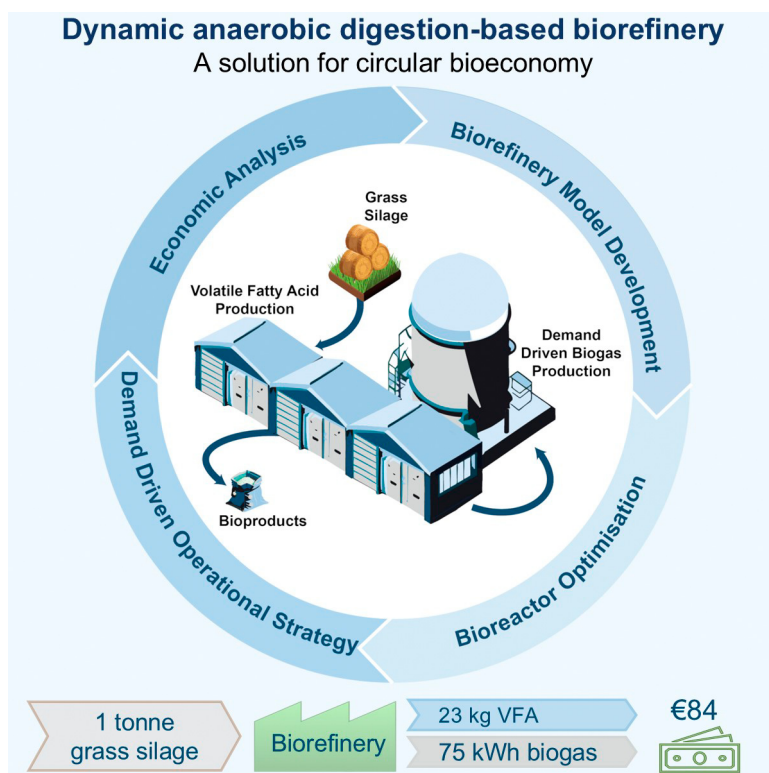


## Research Article

# Dynamic anaerobic digestion-based biorefineries for on-demand renewable energy and bioproducts in a circular bioeconomy



This study proposes a dynamic biorefinery model using anaerobic digestion to convert grass into bioproducts and bioenergy. A demand-driven operational strategy lets the biorefinery adapt outputs to changing market conditions. While currently unprofitable, the study supports anaerobic digestion-based biorefining in diversifying farm incomes and transitioning from fossil resources.

Rajas Shinde, Anga Hackula, Milena Marycz, Archishman Bose, Richard O'Shea, Susanne Barth, Jerry D. Murphy, David M. Wall

[david.wall@ucc.ie](mailto:david.wall@ucc.ie) (D.M. Wall).

## Highlights

Anaerobic digestion-based biorefinery exemplifies a bespoke circular bioeconomy system.

The AD-based biorefinery simultaneously produces high-value organic acids and biogas from grass silage.

A dynamic approach enables the biorefinery to adapt operations to changing market conditions.

A closed-loop operational strategy reduces waste and enhances the circularity of the process.



An economic analysis shows that the proposed model is not profitable under current conditions.

Technological advancements and policy support are vital to ensure system viability.

AD-based biorefineries can support farm-income diversification and reduce reliance on fossil resources.

## Research Article

## Dynamic anaerobic digestion-based biorefineries for on-demand renewable energy and bioproducts in a circular bioeconomy

Rajas Shinde <sup>1,2,3</sup>, Anga Hackula<sup>1,2</sup>, Milena Marycz<sup>1,2,4</sup>, Archishman Bose<sup>1,5</sup>, Richard O'Shea<sup>1,2</sup>, Susanne Barth<sup>3</sup>, Jerry D. Murphy<sup>1,2</sup>, and David M. Wall <sup>1,2,\*</sup>

**Anaerobic digestion (AD) is an important biotechnology for treating biodegradable residues and producing bioenergy, yet its full potential remains untapped. We investigate a two-phase AD system for biorefinery applications, producing valuable bioproducts, such as volatile fatty acids (VFAs) and biogas, from grass feedstock. We introduce a demand-driven operational approach to match market conditions, while minimising water use by reusing the process effluent. The proposed biorefinery model yields ~23 kg of VFAs and 75 kWh of biogas, with a potential gross revenue of €84 per tonne of grass. However, a preliminary economic analysis indicates that this biorefinery model is currently unprofitable. A sensitivity analysis suggests that reducing operating costs through technology advancements and policy support are vital to ensure economic viability. Such biorefineries offer opportunities for the diversification of farmers' incomes and the transition away from fossil resources. Our work exemplifies the role of AD as a key biotechnology in the circular bioeconomy.**

