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Integrated Deep-Lake Water Cooling Infrastructure for Continuous Operation and Monitoring: The Case of a Bioreactor for a Sustainable Lake

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Abstract

This paper presents the fundamentals for the development of a dual-chamber bioreactor integrated into deep-lake water cooling systems. The proposed design enables controlled side-stream operation, continuous monitoring of key water-quality parameters, and automatic bypass mechanisms that default to untreated return water in the event of system deviation. The design incorporates upstream screening and filtration, moderated shear flow, removable fibre cartridges, and operational redundancy. The tannase enzymatic hydrolysis of tannic acid by modified *Aspergillus niger* (*A. niger*) culture bound to a cellulose-based microfiber support provides low hydraulic resistance and allows controlled release into the natural environment. The integrated system is capable of operating continuously under lake-derived hydraulic and thermal conditions while allowing controlled release into the environment. In addition, the proposed integration with the existing deep-lake cooling infrastructure introduces engineering and operational uncertainties, including bio-fouling, membrane degradation, sensor drift, and failure modes under variable flow conditions. This study proposes a solution to the need for a structured infrastructure-integrated system that could help mitigate the effects of climate change by reducing massive carbon emissions.

Keywords

Dual-Chamber Bioreactor, Enzymatic Hydrolysis, *Aspergillus niger*, Infrastructure Integration

1. Introduction

Research conducted by the University of Lausanne (UNIL) revealed that Lac Léman (hereafter Lake Geneva) emits equivalent amounts of carbon dioxide as Lausanne automobile transport [1]. Scientific theories attribute Lake's carbon emissions to the degradation of organic material. However, organic material is not present in abundant quantities in Lake Geneva, researchers at UNIL discovered ***that the unbalanced carbon cycle*** can instead be attributed to the erosion of limestone in the surrounding area. Rainwater causes limestone to erode, releasing bicarbonate and calcium ions into the lake basin. In summer heat, calcium ions precipitate as calcite, triggering carbon emissions. GA is a phenolic acid known to chelate at calcium ion growing sites preventing precipitation, theoretically reducing downstream CO_2 production. The proposed integrated bioreactor should facilitate GA production by naturally inducing tannin hydrolysis in a culture of immobilised, modified *Aspergillus niger* (*A. niger*).

2. Contextual Research

2.1. Biochemical Reaction Pathways

2.1.1. Understanding the Chemical Pathway for Calcite Precipitation in Lake-Water

Lake Geneva is surrounded by a rocky geography that contains large amounts of limestone, chemically recognised as calcium carbonate. Calcium carbonate in the surrounding area erodes with regular rainfall and weathering. This causes the release of bicarbonate and calcium ions that flow into water basins. High temperatures accelerate algae growth, altering pH and thermal conditions. Changes in the lake environment promote catalytic precipitation of these ions into microscopic calcite particles [1]. Calcite precipitation releases CO_2 into the atmosphere. Additionally, the formation of suspended calcite micro-particles results in a milky blue-green coloration.

Although Lake Geneva naturally produces CO_2 as a result of the carbon cycle, algae populations play a vital role in the absorption of CO_2, helping to balance net emissions. The additional output from limestone erosion releases more CO_2 than algae can recycle. Algae are not present in sufficient quantities and excess CO_2 is recycled, creating net gas emissions. Contrary to traditional theories that assume that carbon release comes from biological processes, additional carbon production can be largely attributed to geological processes: limestone erosion and calcite precipitation.

2.1.2. Mechanistic Estimate: GA Chelation Reduces Free Ca^{2+}, Calcite Precipitation, and CO_2 Release

The proposed mechanism is that dissolved GA complexes Ca^{2+}, lowering the free-ion activity that controls calcite supersaturation and precipitation. A simplified net precipitation reaction is:

$$Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3(s) + CO_2 + H_2O$$

so (approximately) each mole of calcite prevented corresponds to one mole of aqueous/atmospheric CO_2 not generated.

For Lake Geneva, dissolved calcium is typically on the order of ~1 mmol·L^{-1} (tens of mg/L) [2]. Let $C_{Ca,T}$ be total dissolved calcium, C_{GA} be the GA concentration in the mixed return stream, and α be the fraction of GA present in a Ca-binding (deprotonated) form under lake pH (order-of-magnitude: $\alpha \approx 0.1 - 0.3$). Assuming a 1:1 dominant complexation stoichiometry and a conditional stability constant K_{cond} (which depends on pH and ionic strength) [3], a first-order speciation estimate is:

$$C_{Ca^{2+},free} \approx \frac{C_{Ca,T}}{1 + K_{cond}\alpha C_{GA}}.$$

Reconciling concentrations: the chelation examples below use *illustrative* μmol·L^{-1} GA to show the sensitivity of $C_{Ca^{2+},free}$ to C_{GA}. However, the **proposed open-return design** release level later in the design is $C_{GA,return} = 0.5 - 2\ \mu g \cdot L^{-1}$, which corresponds to ≈0.003 - 0.012 μmol·L^{-1} (170 g/mol). At those concentrations, the chelation term is very small.

Because calcite saturation and precipitation kinetics scale with free Ca^{2+} activity, the relative reduction in precipitation driving force is approximately the relative reduction in $C_{Ca^{2+},free}$ (all else equal). For example, taking $C_{Ca,T} = 1.0\ mmol \cdot L^{-1}$, $\alpha = 0.2$, and a conservative $K_{cond} = 10^4\ L \cdot mol^{-1}$:

- **Proposed return range:** if $C_{GA} = 0.5 - 2\ \mu mol \cdot L^{-1}$ (≈0.003 - 0.012 μmol·L^{-1}), then $K_{cond}\alpha C_{GA} \approx 6\times10^{-6} - 2\times10^{-5}$ and $C_{Ca^{2+},free}$ decreases by only ~0.0006 - 0.002% (negligible).
- **Sensitivity (not the proposed release):** if $C_{GA} = 5\ \mu mol \cdot L^{-1}$ (≈0.85 mg·L^{-1}), then $K_{cond}\alpha C_{GA} \approx 0.01$ and $C_{Ca^{2+},free}$ decreases by ~1%.
- **Sensitivity (not the proposed release):** if $C_{GA} = 50\ \mu mol \cdot L^{-1}$ (≈8.5 mg·L^{-1}), then $K_{cond}\alpha C_{GA} \approx 0.1$ and $C_{Ca^{2+},free}$ decreases by ~9%.

Conclusion: at the proposed open-return release level (0.5 - 2 μg·L^{-1}), the GA concentration is far too low to plausibly alter free calcium activity at bulk-lake scale; any measurable reduction in free Ca^{2+} would require return-stream GA in the ≳mg·L^{-1} range (or a demonstrable, sustained, highly localised microenvironment at much higher GA prior to dilution).

2.1.3. Carbonate-System Mass Balance: Reduced Calcite Precipitation Is Not Demonstrated Net Atmospheric CO_2 Reduction

To distinguish internal carbonate chemistry from climate impact, we treat the lake/bioreactor return stream as an open system where dissolved inorganic carbon (DIC), total alkalinity (TA), pH, and air-water gas exchange co-evolve. A reduction in calcite precipitation can reduce CO_2 *produced by precipitation*, but net atmospheric CO_2 drawdown requires demonstrating that the air-water CO_2 flux becomes more negative (into the water) over the relevant spatial and temporal boundary.

We track linked outcomes with a minimal mass-balance bookkeeping:

- **DIC** (C_T):

$$\frac{\mathrm{d}C_T}{\mathrm{d}t} = \underbrace{\frac{F_{CO_2}}{h}}_{\text{gas exchange}} + \underbrace{R_{\text{bio}}}_{\text{GA biodegradation(to CO}_2\text{)}} + \underbrace{R_{\text{mix}}}_{\text{mixing/advection}} .$$

Note: calcite precipitation does not remove DIC; it redistributes it into CO_2 + $CaCO_3$.

- TA

$$\frac{dTA}{dt} = -2R_{prec} + 2R_{diss} + \Delta TA_{bio} + TA_{mix}$$

- **pH and speciation**: given (C_T, TA, T, S), carbonate equilibria determine $\left[CO_2^*\right]$, $\left[HCO_3^-\right]$, and $\left[CO_3^{2-}\right]$, and therefore pH. Preventing precipitation primarily preserves TA (avoids the $-2R_{\text{prec}}$ term), which shifts speciation away from CO_2 and can reduce $pCO_2(aq)$.
- **Air-water CO_2 flux**: $F_{CO_2} = k\left(pCO_2(aq) - pCO_2(air)\right)$ (sign convention: positive to the atmosphere). Demonstrated net atmospheric reduction requires measuring/modeling k and showing sustained $F_{CO_2} < 0$ at the system boundary.
- **GA biodegradation**: if GA (or its intermediates) is oxidised microbially, it produces CO_2 and can lower TA depending on net proton production/consumption; therefore, GA persistence/half-life and biodegradation stoichiometry must be tracked alongside any carbonate-chemistry benefit.

This framework makes the evaluation criterion explicit: *reduced calcite precipitation* is an internal pathway change, whereas *net atmospheric* CO_2 *reduction* is an external flux outcome that must be demonstrated via coupled measurements of DIC/TA/pH and air-water gas exchange.

2.1.4. Calcium Carbonate Solubility

The solubility of calcium carbonate in water, illustrated in Figure 1, helps explain this mechanism. In aqueous systems, dissolved CO_2 reacts with water to form bicarbonate. According to Le Chatelier's principle, increased calcium carbonate consumption through precipitation shifts equilibrium to the production of additional bicarbonate, which in turn increases CO_2 generation through associated reaction pathways. Therefore, limiting the precipitation of calcium carbonate micro-particles—by reducing the availability of free calcium ions—would decrease the consumption of bicarbonate and ultimately reduce the production of CO_2 within the system at higher temperatures.

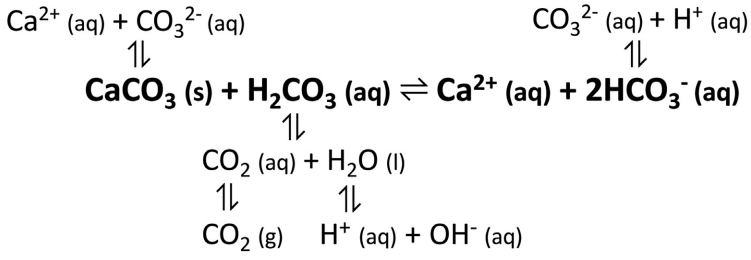

Figure 1. Calcium carbonate solubility [4].

2.1.5. Shikimate Pathway

Polyphenols are a class of chemical compounds that are synthesised naturally by plants, bacteria, fungi, and select archaea [5]. Based on the structure and number of binding sites, polyphenols can be further divided into 4 groups: flavanoids, stilbenes, ligans, and phenolic acids [5]. The shikimate pathway is a 7-step metabolic system that bio-synthesises many aromatic amino acids and polyphenols, including GA [6]. The graphic below outlines the bio-chemical products of the shikimate pathway, illustrating the variety of chemical products given from this metabolic process.

