Interreg
EUROPEAN UNION
North-West Europe
BIOWILL
European Regional Development Fund

BioWILL

‘Integrated "Zero Waste" Biorefinery utilising all fractions of Willow feedstock for the production of high to medium based Bio-Chemicals/Materials, Renewable Energy in the form of Bio Methane production and Natural Fertilisers’

Gas Networks Ireland

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Executive Summary

The BioWILL project was primarily funded by the EU Interreg Northwest Europe agency, additional support funding was also provided for by Gas networks Ireland under its Innovation Fund. The objective of the BioWILL project was to design and undertake a techno-economic evaluation of a zero-waste biorefinery where high value biomolecules, such as salicin extracted from willow bark were be used to produce phytopharmaceutical products such as a skin-cream. For the intended zero-waste system, the willow pulp and waste bark were processed to form catering or food packaging materials. When the packing materials came to the end of their useful life they were used as a feedstock for anaerobic digestion to produce bio-methane and the Anerobic Digestion (AD) digestate was intended to be used as a biofertilizer.

An initial growth trials of 31 varieties of willow was undertaken on four sites in Ireland and France. The willow varieties used in the trial were selected for their properties, notably, yielding potential and bioactives content (e.g. salicin). Once established the plants were harvested at regular intervals over three years and yield and salicin content were measured. The varieties which gave the highest overall yield of both bioactives and biomass were identified for further testing and processing. After harvesting the selected varieties, the bark was separated from the willow stem and the bioactives were subsequently removed using a solvent extraction process and the extractives were then used in the production of a phytopharmaceutical cream. The non bark “pulp fraction” was used as the primary feedstock for the food packaging products. Initially salicin was selected as a key bioactive molecule to be considered in the phytopharmaceutical product but as the project advanced it was observed that the crude extract (willow bark extract) was found to have significant anti-inflammatory and antioxidant activity and it was found that the cost of purification of the crude extract in order to isolate the salicin was prohibitive. Consequently, it was decided to focus on the willow bark extract (WBE) (a mixture of bioactive molecules, including salicin) as the key active ingredient in the phytopharmaceutical cream which was tested and found to be effective against inflammation and oxidation.

Testing of the waste packaging as a feedstock for anaerobic digestion found that the yield of biogas was low and this was compounded by a low concentration of plant nutrients in the residual digestate, limiting its potential as a biofertilizer component. Consequently, it was decided that composting the waste packaging and using the product as a peat substitute in soilless growing medium was a better outlet.

An LCA of the biorefinery identified the energy usage for harvesting, debarking, solvent extraction and production of the packing as the main contributors of environmental impact with no mitigation of this impact from anaerobic digestion.

Net Present Value was used to determine the investment potential of the proposed biorefinery. The scenario modeled included harvesting and extraction on 12500 and 25,000 ha sites and included the costs of purchasing from the farmers, logistics Capex and Opex for producing the willow bark extract, food packaging products and soilless growing media. The economic indicators for the two scenarios suggest that, for a proposed plant with a 15-year lifespan, the NPV is €5 million for the small plant and €14 million for the larger one. The payback period is 1.5 years and 3.5 years plants. The NPV **is highly sensitive to change in willow extract prices**. The Techno economic analysis is provided in Appendix 2. Appendix 3 provides specific economic analysis in relation to the production of biogas as part of the LCA.

Limitations

The findings and recommendations in this report are given in good faith but, in the preparation of this report, we have relied upon and assumed, without independent verification, the accuracy, reliability and completeness of the information made available to us in the course of our work, and have not sought to establish the reliability of the information by reference to other evidence. Any findings or recommendations contained within this report are based upon our reasonable professional judgement based on the information that is available from the sources indicated. Should the scheme elements, external factors and assumptions change then the findings and recommendations contained in this report may no longer be appropriate. Accordingly, we do not confirm, underwrite or guarantee that the outcomes referred to in this report will be achieved. We have not compiled, examined or applied other procedures to any prospective financial information in accordance with Irish, or any other, auditing or assurance standards. Accordingly, this report does not constitute an expression of opinion as to whether any forecast or projection of the scheme will be achieved, or whether assumptions underlying any forecast or projections of the scheme are reasonable. We do not warrant or guarantee any statement in this report as to the prospects of the scheme. There will usually be differences between forecast or projected and actual results, because events and circumstances frequently do not occur as expected or predicted, and those differences may be material. A project and final report overview is given below.

Project Overview:

- **Phytopharmaceuticals** (notably salicin-based creams)
- **Biodegradable packaging**
- **Biomethane production via willow waste feedstock**
- **Natural fertilizers**

Agronomic Trials:

- **39 willow varieties** trialed across **Ireland, UK, and France**.
- Selection criteria: biomass yield, salicin content, bark accessibility.
- **Top performers**: LA970243, LA980348, LA970562, Endurance, LA970253, Meteor.
- **Establishment success**: Loughgall (0.5% failure), Claremorris (0.9%), French sites (<10% survival due to drought).

Extraction & Bioactive Analysis:

- **Salicin** and other bioactives extracted using **ethanol-water solvents**.
- **Best solvent ratio**: 80:20 (water:ethanol).
- **Extraction yield**: Up to 35.16% (S. Uralensis); salicin >2.5% in several varieties.
- **Chromatographic purification** trials showed partial isolation; patent-based protocol (EP1901698) most effective.

Cream Formulation:

- Two variants tested:
    - **Cream 1 (HE)**: Higher extractives, lower salicin.
    - **Cream 2 (HS)**: Lower extractives, higher salicin.

Cream 2 showed better targeted bioactivity.

Bioactivity Testing:

- **Cell lines**: Human keratinocytes & fibroblasts.
- **Assays**: Cytotoxicity, antioxidant, anti-inflammatory, wound healing, skin barrier integrity.

Results:

- Significant antioxidant and anti-inflammatory effects.
- Enhanced wound healing (up to 87.5% gap closure).
- No photoprotection or antibacterial activity observed.

Packaging & End-of-Life:

- Willow pulp used to produce **biodegradable trays and pots**.
- **Anaerobic digestion (AD)** of packaging yielded **low biogas** and **nutrient-poor digestate**.
- **Composting** preferred for peat substitute applications.

Life Cycle Assessment (LCA):

- **Functional Unit**: 1 tube of cream.
- **Hotspots**: Debarking, extraction, packaging.
- **Best harvest cycle**: **1-year** (lower energy use).
- **NPV**: €5M (12,500 ha) and €14M (25,000 ha); payback in 1.5–3.5 years.

Key sensitivities:

- Extractives yield > biomass yield.
- Debarking improves extract efficiency.
- Renewable hydrogen and biomethane reduce environmental impacts.

Pilot & Commercial Scale Facility

- **Pilot plant** proposed at Bangor University.
- **Turbex extraction system** central to process.
- **Commercial facility cost**: ~€27.4M for 2,000 tonnes/year capacity.
- Requires ~100 ha of willow annually.

Key Recommendations

- Focus on **high-extract varieties** (LA970243, LA980348, LA970562, Endurance).
- Use **80:20 ethanol-water** solvent.
- Include **debarking** despite energy cost.
- Minimize packaging material.
- Explore **composting** over AD for packaging waste.
- Consider **biobased ethanol** and **renewable energy** sources for further impact reduction.

1.0 Introduction

The Biowill project aimed to design a biorefinery where high value biomolecules, such as salicin extracted from willow bark will be used to produce topical phytopharmaceutical products. For a zero-waste system, the willow pulp and waste bark were processed to form catering or food packing materials. When the packing materials came to the end of their useful life, anaerobic digestion was used to treat these materials to produce bio-methane and the residual AD digestate was intended to be used as a biofertilizer.

The Biowill project selected 31 varieties of willow for planting establishment and growth. The willow species were selected for their properties, notably suitability for cultivation in Northwest Europe, overall biomass yield and bio-actives content (e.g. salicin). The plants were grown on four different sites, one in Northern Ireland (Loughgall), one in Ireland (Claremorris) and two in France (Noreuil and Gouy-Sous-Bellonne). After becoming established the plants were harvested at regular intervals. After harvesting the bark was separated from the willow stem and the bio-actives were subsequently solvent extracted and the extractives were then used in the production of a phytopharmaceutical cream. The de-barked willow wood was used as the primary feedstock for the food packaging products. Initially salicin was selected as a key bioactive molecule to be considered in the phytopharmaceutical product. As the product advanced it was decided to focus on the crude willow extract (a mixture of bioactive molecules, including salicin) as the key active ingredient in the phytopharmaceutical cream.

1.2 Key Benefits

- Medical Applications – A key focus of BioWILL will be the production of high value natural salicylates from willow bark for use in medical applications. Willow bark is one of the few plant materials to contain substances called salicins that are as effective as synthetic equivalents for pain killing and anti-inflammatory properties, with fewer undesirable side effects.
- Sustainable Packaging - Bark residue and bark-free willow pulp can be converted into biodegradable safe packaging material suitable for food that could replace plastics.
- Renewable Gas - Anaerobic digestion of the end-of-life packaging materials will support the production of biomethane - a carbon neutral renewable gas that can be used in the same way as natural gas for renewable electricity, heating, industry and transport.Organic Soil Fertilizer - The anaerobic digestion process will also produce digestate bio-fertiliser which can act as an organic soil improver, substituting for chemical fertiliser application on farmlands, and in turn reducing greenhouse gas emissions, improving soil fertility and soil carbon sequestration whilst assisting the European Union ambitions for 25% organic farming, 20% reduction in chemical fertiliser application and a 50% reduction in pesticides application by 2030.Support of Rural Economies - This project will also provide benefits to rural communities by demonstrating the commercial viability of rural biorefineries to provide alternative income for farmers, increase employment across a wide skill base and further support the decarbonisation of the agriculture industry.

1.3 Partners

- Coordinated by the University of Limerick, BioWILL consists of 10 project partners in four countries across Northwest Europe (Belgium, France, Ireland and the United Kingdom)
- Coordinated by the University of Limerick, the consortium comprises of: Three universities (University of Limerick, Bangor University and University College Cork)
- Three research institutes (Agri-Food and Biosciences Institute, Institute of Technology Tralee and Materia Nova)
- Four small medium enterprises (Cellulac Plc, Epitheal Ltd, Agriland and Helicon Ltd)

- One consultancy (Crops4Energy)
- One industry forum representing all sectors of the renewable gas industry in Ireland from producer to end-users (The Renewable Gas Forum of Ireland)
- One gas company (Gas Networks Ireland)
- One organisation representing farmers and landowners across the EU (European Landowners' Organisation)

1.4 Salicin

Salicin is a naturally occurring chemical compound found in willow trees (genus Salix). It is a glycoside of salicylic acid, which is closely related to the active ingredient in aspirin. Historically, salicin has been used for its pain-relieving and anti-inflammatory properties, as the body metabolizes it into salicylic acid, which helps alleviate pain and reduce fever. Salicin is an early precursor to modern aspirin and has been used for centuries in traditional medicine to treat headaches, muscle pain, and arthritis. The bark of the willow tree is typically the main source of salicin. The extraction involves processes such as:

- Harvesting willow bark, particularly from species like Salix purpurea and Salix alba.
- Thermal, mechanical, or chemical extraction to isolate salicin from the bark, often through boiling or soaking in solvents like water or alcohol to release the active compound.

Salicin is valued for its ability to reduce inflammation and provide pain relief, making it a key ingredient in herbal remedies and some over-the-counter products designed to manage pain. Salicin has also been explored for its potential in phytopharmaceuticals and other applications due to its natural origin and minimal side effects compared to synthetic aspirin. To date willow has been the ideal candidate for bioenergy production due to its rapid growth and ability to thrive in a variety of conditions. Its unique suitability for short-rotation coppicing (SRC) makes it particularly valuable in renewable energy systems. Willow is managed through a process called short rotation coppicing, where the trees are harvested every 2-4 years, but the root system remains intact. This allows the tree to regrow multiple times from the same rootstock over a span of 20 to 30 years. The process ensures a sustainable and consistent supply of biomass with minimal replanting required. Willow biomass can be converted into biofuels, such as wood chips or pellets, which are used as a renewable energy source in heating systems or further processed into liquid biofuels like ethanol. This biomass provides a cleaner, renewable alternative to fossil fuels, contributing to the reduction of carbon emissions. Willow biomass is highly suitable for combined heat and power (CHP) systems, where it is burned to generate both heat and electricity. This is an efficient energy conversion process as it maximizes energy output from the willow biomass, helping to meet renewable energy targets while reducing reliance on traditional, carbon-intensive energy sources. As willow grows, it absorbs CO_2 from the atmosphere, helping to mitigate climate change. This makes willow bioenergy systems a carbon-neutral or even carbon-negative option, particularly when considering the full life cycle. Willow’s SRC also improves soil health over time, enhancing nutrient cycling and soil structure, making it useful for rehabilitating degraded or marginal lands. Willow bioenergy plantations can create jobs in rural areas, supporting local economies through sustainable land management and energy production. To date willow's combination of fast growth, renewable energy potential, and environmental benefits makes it a key resource in developing sustainable bioenergy solutions.

1.5 European Willow Breeding Partnership (EWBP)

In 1996, a partnership was created between Long Ashton Research Station (LARS based in Bristol, UK), Murray Carter (a willow producer based in Yorkshire, UK) and Swedish Willow (SW). The aim of this breeding programme was to harness greater diversity in crosses from the National Willow Collection (NWC) that was held at LARS. This included over one hundred species and hybrid forms from all over the world totalling over 1,500 accessions.

As the climate in the UK and the Island of Ireland are much more maritime than Sweden, a key focus of this programme was to create varieties that were resistant to the fungal pathogen willow rust. From the beginning, the crossing programme at LARS was much more widely focused than in Sweden. The crosses performed between 1996-2002 led to several interesting varieties that performed well in yield trials. Varieties were named after ships of discovery and exploration such as Endurance, Resolution, Terra Nova, Endeavour and Beagle (Table 1).

The breeding material generated from this programme was extensive. As a result, many diverse and high yielding breeding lines have not yet reached the marketplace.

Table 1. Commercially available varieties produced by the European Willow Breeding Partnership (* indicates some relationship to Tora and Bjorn)

Variety name	Pedigree	Year	Countries tested	Variety details	Typical yield range (odt/ha/yr)
Advance*	S. viminalis Pavainen x (S. schwerinii x viminalis) Bjorn	2014	E, W, NI, IR	Similar to other varieties. Medium-high yielding variety.	8.6-14.4
Beagle	S. viminalis Astrid x S. viminalis	2001	E, W, NI, IR	Medium-high yielding variety. Slight susceptibility to rust.	8.0-12.5
Endurance	S. redheriana x S dasyclados	2015	E, W, NI, IR	Extremely high yielding. Performs well in dry soils.	9.6-14.6
Endeavour	S. schwerinii Hilliers x S. viminalis Jorr	2005	E, W, NI, IR	High yielding variety with excellent wood fuel properties. Loses leaves early in winter season.	8.5-14.4
Meteor	S. viminalis Bowles Hybrid x S. viminalis	2014	E, W, NI, IR	Extremely straight variety. Medium yields.	10.2-12.7
Paramore	S. redheriana x S. dasyclados	2014	E, W, NI, IR	High yielding variety with excellent wood fuel.	9.0-14.0

Resolution	(S. viminalis x (S. schwerinii x viminalis))	2002	E, W, NI, IR	Extremely fast growing and high yielding	8.0 -14.2
Terra Nova	(S. viminalis x triandra) x S. miyabeana Shrubby	2005	UK, NI, IR, GR	Medium yielding variety. Good in hot weather and exposed situations.	6.5-10.4

1.6 Rothamsted Breeding Programme

Following the aftermath of the demise of LARS, the NWC was relocated and breeding work continued at Rothamsted Research (based in Hertfordshire, UK). This was more scientific and less commercial in its rationale with crosses being used to provide information for genetic mapping and to develop marker assisted breeding.

This was the only breeding programme to receive 100% Government funding. Because of the very focussed nature of the work, it was important to use crossing material for which there was a great deal of existing knowledge, understanding and data. Hence, the earliest selections from this programme also are dominated by crosses involving *S. viminalis* and *S. schwerinii* and many have ancestry similar to those produced in Sweden and to a lesser extent at LARS.

Varieties are named after mountains and hill ranges in the UK and have a prefix Roth to show that they were bred at Rothamsted. Released varieties include Roth Cotswold, Roth Chiltern, Roth Hambledon and Roth Mourne.

1.7 Salicin content of willows

Salicin content is not available for every Willow species or variety, however this information is available for a number in the scientific literature these were used when selecting the planting materials for the Biowill project. The consensus amongst these papers is that S. purpurea and related species (S. miyabeana and S. koriyanagi) have elevated levels of salicins. Many of these genotypes were used in the European Willow Breeding Partnership crossing programme and high yielding breeding lines were selected from the progeny. Other species that were used extensively in EWBP crosses such as S. hookeriana, S. rehderiana and S. aegyptiaca also had elevated levels.
The least interesting species for salicin content tend to be those that have been most frequently used for biomass breeding and are present in Swedish and Rothamsted bred varieties. These include S. viminalis, S. schwerinii and S. dasyclados.

1.8 Willows chosen for Biowill Trials

A total of thirty-nine willows were chosen for the Biowill trial plots. The main justifications for selection were the following:

- They are fast growing and produce a lot of biomasses in just two to three years.
- They can be planted and harvested mechanically.
- Some have already been screened and show elevated levels of compounds that are interesting from a medicinal perspective.
- They have a good growth habit with limited side branching, making it easier to strip the bark which is where most of the interesting medicinal compounds are found.

The full list of willows being trialled is shown in Table 2. These willows fall into four distinct categories:

- Commercial biomass varieties
    - We have chosen the most diverse varieties from the European Willow Breeding Programme (EWBP) and Rothamsted willow breeding programme as well as the standard high yielding variety from Sweden called Tora. The EWBP programme had the widest genetic base in crosses (bred by AFBI subcontractor Kevin Lindegaard whilst at Long Ashton Research Station and now of Crops for Energy Ltd) as this had access to the UK National Willow Collection (~1,500 accessions covering 110 species). Crosses included European, Russian, Chinese and North American species.
- Near market breeding lines
    - These are selections from the EWBP that had not been commercialised but show promise. A number of these were included in the trials and include Chinese species in their pedigrees which have shown very interesting qualities in laboratory and field conditions.
- Old commercial clones
    - We included some high yielding named clones (non-commercialised selections) that have a long history of trials. This includes representative species and hybrid forms such as *S. viminalis*, *S. dasyclados*, *S. x sericans* (=*smithiana*).
- Species selections
    - The EWBP also used pure species selections in crosses. Many of these are low yielding exotic equivalents to European willows that have been used extensively in the breeding programme. Species include *S. miyabeana* and *S. redheriana* Chinese), *S. schwerinii* (Russian), *S. aegyptiaca* (Middle East), *S. discolor* (North America). The trial design and randomisation are shown in Figure 1.

Table 2. Full list of willows used in Biowill trials.

Key: BL = Breeding line, CV = Commercial variety, OC = Old commercial clone, SS = Species selections). Yellow highlighting indicates breeding lines covered by a Material Transfer Agreement with Murray Carter, the EWBP rights holder.

No	Name or number	Pedigree	Type	Justification for Inclusion	Potential for Elevated Salicin Level	Source
1	LA970253	115/71 S. vim SW880514 x 052/01 S. miyabeana Purpurescens	BL	High yield with interesting Chinese clone as parent	Y	Cuttings made from AFBI collection. MTA signed between Murray Carter and project team
2	LA980348	S. vim Bowles Hybrid x S. miyabeana (shrubby willow)	BL	High yield with interesting Chinese clone as parent	Y	
3	LA970243	115/71 S. vim SW880514 x 052/01 S. miyabeana Purpurescens	BL	High yield with interesting Chinese clone as parent	Y	
4	LA970523	041/03 S. x dasyclados x 024/02 S. x capreola	BL	Reasonable yield - concentration of sallow species		
5	LA980280	(S. schwer x vim x smith) x Jorr	BL	Good yield - better habit than Endeavour		
6	LA970562	S. vim Romanin x S. miyabeana Purpurescens	BL	High yield with interesting Chinese clone as parent	Y	
7	LA2001155 (Aurora)	LA970540 (vim x miya) x S. miyabeana Purpurescens	BL	Concentrated miyabeana. Good screening genotype with decent yield	Y	
8	LA2001476	S. viminalis Beagle x S. triandra Houghton's Black	BL	Good yield - excellent habit. Good nectar concentration compared to other varieties and genotypes	Y	
9	LA980266	125/01 S. schwer x vim x smith V7535 x 003/01 S. aegyptiaca	BL	Inclusion of aegyptiaca makes this interesting. OK yield but only avg establishment	Y	
10	LA2000339	LA970164 (schwer x Jorr) x S. miyabeana (shrubby willow)	BL	Potentially improved version of Endeavour with greater disease	Y	

				resistance properties because of miyabeana		
11		S. x dasyclados	OC	Used in lots of EWBP crosses		
12	Uralensis	S. purpurea	OC	Higher yielding purpurea clone	Y	Cuttings made from AFBI collection
13		S. triandra	SS		Y	
14	ER65	S. eriocephala	OC		Y	
15		S. purpurea	SS		Y	
16	Shrubby willow	S. miyabeana	OC	Used in lots of EWBP crosses particularly Terra Nova	Y	
17	77056	S. dasyclados	OC	Used in lots of EWBP crosses particularly Endurance		
18	Terra Nova	(S. viminalis x triandra) x S. miyabeana Shrubby	CV		Y	Cuttings supplied by Jamie Rickerby (Willow Energy). All varieties covered by European Plant Breeder's Rights.
19	Endurance	S. redheriana x S. dasyclados	CV	Tends to be highest yielding variety in western UK trials	Y	
20	Endeavour	S. schwerinii Hilliers x S. viminalis Jorr	CV	High yielding variety with strong wood		
21	Meteor	S. viminalis Bowles Hybrid x S. viminalis	CV	Straight viminalis - better than Beagle		
22	Resolution	(S. viminalis x (S. schwerinii x viminalis)) SW930812 x (S. schwerinii S. viminalis) x (S. schwerinii x S.	CV	Very high yielding variety in both west and eastern trials		
23	Cheviot	((S. schwerinii K3 Hilliers x (S. schwerinii x S. viminalis, Bjorn) Discovery x (S. viminalis x (S. schwerinii x viminalis) Quest	CV	Good yield, lovely stem colour		
24	Hambleton	(S. schwerinii x S. viminalis) Tora x S. petiolaris	CV	Unusual pedigree		
25	Mourne	(SW930812 x Quest) Resolution x progeny of a polycross	CV	Unusual pedigree		
26	Tora	S. schwerinii x S. viminalis Orm	CV	Standard European variety used in lots of crosses and related to many varieties		
27	LA990072	S. dasyclados Aud x S.	BL	Short, stout variety with downy	Y	Cuttings provided by Kevin Lindegaard from his

		hookeriana (previously S. candida)		stems and large catkins - very different from other biomass selections		own collection. MTA signed between Murray Carter and
28	LA990073	S. dasyclados Aud x S. hookeriana (previously S. candida)	BL	Short, stout variety with downy stems and large catkins - very different from other biomass selections	Y	
29		S. udensis (=sachalensis) Kioryo	SS	Used in lots of EWBP crosses	Y	Cutting supplied by West Wales Willows & Kevin Lindegaard
30		S. koriyanagi	SS	Used in lots of EWBP crosses	Y	
31		S. miyabeana	SS	Used in lots of EWBP crosses	Y	
32	RR09043	S. vim x schwer	BL	High yielding genotype produced from Marker Assisted Selection - similar pedigree to Tora type hybrids		We were unable to get permission from Rothamsted Research (RRES) to access material from the National Willow Collection due to Covid-19 lockdown. Space was set aside in the trial plots however these were never planted.
33	087/01	S. miyabeana	SS	Used in lots of EWBP crosses	Y	
34	052/01	S. miyabeana (Cordata) Purpurescens	SS	Used in lots of EWBP crosses	Y	
35		S. redheriana	SS	Used in lots of EWBP crosses (e.g. Endurance)	Y	
36		S. schwerinii Hilliers	SS	Used in lots of EWBP crosses (e.g. Endeavour)		
37		S. aegyptiaca	SS	Used in lots of EWBP crosses	Y	
38		S. burjatica Lapin	SS	Used in lots of EWBP crosses		
39		S. discolor	SS	Used in lots of EWBP crosses	Y	

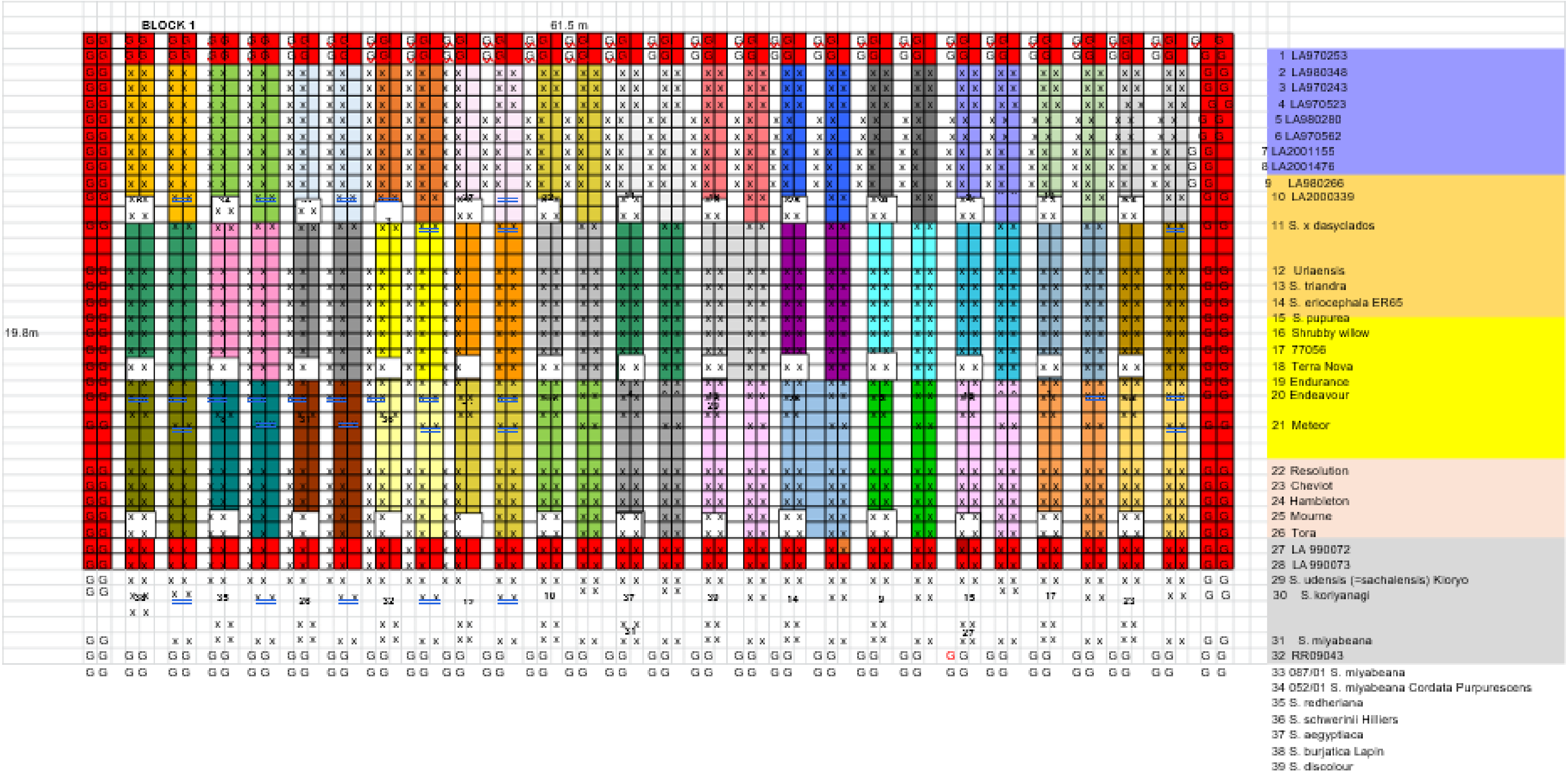


Fig 1: Trial design and example of randomisation design at the Loughgall site (Each trial in NI, Ireland and France consists of three blocks with different randomisations in each case).

1.9 Planting

The willow varieties and clones (Table 3) were sourced in accordance with public procurement. The material came from several different suppliers which included AFBI (in accordance with permission from the owner of the plant breeder's rights (EWBP), West Wales Willow and Rickerby Estates. Access to further material from the Rothamsted Breeding Programme was not possible. within the timeframe of the project

Plot	Materials
1	LA970253
2	LA980348
3	LA970243
4	LA970523
5	LA980280
6	LA970562
7	LA2001155
8	LA2001476
9	LA980266
10	LA2000339
11	S. x dasyclados
12	Uralensis
13	S. triandra
14	S. eriocephala ER65
15	S. purpurea
16	Shrubby willow
17	77056
18	Terra Nova
19	Endurance
20	Endeavour
21	Meteor
22	Resolution
23	Cheviot
24	Hambledon
25	Mourne
26	Tora
27	LA 990072
28	LA 990073
29	S. udensis (=sachalensis) Kioryo
30	S. koriyanagi
31	S. miyabeana

Table 3 – Planting material accessed

The willow cuttings were prepared from one-year-old wood, which had the un-ripened wood at the tip of the harvested rod removed (planting rods). Generally, planting rods of 1.5- 2.5m were supplied by the specialist producers, or cuttings thereof. Cutting material is generally harvested in January – February period when the buds are fully dormant. The planting material was kept in cold storage at AFBI Loughgall until it was transported to the planting site. Refrigeration during transportation was implemented for transporting the material to the French planting sites Dehydration is the most likely problem to be encountered in storage thus the cuttings and rods were protected by wrapping in black 'polythene' film.

All the improved commercial varieties are protected by plant breeders' rights. In practice, this means that it is illegal to produce propagation material for self-use or sale from protected varieties. Derogations are allowed by certain plant breeders which enables the gapping up of establishing crops with the material produced at cutback. It is recommended that the provider of planting material is consulted before doing this.

All the planting material was collected from the different sources and stored at -2 to -4C at AFBI Loughgall, Co Armagh, N.Ireland. Here, the material was prepared (cut, labelled, bundled and stored until it was required right before planting.

The following Protocol (Prescription for ground preparation and willow planting for Biowill) was developed for Biowill and sent to the respective trial establishment teams at Agriland and University of Limerick. AFBI ensured that these activities took place as far as possible however Covid-19 did significantly hamper a lot of site access; especially visits to the two French sites. French visits were not allowed.

- When weeds and grass are actively growing spray the site pre-ploughing with Glyphosate to kill grass and any weeds present. Spray must be applied according to spray manufacture label specifications. Check that there is not too much vegetation on the site prior to spraying, if necessary, cut back and remove vegetation from site.
- Wait at least 14 days after spraying before ploughing to ensure good kill of weeds and grass.
- Plough the site.
- Power harrow the site to make a suitable seed bed as soon as ground conditions allow. Any large stones to be lifted.
- Lightly roll the site
- Trial areas to be marked out using canes and strings as planting guides.
- Hand plant willow according to trial plan
- Willow must be kept in cold store (-2 to -4 ºC) until day of planting
- On the day prior to planting take the willow cuttings from the cold store and soak overnight with bottom of cuttings submerged in water to allow the cuttings to rehydrate and energise

  in preparation for planting (Fig 2). The top of the cuttings will be paint sprayed to assist with variety identification and to ensure cuttings are inserted the correct way into the soil. This negates a lot of potential mix up and confusion when field planting.
- Plan for planting was provided by AFBI (Fig 1.)

Fig 2. Willow cutting hydrating in water overnight just before planting

1.10 Willow planting

The planting followed a defined methodology provided by AFBI to have consistency across all four sites. The sites were prepared and planted at (1) AFBI Loughgall UK, (2) Claremorris, Co Mayo, Ireland, (3) Noreuil (wetter soil) and Gouy-Sous-Bellonne (dryer soil). These trial sites each consisted of randomised plots containing approximately 30 different clones within 3 replicated blocks. It was challenging to get to this point given floods earlier in the season, followed by Covid-19 restrictions followed then by unseasonably dry conditions (Spring 2020) and hard soils.

2.0 Sites

The geographical locations of the 3 trial sites are illustrated in Fig. 3 and Fig 4.

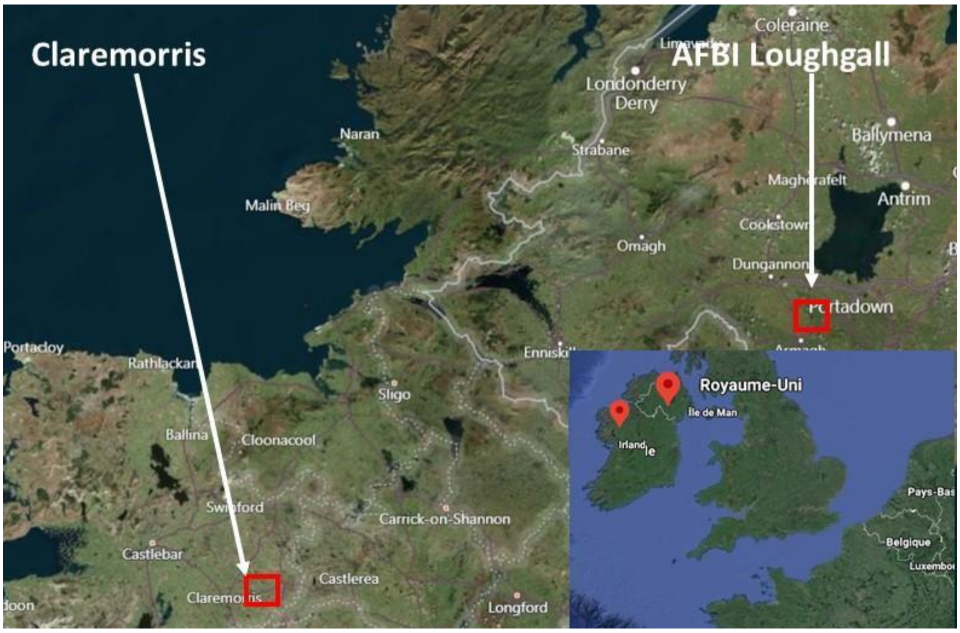
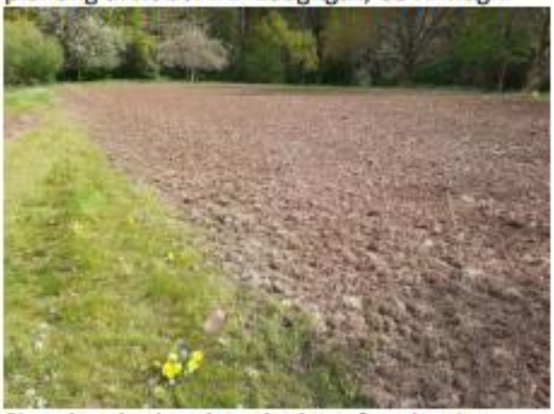


Fig 3. Geographical locations of the trial sites at AFBI Loughgall, Co Armagh (UK) and at Claremorris Co Mayo, Republic of Ireland (University of Limerick)

Fig 4. Geographical locations of the trial sites at Agriland Noreuil and Gouy-Sous-Bellonne

Site prepared for the 3 replicated blocks of the planting trials at AFBI Loughgall, Co Armagh.

Site with grass 'burned off' April 2020



Site ploughed and marked out for planting

Early establishment mid-June 2020

Mid July 2020

End August 2020 block 1

End November 2020 Block 1

Interreg Notice at site

Fig 5. Planting and establishment phases at AFBI Loughgall Co Armagh

Fig 6. Planting and establishment at Claremorris, Mayo, ROI

Fig 7. Planting and establishment phases in Noreuil and Gouy-Sous-Bellonne

<u>Establishment rates in different plots – are there any trends in the failure rates?</u>

Issues were encountered during establishment e.g. dry spring, Covid restrictions, tree falling on NI plot, rabbits and failures (in France).

2.1 AFBI Site, Northern Ireland

AFBI Hillsborough suffered very few failures. In fact, of the approx. 5,000 cuttings planted (including guards), the failure rate amounted to approximately 0.5%. This is an extraordinarily good establishment rate and is a testament to the expertise of the staff involved. There was no real pattern of failure other than perhaps an indication of an issue with S. sachalenensis Kioryo. It was noted that this planting material was poor in terms of it being small and spindly and therefore likely to have low energy reserves but also, harder to establish as difficult to push into hard soils without bending or breakage.

19 Endurance

1 LA970253

25 Mourne

31 S. Miyabeana

6 LA 970562

7 LA 2001155

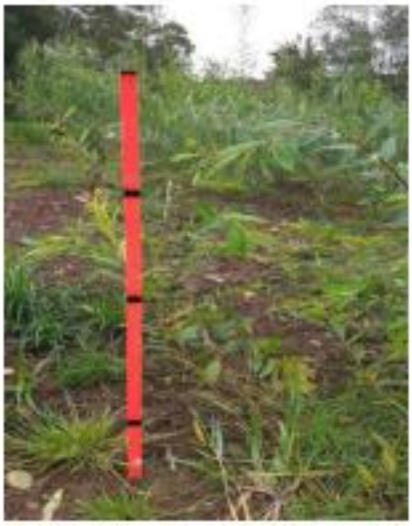
24 Hambledon

20 Endeavour - As you can see the wind blow over slightly, they are a bit top heavy

5 LA 980280

11 S. Dasyclados

11 S. Dasyclados - It is now sending out new shoots, Bk1 is clean and just 2 willows in Bk3 are showing signs of dieback.



29 Kioryo - A few tops snapped off in the wind

30 S. Koriyanagi

8 LA 2001476

10 LA 200339

2 LA 980348

3 LA 970243

22 Resolution

13 S. Triandra

15 S. Purpurea

14 Eriocephala ER65

18 Terra Nova

4 LA970523

9 LA980266

17 77056

26. Tora

27 LA 990072

16 Shrubby willow

21. Meteor

23. Cheviot

12. Uralensis

28 LA 990073

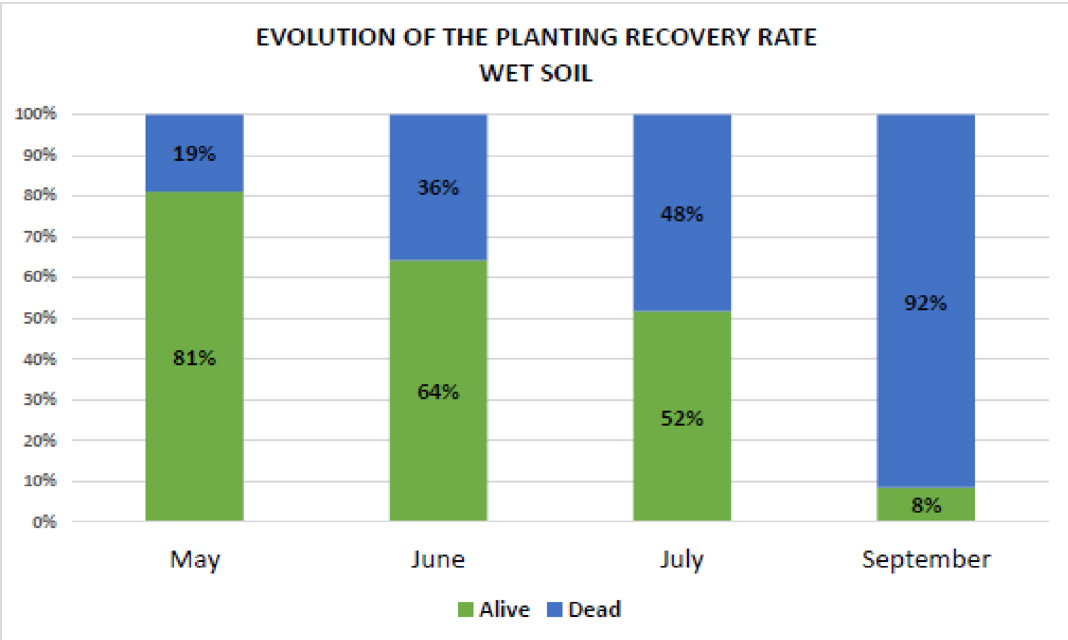
Figure 8. AFBI plantation at establishment

2.2 Claremorris Site, Republic of Ireland

This site showed a 0.9% failure rate (i.e. 45 cuttings out of approximately 5000). There were issues of grass growth which was managed by cutting/mowing every two weeks and a decision was taken not to use any weed/grass killer due to the delicate stage of the plant establishment. It was noted that several of the Welsh cuttings did not take and were still small in comparison to the Rickerby and AFBI & EWBP varieties and clones.

