ethanol production in the yeast cultures.
- *Innovation 3: A3IS gives a hormetic effect with several biofuel producing yeast species such as S. cerevisiae, C. albicans, S. stipitis, E. aerogenes and a brewing yeast.*  
 A3IS treated cell cultures showed a consistent increased in ethanol resistance.

## 2.5 Project Impact

As previously stated, this is the first time the effect of sustained hydrogen peroxide release on fermentation-based ethanol production was investigated. Although alternatives to fossil fuels are

*needed, the sustainability of biofuels was questioned due to substrate shortages, low fermentation yield and relatively high production costs [5-7]. A3IS has shown to increase ethanol resistance and ethanol production when added to the fermentation process. This is a first step in the development process of a fermentation additive which will increase biofuel production, raise its sustainability and efficacy while reducing the production cost.*

*The application of the novel technology will support the Irish government in the transition towards a climate neutral energy system through the development of low-carbon technologies in a fast and cost competitive way. It will also support the SEAI in the transformation of Ireland to a society based on sustainable energy structures, technologies, and practices.*

*From a research aspect, the successful completion of the project is a first step in the development plan of A3IS as a biofuel production enhancer. The completion of the development plan will grow Ireland's national capacity to access, develop and apply international class energy RD&D by enhancing its international credibility in the field of renewable energy.*

*Regarding national guidelines and action plans, the development of Nektr's novel technology as a renewable energy technology will support a vast number of the outlined objectives:*

- *Support the objective of supplying 15% of electricity demands from renewable sources.*
- *Provide for the increased use of biofuels to contribute to the growth of renewable energy and reduction of greenhouse gas emissions in the transport sector.*
- *Support the use of biomass to increase the level of renewable energy in the heat sector.*
- *Support the biofuel blending objectives: Statutory target at 1.11% from 1 January 2019 and 12.360% from 1 January 2020. Blending levels to reach E 10 and B12 by 2030 with statutory blend increasing incrementally. Increases in line with the overall Renewable Energy Source (RES) trajectory set out in the Energy Union Governance Regulation.*
- *Electricity Generation: 55% Renewable Energy Source-Electricity (RES-E) achieved in 2030 and maintained.*

## **2.6 Recommendations**

*This first assessment of A3IS as an ethanol production enhancer yielded very positive results. A3IS is a biological system and therefore many influencing factors have to be investigated and controlled for maximum output. It is recommended that further research is carried out to better understand and augment the impact of A3IS in the biofuel production sector. Furthermore, research is needed to incorporate A3IS in the industrial biofuel production process.*

## **2.7 Conclusions and Next Steps**

*Results obtained by this project indicate that A3IS has the potential to increase ethanol output during the fermentation process. Further research is warranted and needed to maximise A3IS' impact on biofuel production and for the integration of A3IS in the industrial manufacturing process.*

**Note - Both Section 3 and Section 4 of this Final Report are required for SEAI review purposes only and will not be made publicly available.**

## SECTION 3: COMMUNICATION & DISSEMINATION

(max 3 pages)

### 3.1 Communication, Dissemination and Exploitation

*There has been no communication/dissemination of the data at this point.*

#### **Dissemination Summary Tables**

*Please list details of any scientific publications in Table 3.1 on the next page. Please mention papers published in peer-reviewed journals or papers disseminated at conferences (e.g. on the conference website, etc.).*

*Please list details of all dissemination activities in Table 3.2 on the next page (e.g. publications which do not fall under Table 3.1's scope, conferences, workshops, websites/applications, press releases, flyers, articles in press, videos, presentations, exhibitions, thesis, interviews etc.).*

### 3.2 Intellectual Property Management & Exploitation

*If applicable, please provide details of any patents or IP generated as a result of this research award, or patents/IP which you think may eventuate as a result of the project.*

Table 3.1 – List of Scientific Publications

Title	Main Author	Journal Title	Number, Date or Frequency	Publisher	Year of Publication	Is/Will open access be provided? If you marked “will”, provide an estimate of the date	Peer-reviewed (Y/N)?

Table 3.2 – List of Dissemination Activities

Type of Activity	Main Leader	Title	Date/Period	Location	Type of Audience*	Size of Audience
<i>e.g. Conference</i>		<i>e.g. WindEurope Conference &amp; Exhibition</i>	<i>2-4 April 2019</i>	<i>Bilbao, Spain</i>	<i>Industry, Policy makers</i>	50

\*Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias, Other ('multiple choices' is possible).