Gallic acid (3,4,5-trihydroxybenzoic acid) (GA) is formed early in the shikimate pathway. Glucose is catalysed into phosphoenolpyruvate (PEP) and erythrose-4-phosphate (E4P) by glycolysis and the pentose phosphate pathway. PEP is then reacted with E4P to produce 3-dehydroshikimate (3-DHS) via the shikimatic acid pathway, which is used to synthesise GA. The change in 3-DHS to GA results from spontaneous oxidation, alternatively from the dehydroshikimate dehydrogenase enzymes. 3-DHS loses hydrogen and rearranges to become GA in these reactions. As seen in **Figure 2**, the alternative to gallic production is shikimic acid. The natural equilibrium leans towards a higher production of shikimic acid,

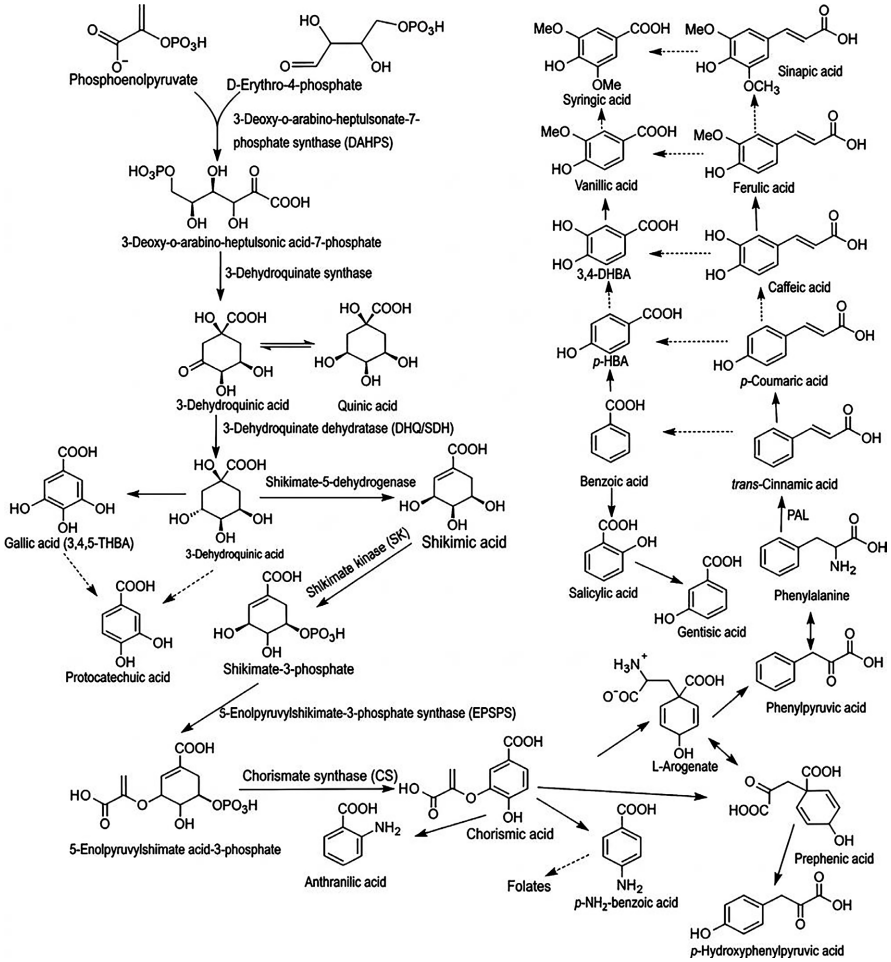

Figure 2. Shikimate pathway [7].

which can be used to synthesise many other phenolic compounds. AroE genes control the expression of shikimate-5-dehydrogenase that competes with the GA-producing reaction. By destroying AroE genes, equilibrium moves in favour of GA.

Moving the equilibrium of the reaction towards GA production may not be the most efficient genetic modification. GA can also be biosynthesised from tannins by hydrolysis. Tannins can be found in degraded organic material and are naturally abundant and can be hydrolysed by tannase enzymes to release GA and glucose, as shown Figure 3. In the shikimate pathway, GA is combined with UDP-glucose or β-Glucogallin to create tannins. In reverse of this process, by initiating the hydrolysis of tannins, GA can be produced more efficiently and with fewer intermediate steps than through the shikimic acid pathway.

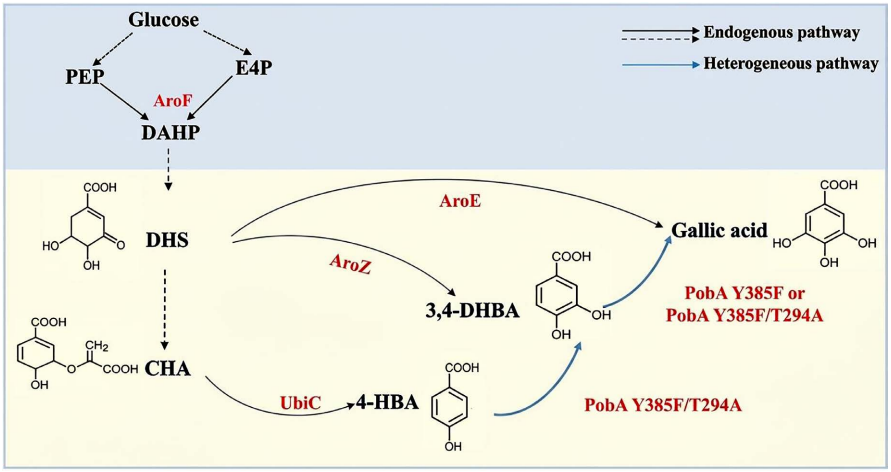

Figure 3. GA endogenous pathway and heterogeneous pathway of *E. coli* [8].

In plants, DHS is associated with dehydroquinase (DHQ), forming a bifunctional enzyme. However, in fungi such as *Neurospora crassa*, the fifth domain in the pentafunctional AROM polypeptide; a polypetide that catalyses five of the seven steps of the shikimate pathway [9]. Although the exact molecular basis for the recognition and reduction of 3-DHS is unknown, analysis of the entire genome of *E. coli* K12 and pathogenic O157:H7 revealed an unknown gene that has a 25% sequence identity share with AroE, potentially concluding that AroE and YdiB are paralogs.

In *Aspergillus niger*, the genes that control GA degradation and tannase expression are better identified. *tanA* has been identified to control extracellular tannase productions and lead to higher conversions of tannins to GA. Additionally, *tanR* activates the transcription of the catabolic tannase and GA genes. Overexpression of both genes could optimise tannin hydrolysis to produce more GA. In addition, *tanX* and FAD-monooxygenase repress the accumulation of GA, allowing the equilibrium to be shifted towards production of tannins instead of GA. Manipulation of these pathways using PglaA and Ptef1 promoters with crisper genome editing to overexpress *tanA* and *tanR*, while destroying FAD monooxygenase and deleting *tanX*, would result in fungi optimised for GA production.

2.2. Microfiber Cell Bioreactors Using Immobilized Microbes

A hollow microfiber cell culture system can be defined as "a 3D cell culture system that uses a bundle of semi-permeable hollow fibres to mimic the physiological environment of blood vessels" [10]. This type of cell culture system is used to optimise the growth and harvest of cells more efficiently compared to traditional 2D cell culture methods. This culture method allows culture media to circulate through the interiors of hollow fibres, allowing nutrients, gases, and waste products to diffuse both ways through the fibre walls. If the genetically modified fungus culture is imobilized within the extra-capillary space of the FiberCell cartridge, as seen in Figure 4, using cellulose walls, then tannins from the surrounding solution of lake water can diffuse across the membrane to interact with tannase and produce GA that diffuses back across the membrane. The GA will then move through this chamber to another where it can be filtered to remove glucose (see Figure 5).

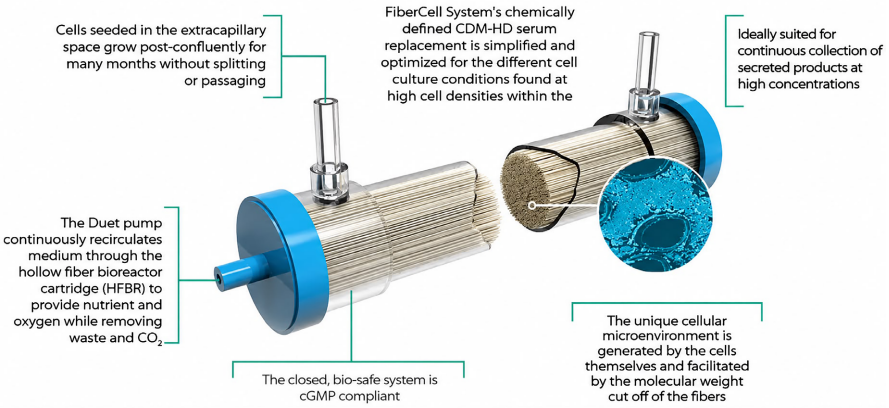

Figure 4. An illustration of the FiberCell systems cartridge [10].

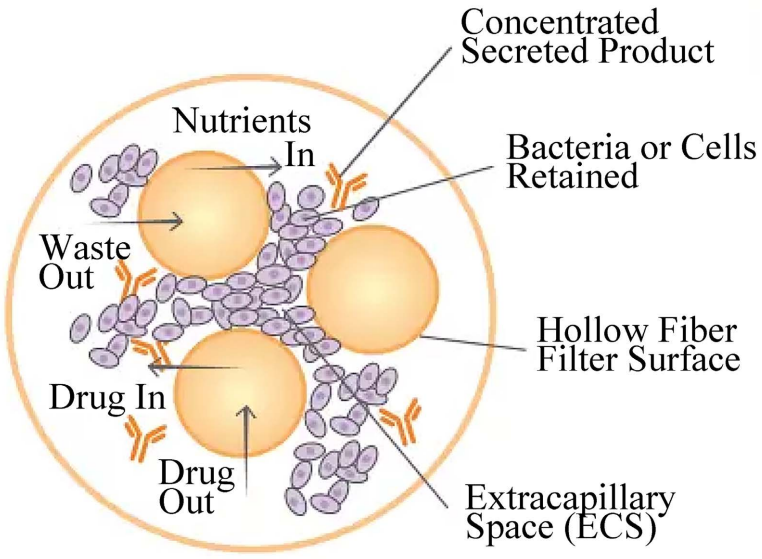

Figure 5. Diagram of how substances move across the membrane in a hollow fiber cell system [10].

If properly adapted, this would provide a simple, coherent mechanism for tannin hydrolysis using a modified fungi culture. It allows for immobilisation and protects the opportunity for rapid diffusion of materials in and out of cells so that biochemical processes can be leveraged.

Synthesis of Tannins from Willow Bark

Willow is a well-researched source of polyphenolic compounds, particularly condensed tannins and similar phenolics. Polyphenols are naturally synthesised as secondary metabolites, acting as defence agents against herbivory, pathogenic, and oxidative stress. When used in bioreactor systems, such as submerged wood-based reactors, tannins are slowly leach into surrounding water.