2.3 French Sites

Within the 20 or so weeks following willow planting, the average temperature was over 17°C with a peak of 24°C and rainfall averaged 2.6mm and 2.9mm per week. Fig 9 summarises the deterioration in the number of surviving plants due to drought. Even though the establishment was relatively good in May, this deteriorated significantly to the extent that by September, only 7 to 8% of the plants had survived.



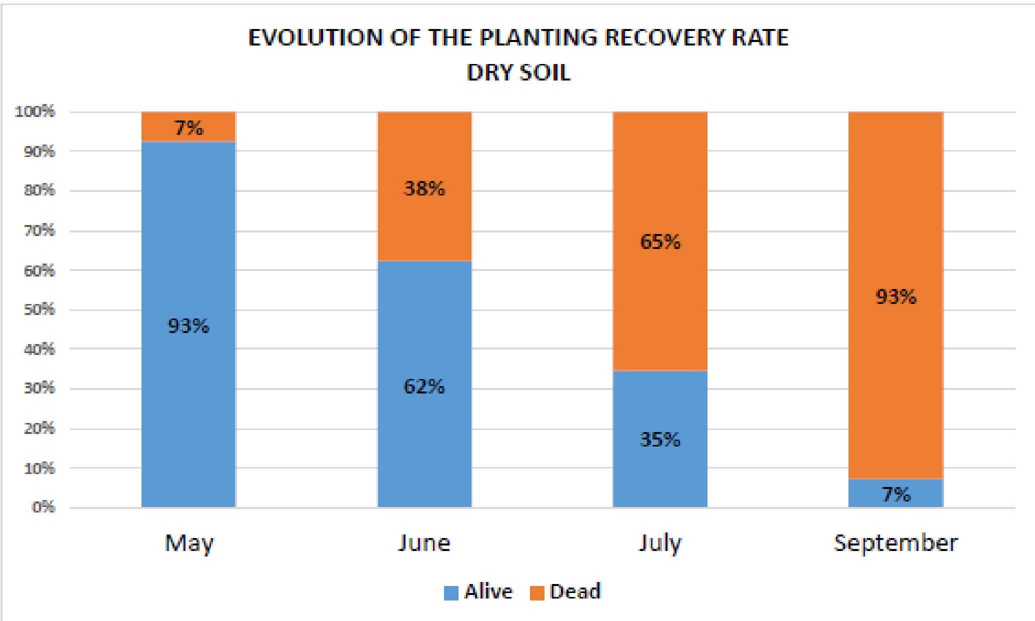


Fig 9. Cutting survival over the months at both French trial sites (both dry and wet soil).

3.0 Harvesting

3.1 Introduction

Willow harvests have been conducted at both the Loughgall and Claremorris sites at the end of season 20-21, 21-22 and 22-23. The material collected at the first harvest, essentially cut-back material, was small generally considered the result of crop husbandry best practice to ensure improved yields for following harvests. This biomass material was used by the project partners to develop protocols. Material cut in 21-22 gave rise to 1 year old biomass and in 22-23, 1 year old and 2-year-old material was harvested and prepared. Figure 10 shows the Loughgall site Autumn 2020 and Autum 2023

Fig 10: Loughgall site Autumn 2020 and Autum 2023

Wet harvest weights are shown in Figures 11 to Fig 14 for both the Claremorris and Loughgall sites. The harvest of 1 year old biomass showed significant differences between the Loughgall and Claremorris sites. These differences were essentially due to difference management practice with Claremorris being managed completely organically with no herbicides, pesticides or fertilisers. As a result, the Claremorris site required considerable manual intervention to achieve the 99%+ establishment rate. The Loughgall site also achieved a 99%+ establishment rate.

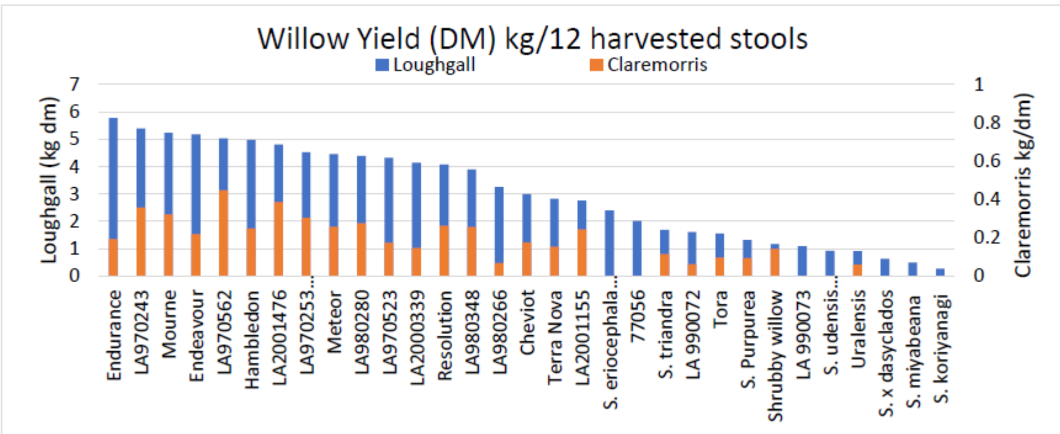


Fig 11. One year old harvested material on 1 year old stools. (cut-back)

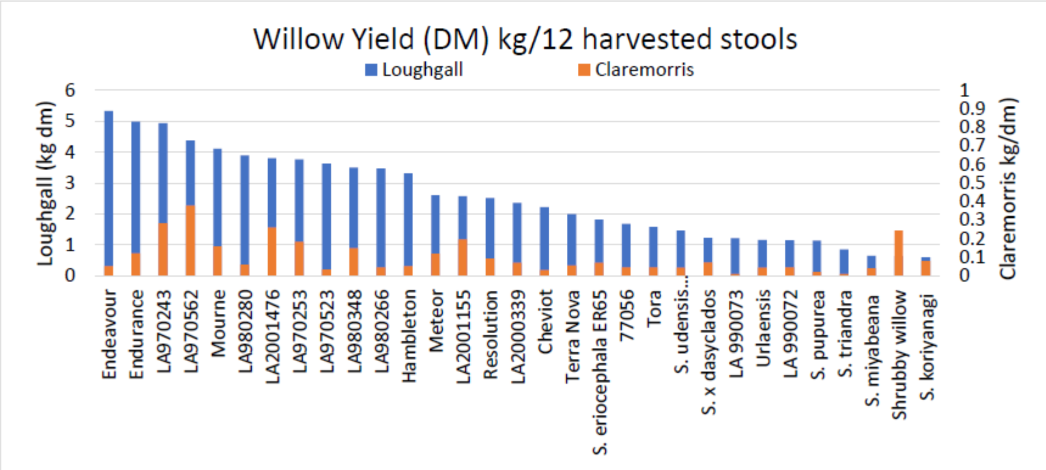


Fig 12. One year old harvested biomass on 2-year-old stools

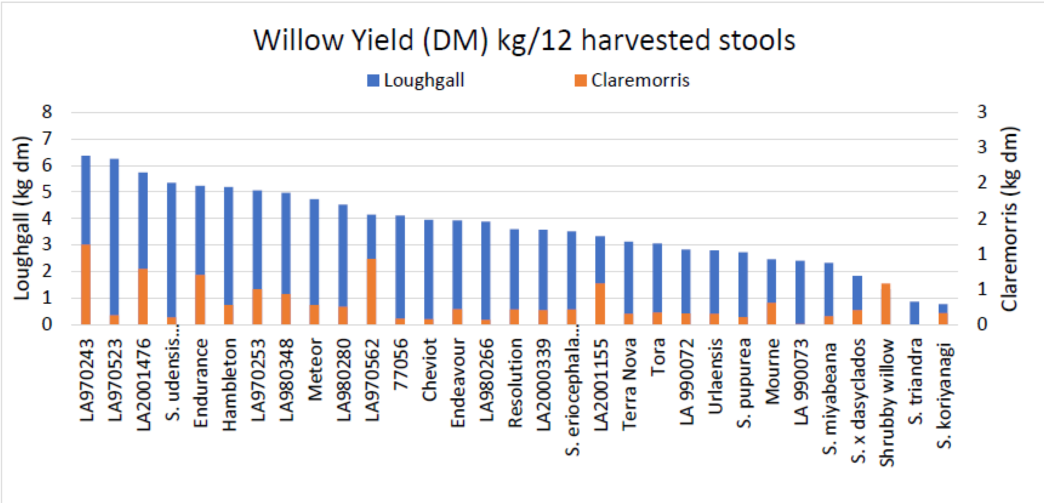


Fig 13. One year old harvested material on 3-year-old stools.

Two-year-old harvest data on a dry weigh basis is presented in Fig 14.

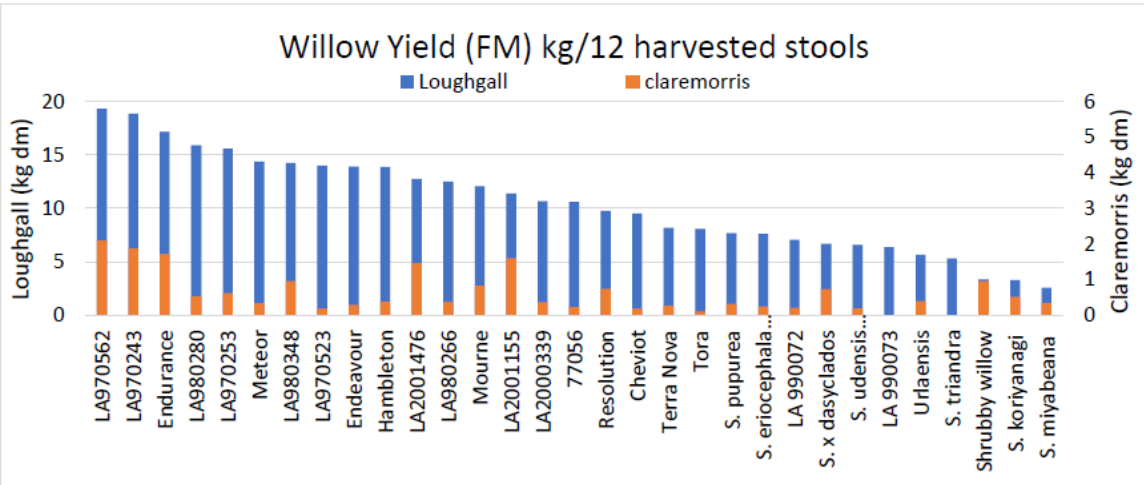


Fig 14. Two-year-old harvested material (dry Weights) on 3-year-old stools.

3.2 Comparison of Yields from different Willow Varieties

As mentioned above, the cut back material was not analysed due to the fact it is considered a crop-husbandry activity to develop improvements in future yields. Aside from that it will be small in biomass quantity and will be unrepresentative of future yields. One year old and two-year-old material is therefore considered.

The top 10 highest yielding varieties from both sites are compared below in Table 4. There are many varieties in common between both sites, as well as the 1- and 2-year-old harvested biomass. Some varieties fall within the 10 highest yielding varieties at both sites and as 1 year old and 2-year-old willow, for example LA970243, LA980348 & Endurance. Other varieties which stand out favourably in this regard are LA970562, Mourne, LA2001476, LA970253. Some varieties seem to have done comparatively better at Claremorris such as Shubby Willow and LA2001155 and others at Loughgall such as LA980280 (also LA970523, Endeavour & Hambleton) while others seem to also do well at both sites such as Meteor.

The data presented in Table 4 suggests that

- Endurance
- LA970243
- LA980348

Highest yields at both sites and for all harvesting intervals.

2021		2023		2023	
1 year Harvest		1 year Harvest		2-year Harvest	
(1 year stem/ 2-year root)		(1 year stem/ 3-year root)		(2-year stem/ 3-year root)	
Loughgall (Normal)	Claremorris (Marginal)	Loughgall (Normal)	Claremorris (Marginal)	Loughgall (Normal)	Claremorris (Marginal)
Endeavour	LA970562	LA970243	LA970243	LA970562	LA970562
Endurance	LA970243	LA970523	LA970562	LA970243	LA970243
LA970243	LA2001476	LA2001476	LA2001476	Endurance	Endurance
LA970562	Shrubby W	Kioryo	Endurance	LA980280	LA2001155
Mourne	LA2001155	Endurance	LA2001155	LA970253	LA2001476
LA980280	LA970253	Hambleton	Shrubby W	Meteor	LA980348
LA2001476	Mourne	LA970253	LA970253	LA980348	Shrubby W
LA970253	LA980348	LA980348	LA980348	LA970523	Mourne
LA970523	Endurance	Meteor	Mourne	Endeavour	Resolution
LA980348	Meteor	LA980280	Meteor	Hambleton	S. x dasyclados

Table 4 – Highest yielding varieties

The following are also viable varieties for maximising yields:

- LA970562
- LA970253
- Meteor
- LA980280 (LG only)
- LA970523 (LG only)
- LA2001155 (CM only)
- Mourne (CM Only)

Endeavour & Hambleton were also considered potentially viable given the strong yields of two-year-old material at Loughgall.

3.3 Bark Proportion from harvested rods

Debarking of the rods was undertaking manually using the freshly harvested willow rods.

Fig 15. Bark stripping and rod preparation

Over the seasons debarking exercises, the average percentage of the biomass yield which was bark is estimated at 20%. Fig 16 shows the % of bark removed from 7 high yielding varieties of 1 year old and 2-year-old biomass.

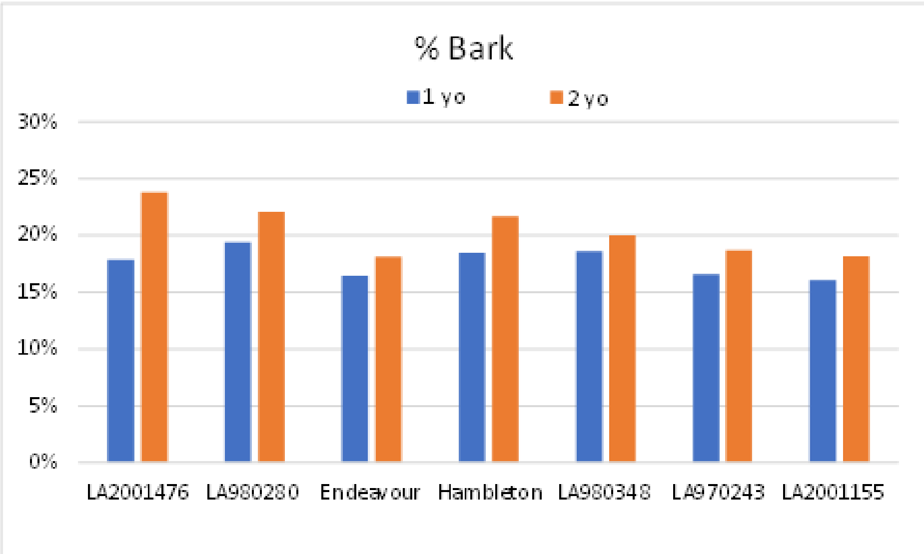


Fig 16. Yield of bark from 7 highest yielding varieties.

4.0 Best performing varieties regarding biomass yield and bioactive constituents

4.1 Loughgall

The yield of bioactives from bark recovered using the ethanol-water solvent extraction (Chapter XXX) process for year 1 and year 2 material ranged from an average of 10.89% (2-year-old Resolution) to 35.16% (2-year-old S.Uralensis). Some of the varieties which indicate high bioactives content however were low yielding in terms of biomass and it was observed that majority of the higher biomass yielding varieties were in the low to mid 20% for extractives.

Incorporating the yield and extraction together and allowing for a 20% w/w bark relative to total biomass, Table 5 illustrates the highest yielding varieties in terms of production of bioactives. The previously selected varieties are highlighted in red (Table 3). Of the varieties which gave high extraction rates for year 1 material most are included as Cheviot and Shubby willow are not high yielding enough for consideration for extractives production from 1 year old willow.

	Y1 Total extractives **	***Y1 Average Extractives***
Variety Name	**kg**	**%**
Endeavour	**0.26**	**24.69**
LA970243	**0.23**	**22.99**
LA970562	**0.21**	**23.83**
Endurance	**0.21**	**20.72**
Mourne	**0.19**	**23.22**
LA970253	**0.18**	**23.8**
LA980348	**0.16**	**22.33**
LA980280	**0.15**	**19.53**
LA2001476	**0.15**	**19.85**
LA970523	**0.15**	**20.61**
Hambledon	**0.14**	**20.82**
LA2001155	**0.12**	**24.01**
Meteor	**0.12**	**23.61**
Resolution	**0.12**	**24.36**
Cheviot	**0.12**	**27.58**
LA980266	**0.12**	**16.82**
LA2000339	**0.1**	**21.83**
Terra Nova	**0.09**	**23.55**
Tora	**0.08**	**23.76**
S. Eriocephala ER65	**0.07**	**20.18**
77056	**0.07**	**21.2**
S. Udensis (=sachalensis) Kioryo	**0.06**	**19.19**
LA 990073	**0.06**	**22.92**
S. Purpurea	**0.05**	**23.3**
LA 990072	**0.05**	**22.98**
S. Uralensis	**0.05**	**22.13**
S. x dasyclados	**0.05**	**20.41**
S. Triandra	**0.04**	**23**
Shrubby Willow	**0.03**	**24.78**
S. Koriyanagi	**0.02**	**20.39**
S. Miyabeana	**0.02**	**15.46**

Table 5. Loughgall Year 1 Bioactive extraction x Biomass Yield

Similar data sets were compiled for year 2 Loughgall and Years 1 & 2 Claremorris material and these are presented in Appendix.

4.2 Variety selection for highest bioactives production

Taking all the evidence of production yields and extraction together, the following varieties were the ones of best promise. These will be harvested for a third and final harvest of one year old and three-year-old material. Given some of the high standard deviation of the averages, extending the list of chosen varieties will serve to proof the already collected data on biomass yields and extractives yields.

No.	**Variety**	**Reason**
3	LA970243	Yields well at both sites and produces high extractives production from both 1- and 2-year material
19	Endurance	Yields well at both sites and produces high extractives production from both 1- and 2-year material
2	LA980348	Yields well at both sites and produces high extractives production from both 1- and 2-year material
6	LA970562	Highest production of extractives at both sites for 1- and 2-year material
1	LA970253	High production of extractives at both sites for 1- and 2-year material
21	Meteor	High production of extractives at both sites for 1- and 2-year material
5	LA980280	Better yields in Loughgall but high bioactives at both sites for 1- and 2-year biomass
4	LA970523 (LG only)	Good in Loughgall
7	LA2001155 (CM only)	High production of extractives in Claremorris, ok in Loughgall
25	Mourne (CM Only)	High production of extractives at both sites for 1- and 2-year-old material
24	Hambledon	High production of extractives at both sites for 1- and 2-year-old material
20	Endeavor	Good 1 year old material in Loughgall
16	Shrubby Willow	High production of extractives in Claremorris.
8	LA2001476	High production of extractives at both sites for 1- and 2-year-old material
9	LA980266	possibly
22	Resolution	possibly

5.0 Solvent Extraction

5.1 Development of extraction method

To effectively extract bioactive compounds from willow bark—particularly salicin and other polyphenols—a tailored extraction method was developed, balancing efficiency with compatibility with available laboratory equipment. This involved optimizing key parameters such as particle size, solvent composition, temperature, and extraction duration. The method was built around the use of a CEM EDGE extraction system, which operates in a closed and pressurized environment and allows extractions to occur at elevated temperatures (up to 200°C) and under controlled pressure. This increases the solubility and diffusion rates of target compounds, significantly improving extraction efficiency without degrading thermolabile components such as salicin. In addition, the CEM EDGE uses minimal solvent volumes through a top-add, bottom-add, and rinse process, which is not only environmentally friendly but also cost-effective. The solvent mixtures can be quickly delivered and recovered, making it easy to perform targeted extractions depending on whether total extractives or specific compounds like salicin are of interest. The system's short extraction cycles (typically less than 30 minutes per sample) allow for high-throughput processing, enabling the analysis of multiple samples per day. This is particularly advantageous during method development and screening of different willow genotypes or environmental treatments.

Method of Extraction: CEM Polyphenols in Plant Feedstock Standard Method of Extraction

- Accessories: S1 Q-Disc, G1 Q-Disc, C9 Q-Discs, Q-Cup
- The S1 Q-Disc is a preassembled sandwich of the G1 Q-Disc between two C9 Q-Discs. The entire sandwich is placed, excluding the blue separator, into the bottom of the Q-Cup, and the Q-Cup is assembled.
- The sample is milled and then weighed into the Q-Cup.
- A Q-Screen is inserted into the Q-Cup using the Q-Screen tool.
- Sample Weight – ~ 0.5 g; Particle Size – ~ 10-40 mm (chips)
- Solvents – 50:50 Ethanol/Water to target total extractives (including salicin); 20:80 Ethanol/Water to target salicin.

Table 6. Cycle 1

Cycle	Top Add (ml)	Bottom Add (ml)	Rinse (ml)	Temp (°C)	Hold (mm:ss)
1	20	10	10	85	20:00

Table 7. Wash Program

Cycle	Solvent	Volume	Temp (°C)	Hold (mm:ss)

1	Ethanol/Water	15	90	00:15
2	Ethanol/Water	15	---	---

5.3 Development of HPLC method

To accurately quantify salicin, key bioactive compound in willow bark, a high-performance liquid chromatography (HPLC) method was developed, drawing on published literature and adapted specifically to the matrix complexity of willow extracts. This method was optimized for speed, sensitivity, and specificity, with particular attention to compound separation, detection reliability, and reproducibility across multiple willow varieties.

The HPLC method utilizes advanced instrumentation and analytical techniques to ensure precise quantification, particularly important for pharmacological and phytochemical evaluations where salicin levels are a key quality marker.

Rationale for Method Conditions

- 1. Instrument Setup
    - Agilent 1260 Infinity HPLC coupled with an Agilent 6530 QTOF (Quadrupole Time-of-Flight) mass spectrometer provides high resolution and mass accuracy. This setup enables both qualitative and quantitative analysis of salicin and potential related metabolites.
    - Detection was performed in both positive and negative electrospray ionization (ESI) modes to maximize ionization efficiency and confirm compound identity via high-resolution mass spectra.
- 2. Column Selection
    - A Luna C18 reversed-phase column was chosen for its robust performance in separating polar to moderately non-polar compounds. Salicin, being a polar glycoside, is well retained and resolved under reverse-phase conditions.
    - The stationary phase offers consistent performance and durability across multiple injections, ideal for routine analysis.
- 3. Mobile Phase Composition
    - A binary solvent system of Acetonitrile and Water (with 0.1% formic acid) was used.
    - Water (0.1% formic acid) improves peak shape and enhances ionization in ESI-MS.
    - Acetonitrile serves as the organic phase, providing efficient elution and sharper peak resolution.
    - The acidic modifier (formic acid) also helps suppress tailing of salicin and enhances its detectability.
- 4. Detection and Monitoring
    - Diode Array Detection (DAD) was set at 267 nm, the absorbance maximum for salicin.

This ensures optimal UV sensitivity and selectivity for quantitation, even at low concentrations.

  - The DAD provides a reliable, non-MS-dependent detection method, useful for routine analysis and method validation.

- 5. Column Temperature and Flow
  - The column temperature was maintained at 30°C, which ensures consistent retention times and improves reproducibility.
  - Isocratic or gradient flow conditions may be applied depending on sample complexity, though for targeted salicin quantification, a short isocratic run is often sufficient.

5.5 Final Analytical Conditions

Parameter	**Value**
Instrument	Agilent 1260 HPLC Infinity + Agilent 6530 QTOF
Ionization Mode	ESI (Positive and Negative)
Column	Luna C18 Reversed-Phase
Column Temperature	30 °C
Mobile Phase	A: Water + 0.1% Formic Acid B: Acetonitrile
Flow Rate	Optimized based on run length (typically 0.5–1.0 mL/min)
Detection (DAD)	267 nm
Mass Detection	Accurate mass monitoring of salicin (m/z ~285 [M–H]^–)

5.6 Advantages of Method

- Rapid and robust: Suitable for high-throughput screening of multiple willow extracts.
- High sensitivity: Capable of detecting low concentrations of salicin.
- Specific and selective: Combines UV and MS data to reduce false positives and increase confidence in quantification.
- Scalable: Can be applied to method validation and adapted for GMP-quality control if required.

This tailored HPLC method ensures accurate, efficient quantification of salicin, forming the analytical backbone for evaluating willow bark extracts in both research and potential commercial applications.

Fig 17 . Equipment used for extraction and analysis (from left to right – Retsch Mill, CEM EDFGE, samples and sample holders, extracts, LC-MS-QToF)

5.7 Chromatogarphic Analysis

Analysis of samples in years 0, 1 and 2

Only three varieties were assessed in year 0, while all available varieties were assessed in years 1 and 2.

In *Loughgall*, the highest **extractives** concentration for Y1 varieties was noted in the variety Cheviot in block 2 with approximately 30% extractives content. High extractives concentrations were observed also in the varieties Endeavour and Tora, respectively in block 3 and block 1. Generally, block 1 and 3 showed the highest extractives concentration trends for most varieties, while the lowest extractives concentration was noted in the varieties S.Miyabeana and LA980266, respectively in block 2 and block 1.

The varieties LA980348, S.Uralenis and LA990073 from Loughgall showed the highest **salicin** concentrations (all of them over 2.5% of the dry biomass weight). Concentrations of salicin over 2% were also noted in LA970253, LA970562 and LA2001155. However, no visible concentration trends were noted between varieties grown at different blocks. The lowest salicin concentration was observed in the varieties LA970523 and Mourne, respectively from block 1 and block 3.

The varieties with the highest **extractives** concentration in *Claremorris* were LA970562, Cheviot and Endurance (approximately 25% of dry biomass weight). Not large differences were noted in extractives content between different blocks. In addition, several samples not harvested due to growth were not feasible for debarking. The lowest concentration of extractives was observed in S. Udensis Kioryo.

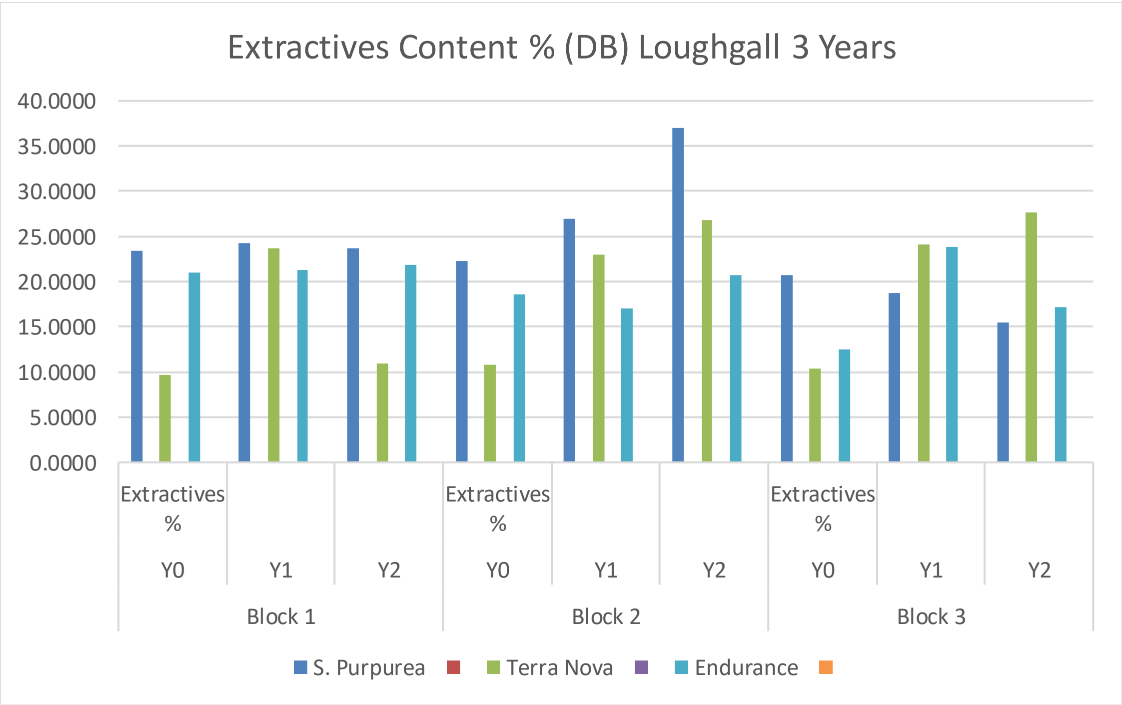
In Claremorris, the highest **salicin** concentration was noted in LA970562 block 2 (over 2.5%). Noteworthy concentrations were also present in LA970253, LA980348, LA2001155 and Endurance (over 1.5%). Not particular trends were noted in salicin content between different blocks, however, block 3 appeared to have slightly lower salicin content for most varieties. The lowest salicin content was noted in LA980266, Tora and Cheviot (all in block 3).

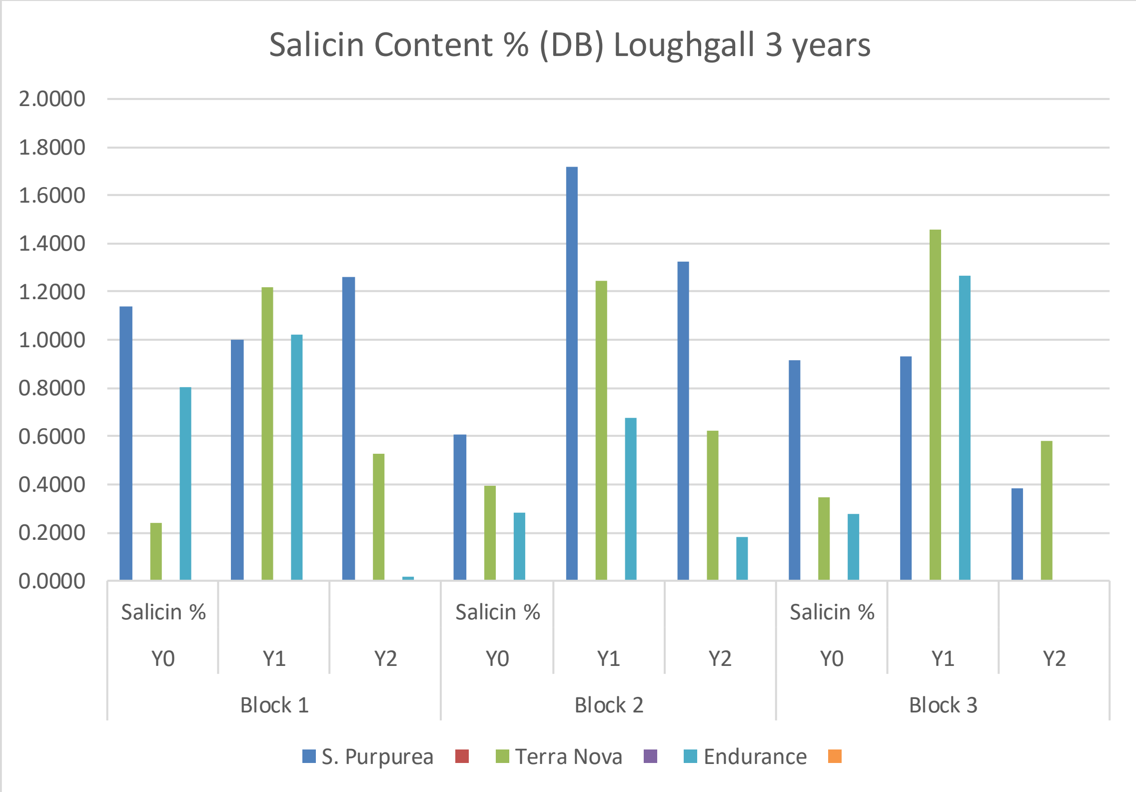
Additional research presented a **comparison** between Y0 extractives and salicin contents with Y1 and Y2 extractives and salicin content was done to assess concentration trends in terms of extractives, or high-value constituents (e.g. salicin) in relation to the year of harvest.

A slight decrease in extractives concentration was noted when comparing Salix Purpurea and Endurance at Year 0 and Year 1 (expect for block 3). In terms of extractives content, the same trend was not noted for Terranova. On the contrary, for Terranova, and more generally for block 3 varieties an increase in extractives concentration was noted from Year 0 to Year 1.

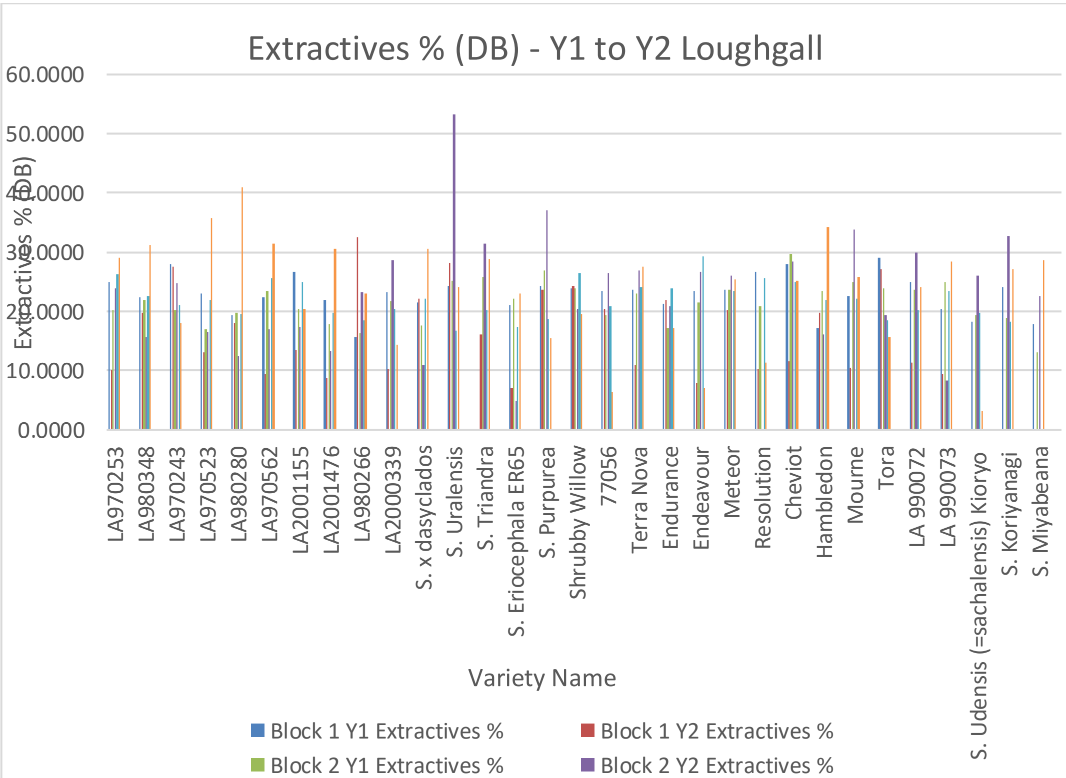
Generally, an increase in salicin concentration was observed when comparing the same variety at Year 0 and Year 1 in Loughgall. On the other hand, a decrease of salicin concentration was noted for Terranova when comparing Y0 to Y1 (block 1) results from Claremorris. No other clear trends were noted due to lack of samples.

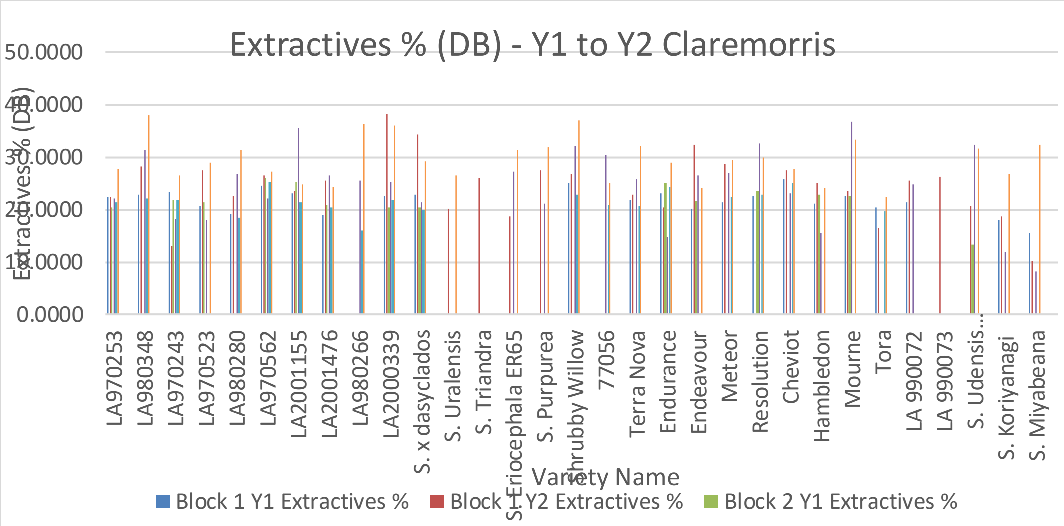
Comparison of three varieties over 3 years





Comparison of all varieties over two years





Considerations

Highest extractives concentration

- Loughgall: Cheviot 30% / High extractives concentration also in Endeavour and Tora (approx. 27%)
- Claremorris: Cheviot 26% / High extractives concentration also in LA970562, and Endurance (25%)

Highest salicin concentration

- Loughgall: LA980348, S.Uralenis and LA99007 (over 2.5%) / High salicin concentrations also in LA970253, LA970562 and LA2001155 (around 1.5%)
- Claremorris: LA970562 (over 2.5%) / High salicin concentrations also in LA970253, LA980348, LA2001155 and Endurance (1.5%)

Not particular trends were noted in salicin and extractives content between different blocks. Results are variable. No relevant information was gained.

Decrease in extractives concentration was noted from Year 0 to Year 1, except for Terranova.

Increase in salicin concentration was noted from Year 0 to Year 1 in Loughgall only. However, a decrease in salicin concentration was observed from Year 0 to Year 1 in Claremorris, for the only variety assessed both years. Results are too variable for clear trends.

Final observations considering both locations:

- Highest extractives content – Cheviot
- Highest salicin content – LA980348, LA99007, LA970253, LA970562, LA2001155 - Trends consistent for LA samples.

From Year 0 to Year 1 a decrease in extractives (except Terranova and block 3) and increase in salicin concentration can be confirmed for certain varieties. Results from data obtained mostly from Loughgall site and variable depending on variety.

Chromatographic Purification of Salicin

The isolation and purification of salicin and related salicylate glycosides from willow bark extracts were conducted through a series of column chromatography experiments. These were designed with reference to the method outlined in European Patent EP1901698, which emphasizes the use of ethanol-water solvent systems based on the favorable solubility characteristics of salicin. The objective of these experiments was to develop an effective chromatographic purification protocol capable of separating salicin from other structurally related phenolic glycosides present in willow extracts.

Experiment 1 – LA970253 (Claremorris, Year 1, Block 2)

The first purification experiment utilized extracts from the Salix variety LA970253, chosen for its relatively high salicin content as established during earlier HPLC analyses. Extraction was performed using an 80:20 water-ethanol solvent, and a pump-assisted elution method was used to push the extract and subsequent solvent mixtures through the resin-packed column. Fractions were collected sequentially based on the changing solvent composition:

Fraction	**Solvent**
1	Crude sample
2	100% H_2O
3	90% EtOH
4	10% EtOH
5	100% H_2O (second)
6	90% EtOH (second)
7	10% EtOH (second)

Following HPLC analysis of each fraction, it became evident that pure salicin was not fully isolated. Instead, fraction 3 and fraction 4 showed the highest salicin concentrations, containing approximately 40% and 30% of the total salicin content from the original extract, respectively. However, several other salicylate derivatives (e.g., salicortin, tremulacin) were also detected within these fractions, confirming that the resins used (e.g., Amberlite by Rohm and Haas) lacked sufficient selectivity for salicin alone.

This result highlights the challenge in achieving complete purification using standard column chromatography and emphasizes that the method was better suited to partitioning rather than isolating salicin in a chemically pure form.

Experiment 2 – Tora (Loughgall, Year 1, Block 3)

For the second experiment, the same 80:20 water-ethanol extract approach was applied to the Tora variety, selected from Loughgall. In contrast to experiment 1, no pump was used, and instead, fixed volumes of solvent mixtures were passed manually through the column. Fractions were collected every 3 minutes, allowing for more detailed tracking of compound partitioning.

Interestingly, the majority of salicin was recovered in the early fractions eluted with 100% water, particularly in fractions F4-13 and F4-14, each containing approximately 20% of the total salicin. This result aligns with salicin's high solubility in water (~40 g/L) and poor solubility in ethanol (~3 g/L), confirming that aqueous washes were most effective at recovering salicin from the resin bed.

Despite the more granular fractionation, this approach did not yield higher selectivity, and like experiment 1, co-elution with other phenolic glycosides was still observed. However, it did enhance the understanding of solvent elution profiles and supported a more strategic application of solvent strength for salicin recovery.