## SECTION 4: PROJECT STATUS & WORK PLAN

### 4.1 Work Plan

Please provide your list of work packages in Table 4.1 below, as detailed in your original Application Form, and include a status update for each.

Table 4.1 – List of Work Packages

No.	Title	Status Update and Completion Status (%)
1	The effect of A3IS on the growth and ethanol tolerance of <i>S. cerevisiae</i>	A3IS does not impact the growth of <i>S. cerevisiae</i> . Hormetic doses of A3IS increase ethanol tolerance. 100% completed.
2	The effect of A3IS on ethanol production	Ethanol production has been achieved. media optimised for maximum ethanol production. Effect of A3IS on ethanol production in small scale cultures has been achieved. Effects on ethanol production in multi-litre setting was not completed. 90% completed.
3	Enzymatic analysis of the A3IS hormetic effect in yeast	Glucose Oxidase activity in A3IS diminishes over time after compound preparation. 80% completed.
4	The hormetic effect of A3IS on other biofuel producing organisms	A3IS gives a hormetic effect with several biofuel producing yeast species such as <i>S. cerevisiae</i> , <i>C. albicans</i> , <i>S. stipitis</i> , <i>E. aerogenes</i> and a brewing yeast. 100% completed.

In Table 4.2, please include details for each work package (copy and replicate the Table for each work package as required). Please provide an update on the progress, the specific milestones and deliverables achieved, and clearly identify any deviations from the original proposed work packages.

Table 4.2 – Summary of Work Packages

<b>WP No. &amp; Title</b>	WP 1. Effect of A3IS on growth and ethanol tolerance of <i>Saccharomyces cerevisiae</i>		
<b>Start Month No.</b>	1	<b>Finish Month No.</b>	3
<b>WP Lead</b>	UCD		
<b>WP Contributors</b>	Nektr UCD		
<b>Objective(s)</b>	WPI -01: Assess impact of various concentrations of A3IS on cell growth.	Completion Update: 100% completed.	
	WP1-O2: Establish the effect of ethanol on A3IS-supplemented yeast cultures.	Completion Update: 100% completed.	
	WP1-03: Monitor A3IS activity over the culturing time	Completion Update: 100% completed.	
<b>Description (max 200 words)</b>	The adaptive response to priming concentrations of hydrogen peroxide, resulting in resistance to later exposure to higher concentrations of the same, has been studied previously. However, the effect of continual exposure to low concentrations of hydrogen peroxide on yeast growth is not known. In this WP <i>S. cerevisiae</i> will be challenged with various concentrations of A3IS and the effect on the growth in standard medium (e.g., yeast extract/peptone/glucose) in multi-well plates will be assessed by OD measurements. Once the impact of A3IS on yeast growth has been established, the hormetic effect can be investigated by cultivating yeast with A3IS supplementation then challenging the culture with various concentrations of ethanol before measuring the effect on growth.		
<b>Milestones</b>	WPI-M1: Establishing yeast cultures. WPI -M2: Conduct ethanol challenge experiment. WP1-M2: Complete A3IS assay in culture.	Completion Status (%): 100 Completion Status (%): 100 Completion Status (%): 100	



<b>Deliverables</b>	WP1-D1: Optimum A3IS concentration for growth WP1-D2: Demonstrate cross-resistance of A3IS-supplemented yeast.	Completion Status (%): 100 Completion Status (%): 100
<b>Deviations from planned WP (if applicable)</b>		
<b>Key Outcomes</b>	A3IS can induce the hormetic effect of increased ethanol resistance.	

<b>WP No. &amp; Title</b>	WP 2. Effect of A3IS on ethanol production		
<b>Start Month No.</b>	4	<b>Finish Month No.</b>	7
<b>WP Lead</b>	UCD		
<b>WP Contributors</b>	Nektr UCD		
<b>Objective(s)</b>	WP2-O1: Measurement of ethanol in A3IS-supplemented small scale yeast cultures.	Completion Update: 100% completed.	
	WP2-O2: Comparison with non-supplemented and hydrogen peroxide supplemented cultures.	Completion Update: 100% completed.	
	WP2-O3: Establish if the hormetic effect is retained in larger scale cultures.	Completion Update: 0% completed.	
<b>Description (max 200 words)</b>	Once the impact of A3IS on the growth of the yeast was understood, the effect of the supplement on ethanol production in yeast cultures was examined. Small scale (50-100 mL) fermentations were established with and without A3IS supplementation, and with hydrogen peroxide addition. Ethanol concentration was monitored enzymatically using a commercial kit (Sigma).		
<b>Milestones</b>	WP2-M1: Measure ethanol in small scale cultures. WP2-M2: Establish 20 L cultures. WP2-M3 Optimisation	Completion Status (%): 100 Completion Status (%): 0 Completion Status (%): 90	
<b>Deliverables</b>	WPI-DI: Measurement of ethanol in A3IS supplemented/ un-supplemented cultures. WP1-D2: Optimised multi-litre fermentation.	Completion Status (%): 100 Completion Status (%): 0	
<b>Deviations from planned WP (if applicable)</b>	M2 & D2 were not achieved. Due to variation in ethanol production and tolerance in small scale lab cultures the team did not progress to 20L cultures and continued to work at small scale batches. M3: 90% completed, the system was optimised but repeatability between testing replicates was difficult to achieve.		
<b>Key Outcomes</b>	A3IS supplementation increases ethanol production in small scale cultures. A3IS is a biological system and more research work is to be completed to control all influencing factors to make outcomes more repeatable.		