In an aqueous environment, tannins are mobilised mainly by diffusion and hydrolysis of bark tissues. Water penetrates the bark matrix and dissolves soluble phenolic compounds, while microbial activity further enhances tannin release by decconstructing the lignocellulosic structures that bind polyphenols. Both fungal and bacterial decomposers enable the depolymerisation of complex phenolic compounds. Increasing tannin availability in the water column. The release rate depends on the conditions and the hydraulic residence time within the bioreactor.

Willow bark bioreactors leverage these natural processes to introduce tannins into aquatic systems in a controlled manner. Tannins are chemically reactive and can inhibit calcite precipitation by chelating calcium ions, thus indirectly influencing carbonate equilibrium and CO_2 dynamics in water bodies.

Willow bark bioreactors provide a sustainable, nature-based solution to regulate water chemistry over extended periods, integrating biological, chemical, and microbial processes.

2.3. Optimal Concentrations of Tannic Acid

Experimentation with optimal concentrations of tannic acid for enzyme hydrolysis using tannase revealed that the optimal concentration lies lower. This is because high concentrations of tannins can form complexes that inhibit enzyme activity.

- Unmodified *Aspergillus niger* and *A. fumigatus* in culture medium: 2% tannic acid was optimal for tannin release [11].
  - 15 g/L (1.5%) tannic acid concentration resulted in high tannase activity of 26.4 U/mL for a strain producing tannase [11].
- *A. awamori* tannase: 25.3 mg/ml or 2.53% tannic acid was the optimal concentration to maximise the release of GA liberation [11].

2.4. Commentary on Previous Research

Research such as Hamad & Soliman's investigation into Congo red dye decolorization using alginate-immobilised *Aspergillus niger* found a high removal efficiency based on Langmuir and Freundlich isotherms and kinetic modelling, demonstrating effective biotreatment of textile dyes. Again, research conducted by Prof. Dr. Ülkü Mehmetoglu at Ankara University investigated the application

of immobilised conidia of *A. niger* for citric acid production.

However, no investigation has been done on the use of a dual compartment bioreactor for the production of GA using immobilized *A. niger* in a fiber cell system. In addition, the application of naturally synthesised compounds produced in a bioreactor integrated into deep-lake cooling systems is a unique and promising proposal that could mitigate climate change effects by reducing carbon emissions.

2.5. CRISPR-Cas9 (CRISPR-Associated Protein)

CRISPR-Cas9 (CRISPR-associated protein 9) is a gene editing enzyme that uses CRISPR sequences as a guide to recognise and open specific strands of DNA that are complementary to the CRISPR sequence [12]. This technology can be used to edit genes within living organisms [13]. Guide RNA is made to match the specific sequence and is combined with the Cas9 protein; together it cuts DNA. This opening can then be closed by cell regeneration, causing the gene to be inactivated, or scientists can insert a modified gene and consequently alter the DNA sequence [12].

3. Problem Statement and Requirements for Dual Chamber Bioreactor Design

3.1. Problem Statement

Current synthetic biology approaches to managing carbon emissions in freshwater systems focus on engineering specific algae to act as carbon sinks, instead of addressing the root of the problem. Additionally, current approaches to in-situ carbon management in freshwater systems do not have the possibility to integrate scalable and controllable pathways into existing infrastructure. Specifically, there are no proposed processes that couple biochemical conversion and integration without negatively impacting water quality, ecological health, or public health. This paper presents a solution to the need for a patented infrastructure-integrated contained system operating under lake-derived hydraulic and thermal conditions while controlling release.

3.2. Design Requirements

The system proposed in this paper is subject to the following functional and safety requirements:

3.2.1. Safe and Manageable Genetic Modification of Immobilised Fungi

The development of this system beyond theoretical applications is contingent on the development of a safe and manageable modified fungus culture. Culture should pose no risk to the lake ecosystem and should effectively complete the proposed pathway under simulated lake conditions.

3.2.2. Continuous Operation Compatibility

The system should operate continuously within the flow rates and temperature ranges of the existing deep-lake cooling system, without imposing hydraulic losses or disrupting the cooling performance.

3.2.3. Preservation of Water-Quality

The return stream should remain within predefined water-quality constraints, relative to the intake conditions, and this will include but is not limited to pH stability, dissolved and total organic carbon (DOC/TOC), optical properties (colour and UV absorbance), and dissolved oxygen demand. Indicative target ranges are summarised in Table 1.

Table 1. Acceptable value ranges for quantifiable water-quality variables according to CIPEL and Swiss WPO [14].

Quality	Acceptable Range
pH	6.5 - 8.5
DOC/TOC	<1.5 to 2.0 mg/L
Turbidity	<1.0 - 2.0 NTU
Phenolics	≤2 µg/L
Dissolved Oygen (DO)	>4.0 mg/L

3.2.4. Containment and Fail-Safe Operation

This system should include passive and active fail-safes; this includes automated bypass to untreated cooling return flow in the event of deviation from a parameter, equipment failure, and data collection for observation and analytical purposes.

3.2.5. Monitoring and Controllability

This system should include continuous monitoring of key processes and water quality to allow real time control, fault detection, and data collection for validation purposes.

3.2.6. Staged Validation Capability

This design should enable progressive validation and development, including bench-scale testing, closed-loop pilot operation, and mesocosm evaluation, without direct lake release prior to any development of an open pilot system. The development of an open lake system integration is contingent on the fulfilment of predefined go/no-go criteria at each stage of validation.

3.2.7. Regulatory and Ethical Compliance

The system should be designed to provide the opportunity for regulatory re-view and ethical oversight by enabling conservative operation, reversibility, and transparent documentation of performance and potential risk.

4. Genetic Modification of *A. niger*

Genetic modification of *A. niger* is the basis for the core process—tannin hydolysis. While tannase, the key enzyme that catalyses the hyrolysis of tannins, is produced in unmodified *A. niger*, it is normally used (net direction) to convert GA into hydrolysable gallotannins. Genetic modifications of *A. niger* would be to optimise tannase production, facilitate GA accumulation, and adapt the fungi to operate in low temperatures. The main proposed edits and their expected system-

level effects are summarised in Table 2.

4.1. Over-Expression of *tanA*, *tanR* and *tanX* Genes

In *A. niger*, the genes that regulate the expression of tannase have been identified as *tanA* and *tanR*. Overexpression of the *tanA* gene would increase the production of extracellular tannase, leading to higher conversion rates of tannins to GA. Using a CRISPR promoter mechanism, this gene can be selected and overexpressed. The Ptef1 promoter is a constitutive promoter that is continuously active and enables high expression of the selected gene. This type of promoter is best suited for cases where continuous gene expression is needed. Given these facts, the Ptef1 promoter is the best suited promoter for the *tanA* gene.

To optimise transcription, mimicry of natural gene regulation using *tanR* native co-expression is likely to be the most effective promotion type. The use of a native promoter would work differently from a non-native promoter in that it would replicate the relationship between the tannin concentration of the medium and enzymatic production. In other words, in tannin-rich media, the fungi will respond to the increase in concentration by producing and transcribing tannase enzymes and GA conversion. The co-expression would enable both genes to be expressed in a balanced ratio that mimics the natural functioning of the shikimate pathway.

Table 2. Compact reconciliation of the manuscript's gene-editing logic for tannase-driven hydrolysis and GA accumulation in *A. niger*.

Target	Native role (as used here)	Edit	Expected system-level effect
tanA	Encodes extracellular tannase (key hydrolysis catalyst).	Overexpress (e.g., strong/ constitutive promoter).	Faster tannin → GA + glucose conversion; higher throughput and lower required residence time.
tanR	Transcriptional activator coordinating tannase/GA catabolism genes in response to tannin signals.	Overexpress/ co-express with *tanA* using native regulation where possible.	Better coupling of enzyme production to tannin availability; stabilises performance under variable feed.
tanX	Regulator influencing tannase-related transcriptional response to tannin derivatives.	Tune (downregulate or delete).	Allows for accumulation of gallic acid independent of the environmental conditions.
creA	Global carbon catabolite repressor; suppresses tannase/hydrolase expression when glucose is present.	Down-regulate (e.g., RNAi/ CRISPRi).	Reduces glucose-mediated repression; maintains hydrolysis even as glucose accumulates downstream.
FAD-monooxygenase	Contributes to GA degradation (negative to GA accumulation).	Knockout or strong down-regulation.	Increases GA accumulation/export by slowing intracellular GA breakdown; improves net GA yield.

tanX is a regulatory protein whose role is to help control the expression of genes that encode tannase, such as *tanA*, in response to environmental conditions. It acts as a transcriptional activator or co-regulator, responding to the presence of tannins or their derivatives. When induced, *tanX* promotes the transcription of tannase genes by binding directly to promoter regions or interactions with other transcription factors involved in the regulation of the carbon source. Regulation of *tanX* can help optimise the transcription reaction and improve the fungi sensitivity to tannin availability. Effectively *tanX* regulates the tannase enzymes which make gallic acid depending on the environmental conditions of tannic and gallic acids and so by deleting or downregulating *tanX*, gallic acid production will continue independent of the environmental conditions: allowing for accumulation of gallic acid [15].

4.2. Down-Regulation and Knockout Genes

In the genome of *A. niger*, specific genes are designed to maintain homeostasis and manage the over-production of GA by providing negative feedback or degrading the acid. Deletion or down-regulation of these genes can help improve the GA capacity that can be produced and contained within the fungi cell.

FAD-monooxygenase controls GA degradation; deletion of this gene using CRISPR-Cas9 will slow GA degradation, allowing it to accumulate.

creA is a DNA-binding repressor that binds to consensus CreA-binding sites in the promoter regions of tannase genes and other extracellular hydrolase genes. It acts to prevent the fungus from producing enzymes for complex carbon sources when an easily metabolisable carbon source such as glucose is available. When bound, it blocks transcription and overrides induction signals despite the presence of tannin. Repression of this gene using RNA interference techniques would impair its transcription and thus remove tannase transcription repression.

5. System Overview

The proposed system can be divided into six steps, including infiltration from the deep-lake cooling loop;

1) Chamber 1: tannin production/extraction/conditioning
2) Transfer with lake water to hydrolysis cell: carrier stream
3) Chamber 2: microfiber hydrolysis cell that produces GA and glucose
4) Separation: glucose removal
5) Return to the lake through the existing outflow stream

The schematic below, **Figure 6**, indicates the theoretical movement of water through the cooling system and indicates the several steps of the bioreactor processing.

5.1. Integration with Deep-Lake Cooling Systems

The first step, the intake of the deep-lake cooling loop, is the primary determinant of the functioning of this system. The reactor should be located on the outflow.