Experiment 3 – Endurance (Loughgall, Year 1, Block 3)

The third experiment followed the same overall framework as experiment 2 but focused on the Endurance variety. Notably, only two solvent mixtures (10:90 and 90:10 water-ethanol) were used, and longer elution times were applied. No pump assistance was used, and the system relied solely on gravity-based elution.

Unlike the previous two experiments, significant salicin concentrations were detected in later-stage fractions, particularly during the final 90:10 water-ethanol washes. The last nine fractions together accounted for approximately 90% of the total salicin recovered, with each containing around 10% of the total salicin.

This pattern deviates from expectations based solely on salicin's water solubility, suggesting that resin-salicin interactions and flow rate might play a role in retention and delayed elution, particularly under conditions of limited solvent polarity.

Experiment 4 – Patent-Based Protocol

The final purification trial followed the step-by-step procedure outlined in EP1901698 in full, with minor adjustments to scale. The multi-step process included:

- Accelerated solvent extraction (0.5 g bark with 20 mL ethanol-water).
- Concentration via evaporation (to 25% of original volume).
- Precipitate removal by centrifugation (Step S).
- PVPP treatment to bind polyphenols and remove phenolic impurities.
- Column adsorption of the clarified extract onto resin.
- Sequential washing:
    - 50 mL water (removes weakly bound impurities)
    - 150 mL 90% ethanol (elutes salicin and salicylates)
    - Final 150 mL pure water rinse.
- Evaporation and resuspension of eluate in ethanol-water.

This approach yielded clearer fractionation, and the eluate recovered from the 90% ethanol wash was particularly enriched in salicin. Analytical validation via HPLC confirmed that, while not chemically pure, the eluted fraction contained a significantly higher proportion of salicin relative to other co-extracted compounds. This stepwise protocol proved to be the most refined and effective of the four, closely approximating semi-purified salicin extract.

Considerations

Across the four chromatography experiments, several conclusions were drawn:

- Complete purification of salicin alone was not achieved using standard resins (e.g., Amberlite), largely due to their non-specific affinity for phenolic compounds and the structural similarity of salicin to other salicylates.
- Nonetheless, the protocols effectively partitioned salicin into select fractions, improving downstream purity and simplifying extract composition.
- Salicin was most effectively eluted in high water-content fractions, which corresponds with its known hydrophilicity. However, experiments also showed variability in elution behaviour, suggesting the importance of resin type, flow rate, and solvent sequence.
- The patent-based protocol was the most comprehensive and effective, offering the highest degree of refined enrichment, though not absolute purification.

Future efforts may benefit from exploring more selective stationary phases (e.g., affinity resins or molecularly imprinted polymers) or employing preparative HPLC for final purification stages, particularly if pharmaceutical grade salicin is the target.

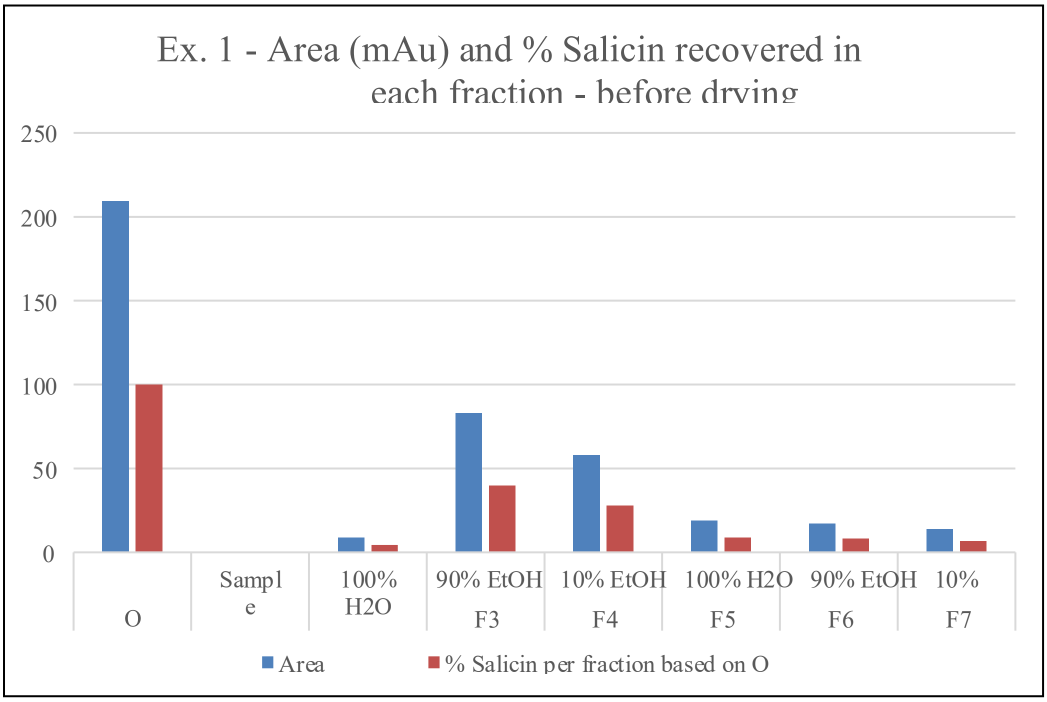


Fig 17 - % Salicin recovered in each purification fraction before drying

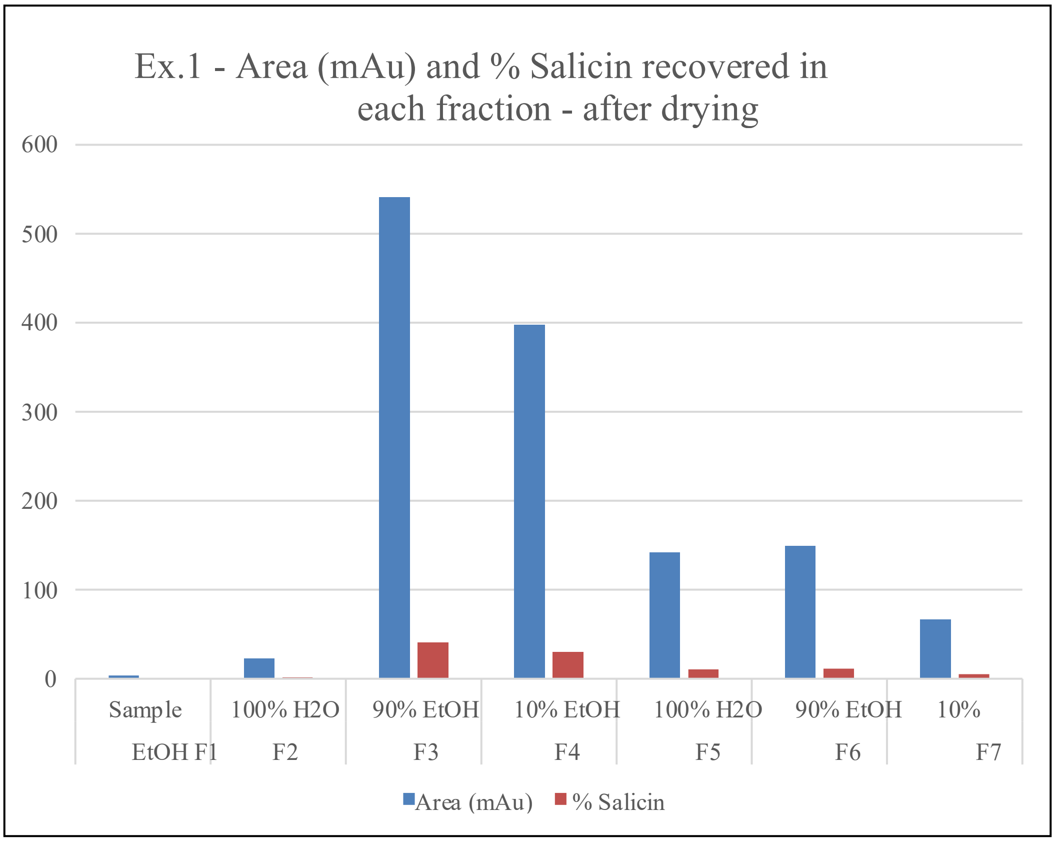


Fig 18 - % Salicin recovered in each purification fraction after drying

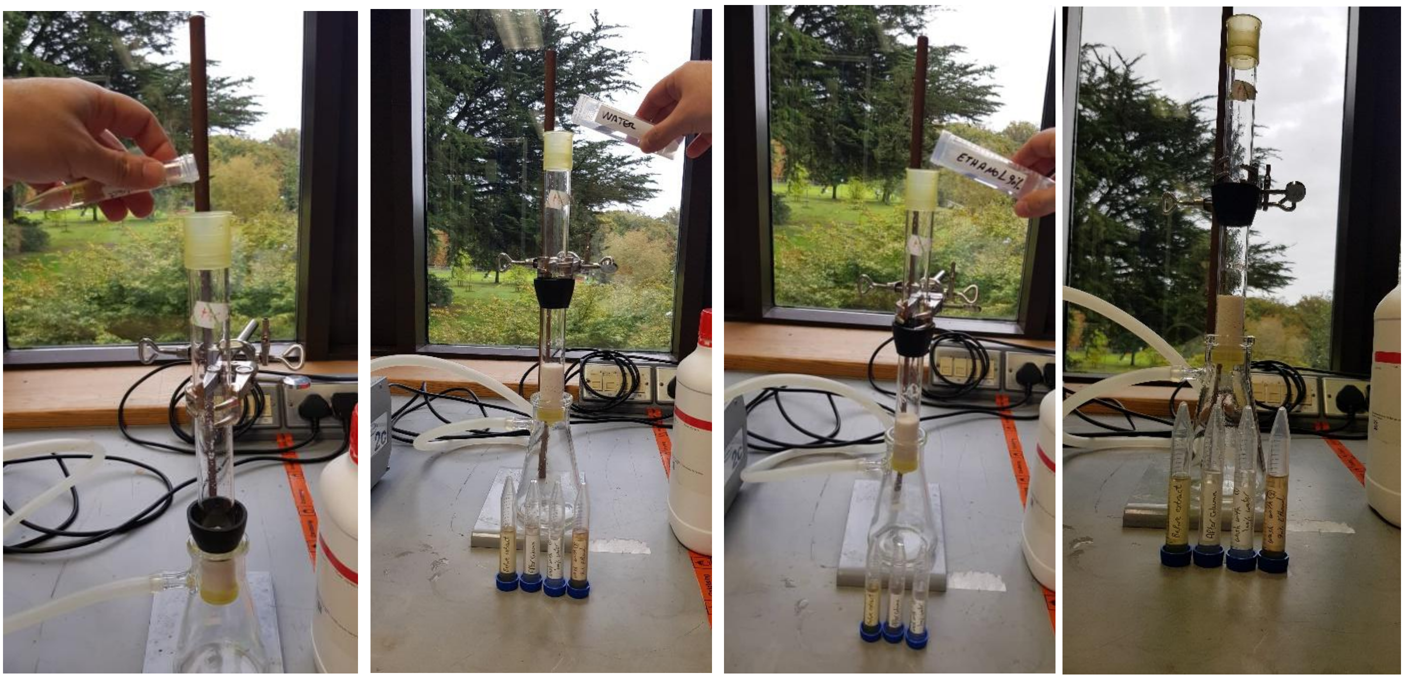


Fig 19 - Purification apparatus

Large-Scale Experiments

Rationale

To evaluate the feasibility of upscaling willow bark extraction processes for potential industrial application, a series of large-scale extraction experiments were conducted. These experiments were designed to mirror the conditions and insights gained from prior lab-scale extractions while accounting for the practical limitations and engineering constraints of operating at a larger volume.

Setup and Design

The large-scale setup comprises three stainless steel extraction vessels, each with a capacity of 50 Liters. These vessels were selected for their chemical resistance and thermal stability and are equipped with heating jackets and mechanical stirring to ensure homogeneous temperature distribution and proper solvent-biomass contact. Each vessel was loaded with 10 kilograms of milled willow bark, resulting in a solid loading of 20%, a value chosen based on a balance between extraction efficiency and mixing feasibility.

Three different solvent systems were tested in parallel:

- Recycled solvent extraction: One vessel was filled with an 80:20 water/ethanol mixture that had been previously used in lab-scale extractions. This setup aimed to evaluate the efficiency of recycled solvents and their potential for reuse in reducing solvent waste and operational costs.
- Fresh 50:50 water/ethanol extraction: A second vessel was used with a fresh solvent mixture composed of equal parts water and ethanol. This mixture was selected based on its performance in targeting a broad range of extractives, including both polar and semi-polar compounds, and reflects standard conditions used in previous EDGE extraction cycles.
- Fresh 80:20 ethanol/water extraction: The third vessel employed an 80:20 ethanol-rich mixture, intended to explore the selective extraction of less polar components and compare the efficiency of higher ethanol content against aqueous extractions.

6.0 Process Conditions

Unlike lab-scale extractions—where temperature and pressure could be finely controlled using the CEM EDGE system—the large-scale vessels operate under atmospheric pressure, with temperature capped at 60°C due to equipment constraints and energy considerations. This temperature represents a practical upper limit for many industrial extraction systems and was chosen to approximate the thermal energy input used in the pressurized lab system, while avoiding solvent degradation or excessive evaporation.

To understand how time affects extraction efficiency under these milder conditions, three different extraction durations were tested:

- 30 minutes.
- 1 hour.
- 2 hours.

These time points were selected to assess how the kinetics of extraction vary without pressure enhancement, and to determine the optimal duration for maximum extractive yield without unnecessary energy or time expenditure.

6.1 Comparison to Lab-Scale Experiments

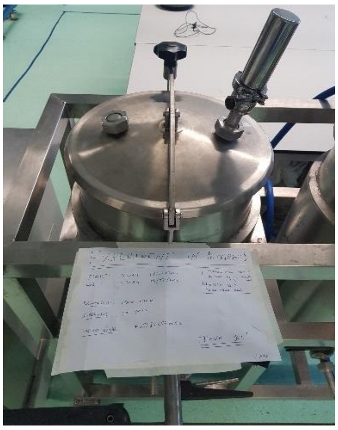
It is important to note that direct comparisons between lab- and large-scale extractions are not straightforward. Lab-scale extractions were performed using accelerated solvent extraction (ASE) with pressurized systems, resulting in shorter extraction times and potentially more efficient penetration of the solvent into plant tissue. The EDGE system used in lab conditions also operated at temperatures as high as 85°C, further enhancing solubilization of targeted constituents such as salicin and polyphenolic compounds.
In contrast, the large-scale extractions rely entirely on temperature-driven diffusion and mechanical agitation, with no pressure enhancement. Consequently, the kinetic profile of solute release is slower, necessitating longer contact times and potentially affecting compound stability, particularly for heat-sensitive phenolics.

Objectives

The key aims of these large-scale experiments are to:

- Validate the scalability of lab-developed methods under more realistic industrial conditions.
- Compare the performance of fresh vs. recycled solvent systems, especially regarding extractive yield and compound integrity.
- Identify the optimal extraction time that balances efficiency and resource use.
- Assess the effect of solvent composition on the selectivity of extraction (e.g., salicin-rich vs. polyphenol-rich profiles).

The results of these trials inform decisions around process optimization, solvent recycling strategies, and economic viability for full-scale willow bark valorisation. They will also serve as a foundation for assessing purification feasibility following bulk extraction and for tailoring downstream processing depending on the target bioactive fraction.



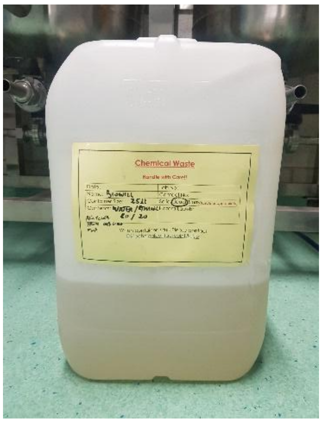


Fig 20 – Large Scale Extraction apparatus and extracts

7.0 Bioactives Identification

The analysis of willow bark and wood extracts across several Salix varieties revealed a diverse array of bioactive compounds, beyond the well-known salicin. These compounds were extracted using a 20:80 Water/Ethanol solvent system, which has shown a broader solubilization potential for both polar and moderately polar phytochemicals. The identification of the compounds was performed through advanced chromatographic and mass spectrometric techniques, detecting both positive and negative ionization modes to capture a wide spectrum of molecular species.

Notably, **salicin**, the characteristic phenolic glycoside of willow, was consistently detected in the bark across all varieties, reaffirming its role as a chemotaxonomic marker for Salix species. Alongside salicin, **salicortin**—another potent phenolic glycoside—was also found ubiquitously, often accompanied by its formate adduct in mass spectra, indicative of formic acid traces from extraction buffers.

One of the most recurrent flavanols identified was **catechin**, which was present in both bark and wood extracts of every tested variety. Its prevalence suggests a potential role in the antioxidant profile of the extracts. In several cases, catechin was found complexed with gallic acid or as its dimerized or gallated forms (e.g., **catechin gallate**), especially in varieties like Cheviot and Resolution.

Further, several **flavonoid glycosides** were discovered, such as **acacetin-5-O-xyloside**, **luteolin-7-glucoside**, and **apigenin-7-O-glucoside**, indicating the presence of specialized metabolites with known anti-inflammatory and antioxidant activities. These were especially abundant in varieties like Tora and Resolution.

Triandrin, a lesser-known stilbene derivative, was detected consistently in the wood extracts, suggesting a potential for industrial valorization of what is often considered waste material. Meanwhile, additional minor constituents, such as **picein**, **kaempferide**, and **gibberellic acid**, appeared selectively in certain cultivars, hinting at unique chemotypes.

Below is a consolidated summary table of the main identified compounds from representative varieties:

Variety	Fraction & Ionisation	Compound	Formula
S.X. Dasyclados	Bark (-)	Salicin	$C_{13}H_{17}O_7$
		Salicortin	$C_{20}H_{23}O_{10}$
		Catechin	$C_{15}H_{13}O_6$
	Bark (+)	Rosin	$C_{15}H_{20}O_6$
	Wood (-)	Triandrin	$C_{15}H_{19}O_7$
Endeavour	Bark (-)	Acacetin-5-O-xyloside	$C_{21}H_{19}O_{19}$
Cheviot	Bark (-)	Catechin (gallic acid)	$C_{15}H_{13}O_6$
		Salicortin	$C_{20}H_{23}O_{10}$
Tora	Bark (-)	Luteolin-7-glucoside	$C_{21}H_{20}O_{11}$
		Picein	$C_{14}H_{19}O_7$
Resolution	Bark (-)	Apigenin-7-O-glucoside	$C_{21}H_{19}O_{10}$
		Vitexin-2-rhamnoside	$C_{27}H_{29}O_{14}$
Salix purpurea	Bark (-)	5-Methoxysalicylic acid	$C_8H_8O_4$
Terranova	Bark (-)	6-Prenylnaringenin	$C_{20}H_{19}O_5$
	Bark (-)	Quercitrin/Kaempferol-7-O-glucoside	$C_{21}H_{20}O_{11}$
Endurance	Bark (+)	Kaempferide	$C_{16}H_{13}O_6$
		Gibberellic acid	$C_{19}H_{23}O_6$

Table 8 – Main compounds (other than salicin) identified in Bark ethanolic extracts

This broad-spectrum profiling indicates that willow extracts—especially those obtained with ethanol-rich solvents—contain a complex and rich matrix of phytochemicals, many with potential health-related or commercial applications. Such findings pave the way for targeted valorisation strategies based on cultivar-specific bioactive content.

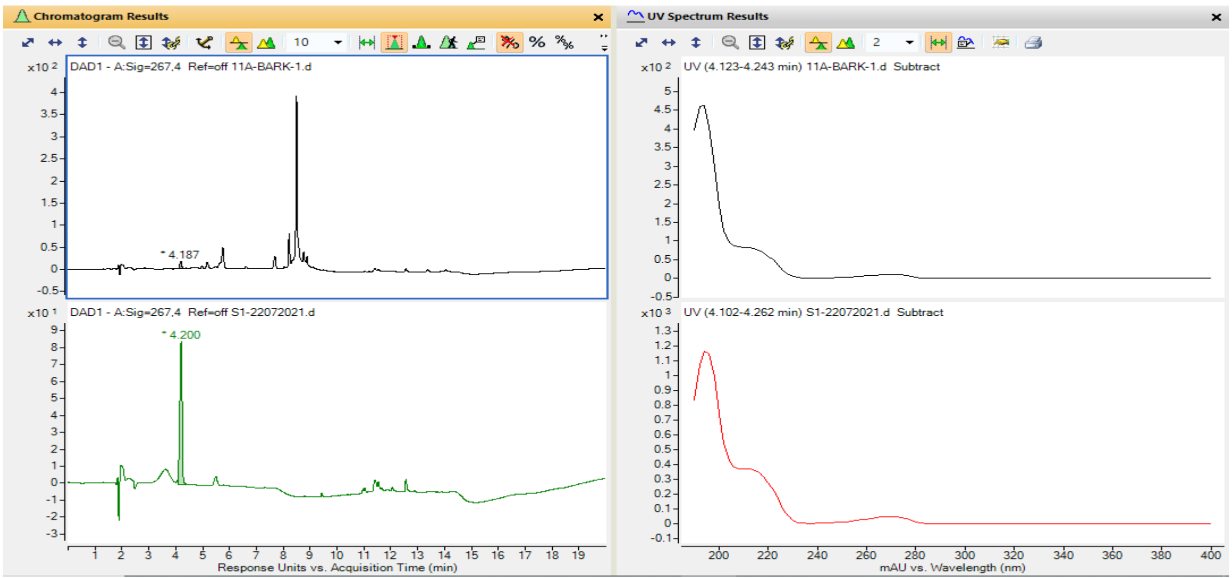


Fig 20 – Example of UV chromatogram of salicin standard (above) and salicin in willow bark extract (below)

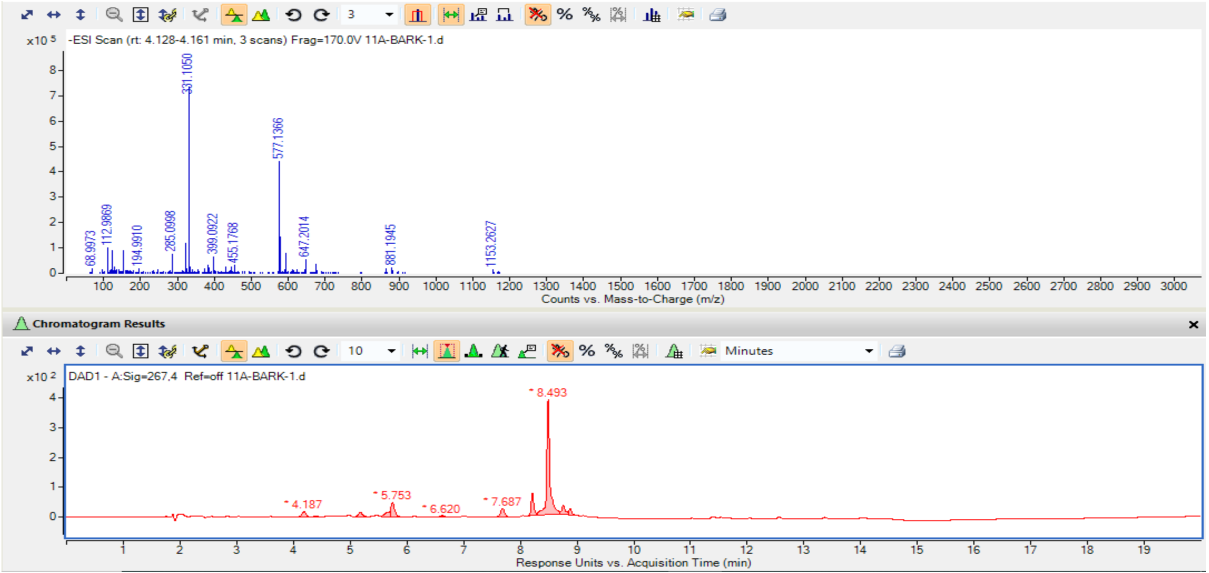


Fig 21 – Example of UV chromatogram of salicin in willow bark extract (below) and m/z fragmentation of peak identified as salicin

8.0 Cream Formulation

The initial formulation of a willow-based topical cream was conducted to assess the feasibility of incorporating bioactive-rich extracts into a commercially viable dermal base. This trial, under the **BioWILL** project, utilized **Silcock's base**, a commonly used pharmaceutical-grade cream base known for its stability and skin compatibility.

The process comprised several key stages:

Step 1 – Base Preparation

The cream formulation began with the use of **Silcock's Base**, weighed into a large beaker under controlled laboratory conditions. This base serves as the neutral matrix to which plant-derived active compounds are introduced.

Step 2 – Addition of Willow Extracts

Measured quantities of willow extracts—previously prepared using a 20:80 Water/Ethanol extraction protocol—were added directly into the base. These extracts had been quantified for total extractives and **salicin**, a signature active component of willow bark known for its analgesic and anti-inflammatory properties.

Two different extract samples were tested:

- **Cream 1 (HE)**: Higher extractive load
- **Cream 2 (HS)**: Lower extractive content, higher salicin proportionally

Step 3 – High-Shear Mixing

The mixture was subjected to high-shear homogenization at **4500 RPM** using a Silverson mixer. This step ensured uniform distribution of the willow extractives throughout the cream matrix, leading to a stable and consistent final product. The choice of high RPM mixing is essential for optimal dispersion and emulsification of plant compounds in oil-in-water emulsions.

Step 4 – Final Product

The homogenized creams were transferred to clean containers and tubes, labeled accordingly for further testing and evaluation. Visual inspection confirmed good texture and homogeneity, suggesting successful incorporation of the botanical ingredients without phase separation.

Step 5 – Sample Tubes

The creams were portioned into clearly labeled tubes for subsequent trials or stability analysis. Each tube was annotated with its formula code and extract dosage.

Creams Composition Summary

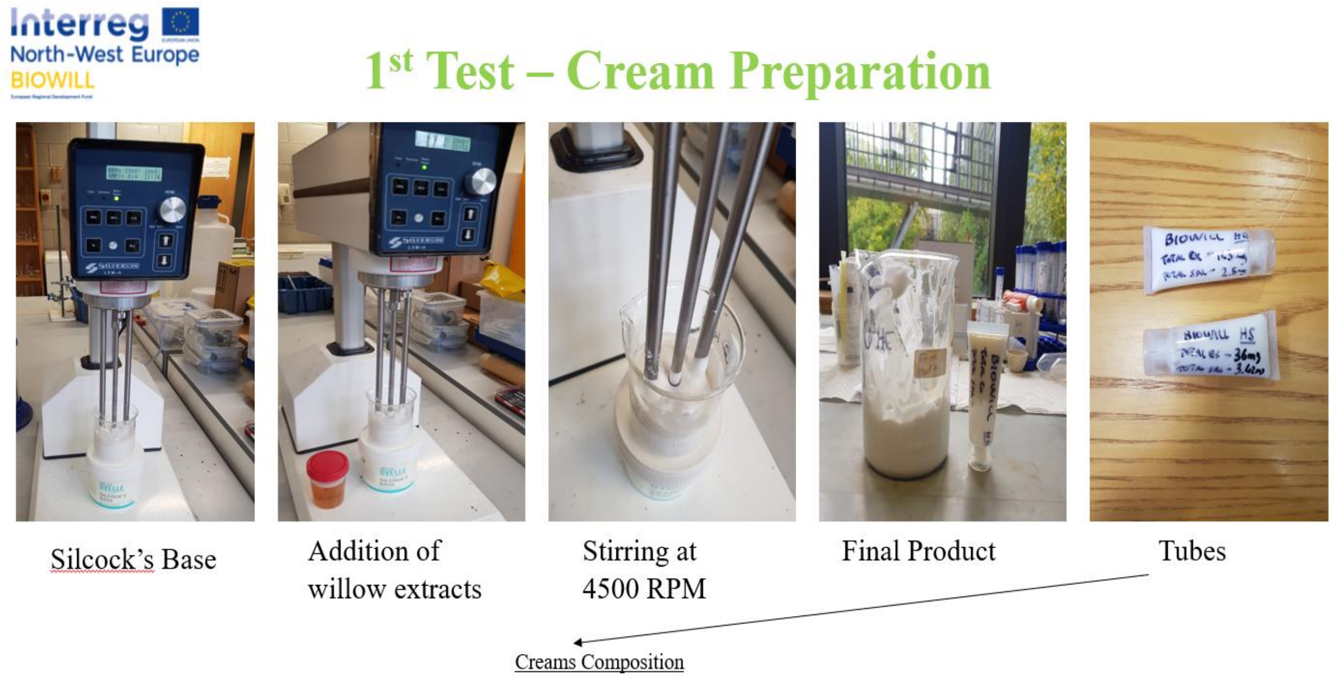
Cream	Cream Mass (g)	Extractives (g)	Salicin (g)	Extractives % in Cream	Salicin % in Cream
Cream 1 – HE	232.6867	0.123881515	0.002509	0.05323962	0.001078305
Cream 2 – HS	82.7267	0.036485959	0.003425	0.044104211	0.004139807

Key Observations:

- **Cream 1 (HE)** contained a higher total amount of extractives but had a lower salicin concentration per gram of cream, indicating a broader mix of other phytoconstituents besides salicin.
- **Cream 2 (HS)**, despite lower total extractives, had a higher **salicin percentage**, which may result in a more targeted bioactive cream if salicin is the primary functional component.
- The differences between these formulations offer a useful experimental contrast for studying the roles of total phenolics versus isolated actives in topical efficacy.

This cream formulation trial demonstrates a successful proof-of-concept for incorporating **willow-derived actives into dermatological products**. Future development could focus on:

- **Stability testing** over time and under different storage conditions.
- **In vitro/in vivo bioavailability** of salicin from the cream matrix.
- **Sensory evaluations** and **consumer acceptability testing**



	Cream (g)	Extractives (g)	Salicin (g)	Extractives % in cream	Salicin % in cream
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Fig 22 – Overview of cream formulation process

9.0 Fibre & Pulp Processing

9.1 Pulp

The **BioComposites Centre at Bangor University** has established itself as a national and international leader in biomass processing and materials development, with over three decades of specialized experience in the **pre-treatment and valorisation of agri-forestry residues**. These residues — including materials such as straws, grasses, and softwoods — have historically been underutilized or discarded as waste. The Centre's work aims to turn them into valuable raw materials for sustainable product manufacturing.

A wide range of **pilot-scale equipment** is housed at the Centre, designed to replicate and optimise industrial processes for the transformation of lignocellulosic biomass into usable fibres. These fibres have been successfully applied in diverse sectors, including:

- **Construction** (e.g. medium-density fibreboard [MDF], oriented strand board [OSB]),
- **Thermal insulation** (natural fibre panels),
- And more recently, in **sustainable packaging**, particularly in the form of **moulded pulp products (MPPs)**.

With increasing market and regulatory pressure to phase out single-use plastics, MPPs have garnered significant interest as biodegradable and compostable alternatives. These pulp-based materials are especially suited for **food**

contact applications, such as fresh produce trays, egg boxes, and general-purpose food containers.

9.2 Pulp Moulding and Thermoforming Capabilities

In support of this transition to bio-based packaging, Bangor University has developed **bespoke pulp moulding-thermoforming equipment** that simulates the techniques and tooling used in commercial MPP production lines. This equipment was designed specifically to handle not only conventional pulp sources (like **recycled paper and cardboard**) but also a broad range of **non-wood fibres and agricultural residues**, such as those generated in forestry, farming, or land management.

The process used at Bangor involves two major stages: **wet-forming** and **thermoforming**. These steps mirror industry practice but have been tailored to facilitate **flexible, small-batch research and prototyping**.

1. **Wet-forming Phase**:
    - A defined quantity of pulp is suspended in a water medium in a **pulp tank**. The pulp is traditionally derived from materials like **old newsprint (ONP)** or **recycled corrugated cardboard**, but in this project, it includes novel feedstocks such as willow.
    - The suspension is kept in constant motion using agitation to prevent settling and ensure uniform fibre distribution.
    - A two-part mould is submerged in the tank. The **lower half of the mould** is perforated and connected to a vacuum system. This vacuum draw pulp fibres onto the mould surface over a set period, forming the basic shape of the product.
    - Once a sufficient fibre layer is formed, excess water is removed by further vacuum dewatering.

2. **Thermoforming Phase**:
    - The semi-formed product is transferred to a **heating mould** — an exact replica of the forming mould — which contains **integrated heating elements**.
    - The heated mould is closed under pressure, and thermal energy is applied to **dry, cure, and consolidate** the moulded pulp product.
    - This step not only removes residual moisture but also helps bind the fibres through a process of thermo-mechanical bonding.
    - Critically, it produces a **smooth, uniform surface finish**, which is an essential characteristic for consumer-facing applications like food packaging.

9.3 Tooling Versatility and Product Prototyping

The versatility of the equipment allows for the development of a variety of prototypes. At present, Bangor University's system can fabricate:

- **Food trays** (e.g. 600 mL capacity)
- **Seedling pots**
- **Hard fruit trays**
- **Egg cartons**

Each tool can be swapped easily to accommodate different forms and geometries, which has enabled the Centre to prototype and evaluate multiple product types within the project. Figure 23 details the layout of the process, the flow of materials from pulp tank to final product, and the dimensions of prototype trays.

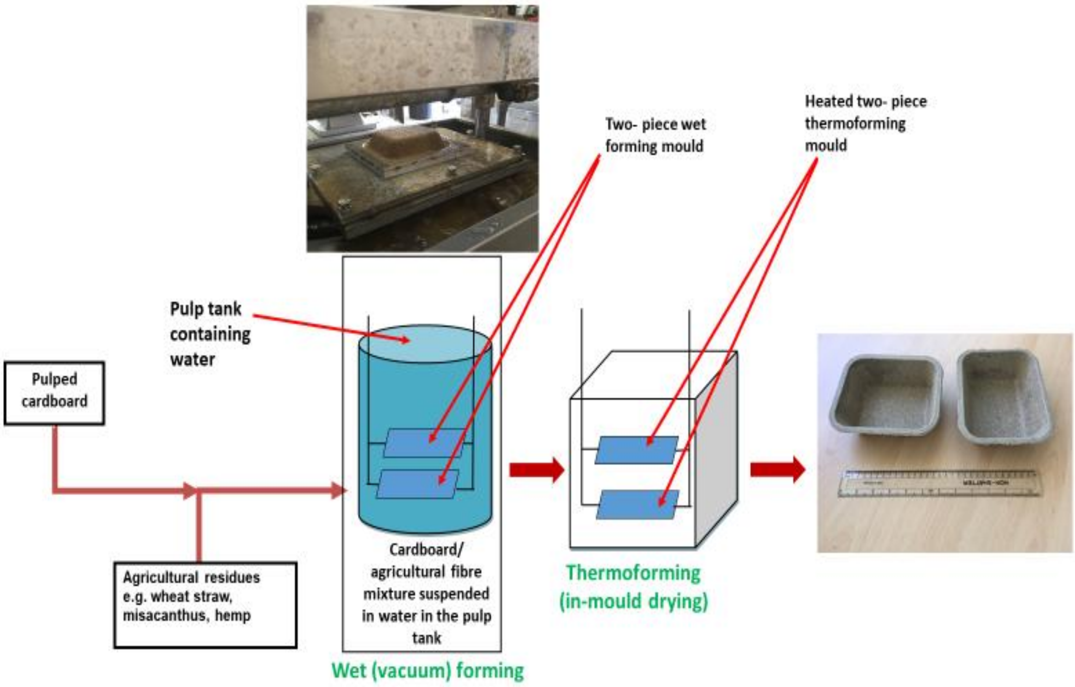


Fig 23 – Schematic showing the pulp moulding- thermoforming process

9.4 The Rising Cost of Recycled Fibre and the Case for Alternative Feedstocks

Most of the moulded pulp packaging on the market today is produced from **recycled newspaper (ONP)** or **recycled cardboard**. While these sources have historically provided a low-cost, reliable pulp stream, **market volatility**, **supply chain limitations**, and **increased global demand for packaging** have driven up the cost and reduced the availability of recycled fibre.

This trend has prompted packaging manufacturers and research centres alike to search for **alternative, cost-effective, and locally sourced fibre options**. Agricultural and forestry residues — such as **willow, miscanthus, hemp, and straw** — are now under serious investigation as viable replacements.

9.5 The Role of Willow in the Biowill Project

The **Biowill Project** specifically investigates **willow (Salix spp.)** as a potential feedstock for MPP production. Willow is an ideal candidate due to its:

- Rapid growth rate (harvestable within 2–3 years),
- Regrowth capability from coppiced stumps,
- Compatibility with marginal or underutilised land,
- And favourable fibre characteristics, especially in certain high-cellulose genotypes.

Within the scope of this research, Bangor University's infrastructure plays a key role in evaluating **how different willow processing methods** — such as **steam explosion** or **solvent extraction** — affect pulp quality, mouldability, and final product performance.
By integrating willow into existing pulp moulding systems, the Biowill team aims to demonstrate that **a renewable, low-input crop** can be effectively converted into **commercial-grade, biodegradable packaging products**, thus reducing dependence on both virgin wood pulp and imported recycled fibres.

9.6 Preliminary Pulp Moulding Trials

The first phase of experimental work conducted as part of the Biowill Project took place between August and September 2021. This initial stage focused on the processing and characterisation of willow biomass supplied by the University of Limerick, with the objective of assessing its suitability for pulp production and downstream moulded product manufacturing.
Source and Composition of Willow Samples

The willow material originated from a mixed batch composed of different cultivars — including *Salix purpurea*, *Terra Nova*, and *Endurance* — all harvested from the Claremorris experimental site in Ireland on 23 February 2021. The material was subsequently shipped to Bangor University in July 2021, where it arrived in chipped form and included a substantial proportion of bark. The presence of bark is a notable consideration, as it may affect both pulping efficiency and final fibre quality due to its higher lignin and ash content compared to the inner stem wood.
Three main categories of this biomass were defined and labelled for experimental purposes:

- Raw willow chips (untreated)
- Solvent-extracted willow
- Steam-exploded willow (produced from the raw chips)

Each of these sample types was subjected to compositional and physical analysis, as well as different pre-treatment and moulding trials, to determine their processability and functional performance.

9.7 Pre-treatment: Steam Explosion and Solvent Extraction

Preliminary pre-treatment experiments began in August 2021 and involved the application of batch steam explosion at varying pressures, primarily between 2 and 4 bar. These pre-treatments were conducted to evaluate how thermal and mechanical disruption of the biomass would alter the chemical composition of the fibres — particularly in relation to their cellulose, hemicellulose, and lignin content.

Steam explosion is a well-established biomass treatment method that uses high-pressure steam followed by rapid decompression to rupture the plant cell walls. This facilitates fibre separation, increases porosity, and enhances the digestibility or mouldability of the material.

In parallel, solvent extraction was carried out to remove waxes, resins, and other extractives that might interfere with fibre bonding or surface quality in the moulded products. The extracted samples provided a cleaner, more homogeneous fibre profile for comparative analysis.

9.9 Methodology and Trials

Compositional Analysis Methodology

To understand the impact of these treatments, a detailed fibre composition analysis was conducted on all three sample types: raw willow, solvent-extracted willow, and steam-exploded willow. The methodology followed included:

1. Moisture Content Determination:
   - 5 g samples were analysed using a moisture analyser.
   - Drying continued until the rate of moisture loss dropped below 20 mg per minute.
2. Sample Preparation:
   - Dried samples were milled into a fine powder using a ball mill.
   - 0.5 g of milled powder was then sealed into Ankom fibre analysis bags.
3. Sequential Detergent and Acid Washes:
   - Neutral detergent: to remove soluble cell components and determine non-fibre content.
   - Acid detergent: to isolate cellulose and lignin fractions.
   - 72% sulfuric acid: to further separate lignin and quantify cellulose.
   - Ash content was measured by combusting 0.5 g samples at 600°C for four hours in a muffle furnace, following NREL standard protocols.

Results of Fibre Composition Analysis

The key findings of the compositional analysis are summarised below, with all values presented as percentages of dry weight. These figures reveal important trends in how different pre-treatments affect fibre properties:

Sample	Non-fibre	Hemicellulose	Cellulose	Lignin	Ash
Raw willow chips	19.08 ± 2.03	19.63 ± 0.70	46.56 ± 1.10	12.57 ± 0.22	2.15
Solvent extracted willow	13.56 ± 0.18	16.99 ± 0.44 ($P < 0.05$)	53.10 ± 1.18 ($P < 0.05$)	14.12 ± 1.80 ($P < 0.05$)	2.22
Steam exploded willow	13.41 ± 0.39	16.02 ± 0.66 ($P < 0.05$)	48.20 ± 0.22 ($P > 0.05$)	19.52 ± 0.06 ($P < 0.05$)	2.85

The data indicate that:

- Solvent extraction increased cellulose content significantly while reducing non-fibre and hemicellulose fractions.
- Steam explosion resulted in lower hemicellulose and increased lignin concentration, likely due to hemicellulose degradation during high-temperature exposure.
- Ash content was highest in steam-exploded willow, reflecting the concentration of inorganic material after volatile losses.