<b>WP No. &amp; Title</b>	WP 3. Enzymatic analysis of the A3IS hormetic effect in yeast		
<b>Start Month No.</b>	1	<b>Finish Month No.</b>	12

<b>WP Lead</b>	UCD		
<b>WP Contributors</b>	Nektr UCD		
<b>Objective(s)</b>	WP3-O1: Assay of key enzymes throughout culture growth in supplemented and non-supplemented flasks.	Completion Update: 100% completed.	
	WP3-O2: Proteomic analysis of yeasts grown in the absence/presence of A3IS.	Completion Update: 0% completed.	
<b>Description (max 200 words)</b>	An earlier study by Godon et al. (1998) revealed that when challenged with hydrogen peroxide synthesis of 115 proteins increased and 52 decreased. Enzymes associated with antioxidant scavenging (catalase, superoxide dismutase) and carbohydrate metabolism via the pentose phosphate pathway were stimulated by hydrogen peroxide. Although this experiment was revealing, it was conducted under narrow parameters of hydrogen peroxide addition. Thus, the impact of A3IS on key enzyme activity will be measured here.		
<b>Milestones</b>	WP3-M1: Establish enzyme assays WP3-M2: Measure enzyme activity.	Completion Status (%): 100	
	WP3-M3 Mass spec analysis of proteins	Completion Status (%): 100	
	WP3-M4 Protein identification/quantification	Completion Status (%): 0	
		Completion Status (%): 100	
<b>Deliverables</b>	WP4-D1: Key enzyme activity established at different times in the presence/absence of A3IS.	Completion Status (%): 100	
	WP4-D2: Proteomic differences identified in cultures with and without A3IS	Completion Status (%): 0	
<b>Deviations from planned WP (if applicable)</b>	Mass spec analysis of proteins (M3) and therefore proteomic differences between treated and untreated samples (D2) was not carried out due to time constraints and delays with the delivery of consumables needed to complete M1 and M2 tasks. Instead, enzyme quantification and activity analysis were carried out to determine the activity of the glucose oxidase over time when used with the yeast cultures which was identified during the course of the project as a key variable. Tasks previously outlined in WP2 and WP3 were carried out again also as repeatability between some experiments could not be established.		
<b>Key Outcomes</b>	Glucose Oxidase activity in A3IS diminishes over time after compound preparation.		

<b>WP No. &amp; Title</b>	WP 4. The hormetic effect of A3IS on other biofuel-producing microorganisms		
<b>Start Month No.</b>	1	<b>Finish Month No.</b>	3

<b>WP Lead</b>	UCD	
<b>WP Contributors</b>	Nektr UCD	
<b>Objective(s)</b>	WP4-01: Establish if the hormetic effect extends to other biofuel producing microorganisms.	Completion Update: 100% completed.
<b>Description (max 200 words)</b>	To extend the potential application of A3IS, the hormetic effect on other biofuel producing microorganisms will be investigated. The experiments will follow the approach outlined in WPI and will focus on other yeasts known for their efficient ethanol production.	
<b>Milestones</b>	WP4-M1: Complete screen of other microorganisms	Completion Status (%): 100
<b>Deliverables</b>	WP4-D1: Hormetic effect established in other microorganisms	Completion Status (%): 100
<b>Deviations from planned WP (if applicable)</b>	N/A	
<b>Key Outcomes</b>	A3IS induces the hormetic effect with several biofuel producing yeast species such as <i>S. cerevisiae</i> , <i>C. albicans</i> , <i>S.stipitis</i> , <i>E. aerogenes</i> and a brewing yeast.	