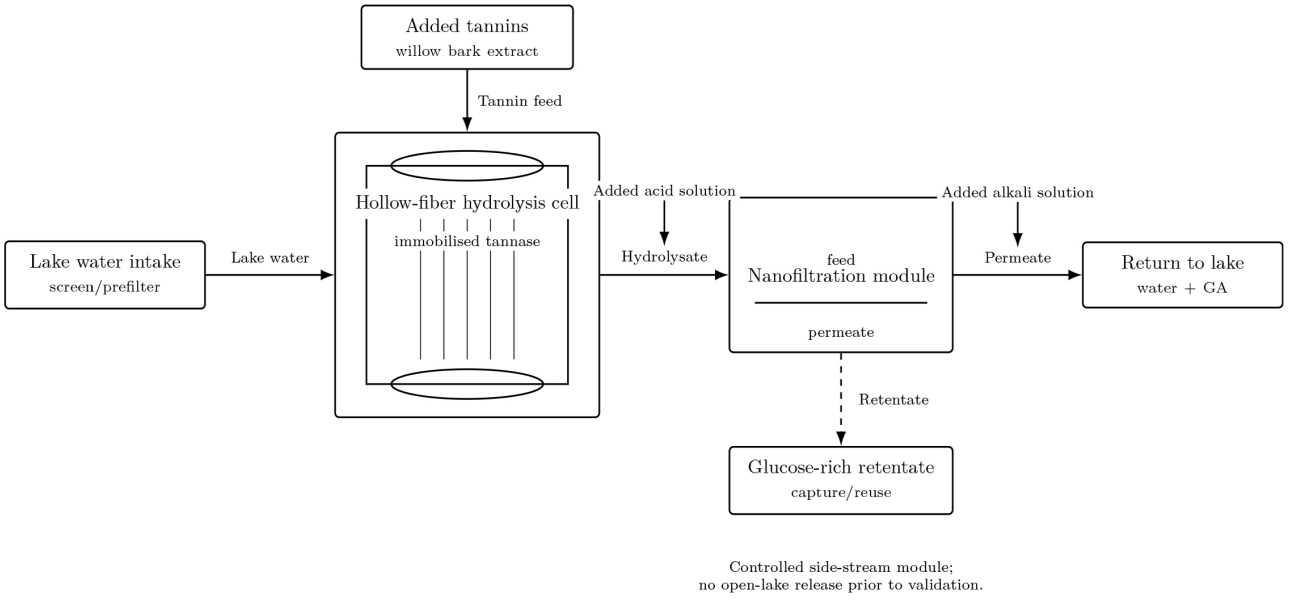

Figure 6. Schematic of the proposed side-stream process integrated into a deep-lake cooling loop. Hydrolysable tannins are converted to GA (GA) and glucose in a hollow-fibre hydrolysis cell (immobilised tannase), followed by nanofiltration to retain glucose and return a permeate stream containing GA to the lake.

stream of the cooling system, and water will branch from this stream to be moved through the bioreactor chambers. Although it may be warmer than the inlet stream, outlet streams are should still be relatively cool because deep-lake cooling systems have a limited impact on water temperature. Cooler water conditions will slow the rate of reaction, potentially leading to slower diffusion and hydrolysis of tannins.

The research conducted by the House of Switzerland concluded that tannase production under submerged fermentation conditions of *A. niger* peaks at approximately 35˚ Celsius after 96 hours of fermentation. The water circulated through the proposed bioreactor system is likely to be in the range of 7˚C - 8˚C [16]. As such, metabolic rates will be significantly slower at these low temperatures, making tannase production less efficient. For this reason, the modified fungi culture will need to undergo further modification to optimise its metabolism at such low temperatures. However, the hydrolysis chamber could be fitted with a heating system to warm the water within the chamber.

Consequently the stream would require subsequent cooling in the outlet stream prior to filtration of glucose molecules. Although this may be mechanically more complex, it reduces the need for additional genetic modifications that could impair the efficiency of the fungi. To mitigate the massive energy need, a solar panel battery system could be used to sustainably source electricity for heating and cooling. Taking this into account, genetic manipulation of fungi enzymes for their function at low temperatures may be the most sustainable approach to navigate the low water temperatures of deep-lake cooling outlet streams.

The side-stream attachment of the bioreactor system to the outlet streams of the deep-lake cooling system is a minimal risk approach to integration. Adjoining

the two systems in a branched structure facilitates easy monitoring, regulation, and gradual scaling. This approach should ensure that there is the opportunity to adjust and optimise the release and intake to control ecological impacts. Automated valves enable isolation of the reactor system; in case a problem arises, the reactor can be shut down independently. Additionally the inlet stream must have a disposable 5-micron filter to retain algae and sub-micron organic colloids that would interfere with the molecular weight cut-off (MWCO) filtration in the outlet stream.

5.2. Chamber 1: Tannin Generation Stage

Definition (used consistently here): Chamber 1 is a *willow-bark extraction and conditioning unit*, not a fermentation unit. Its function is to leach water-soluble *hydrolysable gallotannins* from milled willow bark into a circulating aqueous extract, then remove solids before the extract is metered into Chamber 2.

Representative material-flow description: dry willow bark is loaded into a screened basket (or packed cartridge) inside Chamber 1. A slipstream of lake water (or prefiltered cooling-loop water) is recirculated through the bark bed at controlled temperature and residence time to extract dissolved tannins. The resulting extract is clarified by an internal screen/inline cartridge filter (to prevent bark fines and biomass from passing forward), producing a *tannin-rich hydrolysate feed* that is transferred to Chamber 2 under flow control. Inline UV-vis/TOC (or Folin-Ciocalteu equivalents) provides a concentration signal used to regulate the extraction flow and to maintain a consistent tannin-equivalent feed to the hydrolysis cell.

Note: hydrolysable tannins are water-soluble and therefore extract readily; however, in open lake water they are also biodegradable and do not accumulate at high background levels, so the inlet lake-water stream is treated as having negligible tannin content.

5.3. Chamber 2: Microfiber Hydrolysis Cell

The purpose of this chamber is to hydrolyse the tannins produced from the primary chamber to release GA and glucose, using a microfiber cell reactor to optimise enzymatic hydrolysis and thus GA production. The microfiber cell architecture has been designed and tested to in commercial settings to maximise the surface area for diffusion of tannins across the membrane, is compatible with fungi immobilisation, and provides low hydraulic resistance relative to packed-bed alternatives. The large active surface area to volume ratio enables for high effective catalyst loading without an excessive pressure drop. This architecture is particularly beneficial for integration into lake-water side streams, because it maintains stable flow and minimises head loss.

Immobilisation confines catalytic activity within the bioreactor, preventing fungal spread into lake water, ensuring the safe and ethical use of an active fungi culture. The dominant pathway is the cleavage of galloyl ester bonds in hydrolys-

able tannins, or gallotannins, which yield GA and glucose. The stream travelling from chamber 1 and entering Chamber 2 is assumed to have a 2% w/v concentration.

$$\text{Gallotannin}\left(\text{Glc-}\left(\text{O-CO-Gal}\right)_n\right)+n\ \text{H}_2\text{O}\rightarrow\text{Glucose}+n\ \text{GA} \tag{1}$$

Due to the heterogeneous chemical nature of gallotannins, the degress of galloylation " n " is treated as an effective average. This allows the reactor to be analysed on a gallic-acid-equivalent basis. It is important to note that immobilisation does not alter the reaction stoichiometry but allows for the separation and containment of live cultures.

Reaction performance in this section is determined mainly by hydraulic residence time rather than batch exposure because of the continuous contact axial flow of liquid through the fixed microfiber matrix. This enables stable and predictable operation, residence time can be defined as:

$$\tau_{\text{h}}=\frac{V_{\text{h}}}{Q_{\text{h}}} \tag{2}$$

where τ_{h} is the residence time of hydrolysis, V_{h} is the effective void volume of the microfiber matrix, and Q_{h} is the volumetric flow through the reactor. This architecture separates reaction control from environmental mixing and allows the conversion to be tuned through flow adjustment without altering the enzyme loading or chemistry.

The geometry of microfiber cells promotes short diffusion distances between the tannin-lake water mixture and immobilised fungi, reducing internal mass transfer limitations. The reactor is designed to operate under conditions where convective transport dominates, and this minimises concentration gradients along the microfiber surface. In this stage no specific kinetic model is used, yet conversion behaviour can be interpreted using residence-time-based relationships common to plug-flow-like systems:

$$X_T=1-\exp\left(-k_{\text{eff}}\tau_{\text{h}}\right) \tag{3}$$

In this expression k_{eff} is an effective first-order hydrolysis constant.

The conversion of tannins in the hydrolysis cell can be modelled using the following equation:

$$X_T=\frac{C_{T,\text{in}}-C_{T,\text{out}}}{C_{T,\text{in}}} \tag{4}$$

where $C_{T,\text{in}}$ and $C_{T,\text{out}}$ are the inlet and outlet concentrations of hydrolysable tannins (or equivalent tannins), respectively. Additionally, the rate of GA, when framed as reactor output, can be expressed through the following equation.

$$r_{\text{GA}}=\frac{Q_{\text{h}}\left(C_{\text{GA,out}}-C_{\text{GA,in}}\right)}{V_{\text{h}}} \tag{5}$$

where r_{GA} is the volumetric GA production rate. To relate tannin conversion to predicted GA output, the following equation is to be applied:

$$\frac{\Delta n_{GA}}{\Delta n_{Glc}} \approx n \tag{6}$$

relates the effective degrees of galloylation of the tannin mixture to those of GA produced.

Finally, contamination risks and mitigation strategies are imperative for designing a safe integrated bioreactor system. Lake water contains suspended solids, colloids, and natural organic matter, making contamination a major concern. Contamination in the microfiber cell may arise from particulate deposition, biofilm formation, or adsorption of humic substances onto fibre surfaces. The proposed design incorporates upstream screening and prefiltration, moderated shear flow, removable fibre cartridges, and operational redundancy. Fouling is treated as an expected operational phenomenon rather than a failure mode, and maintenance intervals are incorporated into the system design from the outset. Each design feature enables filtration and cleaning of the microfiber cell system to reduce the influence of contamination.

In summary, microfiber hydrolysis cell systems serve as the biochemical reactive core of the process. By confining the reaction and immobilising catalytic activity, precise control of GA release and operation under continuous flow conditions is allowed, whilst minimising ecological and regulatory risk.

5.4. Transfer and Mixing of Tannins with Lake Water

The transfer and mixing stage serves as a connection point between the fermentation process and hydrolysis. It acts as a concentration-control and conditioning step in between the two-chamber processes. Its primary function is precise control tannin concentration and acts secondarily to control GA concentrations, hydraulic residence times, and mass transfer conditions using lake water as a carrier stream.

The relative flow rate of hydrolysate and carrier stream define the resulting GA concentration according to the mass-balance relationship shown below:

$$C_{mix} = \frac{Q_h C_h + Q_{lw} C_{lw}}{Q_h + Q_{lw}} \tag{7}$$

In this equation C_{mix} is the GA concentration in the mixed stream. Q_h and C_h are the volumetric flow rate and GA concentration of the hydrolysate. Lastly, Q_{lw} and C_{lw} represent the corresponding quantities for the lake-water carrier stream. Given that background GA concentrations in lake water are negligible, $C_{lw} \approx 0$, and dilution is primarily governed by the ratio Q_h / Q_{lw}.