These shifts in composition have direct implications for pulp quality, strength, and water resistance — all critical parameters for packaging applications.

Pulp Refining and Prototype Production

Following the chemical analysis, a batch of steam-exploded raw willow chips (4 bar, 1 hour) was processed using an atmospheric disc refiner to prepare fibres suitable for moulding trials. Several refining passes were conducted:

- First pass: 15 µm plate gap
- Two additional passes: 10 µm gap for finer defibrillation

Once refined, attempts were made to blend willow pulp with recycled cardboard pulp in two different ratios:

- 50:50 (willow: cardboard)
- 80:20 (willow: cardboard)

These blends were used to create demonstration trays via the Bangor thermoforming system, although this phase primarily served as a comparative benchmark for 100% willow pulp trials.

Moulding Trials Using 100% Willow Pulp

A series of controlled pulp moulding experiments were performed using 100% willow pulp (4% fibre consistency). The objective was to assess how unblended willow pulp performed in standard tray moulds under real processing conditions.

A total of 37 trays were successfully produced:

- 21 trays exhibited no defects, with an average dry weight of 19.11 g.
- 16 trays had minor defects (e.g., surface irregularities or incomplete edges), averaging 16.95 g in weight.

Despite some imperfections, most products were structurally sound and demonstrated that pure willow pulp could be moulded into rigid forms without fibre blending — a significant milestone for the project.

Sample Distribution for Anaerobic Digestion (AD) Studies

In early 2022, trays and control samples were dispatched to University College Cork (UCC) for anaerobic digestion trials, assessing their biodegradability and biomethane potential.

The following items were submitted:

- Trays from 100% steam-exploded willow:
    - 10 non-defective trays
    - 16 defective trays
- Solvent extracted willow controls (sent December 2021):

1. 1.0 kg of untreated, solvent-extracted willow
2. 1.1 kg of steam-exploded solvent-extracted willow (4 bar, 1 hour)

These materials were used to evaluate how pre-treatment methods influence digestibility and gas yield, an important factor for end-of-life composting or biogas applications.

Biocomposites for Sustainable Horticulture and Packaging: Pilot Trials and Pulping Process

Bangor University collaborates with a major commercial nursery supplying plants globally. Currently, this nursery relies on single-use plastic pots and trays in its greenhouses. As part of Task 3 activities under the Biowill project, the BioComposites Centre explored sustainable alternatives, evaluating plant pots made from lignocellulosic biomass.

A series of moulded pots were developed using a blend of willow pulp and recycled cardboard (80:20 ratio) – see Figure 24. These were presented to the commercial nursery in June 2022, which expressed strong interest in trialling the pots in greenhouse conditions. The nursery indicated a need for a batch of 100 pots for dedicated testing. Additionally, they were keen to trial a 40-litre batch of extracted willow bark as a peat-free growing medium. Due to technical limitations, this material could not be supplied within the current project timeframe but remains a focus for future research.

Fig 24. Horticultural pots made from willow pulp and recycled cardboard.

To meet additional material demands from project partners, Bangor University conducted pilot-scale willow pulping trials in September 2022. These trials supported partners requiring material for upscaled anaerobic digestion tests. Due to limited moulding capacity, only refined pulp was provided rather than final moulded products (trays and pots).

AFBI supplied 280 kg of whole, chipped willow (30% moisture, equivalent to 196 kg dry matter), harvested at their Hillsborough site (April–May 2022) and consisting of multiple willow varieties. The material was processed using a pressurised disc refiner at Bangor University under the following conditions:

- Pressure: 6 bar
- Retention time: 1.5 minutes
- Plate gap: 15 µm
- Drying: Flash dried at 200°C to ~15% moisture
- Output: 171 kg of dry, refined fibre
- Additional data: Energy consumption recorded and supplied to Materia Nova for LCA

Refined willow fibre was distributed to project partners as follows:

- University of Limerick: 110 kg (anaerobic digestion)
- University College Cork: 15 kg (anaerobic digestion)
- AFBI: 15 kg (pelletising trials)
- Technical University of Lodz, Poland: 12 kg

Pulping and Fibre Production Methods

For small-scale willow processing (1–5 kg), Bangor University employed a two-step method: batch steam explosion to soften the biomass, followed by atmospheric disc refining to defibrillate the material. For larger batches (>100 kg), pilot-scale pressurised disc refining was used exclusively.

Effective pulping is critical for producing fibres suitable for paper and packaging applications. The process typically involves chemical, mechanical, or thermo-mechanical methods. Initial willow samples from Claremorris (supplied by University of Limerick) were too small for the continuous pressurised refiner (minimum input >80 kg), necessitating batch steam explosion followed by atmospheric disc refining.

9.10 Steam Explosion Process

Steam explosion, a batch pre-treatment method, involves placing biomass in a pressure-rated digestor and injecting steam (2–12 bar). The sample is held at pressure to initiate hemicellulose hydrolysis and then rapidly depressurized. This explosive decompression disrupts the biomass structure, improving accessibility for subsequent processing. The 60 L digestor at Bangor University (see Figure 25) is part of the continuous pressurised system but can operate independently for batch processing. Post-treatment, the biomass darkens due to partial degradation of hemicellulose and lignin.

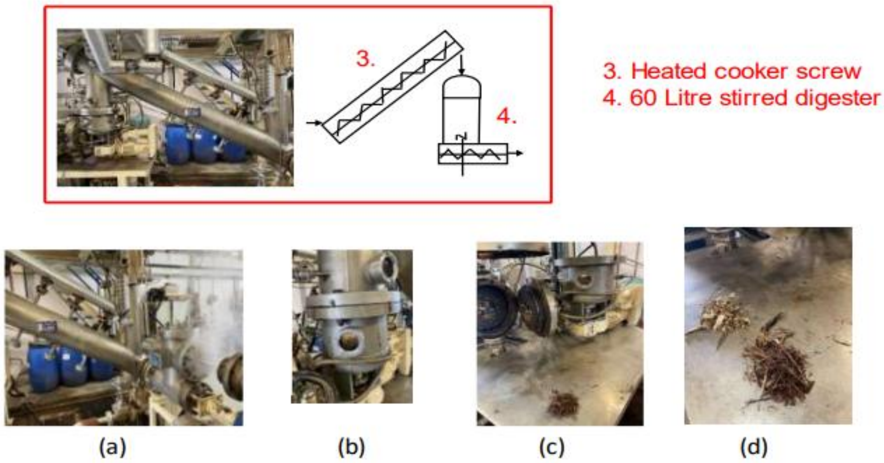


Fig 25. Batch steam explosion equipment at Bangor University

(a)–(d): Equipment and appearance of treated material.

Atmospheric Disc Refining

Following steam explosion, mechanical disc refining was used to pulp the willow. Disc refining, standard in the pulp and paper industry, enhances fibre surface area, inter-fibre bonding, and structural uniformity by external fibrillation, internal delamination, and shearing. This results in ribbon-like fibre shapes ideal for forming strong, uniform networks.

The atmospheric disc refiner (Figure 26) consists of:

- A rotating disc plate (motor-driven)
- A stationary disc plate
- Enclosed housing with grooved and barred surfaces

The refining process is influenced by:

- Biomass slurry consistency (solid-to-liquid ratio)
- Refiner plate configuration and gap
- Number of passes through the refiner

Low-consistency slurries are typically fed through an inlet pipe, while high-consistency slurries use a screw feed. Multiple refining passes further break down the biomass matrix, enhancing fibre quality.

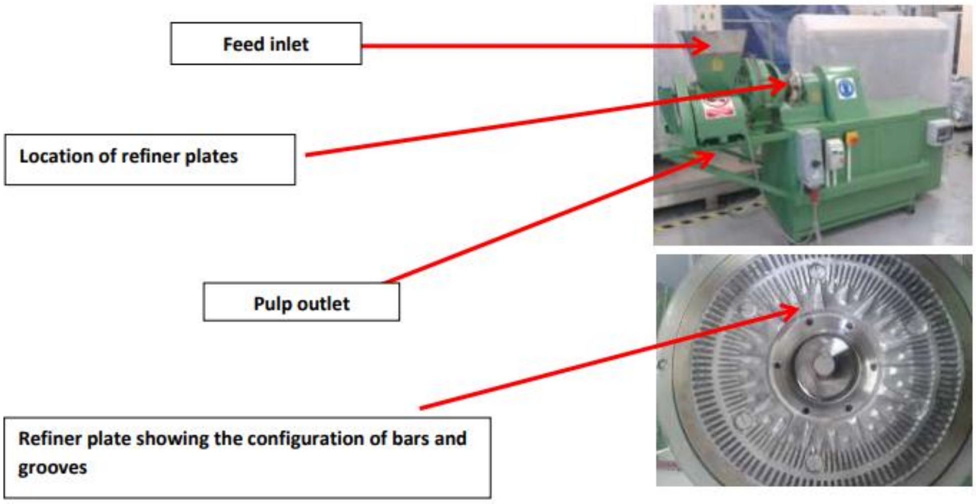


Fig 26 - Atmospheric disc refining equipment at Bangor University.

Processed willow fibres were subsequently used to produce moulded pulp items such as trays and pots via thermoforming, as detailed in Task 3 deliverables.

Processing and Fibre Characterisation of Willow Samples

In the early phase of the project (2021), small batches (<10 kg) of willow harvested from the Claremorris trial site in Ireland and supplied by the University of Limerick were processed at Bangor University using a combination of batch steam explosion and atmospheric disc refining, as previously described.

As the project progressed, larger batches (~200 kg) of willow were harvested from the Loughgall trial site in Northern Ireland and supplied by AFBI. To accommodate this scale, Bangor University utilised its pilot-scale continuous pressurised disc refining equipment (Figure 27).

Fig 27 - Pilot scale (Andritz Sprout-Bauer 12 inch - 30.5cm) continuous pressurised disc refining equipment (Bangor University)

Continuous Pressurised Disc Refining

This equipment—a 12-inch (30.5 cm) Andritz Sprout-Bauer unit—integrates high-pressure steam softening with mechanical refining in a continuous process (Figure 28). Chipped willow biomass is introduced via a feed hopper (1) into a modular screw device (MSD, 2), which compacts the material into a plug. This plug not only facilitates feed transport but also acts as a pressure barrier between atmospheric and elevated pressures within the refining system.

The compacted biomass is transferred into a diagonally mounted steam-injected cooker screw (3), where it is treated at 6–10 bar (~160–180 °C). This thermal pre-treatment initiates hemicellulose hydrolysis, lignin redistribution, and cellulose decrystallisation, increasing fibre surface area and accessibility.

The softened biomass passes into a 60-litre digestor (4), where it is retained under pressure, and is subsequently transferred into the refining zone (5). Here, it is processed between two 30 cm diameter refiner plates—one rotating at up to 2500 rpm and the other stationary (Figure 28). The biomass is sheared and compressed as it moves through a series of grooves and bars on the plates, generating refined fibres.

Key process parameters include:

- Refiner plate gap and separation
- Steam pressure and temperature

- Cooker screw speed (affecting residence time)

Refined fibre can be discharged either directly (wet) or via a 1.5 m blowline (7) into a 100 m flash dryer (8) operating at up to 300 °C. Dried fibres (typically 15–20% moisture) are collected using a cyclone (9), then bagged for dispatch to project partners.

Fig 28 - Refiner plates located inside the refining zone (Note: left plate rotates when operating; right plate is stationary; arrows indicate direction of travel of the biomass through the plates once it exits the digestor

Initial Willow Samples and Pre-treatment Trials

The first willow samples received in July 2021 included:

- “Raw willow” (whole chipped rods including bark)
- “Solvent-extracted willow” (fine-chipped to increase extraction surface area)

Both originated from a mix of *Salix purpurea*, ‘Terra Nova’, and ‘Endurance’ varieties.

Moisture content and compositional analysis were performed to determine cellulose, hemicellulose, lignin, and ash content. These samples underwent a series of batch steam explosion and disc refining experiments.

Raw Willow

A 1.14 kg (dry equivalent) sample was treated in the 60-litre digestor:

- Steam at 2 bar for 30 minutes (extended to 1 hour)
- Increased to 4 bar for 30 minutes
- Yielded 2.657 kg wet material (moisture ~152.8%)

The material darkened significantly post-treatment but remained too coarse for direct disc refining. A hammer milling step was introduced to reduce particle size prior to further processing.

Following this, 1 kg of hammer-milled raw willow was steam exploded (2 bar, 1 hour; 4 bar, 30 minutes), yielding 778 g dry equivalent material (Figure 29), which was frozen for subsequent use.

Fig 29 - Hammer milling (a) to reduce particle size of the 'raw willow' (b) compared to the starting material (c)

Solvent-Extracted Willow

Due to its finer particle size, this sample required no milling. A 1 kg batch was steam exploded under identical conditions and yielded 1194 g (moisture ~150%).

Both pre-treated samples were then pulped using atmospheric disc refining:

- Raw willow: 3 passes (15 µm, then 2x at 10 µm plate gap)
- Solvent-extracted willow: Required a wider initial gap (21 µm) to break up fibres, followed by 2x at 10 µm

The more resistant behaviour of the solvent-extracted willow was likely due to hornification during prior drying. Samples (1 kg each) of untreated and steam-exploded solvent-extracted willow were sent to University College Cork for anaerobic digestion studies.

Fibre

Initial fibre analysis showed willow chips from Claremorris had higher cellulose content than other hardwood and softwood samples (Baker et al., 2017).

To assess treatment effects, compositional analysis was performed on milled willow, solvent-extracted willow, and steam-treated willow (Table 9).

Key findings included:

- Solvent extraction significantly increased lignin content
- Steam treatment significantly reduced hemicellulose, while increasing lignin and ash content

Table 9. Fibre Composition of Debarked Willow Chips (2021)

Sample	Non-fibre (%)	Hemicellulose (%)	Cellulose (%)	Lignin (%)	Ash (%)
Milled willow pulp	23.47 ± 1.12	18.15 ± 0.71	43.83 ± 1.65	12.92 ± 0.19	1.62 ± 0.06
Solvent-extracted	16.18 ± 0.15	15.93 ± 0.10	51.31 ± 0.05	14.75 ± 0.20	1.83 ± 0.12
Steam-treated	19.51 ± 5.51	7.04 ± 1.05	38.05 ± 4.24	31.90 ± 0.25	3.49 ± 0.18

Further steam treatment experiments were conducted on a 200 kg batch of un-debarked willow chips (September 2022). Samples were steam-treated under increasing pressure and packed into 2.2 kg substrate bags for mushroom cultivation trials.

Results demonstrated a clear decrease in hemicellulose with increasing pressure and time, while lignin content increased significantly, particularly under prolonged 4-bar treatments (Table 10).

Table 10. Fibre Composition of Willow Chips (2022) Under Varying Steam Treatments

Treatment	**Non-fibre (%)**	**Hemicellulose (%)**	**Cellulose (%)**	**Lignin (%)**	**Ash (%)**
Milled willow	10.6 ± 1.4	17.9 ± 0.2	53.4 ± 0.9	16.5 ± 0.3	1.6 ± 0.2
2 bar – 1h	12.2 ± 0.2	16.1 ± 0.1	50.2 ± 0.2	19.7 ± 0.5	1.7 ± 0.3
2 bar 0.5h → 4 bar 0.5h	15.7 ± 0.4	15.2 ± 0.6	50.3 ± 0.5	17.4 ± 1.6	1.4 ± 0.2
4 bar – 1h	12.7 ± 0.1	9.3 ± 0.4	53.1 ± 0.4	23.5 ± 0.1	1.2 ± 0.4

These findings highlight the potential to tune fibre composition through steam pre-treatment, enabling the production of fibres suitable for various bioproduct applications such as moulded packaging and bio-based substrates.

Willow Harvesting, Processing, and Analysis – February 2022

In February 2022, material was harvested from the AFBI willow trial plot located at Loughgall, Northern Ireland. The focus was on seven willow genotypes known for producing bark with elevated salicin levels.

Following harvest, the rods were manually debarked at AFBI (Figure 30). The bark was sent to the University of Limerick for salicin extraction experiments, while the debarked rods (Figure 31) were dispatched to Bangor University for downstream processing. Table 11 provides details on the harvested varieties, including the quantity of bark and rods obtained from each.

Fig 30. (Up) Willow trial plot at AFBI Loughgall; (left) debarking process; (right) debarked rods prepared for dispatch to Bangor University.

The willow rods supplied (~36 kg) had a high moisture content (ranging from 68% to 108%). To prevent material degradation during storage, the rods were chopped and then dried.

Processing at Bangor University

The rods were chopped using a pilot-scale forage chopper set to a 1" cutting length. The chopped material was then dried using a 100-meter-long flash dryer connected to a refiner. Material was fed into the dryer via a transfer fan and collected through a cyclone. The flash dryer operated at an inlet temperature of 200°C and an average outlet temperature of 134°C.

Fig 31. Chopping and drying of harvested willow rods prior to storage and downstream processing.

After the initial drying pass, moisture levels remained too high, necessitating a second drying cycle to bring the material down to ~40% moisture content.

Variety	Mass wood (kg)	Moisture content of the wood (%)	Mass - After chopping (kg)	Mass - after 1st dry (kg)	Moisture content - after 1st dry (%)	Mass - after 2nd dry (kg)	Moisture content - after 2nd dry (%)	Mass dry wood (kg)
LA2001476	6.28	108.2	6.16	5.98	72.1	5.52	45.6	3.0
LA980280	4.32	122.0	4.22	4.02	51.0	3.64	39.5	2.2
Endeavour	5.08	108.0	5.02	4.82	61.1	4.40	47.6	2.31
Hambledon	4.44	83.6	4.44	4.40	87.8	3.98	53.8	1.84
LA980348	2.98	118.0	2.98	2.84	62.2	2.58	48.8	1.32
LA9702423	4.58	87.4	4.48	4.22	76.7	3.40	51.6	1.65
LA2001155	3.56	68.5	3.46	3.32	53.9	3.04	46	1.64

Table 11. Mass balance and moisture content of willow batches processed at Bangor University

To support life cycle assessment (LCA) efforts coordinated by Materia Nova (Belgium), energy usage data for chopping and flash drying was collected. Bangor University's pilot-scale equipment is fitted with OWL USB energy monitors and gas/water meters. Electricity consumption was logged in real-time and later downloaded for analysis. Gas usage for the steam boiler was measured with a dedicated flow meter, while water usage was monitored through a facility inlet meter.

Limitations in Energy Data Collection:

- Each batch was small (3.0–6.3 kg) and processed rapidly (chopping: <45 seconds; drying: <90 seconds).
- OWL energy monitors collect data every 60 seconds, limiting batch-specific resolution.
- Data from the forage chopper was lost; average energy usage from prior wheat straw trials was used as a proxy.

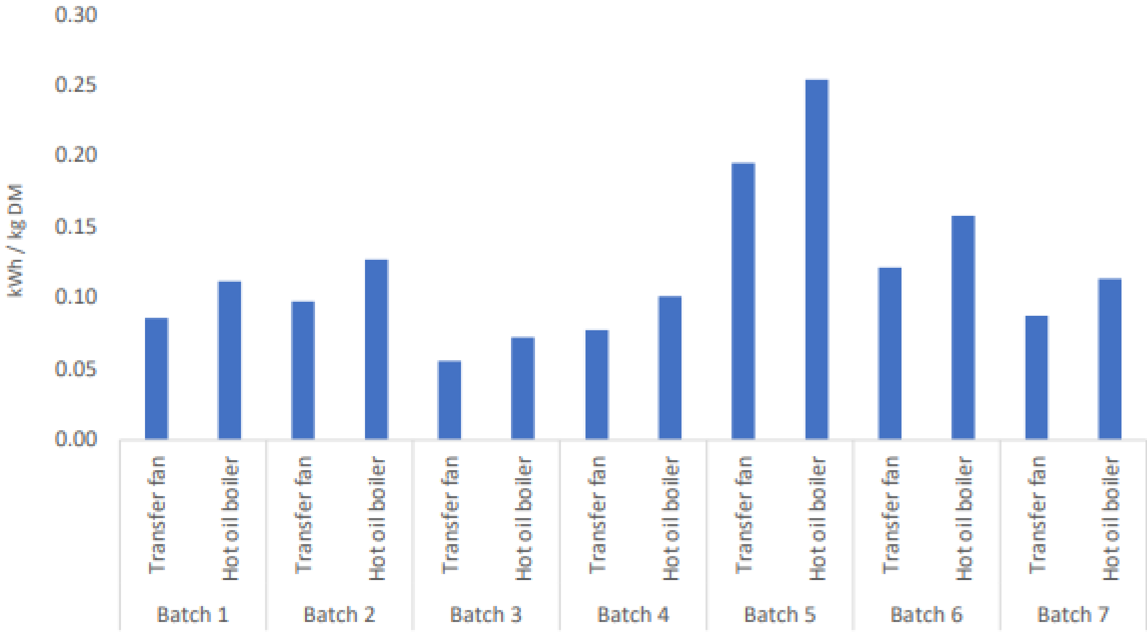


Fig 32. Energy usage per batch during chopping and first-pass drying. (Drying round 1 only)

Manual feed rates significantly impacted energy consumption. Operators experience improved efficiency in batches 3 and 4 but resulted in jamming, leading to slower processing in batches 5 and 6. Batch 7 is considered the most representative of sustainable operational throughput.

Table 12. Estimated energy consumption per dry kilogram:

Process Step	Energy Use (kWh/kg DM*)
Forage chopping	0.033
Flash drying (1st)	0.201
Flash drying (2nd)	0.201
Total	0.434

*DM = Dry Matter

While this energy data was shared with Materia Nova, AFBI later indicated that future larger-scale processing would involve pre-chipped material. This would be immediately processed via continuous pressurized disc refining at Bangor, followed by flash drying to ~20% moisture.

Steam Explosion and Supply to UCC

Two batches (LA980348 and LA2001155) totalling 2.96 kg (dry weight) were subjected to steam explosion (6 bar, 15 min). The material was then suspended in water and passed three times through an atmospheric disc refiner (plate gaps: 15 µm for the first pass, 10 µm for the next two). The resulting wet, refined biomass (18.28 kg at 97.3% moisture) was bagged and shipped to University College Cork (UCC) for anaerobic digestion trials. These trials aimed to compare biomethane yields from chemically pre-treated (UCC) and thermomechanical pre-treated (Bangor) willow biomass.

Fibre Composition of Willow Genotypes

Fibre analysis was conducted on pulps and barks from multiple willow genotypes harvested from another trial at Loughgall on 26 March 2022. Notable differences were observed in cellulose and non-fibre content. While hemicellulose, lignin, and ash contents varied less across genotypes, other studies have reported broader variability depending on the analytical methods used.

Pulp generally contained higher levels of cellulose and hemicellulose, whereas bark had higher lignin and ash contents. No direct correlation was found between genotypes with high cellulose in both pulp and bark.

Table 13. Fibre composition of selected willow genotypes (mean ± SD)

Component	Genotype	Non-Fibre (%)	Hemicellulose (%)	Cellulose (%)	Lignin (%)	Ash (%)
Bark	LA970243	56.7 ± 1.6 a	7.1 ± 0.3 a	15.6 ± 0.8 a	15.6 ± 2.1 a	5.8 ± 0.2 a
	LA980280	46.0 ± 2.5 b	9.2 ± 2.2 a	21.9 ± 1.8 bc	18.9 ± 2.2 a	5.2 ± 0.5 b
	LA2001155	55.2 ± 1.0 a	7.0 ± 0.8 a	18.6 ± 0.6 ab	13.4 ± 0.4 a	4.1 ± 0.2 ab
Pulp	Endeavour	13.4 ± 0.1 ab	19.0 ± 0.6 a	54.1 ± 0.9 c	12.7 ± 0.3 a	0.8 ± 0.1 a
	LA980348	19.5 ± 1.1 d	17.9 ± 0.8 a	48.7 ± 0.5 a	12.3 ± 0.8 a	1.5 ± 0.0 a
	LA970243	15.0 ± 0.2 abc	18.0 ± 0.4 a	53.1 ± 0.4 bc	12.5 ± 0.7 a	1.4 ± 0.5 a

Note: Full data table includes additional genotypes (Hambledon, LA2001476, LA2001155).

Among the genotypes, LA970243 consistently ranked in the top three for biomass yield at both the Loughgall and Claremorris sites. Its pulp showed high cellulose content (53.1%) and moderate non-fibre content (15%). The bark, however, had a high non-fibre content (56.7%), resulting in lower relative cellulose, hemicellulose, and lignin levels. Additionally, ash content was lower in pulp (1.1–2%) than in bark (3.9–5.8%).

Pilot-Scale Refining of Willow: Process Overview and Downstream Applications

A larger batch of chopped whole willow rods (280 kg at 30% moisture; equivalent to 196 kg dry matter) was supplied by AFBI for use in pilot-scale pressurised refining trials at Bangor University. The willow, comprising mixed varieties harvested at AFBI Hillsborough in April/May 2022, was chopped to ~2 cm length using a Ny-vra PTO-driven harvester prior to dispatch.

Fig 33. Larger batch of chopped willow supplied by AFBI (September 2022)

A portion of this material was retained for other experiments. For the refining trial, 251 kg (wet weight) of willow was processed as a single batch using the following conditions:

- Pressure: 6 bar
- Retention time: 1.5 minutes
- Plate gap: 15 µm
- Refiner plates: D2-503 (30 cm diameter)

The refined fibre was flash-dried at 200 °C to ~15% moisture, collected, compressed, and bagged. A total of 171.8 kg of dry fibre was recovered from 313 minutes of continuous operation. Energy use for each process component was monitored (see Figure 34 and Table 14) and reported to Materia Nova by Campbell Skinner (Bangor University).

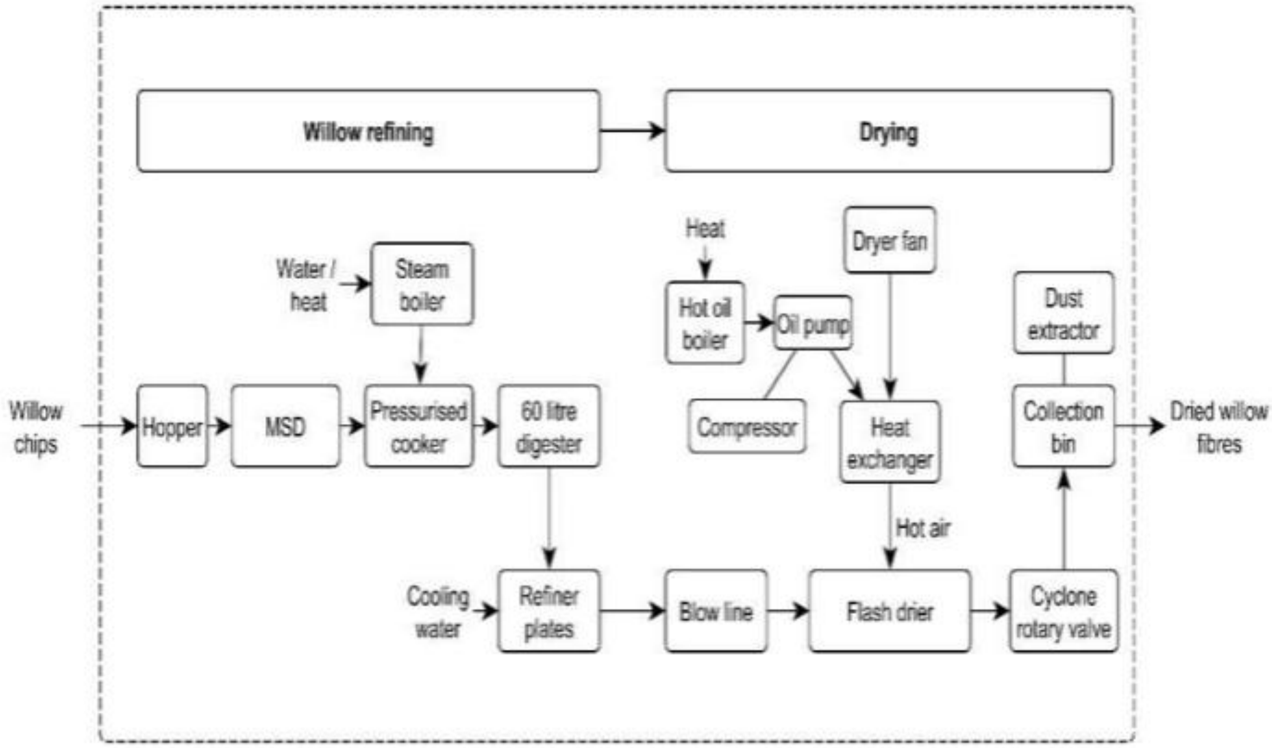


Fig 34. Process schematic – continuous pressurised disc refining of willow

Process Step	Energy/Gas Consumption (per kg DM fibre)
Refiner	0.908 kWh
Steam boiler	0.071 kWh
Compressor	0.164 kWh
Hot oil boiler	0.407 kWh
Hot oil pump	0.118 kWh
Dryer fan	0.470 kWh
Cyclone RV	0.051 kWh
Extractor fan	0.152 kWh
Gas	0.200 m^3

Table 14. Energy/ gas usage for the pressurised refining of the larger batch (Results per kg DM* yield fibre)

Water use during refining was 44.58 L per kg DM fibre, primarily for cooling refiner plate seals and steam generation.

Fibre Characteristics and Fines Analysis

The refined willow fibre (Figure 35) had a high proportion of fines (particles <5 mm), due to:

1. Inclusion of bark, high in lignin, which forms dust during refining
2. Refiner plate type (cutting rather than shearing design)

For future trials, plates were replaced with ones offering greater compressive and shearing action.

A sieving test was conducted on a 3 kg sample (6.8% moisture) using 2 mm and 1 mm mesh screens:

- Mass of sieved fines: 1.92 kg
- Mass of retained fibre: 1.08 kg
- Fines proportion: 64%

Total dry willow refined: 171.79 kg

Refined fibre collected: 167.17 kg

Material loss attributed to:

1. Condensate drain from cooker screw (every 20 min)
2. Dust escaping via dryer cyclone
3. Airborne dust/fibre extracted through LEV system
4. Material retained in the MSD plug during shutdown

Losses associated with (4) are considered scale-dependent and expected to diminish with industrial upscaling.

Fig 35 - Willow fibres produced during pilot scale continuous pressurised disc refining trials

Distribution of Refined Willow for Further Studies

Refined willow fibre was distributed for diverse applications:

- University of Limerick: 110 kg for pilot-scale anaerobic digestion
- University College Cork: 15 kg for lab-scale digestion
- AFBI: 15 kg for pelletisation trials
- Technical University of Lodz (Poland): 12 kg for digestion studies
- Bangor University: Steam explosion trials for mushroom cultivation

Steam Explosion for Mushroom Cultivation

Lentinus edodes (Shiitake) cultivation was tested using steam-exploded willow:

- Steam treatments:
    - 2 bar for 1 h
    - 2 bar for 30 min + 4 bar for 30 min
    - 4 bar for 1 h
- Additional: 5 autoclaved bags (121 °C for 1 h)

All substrates received 150 g wheat bran + 8 g gypsum and were incubated at 22 °C with hourly misting. Biological efficiency (BE) after the first flush averaged 4.5 ± 2.8%.

Shiitake fruiting on steam-exploded willow at 4 bar

Chemical analysis showed:

- Hemicellulose content decreased with increasing pressure
- Lignin content increased inversely
- Mildest treatment (2+4 bar, 30 min each) preserved more hemicellulose than 4 bar for 1 h

Lignin-Degrading Enzyme Production Using T. versicolor

The goal was to produce ligninolytic enzymes (especially manganese peroxidase, MnP) for willow pulp delignification. Microcosms were prepared using wheat bran at varying moisture levels (42.8%–60%) and inoculated with *Trametes versicolor* CM13.

Key findings:

- MnP activity peaked at lowest (42.8%) and highest (60%) moisture levels
- Laccase activity varied less with moisture
- Fibre degradation increased with moisture; lowest moisture favoured soluble compound breakdown

A second experiment using 100 g wheat bran and spore suspension inoculation accelerated colonisation (Figure 36) compared to agar plug inoculation.

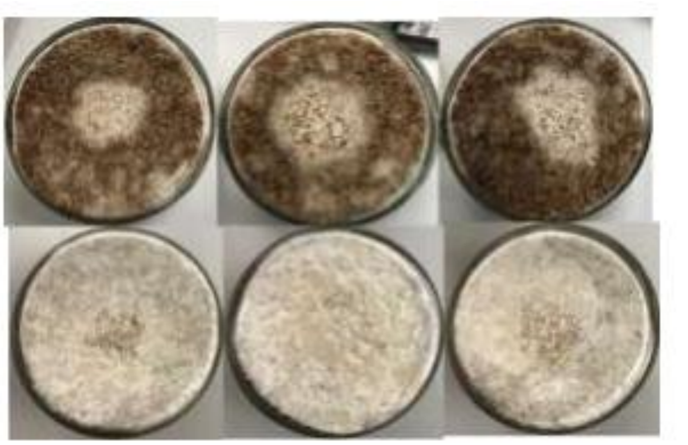


Fig 36. Growth of CM13 on 100 g wheat bran after 1 and 2 weeks

Time-Series Enzyme and Fibre Analysis in Microcosms

Microcosms (20 g wheat bran, 47.4% moisture) were sampled over 37 days:

- pH: declined then rose, tracking fungal growth
- Laccase: peaked on day 13
- MnP: peaked on days 25 and 29
- Lignin content: remained stable or increased despite enzyme activity
- Fibre analysis:
    - Hemicellulose declined
    - Cellulose and ash increased over time

Table 15 – Summary of enzyme activity and fibre composition data.

days	pH	moisture	laccase	manganese peroxidase
7	6.49 ± 0.29 [a]	55.7 ± 12.4 [a]	0 ± 0 [a]	
11	5.54 ± 0.04 [bc]	48.3 ± 0.3 [a]	85 ± 21 [ab]	7 ± 0 [a]
13	5.42 ± 0.06 [bc]	50.3 ± 1.8 [a]	216 ± 21 [bcd]	3 ± 0 [a]
15	5.22 ± 0.03 [c]	52.5 ± 1.4 [a]	290 ± 11 [cd]	0 ± 1 [a]
18	5.74 ± 0.07 [b]	50.3 ± 1.9 [a]	218 ± 41 [bcd]	3 ± 1 [a]
25	6.30 ± 0.01 [a]	55.4 ± 3.3 [a]	265 ± 52 [bcd]	37 ± 33 [a]
29	6.21 ± 0.07 [a]	46.1 ± 9.2 [a]	189 ± 62 [bcd]	33 ± 16 [a]
33	6.38 ± 0.06 [a]	60.6 ± 5.1 [a]	344 ± 40 [d]	27 ± 20 [a]
37	6.33 ± 0.11 [a]	51.5 ± 2.0 [a]	127 ± 92 [abc]	12 ± 13 [a]

NB Values having the same letter are not significant and those with different letters are significantly different at P > 0.5.

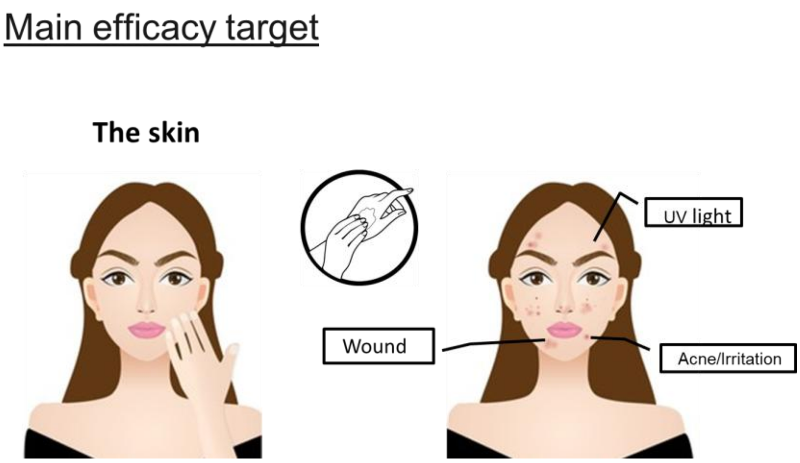
time (days)	non-fibre	hemicellulose	cellulose	lignin	ash
0	62.4 ± 1.5 [ab]	22.6 ± 0.9 [ab]	6.8 ± 0.3 [ab]	3.2 ± 0.2 [a]	5.0 ± 0.0 [a]
7	61.2 ± 1.3 [a]	24.3 ± 0.5 [a]	6.5 ± 1.8 [a]	3.3 ± 0.0 [a]	4.7 ± 0.0 [a]
11	61.2 ± 1.6 [a]	22.0 ± 0.7 [abc]	7.7 ± 0.8 [ab]	4.4 ± 0.1 [abc]	4.7 ± 0.0 a
13	61.2 ± 1.9 [a]	21.5 ± 1.3 [c]	7.7 ± 0.7 [ab]	4.8 ± 0.1 [c]	4.8 ± 0.0 [a]
15	59.8 ± 0.1 [abc]	21.8 ± 0.3 [abc]	8.8 ± 0.2 [abc]	4.3 ± 0.2 [abc]	5.4 ± 0.3 [ab]
18	56.3 ± 2.0 [abc]	21.7 ± 0.2 [abc]	9.7 ± 0.9 [abc]	3.4 ± 1.0 [ab]	5.4 ± 0.4 [ab]
25	56.3 ± 0.6 [abc]	21.4 ± 0.3 [c]	11.2 ±0.4 [c]	4.4 ± 0.1 [abc]	6.7 ± 0.4 [bcd]
29	57.8 ± 0.4 [abc]	21.7 ± 0.9 [abc]	10.1 ± 1.2 [bc]	4.3 ± 0.1 [abc]	6.0 ± 0.6 [abc]
33	55.6 ± 0.6 [abc]	21.2 ± 0.2 [c]	11.1 ± 0.1 [c]	4.6 ± 0.2 [abc]	7.6 ± 0.3 [d]
37	55.4 ± 0.1 [c]	21.3 ± 0.2 [c]	11.5 ± 0.1 [c]	4.7 ± 0.2 [bc]	7.2 ± 0.2 [cd]

NB Values having the same letter are not significant and those with different letters are significantly different at P > 0.5.

Further work increased microcosm size to 50 g and improved homogenisation via blending, which reduced variability and better captured enzymatic and chemical trends.

10.0 Salicin Testing

The liquid arising from the extraction of the willow bark was tested for its bioactivity. As the main objective of the Biowill project was to produce an extract that could be formulated into a phytopharmaceutical topical cream the bioactivity was assessed using skin cells. Following an initial screening selected varieties of willow bark extracts (WBE) were tested for their benefits in human keratinocyte cell line and human dermal fibroblast cell line using the following assays: cytotoxicity, anti-inflammatory activity, wound healing, antioxidant, ultraviolet light protection, and moisture retention. The willow varieties which were tested were S. x dasyclados, Endeavour, Resolution, Cheviot, S. Purpurea, Tora, Endurance, Terranova, as each of these showed were high in overall extractives but showed up to 10% salicin content within the extractives during chemical testing.