## Introduction

Agriculture is responsible for over 11% of total **greenhouse gas (GHG)** (see [Glossary](#)) emissions in the EU [1]. In countries with expansive livestock farming, such as Ireland, this figure is as high as 37%, owing to the high emissions from enteric fermentation and manure management [2]. With the current policies and measures in place, agricultural emissions in the EU are projected to reduce by a mere 1.5% between 2020 and 2040, making agriculture a hard-to-abate sector in achieving climate-neutrality goals [3]. Farmers face a significant challenge to be climate compliant in an ever-changing policy landscape, increasing their financial strain and impacting their well-being [4]. This, among other reasons, has led to recurring protests by farmers across the EU. Addressing these issues requires urgent action, including the diversification of farm incomes, while transitioning away from emission-intensive farming practices. Cascading biorefineries converting biomass to bioproducts and bioenergy in a multiproduct process could be a potential solution. Agricultural grasslands, covering 13% of the EU area in 2018, and over 90% of the agricultural land in Ireland<sup>#</sup>, are recognised for their role in providing livestock feed, ecosystem services, and carbon sequestration under the EU's Common Agricultural Policy [5]. With the EU cattle herd size declining [5], surplus grass and grass unfit as animal feed can be used for biorefining. Supplying feedstocks, such as grass, for biorefineries offers a dual solution: creating alternative sources of income for farmers and reducing emissions associated with traditional farming systems [6].

Conventionally, **AD** has been used to treat biodegradable residues and produce biogas (bioenergy) and digestate (biofertiliser). However, the economic viability of AD systems is

## Technology readiness

The proposed two-phase anaerobic digestion (AD) system for biorefinery applications combines a leach bed reactor (LBR) and an upflow anaerobic sludge blanket (UASB) reactor. Both reactors are commercially developed and widely used: LBR for high-solid content organic waste and UASB for high-strength industrial effluents and sewage. Therefore, independently, both reactors can be considered at a TRL of 9. However, the combination of LBR and UASB for the simultaneous production of volatile fatty acids (VFAs) and biogas is largely unexplored. Our research identified a few LBR–UASB combinations primarily for biogas, with one industrial and one pilot-scale attempt at VFA and biogas production ceasing operations due to process failure or lack of policy/market support. Therefore, the integrated biorefinery system we propose can be considered at TRL 6.

Our analysis indicates high operating costs as a barrier to the economic feasibility of the proposed biorefinery model. Specifically, costs associated with feedstock procurement and utilities resulted in operating costs exceeding revenues. Reducing these costs through technology optimisation and policy interventions is essential to enhance economic performance. It is also crucial to create a conducive environment for the development of biobased technologies within an economy dominated by fossil-based resources. This could be achieved through policy mechanisms, such as subsidies, incentives for renewable resource utilisation, and promotion of markets for bio-based products.

complex, influenced by factors such as scale, technology choice, feedstock availability, and product end-use [7]. Advanced AD technologies offer opportunities to unlock higher-value products in a biorefinery approach and potentially improve financial feasibility [8]. For instance, the possible extraction of intermediate products of the AD process, namely **VFAs**, represents an additional revenue stream. VFAs, such as acetic acid, propionic acid, butyric acid, and caproic acid, serve as chemical building blocks in various industries [9]. Reports indicate that VFA market prices are at least two to three times higher compared with biogas, which is a lower-value commodity primarily used for energy generation, with its prices constrained by the price of natural gas [10,11]. Furthermore, VFAs also serve as precursors for producing biobased commodities of growing interest, such as **polyhydroxyalkanoates (PHAs)** [12]. PHAs are considered a promising alternative to conventional plastics due to their suitable properties, but remain expensive to produce, with the carbon source accounting for up to 50% of the production cost. Biomass-derived low-cost VFAs are considered a promising way to reduce these costs [9]. Thus, harvesting these intermediate products of AD epitomises a circular bioeconomy, where bioproducts and bioenergy are produced from biomass in a cascading manner [8].

In single-phase AD systems, such as conventionally used **continuous stirred-tank reactors (CSTRs)**, VFAs are consumed by the microbial consortia for biogas production [13]. A **two-phase AD** system facilitates the extraction of VFAs as intermediate products. An example of a two-phase AD system is a **leach bed reactor (LBR)** combined with an **upflow anaerobic sludge blanket (UASB)**. In the LBR–UASB system, VFA production takes place in the LBR, whereas conversion of VFAs to biogas takes place in the UASB, thus separating and optimising the two processes [14]. Such a system also allows for the digestion of high-solid content feedstocks, such as grass, with little to no pretreatment [15]. Despite the widespread industrial-scale use of both LBRs and UASBs independently, the combined configuration for producing VFAs and biogas in a biorefinery approach has been largely unexplored.

The LBR–UASB system has previously been evaluated primarily for improving the efficiency of biogas production [14–16]. Hackula and colleagues found that producing both VFAs and biogas from whiskey distillery by-products in a similar two-phase AD system could increase revenues compared with biogas production alone [17]. Furthermore, such two-phase AD systems may facilitate demand-driven operations. Biogas may be produced only at times of high electricity demand and, at other times, high-value VFAs can be produced to maximise the total revenue [18]. In future energy systems dominated by variable intermittent renewables, dispatchable renewable energy sources will have a crucial role in balancing the power grid [19]. Demand-responsive AD systems can adjust biogas and electricity production to provide both positive and negative balancing power [20]. In previous work, we developed a strategy to align biogas production to peak electricity demand hours using the LBR–UASB system; VFA-rich leachate produced from the LBR was fed to the UASB for quick conversion to biogas to generate electricity only at times of high electricity demand [21]. However, the implications of operating the LBR–UASB system for producing both VFAs and biogas in a demand-driven manner are yet to be investigated.