This approach enables the management of concentration to be regulated through flow control instead of through chemical addition, preserving conservative operation in early validation stages and rapid response to condition variations.

Design release range (GA): For any open-return operation, a single conservative design criterion is adopted at the lake return boundary:

$C_{GA,return} = 0.5 - 2\ \mu g/L$ (consistent with the phenolics constraint in Table 1). Using $C_{lw} \approx 0$, this corresponds to $C_{GA,return} \approx C_h \frac{Q_h}{Q_{lw}}$, so for the upper-bound hydrolysate estimate $C_h = 18\ g/L$ the required dilution is $\frac{Q_h}{Q_{lw}} \approx 2.8\times10^{-8} - 1.1\times10^{-7}$.

Mechanistically, only a fraction of this GA is expected to contribute to Ca^{2+} complexation: $C_{GA,eff} \approx f_\alpha f_{lig} C_{GA,return}$, where f_α captures pH-controlled deprotonation (order 0.1 - 0.3) and $f_{lig} < 1$ captures competition from natural ligands (e.g., DOC/humics and carbonate). Therefore, lake-scale impact claims must be tied to measured $C_{GA,return}$, inferred $C_{GA,eff}$, and observed changes in calcite precipitation/CO_2 flux.

Representative Operating Scenario (Baseline)

To enable consistent feasibility calculations, we define a single representative baseline operating scenario for the integrated side-stream system and use it throughout the discussion:

- **Side-stream flow (treated return):** $Q_{ss} = 50\ m^3 \cdot h^{-1}$ operated continuously (24 h/day), so $V_{ss,day} = 1.2\times10^3\ m^3/day$.
- **Return location and mixing:** discharge to the lake at an existing cooling-system outfall with a diffuser at 30 - 40 m depth (below the summer mixed layer) to promote rapid dilution while avoiding surface-water optical impacts.
- **Seasonal lake-water temperature (summer case):** intake/side-stream feed $T_{lw} \approx 6^\circ C - 8^\circ C$ (deep-lake water), with ambient near-outfall receiving water at the return depth ≈ 8˚C - 10˚C.
- **Heating/cooling demand:** operate the extraction/hydrolysis stages at a moderate setpoint of 45˚C, using heat recovery from the cooling infrastructure where available; the representative net thermal/electrical requirement is taken as $E_{th} = 10\ kWh/day$ (excluding building HVAC loads).
- **GA return concentration and mass released:** adopt the upper-bound design setpoint $C_{GA,return} = 2\ \mu g/L$. The corresponding GA mass returned is $m_{GA,day} = C_{GA,return} V_{ss,day} \approx 2.4\ g/day$.

To go further, the mixing section provides a defined hydraulic residence time that enables the mixed stream to reach chemical and physical equilibrium prior to separation. Residence time is expressed in the following equation:

$$\tau_{mix} = \frac{V_{mix}}{Q_{tot}} \tag{8}$$

In this expression τ_{mix} is the hydraulic residence time of the mixing section and V_{mix} is the effective mixing volume. The residence time must be sufficient for homogenisation while minimising unnecessary exposure of GA to conditions that can promote degradation or microbial consumption.

The mixing is structured to occur under controlled turbulent conditions. This ensures rapid homogenisation without introducing excessive shear. Excessive

shear can damage membranes or promote downstream fouling of the system. Turbulence levels are chosen to achieve a high Péclet number regime, where advective transport dominates over molecular diffusion. As a result, concentration gradients can be kept to minimums. Notably, turbulence in this stage is used as the mass-transfer tool, and not as a dispersive release mechanism.

The main design feature of this section is controlled exposure. In contrast with typical uncontrolled discharge scenarios, the proposed design offers deterministic, reversible, and continuous concentration control.

In summary, this stage acts as the interface between reaction and separation, ensuring that the filtration operates under stable conditions and the feed remains within the concentration limits. As such, it acts as a key safety and control feature in the overall process architecture.

5.5. Nanofiltration

Nanofiltration was selected for this system to separate glucose from lake water and GA after tannin hydrolysis because of the challenges of separating glucose from an aqueous solution without heating the mixture. It is imperative that the stream that is returned to the lake remains within stable temperatures relative to the inlet conditions. As such, crystallisation or evaporation cannot be used. This stage aims to isolate glucose to prevent the growth of microbials and their ecological consequences.

Mixed-diamine-based nanofilm membranes are engineered with a precise pore size and molecular weight cut-offs. Given that the two molecules have similar diameters, molecular weight-based filtration is a more efficient solution. Mixed-diamine-based nanofilm membranes in this system would be designed with a sharp cut-off at approximately 170 Da. Even more so, mixed-diamine-based nanofilm membranes weight cut-offs are customisable, making them the most suited for high precision filtration.

Retained glucose can be collected from the filtration system and sold for commercial purposes, providing a source of revenue from this system, creating potential for it to be self-paying, giving companies an incentive to choose to implement this model in their cooling system.

Because the nanofilm mebrane is molecular weight based, no spores or mycelia should be able to escape the system. Spores and mycelia are much heavier than glucose molecules, lying in the gigadalton range, meaning that the existing nanofilm memebrane should have 100% retention of *A. niger* [17]. To flag compromise of the microfiber cell and the potential leakage of spores and mycelia into the outlet stream, sensor will be used after the nanofiltration step. These sensors include TOC monitors, indicating the potential release of spores if there is a tear in the filtration and triggering the system to stop. A tear in the nanofilm would allow glucose to pass into the outlet stream and this sensor would notice a sharp spike in TOC and trigger a closure of the system.

However, given the neutral pH of the lake water passing through the nanofilm,

MWCO filtration is insufficient to retain glucose effectively, while allowing gallic acid to be released into the outlet stream. In pH neutral solutions gallic acid deprotonates into gallate ions, which are negatively charged, and would be repelled by the electrostatic forces of the nanofilm MWCO filter; the repulsion would impeded. To mitigate potential inefficiencies of gallic acid release, treating the outlet stream from chamber 2 with a pH solution to drop pH to a range of 3.0 - 3.5, would allow for the gallic acid molecules to remain neutral and the highly hydrophilic glucose molecules would remain neutral. The filtrate would then be treated with a base solution to return the pH to 6.5 - 8.5. Moreover, the transmembrane pressure must remain lower due to the compressibility of sugar molecules in high pressure environments; compressed glucose molecules may become small enough in diameter to fit through the 170 Da pores and be released into the water system. This approach would ensure that gallic acid molecules are not retained in the filter and can be passed into the outlet stream.

5.6. Return Stream Quality Control

Return water quality must be strictly monitored and restricted with predefined criteria relative to the inlet stream. Significant deviation from these criteria can result in adverse ecological effects and changes in the ecosystem.

pH must remain within a specific range that is harmless to all aquatic life, so as not to impact microbial activity and larger food webs. Changes in pH can cause enzyme denaturation or promote microbial growth, causing damage to the lake ecosystem. By confining the pH within a conservative range, changes in the environmental condition due to the proposed system can be limited.

Dissolved oxygen is also an important parameter to monitor, it assesses the potential increase in microbial respiration associated with residual organic compounds. Changes in the oxygen uptake rate are treated as a conservative proxy of biological oxygen demand. This enables early detection of adverse biogeochemical responses prior to release to the lake.

The total organic carbon (DOC/TOC) and dissolved carbon are monitored as integrative indicators of organic loading. DOC can be used to assess the potential carbon availability, while TOC gives a mass-balance constraint on the TOC introduced by the process.

Properties, including colour and turbidity, must be monitored to detect residual phenolic compounds, colloidal material, or membrane breakthrough. These parameters serve as early indicators of treatment inefficiency and potential impacts on water clarity and treatability. In addition, residual tannin and total phenolic concentrations are monitored to verify effective hydrolysis and separation. This provides a safeguard system against the return of reactive precursors that may exhibit a different ecological behaviour than GA.

Through continuous monitoring and analysis of these parameters, the ecosystemic conditions will most likely remain relatively stable. In the event that these parameters are significantly deviated from, the system automation will self-reulate us-

ing the integrated software, and control inlet and outlet flow. Closed-loop pilot validation will provide a basis for these parameters but also provide the opportunity to integrate fail-safes and optimisation of the system to remain within safe parameters.

6. Monitoring, Automation, and Fail-Safes

In order for this system to operate autonomously, continuous data collection and extensive fail-safe measurements will need to be used.

Sensors will be placed in the intake, outlet and within various points within the body of water to ensure that there is no significant deviation from standard ecological markers from the initial intake prior to introduction of the system. Sensors will continuously measure pH, temperature, flow, ultraviolet-visible (UV-vis) absorbance, conductivity, and turbidity and compare them against a database of historical value ranges. Data will be collected years before the introduction of this system to observe how the lake ecosystem operates without influence. If a value is recorded and appears outside the predetermined acceptable range of values, the fail-safes of the systems will be activated. Automated valves will allow remote control of the intake and outlet, allowing for management of the product released into the lake. As such, the system automation will be able to self-regulate based on changes in the lake environment and will be able to cope and adapt to moderate these changes. If the deviation from the standard values is too large, the system will alert the monitors and stop: closing the inlet and outlet valves.

Monitoring and sampling of water quality and ecological health will enable operators to fully understand the impacts of this system on ecosystems and prevent irreversible damage. Transparent data collection and reports on the health and environmental conditions of the lake ecosystem should be publicly available, and all relevant investigations of the effects of the system on the ecosystem should also be publicly available.

7. Model-Based Theoretical Performance

7.1. Willow Bark Input to Tannin Concentration

Willow bark (*Salix spp.*) is a well-known tannin source. Depending on species, harvest season, and drying method, tannin concentrations can range from 5% - 15% w/w. For air-dried willow bark willow, a middle range of 8% - 12% with median values of 10% is typical.

Hydrolysable vs. condensed tannins (design assumption): willow bark contains a mixture of *hydrolysable tannins* (gallotannins/ellagitannins) and *condensed tannins* (proanthocyanidins). Only the hydrolysable gallotannin fraction can yield gallic acid via tannase-catalysed cleavage of galloyl ester bonds; condensed tannins do not produce GA under tannase hydrolysis. To keep the following mass-balance tractable, we define a single parameter f_{GT} as the mass fraction of the total "tannin" content that is present as hydrolysable gallotannins. In the *upper-bound* feasibility estimate below we take $f_{GT} = 1$ (*i.e.*, all extracted tannin is treated as tannic-acid-equivalent gallotannin), which maximises GA

yield and should be interpreted as a conservative, best-case bound.

Tannic acid is the most common and best understood model compound for hydrolysable gallotannins; the stoichiometry is dependent on the type of tannin available to the reaction, making reaction equations and yield calculations difficult to produce and variable. Given that hydrolysable tannins are sugar molecules esterified with phenolic acids (gallic and egallic), under hot, alkaline, or enzyme regulated conditions they undergo hydrolysis and release the phenolic acids: separating the gallic acid groups from the sugar. For these reasons, tannic acid is used as a proxy to hydrolysable tannins in the stoichiometric calculations below to give validity to the proposed theory.