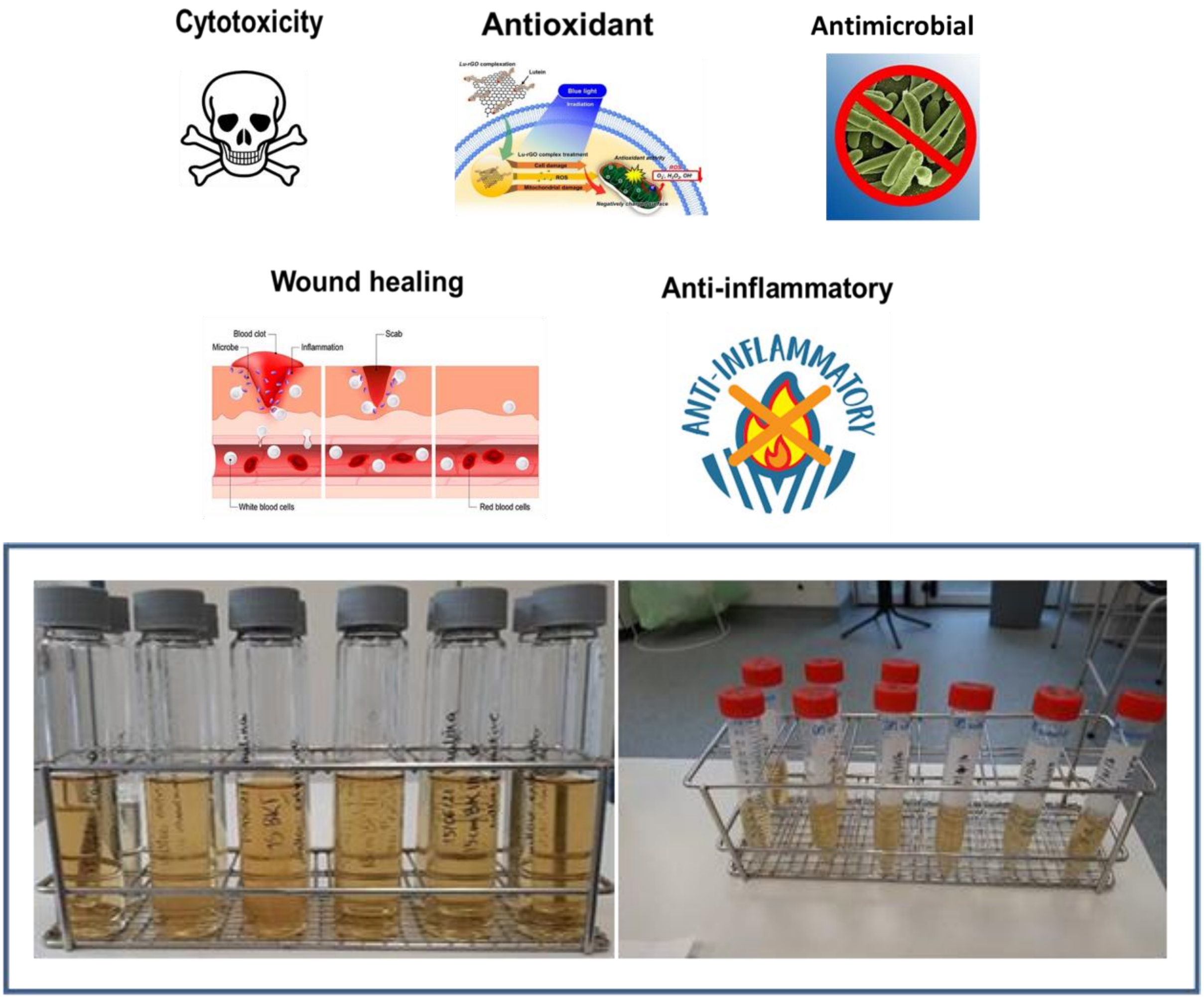


Fig 37. Willow bark extract used for bioactivity tests.

All materials can be cytotoxic if the concentration is sufficiently, so initial testing established an operational range for testing the WBE for anti-inflammatory activity, wound healing, antioxidant indications. This concentration differed depending on the ratio of ethanol to water used during extraction suggesting that ethanol may have a cytotoxic contribution.

10.1 Antioxidant activity

Table 16 shows antioxidant activity of the willow varieties extracted using 80:20 ethanol to water where it can clearly be observed that the antioxidant effect of crude willow extracts demonstrated a significant protective effect against pro-oxidant challenge (hydrogen peroxide) in keratinocytes and fibroblasts cells at various concentrations, when compared against the untreated control ($p< 0.05$).

Willow variety (80:20 EtOH/Water)	**Keratinocytes** Concentration (mg/ml)	**Fibroblasts** Concentration (mg/ml)
S. x dasyclados	0.1	Nil
Endeavour	0.1, 0.01	Nil
Resolution	0.1, 0.01, 0.001, 0.0001	0.001, 0.0001
Cheviot	0.1, 0.01, 0.001, 0.0001	0.001
Tora	0.1, 0.01, 0.001, 0.0001	0.0001
S. Purpurea	0.1,	0.1, 0.01
Endurance	0.1	Nil
Teranova	1, 0.1	1, 0.1
High salicin	0.1, 0.01, 0.001, 0.0001	0.1, 0.01, 0.001, 0.0001
High extractive	0.1, 0.01, 0.001, 0.0001	0.1, 0.01, 0.001, 0.0001

Table 16. The willow varieties extracted using 80:20 ratio of ethanol to water, and their corresponding concentrations which displayed antioxidant activity.

10.2 Anti-inflammatory activity

The bioactivity of crude willow extracts elucidated a significant protective effect against inflammation in keratinocytes and fibroblasts cells at various concentration compared with an untreated control (p< 0.05) (Table 17). Para-Methoxyamphetamine and IL 1-beta were used to initiate an inflammatory response in both keratinocytes and fibroblasts cell lines. This suggest that the crude extracts at their respective effective concentration could offer high value protective effects against inflammation**.**

Willow variety	**Keratinocytes** Concentration (mg/ml)	**Fibroblasts** Concentration (mg/ml)
S. x dasyclados	0.01, 0.001,	0.1, 0.01, 0.001, 0.0001
Endeavour	0.1, 0.01	0.1, 0.01, 0.001, 0.0001
Resolution	0.01, 0.0001	0.1, 0.001, 0.0001
Cheviot	0.01, 0.001, 0.0001	0.1, 0.01, 0.001, 0.0001
Tora	0.01, 0.001, 0.0001	0.1, 0.01, 0.001, 0.0001
High salicin	Nil	0.1, 0.01, 0.001, 0.0001
High extractive	Nil	0.1, 01

Table 17. The willow species and the corresponding concentration which were found effective.

10.3 Wound Healing

Would healing was quantitatively assessed by measuring wound closure following an incision. The results for crude willow extract on wounds on keratinocytes and fibroblast cells found that it had a much more positive healing effect as compared to the untreated control from a concentration range of 0.1 to 0.0001mg/ml after 24 hours. There was a significant healing effect ($p<0.05$) observed for the extracts at a concentration of 0.001 and 0.0001mg/ml with an average of 78% and 87.5% gap closure respectively compared against the positive control (63.5% gap closure). The exposure of extracts to the cells at a concentration range of to 0.0001mg/ml helps in wound recovery as compared to the untreated wound. This illustrates that the extract is capable of promoting wound healing in skin cells and may be of therapeutic value.

10.4 Skin barrier integrity

Skin barrier integrity tests are required by regulatory bodies in various contexts, particularly for studies involving dermal absorption and bioequivalence. These tests are essential to ensure the validity of in vitro skin absorption studies and to comply with guidelines from organizations like the OECD, FDA, EMA, and SCCS. The tests assess the ability of the skin's outermost to function as a barrier, preventing water loss and protecting against external threats. These tests are used to identify if the skin barrier is compromised and to evaluate how it might be affected by various substances or conditions. The effect of an ingredient such as willow bark extracts on skin barrier integrity may be assessed on skin explants or 3D reconstructed skin models (epidermis or full thickness) where the strength and integrity of these barriers is assessed via measurements of the electrical resistance across the cell layer.

Selected WBEs with extractive concentrations of 0.01 and 0.1 mg//mL were tested on a 3D *in vitro* human skin equivalent using real time TEER (transepithelial electrical resistance) impedance measurements to assess skin integrity. The higher concentration of extract (0.1 mg/mL) was observed to be very effective but no additional effect was seen at the lower concentration of extract.

10.5 Photoprotection

The results of testing the extracts for protection of skin cells against ultra-violet radiation showed that there was no significant photoprotective effects from the extracts in keratinocytes.

10.6 Moisture retention

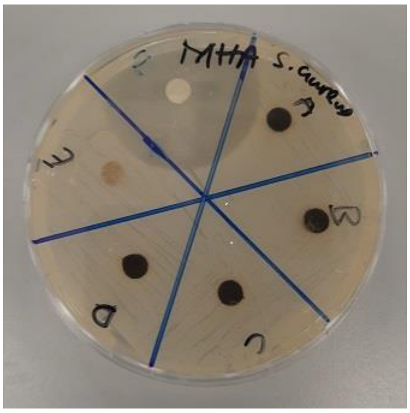
A cream was formulated using Silcoks base and a willow bark extract with high salicin and high extractives (0.1 and 0.01mg/ml) concentrations was tested on a 3D *in vitro* human skin equivalent for moisture retention properties.(Tewitro device https://www.courage-khazaka.de/en/scientific-products/efficacy-tests/in-vitro?view=article&id=159&catid=16). The cream demonstrated high moisture retention, however no significant additional moisture retention was observed beyond that of the Silcox base.

Anti-bacterial activity testing of trays made from processed willow

The extracts were not tested for anti-bacterial activity on skin; however, packaging trays produced from willow bark by partners at Bangor University were tested for activity. This was undertaken to determine if the trays could extend the shelf-life of fruit for example which was packaged using the trays. Two common methods were used for these tests, the disk diffusion method and the broth dilution method using two bacterial strains, the gram-positive bacterium *Staphylococcus aureus* and the gram-negative bacterium *Escherichia coli*, were used for this work.

The diameter of zone of inhibition of different test sample disks was measured to analyse the antibacterial activity.

a. *Staphylococcus aureus*



b. *Escherichia coli*

Fig 38. The disks from processed willow tray (A, B, C, D) and control tray disk (E).

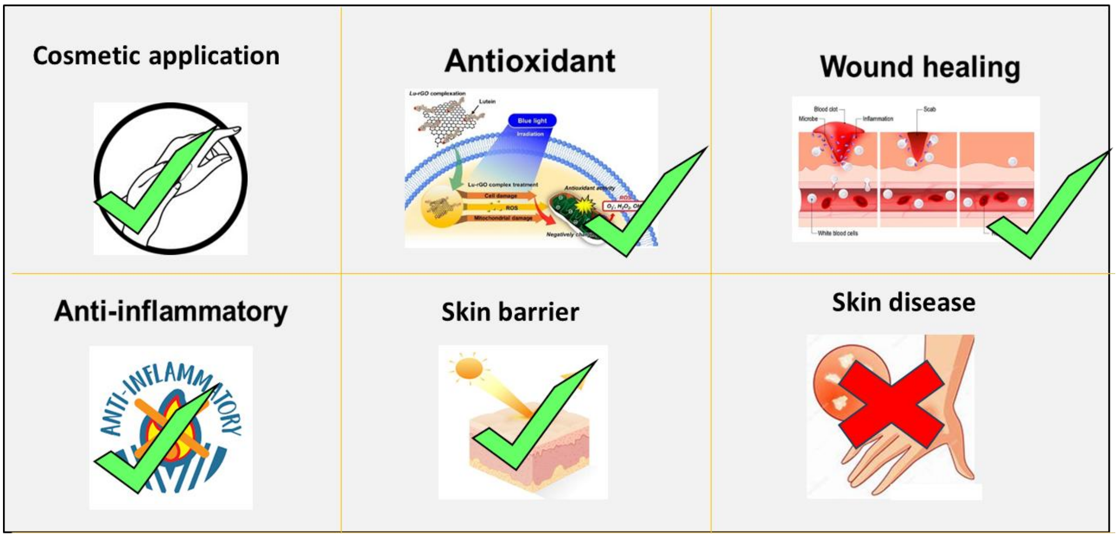
The discs from the willow trays and a control tray did not exhibit antibacterial activity against Gram-positive bacterium *Staphylococcus aureus* in agar plates. Antibiotic control, 1 mg/ml Penicillin-Streptomycin (F) produced a zone of inhibition (diameter 40mm) against the bacterium *Staphylococcus aureus*. b. Processed willow tray disks (A, B, C, D) and control tray disk (E) activity on Gram-negative bacterium *Escherichia coli.* (F) Antibiotic control, 1 mg/ml Penicillin-Streptomycin activity on *Escherichia coli.*

Only the antibiotic control had an antibacterial effect against *Escherichia coli* (a zone of inhibition diameter 20mm).

Similarly, only the antibiotic control had an antibacterial effect on *Escherichia coli* in Mueller-Hinton broth. The willow wood trays did not exhibit antibacterial activity on gram-positive bacterium Staphylococcus aureus or gram-negative Escherichia coli.

10.7 Considerations

This study has provided significant *in vitro* evidence that the willow bark crude extracts from Biowill possess antioxidant, anti-inflammatory and wound repair properties in human skin cell lines representing the dermal and epidermal layers of skin, respectively. Although further work is required to elucidate the detailed mechanisms involved, this report supports the use of will bark extracts isolated from Biowill for skincare applications.



The willow bark crude extracts were found to be non-cytotoxic in the cell cultures tested at a concentration range of 0.1 to 0.0001 mg/ml, and toxic at 1mg/ml. The non-cytotoxic extract concentrations were used in the various bioassays to evaluate their potential benefits. Skincare-relevant bioactivities were detected from both crude extracts from individual willow varieties across different solvent ratios (ethanol), as well as in crude extract mixtures of varieties e.g. 'high salicin' samples in both epidermal- and dermal-relevant human cell lines. This suggest that the extracts are capable of elucidating various benefits to skin which may hold therapeutic value once incorporated into a skincare cream.

11.0 Overall LCA of willow based zero wate

Acronyms

AC	Acidification
CC	Climate Change
CTUe	Comparative Toxic Unit for ecosystems
CTUh	Comparative Toxic Unit for humans
FE	Freshwater Eutrophication
FU	Functional Unit
FWT	Freshwater Ecotoxicity
GHG	Greenhouse Gas
GWP	Global Warming Potential
HT-c	Human Toxicity, cancer effects
HT-nc	Human Toxicity, non-cancer effects
ILCD	International Reference Life Cycle Data System
IR	Ionizing Radiation
ISO	International Organisation for Standardisation
LCA	Life Cycle Assessment
LCI	Life Cycle Inventory
LCIA	Life Cycle Impact Analysis
LCS	Life Cycle Stage
LU	Land Use
ME	Marine Eutrophication
NMVOC	Non-Methane Volatile Organic Compounds
NREU	Non-Renewable Energy Use
OD	Ozone Depletion
PEF	Product Environmental Footprint
PM	Particulate Matter
POF	Photochemical Ozone Formation
RD	Resource Depletion
REU	Renewable Energy Use
SRC	Short Rotation Coppice
TE	Terrestrial Eutrophication
USP	Unique sell point
WS	Water Scarcity

Units:

ha	Hectare
kg	Kilogram
p	Piece
y	Year

Geographical representativeness acronyms:

{CH}	Switzerland
{EU or RER}	European Union
{GLO}	Entire World
{RoW}	Rest of the World (the geographic zone described by {RoW} may vary depending on the items of the background database)

11.1 Preface

Overall life cycle assessment of willow-based zero waste biorefinery

11.2 Introduction

The Biowill project aimed to design a biorefinery where high value biomolecules, such as salicin extracted from willow bark will be used to produce topical phytopharmaceutical products. For a zero-waste system, the willow pulp and waste bark were processed to form catering or food packing materials. When the packing materials came to the end of their useful life, anaerobic digestion was used to treat these materials to produce bio-methane and the residual AD digestate was intended to be used as a biofertilizer.

The Biowill project selected 31 varieties of willow for planting establishment and growth. The willow species were selected for their properties, notably suitability for cultivation in Northwest Europe, overall biomass yield and bio-actives content (e.g. salicin). The plants were grown on four different sites, one in Northern Ireland (Loughgall), one in Ireland (Claremorris) and two in France (Noreuil and Gouy-Sous-Bellonne). After becoming established the plants were harvested at regular intervals. After harvesting the bark was separated from the willow stem and the bio-actives were subsequently solvent extracted and the extractives were then used in the production of a phytopharmaceutical cream. The de-barked willow wood was used as the primary feedstock for the food packaging products. Initially salicin was selected as a key bioactive molecule to be considered in the phytopharmaceutical product. As the product advanced it was decided to focus on the crude willow extract (a mixture of bioactive molecules, including salicin) as the key active ingredient in the phytopharmaceutical cream.

11.3 Objectives

The goal of the Life Cycle Analysis (LCA) is to provide a quantitative cradle-to-grave environmental analysis for the Biowill biorefinery, where willow cultivation was modelled for a plantation lifetime of 25 years. The LCA study aimed to identify the potential environmental hotspots and identify the optimum harvest cycle (1, 2 or 3 years). As with all LCA analyses there is a requirement to identify the Functional unit (FU) so as the USP of Biowill as extract bioactives with anti-inflammatory activity and formulate the extract in the form of a phytopharmaceutical cream, the FU is expressed as 1 tube of phytopharmaceutical cream but includes the production of the biorefinery co-products: willow packaging, biomethane (electricity production) and digestate (soil conditioner). The LCA study was expanded to include all the environmental burdens within the system being studied. Furthermore, the environmental wastes are the responsibility of the producer, so no burdens are attributed to the recycled product.

11.4 Systems Boundaries

The system includes all biorefinery gate-to-grave environmental life cycles stages for the biorefinery.

The flow chain starts with planting using willow cuttings followed by establishing a willow plantation and the associated operations including harvesting, transport, site termination and debarking over the plantation lifetime (25 years).

Harvested willow stems are debarked, and the bark is used as the feedstock for solvent extraction to yield crude willow extractives which are subsequently used as the active ingredient in the phytopharmaceutical cream. Debarked wood, a by- product produced during the debarking process, is pretreated and pulp moulded to make willow food packaging. Used (waste) packing is collected and treated by AD. The value chain ends with electricity produced from biomethane and treating agricultural land with digestate pellets. The principal stages are summarised in Table 18.

Life Cycle Stage	Short Description of the Processes Included
Cuttings	Willow cuttings are harvested, packed in black plastic, and kept cool until dispatched.
Transport (Cuttings)	The cuttings are transported to the planting site in a refrigerated lorry.
Preliminary Phase	The cuttings are kept in cold store until needed for planting.
Establishment Year	Site is prepared by herbicide application, ploughing, and harrowing. After planting, the site is rolled, and another herbicide treatment is applied.
Cutback	At the end of the establishment year, willow plants are coppiced to initiate multiple stem growth.
Growth	Post-coppicing, herbicide treatment and mowing may be necessary. Plants are left to grow for 1–3 years between harvests.
Rod Harvesting	Willow rods are harvested using a rod harvester and transported to a barking centre.
Debarking	Bark is removed from willow stems.
Drying	Bark and debarked rods are left to dry naturally.
Transport (Rods and Bark)	Bark is baled and transported to the biorefinery. Debarked rods are chipped and transported in 1 m^3 bags.
Site Termination	After 25 years, the plantation is terminated by herbicide application following the final harvest.
Milling	Necessary to optimize extraction.
Solvent Extraction	Bark extracted at 80 °C in a water-ethanol solvent mixture.
Cream Production	Ingredients are blended and gently heated.
Cream Packaging	Cream is packaged in plastic tubes and cardboard boxes, ready for dispatch.
Cream End-of-Life	Empty tube is treated as municipal waste and incinerated.
Steam Explosion and Disc Refining	Debarked wood chips processed into natural fibres.
Pulp Moulding	Fibres mixed with water to form a pulp, moulded using presses, then

	dried.
Boxing for Distribution	Willow packaging products are packed for distribution.
Distribution Transport	Out of scope.
Use	Out of scope.
Waste Management	Collected packing returned to the biorefinery for AD; unrecovered packaging undergoes alternative treatment.
Recovery and Transport to the Biorefinery	Out of scope.
Shredding	Willow packaging is shredded to reduce particle size.
Anaerobic Digestion	Willow packaging broken down by bacteria to produce biogas.
Upgrading and Compression	Biogas purified using chemical absorption (amine scrubbing).
Methanisation	CO_2 removed in upgrading is converted to biomethane using hydrogen via the Sabatier reaction.
Completion Step	Biomethane temperature and pressure adjusted for gas grid injection.
Pressure Reduction	Grid biomethane pressure is reduced for micro gas turbine (100kW) use.
Electricity Low Voltage Production	Low-pressure biomethane is used as fuel to generate electricity.
Digestate Dewatering	A decanter centrifuge reduces the digestate's water content.
Pelletizing	Dewatered digestate solid is dried and formed into pellets.
Transport (Pellets)	Digestate pellets are transported to agricultural sites.
Field Application	Pellets used as soil improver (not fertilizer) and spread on agricultural land.

Table 18 - Summary of process steps for the biorefinery.

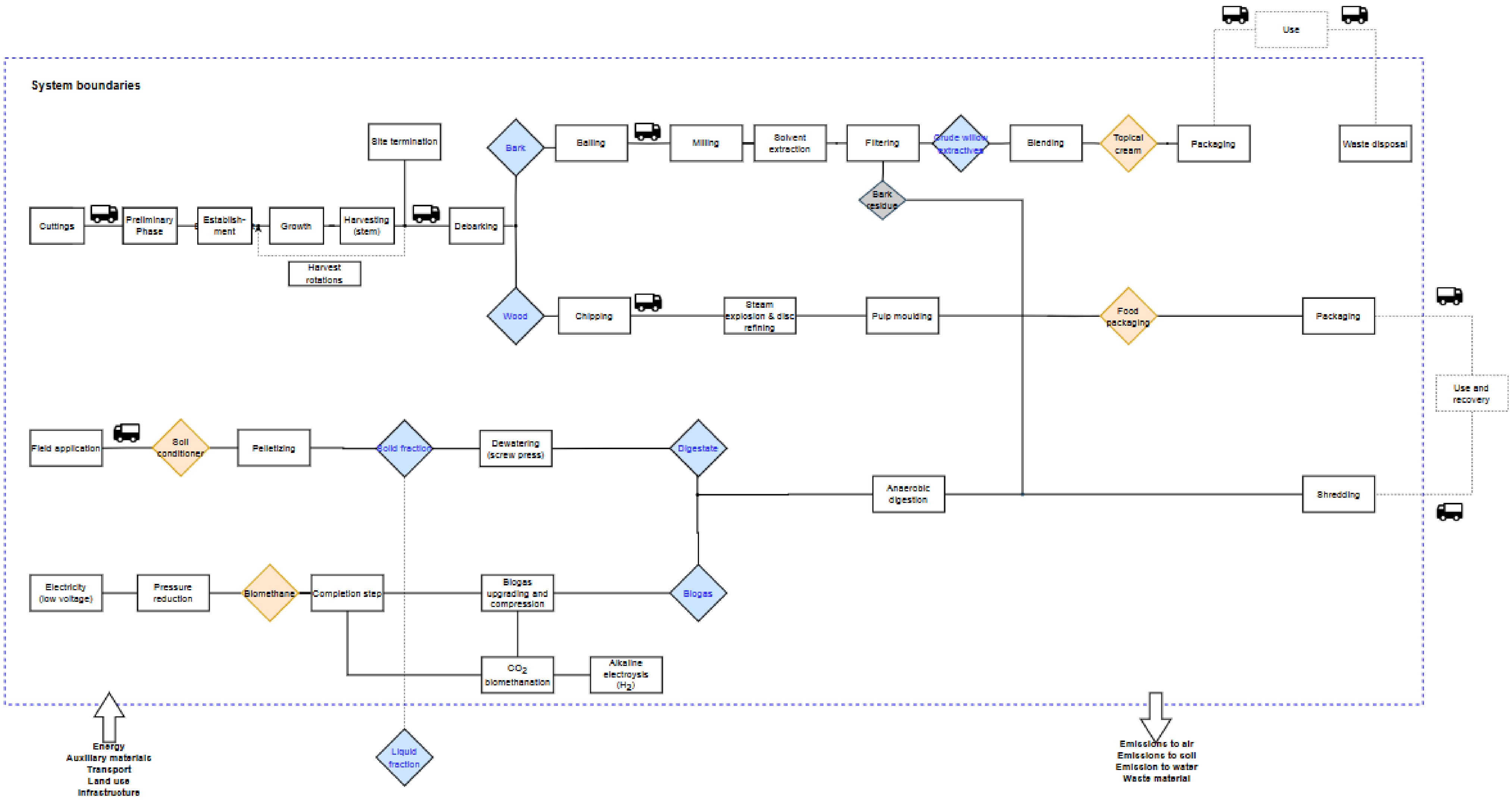


Fig 39 - Life cycle flow diagram for the biorefinery.

11.5 Base scenario

The base scenario assessed is based on the following parameters:

- Loughgall site using the Endurance willow plant with a 1 year harvesting cycle
- Bark is extracted with water and ethanol (80:20) as solvent using 100 L solvent extraction vessels
- the phytopharmaceutical cream is formulated in a 1000 L vessel using 5% crude willow extractives
- 70% packaging recycling rate
- Biogas composition of 40% CH_4 and 60% CO_2
- AD methane slip of 1%

11.6 LCIA methodology and software

This LCA used ISO 14040 and 14044 methodologies and SimaPro 9.5.0.0 Software using the environmental impact categories recommended in the EF3.0 method of the European Commission.

The LCIA methods for each impact category are given in Table 19. The recommended characterisation models and factors are classified according to their quality. There are three quality levels: "I" (recommended and satisfactory), level "II" (recommended but in need of some improvements) or level "III" (recommended, but to be applied with caution).
The study was based on laboratory data and experience from different Biowill project partners. For an upscaled process additional inventory data was taken from experts' data and opinions, literature, notably scientific publications, and information from existing datasets from Ecoinvent database, version 3.9.

Ab.	*Impact category*	*LCIA method*	*Midpoint unit*	*Reference*	*Class.*
CC	Climate Change	Bern model – Global Warming Potentials	kg CO_2 eq.	Intergovernmental Panel on	I
OD	Ozone Depletion	EDIP model based on the ODPs of the World Meteorological	kg CFC^{-11} eq.	WMO, 1999	I
PM	Particulate Matter/Respira	UNEP recommended	Disease	Fantke *et al.*, 2016	I
IR	Ionising Radiation –	Human Health effect	kBq U235 eq. (to	Dreicer *et al.*, 1995 ; Frischknecht *et*	II
POF	Photochemical Ozone	LOTOS-EUROS model	kg NMVOC eq.	Van Zelm *et al.*, 2008 as applied	II
AC	Acidification	Accumulated	mol H^+ eq.	Seppälä *et al.*,2006; Posch *et al.*, 2008	II
FE	Eutrophication –	EUTREND model	kg P eq.	Struijs *et al.*, 2009 as implemented in	II
ME	Eutrophication – marine	EUTREND model	kg N eq.	Struijs *et al.*, 2009 as implemented in	II
TE	Eutrophicatio	Accumulated	mol N eq.	Seppälä *et al.*,	II
WS	Water scarcity	Available WAter REmaining (AWARE)	m^3 water use related	Boulay *et al.*, 2016	III
LU	Land Use	Soil quality index	Dimensionl	Beck *et al.*,	III
RD, e	Resource	CML 2002 – Abiotic	MJ	Guinée *et al.*,	III
RD, m	Resource depletion,	CML 2002 - Abiotic resource depletion,	kg Sb eq.	Guinée *et al.*, 2002 ; van	III
HT, nc	Human Toxicity – non-	USETox 2.1 model	CTUh (Comparative Toxic	Rosenbaum *et al.*, 2008	III
HT, c	Human Toxicity -	USETox 2.1 model	CTUh (Comparative Toxic	Rosenbaum *et al.*, 2008	III
FWT	Ecotoxicity for aquatic	USETox 2.1 model	CTUe (Comparative Toxic	Rosenbaum *et al.*, 2008	III

Table 19 - LCIA methods for each impact category.

In the calculations, a distinction is made between biogenic and fossil CO_2. Biogenic carbon is considered as CO_2 neutral and so CO_2 uptake is not considered.

11.6 Life cycle inventory

Milling

Waste bark is used as a feedstock in anaerobic digestion.

Amount	*Unit*	*Process step*	*Dataset (ecoinvent 3.8)*
0.016	kWh	Milling	*Electricity, medium voltage {RER}|*
0.05	kg	Waste bark	*AD (Deliverable T3.9.4)*
$6.51E^{-3}$	kg	Waste plastic	*Mixed plastics (waste treatment)*

Table 20 - Inventory data for milling 1kg of bark

12.8 Solvent extraction

Waste bark after solvent extraction is treated by anaerobic digestion.

Amount	*Unit*	*Process step*	*Dataset (ecoinvent 3.8)*
4	kg	Water	*Water, deionised {Europe without*
0.789	kg	Ethanol	*Ethanol, without water, in 99.7% solution state, from ethylene {RER}| market for*
0.431	kWh	Heating energy	*Electricity, medium voltage {RER}| market group for | Cut-*
$3.59E^{-4}$	kWh	Stirring energy	*Electricity, medium voltage {RER}| market group for | Cut-*
$8.26E^{-3}$	kWh	Filtering energy	*Electricity, medium voltage {RER}| market group for | Cut-off, U*
0.2072	kg	Crude willow extractives	
0.7928	kg	Waste bark	*AD (Deliverable T3.9.4)*

Table 21 - Inventory data for solvent extraction in a 100L vessel for 1 kg bark (dry mass) in a solvent solution of water and ethanol 80:20 using data for Year 1 Endurance plant from Loughgall.

11.7 Biorefinery material mass flow

The Biowill project determined the yield of crude willow extractives for each willow variety, the different sites and harvest cycles. Using 1 tube of cream as the reference unit, the extractives yield determines the amount of material moving through the bio-fibre chain. This fibre material is then used to make packing products, and biomethane and potentially a soil conditioner using AD.

To assess the whole biorefinery it was necessary to calculate the mass flow for each variety and experimental condition (plantation site, harvest cycles etc). As an example, the mass flow for the base case is given in **Annex 9.1**. A table for the co-product quantities for the difference varieties and scenarios tested are given in **Annex 9.2**.

12.0 Results

Graphs of hotspots and tabulated data (Table 22) are presented for the base scenario for the 5 most significant environmental indicators, i.e. climate change (CC), freshwater eutrophication (FE), fossils resource use (RES, e), minerals and metals resource use (RES, m) and freshwater ecotoxicity (FWT).

12.1 Eco-profile for base case

Impact category	*Unit*	*Base scenario*
Climate change	kg CO_2-eq	2,70E-01
Ozone depletion	kg CFC11 eq	8,63E-09
Particulate matter	disease inc.	9,12E-09
Ionising radiation	kBq U-235 eq	6,89E-02
Photochemical ozone formation	kg NMVOC eq	9,13E-04
Acidification	mol H+ eq	1,23E-03
Eutrophication, freshwater	kg P eq	1,29E-04
Eutrophication, marine	kg N eq	3,06E-04
Eutrophication, terrestrial	mol N eq	2,47E-03
Water use	m^3 depriv.	9,37E-02
Land use	Pt	1,55E+00
Resource use, fossils	MJ	6,00E+00
Resource use, minerals and metals	kg Sb eq	1,57E-06
Human toxicity, non-cancer	CTUh	4,57E-09
Human toxicity, cancer	CTUh	1,36E-10
Ecotoxicity, freshwater	CTUe	1,48E+00

Table 22: Biorefinery gate to grave impact assessment results for 1 tube of cream.

12.2 Hotspots

Analysis of the phytopharmaceutical cream value stream identified the production of extractives and cream formulation as large contributors to the impact factors. The packaging impacts were associated with the pulp moulding process where electricity consumption is high.

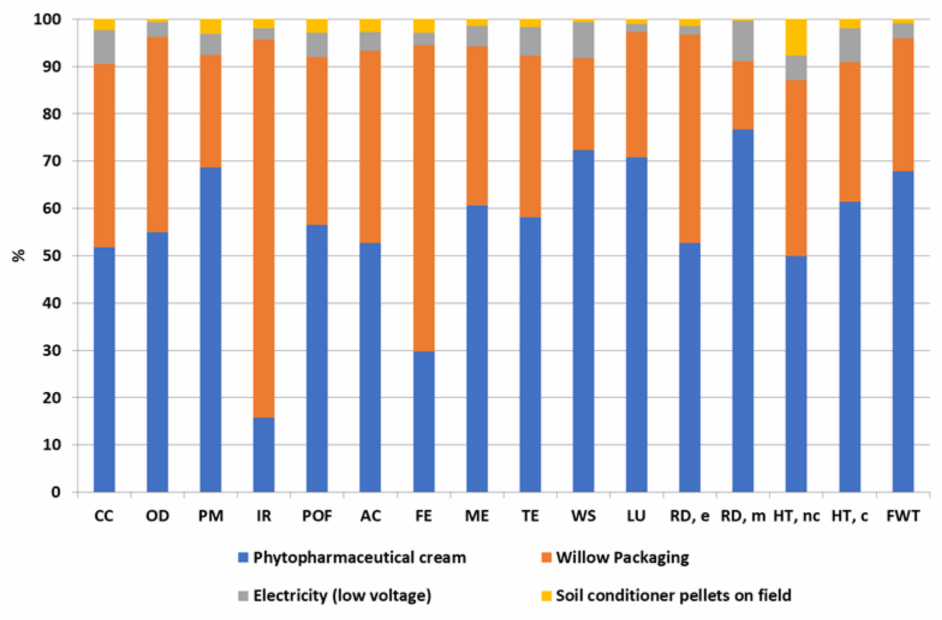


Fig 40. Contribution analysis for biorefinery

The sensitivity of the LCA model was assessed with respect to biomass yield and quantity of extractives recovered is presented in Figure 41. The data suggests that the recovery of high extractive recovery is important to reduce environmental impact significant as the food packaging component of the biorefinery has a high contribution to the impacts. A system with a lower yield of extract means using more willow for the cream and pushing the residue towards the most impacting branch.

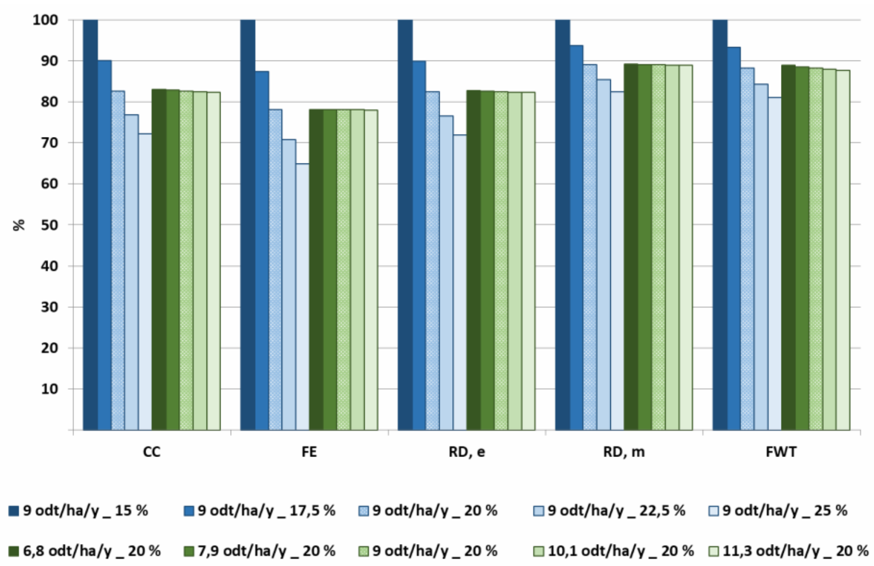


Fig 41 - *Compares impacts for 1 pot of cream with variable the biomass and extractives yields.*

When the impact of harvest cycles are compared, it was observed that a 1-year harvesting is best for the majority of impact categories, this is because less electricity is needed to debark younger rods as they are smaller and lighter, which means a higher debarking rate.

12.3 Optimum Variety and harvest cycle

The five willow varieties with the highest extractive content for a 1- and 2-year harvest cycle shown in Table 22 and base biomass yields were compared for the Loughgall site and clearly showed that extractives content is a key parameter. The lowest environmental impacts were observed for the variety and harvest cycle with the highest extractives content. The sensitivity of the system to extractive content is accentuated due to push of materiel through the bio-fibre chain where the willow packaging process is a high contributor to the overall environmental burden.

Variety Name	*Y1 Average extractives*	*Y1 Extractives SD*
	%	
Cheviot	27,58	2,5
Shrubby Willow	24,78	1,5
Endeavour	24,69	4,1
Resolution	24,36	3,1
LA2001155	24,01	3,2

Variety Name	*Y2 Average extractives*	*Y2 Extractives SD*
	%	
S. Uralensis	35,16	15,9
S. Koriyanagi	29,91	3,9
LA980266	26,27	5,4
S. Miyabeana	25,60	4,2
S. Triandra	25,44	8,2

Table 22 - Loughgall extractives yield from highest to lowest for each harvest cycle.

The LCA also compared two solvent mixtures for recovery of the extracts and found no difference between a water to ethanol ratio of 80:20 or 20:80 which gave poor extractives yield. This suggests that a water to ethanol ratio of 80.20 is preferable.

The effect of not debarking was also assessed as an option whereby willow harvested as chips in the field is sent directly to the biorefinery for extraction. After solvent extraction the residue (bark and wood) can be used as the packaging feedstock. Laboratory tests on bark and willow chips indicated that the extractives are found almost exclusively in bark with negligible amounts in the wood. Measurements also showed that the average willow rod comprises of 20% of bark and 80% wood, which means that 5 times more material is needed for the extraction process using willow chips in comparison to bark suggesting that debarking is advisable as otherwise more material needs to be processed for no increase in extractives.
Assessment of the sensitivity of the LCA to biogas composition found that a higher biomethane to carbon dioxide ratio was marginally better environmentally.

The effect of using biomethane derived electrical power on global environmental impacts rather than natural derived power was assessed and the results are shown in Figure 42.

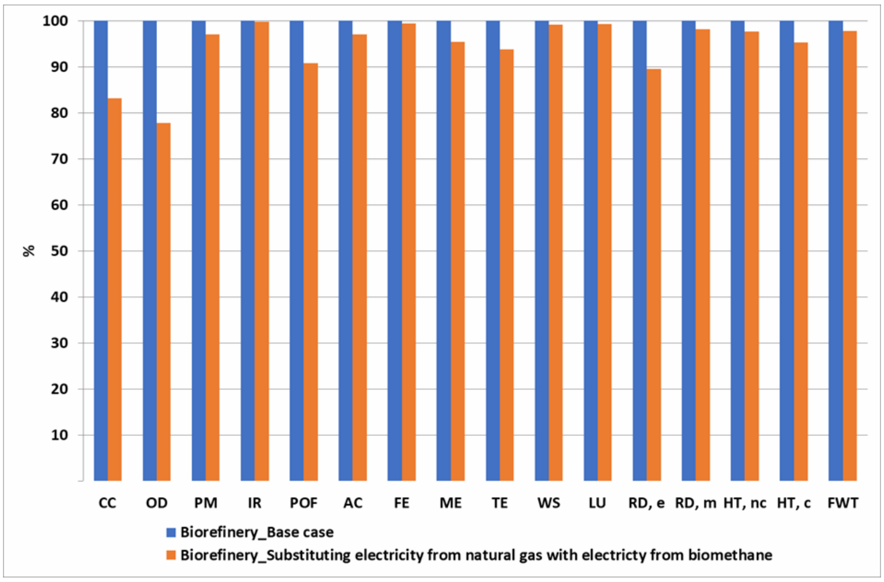


Fig 42 - Effect of the avoidance of electricity derived from biomethane on the global impacts

Avoiding electricity produced from natural gas is beneficial. All of the environmental impact categories are reduced, particularly for climate change and ozone depletion. If the AD unit was local to the biorefinery then it is possible that heat produced during the methanisation process could be used in the biorefinery instead of using heat derived from natural gas. In this case impacts could be reduced for the biorefinery particularly for the impact categories climate change, particulate matter, photochemical ozone depletion, as well as human toxicity (non-cancer).

The potential use of renewable hydrogen was examined, and the contribution analysis (Figure 43) shows a general shift to lower environmental impacts. The impacts are considerable higher for the category's fossil resource use and for ionisation radiation for the general electricity mix. This is likely to be due to electricity originating from fossils fuel and nuclear power sources respectively.

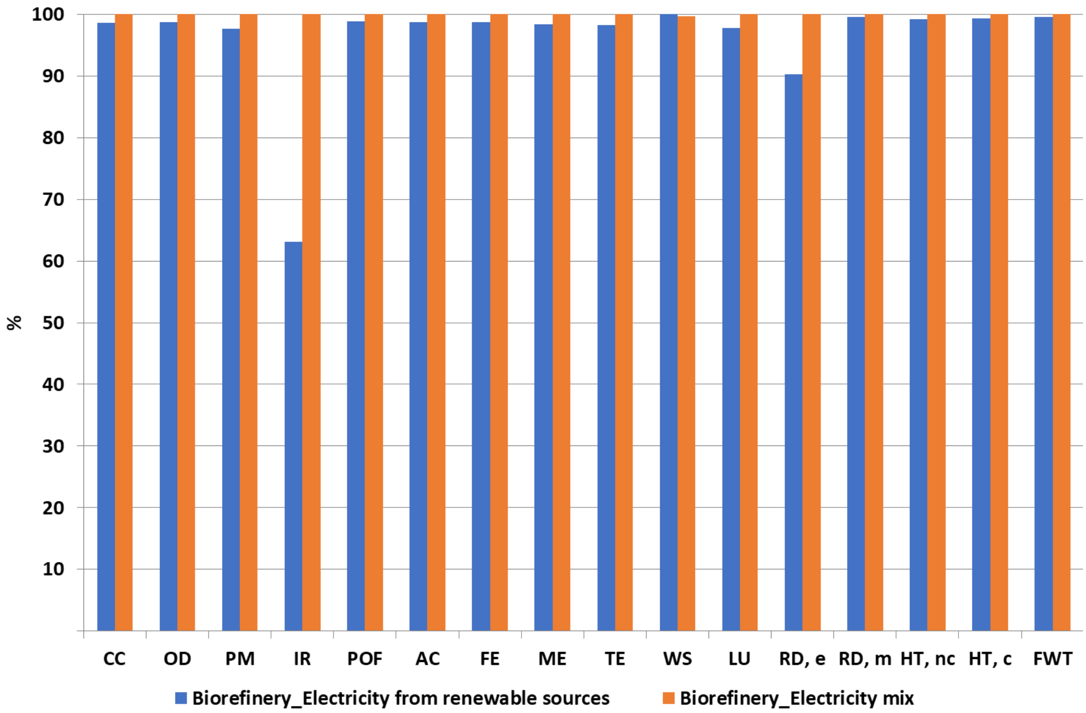


Fig 43 - Sensitivity analysis – type of electricity source for the electrolysis step.