To enhance the circularity of a biorefinery, it is also imperative that any effluent streams are reutilised. Previous studies investigated the recirculation of the effluent generated from the UASB back to the LBRs for leaching purposes. However, this ultimately had the undesired effect of methanogenic archaea migrating from the UASB to the LBRs and, subsequently, inefficient consumption of VFAs and the production of biogas in the LBRs [16]. To make the biorefinery process a closed loop (zero-discharge) and reduce the freshwater requirements of the biorefinery, a strategy for effluent recirculation without a detrimental impact on VFA and biogas production is needed.

<sup>1</sup>SFI MaREI Centre for Energy, Climate and Marine, Environmental Research Institute, University College Cork, Cork, Ireland

<sup>2</sup>Civil, Structural and Environmental Engineering, School of Engineering and Architecture, University College Cork, Cork, Ireland

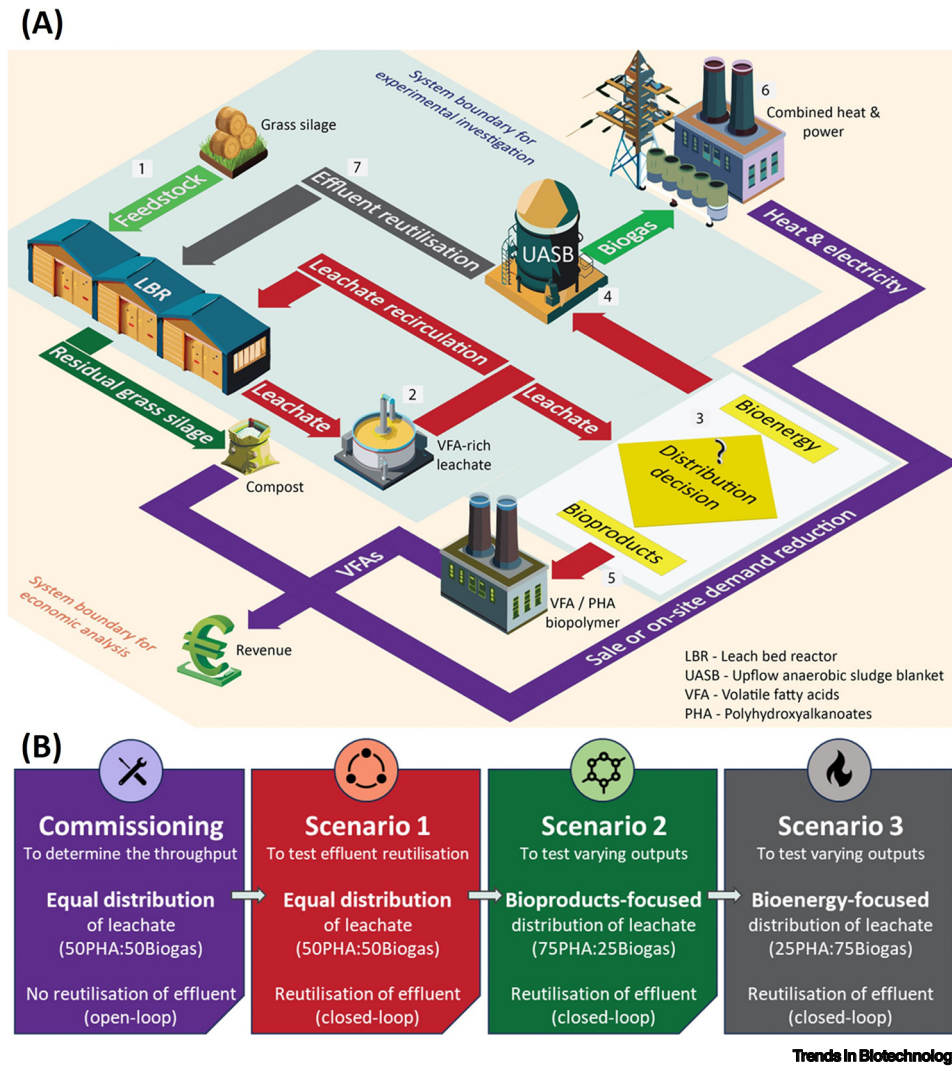
<sup>3</sup>Crops, Environment and Land Use Programme, Crop Science Department, Teagasc, Oak Park, Ireland

<sup>4</sup>Faculty of Electronics, Telecommunications and Informatics, and Advanced Materials Centre, Gdansk University of Technology, Gdańsk, Poland

<sup>5</sup>Process and Chemical Engineering, School of Engineering and Architecture, University College Cork, Cork, Ireland

\*Correspondence: david.wall@ucc.ie (D.M. Wall).