The input willow bark can be assumed to have a 10% w/w tannin content [18]. Given that 5 kg of dry willow bark is added to Chamber 1, approximately 0.5 kg of total "tannins" will be available, of which $0.5 f_{GT}$ kg is treated as hydrolysable gallotannin-equivalent for GA-yield calculations. Willow tannins are polyphenols that are readily soluble in water. Hot-water or dilute acid extraction typically achieves 70% - 90% efficiency. If adequate residence time is allowed, it can be assumed that the extraction is 80% efficient.

Using 0.5 kg of available tannin and $f_{GT} = 1$ for the upper-bound case, approximately 0.4 kg will be extracted as hydrolysable gallotannin-equivalent. Thus, if 5kg of willow bark is submerged in 20 L of water, then:

$$\left[\text{Tannin}\right]_1 = \frac{0.4\ \text{kg}}{20\ \text{L}} = 20\ \text{g/L}\ \left(\text{or } 2\%\ \text{w/v}\right)$$

This concentration is standard for boiling extraction solutions and lies within the solubility range. *However, given the variability in tannin structure and the presence of condensed tannins* (*i.e.*, $f_{GT} < 1$ *in reality*), *the actual GA-yielding hydrolysable fraction and therefore GA production may be substantially lower than this upper-bound estimate*.

7.2. Tannin to GA and Glucose

Willow bark is composed of both condensed and hydrolysable tannins. In this process, only *hydrolysable gallotannins* are assumed to undergo tannase-catalysed hydrolysis to yield GA and glucose; condensed tannins are treated as non-GA-yielding and are excluded from the GA stoichiometry. The stoichiometry is dependent on tannin structure, but a representative reaction for tannic acid can be observed as follows:

$$\text{Tannic acid} + n\ \text{H}_2\text{O} \xrightarrow{\text{tannase}} 10\ \text{GA} + \text{Glucose} + \text{byproducts}$$

$$\left(\text{MW} \approx 1700\right) \rightarrow 10\left(\text{MW} \approx 170\right) + \left(\text{MW} \approx 180\right) + \text{other fragments}$$

The literature on enzymatic hydrolysis of tannase and acid-catalysed hydrolysis of willow tannins observes yield ranges of 40% - 65% w/w glucose recovery and proportional GA formation.

The molecular weights of each reactant and product can be assumed to be:

- Tannic acid (parent gallotannin): MW ≈ 1700 g/mol (polymer of $\tilde{1}0$ galloyl

units on glucose)

- GA: MW = 170 g/mol
- Glucose: MW = 180 g/mol
- Water added per hydrolysis: 18 g/mol per ester bond (10 bonds → 180 g H_2O)

Theoretical stoichiometry gives 10 mol of GA and 1 mol of glucose per mol of tannic acid when 180 g of water is consumed. The molar ratio of this reaction is 1:10, if 1700 g of tannic acid are hydrolysed, then 1700 g of GA should be released, assuming 100% efficiency From, the yield coefficient is 1 w/w or a 1:1 ratio. Using 0.4 kg of tannic acid, 0.4 kg of GA can theoretically be produced.

For GA:

- Molar ratio from equation: 1 mol tannic acid → 10 mol GA
- Mass basis: 1700 g TA → 10 × 170 g = 1700 g GA
- Yield coefficient: 1700 g GA per 1700 g TA = 1.0 (w/w or 1:1 ratio)
- For input of 0.4 kg: 0.4 kg × 1.0 = 0.4 kg GA (theoretical maximum)

For glucose, the reaction ratio is 1:1 mol of tannic acid to glucose. In terms of mass, this is 1700 g of tannic acid for 180 g of glucose, making the yield coefficient 0.106 w/w or 10.6%. Again, if 0.4 kg of tannic acid is input, the theoretical maximum glucose yield is 0.0424 kg.

For glucose:

- Molar ratio from equation: 1 mol tannic acid → 1 mol glucose
- Mass basis: 1700 g TA → 180 g glucose
- Yield coefficient: 180 g glucose per 1700 g TA = 0.106 (w/w or 10.6:1)
- For your 0.4 kg input: 0.4 kg × 0.106 = 0.0424 kg = 42.4 g glucose (theoretical maximum)

Applying the practical enzyme conversion efficiency of 80% - 90% in industrial and pilot systems, the GA yields will be lower than theoretical predictions due to incomplete hydrolysis, contamination, and insufficient residence time. If a conversion rate of 90% is assumed:

$$\begin{aligned}&\text{Practical GA} = 0.4\ \text{kg} \times 1.0 \times 0.9 = 0.36\ \text{kg GA} = 360\ \text{g},\\&\text{Practical glucose} = 0.4\ \text{kg} \times 0.106 \times 0.9 = \mathbf{0.0382}\ \text{kg glucose} = \mathbf{38.2}\ \text{g},\\&\text{Residual TA} = 0.4\ \text{kg} \times (1-0.9) = \mathbf{0.04}\ \text{kg} = \mathbf{40}\ \text{g}.\end{aligned} \tag{9}$$

The residual tannic acid will later be removed by the nanofilm.

7.3. Outlet Stream Concentrations

The concentrations of the outlet stream product can be calculated under the assumption that chamber 2 contains the hydrolysed broth at a total volume of 20 L.

$$\begin{aligned}&[\text{GA}] = \frac{360\ \text{g}}{20\ \text{L}} = 18\ \text{g/L},\\&[\text{Glucose}] = \frac{38.2\ \text{g}}{20\ \text{L}} = \mathbf{1.91}\ \text{g/L},\\&[\text{Residual TA}] = \frac{40\ \text{g}}{20\ \text{L}} = \mathbf{2.0}\ \text{g/L}\end{aligned} \tag{10}$$

7.4. Literary Validation

Through cross reference to the published literature, the calculation based predictions of tannin conversion and tannase performance can be supported. Table 3 below shows the yields from various published papers and the consistency with the predicted values from the proposed process. It is important to note that the published systems assume 90% conversion rates. These results are within a close range of those of published laboratory results, which validates the order of magnitude.

Even when considering margins of error, the proposed hydrolysate concentration should be treated as an *upper-bound design estimate* rather than a universal literature-consistent value.

Table 3. Comparison of GA (GA) production with literature.

Source	Substrate	Product	Concentration/Yield	Match?
Proposed system	Tannic acid	GA	18 g/L	–
Ebune *et al.*	Tannic acid	GA	18.32 g/L	Excellent
Aftab *et al.*	Plant tannin	GA	225 μg/mL	Order consistent
Selim *et al.*	Mixed tannins	GA	(0.225 g/L) ~85% - 100% conversion	Within range

Based on tannic acid stoichiometry and tannase conversion efficiencies in the literature, enzymatic hydrolysis of 0.4 kg of extracted tannin (in 20 L broth) produces approximately 360 g of GA (18 g/L) and 38 g of glucose (1.9 g/L) at conversion rates of 90% [19]-[21]. The fact that some published results (e.g., Aftab *et al.*) report substantially lower GA concentrations indicates sensitivity to (among others) substrate composition (tannic acid vs. plant tannin mixtures), operating conditions, enzyme source, and downstream losses; therefore, a pilot should report measured GA in the hydrolysate and in the post-dilution return stream. Residual tannin (40 g, 2 g/L) is removed by nanofiltration. Sensitivity analysis confirms that ±10% variations in enzyme efficiency yield 320 - 380 g of GA and 34 - 42 g of glucose, preserving the internal stoichiometric scaling.

7.4.1. Nanofilm Glucose Removal

Glucose is a highly soluble molecule and a readily biodegradable substrate. Upon decomposition, one gram of glucose consumes 1.07 g of dissolved oxygen, allowing for eutrophication and anaerobic zones [22]. If the glucose concentration entering the nanofilm is 1.9 g/L and the membrane is between 85% - 95% efficient, then the post filtration concentration should be within the range of 0.10 - 0.29 g/L. Using the information for BOD of glucose, the ecological impact can be estimated. If 0.19 g/L glucose is released, then the BOD should be close to 0.2 mg/ml.

The membrane rejection coefficient can be defined as

$$R = \left(1 - \frac{C_{\text{feed}}}{C_{\text{permeate}}}\right) \times 100\% \tag{11}$$

The rejection coefficient is important for the determination of the residual glucose in the outlet stream. A high rejection coefficient indicates low amounts of glucose released into the environment, and therefore a lower ecological impact.

These results show that the discharged water has a minimal glucose content and remains within the BOD limits of fresh water (<1 mg/ml) according to the EU Commission [23].

7.4.2. Residence Time Reasoning

Willow tannins are polar polyphenols that are soluble in water. Their extraction kinetics are dominated by solid-liquid diffusion and follow typical leaching profiles. Within the first 2 hours, the rapid extraction phase is followed by a slower diffusion-limited phase. The literature on willow bark hot-water extraction reports a nearly complete extraction in 4 - 6 hours at 40˚C - 60˚C. The residence time of chamber 1 can be defined as a function of the volume and flow rate.

$$t_1 = \frac{V_1}{Q} \tag{12}$$

where V_1 is the volume and Q is the flow rate. Through calculation, the mean residence time can be estimated. For residence times longer than 8 hours, the further extraction gain is marginal and increases in temperature, reduction of bark particle size, or mechanical aggregation should be considered.

The fibre cell provides high specific surface area for solid-liquid contact and facilitates batch recirculation or continuous leaching. Estimate fibre surface area: if using synthetic fibre medium (SSA ≈ 150 - 250 m^2/m^3), a 25 L chamber offers 3.75 - 6.25 m^2 of contact area, accelerating diffusion relative to stagnant leaching. The liquid left in the nanofilm chamber can be recirculated through chamber 2 to increase residence time and improve conversion should any tannins remain unhydrolyzed.

The enzymatic hydrolysis of tannic acid by tannase follows the kinetics of Michaelis-Menten, with reported Michaelis constants (K_m) for tannic acid ranging from approximately 3.81 to 7.3 $mg{\cdot}mL^{-1}$, indicating a strong substrate affinity between different sources of enzymes and operating conditions [24]-[26]. The maximum reaction velocities reported (V_{max}) span several orders of magnitude (0.0232 - 3333.33 $\mu mol{\cdot}mL^{-1}{\cdot}min^{-1}$), reflecting variations in enzyme origin, purity, concentration, and immobilisation; however, the qualitative kinetic behaviour is consistent, with first-order dependence on substrate concentration at low tannin levels and an approach to zero-order kinetics under substrate-saturated conditions. In the proposed system, the tannin concentration entering the second reaction chamber is approximately 20 g L^{-1} (20 $mg{\cdot}mL^{-1}$); well above the reported range K_m, so it is expected that the reaction will proceed near V_{max}, with overall conversion governed primarily by enzyme loading and residence time rather than substrate availability. The enzyme dosage is specified to achieve an assumed conversion of 90% within 8 - 12 h, consistent with reports from the literature such as Ebune *et al.*, who achieved 18.32 $g{\cdot}L^{-1}$ GA from tannic acid using *Debaryomyces*

hansenii tannase under optimised conditions [25]. For a representative chamber volume of 50 L and a volumetric flow rate of 5 $L \cdot h^{-1}$, the resulting residence time of 10 h lies within the range required to approach high conversion under substrate-saturated conditions, supporting the kinetic and design feasibility of the proposed process. The residence time in the fibre zone can be similarly estimated through the expression:

$$t_{\text{fiber}} = \frac{V_{\text{fiber}}}{Q} \tag{13}$$

Optimal operating conditions for enzymatic hydrolysis lie between 40˚C - 50˚C with a pH between 5.0 - 6.0. Since tannase is primarily an intracellular or surface-associated aeration in immobilised form, aeration is not required; however, moderate agitation will ensure substrate-enzyme contact and product removal. Moderate mixing will be achieved through continuous flow between chambers as a function of the side stream architecture.