## ANNEX 1 – CASE STUDY TEMPLATE

Please complete the SEAI Case Study Template below. The details below may be used for SEAI promotional activities, e.g. project dissemination on SEAI Website or SEAI Twitter account.

<b>Project Title</b>	
A novel technology to maximise biofuel efficiency	
<b>Project Summary – Please provide a brief and high-level summary of your project. (Max 3 sentences)</b>	
The aim of this project was to investigate the potential of the novel compound A3IS as a biofuel production enhancer. The technologies' sustained hydrogen peroxide release induces hormetic effects in yeast cultures increasing ethanol tolerance and ethanol production.	
<b>What challenges did you face? Challenges can be technical (e.g. sensor failure), managerial (e.g. delay in the hiring process), financial (e.g. unexpected costs), etc.</b>	
<ul style="list-style-type: none"> <li>• <b>Delay in delivery of materials</b></li> <li>• <b>Change of responsible person internally</b></li> </ul>	
<b>Three key statistics – If applicable, please provide three key statistics related to your RD&amp;D Project: e.g. X kW generation capacity; X Papers Published; X Communities/Users involved; X potential energy/cost savings</b>	
1.	12.5% improvement in ethanol production
2.	
3.	
<b>What would you regard as the three most significant achievements or impacts enabled by this SEAI funding?</b>	
1.	Successful pilot investigation of the novel compound A3IS
2.	Established 12.5% improvement in small scale ethanol production
3.	Clarification of further research steps for A3IS market approach
<b>Other</b>	
<b>How has this or will this research project be of benefit to Ireland?</b>	
<p><i>From a research aspect, the successful completion of the project is a first step in the development plan of A3IS as a biofuel production enhancer. The completion of the development plan will grow Ireland's national capacity to access, develop and apply international class energy RD&amp;D by enhancing its international credibility in the field of renewable energy.</i></p> <p><i>The application of the novel technology will support the Irish government in the transition towards a climate neutral energy system through the development of low-carbon technologies in a fast and cost competitive way. It will also support the SEAI in the transformation of Ireland to a society based on sustainable energy structures, technologies, and practices.</i></p>	
<b>What was the biggest learning outcome throughout the project?</b>	
A3IS is a biological system which means that many factors can influence its activity. While a 12.5% improvement in ethanol production was achieved, the output can be further maximised by influencing and controlling all important variables. Further research is warranted.	
<b>What has this SEAI funding enabled for you/your organisation? (e.g. building capacity, developing a product, opening new markets, growth in revenue). Please be specific and quantify your responses where possible.</b>	

- Enabled further research and understanding of the novel compound A3IS.
- Growing our research network.
- Gained understanding of the future research path.

#### What advice would you give to other researchers?

Emphasize a structured communication pathway with weekly/ monthly meetings between project partners.

If you wish, please describe your overall experience working on this project (e.g. are you happy with its success, what was the highlight for you, and/or what do you have planned next)

Please submit a separate e-copy of any pictures / maps / images / graphics inserted into the text above, as individual .jpeg, .tiff, .csv files to ensure good quality printing.

Please also submit 3 **research project cover pictures** (e.g., team photo, site photos, prototype photos, research lab photos etc). Please ensure the below picture requirements are met:

- Pictures must be of high quality to ensure good quality printing
- Layout: Landscape
- Format: Jpeg
- Size: Minimum 1200 x 1200 pixels

## ANNEX 2 – PROJECT COMPLETION SURVEY

### Workforce Statistics

Please indicate in the table below the number of people who worked on this project within the Lead/Partner Organisation Types listed

Project Staff (By Lead/Partner Organisation Type)	Number of Women	Number of Men
Industry and SMEs (if applicable)	1	2
Academia or publicly funded research institutes (if applicable)	0	2
Of which, number of PhD Students	0	0
Other Public Sector or Semi-state Organisations (if applicable)	0	0
How many of the above staff were recruited specifically for this project?	0	1

### Engagement – Civil Society and Policy Makers

Did your project involve working with students and/or school pupils (e.g. open days, participation in science)?

Yes – Please specify

No X

Did your project engage with societal actors beyond the research or industrial community?

Yes – Please specify

No X

If yes, did you engage with citizens or organised societal groups (select from the below options)?

No X

Yes – in determining the research to perform

Yes – while implementing the research

Yes – in communication/dissemination of research results

Did you engage with government / public bodies or policy makers (including international organisations)?