In this study, we addressed key research gaps in implementing a biorefinery model based on two-phase AD (Figure 1). The proposed biorefinery model comprises a central LBR–UASB system treating grass silage to produce VFAs and biogas (experimentally validated). The VFAs were assumed to be used for PHA biopolymer production and biogas for electricity and heat generation (outside the scope of experimental validation, but considered for economic analysis). First, we commissioned a laboratory-scale LBR–UASB system for stable long-term VFA and biogas



**Figure 1.** Overview of the proposed anaerobic digestion (AD)-based biorefinery approach and the experimental design of this study. (A) Process flow: (1) grass silage feedstock is treated in the leach bed reactor (LBR); (2) intermediates of the AD process, volatile fatty acids (VFAs), accumulate in the leachate; (3) dynamic biorefinery operation allows variable distribution of the VFA-rich leachate as per demand; (4) at times of high energy demand, bioenergy (biogas) can be produced through the upflow anaerobic sludge blanket (UASB); (5) at other times, the biorefinery can focus on producing high-value bioproducts, such as VFAs/polyhydroxyalkanoate (PHA) biopolymer for revenue; (6) electricity and heat produced from the biogas can either be sold to the grid (earning revenue) or used on-site to reduce the demand (for savings); and (7) leachate treated in the UASB can be reutilised for leaching in the LBR, reducing freshwater requirement and enhancing circularity. (B) Experimental design of this study to develop the LBR–UASB dynamic biorefinery approach: After commissioning, a closed-loop operation and demand-driven production of bioproducts (PHA) and bioenergy (biogas) were tested through three operational scenarios.

## Glossary

**Anaerobic digestion (AD):** process in which microorganisms break down organic materials in the absence of oxygen, producing biogas (energy) and digestate (biofertiliser).

**Capital expenditure (CAPEX):** initial capital costs associated with acquiring and setting up a project, such as equipment and infrastructure.

**Chain elongation:** biochemical process that extends the carbon chains in VFAs/organic acids, producing longer-chain fatty acids, such as caproic acid and valeric acid, which serve as platform chemicals in various industries.

**Chemical engineering plant cost index (CEPCI):** index used to estimate the cost of constructing or updating chemical process plants based on inflation and other economic factors.

**Chemical oxygen demand (COD):** common metric used to quantify the amount of organic matter in liquid samples. It measures the amount of oxygen required if all organic matter in the sample was chemically oxidised, serving as a proxy for the concentration of organic matter present.

**Combined heat and power (CHP):** generator system that produces both electricity and useful heat from the same energy source, such as biogas.

**Continuous stirred-tank reactor (CSTR):** type of reactor in which contents are continuously mixed, widely used in industrial fermentation and digestion processes.

**Greenhouse gas (GHG):** gases, such as carbon dioxide and methane, that trap heat in the Earth's atmosphere, contributing to global warming and climate change.

**Hydraulic retention time (HRT):** average time that liquid remains in a reactor or treatment system, influencing the efficiency of processes, including AD.

**Leach bed reactor (LBR):** type of reactor used in AD to treat high-solid organic feedstocks, such as the organic fraction of municipal solid waste, through leaching.

**Net present value (NPV):** financial metric that calculates the present value of all cash flows of a project, determining its profitability at the end of the project duration. A positive NPV indicates the project is expected to generate profits, while a negative NPV indicates potential losses.



production. Second, we presented a closed-loop operational strategy that allows the reuse of any effluent generated to enhance the circularity of the system. Third, we investigated a demand-driven approach to operation by varying the (assumed) PHA and biogas outputs in the biorefinery to match different demand scenarios. Finally, we conducted a preliminary assessment of the economic performance of the proposed biorefinery model to identify areas for potential improvement. Through this, we demonstrated a dynamic AD-based biorefinery approach for on-demand renewable energy and bioproducts production, promoting a circular bioeconomy, and supporting diversification of farmers' incomes.

## Results

### Developing a stable LBR–UASB biorefinery process

An overview of the proposed biorefinery approach using the LBR–UASB system and the experimental design is provided in [Figure 1](#). The laboratory reactor setup designed for experimental trials is presented in [Figure 2](#), with a detailed description in the STAR★Methods.

The leaching of grass silage feedstock in the LBR extracted the organic matter from the grass silage. This accumulated organic matter in the leachate was quantified in terms of the **chemical oxygen demand (COD)** concentration of the leachate. The VFAs formed a portion of this COD in the leachate. Maintaining a stable level of COD-VFA concentration in the leachate over long-term operation is crucial for the biorefinery to ensure sustained production of downstream products derived from VFAs. During the commissioning stage, a stable COD-VFA concentration in the leachate was achieved by balancing COD production and consumption rate. This essentially determined the throughput of the system, which is the rate at which the leachate can be extracted for downstream applications. This throughput of the system was determined through trial and error during the commissioning stage of the experiments, targeting a COD level of ca.  $20 \text{ g l}^{-1}$  in the leachate. A throughput of  $0.6 \text{ l day}^{-1}$  for a leachate volume of 10 l was found to be suitable, resulting in a **hydraulic retention time (HRT)** of 17 days for the leachate tank ( $10 \text{ l} / 0.6 \text{ l day}^{-1}$ ) (for further explanation see the STAR★Methods). This throughput rate was then maintained for further operation. The system was commissioned in an open-loop operation, that is, without reutilisation of the UASB effluent for leaching in the LBR. This was because literature and preliminary trials indicated that reutilising the UASB effluent for leaching would lead to a decline in VFA production in the long term. During the commissioning stage, the VFA-rich leachate was equally distributed towards (assumed) PHA and biogas production. The last HRT in the commissioning stage (considered stable operation) was termed the 'Baseline' operation for comparison with further experimental trials using a closed-loop operation ([Table 1](#)).