7.4.3. Energy Requirements

Assuming that there is a continuous flow rate of 5 L/h and the chamber circulation is within 1 - 2 bar gauge pressure range and nanofilm filtration remains between 10 - 20 bar:

$$\begin{aligned} P_1 &= \frac{Q \times \Delta P}{\eta} = \frac{\left(\frac{5\ \text{L/h}}{3600}\right) \text{m}^3/\text{s} \times 20000\ \text{Pa}}{0.7} \approx 0.04\ \text{W}\ \left(\text{negligible}\right) \\ P_2 &= \frac{Q \times \Delta P}{\eta} = \frac{\left(\frac{5\ \text{L/h}}{3600}\right) \text{m}^3/\text{s} \times 150000\ \text{Pa}}{0.75} \approx 0.28\ \text{W} \end{aligned} \tag{14}$$

where P_1 is the pump power for low-pressure chamber circulation and P_2 is the pump power for nanofilm pressurisation. Given the negligible pressure drop across the chamber circulation (≤0.04 kWh/day) and the minimal pumping of nanofilms (≈0.007 kWh/day), the additional energy requirements for the proposed system are relatively minor and can be integrated with the cooling system power budget.

The thermal energy requirements for tannin extraction in chamber 1 at 50˚C and a starting temperature of 20˚C at a constant volume of 25 L can be expressed as follows.

$$Q = m \times c_p \times \Delta T = 25\ \text{kg} \times 4.18 \frac{\text{kJ}}{\text{kg} \cdot \text{K}} \times 30\ \text{K} \approx 3135\ \text{kJ} \approx 0.87\ \text{kWh}\ \left(\text{one-time startup}\right) \tag{15}$$

$$P_{\text{maintained}} \approx 0.087\,\text{kWh/h} \approx 2.1\,\text{kWh/day} \tag{16}$$

The additional energy for heating chamber 2 to optimise the enzyme condition can be modelled as follows:

$$Q_{\text{initial}} = 50\ \text{kg} \times 4.18 \frac{\text{kJ}}{\text{kg} \cdot \text{K}} \times \left(60 - 20\right)\text{K} \approx 8360\ \text{kJ}\ \text{ and }\ Q_{\text{cont}} \approx 2 \text{ - } 4\ \text{kWh/day} \tag{17}$$

Using the representative baseline operating scenario defined above (summer

case; $Q_{ss} = 50\ m^3 \cdot h^{-1}$; reactor setpoint 45˚C), the feasibility discussion adopts a single representative net thermal/electrical requirement of $E_{th} \approx 10$ kWh/day (plus minor pumping power).

8. Validation Plan

Validation would be structured as a "stage-gated" (see Table 4) program that allows for a controlled progression from bench experiments to closed-loop pilot operation and mesocosm assessment. Movement to the next stage of validation is contingent on meeting predefined criteria for conversion performance, separation reliability, fouling control, and ecological safety. These thresholds will ensure the protection of the lake ecosystem and drinking water.

8.1. Stage 1—Bench Chemistry and Proof of Chemistry

8.1.1. Objective and Chemical Basis

The goal of this phase is to confirm the reaction pathway and quantify the reaction yields. Stage 1 is designed to establish the chemical feasibility of the proposed modifications, without considering scale and ecological conditions. In this stage,

Table 4. Parameter-specific GO/NO-GO triggers for staged validation. Thresh-olds shown are initial design targets and must be refined and justified with pilot measurements and site-specific regulatory limits [27] [28].

Category	Sensor/metric	GO criterion (continue)	NO-GO trigger (trip condition)	Automatic action
Conversion (hydrolysis)	Y_{GA}, X_T; GA and glucose concentrations	Y_{GA} and X_T remain within the stage setpoint band for ≥8 h; GA:glucose stoichiometry consistent with expected n within tolerance	Drift outside setpoint band for >30 min, or GA:glucose stoichiometry indicates side reactions/measurement failure	Isolate reactor; divert to hold tank; require re-sampling before restart
Membrane performance	Glucose rejection R; TMP/flux trend; TOC downstream of membrane	$R_{glucose} \geq 90\%$ and TMP/flux remain within baseline envelope (no sustained deterioration)	Any sustained loss of rejection (e.g., $R_{glucose} < 85\%$), abnormal TMP rise, or downstream TOC step-change consistent with breakthrough/tear	Trigger bypass; close discharge valve; alarm + event log
Return-water chemistry	pH, DO, turbidity, DOC/TOC, phenolics	pH 6.5 - 8.5; DO > 4 mg/L; turbidity < 2 NTU; DOC/TOC < 2 mg/L; phenolics ≤ 2 μg/L (Table 1)	Any limit exceedance persisting > 10 min (or faster for abrupt spikes), or rate-of-change exceeds a defined alarm slope	Trigger bypass to untreated return water; divert treated stream to hold tank; alarm
Containment/ biosafety	Downstream particle/ATP/CFU/qPCR (as applicable); filter integrity ΔP	No detectable viable organism marker downstream above method LOD; integrity signals stable	Positive viability signal, integrity anomaly (sudden ΔP drop), or leak sensor trip	Immediate isolation and kill/hold procedure; stop operation; incident report
Bypass logic	Interlock state and valve position feedback	All interlocks satisfied; sensors healthy; power and comms stable	Any sensor fault, power loss, comms loss, or any NO-GO trigger above	Fail-safe: default to untreated bypass; lockout until cleared

the primary objective is to verify that the hydrolysable tannins produced from the fermentation of willow bark are converted to GA under conditions similar to and compatible with lake conditions. This stage would isolate the core chemical transformations and provide a proof of concept for our proposed biochemical reaction pathways, making it a crucial step in our developmental process. Stage 1 establishes the chemical credibility of the proposed system; by confirming that the fundamental transformation from tannins to GA occurs under realistic conditions and is predictable, it gives a valid justification for the following experiments. Without success in this stage, further validation would be scientifically unjustified.

The chemical reaction on which the proposed process is focused is the hydrolysis of hydrolysable tannins, also known as gallotannins. Gallotannins are composed of a glucose core esterified with multiple GA units. In hydrolytic conditions, cleaving these ester bonds results in the release of GA and glucose. This process is represented by the generalised reaction shown in the following. Here, η represents the effective degree of galloylation. This value is treated as an average value because of the heterogeneous nature of tannin mixtures, allowing reaction behaviour to be analysed without reliance on a single molecular structure.

$$\text{Gallotannin}\left(\text{Glc-}\left(\text{O-CO-Gal}\right)_n\right) + n\ \text{H}_2\text{O} \rightarrow \text{Glucose} + n\ \text{GA} \tag{18}$$

In the microfiber cell system, the reaction is catalysed by the tannase enzyme. This reaction can be seen in the following equation. Catalysis by tannase enables a faster and more efficient conversion of gallotannins to GA and glucose. Manipulation of genes that express tannase is the basis for genetic modification in the proposed process.

$$\text{Galloy lester} + \text{H}_2\text{O} \xrightarrow{\text{tannase}} \text{GA} + \text{polyol fragment} \tag{19}$$

8.1.2. Conceptual Experimental Approach

Bench validation experiments would be conducted using a microfiber hydrolysis cell that would be operated in a continuous configuration. The reactor would be configured to mimic the surface-area-driven contact and hydrodynamic traits of the proposed system, while providing the possibility of controlled variation of residence time. Residence time can be defined using the equation:

$$\tau = \frac{V}{Q} \tag{20}$$

where τ is the residence time, V is the effective reactor volume, and Q is the volumetric flow rate. This formula gives a scale-independent architecture for the interpretation of the conversion behaviour.

The experiments would evolve from model tannin solutions to willow bark tannin extracts and finally diluted solutions of lake water and willow bark tannins. This approach is intended to distinguish intrinsic reaction limitations from arising buffering capacity, ionic strength, or background dissolved organic matter.

8.1.3. Analytical Metrics and Performance Indicators

The reaction performance would be evaluated against the selective quantification

criteria of GA and glucose as the expected products of the hydrolytic reaction. These calculations would be supported by measurements of the total phenolic content and basic water-chemistry parameters. Tannin conversion would be expressed in terms of inlet and outlet concentrations according to the equation:

$$X_T = \frac{C_{T,\text{in}} - C_{T,\text{out}}}{C_{T,\text{in}}} \tag{21}$$

where $C_{T,\text{in}}$ and $C_{T,\text{out}}$ denote the inlet and outlet concentrations of hydrolysable tannins, expressed either as molar concentration or as equivalents of GA.

The formation of GA would be quantified using a yield metric that would be normalised to the input of tannin, shown in the equation.

$$Y_{\text{GA}} = \frac{C_{\text{GA,out}} - C_{\text{GA,in}}}{C_{T,\text{in}}} \tag{22}$$

These calculations cited above allow for the comparison of reaction performance across different feed matrices and operating conditions without obliging absolute optimisation in the early experimental stages.

8.1.4. Stoichiometric Consistency and Internal Validation

Because of the molecular structure of gallotannins, which contain a single glucose core, the stoichiometric consistency between GA and glucose formation provides an internal validation of the tannin hydrolysis pathway. The ratio of moles of GA produced to moles of glucose released can be used as an approximation of the effective degree of galloylation, where n represents the effective degree of galloylation:

$$\frac{\Delta n_{\text{GA}}}{\Delta n_{\text{Glc}}} \approx n \tag{23}$$

Significant deviation from this expected relationship would indicate incomplete hydrolysis, secondary degradation pathways, or analytical interference. This would trigger a need to reassess the reactor structure or operating conditions.

8.1.5. Evaluation Criteria and Scope

Success in Stage 1 experimentation would require reproducible detection of GA and glucose above analytical detection limits, stable conversion trends, and performance in lake-water matrices relative to model systems. No kinetic order or rate constants are assumed or imposed. The kinetic relationships are included solely to illustrate how the reactor parameters may be inferred from the experimental data.

Stage 1 is limited to chemical validation of efficiency and ignores membrane separation, fouling behavior, ecological toxicity, or net carbon outcomes. These aspects are delayed to the following validation stages to ensure that the fundamental chemical pathways are established prior to broader system-level evaluation and interference.