No X

Yes – in framing the research	
Yes – while implementing the research	
Yes – in communication/dissemination of research results	
If you marked yes above and engaged with international organisations, please specify which organisation and which country here:	
<b>Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?</b>	
Yes – as a primary objective	
Yes – as a secondary objective	
No	X
If you marked yes above, please add details here	
<b>If yes, at which level?</b>	
Local / Regional Level	
National Level	
European Level	
International Level	

<b>Dissemination and Market Readiness</b>	
How many articles were published/accepted for publication in peer-reviewed journals?	0
How many articles were presented and published in conference proceedings?	0
How many new patent applications have been made?	0
How many spin-off companies were created/are planned as a direct result of this project?. If you marked “are planned”, please give an estimation of the date of creation.	0
Did the project result in a market ready solution (e.g. a product, a service)? (Yes/No)	No

<b>Communication Statistics</b>	
<b>Which of the following have been used to communicate information about your project? (tick as appropriate)</b>	
<input type="checkbox"/> Press Release <input type="checkbox"/> Communication via social media (Twitter, LinkedIn, Applicant website, etc.) <input type="checkbox"/> Media Briefing <input type="checkbox"/> TV coverage / report <input type="checkbox"/> Radio coverage / report <input type="checkbox"/> Brochures / posters / flyers <input type="checkbox"/> DVD / Film / Multimedia <input type="checkbox"/> Other (please specify):	<input type="checkbox"/> Coverage in specialist press <input type="checkbox"/> Coverage in general press <input type="checkbox"/> Coverage in national press <input type="checkbox"/> Coverage in international press <input type="checkbox"/> Website for the general public <input type="checkbox"/> Event targeting general public (Festival, conference, exhibition) <input type="checkbox"/> Scientific conferences <input type="checkbox"/> Other (please specify):

<b>SEAI National Energy RD&amp;D Funding Programme - Feedback</b>
<b>If you have any feedback or suggestions in relation to the SEAI National Energy RD&amp;D Funding Programme, please provide below:</b>

It would be helpful for project to be able to apply for a follow on grant as part of the SEAI RD&D funding opportunity.

## References

1. Zabed, H., et al., *Bioethanol production from renewable sources: Current perspectives and technological progress*. Renewable and Sustainable Energy Reviews, 2017. **71**: p. 475-501.
2. Vanhala, P., et al., *Boreal forests can have a remarkable role in reducing greenhouse gas emissions locally: Land use-related and anthropogenic greenhouse gas emissions and sinks at the municipal level*. Science of The Total Environment, 2016. **557-558**: p. 51-57.
3. Abnisa, F., et al., *Characterization of Bio-oil and Bio-char from Pyrolysis of Palm Oil Wastes*. BioEnergy Research, 2013. **6**(2): p. 830-840.
4. Nigam, P.S. and A. Singh, *Production of liquid biofuels from renewable resources*. Progress in Energy and Combustion Science, 2011. **37**(1): p. 52-68.
5. Berndes, G., M. Hoogwijk, and R. van den Broek, *The contribution of biomass in the future global energy supply: a review of 17 studies*. Biomass and Bioenergy, 2003. **25**(1): p. 1-28.
6. Alvira, P., et al., *Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review*. Bioresource Technology, 2010. **101**(13): p. 4851-4861.
7. Hakizimana, O., E. Matabaro, and B.H. Lee, *The current strategies and parameters for the enhanced microbial production of 2,3-butanediol*. Biotechnology Reports, 2020. **25**: p. e00397.
8. Ludovico, P. and W.C. Burhans, *Reactive oxygen species, ageing and the hormesis police*. FEMS yeast research, 2014. **14**(1): p. 33-39.
9. Martins, I., L. Galluzzi, and G. Kroemer, *Hormesis, cell death and aging*. Aging, 2011. **3**(9): p. 821-828.
10. Semchyshyn, H.M., *Hormetic concentrations of hydrogen peroxide but not ethanol induce cross-adaptation to different stresses in budding yeast*. Int J Microbiol, 2014. **2014**: p. 485792.
11. Semchyshyn, H.M. and B.V. Valishkevych, *Hormetic Effect of H<sub>2</sub>O<sub>2</sub> in Saccharomyces cerevisiae: Involvement of TOR and Glutathione Reductase*. Dose-response : a publication of International Hormesis Society, 2016. **14**(2): p. 1559325816636130-1559325816636130.
12. Davies, J.M., C.V. Lowry, and K.J. Davies, *Transient adaptation to oxidative stress in yeast*. Arch Biochem Biophys, 1995. **317**(1): p. 1-6.