After successful commissioning, the system was tested for closed-loop and demand-driven operations through three operational scenarios. First, in Scenario 1, to achieve a closed-loop operation, all of the UASB effluent was reutilised for the leaching process in the LBR, after acidifying the effluent to inactivate the methanogenic archaea. This strategy of lowering the pH of the effluent before recirculation by acid dosing was effective (see below) and was continued during the subsequent stages of operation. As in the Baseline operation, the leachate was equally distributed towards (assumed) PHA and biogas production in Scenario 1. Therefore, Scenario 1 is referred to as the 'Equal distribution' or '50PHA:50Biogas' scenario.

Following this, the ability of the system to vary the PHA/biogas outputs in a demand-driven approach was tested in Scenarios 2 and 3. In Scenario 2, the system was operated in a 'bioproducts-focused' scenario, where 75% of the VFA-rich leachate extracted daily was used for (assumed) PHA production and 25% was used for biogas production in the UASB (75PHA:25Biogas). In Scenario 3, the system was operated in a 'bioenergy-focused' scenario,

#### Operating expenditure (OPEX):

ongoing costs required to run and maintain a project, such as utilities, labour, materials, and waste disposal.

**Polyhydroxyalkanoate (PHA):** type of biodegradable polymer synthesised by certain microbes using organic feedstocks in a fermentation process.

**Two-phase AD:** method of anaerobic digestion in which the process is split into two parts to enhance efficiency. Partial digestion or the hydrolysis process for acid production occurs in the first phase and conversion of acids to methane occurs in the second. This allows optimisation of treatment and harvesting of valuable intermediate products of AD.

#### Upflow anaerobic sludge blanket

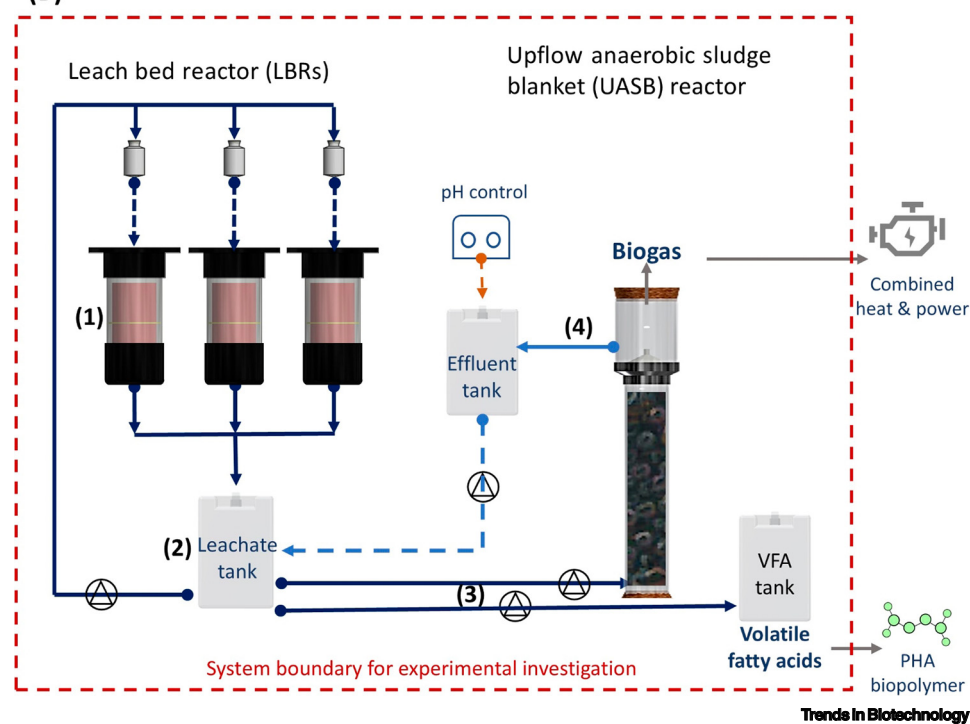
**(UASB):** reactor design used in AD where wastewater flows upward through a sludge blanket containing anaerobic bacterial biofilm, called granular sludge, facilitating digestion of organic matter and biogas production.

**Volatile fatty acids (VFAs):** organic acids (fatty acids) produced during AD or fermentation, used as precursors for biofuels and chemicals (e.g., acetic acid, butyric acid, caproic acid, etc.).

(A)



(B)



**Figure 2.** Leach bed reactor (LBR)–upflow anaerobic sludge blanket reactor (UASB) reactor setup. (A) Reactor setup in the laboratory. (B) Reactor operation schematic: (1) grass silage was loaded in the LBRs; (2) water/leachate was stored in the leachate tank and recirculated over the grass silage in the LBRs; (3) volatile fatty acid (VFA)-rich leachate was pumped to the UASB for biogas production and the VFA tank for VFA utilisation; and (4) effluent exiting the UASB was collected in the effluent tank, where it underwent pH adjustment to inactivate methanogens and was subsequently pumped back to the leachate tank, where it was reused for the leaching process in the LBRs, closing the process loop. Abbreviation: PHA, polyhydroxyalkanoate.

where 25% of the leachate was used for (assumed) PHA production and 75% for biogas production (25PHA:75Biogas). Additionally, in all operational scenarios, the UASB was fed only between 14:00 and 16:00 h to align biogas production with peak electricity demand hours of 16:00–20:00

h, as demonstrated in our previous work [21]. Testing the ability to vary the output quantities on demand demonstrated the dynamic capabilities of this biorefinery approach. The performance of the system during these operational scenarios is presented in Table 1 and further elaborated upon in the following sections.