8.2. Stage 2—Membrane Separation Performance and Fouling Assessment

Stage 2 is designed to validate the selective separation performance of the proposed mixed-diamine-based nanofilm filtration step. The main objective is to demonstrate an effective removal of glucose from GA and glucose solution while maintaining the passage of GA and preserving overall water-quality. This stage addresses uncertainties related to separation efficiency, fouling propensity, and stability under realistic lake-water matrices.

8.2.1. Conceptual Approach

The performance of the nanofilm would be judged using the feed streams created during stage 1. Filtration would operate under constant trans-membrane pressure or constant flux conditions to characterise performance across representative operating regimes. Instead of basing fouling dynamics on rejection behaviour alone, the system would be operated for extended durations.

8.2.2. Analytical Metrics

The performance of separation would be quantified using a species-specific rejection coefficient:

$$R_i = 1 - \frac{C_{p,i}}{C_{f,i}} \tag{24}$$

where R_i is the rejection of the species i, and $C_{p,i}$ and $C_{f,i}$ are the concentrations of permeate and feed, respectively. Flux stability was evaluated by monitoring the normalised permeate flux:

$$R_i = 1 - \frac{C_{p,i}}{C_{f,i}} \tag{25}$$

$$\frac{J}{J_0} \tag{26}$$

where J is the instantaneous permeate flux and J_0 is the initial clean-water flux under equivalent conditions.

8.2.3. Evaluation Criteria and Scope

Successful validation in this step would require sustained glucose rejection above a predefined target range, manageable flux decline over time, and demonstrable flux recovery following cleaning. The unexpected concentration of compounds that could exacerbate the downstream ecological risk would result in an unacceptable outcome. In this stage, ecological effects are not considered as they are considered in later validation stages.

8.3. Stage 3—Eco-Toxicological Screening and Oxygen Demand Assessment

This stage is intended to identify potential biological and bio-geochemical risks associated with exposure to return streams containing GA. The objective is to

screen for effects on representative aquatic organisms and to quantify microbial mediated oxygen demand. Microbial mediated oxygen demand represents a critical risk pathway in stratified lake systems.

8.3.1. Conceptual Approach

Validation would use controlled microcosm assays using lake water and sentinel species selected to represent primary producers and consumers. Parallel assays would be conducted to quantify microbial respiration in response to exposure to GA and hydrolyzate, with and without glucose removal.

8.3.2. Analytical Metrics

Biological responses would be measured using growth, immobilisation, or reproduction endpoints depending on the type of organism. The oxygen demand would be evaluated through changes in the concentration of dissolved oxygen over time and would be expressed as volumetric oxygen consumption.

$$\mathrm{OUR} = -\frac{\mathrm{d}[\mathrm{DO}]}{\mathrm{d}t} \tag{27}$$

where $[\mathrm{DO}]$ is the concentration of dissolved oxygen and OUR is the oxygen uptake rate.

8.3.3. Evaluation Criteria and Scope

Progress from this stage would require the absence of statistically significant adverse biological responses and oxygen consumption rates that remain within conservative thresholds relative to untreated lake-water controls. Chronic and community-level effects are deferred to later-stage mesocosm testing.

8.4. Stage 4—Closed-Loop Integrated Pilot Operation

In stage 4, system-level performance and control were evaluated by operating the full process train in a closed-loop configuration without environmental release. In this stage, the goal is to demonstrate stable integration of tannin hydrolysis and separation. Additionally, this stage aims to provide evidence for stable monitoring under realistic hydraulic and thermal conditions while maintaining the quality of the outlet water within predefined constraints.

8.4.1. Conceptual Approach

The integrated system would circulate lake water and return it to a holding reservoir rather than directly re-introducing it into the lake. Operation over extended periods would provide the opportunity to capture cumulative effects such as fouling, sensor drift, and control responsiveness.

8.4.2. Analytical Metrics

The performance of the system would be judged by adjusting the inlet water quality and outlet water quality parameters. This includes pH, dissolved organic carbon, turbidity, and dissolved oxygen. An overall consistent balance of materials would be evaluated using:

$$\Delta C_i = C_{i,\text{out}} - C_{i,\text{in}} \tag{28}$$

where C_i represents the concentration of species i.

8.4.3. Evaluation Criteria and Scope

In this phase, successful validation would require that the quality of the outlet water relative to the quality of the input water remain within the predefined deviation limits under steady and perturbed operation. Any persistent deviation that triggers automatic bypass or shutdown would constitute an unacceptable outcome for environmental testing, thus blocking the progression to the final testing stage.

8.5. Stage 5—Mesocosm-Scale Environmental Validation

This final stage of validation takes ecosystem level concerns into consideration. Stage 5 aims to assess the integrated effects of the treated return stream on the physical, chemical, and biological dynamics of a simplified lake analogue prior to consideration of open-system introduction.

8.5.1. Conceptual Approach

Mesocosms containing lake water, sediment and natural biota would be managed under natural light and temperature cycles. Treatments would consist of untreated control groups and exposure to bioreactor return streams.

8.5.2. Analytical Metrics

The endpoints for this stage would include the dynamics of dissolved oxygen, pH stability, dissolved organic carbon trends, and indicators of primary producer productivity. The net system response would be analysed using relative change metrics:

$$\Delta X = \frac{X_{\text{treatment}} - X_{\text{control}}}{X_{\text{control}}} \tag{29}$$

in this equation X represents a monitored ecological or chemical parameter.

8.5.3. Evaluation Criteria and Scope

The progression from this stage to an open system would require that no sustained deterioration of water quality or biological indicators, relative to the controls, is observed. However, this stage does not establish regulatory compliance, but informs risk-benefit assessment and decision-making.

9. Ethics and Limitations

9.1. Ethical Considerations

The proposed system involves the intentional introduction of a biologically active organic compound into a natural freshwater ecosystem. Lake Geneva is not only a complex ecological system but also a critical drinking water source and a shared trans-boundary resource. Therefore, any intervention raises substantial ethical responsibilities related to environmental stewardship, public health, and intergenerational equity.

A primary ethical concern is the risk of unintended ecological harm. GA is a phenolic compound that can influence microbial activity, oxygen demand, metal mobility, and organismal health. Even low-concentration continuous input may produce cumulative or nonlinear effects that are not readily predictable from laboratory studies alone. Ethical implementation therefore requires the adherence to the precautionary principle, in which the absence of complete scientific certainty cannot justify environmental release without robust safeguards.

A second ethical issue concerns irreversibility and consent. Once introduced into an open lake system, chemical additions cannot be easily retrieved, and downstream impacts may affect communities and ecosystems beyond the immediate release zone. Ethical deployment would require transparent stakeholder participation, regulatory approval, and international coordination, particularly given the shared governance of the lake.

The proposed design addresses these concerns by emphasising containment, monitoring, and reversibility. Integration into an existing deep-lake cooling infrastructure enables controlled side-stream operation, continuous monitoring of key water-quality parameters, and automatic bypass mechanisms that default to untreated return water in the event of system deviation. Furthermore, this work explicitly frames environmental introduction as conditional and experimental, contingent upon prior validation in closed-loop, laboratory, and mesocosm settings.

Finally, ethical responsibility extends to scientific integrity. This study does not claim a demonstrated environmental benefit, but rather proposes a theoretically grounded system whose efficacy and safety must be empirically established. Claims regarding CO_2 mitigation are therefore presented as hypotheses rather than confirmed outcomes.

9.2. Limitations

This work is subject to several important limitations inherent in its conceptual and model-based nature. First, no experimental or field data are presented to validate GA production rates, hydrolysis efficiency, membrane separation performance, or ecological responses. Reactor performance, conversion efficiency, and long-term operational stability are inferred from established chemical principles and analogous systems rather than direct measurement.

Second, the mechanism by which GA may contribute to net CO_2 reduction remains uncertain. Although several plausible pathways are discussed, GA is also readily biodegradable and microbial mineralisation could result in CO_2 release rather than sequestration. Without empirical measurements of carbon fate, residence time, and long-term storage, a net benefit to the climate cannot be assumed.

Third, ecological responses are highly system-specific. Lake Geneva exhibits spatial and seasonal variability in temperature, stratification, nutrient availability, and biological communities. These factors may significantly influence the fate and impact of the GA introduced, limiting the generalisability of this proposal to other lakes or even to different zones within the same lake.

Fourth, the proposed integration with the deep-lake cooling infrastructure introduces engineering and operational uncertainties, including bio-fouling, membrane degradation, sensor drift, and failure modes under variable flow conditions. Although mitigation strategies are described, their effectiveness remains untested.

Finally, regulatory and social constraints represent a non-technical limitation. Even if the system proves chemically and biologically viable, regulatory approval for chemical addition to a drinking-water source may not be attainable, and public acceptance may limit the deployment irrespective of technical merit.

9.3. Summary

Together, these ethical considerations and limitations underscore that the present work should be viewed as a framework for hypothesis generation and system design, rather than as a deployable solution. The primary contribution of the proposal lies in its integrated and infrastructure-coupled approach and in identifying the critical scientific, ecological, and ethical questions that must be resolved before any environmental application can be considered.

10. Conclusion

The proposed system is a unique ***hypothetical system*** to directly address the root of this particular carbon problem while integrating into existing infrastructure. The ***proposed*** biochemical approach gives the system a sustainable and natural aspect, theoretically making it safer to introduce into a natural environment. The system could be easily scaled to large applications by replicating the dual-chamber bioreactor in multiple areas of the lake periphery. In addition, this process ***would*** allow for adaptations and control based on environmental feedback, making it a tailored approach to each lake ecosystem. A dual chamber bioreactor system, such as the one proposed in this paper, can not only be adapted to have integration with existing infrastructure, but also to be adapted to different types of infrastructure such as water treatment outlets and dams. It is in this fact that this system ***proposes*** a realistic solution to the bio-geochemical issues that result in large carbon emissions ***that must be verified through experimental trials***. Continuous data collection and passive fail-safes would be implemented to ensure safe operation and monitoring of ecological conditions to avoid any adverse effects.

11. Next Steps

This research is purely speculative and does not include any experimentation that validates the proposed solutions. For this reason, the next step in validating this research would be bench-scale experimentation with the modified *A. niger* cultures to ensure that the proposed gene edits function in the desired way. The first step to validating this approach is to experiment with different gene promoters to test their efficiency in overexpressing the *tanA* and potentially the *tanR*. Validation of the synthetic biology modifications is the basis for this system proof of concept. All genetic modification will need to undergo extensive testing to ensure

that the fungi are not harmful to the ecosystem but effective in producing tannase and allowing GA accumulation. Next, bench-scale experiments validating the proposed tannin hydrolysis process, followed by membrane specificity screening, ecological risk assessment, and closed-loop pilot systems are essential before progression to an integrated approach.

Author Contributions

Olympia Betts conducted the experiments and wrote the paper, Tamás Kovács supervised the research and wrote the paper.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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