12.4 Limitations of the study

The lack of primary data due to the early status of the biorefinery development is a major limitation to this study, specifically for the absence of the phytopharmaceutical formulations, end use, packaging and distribution, as well as the end function for the willow packaging, waste collection scenario and the downstream processing of biogas and digestate. Primary data was unavailable for these processes. Much of the data used for the model comes from literature and it was necessary to make numerous approximations. It is therefore likely that some elementary flows were omitted because of data gaps in the inventory. Furthermore, there are uncertainties for the end use of digestate pellets as it is not sure it would meet regulatory requirements.

12.5 Pilot Scale Facility

Willows of the genus Salix are a versatile and adaptable species of tree making them an ideal candidate feedstock for a manufacturing facility designed to produce a range of bio-based materials, including pharmaceuticals, bio-composites, fuels and fertiliser.

Valuable substances can be extracted from the bark of the willow tree that are known to be relevant in topical medication. The remainder of the tree plus the residual bark post-extraction provide materials that are of interest in bio-composite manufacturing, fuel and energy production and the generation of fertilisers.

Source: Industrial Crops and Products, Volume 189, 1 December 2022, 115823, A review of Willow (Salix spp.) as an integrated bio-refinery feedstock

12.6 Process Description

A facility designed and built for the processing of harvested willow trees will consist of the following manufacturing and logistics areas:

- Receiving and Drying Store
- De-barking and Chipping Hall
- Pressure Refining Hall
- Extraction Solvent Drum Dispensary
- Bio-refinery
- Extracted Fluid Concentration, Crystallisation and Dispensing Hall
- Formulation Hall
- Fibre Product Packaging Hall
- Warehousing
- Waste Treatment

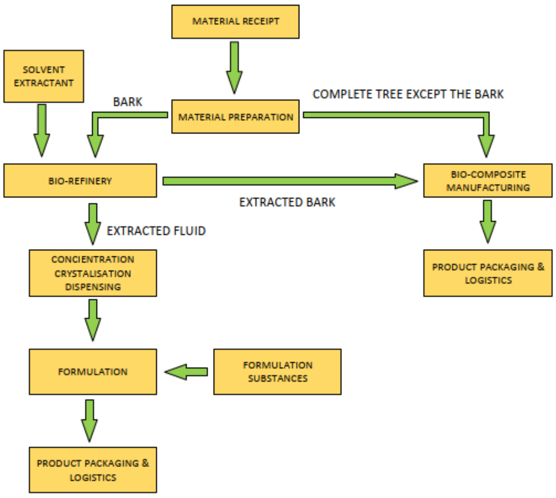


Fig 44 – Process Flow Diagram

Besides the manufacturing areas listed above, the facility will also be designed and built with administration, welfare and operational centres required to provide a fully functioning business unit for the manufacturing, marketing and supply of willow bark extracts, bio-composite materials, fuels and fertilisers.

12.7 Harvest Receipt and Feedstock Preparation

Harvested willow tree will be delivered to the facility as rods. Each willow tree stump will furnish 5-6 rods in a growing season. One hectare (10,000m^2) of utilised land can support approximately 15,000 willow tree stumps with the result that 90,000 willow tree rods can be produced from each hectare of utilised land per growing season.

The mass of rods delivered to the facility per hectare of utilised land will be approximately 20 metric tonnes (20,000 kg).

The rods will be placed in dry storage bunkers within the Receiving and Drying Store upon arrival at the facility. Here they will remain until the moisture content of the rods reduces from approximately 55% to no more than 20%.

When the willow tree rods have dried sufficiently, they will be transferred to the De-barking and Chipping Hall where the rods will first be stripped of bark.

The de-barked rods will be fed into a woodchipper and the wood chip will transfer to the Pressure Refining Hall where bio-composite materials will be manufactured.
The bark stripped from the willow tree rods will be transferred to the Biorefinery.

The mass of bark generated from a 20,000 kg delivery of willow tree is approximately 4,000 kg. The remaining 16,000 kg is wood chip.

12.8 Bio-Refining & Downstream Processing

Bio-refining of the willow tree bark involves the formation of a paste that is then exposed to a solvent composition. The solvent composition is designed to maximise the yield of those substances of interest contained within the bark.

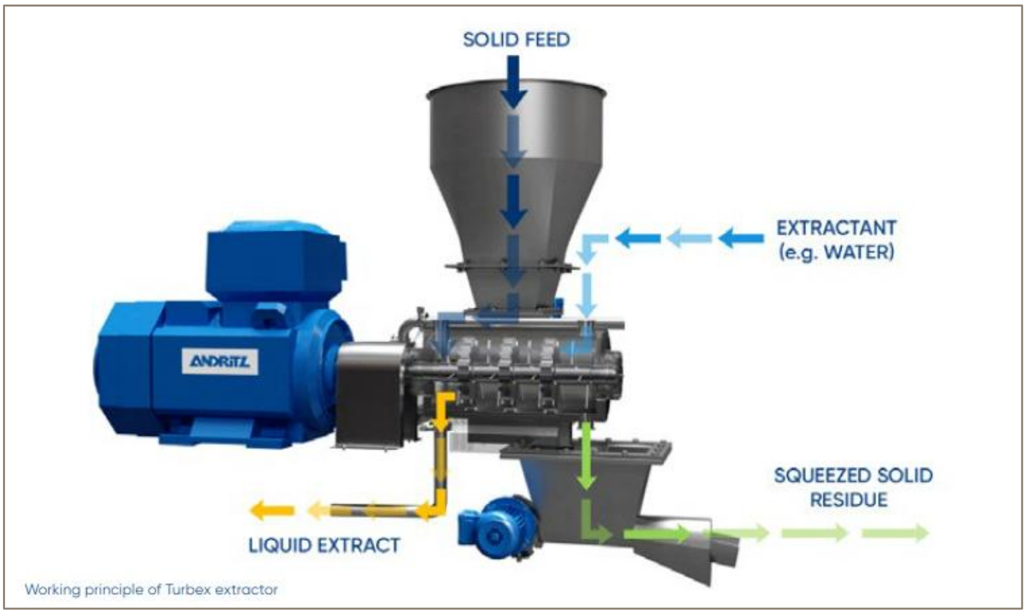


Fig 45 – Working principle of Turbex extractor

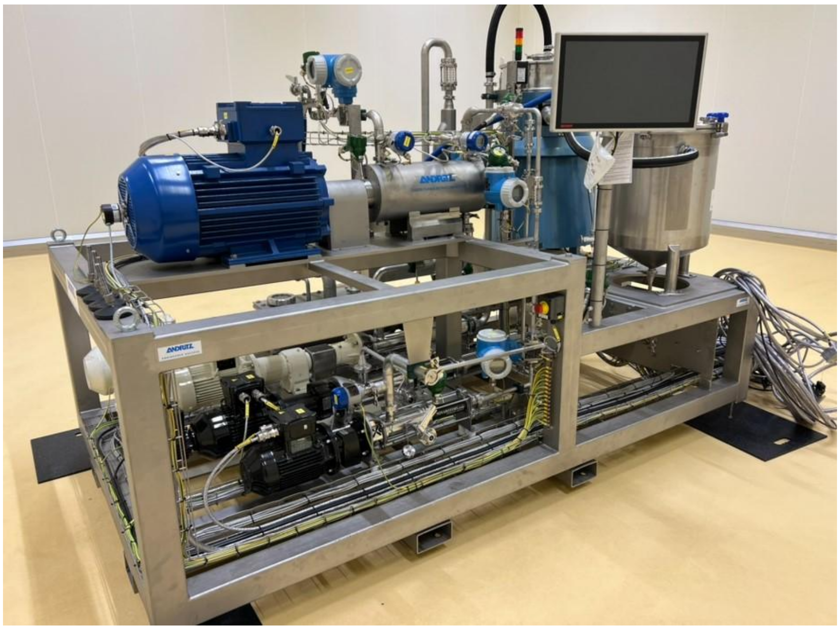


Fig 46 – Photo courtesy of Andritz Group

The extraction process is centred on a solvent extraction unit that facilitates the extraction of those substances of interest from the bark into the extractant fluid (ie: the solvent composition).

The diagram and photograph above are of a Turbex solvent extraction unit from Andritz Group.

Andritz Group is an internationally technology group with headquarters in Graz, Austria.

The Turbex system is a disruptive extraction system designed to perform solid/liquid extraction processes. At the heart of the Turbex system is the extraction chamber. Multiple rotors and stators operate to produce intense turbulence, shearing and cavitation within the chamber which together act on the bark paste.

The bark paste moves counter-currently to the flow of solvent through the extraction chamber. The flow of liquid solvent through the extraction chamber captures substances extracted from the bark that have an affinity for the solvent composition and this liquid extract is discharged from the chamber into storage tanks from where it is sent for downstream processing. The spent bark discharged from the Turbex extraction chamber is likewise collected and it is sent for processing into bio-composites, fuel or fertiliser.

The substances of interest are contained within the liquid extract that discharges from the solvent extraction unit. Downstream processing is required to concentrate, crystallise and dispense the substances of interest so that they can be blended with formulation substances before being packaged for storage and shipment.

The spent bark from the solvent extraction unit is transferred to the Pressure Refining Hall for processing into bio-composite materials, fuel or fertiliser.

To fully develop the manufacturing processes, it is necessary to undertake a period of extensive research using pilot scale versions of the equipment that will be used in the full-scale manufacturing facility. At this stage the process flow has been developed and the equipment necessary to delivery that process flow has been identified. Work is needed to validate both the process flow and the equipment and to determine the optimum process parameters for maximum product yields.

To conduct this work, it is proposed that pilot scale equipment is obtained, either by purchase of the equipment or through a borrow-for-trial programme with the identified providers of the equipment. This would enable the establishment of a pilot scale production facility. It is proposed that the equipment is established at a location associated with the University of Bangor, in Wales.

If it is not possible to obtain equipment that can be installed to the proposed pilot scale facility in Wales, an arrangement can be made with equipment providers to utilise test facilities operated by those providers where test programmes can be undertaken.

A process flow diagram that illustrates the bio-refinery process is shown below. The diagram provides preliminary data that relates the input of solid feedstock to the amount of product material that can be produced using this process.

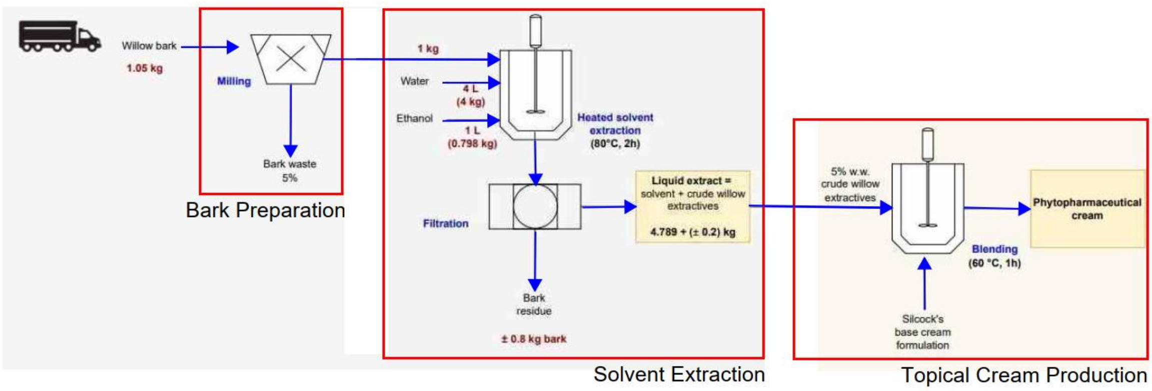


Fig 47 – Detailed Process Flow Diagram

For every 1 kg of bark entering the Solvent Extraction process, the Andritz Turbex extraction unit uses 5 litres of liquid solvent (4 litres water and 1 litre ethanol) to achieve extraction.

4.8 kg of liquid extract is collected because of the extraction process. The extract liquid is comprised of liquid solvent and extractives.

96% (4.6 kg) of the liquid extract collected is solvent and 4% (0.2 kg) is dry extracts.

The willow tree bark contains approximately 20% DB (dry weight) of extractives in the bark samples.

20% of 1 kg of bark would therefore be expected to deliver 0.2kg of extractives.

The liquid solvent to liquid extract ratio for the solid - liquid extraction is 24 to 1. For every 1 kg of dry bark extracted, 4.8 kg of liquid extracts consisting of 96% (4.6 kg) solvent and 4% (0.2 kg) extractives.

12.9 Process Development

Research work to date has focused on the identification of technologies that together will deliver a range of commercial products from willow tree feedstock.

The next step is to build on that initial research work with pilot scale experiments that are designed to optimise each step in the process.

Pilot scale work also serves to prove the process concept, the choice of unit operation and the equipment selection. It is a platform that leads to the detailed design of a fully operational commercial scale manufacturing plant.

A pilot plant continues to be a valuable asset well into the future with respect to the further optimisation of established commercial process as well as the development of new processes, techniques and product lines.

At this stage of the project, a pilot plant serves the design and execution of experiments with the objective of:

- Developing a fast and efficient means of stripping bark from the willow tree feedstock and preparation of the remaining material for supply into the bio-composite, fuel and fertiliser manufacturing process
- Preparing an ideal consistency of bark paste for extraction
- Identifying ideal solvent compositions to maximise substance extraction and yield
- Optimisation of flow, pressure, temperature, and speed of throughput within the Turbex solvent extraction system
- Optimise downstream processing techniques and parameters with the aim of delivering the maximum possible yield of specific substances of interest through crystallisation and purification.
- The development of commercial formulations containing the purified substances extracted from the willow tree feedstock.

It is proposed that a pilot plant facility is developed within a serviced building linked to the University of Bangor in Wales. A facility of this type can be fitted out with pilot scale equipment that is either provided on loan from suppliers/vendors or is purchased outright.

At the heart of this facility will be the Andritz Turbex solvent extraction system. The Andritz Group has offered to supply a pilot scale skid system of the type should in the photograph from earlier in this report.

The Andritz Group has proposed three options regarding the provision of a Tubex pilot scale solvent extraction system:

- 2 days of trial work at the Andritz Group test centre, FIX (Food Innovation Xperience), in Gouda, the Netherlands.

  Cost: €10,500

- Rental of the pilot plant for 1 week plus 1 week transportation to the University of Bangor pilot test centre and 2 days of mandatory instruction at that location.

  Cost: €12,000

- Purchase of the pilot plant.

  Cost: €450,000

The pilot plant will permit up to 200kg per hour of dry feedstock to be processed.

To fully develop the pilot plant with equipment for bark paste production and pilot plant for crystallisation, purification, laboratory testing and liquid solvent preparation and storage, a budget sum of €1,000,000 to €1,500,000 is indicated.

12.10 Commercial Scale Manufacturing Facility

A fully functioning commercial scale manufacturing facility will require not only a manufacturing centre with processing and utility equipment. It will also require office and welfare centres plus warehousing, storage and waste management

Current build cost indexes provide insight in the type of costs to be expected to build types of developments per square meter of floor space. Cost figures relevant to the building of a manufacturing facility are shown below:

- High Specification Manufacturing Development:
  €1,300 - €1,500 per m^2

- Office and Welfare Accommodation:
  €300 - €500 per m^2

- Warehousing:
  €1,000 - €1,400 per m^2

Based on the above figures and indicative process and utility costs and specialised contractual services in the current marketplace, the following table gives an indication of the costs to be expected for the development of a commercial manufacturing facility.

FACILITY COST ELEMENT	COST (€)
Warehousing structure including storage racking fit out: 30m x 20m	840,000
Office and Welfare Accommodation structure including fit out: 40m x 40m (2 floors)	1,600,000
Manufacturing Facility structure and fit out less equipment: 60m x 30m (2 floors)	5,400,000
Waste Treatment Facility including storage and effluent treatment equipment	800,000
Processing Equipment[1]	13,250,000
Utility Equipment	1,500,000
Specialised mechanical and electrical installation	1,500,000
Design & Project Management (10%)	2,849,000

Total indicative cost for a Commercial Manufacturing Facility: €27,379,000

Notes:
1. Processing Equipment includes for:

- Bio-refining Andritz Turbex System: €3,500,000
- Andritz Pressure Disc Refining system: €8,000,000
- Agitated Storage Tanks: €800,000
- Conveyors: €300,000
- Transfer Pumps: €50,000
- Purification chromatography column: €200,000
- Crystallisation system: €150,000
- Charging and Dispensing Equipment: €100,000
- Blending Equipment for formulation: €150,000

The total indicative cost figure of €27,379,000 is representative of a facility with a capacity to handle up to 2,000 metric tonnes of feedstock per annum.

To produce this amount of willow tree as feedstock, 100 hectares of land would be required to grow sufficient material per annum assuming a single harvest of willow tree per hectare of land each year.

This would yield up to 80,000 kg of extractives per annum that can be blended into formulations for commercial sale and 1,920,000 kg per annum of wood chip and spent bark for use in the manufacture of bio-composites, fuel and fertilisers.

13.0 Conclusions

In this study, the environmental impacts of the biorefinery from a cradle to grave perspective was investigated through life cycle assessment.

Based on this model and current knowledge:

- Hotspot analysis of the biorefinery system identifies the topical cream and willow packaging products as important contributors to environmental impacts. For the cream value stream efforts should be made to reduce impacts arising from the cream formulation, debarking energy requirements and the quantity of ethanol per unit of feedstock. The packaging produced from debarked willow is energy intensive the energy usage for the pulp moulding process and needs to be revised.

- Depending on the willow variety and on the harvesting frequency, the overall yield of both biomass and crude extract vary. The extractive yield is a more significant parameter than the global biomass yields partly because the food packaging branch of the biorefinery has a high contribution to the impacts. A lower yield of extract requires using more willow for each tube of cream pushing more material to the more environmentally damaging packaging process. Overall, a high extractives yield is preferential

- From a business perspective the extractives yield for a willow variety could be important. Using Biowill primary data for biomass yield, extractive content and bark 4 promising varieties were identified (LA970253, LA980348, LA970243, LA970562), which were in the top 10 varieties for total extractives yield for both the Loughgall and Claremorris plantation sites and for both harvest cycles (1, 2 year).

- For the same extractives content range, the water to ethanol mixture of 80:20 is recommended.

- It is advisable to include a debarking step even though the impact of debarking is not marginal because skipping this step means using higher volumes of solvent etc for the same yield of extract.

- Using an alternative, biobased source of ethanol has the potential to lower some environmental burdens for specific impact categories such as resource use (fossil, mineral and metal), but it can also shift impacts to other categories such as the land use and water scarcity.

- For the product packaging, it is better to keep packaging materials to a minimum.

- The waste management strategy is an important step in the biofibre value chain. Before the packaging end-of-life is validated, the packing end user, function and collection options needed to be known before the relevance the of using AD can be ascertained.

- The avoidance of electricity from natural gas through the production of biomethane derived electricity reduces environmental impacts for all the impact categories.

- Work carried out by project partner UCC confirms the minimum selling price of biomethane is more than 35.7 c€/kWh; 7.8 times higher than the EU weighted average price using willow as a main feedstock, confirming willow not commercial option for AD feedstock.

- According to current regulations it was not possible to compare Bio WILL digestate products as a fertilizer against mineral fertilizer.

- The pertinence of using AD as the waste management strategy for willow packaging is seen an opportunity and should be considered. Alternative routes such as composting should be investigated.

- Using heat produced as a process by-product (for example carbon dioxide methanisation) is advantageous and reduces the necessity to use heat derived from natural gas.

- Recalcitrant carbon present in willow, and the fraction that remains in willow digestate, could potentially be used to capture and store carbon, which could lower impacts for climate change. Before firm conclusions can be made on this subject further work is needed to determine the carbon stability (> 100y is required) and the digestate/pellet carbon content.

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Appendix 1

Mass flow for the base case

	Mass flow	Units	Value
	FU	tube	1
Extraction + Cream	Tube Cream	ml	75
	Cream density	kg/L	1
	Cream in tube	kg	0,0750
	Extractives in cream	%	5
	Extractives in cream	L	0,0038
	Extractives density	kg/L	1
	Extractives in cream	kg	0,0038
	Extractives yield from feedstock (bark)	%	20,72
	Feedstock for solvent extraction	kg	0,0181
	Bark waste residue	%	79,28
	Bark waste residue	kg	0,0143
	Milling waste	%	5
	Milling waste	kg	0,0010
	Milling feedstock (bark)	kg	0,0191
Food packaging	bark portion	%	20
	wood portion	%	80
	Debarked wood feedstock	kg	0,08
	Fibre yield (steam explosion/refining)	%	97,40
	Fibre yield (steam explosion/refining)	kg	0,07
	Fibre waste	%	2,60
	Fibre waste	kg	0,0020
	Tray yield	%	97,09
	Tray yield	kg	0,0721
	Tray waste	%	2,91
	Tray waste	kg	0,0022
	Tray weight	kg	0,025
	N° trays		2,9
AD	Recycling rate	%	70
	AD feedstock (recycled trays + bark waste from extraction and milling)	kg	0,07
	CH4 emissions from AD methane slip	kg	5,14E-05
	CO2 emissions from AD methane slip	kg	2,13E-04
	AD Digestate	kg DM	3,93E-02
	Digestate moisture content	%	90
	AD Digestate	kg f.w	0,39
	Biogas	kg	2,62E-02
Upgrading	CH4 emissions from upgrading methane slip	kg	5,09E-06
	CO2 emissions from upgrading methane slip	kg	2,11E-05
	Unrecovered biomethane	kg	4,58E-05
	Biomethane from biogas	kg	5,08E-03

	CO2 from biogas	kg	2,10E-02
Methanisation	Biomethane from CO2	kg	7,59E-03
	Water	kg	1,34E-02
Completion	Unrecovered biomethane	kg	2,53E-04
	Biomethane for grid injection	kg	1,24E-02
	Total biomethane for grid injection	m3	0,02
EoL	high pressure CH4 to electricity from low pressure CH4 conversion factor		3,58
	Electricity	kwh	0,0621
Dewatering	Decantering centrifuge solid fraction separation efficiency	%	60
	Solid fraction moisture content	%	70
	Digestate Solid fraction (centrifuge)	kg d.w	2,36E-02
	Digestate Solid fraction (centrifuge)	kg f.w	0,08
	Digestate Liquid fraction fraction (centrifuge)	kg d.w	0,02
	Digestate Liquid fraction fraction (centrifuge)	kg f.w	0,31
Pelletizing	Digestate pellets	%wt	100,0
	Pellet digestate	kg d.w	0,02
EoL	Soil conditioner Application rate	kg/ha	6000
	Field treated	ha	3,93E-06

The AD values were calculated from the AD mass flow given below for 1 kg (d.w.) willow feedstock.

	Mass flow	**Units**	**Value**
AD	CH4 emissions from AD methane slip	kg	7,8237E-04
	CO2 emissions from AD methane slip	kg	3,2453E-03
	AD Digestate	kg	0,5972
	Biogas	kg	0,3987
Upgrading	CH4 emissions from upgrading methane slip	kg	7,7455E-05
	CO2 emissions from upgrading methane slip	kg	3,2129E-04
	Unrecovered biomethane	kg	6,9651E-04
	Biomethane from biogas	kg	7,7261E-02
	CO2 from biogas	kg	0,3194
Methanisation	Biomethane from CO2	kg	0,1155
	Water	kg	0,2039
Completion	Unrecovered biomethane	kg	3,8549E-03
	Biomethane input for completion	kg	0,1927
	Biomethane density	kg/m^3	0,716

Biorefinery products for different scenarios

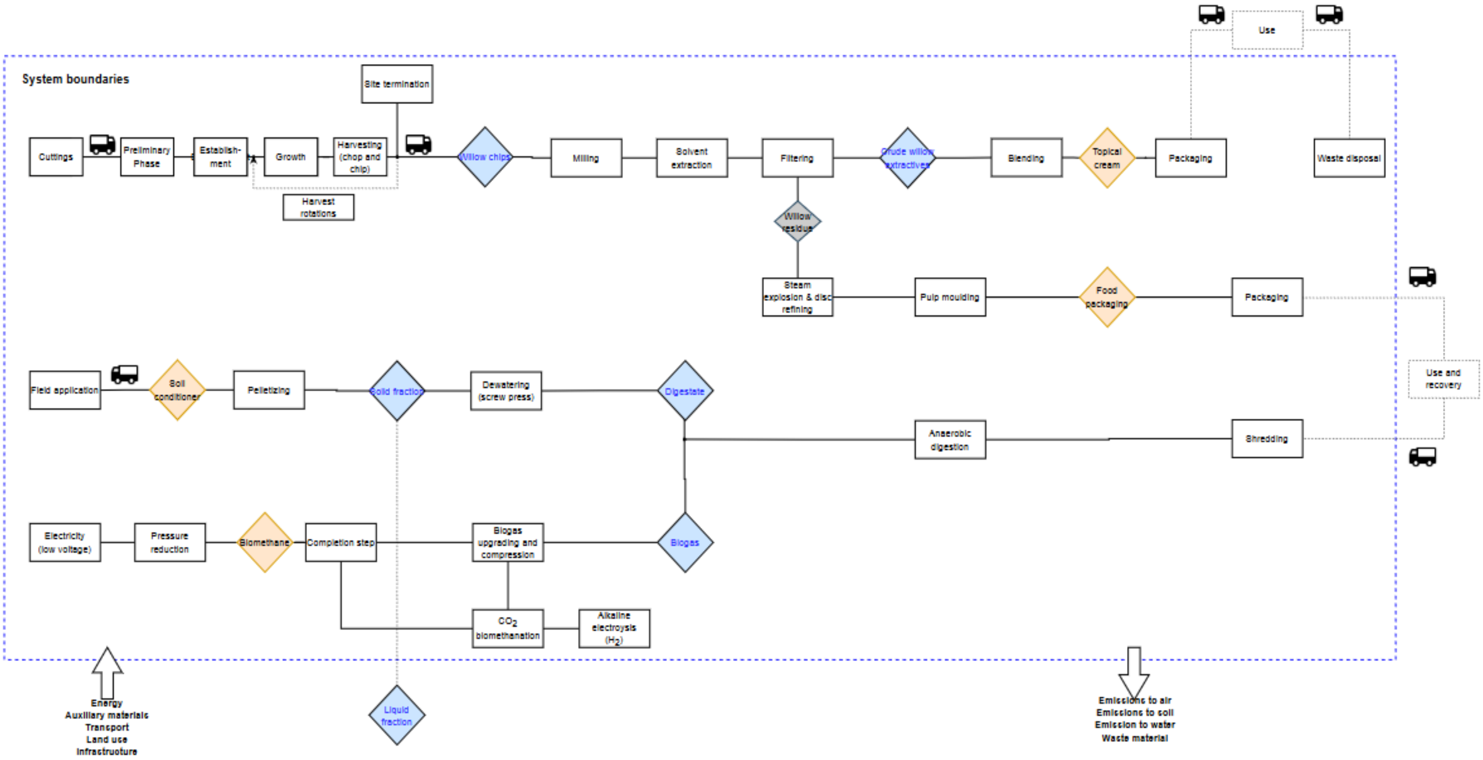
					Y0 harvest					
Biorefinery parameters		**Base scenario**	**Chips**	**Biogas composition 60:40**	**Solvent system 80:20**	**Solvent system 80:20**	**Solvent system 80:20**	**Solvent system 20:80**	**Solvent system 20:80**	**Solvent system 20:80**
Variety		Endurance	Endurance	Endurance	Endurance	Salix Purpurea	Terranova	Endurance	Salix Purpurea	Terranova
Extractives content	(% dry mass)	20,72	4,14	20,72	17,38	22,14	10,29	15,40	25,82	9,65
Site		Loughgall	Loughgall	Loughgall	Loughgall	Loughgall	Loughgall	Loughgall	Loughgall	Loughgall
Harvest cycle	y	1	1	1	1	1	1	1	1	1
Biomass base yield	t DM/ha /y	9	9	9	9	9	9	9	9	9
Number of harvests		24	24	24	24	24	24	24	24	24
Feedstock		Bark	Chips	Bark	Bark	Bark	Bark	Bark	Bark	Bark
Solvent system: water to ethanol		80:20	80:20	80:20	80:20	80:20	80:20	20:80	20:80	20:80
Extractives in cream	%	5	5	5	5	5	5	5	5	5
Packaging recovery rate	%	70	70	70	70	70	70	70	70	70
AD fugitive emissions	%	1	1	1	1	1	1	1	1	1
Biogas composition: CH_4 to CO_2		40:60	40:60	60:40	40:60	40:60	40:60	40:60	40:60	40:60
Biorefinery products										
Tube cream	(p)	1	1	1		1	1	1	1	1
Tray	(kg)	0,0721	0,0863	0,0721	0,0859	0,0674	0,1451	0,0970	0,0578	0,1547
Electricity	(kWh)	0,0621	0,0571	0,0414	0,0747	0,0578	0,1285	0,0847	0,0491	0,1373
Soil conditioner	(ha)	3,93E-06	3,61E-06	5,11E-06	4,72E-06	3,66E-06	8,13E-06	5,36E-06	3,10E-06	8,68E-06

Biorefinery parameters		**Varieties in top five for each harvest cycle**									
Variety		Cheviot	Shrubby Willow	Endeavour	Resolution	LA2001155	S. Uralensis	S. Koriyanagi	LA980266	S. Miyabeana	S. Triandra
Extractives content	(% dry mass)	27,58	24,78	24,69	24,36	24,01	35,16	29,91	26,27	25,60	25,44
Site		Loughgall									
Harvest cycle	y	1	1	1	1	1	2	2	2	2	2
Biomass base yield	t DM/ha /y	9	9	9	9	9	10	10	10	10	10
Number of harvests		24	24	24	24	24	12	12	12	12	12
Feedstock		Bark	Bark	Bark	Bark	Bark	Bark	Bark	Bark	Bark	Bark
Solvent system: water to ethanol		80:20	80:20	80:20	80:20	80:20	80:20	80:20	80:20	80:20	80:20
Extractives in cream	%	5	5	5	5	5	5	5	5	5	5
Packaging recovery rate	%	70	70	70	70	70	70	70	70	70	70
AD fugitive emissions	%	1	1	1	1	1	1	1	1	1	1
Biogas composition: CH_4 to CO_2		40:60	40:60	40:60	40:60	40:60	40:60	40:60	40:60	40:60	40:60
Products											
Tube cream	(p)	1	1	1	1	1	1	1	1	1	1
Tray	(kg)	0,0541	0,0603	0,0605	0,0613	0,0622	0,0425	0,0499	0,0568	0,0583	0,0587
Electricity	(kWh)	0,0457	0,0513	0,0515	0,0522	0,0531	0,0351	0,0419	0,0482	0,0495	0,0499
Soil conditioner	(ha)	2,89E-06	3,24E-06	3,26E-06	3,30E-06	3,36E-06	2,22E-06	2,65E-06	3,05E-06	3,13E-06	3,15E-06

Biorefinery parameters		Varieties in top 10 for both sites and harvest cycles considering total extractives yield							
Variety		LA970253	LA980348	LA970243	LA970562	LA970253	LA980348	LA970243	LA970562
Extractives content	(% dry mass)	23,80	22,33	22,99	23,83	20,98	22,26	23,42	19,26
Site		Loughgall	Loughgall	Loughgall	Loughgall	Loughgall	Loughgall	Loughgall	Loughgall
Harvest cycle	y	1	1	1	1	2	2	2	2
Biomass base yield	t DM/ha /y	9	9	9	9	10	10	10	10
Number of harvests		24	24	24	24	12	12	12	12
Feedstock		Bark	Bark	Bark	Bark	Bark	Bark	Bark	Bark
Solvent system: water to ethanol		80:20	80:20	80:20	80:20	80:20	80:20	80:20	80:20
Extractives in cream	%	5	5	5	5	5	5	5	5
Packaging recovery rate	%	70	70	70	70	70	70	70	70
AD fugitive emissions	%	1	1	1	1	1	1	1	1
Biogas composition: CH_4 to CO_2		40:60	40:60	40:60	40:60	40:60	40:60	40:60	40:60
Products									
Tube cream	(p)	1	1	1	1	1	1	1	1
Tray	(kg)	0,0627	0,0669	0,0649	0,0627	0,0712	0,0671	0,0638	0,0775
Electricity	(kWh)	0,0536	0,0573	0,0556	0,0535	0,0612	0,0575	0,0545	0,0670
Soil cond.	(ha)	3,39E-06	3,62E-06	3,51E-06	3.38E-06	3,87E-06	3,64E-06	3,45E-06	4,24E-06

Biorefinery parameters		Varieties in top 10 for both sites and harvest cycles considering total extractives yield							
Variety		LA970253	LA980348	LA970243	LA970562	LA970253	LA980348	LA970243	LA970562
Extractives content	(% dry mass)	21,30	22,48	22,32	25,25	24,02	32,50	19,31	25,16
Site		Claremorris	Claremorris	Claremorris	Claremorris	Claremorris	Claremorris	Claremorris	Claremorris
Harvest cycle	y	1	1	1	1	2	2	2	2
Biomass base yield	t DM/ha /y	2,5	2,5	2,5	2,5	6	6	6	6
Number of harvests		24	24	24	24	12	12	12	12
Feedstock		Bark	Bark	Bark	Bark	Bark	Bark	Bark	Bark
Solvent system : water to ethanol		80:20	80:20	80:20	80:20	80:20	80:20	80:20	80:20
Extractives in cream	%	5	5	5	5	5	5	5	5
Packaging recovery rate	%	70	70	70	70	70	70	70	70
AD fugitive emissions	%	1	1	1	1	1	1	1	1
Biogas composition: CH_4 to CO_2		40:60	40:60	40:60	40:60	40:60	40:60	40:60	40:60
Products									
Tube cream	(p)	1	1	1	1	1	1	1	1
Tray	(kg)	0,0701	0,0664	0,0669	0,0591	0,0622	0,0459	0,0773	0,0593
Electricity	(kWh)	0,0603	0,0569	0,0573	0,0503	0,0530	0,0383	0,0668	0,0505
Soil cond.	(ha)	3,81E-06	3,60E-06	3,63E-06	3,18E-06	3,35E-06	2,42E-06	4,23E-06	3,19E-06

Flow diagram for biorefinery without debarking step



Appendix 2

Techno Economic Analysis

Techno-Economic Analysis Report BIOWILL

Table of Contents

Introduction

Background on willow utilization.

Currently, willow is grown for bioenergy production due to its rapid growth and ability to thrive in a variety of soil/climatic conditions. Its suitability for short-rotation coppicing (SRC) makes it particularly desirable in sustainable energy systems. Willow short- rotation coppicing is typically managed by harvesting the trees every 2-4 years, but the root system remains intact. This allows the tree to regrow multiple times from the same rootstock over a span of 20 to 30 years. The process ensures a sustainable and consistent supply of biomass with minimal replanting required.

Willow biomass can be converted into solid biofuels, such as wood chips or pellets, which are used in heating systems, or further processed into liquid biofuels like ethanol or SAF. Growing willow for bioenergy provides a relatively poor income for growers as they are usually paid for the heating value of the harvested material which can often have a high moisture content.
Salicins are a naturally occurring chemical compounds found in the bark of willow trees (genus Salix) from which they are easily extracted similar to coffee from ground coffee beans. These molecules constitute a class of compounds which are glycosides of salicylic acid (the active ingredient found in aspirin). Historically, willow extracts were used for their pain-relieving and anti-inflammatory activity, as the body metabolizes ingested salcins into salicylic acid which helps alleviate pain and reduce fever. The bark of the willow tree is typically the main source of salicin. There are more than 2000 varieties of willow and the salicin concentration and chemical profile varies among these. In general, the exact chemical structure of such naturally occurring plant bioactives is determined by both genetic and environmental factors. For this reason, plants of the same species grown under natural conditions can differ in their content of bioactive metabolites. *Salix purpurea*, for example listed as widespread and common in many countries, belongs to the willow class with the highest content of salicylic compounds. Salicin, the best known salicylic metabolite, because of its ability to reduce inflammation and provide pain relief, makes it a key ingredient in herbal remedies and some over-the-counter products designed to manage pain. Salicin has also been trialled in phytopharmaceuticals and other pain management applications due to its natural origin and minimal side effects compared to synthetic aspirin (salicylic acid). It is permitted in topical applications at low concentrations by the European medicines Agency (EMA). “The EMA has assessed willow bark preparations for their "well-established use" in treating lower back pain, indicating that there is sufficient evidence of their effectiveness and safety based on long-standing use.”
Salicins are recovered from willow through a process of extraction which involves: harvesting willow and removing the bark, followed by a thermal, mechanical, or chemical extraction process to isolate the salicin from the bark, often through boiling or soaking in water or alcohol to release the biologically active compounds.

Techno-economic analysis of salicin extraction from willow bark
A survey of the scientific literature found that there are no reported the techno-economic evaluations of the salicin extraction. Reports do exist for willow fibre for use in composite products for the automobile industry.

Scope and objectives of the report.
This report evaluates and compares two scenarios for willow utilization using the well-established Net Present Value (NPV) financial analysis protocol:

1) the base scenario is the current utilization of willow as fuel for heating/bioenergy production,

2) an alternative scenario is when willow rods are harvested, bark is removed, and bioactive compounds are extracted. The remaining residue will be transformed into pulp (pulp moulding) that could be used to produce eco-friendly horticultural pots and food packaging.

Methodology

Net Present Value (NPV)
The calculation of NPV as a profitability indicator was carried out according to:

$$NPV(r,n) = -I + \sum_{t=1}^{n} \left(\frac{CF_t}{(1+r)^t} \right)$$

where:
CF = Net Cash flow at time t (inflows minus outflows)
r = Discount rate (cost of capital or required rate of return)
t = Time period (year, quarter, etc.)
n = Total number of time periods
Net cash flow represents expected future net cash flows: Cash inflows can be revenues, cost savings, or investment returns while Cash outflows include Initial Investments (I, CAPEX) and operating expenses (OPEX).
The value of NPV is the simplicity of interpretation of the result:
NPV > 0** → The project is expected to generate more value than its cost (**profitable**).
NPV = 0** → The project breaks even (**neutral**).
NPV < 0** → The project is expected to lose money (**not viable**).

In this model it was assumed that the capital investment will be paid from a loan. The payment for a loan based on instalment payments and a fixed interest rate was calculated and included in the calculations of NPV.

$$NPV(r,n) = -I + \sum_{t=1}^{n} \left(\frac{CF_t}{(1+r)^t} \right) - \sum_{t=1}^{n} \left(\frac{Loan\ Repayments_t}{(1+r)^t} \right)$$

Internal Rate of Return (IRR)
As an alternative profitability measure, the NPV was set equal to zero to determine the IRR on the investment:

$$NPV(r,n) = -I + \sum_{t=1}^{n} \left(\frac{CF_t}{(1+r)^t} \right) = 0$$

Interpreting IRR:
IRR > Discount Rate** ⟶ The project is attractive and should be considered.
IRR = Discount Rate** ⟶ The project breaks even.

IRR < Discount Rate** → The project is not financially viable.

Payback Period (PP)
The Payback Period was used to determine the amount of time required for an investment to recover its initial cost from the cash inflows it generates. PP was calculated according following formulae:

$$PP = \frac{\text{Initial Investment}}{\text{Annual Cash Inflows}}$$

Annual Cash Inflows refers to the cash generated from the investment each year; it can come from revenues, cost savings, or other financial benefits. Initial Investment includes capital expenditures and other startup costs.