The stability and performance of the UASB are typically determined based on its capacity for COD/VFA removal. Throughout the experimental trials, COD/VFA removal in the UASB was close to 100% (Table 1). Although the UASB was operating at lower daily organic loading rates than what is considered typical for UASBs ( $4\text{--}15\text{ g COD } I_{\text{reactor}}^{-1}\text{ day}^{-1}$ ) [22], for the demand-driven operation of the UASB, the entire daily load of COD was fed in a 2-h period between 14:00 and 16:00 h, resulting in a shock loading of the UASB [23]. Nevertheless, the UASB performance was excellent, as evident from the COD removal in the UASB (Table 1). Moreover, gas composition in both the LBR and the leachate tank was periodically checked, and no methane was detected throughout the operation. This indicated that the previously reported issue of VFA consumption in the LBR and associated methane production by methanogenic archaea transported through the recirculation of effluent from the UASB was addressed by the pH adjustment method used. Hence, a stable operation of the LBR–UASB system for biorefinery applications was achieved.

The LBR and UASB operated at two different pH conditions suitable for VFA and biogas production, respectively. The LBR operated at a pH of 4.6–4.7 resulting from the accumulation of VFAs from the hydrolysis and acidogenesis of grass silage (Table 1). VFAs were consumed during the methanogenesis process in the UASB reactor to produce biogas, resulting in a neutral pH range of ca.  $7.6 \pm 0.2$  within the UASB. The respective pH conditions of the LBR and the UASB were within the reported ranges for an acidogenic and a methanogenic reactor across the different scenarios of operation [24]. This indicated that the closed-loop operation or varying the distribution of leachate towards PHA and biogas outputs in Scenarios 1–3 did not affect pH conditions in the system.

#### Performance of the LBR–UASB system for VFA production

The COD of the leachate is representative of the VFA content, which can subsequently be used for the production of biogas or high-value products, such as PHA biopolymer. The objective of maintaining COD levels consistent at ca.  $20\text{ g l}^{-1}$  was achieved because there was no significant difference in the COD concentration across the different scenarios of operation in the experiments (Table 1) [ANOVA:  $F(3,22) = 1.29$ ,  $p = 0.29$ ].

The average total VFA (TVFA) concentration in the leachate was  $6.6\text{ g l}^{-1}$ , with no significant difference observed across the Baseline and three operational scenarios [ANOVA:  $F(3,22) = 1.9$ ,  $p = 0.16$ ]. Acetic acid, butyric acid, and caproic acid were the predominant VFAs, constituting, on average, ca. 20%, 53%, and 12% of the total VFAs in the leachate, respectively. Other VFAs, such as propionic acid, isobutyric acid, and isovaleric acid, made up the remainder of the total VFAs (see Figure S1 in the supplemental information online for the complete VFA profile). While no particular trend in the concentrations of acetic acid and butyric acid was evident across the different operational scenarios, caproic acid concentrations increased over the course of a ~5-month operation. The caproic acid share in the total VFA concentration increased from ca. 9% during the Baseline operation to ca. 12–14% over Scenarios 2 and 3, and the difference was statistically significant ( $p = 0.008$ ). This could be ascribed to **chain elongation** occurring in the leachate, whereby shorter-chain VFAs, such as acetic acid and butyric acid, combine to form longer-chain VFAs [25]. This process is speculated to be mediated by *Caproiciproducens* bacteria through the reverse  $\beta$ -oxidation pathway using lactate as the electron donor and short-chain VFAs, such as acetate, as the electron acceptor [26].

Table 1. Performance of the LBR–UASB system for VFA and biogas production<sup>a</sup>