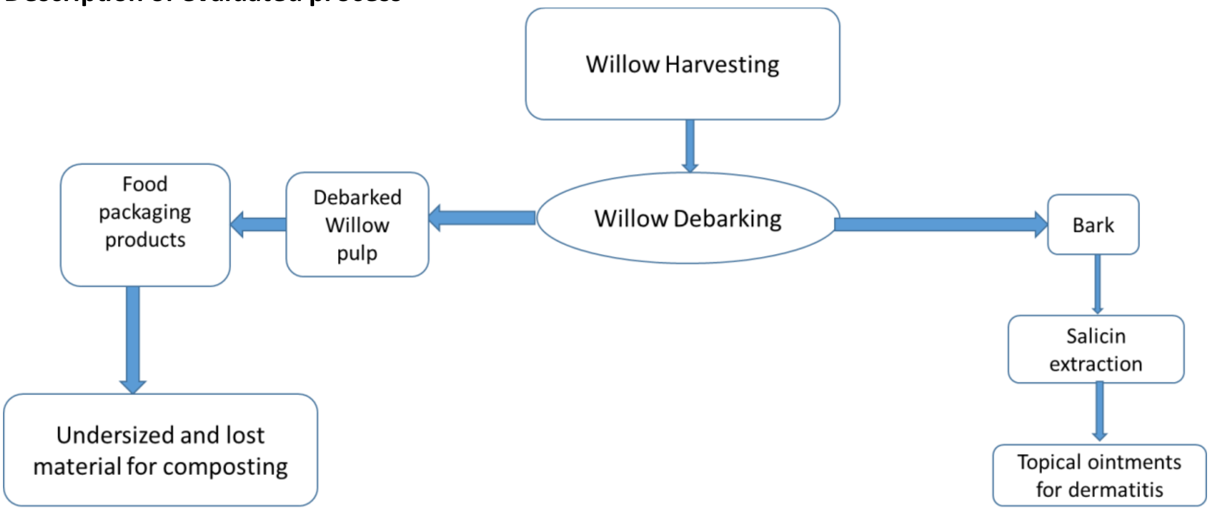
Sensitivity analysis
The objective of the sensitivity analysis was to analyze the influence of the parameters that could have the most significant effect the project viability based on the NPV variation. We tested the sensitivity of the profitability for harvesting and price of willow bark extract.
The reference value of each parameter was used to initially obtain the NPV and then a variation of between 40% and 140 % of the reference value was used to assess the impact of these factors on the NPV. To draw more representative conclusions, the selected parameters were varied individually.

Data sources and assumptions: The analysis was based both on data directly from the Biowill project but in some cases in was necessary to benchmark against published information in the scientific literature.

Technology Description

The analysis is based on the extraction of a cocktail of bioactives from willow bark which will be referred to "willow bark extract" (WBE) rather than the purified single component salicin.

Description of evaluated process



The process evaluated includes the harvesting and debarking of willow trees. The bark from the trees is used for extraction of salicin and the non-bark material referred to as pulp is used for producing packaging materials. Undersized and fines lost during processing the pulp are used to produce compost. The costs for harvesting and debarking were obtained directly from partners in Biowill who are agricultural contractors involved in commercial harvesting of the crop. The costs for producing the food packaging are based on the trials undertaken using the debarked pulp at the University of Bangor pilot scale facility. Costs for the scaled up solvent extraction and bio-actives recovery were provided from

Helicon Process Development Solutions as part of their design.

Assumptions used in analysis:

- The cost of establishment of the willow plantation presented in Table 1 are not considered in the model but referenced for the farmer. The costs were obtained from AFBI and from Agriland in France who are agricultural contractors for harvesting and transportation of willow for power utilities.
- Packaging costs were provided by the University of Bangor using a model derived from commercial pilot runs for industrial packaging companies in the UK.

Table 1 Estimated initial plantation costs per hectare of willow plantation.

Item	Amount	Unit
Plantation Costs	1800	€
Ploughing	94	€
Soil Preparation	250	€
Herbicide protection	395	€
Fertilisation	260	€
Initial Costs per ha	**2799**	€

- The willow properties at harvest are presented in Table 2. In the calculations the moisture content of the willow at harvest was assumed to be 50 %.

Table 2. Willow properties at harvesting.

Item	Unit
Crop density	13,000 – 15,000
Harvesting cycle	2-3 years
Stump diameter	3.5 - 5 cm
Growing stock at harvest	35 – 60 fresh t/ha
Water content	45 – 60%
Rods harvested lengths	up to 8m
Willow yield	10.7 - 13.3 t/ha/yr

- We did not consider any taxes in the NPV calculations.

Technology specification and energy consumption.

Currently, the price of willow per tonne for heat and power production varies from €25–€34/tn but in the model it was assumed to be €57 in order to provide farmers with an income sufficient to encourage planting.

The pulp has a moisture content of 50% and before processing into packaging material it is dried to moisture content of 20%.

Table 3 Process specification, assumptions, and energy consumption.

Assumption	Value	Unit
Operating Hours per Year (260 days, 24-hour processing)	6,240	h
Price of Willow per Tonne	57	€/tn

Assumption	Value	Unit
Wages for Plant per Hour of Operation	60.00	€
Ratio of Bark to Willow Pulp	0.15	
Ratio of Pulp to Willow	0.85	
Drying of willow pulp for packaging		
Moisture Content of pulp	50	%
Heat required for drying per tonne	250	kWh
Cost of Thermal Heat per kWh	0.06	€/kWh
Density of Chips	0.28	Tonnes per m^3
Heat Required to Evaporate 1 Tonne of Water	0.865	MW
Total quantity of Water in Pulp	0.425	Tonnes/tn fresh willow
Quantity of Water to Evaporate to moisture content of 20%	0.255	Tonnes/tn fresh willow
Cost of Evaporating Heat	60	€/MW/hr
Cost per Tonne of Chip	13.23	€

Economic Analysis

Capital and Operating Costs

The economic break down of CAPEX and OPEX is presented in this section.

CAPEX is split into: 1) initial cost of plantation (Table 1); 2) costs of building the processing facility and 3) costs of plant and machinery (Table 4). It was assumed that the process will be mainly skid mounted in a warehouse environment.

The OPEX is split into: a) preparation for extraction that includes harvesting, transporting, debarking and storage (Table 5); b) extraction of bio-actives and associated processes for pulp preparation (Table 6)

Table 4 Estimated CAPEX

Item	**Amount**
Building Facility Costs	
Factory Building Size	15,000 sq ft
Cost per Sq Ft	€50.00
Building Cost	€750,000.00
Site Development Cost	€200,000.00
Planning Fees	€50,000.00
Development Charges	€30,000.00
Design Costs	€61,800.00
Total Costs (Buildings/Facility)	€1,091,800.00
Plant & Machinery Costs	
Mulch Formation	€35,000.00
Solvent Extraction System	€265,000.00
Vacuum Evaporation	€50,000.00
Crystallization	€50,000.00
Drying	€60,000.00

Dispensing	€20,000.00
Mobile Storage Vessels	€20,000.00
Additional Equipment (pumps/hoses etc.)	€50,000.00
Storage Systems	€50,000.00
Installation Costs	€300,000.00
Total Costs (Plant & Machinery)	€900,000.00
Grand Total CAPEX	€1,991,800.00

The total cost of preparation of willow for extraction is €831 per hectare per year (Table 5). Among the different costs involved, the cost of harvesting is the largest accounts for 65% of all the cost at this pre-processing stage.

A transportation distance of 25 km was assumed, corresponding to the area required to produce a sufficient amount of willow for both small and large scale facilities, i.e 1,000 hectares and 2,000 hectares, respectively.

Table 5. Estimated OPEX of willow preparation for extraction (this is per hectare).

Item	**Costs**	**Unit**
Maintenance	85	€/ha/yr
Harvesting	**540**	€/ha/yr
Transport	105	€/ha/yr
General management	75	€/ha/yr
Storage	10	€/ha/yr
Debarking	16	€/ha/yr
OPEX Costs per ha	**831**	€/ha/yr

The total cost of willow processing is €495 per tonne (Table 6). The extraction of bioactives, is the single most expensive process operation, as it accounts for 92 % of the total processing.

Table 6. Estimated OPEX for processing of one tonne of willow, including extraction of WBE, packaging and composting..

Item	**Processing cost**	**Unit**
Assuming willow yield	11	t/ha/yr
Pulp for Packaging	29	€/t
Bark to Extract (WBE)	455	€/t
Bark to Compost	11	€/t
Total processing cost	**495**	**€/t**

Table 7 summarises the total costs inclusive of purchasing, materials preparation and processing into products and indicates a cost per tonne of €627.

Table 7. Summary of costs for purchasing, and processing willow assuming yield of willow **11 t/ha/yr**.

Processing Costs	per ha	Per tonne	Unit
Price to Farmer	627	57	€
OPEX (preparation)	831	76	€
Processing to products	5,442	495	€
Total cost	**6,900**	**627.30**	**€**

Revenue Streams

Market prices for products

There are three main products with market value: willow bark extract (WBE), pulp and compost. In the calculations we assumed a price of willow bark extract of 60 €/kg (Table 8) based on 10% of the wholesale price of "salicins". Price for compost and pulp are from industry sources and may be an underestimation. There is currently a diminishing availability of waste paper from the newsprint industry, which was one of the main feedstock sources.

Table 8 Estimated price of products.

Product	Price	Unit
Pulp for packaging	100	€/t
Compost	45	€/t
Bark extract (Bioactives)	60	€/kg

Table 9 Estimated yield of willow, bark and the willow bark extract per hectare.

Land/ha	Willow/t	Willow Bark/t	Extractives/t
1	11	1.65	0.132

Table 10 Estimated yield of products per tonne of willow and their value.

Product	Yield, %	Yield, kg	Per Tonne of Willow
Pulp	84.6%	846	€85
Compost	14.20%	142	€6
Bark extract (BioActives)	1.20%	12	€720

Streams and costs of products

The quantities of products produced annually from willow and processing costs are presented in Table 11.

Table 11. The quantity of products produced per year and processing costs.

Annual input fresh willow	25,000	12,500	t/year
Willow bark extract	300	150	t/year
Revenue from willow bark extract	18,000,000	9,000,000	€/year
Willow pulp	21150	10575	t/year
Revenue from pulp	2,115,000	€1,057,500	EUR/year
Pulp for compost	3550	1775	t/year
Revenue from compost	€159,750	€79,875	EUR/year
Willow from Farmer	1,425,000	712,500	EUR/year
Total drying cost of pulp	330,863	165,431	EUR /year
Annual Maintenance cost	800,000	400,000	EUR/year
Processing cost	15,682,386	7,841,193	EUR/year
Total variable costs	€15,682,386	€7,841,193	EUR/year
Loan payback	-€344,223	-€344,223	EUR/year

Loan for CAPEX

In the calculations we assumed that

- Loan amount: €1,991,800.00
- Annual interest rate: 5%
- Loan term: 7 years
- Project duration: 15 years or 25 years
- Discount rate: 5%

The payment for a loan based on a fixed repayment monthly schedule and fixed interest rate was calculated to be €344,223.00 for each year. Loan payment was included in the calculations of NPV.

Economic Performance Metrics and Sensitivity analysis for key economic drivers

The aim of this study was to evalthree common factors used in decision making. Table 12 presents the main assumptions considered in the economic viability study.

Table 12 Main assumptions in viability study.

Parameter	Value	Unit
Discount rate	5	%
Inflation rate	2	%
Operating lifetime	15 and 25	years

Spreadsheet-based calculations were developed to calculate NPV, IRR and PP for two scenarios, 15 years and 25 years.

Case 1

15 year operational cycle: The operating lifetime of the project is 15 years. It is assumed that the CAPEX (total cost of facility, Table 4) will be paid from a loan with a 5% interest rate and loan payback time of 7 years. The NPV of the investment was calculated by considering the cash inflows from the investment and the cash outflows and the cost of loan repayment. Additionally, the Internal Rate of Return (IRR) and Payback Period (PP) were calculated and are presented in Table 13.

Cash outflows were CAPEX and OPEX in each year as well as loan repayments
Cash inflows were from the sales of willow bark extract, pulp and compost.

Table 13. Main economic results for project lifetime 15 years.

Project duration, years	Willow production (fresh), tonnes	NPV, €	IRR, %	Payback period, years
15	25,000	€21,851,746	87.45	1.17
15	12,500	€8,876,362	38.33	3.9

NPV is positive for both processing capacities of 25,000 t/y and 12,500 t/y suggesting that it is expected to be profitable, with the PP is less than 1.5 year for the higher willow throughput and 3.8 years for the lower throughput. Also, IRR's of 87% and 38 % for the 25,000 tonnes and 12,500 tonnes respectively suggests an attractive return on investment.
To test this conclusion, a sensitivity analysis was conducted because of the imprecision and uncertainty of some of the assumptions in Table 8, in particular price of willow bark extract. The value of willow bark extract will depend on its composition, and it may vary significantly with willow variety, harvest frequency etc.

The price of willow bark extract for the sensitivity analysis was varied from €15 to €120 per kg to evaluate how sensitive the investment is to it's price variation and the results of sensitivity analysis are presented in Fig. 1. which shows that a decrease in willow bark extract (WBE) prices below €55 per kg makes the profitability of the plant negative only for both processing capacities.

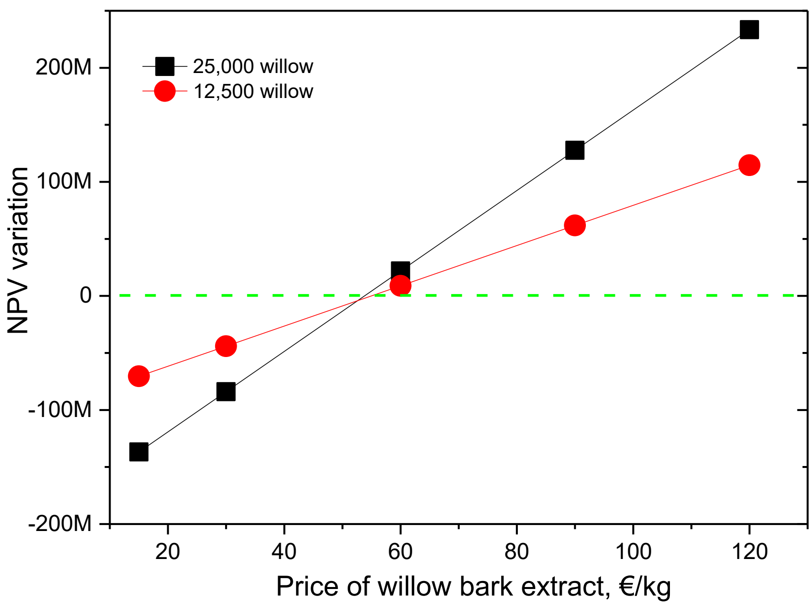


Fig. 1 Sensitivity of NPV to willow extract price for 15 years operation timeline.

The sensitivity of NPV to the cost of willow debarking (another operation where it is difficult to obtain robust data regarding costs) for a 15 years operation timeline is presented in Fig. 2. An increase of debarking cost from €16 to €296 per hectare per year decreased profitability of the investment. A 10-fold increase in the cost reduced the NPV by 14% for the larger scale and by 17 % for the smaller processing capacity.

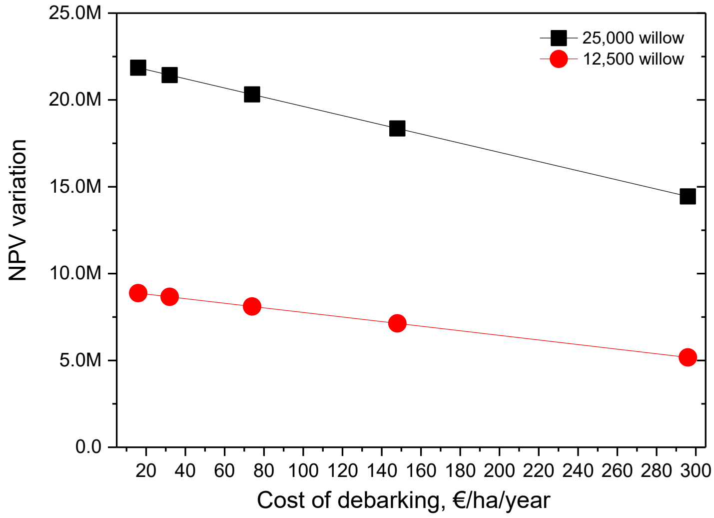


Fig. 2 Sensitivity of NPV to the cost of willow debarking for 15 years operation timeline.

The sensitivity of NPV to the moisture content of fresh willow is presented in Fig. 3. An increase in moisture content showed a relatively minor decrease in profitability of the investment. An increase from 50% to 60% of moisture reduced the NPV by 3.5% for the larger scale and by 4.3 % for the smaller scale processing capacity.

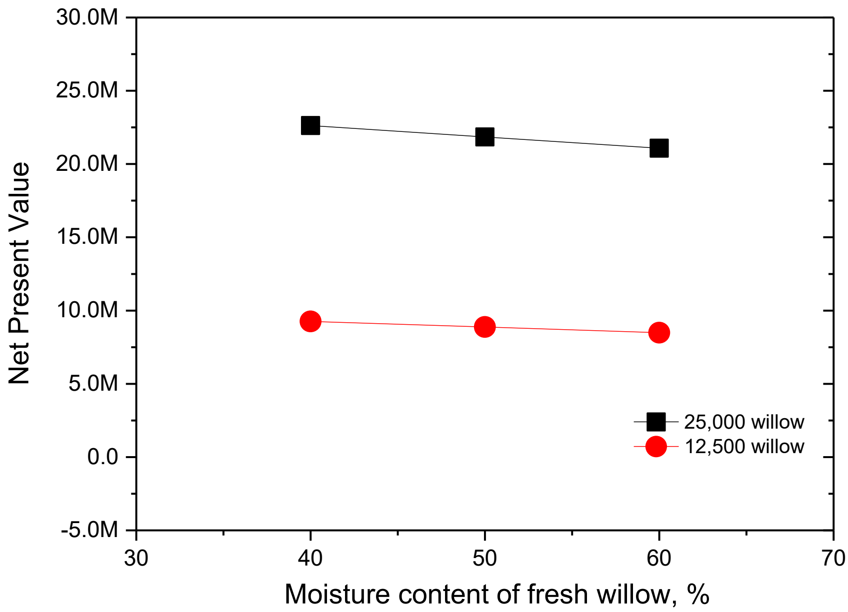


Fig. 3 Sensitivity of NPV to the moisture content of fresh willow for 15 years operation timeline.

The sensitivity of the NPV to the cost of harvesting is presented in Fig. 4. A 50% increase in harvesting costs reduced the NPV by 32% for the larger scale and by 40% for the smaller scale processing capacity.

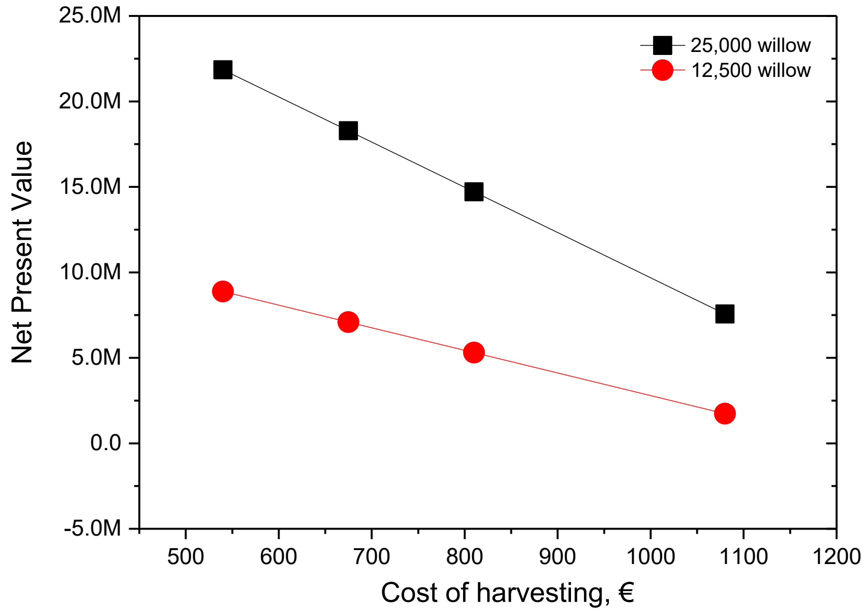


Fig. 4 Sensitivity of NPV to the cost of willow harvesting for 15 years operation timeline.

Case 2

Operating lifetime of the project is 25 years and the NPV, IRR and PP calculations are presented in Table 14.

Cash outflows were CAPEX and OPEX in each year as well as loan repayments

Cash inflows were from the sales of willow bark extract, pulp and compost.

Table 14. Main economic results for project lifetime 25 years.

Project duration, years	Willow production (fresh), tonnes	NPV, €	IRR, %	Payback period, years
25	25,000	€34,852,597	87.46	1.20
25	12,500	€15,159,427	38.85	3.90

Similar to a project lifetime of 15 years, a decrease of willow extract prices below €55 per kg of extract makes the profitability of the plant negative for both processing capacities when a 25 year project lifetime is considered.

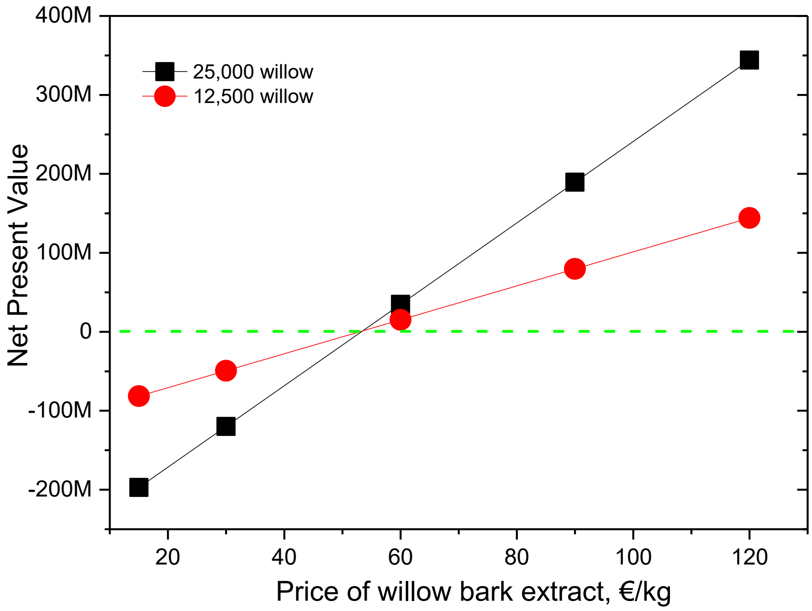


Fig. 5 Sensitivity of NPV to willow extract price for 25 years operation timeline.

Risk Assessment

Technical, economic, and regulatory risks of integrating of HTC within WWTP and mitigation strategies for the identified risks are presented in Table 11.

Table 15 Risks and mitigation strategies for the identified risks.

Technical risks	**Mitigation strategy**
The development of technology for automation of debarking of dry willow rods is very slow.	Debarking may have to undertaken using freshly harvested material
Economic risks	**Mitigation strategy**
Entire project very highly dependent on price for Willow Bark extract which has a limited market	Develop alternative potential markets other than for topical ointments such as a food ingredient
Regulatory risks	**Mitigation strategy**
European medicines agency may disapprove use of WBE as phytopharmaceutical ingredient	Find alternative markets such as food ingredient

Conclusion and Recommendations

The economic indicators for the two scenarios suggest that, for a proposed plant with a 15-year lifespan, the NPV is €5 million for the small plant and €14 million for the larger one. The payback period is 1.5 years and 3.5 years plants. The NPV **is highly sensitive to change in willow extract prices**.

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Coláiste na hOllscoile Corcaigh

Interreg North-West Europe BIOWILL

European Regional Development Fund

BIOWILL REPORT

WPT 2.4 Optimum Biogas/Biofertiliser production from willow residue

Chen Deng, Benteng Wu, Xihui Kang, David Wall, Richen Lin, Jerry Murphy

MaREI Center | University College Cork

Deliverable T2.4.1 Report on effect of steam hydrolysis pretreatment technologies on biogas yield from bark and pulp biofibers, enzymatic hydrolysis (UCC)

(1) Optimization of the hydrothermal hydrolysis of lignocellulosic biomass for sugar production [1].

Hydrothermal hydrolysis at different temperatures (100 to 180 °C) was investigated to improve sugar (a biomass energy precursor) production from lignocellulosic grass silage. The optimal conditions (140 °C for 20 min duration) showed the highest sugar yield of 0.29 g/g volatile solid (VS) of grass silage. Further increasing the temperature to 180 °C favored the degradation of sugars to by-products (such as furfural and hydroxymethylfurfural). A first-order reaction model confirmed a two-step reaction with the first step hydrolysis and the second step degradation. The apparent activation energy (5.89 kJ/mol) for the hydrolysis reaction was higher than the one (1.85 kJ/mol) for the decomposition reaction, indicating that the hydrolysis reaction is more difficult to take place than the decomposition reaction in the temperature range used in the experiments. An energy balance calculation indicated that pretreatment at 140 °C required an energy input of 16.5 kJ/g VS, which could be significantly reduced to 5.1 kJ/g VS through efficient heat recovery. This study advanced the understanding of hydrolysis kinetics and would facilitate the optimization of willow pretreatment for anaerobic digestion.

(2) Optimization of the acid hydrolysis conditions to maximize reducing sugar yields; evaluation of the effects of acid hydrolysis pretreatment on the specific biohydrogen and biomethane yields in single-stage and two-stage fermentation of lignocellulosic biomass; and assessment of the energy conversion efficiency for both processes [2, 3].

The rigid lignocellulosic structure of willow makes it resistant to microbial metabolism, resulting in a sub-optimal production of biohydrogen and biomethane in fermentative processes. Acid pretreatment is an effective method to enhance the conversion of lignocellulosic components. In this study, the effects of acid pretreatment with mild thermal treatment conditions on biohydrogen and biomethane production from grass silage (as a substitute feedstock for willow when the delivery of willow was delayed due to the COVID pandemic) was assessed through single-stage (biomethane) and two-stage (biohydrogen + biomethane) fermentation. Microstructural characterization showed that pretreatment significantly reduced the recalcitrance and enlarged the specific area of grass silage. The optimal pretreatment with 2% H_2SO_4 at 135 °C for 15 min achieved a total reducing sugar yield of 0.33 g/g volatile solid (VS) of grass silage. The pretreated silage led to a hydrogen yield of 68.26 ml/g VS in the first-stage hydrogen fermentation, a 3-fold increase compared to untreated silage. The production of volatile fatty acids accordingly increased by 29.2%. In the second-stage anaerobic digestion, untreated silage achieved the highest biomethane yield of 392.84 ml/g VS, with a corresponding highest total energy conversion efficiency of 83.5%. Due to a lower biomethane yield, the pretreated silage presented a decreased total energy efficiency of 68.4%. In comparison, single-stage anaerobic digestion showed lower energy conversion efficiencies of 49.7% and 54.2% for the pretreated and untreated silage, respectively. Despite the slight decrease in biomethane yield, the pretreatment led to decreased energy consumption for the operation of anaerobic digestion processes due to the shorter digestion duration. This study identified the optimal acid hydrolysis conditions for the release of sugar monomers from lignocellulosic biomass (represented by grass silage and willow) to enhance biogas production in the anaerobic digestion process, which could provide a reference for the determination of pretreatment conditions for willow materials.

(3) Steam explosion pretreatment of willow for efficient biomethane production.

Steam explosion can depolymerize the fiber bundles of lignocellulose by the chemical forces of self-hydrolysis of hemicellulose at high temperatures, and by the shearing action of saturated steam at a quickly reduced pressure through explosive decompression. A steam explosion at 6 bar and 165 ºC for 15 min was applied to willow pretreatment. Results revealed that the biomethane yield of steam-exploded willow was 139 ± 0.8 mL/g VS, which was effectively enhanced by 52% compared with that of the raw willow. Furthermore, similar biomethane production (126 ± 8.9 mL/g VS) was obtained from food trays which were the end use of packaging products made from steam-exploded willow. This study demonstrated that steam explosion pretreatment could be a promising method for the optimal conversion of willow into biomethane, and the downstream manufacturing process of packaging products did not affect the biomethane yield from willow.

(4) Evaluation of the performance of mono-digestion of steam-exploded willow.

This study further evaluated the mono-digestion of steam-exploded willow from batch mode to continuous mode with a specific focus on the stability of digester performance over a long-term operation. Results revealed that the specific biomethane yield of steam-exploded willow reduced over time when the organic loading rate was 2 g VS/L-reactor/day, decreasing from 114 ± 7.4 to 66 ± 1.7 mL/g VS over 63 days; this was a 42% reduction. One possible explanation for this is that the extremely high C/N ratio (460) of willow led to the lack of nitrogen for the growth of the microbial consortium and the proceeding of metabolic activities. The results indicated that mono-digesting steam-exploded willow may encounter instability and other strategies such as co-digestion with high nitrogen content feedstocks (such as food wastes) might offer a solution.

Deliverable T2.4.2 Report on the effect of chemical hydrolysis pretreatment technologies on biogas yield from bark and pulp biofibers (UCC)

(5) Optimization of deep eutectic acid pretreatment of willow for efficient biomethane production [4].

Lignin extraction from lignocellulosic biomass can enhance its bioconversion efficiency, whilst the recovered lignin can provide added economic value. This study comprehensively investigated the impacts of short-chain carboxylic acid-based deep eutectic solvents (DESs) pretreatments on lignin extraction from willow and assessed subsequent biomethane production through anaerobic digestion. Process parameters (including the DES type, molar ratios of DES components, temperature and reaction time) in the DES pretreatments of willow for lignin extraction were optimized using the central composite surface response methodology. Results showed that lactic acid-based DES pretreatment outperformed acetic acid and propionic acid-based DES pretreatments in terms of lignin removal efficiency and methane production. Under the optimal conditions (choline chloride:lactic acid with a molar ratio of 1:10 at 160 ◦C for 15 min) lactic acid-based DES pretreatment retained over 94% of the glucan content in the raw willow whilst achieved the highest lignin removal of 80%. The recovered lignin showed a purity of above 81%. Compared with the biomethane production from raw willow, the biomethane production significantly increased by 36.3% after the lactic acid-based DES pretreatment. The optimal condition reduced the digestion time from 22 to 10 days. The overall energy conversion efficiency of 62.7% demonstrated that lactic acid-based DES pretreatment of willow could be a promising method to co-produce renewable gaseous fuels and lignin in a sustainable approach.

(6) Valorization of willow lignin to high-quality biochar and activated carbon and utilization of the obtained carbon materials to enhance biomethane production from willow [5-8].

Lignocellulosic biomass can add to the worldwide resource of biogas; however, the aromatic structure of lignin is recalcitrant which impairs biodegradation. Direct interspecies electron transfer (DIET) may overcome limitations in the biodegradation of lignin derivatives. Within a circular bioeconomy system, lignin-derived biochar and activated carbon were assessed for their ability to enhance the digestion of a typical lignin monomer – syringaldehyde. Biochar at 5–10 g/L significantly reduced the lag-phase time by 33–42% possibly due to the enhancement of syntrophic hydrogenotrophic methanogenesis. In comparison, activated carbon at 1–10 g/L reduced the lag-phase time by 46–85% and significantly accelerated the degradation of volatile fatty acids, due to a combinational effect of enhanced syntrophic oxidation and DIET. When activated carbon was added at a higher dosage of 20 g/L, the highest biomethane yield (426.6 ml/g) was achieved; an increase of 33% compared to the digestion of syringaldehyde alone. The enhancement was ascribed to the metabolic shift from the hydrogenotrophic to the DIET pathway, which could be implied from the microbial community dominated by Methanosaeta. The superior function of activated carbon over biochar was speculated to be associated with its larger surface area and higher abundance of the C=O group. When applied in the digestion of willow, biochar significantly enhanced the biomethane yield, whereas activated carbon did not significantly enhance the biomethane yield beyond that of biochar.

Deliverable T2.4.3 Report on the effect of enzymatic hydrolysis pretreatment technologies on biogas yield from bark and pulp biofibers (UCC)

(7) Combined steam explosion and enzymatic pretreatment of willow for efficient biomethane production.

Fungal laccases are ligninolytic enzymes that are capable of oxidizing both phenolic and non-phenolic compounds in lignin, and effectively catalyze the degradation of steam-exploded willow. To combine the strength of steam explosion and laccase catalysis, steam explosion at 6 bar and 165 ºC for 15 min was applied to willow and then the exploded willow was subjected to laccase treatment. Results revealed that laccase pretreatment did not further increase the biomethane yield. Nevertheless, it increased the biomethane production rate by 20% and shortened the lag-phase time by 20% as compared to the digestion of steam-exploded willow. This study demonstrated that combined steam explosion and laccase pretreatment could benefit the optimal conversion of willow residue into biomethane.

Deliverable T2.4.4 Report on the properties of digestate / digestate derived fertilizer as soil fertilizers to meet the threshold values of the organic fertilizer regulation. (UCC & UL)

(8) Evaluation of the fertilizer equivalence of willow digestate.

After the biomethane potential assays of both the raw and DES pretreated willow, the content of nitrogen (N), phosphorus (PO_4), and potassium (K) in the digestate was tested. In the digestate from raw willow, the content of total N, K and PO_4 was approximately 1200 mg/L, 600 mg/L, and 20 mg/L, respectively. Compared to the seed inoculum sourced from cattle manure, the N content in the digestate decreased by 20% due to the consumption of microbial metabolism, whilst the content of K and PO_4 remained unchanged. In the digestate from DES pretreated willow, the N content was further decreased by 8% compared to digestate from raw willow. The DES pretreatment facilitated the degradation of willow and enhanced the microbial metabolism, therefore, leading to a further decrease in N content.

Continuous trials for AD of steam-exploded willow were conducted to produce digestate in practical conditions. The inoculum was sourced from cattle manure. The continuous anaerobic digestion was carried out in two reactors each with a working volume of 4 L. The hydraulic retention time was set at 35 days based on the batch trails. The organic loading rate was initially set at 2 g VS/L-reactor/day, but it was reduced to 1.5 g VS/L-reactor/day after day 63 due to the deterioration of the AD performance. After 180 days of continuous operation, about 20 litres of digestate (containing 1.3 kg of total solids) were delivered to the University of Limerick for further studies.

Deliverable T2.4.5 Report on the techno-economic analysis of the proposed anaerobic digestion system (UCC/GNI)

(9) Techno-economic analysis of the anaerobic digestion system based on willow feedstock. A techno-economic analysis was carried out to evaluate the economic feasibility of the anaerobic digestion system consisting of a biogas plant and an ex-situ biogas upgrading plant. Steam-exploded willow which is also the material for food packaging production was assumed to be the feedstock for biogas production. A 4000 m^3 digester with a 90% of working volume was selected. The organic loading rate of willow and the hydraulic retention time of the digester were 2 kg VS/m3/d and 35 d, respectively, based on the continuous trial. As such liquid digestate was recycled to adjust the TS of feeding to give the desired hydraulic retention time. The plant life was assumed to be 20 years and the straight-line depreciation method was used. The feedstock price was assumed to be 4 €/t (transportation costs only) and the electricity and natural gas price was based on the commercial fuel cost. Wind energy-sourced renewable hydrogen was assumed to be used for biogas upgrading with a price of 3.8 €/kg. Solid digestate was sold as a biofertilizer for farmers at a cost of 4 €/t (transportation costs only). Two scenarios were considered for analysis. For the base scenario, the biomethane yield of willow was assumed to be 71.9 Nm^3 CH_4/t VS willow based on the continuous trial, while for the optimal scenario, this figure was 111.5 based on the batch experiment.

The minimum selling price of the biomethane was calculated when the net present value was zero. Results showed that the minimum selling price of biomethane in the optimal case was 35.7 c€/kWh which is 24.7% lower than that of the base case but 7.8 times higher than the EU weighted average price of natural gas (4.6 c€/kWh) in 2021. Sensitivity analysis showed that capital cost, operating hours and H_2 price were the three most sensitive parameters. The bigger the plant, the lower the capital costs per unit output of energy. As such when the system capacity of the optimal case was scaled up by 5.9 times to 3.96 million Nm^3/year based on a commercialized biomethanation plant, the minimum selling price of biomethane was reduced by 51% to 17.5 c€/kWh. However, this figure is still 3.5 times higher than the EU-weighted average price. Overall, this study showed that mono-digestion of willow to produce biomethane is not economically feasible in the long run, and other strategies such as co-digestion with food wastes or grass silage should be considered to increase the potential economic benefits of willow-based anaerobic digestion systems.

Deliverable T2.4.6 Report on steps for upgrading of bio-gas to meet the requirements for biomethane for gas grid injection (GNI)

(10) *Ex-situ* biogas upgrading from a novel biomethanation system to produce natural gas standard biomethane [9].

A novel *ex-situ* biomethanation system ($CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$) was designed and commissioned to upgrade biogas into biomethane. The biomethanation system consisted of three reactors that were connected in sequence. The inoculum was sourced from cattle manure and was sieved over a 150 μm mesh before being added to the reactor. The temperature was set at 57 ± 1 ºC. The high-performance ceramic gas diffusing system was adopted to increase hydrogen concentration for methanogenic utilization. Results showed that when the hydrogen loading rate was 34.6 ± 0.8 L/L-reactor/day, the methane concentrations in the output gas were more than 92%, indicating the high efficiency of the upgrading system.

Fluctuations in variable renewable electricity may lead to intermittent hydrogen supply, which is shown to adversely affect the microbial activity and performance of the biomethanation process. Carbonaceous materials may act as an abiotic additive to enhance microbial robustness and improve system performance. Nanomaterial graphene and pyrochar were compared to assess their effects on biomethanation systems with an intermittent supply of hydrogen. Results revealed that intermittent gas supply caused deterioration in the restart performance with only

66% of theoretical methane production obtained in the control compared with 84% under steady state conditions. The addition of graphene in biomethanation led to 78% of the theoretical methane production after repetitive intermittent supply; this improvement is postulated to be due to its high electrical conductivity and large specific surface (500 m2/g). In comparison, pyrochar amendment did not lead to a significant improvement in upgrading performance. Microbial analysis showed that the OTUs affiliated to bacteria withinin the order SHA-98 (42.9% in abundance) and archaea from the genus Methanothermobacter (99%) may result in the establishment of a new syntrophic relationship to improve the robustness of biomethanation process.

(11) Development of cascading circular bioenergy systems [10, 11].

Producing advanced fuels (such as biomethane) and bio-based valorized products (such as biochar) may offer a solution to significantly reduce greenhouse gas emissions associated with energy and agricultural circular economy systems. Biological and thermochemical bioenergy technologies, together with power-to-gas (P2G) systems can generate green renewable gas, which is essential to reduce the greenhouse gas footprint of the industry. However, each technology faces challenges with respect to sustainability and conversion efficiency. This study identifies an optimal pathway, leading to a sustainable bioenergy system where the carbon released in the fuel is offset by the greenhouse gas savings within the circular bio-based system. It provides a state-of-the-art review of individual technologies and proposes a bespoke circular cascading bio-based system with anaerobic digestion as the key platform, integrating electro-fuels via P2G systems and value-added pyrochar via pyrolysis of solid digestate. The mass and energy analysis of the system suggests that a reduction of 11% in digestate mass flow with the co-production of biochar, bio-oil and syngas leading to an increase of 70% in biomethane production with the utilization of curtailed or constrained electricity can be achieved in the proposed bio-based system, enabling a 70% increase in net energy output as compared with a conventional biomethane system. However, the carbon footprint of the electricity from which the hydrogen is sourced is shown to be a critical parameter in assessing the GHG balance of the bespoke system.

(12) A detailed techno-economic and environmental assessment of biomethane or biomethanol production in cascading circular bioenergy systems [12].

Production of renewable C1 transport biofuels (such as biomethane and biomethanol) through the integration of anaerobic digestion (AD) with carbon capture, utilization and sequestration technologies may offer a solution to reduce greenhouse gas (GHG) emissions. This study presented a detailed techno-economic and environmental assessment of four cases of biomethane or biomethanol production by incorporating AD, CO_2 utilisation via biomethanation (CU), solid digestate pyrolysis (Py) and methanol synthesis (MeOH). The results reflected the current state of technologies and potential future scenarios with improved development. Under optimistic scenarios (scaled-up systems and reduced hydrogen prices of 1 €/kg), the minimum potential GHG abatement cost for the AD-Py-CU case was –111.1 €/t $CO_{2\text{-}eq}$ when biomethane was sold at 1.03 €/Nm^3 (a contract gas price in 2022), while the abatement cost rose to –58.2 €/t $CO_{2\text{-}eq}$ when H_2 was purchased at €3.40/kg. When methanol was sold at 425 €/t (global weighted average value), the marginal abatement cost for the AD-Py-CU-MeOH (with H_2 at 1€/kg) case was 136.5 €/t $CO_{2\text{-}eq}$, which is higher than current carbon credits at 33.5 €/t CO_2. This study suggests that biomethane produced by incorporating AD, CO_2 biomethanation and pyrolysis technologies may be economically and environmentally competitive over natural gas.

Publications:

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USE OF WILLOW AND WILLOW BASED BIO FIBRE PRODUCTS IN ANAEROBIC DIGESTION

The following report was delivered by the UCC partner in defining the AD opportunity for willow

Research scope of BioWILL

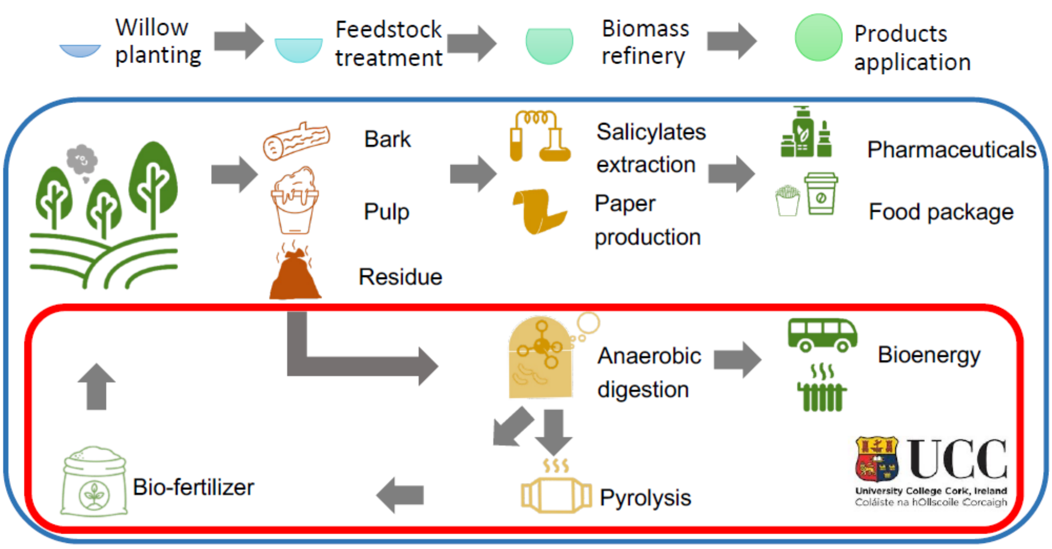


Fig. 1. Scope of BioWILL project.

Research flowchart

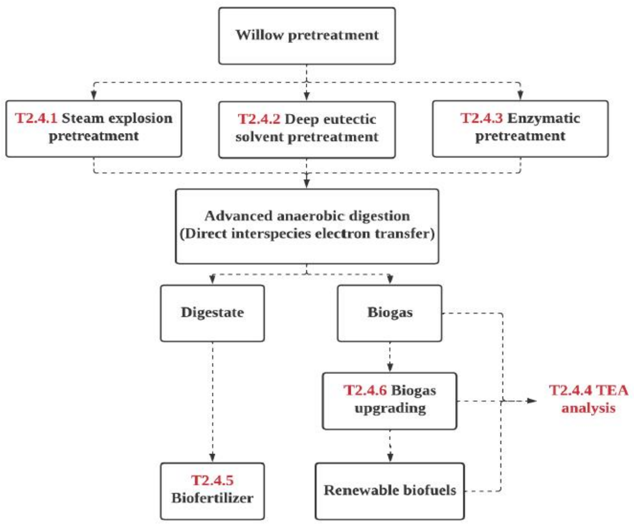
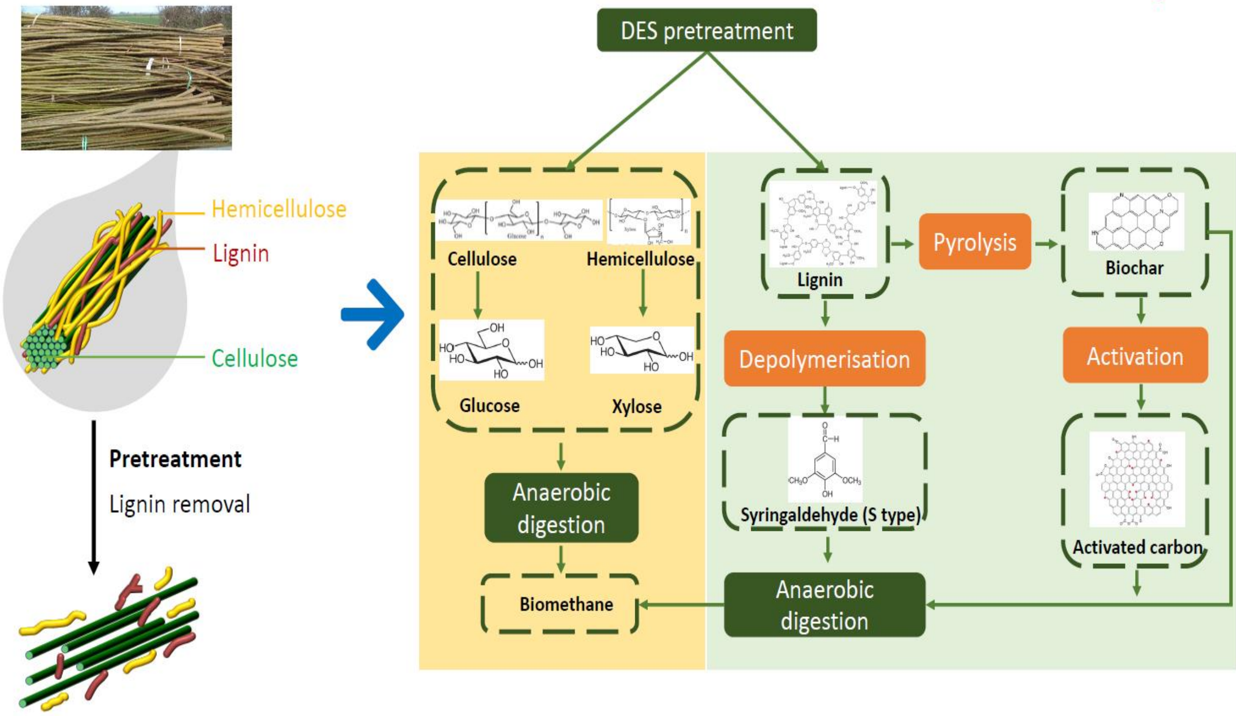


Fig. 2. Flowchart of our research.

Research questions:

- What are the optimal pretreatment methods for biogas production from willow?
- What may a circular biorefinery system look like by integrating anaerobic digestion with pyrolysis?
- How economically sustainable is the anaerobic digestion of willow?

WP T2.4.2 Deep eutectic solvent pretreatment



WP T2.4.2 Deep eutectic solvent pretreatment

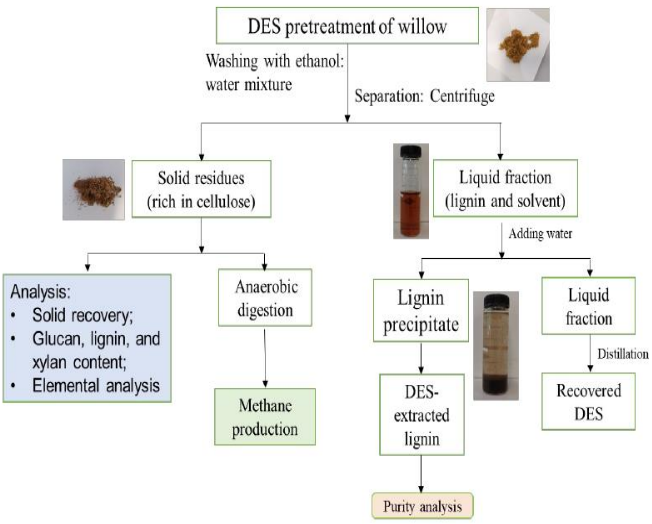


Fig. 3. Scheme of the DES pretreatment and methane production assessment.

Objectives:

1) **To optimise pretreatment conditions**
2) **To improve biomethane production**

Box1:
Deep eutectic solvent (DES) is mixed of two or more chemicals acting as either Hydrogen-Bond Donors (such as the acetic acid, lactic acid, and propionic acid used in this study) or Hydrogen-Bond Acceptors (such as choline chloride).

WP T2.4.2 Deep eutectic solvent pretreatment

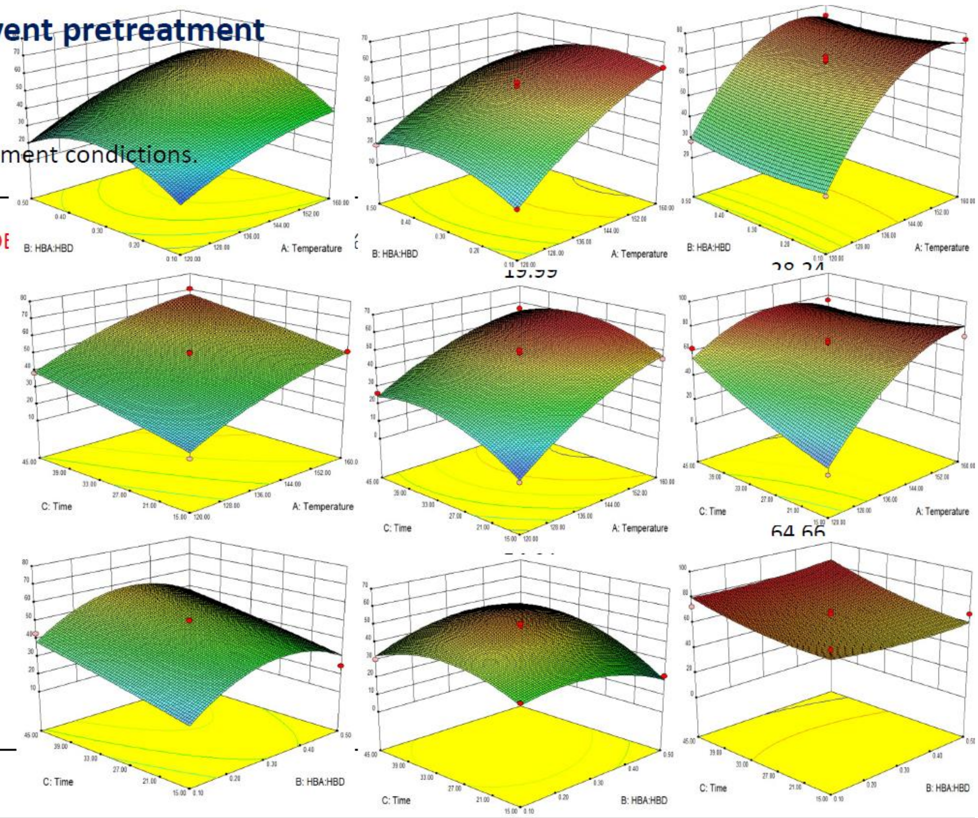


Table 2. Set-up of the DES pretreatment conditions.

Run	Factor 1 Temperature	Factor 2 Mixing ratio of DE
8	120	1:2
7	120	1:4
2	120	1:4
13	120	1:10
9	140	1:2
14	140	1:2
3	140	1:4
4	140	1:4
5	140	1:4
1	140	1:4
16	140	1:4
6	140	1:10
11	140	1:10
10	160	1:2
17	160	1:4
12	160	1:4
15	160	1:10

Outcome 1: Optimal conditions for lignin removal

Table 3. Lignin removal efficiency at the optimised condition for each type of DES pretreatment.

Type of DES	Temperature (℃) (Predicted/actual)	Mixing ratio of HBA:HBD (Predicted/actual)	Time (min) (Predicted/actual)	Predicted lignin removal (%)	Actual lignin removal (%)	Variability (%)
Acetic acid-DES	160/160	1:4/1:4	45/45	68.04	72.27	6.22
Propionic acid-DES	160/160	1.05:4/1:4	32/32	61.47	70.48	14.66
Lactic acid-DES	159.89/160	1:10/1:10	15/15	86.73	79.72	8.08

Outcome 2: Biomethane production from willow

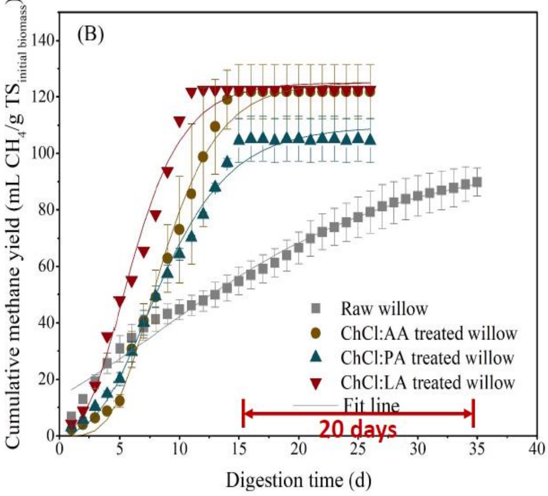


Fig. 4. Biomethane yield from willow with different types of DES pretreatment.

- **Acetic acid-DES pretreatment led to an increase of 35.5% in biomethane yield.**
- **Propionic acid-DES led to a 17.0% increase in biomethane yield.**
- **Lactic acid-DES led to a 36.3% increase in biomethane yield.**
- **Pretreatment significantly shortened the digestion period of willow**

Outcome 4: Efficiency of willow digestion

Renewable deep eutectic solvents pretreatment improved the efficiency of anaerobic digestion by lignin extraction from willow

Xihui Kang[1,2], Chen Deng[1,2,*], Richen Lin[1,2,3], Jerry D Murphy[1,2]

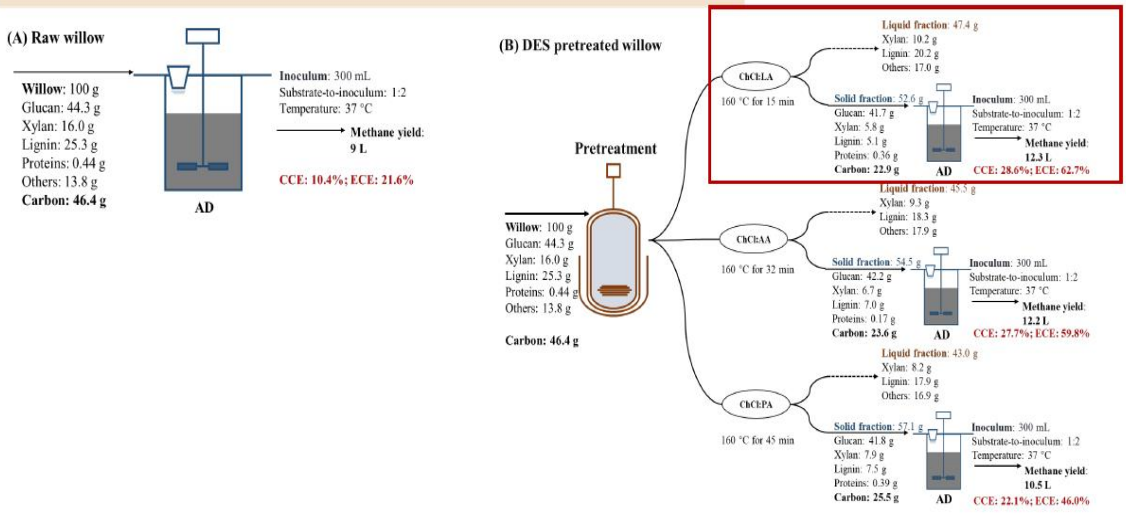


Fig. 5. Biomethane yield from willow with different types of DES pretreatment.

- **Lactic acid-DES led to a 175% increase in carbon conversion efficiency of willow in AD.**

WP T2.4.2 Lignin-derived carbon materials enhance biogas production

Outcome: Enhanced biomethane production from willow

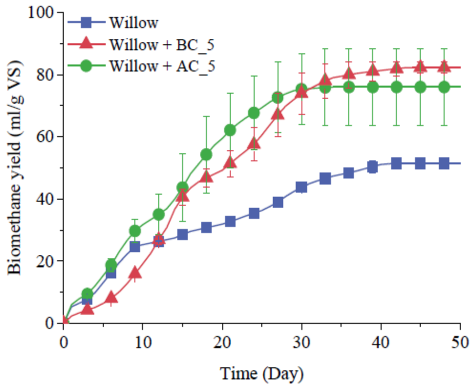
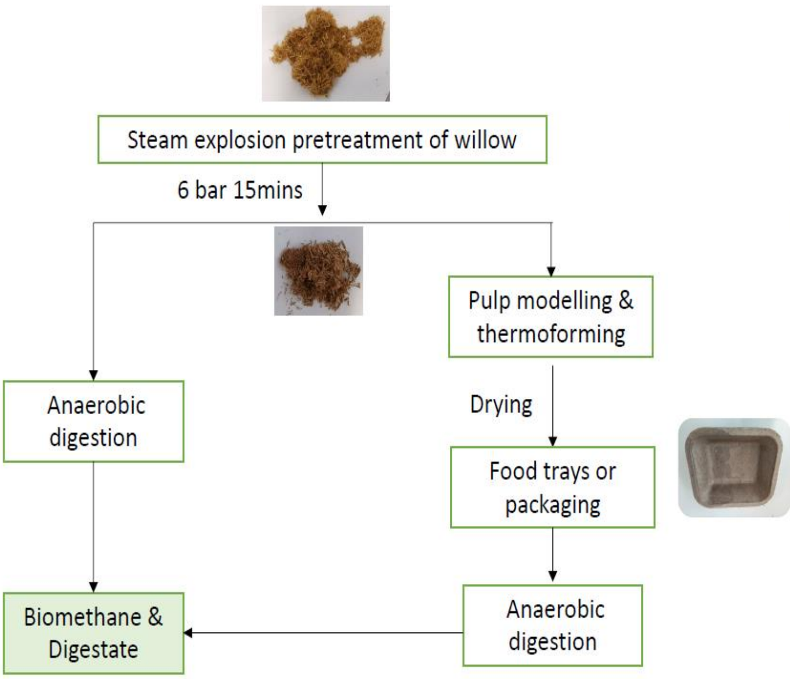


Fig. 10. Biomethane production from willow with the addition of biochar and activated carbon.

- **The addition of 5 g/l biochar significantly improved the biomethane yield by 60%.**
- **Difficulty in enhancing biogas production from willow was due to the low hydrolysis efficiency of lignin components.**

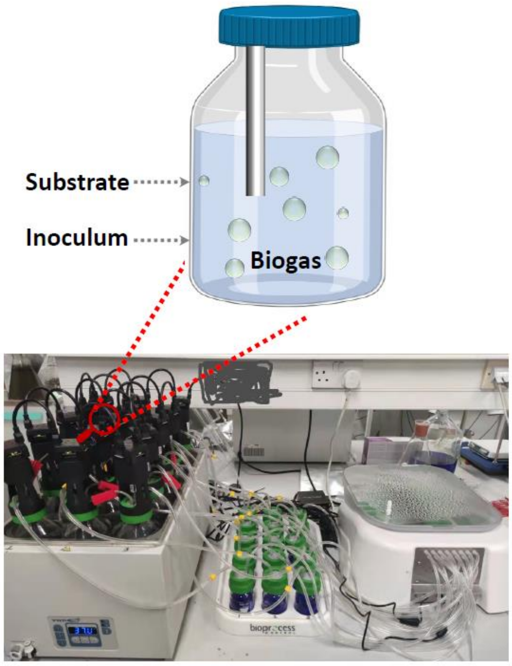
WP T2.4.1 Steam explosion pretreatment



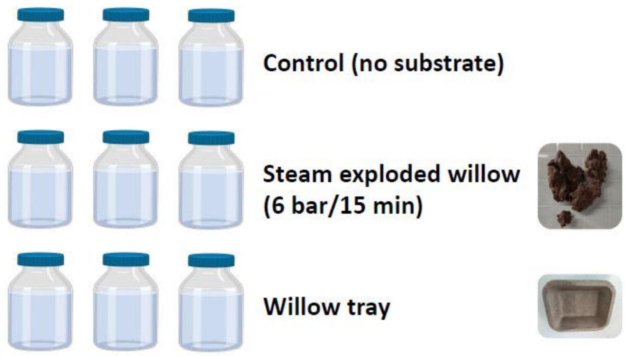
Objectives:

1) **Effect of steam explosion pretreatment**
2) **Biomethane production in the continuous operation**

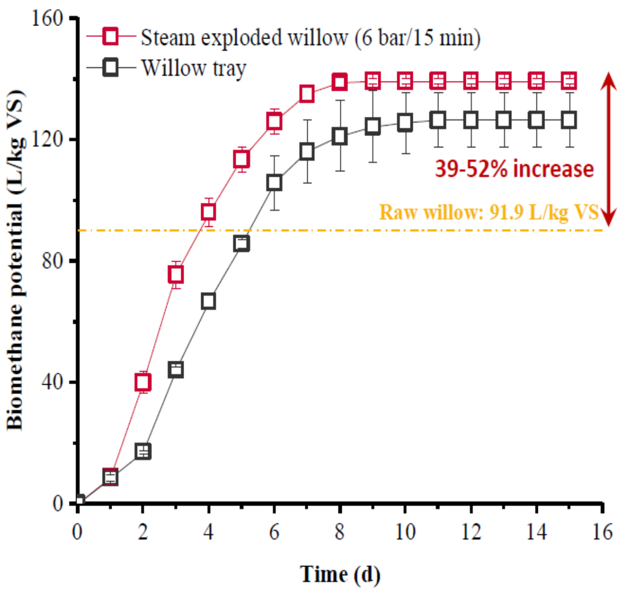
Experimental design of the batch experiment



- T = 37 ^{o}C
- Inoculum (g VS): substrate (g) = 2:1
- Working volume = 400 mL



Effect of steam explosion pretreatment on biomethane production



- **No significant difference between the steam exploded willow and willow tray groups was observed (p>0.05).**
- **Compared with raw willow, steam explosion enhanced biomethane production by 39 to 52%.**

Fig. 12. Biomethane potential of different batches of pretreated willow.
Error bars represent the standard deviation from experimental triplicates.

Experimental design of the continuous experiment

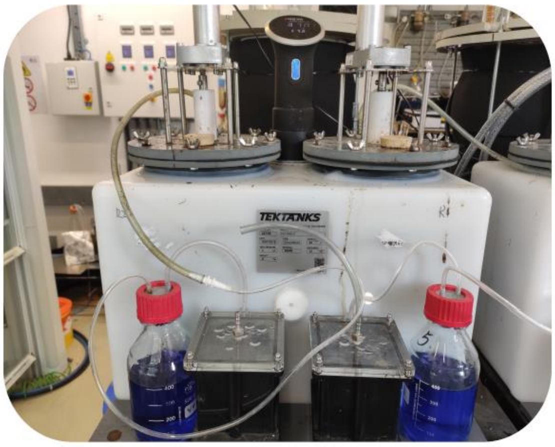


Table 4 Experimental design of the continuous anaerobic digestion system.

Parameters	
Feedstock	Steam exploded willow
Inoculum concentration (g VS/L)	17.8 ± 0.2
Organic loading rate (g VS/L/d)	2.0-1.5
Volume (L)	4.0 * 2
Hydraulic retention time (d)	35
Initial pH	7.44

- **Digester performance for mono-digestion of pretreated willow.**
- **Production of digestate derived fertilizer for characterization.**

Outcome: Specific methane yield of steam exploded willow in continuous operation

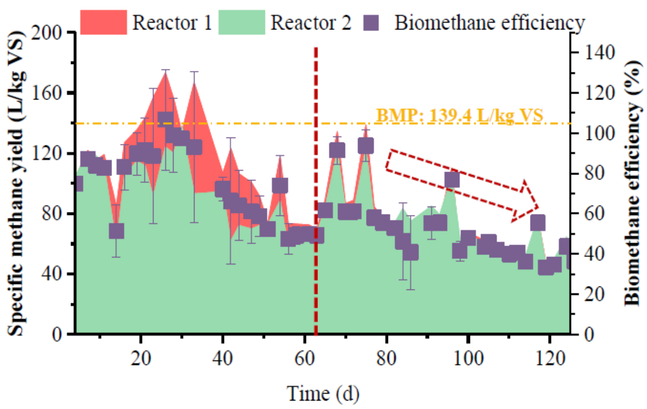
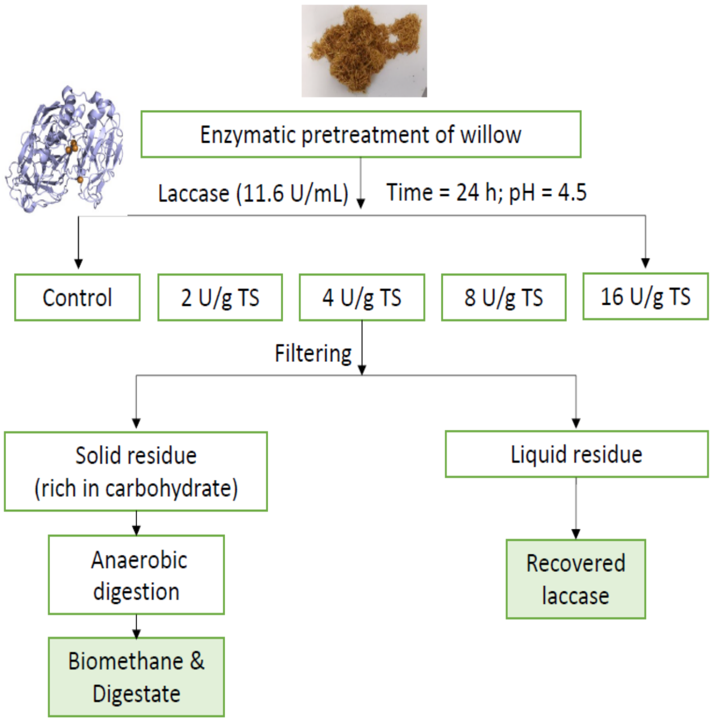


Fig. 13. Specific methane yield of steam exploded willow under continuous operation.

- **Compared with the BMP, the biomethane efficiency decreased gradually to ca. 40%, possibly due to the high C/N ratio (460) of willow.**

WP T2.4.3 Combined steam explosion and enzymatic pretreatment



Outcome: Optimal pretreatment conditions

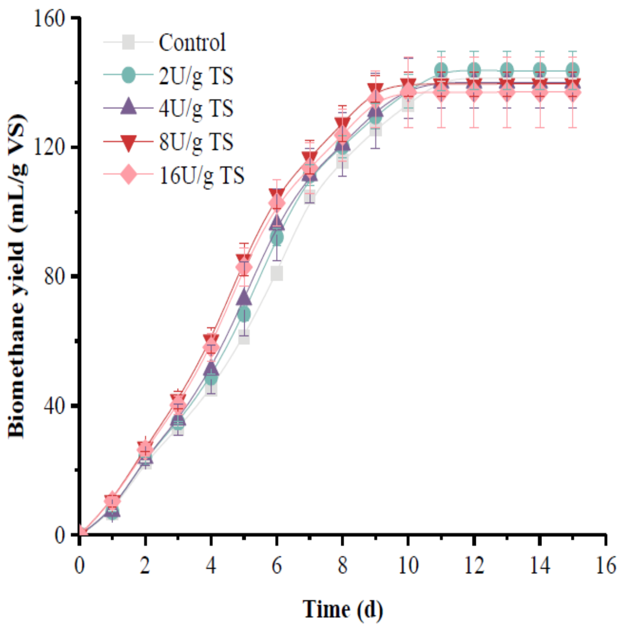


Fig. 14. Biomethane yield of steam exploded willow under different laccase dosages. Error bars represent the standard deviation from experimental triplicates.

Gompertz equation

$$M = P \times \exp\left\{-\exp\left[\frac{R_{max} \times e}{P}(\lambda - t) + 1\right]\right\}$$

- **Compared with the biomethane yield of the control, no significant difference was observed from laccase added groups;**
- **Laccase added at 8 U/g TS and 16 U/g TS enhanced the maximum production rate by ca. 21%;**
- **8 U/g TS and 16 U/g TS shortened the lag time by 17% and 21% respectively.**

WP T2.4.5 TEA analysis of the AD system

Table 5. Basic economic parameters for the considered systems.

Parameters	Value	Note
Base year	2021	
Plant life	20 years	
Plant annual operating hours	7920 h	
Discount rate	10%	[39]
Depreciation method	Straight-line	
Depreciation period	20 years	[40]
Salvage/administration cost	5% of capital expenditure	
Debt ratio	60%	Assumed
Interest on debt	2%	
Loan period	10 years	
Income tax	12.5%	[26]
Inflation	2%	Yearly increment of OPEX and product selling prices; [41]
Yearly decrease in product yields	2%	

Renewable energy discount rate survey results, 2018
Verbeeck et al. Energy Environ Sci, 2018
Vo et al. Appl Energy, 2018
Statista Ireland, 2021

Table 6. Summary of the major economic inputs for the considered systems.

Items	Value	Note
Feedstock prices		
Willow food packaging	4 €/t	Transportation cost only; Vo et al. and Rajendran et al.
Utility prices		
Electricity	241.7 €/MWh	Based on Commercial Fuel Cost Comparison SEAI
Natural gas	82.3 €/MWh	
Raw material prices		
Renewable hydrogen	3.8 €/kg	Assuming from water electrolysis using wind energy; Jacobs and Parkinson et al.
Product selling prices		
Biomethane	4.6 c€/kWh	EU weighted average in 2nd semester 2021 (ex-VAT)
Biofertilizer	4 €/t	Transportation cost only; Bose et al.

Vo et al. Appl Energy, 2018
Rajendran et al. Renew Energ, 2019
Jacobs, Towards a zero carbon future, 2020
Parkinson et al. Energy Environ Sci, 2019
Bose et al. Renew Sust Energ Rev, 2022

Mass balance

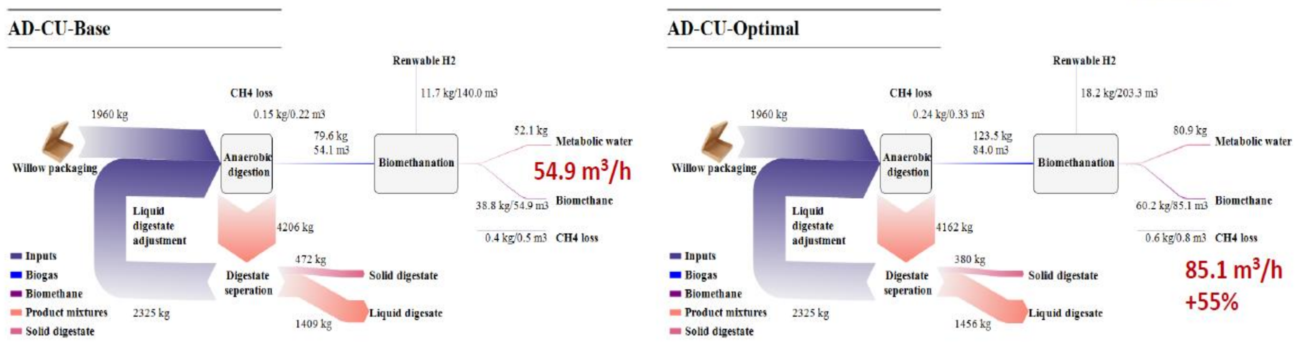


Fig. 16. Hourly mass balance of the AD-CU-Base and AD-CU-Optimal cases. AD represents anaerobic digestion; CU represents biogas upgrading.

Table 7. Summary of mass and energy balances for the considered systems.

Items	AD-CU-Base	AD-CU-Optimal	Note
Plant inputs			
Willow (kg/h)	1960	1960	LHV = 13.4 MJ/kg
Renewable hydrogen (kg/h)	11.7	18.2	Assuming 4.4 kWh/Nm³ H_2
Electricity (kW_{el})	73.7	90.8	PEF = 1.952
Natural gas (kW_{th})	121.8	122.1	PEF = 1.1
Plant outputs			
Biomethane (Nm^3/h)	54.9	85.1	LHV = 34.9 MJ/Nm^3
Efficiency			
Plant energy efficiency (%)	27.0	35.5	

CAPEX and OPEX

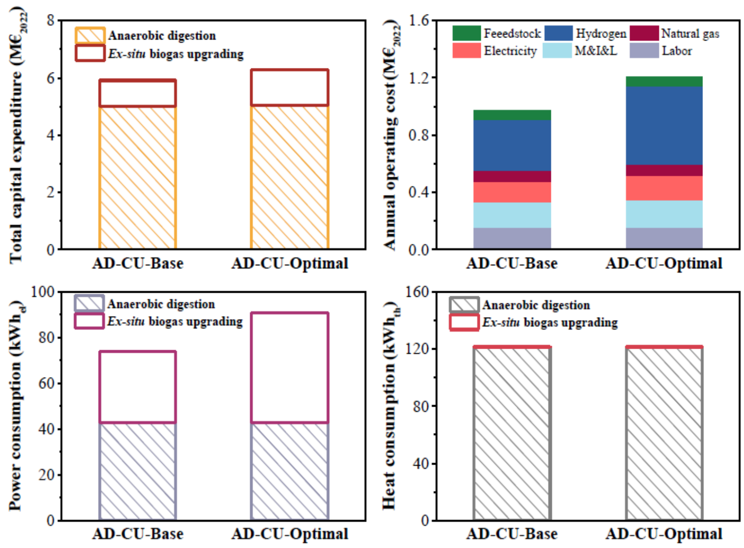


Fig. 17. Total capital expenditure (A), annual operating costs (B), hourly power consumption (C) and hourly heat consumption (D) of all four evaluated systems.

Table 8. Summary of overall economics of two cases.

Items	AD-CU-Base	AD-CU-Optimal
Capital expenditure (M€)	5.91	6.29
Operating costs (M€/year)	0.97	1.21
Biomethane production (MNm^3/year)	0.43	0.67
MSP of biomethane (c€/kWh)	47.4	35.7

- **Minimum selling price of biomethane is more than 35.7 c€/kWh; 7.8 times higher than the EU weighted average price in 2021.**

Electricity & Gas Prices in Ireland - 2nd Semester (July – December) 2021, SEAI

Scaled-up cases

The six-tenths factor rule was used to estimate the capital costs of the scaled-up systems according to Dimitriou et al. [5], as follows:

$$C_2 = C_1 \times \left(\frac{S_2}{S_1}\right)^{0.6} \quad \text{(S8)}$$

Where C_1 and C_2 are the CAPEX of the base and large systems, respectively, S_1 and S_2 are the capacities of the base and large systems, respectively, and 0.6 is the scaling factor. In this study, the same contribution percentages were assumed in estimating the operating costs of the scaled-up systems.

- System capacities are scaled up **5.9 times** based on the capacity of a commercialized upgrading plant;
- The six-tenths factor rule is applied to estimate the CAPEX of the scaled-up systems;
- Same contribution percentages of OPEX to CAPEX are assumed.

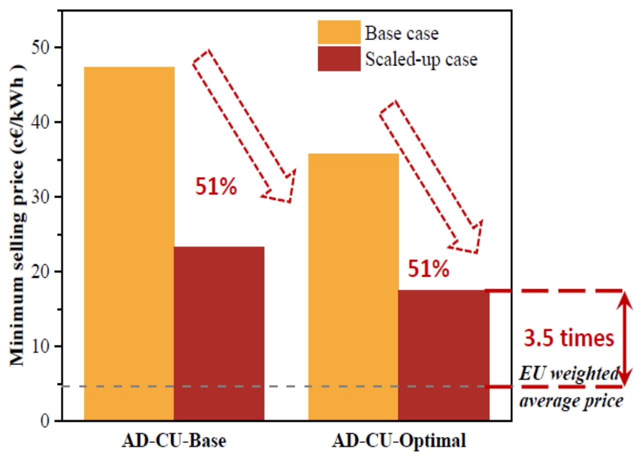


Fig. 19 Minimum selling prices of biomethane of the scaled-up system in comparison with the base case.

OUTCOMES

- Research plan on valorisation of willow had been discussed between UCC and UL before the experiments were started. Lignin was removed from willow before it is digested to produce biomethane. The extracted lignin was valorised to carbon materials (biochar and activated carbon). The carbon materials was then added into the digester to enhance biomethane production from lignin derivatives in a circular approach.
- Deep eutectic solvents (DES) pretreatment of willow was conducte. Three types of DESs were synthesized and applied to the pretreatment of willow to remove lignin. The pretreatment conditions (such as pretreatment temperature, reaction time, and molar ratio in different DESs) were optimized using response surface methodology in order to achieve the highest lignin removal. Under each optimal condition with different DESs, pretreated willow was subjected to AD; the digestion period lasted for more than 30 days. Once the most suitable type of DES is defined, it will be employed to extract lignin from willow for further use. The fertility of the digestate after AD experiments will also be analysed.
- Biochar and activated carbon were produced from lignin and applied to the AD of lignin derivatives. The AD experiments lasted for more than 30 day. Once the better additive (either biochar or activated carbon) is determined, it was applied into the AD of willow extractives.

Optimised DESs pretreatment of willow for efficient biomethane production.

- Three different DESs systems (choline chloride and acetic acid (CA), choline chloride and propionic acid (CP), and choline chloride and lactic acid (CL)) were applied to willow pretreatment at varied reaction temperatures (120, 140 and 160 °C) and time (15, 30 and 45 min). Results showed that all the DESs pretreatment could effectively remove lignin and increase the subsequent biomethane production from willow. The raw willow contained 25.33% lignin and presented a biomethane production of 89.9 mL/g total solids (TS). Under the optimal pretreatment conditions, DESs pretreatment with CA, CP, and CL removed 72%, 71%, and 80% of the lignin in raw willow, respectively. In the subsequent biomethane potential assays the pretreatment with CA, CP, and CL led to an increase of 36%, 17%, and 36% in biomethane yield, respectively.

Enhanced biomethane production from willow through the addition of biochar/activated carbon derived from willow lignin.

- High-quality biochar and activated carbon were produced from the lignin extracted from willow and applied in the digestion of lignin monomer and willow. Biomethane potential assays of the lignin monomer syringaldehyde (the major component in willow lignin) and willow were conducted to compare the effects of biochar and activated carbon on biomethane yield. With the optimal addition of biochar and activated carbon, the biomethane yield from both syringaldehyde and willow increased by 20 – 40%. Simultaneously, the lag-phase time of the digestion process was significantly reduced. This study demonstrated that both biochar and activated carbon could significantly enhance the anaerobic digestion of lignin derivatives and willow.

Improved biohydrogen and volatile fatty acid production from seaweed through the addition of conductive carbon materials.

- Fermentative production of biohydrogen and volatile fatty acids (VFAs) from advanced feedstocks such as seaweed provides opportunities in the carbon-neutral bioeconomy. This study evaluated the effects of

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European Regional Development Fund

BioWILL

Integrated "Zero Waste" Biorefinery utilising all fractions of Willow feedstock for the production of high to medium based Bio-Chemicals/Materials, Renewable Energy in the form of Bio Methane production and Natural Fertilisers.

WP. T3.9.5: Report of Environmental Life Cycle assessment of the AD digestate biofertilizer.

September 2023

MATERIA NOVA Materials R&D Center

Preface

T3 – Optimise Product Development of Willow Residues

WP T3 – Activity 9
Environmental Sustainability and economic viability assessment of a zero-waste willow based biorefinery.

Deliverable 9.5 Report of Environmental Life Cycle assessment of the AD digestate biofertilizer. Description of the Life Cycle Assessment (LCA) methodologies and outcomes from the bio-fertilizer value chain processes compared to mineral fertilizer. It will incorporate the GHG data and carbon sequestration modelling from the pot trials.

Introduction

BioWILL aims to design a biorefinery where high value biomolecules, such as salicin extracted from willow bark will be used to produce phytopharmaceutical products. For a zero-waste system, the willow pulp and waste bark will be processed to form catering or food packing materials. When the packing materials come to the end of their useful life, anaerobic digestion will be used to treat these materials to produce and valorise bio-methane and a digestate product.

Objective and limitations

The objective for this deliverable was to provide a quantitative environmental analysis for the comparison between BioWILL AD digestate biofertilizer and mineral fertilizer using primary data from GHG measurements and carbon sequestration modelling.

As the BioWILL project advanced it become apparent that the BioWILL fertiliser would not class as a biofertilizer. In fact any digestate products would not be accepted by current regulations and any fertilising qualities were said to originate principally from inoculum coming from an AD plant using dairy manure as a feedstock. Without the AD digestate being able to fulfil the function of being a fertiliser, the LCA comparison and study against mineral fertiliser was not possible.

The project partner Profession J.J Leahy from the University of Limerick confirmed that according to "*FPR 2019/1009 the revised fertilizer regulation currently does not permit the use of Animal By-Products in fertilizing products, (they are covered separately by the Animal by-product regulation). Animal manures which provided our inoculum and are used as feedstocks in the majority of AD plants in the EU but the digestate will not qualify unless there is a change to the regulation*." Following further discussion with the project partner Chen Deng from the University College Cork, in her professional opinion "*the fertilizer equivalence is highly dependent on the inoculum. The nitrogen content in raw willow has little impact on it as nitrogen content in willow is too low*".

Furthermore, carbon sequestration modelling and GHG data were not available and the time of writing this report. Partners at the University of Limerick had commenced litter bag experiments but explained that "*to have sufficient datapoints to develop a kinetic model will require sampling for an estimated 5 years. This was unforeseen. The EU requires us to estimate the residual carbon after 100 years, therefore a robust kinetic model is warranted.*"

Conclusions

According to current regulations it was not possible to compare BioWILL digestate products as a fertiliser against mineral fertiliser. The pertinence of using AD as the waste management strategy for willow packaging should be considered. Alternative routes such as composting should be investigated.