Parameters	Baseline: commissioning (50PHA: 50Biogas, open-loop)	Scenario 1: equal distribution (50PHA: 50Biogas, closed-loop)	Scenario 2: bioproducts-focused distribution (75PHA: 25Biogas, closed-loop)	Scenario 3: bioenergy-focused distribution (25PHA: 75Biogas, closed-loop)
LBR: leachate tank				
Leachate volume (l)	10			
Total leachate extracted daily (throughput) (l d <sup>-1</sup> )	0.6			
Effluent recirculation for leaching in LBR	No	Yes	Yes	Yes
Leachate used for PHA production (l d <sup>-1</sup> ) (% of total leachate extracted per day)	0.3 (50%)	0.3 (50%)	0.45 (75%)	0.15 (25%)
Leachate used for biogas production (l d <sup>-1</sup> ) (% of total leachate extracted per day)	0.3 (50%)	0.3 (50%)	0.15 (25%)	0.45 (75%)
COD in leachate (g l <sup>-1</sup> ) <sup>b</sup>	22 ± 2	21 ± 2	20 ± 2	20 ± 2
pH of leachate	4.7 ± 0.1 <sup>+</sup>	4.6 ± 0.1	4.6 ± 0.1	4.6 ± 0.1
Total VFA in leachate (g l <sup>-1</sup> ) <sup>b</sup>	6.2 ± 1.2	7.2 ± 0.3	6.6 ± 0.3	6.6 ± 0.7
Acidification (%) <sup>b</sup>	50 ± 12	60 ± 6	60 ± 11	59 ± 8
Acetic acid in TVFA (%) <sup>b</sup>	20 ± 3	19 ± 2	23 ± 4	18 ± 3
Butyric acid in TVFA (%)	55 ± 2 <sup>#</sup>	52 ± 1	47 ± 2 <sup>+</sup>	56 ± 2 <sup>#</sup>
Caproic acid in TVFA (%)	9 ± 2 <sup>#</sup>	14 ± 1	14 ± 2 <sup>+</sup>	12 ± 1 <sup>+</sup>
UASB				
Organic loading rate (gCOD l <sub>reactor</sub> <sup>-1</sup> day <sup>-1</sup> )	3.2 ± 0.2	3.1 ± 0.2	1.5 ± 0.1	4.4 ± 0.4
pH inside UASB	7.9 ± 0.1	7.6 ± 0.1 <sup>+</sup>	7.6 ± 0.0 <sup>+</sup>	7.3 ± 0.2 <sup>#</sup>
Methane yield (based on COD fed to UASB through leachate) (l CH <sub>4</sub> kg <sup>-1</sup> COD) <sup>b</sup>	323 ± 28	347 ± 19	350 ± 61	320 ± 35
Methane content in biogas (% <sub>v,v</sub> )	71 ± 0.6 <sup>+</sup>	72 ± 0.5	76 ± 0.5 <sup>#</sup>	68 ± 0.5 <sup>@</sup>
COD removal in UASB (%)	98 ± 0.5 <sup>+</sup>	98 ± 0.2 <sup>+</sup>	98 ± 0.2 <sup>+</sup>	97 ± 1.1

<sup>a</sup>In all scenarios of operation, the UASB was fed between 14:00 h and 16:00 h to produce most biogas during peak electricity demand hours between 16:00 h and 20:00 h. The symbols <sup>+</sup> # <sup>@</sup> indicate statistical differences as per one-way ANOVA and post-hoc analysis. Two groups with the same symbol indicate no statistical difference between the pair, and vice versa.

<sup>b</sup>Parameters with no statistically significant differences within the groups.

Acidification represents the fraction of the COD contributed by the VFAs. On average, an acidification rate of 57 ± 9% was obtained in the leachate over the entire operation. The acidification increased from 50% during the Baseline commissioning stage to 59% during the last operational Scenario 3 (although the difference was not statistically significant;  $p = 0.15$ ). Increasing acidification with no particular increase in the overall total VFA concentration may be another indication of chain elongation. Longer-chain VFAs have a higher COD compared with shorter-chain VFAs. For example, the COD equivalent of acetic acid is 1.07 g COD g<sup>-1</sup> VFA, while that for caproic acid is 2.21 g COD g<sup>-1</sup> VFA [27]. Considering that longer-chain VFAs are easier to separate from an aqueous mixture (leachate in this case), a VFA profile shifting towards longer-chain VFAs could be beneficial for a biorefinery producing VFAs [28,29]. However, shifting to longer-chain VFAs would require longer retention times and, consequently, lower throughput rates for extracting leachate, because the production of longer-chain VFAs involves additional chain elongation steps [25]. By contrast, for applications such as PHA production, a consistent VFA profile is preferred [9].

Overall, the LBR–UASB system demonstrated stable performance for VFA production over 136 days of the commissioning stage and three different operational scenarios. Reutilising the



UASB effluent for leaching in the LBRs after lowering the pH (details in the STAR★Methods), effectively mitigated the previously reported problem of reduced VFA yield by inactivating methanogenic archaea in the recirculated effluent [16]. The system performance was stable throughout the open-loop (Baseline), closed-loop, and variable output scenarios (Scenarios 1–3) (Table 1). This implies that the effluent can be reutilised with a pH control strategy without any detrimental effects on reactor performance, thereby enhancing the circularity of the biorefinery process. Similarly, varying the quantities of leachate extracted for PHA and biogas production as per demand, while maintaining the overall throughput rate constant, should not negatively impact the reactor performance.

#### Performance of the LBR–UASB for biogas production

A portion of the VFA-rich leachate extracted daily was fed to the UASB for biogas production. The methane yield from the UASB, based on the COD fed through the leachate, ranged between 320 and 350 l CH<sub>4</sub> kg<sup>-1</sup> COD (Table 1), with no significant difference between the operational scenarios [ANOVA: F(3,56) = 2.9, *p* = 0.07]. The methane content in the biogas ranged from 67 to 75%<sub>volume</sub> throughout all operational scenarios (Table 1). In Scenario 3, the COD removal, methane yield, and methane content of biogas were slightly lower than that in the other operational scenarios (Table 1). The difference was significant for COD removal in the UASB [ANOVA: F(3,64) = 26.0, *p* < 0.001] and methane content in biogas [ANOVA: F(3,64) = 680.2, *p* < 0.001], but not for methane yield [ANOVA: F(3,56) = 2.9, *p* = 0.07]. This marginal drop in the performance of the UASB could have resulted from the higher loading rates applied in Scenario 3 compared with the other scenarios (Table 1). Nevertheless, the UASB performance was in line with values reported in the literature for UASBs [30] and with our previous studies [18,21] in terms of the COD removal (>80%), methane yields (250–350 l CH<sub>4</sub> kg<sup>-1</sup> COD), and methane content in biogas (ca. 70%).

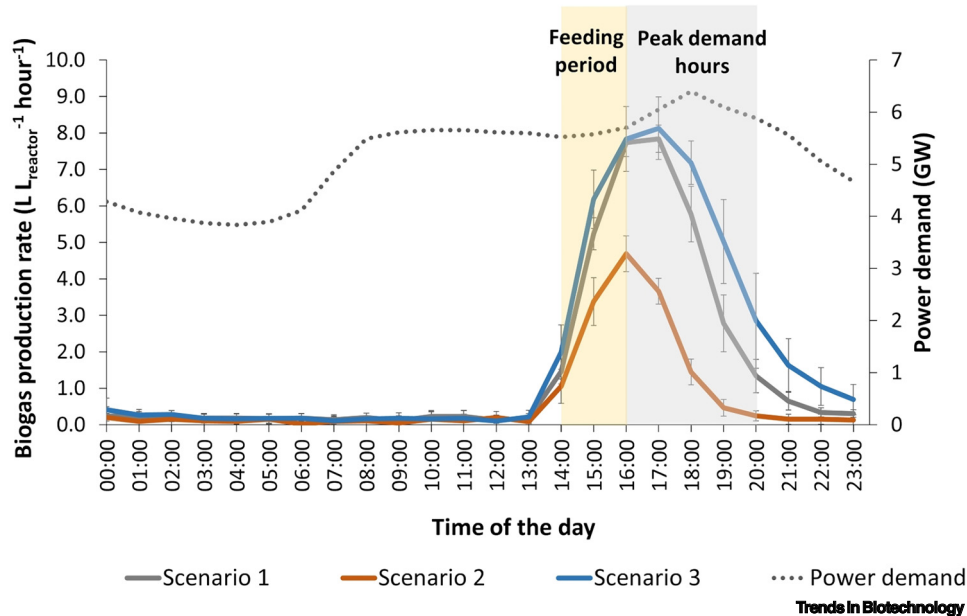
With the demand-driven feeding regime for the UASB to align the biogas output with peak electricity demand hours, over 60% of the daily biogas production was obtained between 16:00 h and 20:00 h. Variations in peak gas production rates were observed due to different organic loading rates resulting from varying quantities of leachate supplied to the UASB in different operational scenarios (Figure 3). However, the biogas production curve generally followed a similar pattern, peaking between 16:00 h and 17:00 h, and coinciding with the peak electricity demand hours.

Aligning biogas production from an AD-based biorefinery, as proposed herein, can have several advantages. By producing biogas and subsequently operating a **combined heat and power (CHP)** unit during peak demand hours when electricity tariffs are typically higher<sup>iii</sup>, a limited feedstock (VFA-rich leachate) can be strategically used to maximise revenues. Producing biogas only when it is to be used in the CHP could reduce the need for gas storage (saving costs) and potential methane leakage during storage (reducing GHG emissions) [21,31].

Overall, the UASB performance was largely consistent across the different operational scenarios in terms of biogas quality and yield. Thus, the findings suggest that the closed-loop operation of the LBR–UASB, demand-driven approach to vary output distribution or aligning biogas output to specific hours of the day should not adversely affect the system performance in terms of biogas production.

#### Economic analysis of the LBR–UASB biorefinery system

A preliminary economic analysis was conducted to assess the feasibility of the AD-based biorefinery approach based on the LBR–UASB system. A detailed description of the proposed biorefinery model, assumptions, estimation of costs, and calculation of profitability indicators is provided in the STAR★Methods and the supplemental information online. Figure 1 provides a schematic of the proposed AD-based biorefinery approach.



**Figure 3.** Demand-driven biogas production in the upflow anaerobic sludge blanket reactor (UASB) reactor: aligning biogas production rate to match peak electricity demand hours. Three different curves correspond to the three organic loading rates (OLRs) as per leachate distribution scenarios tested for demand-driven operation of the biorefinery: Scenario 1: OLR 3.1 gCOD  $l_{\text{reactor}}^{-1} \text{ day}^{-1}$ , equal distribution (50PHA:50Biogas); Scenario 2: OLR 1.5 gCOD  $l_{\text{reactor}}^{-1} \text{ day}^{-1}$ , bioproducts-focused distribution (75PHA:25Biogas); Scenario 3: OLR 4.4 gCOD  $l_{\text{reactor}}^{-1} \text{ day}^{-1}$ , bioenergy-focused distribution (25PHA:75Biogas). ‘Feeding period’ represents the time when the UASB was fed with the leachate (14:00–16:00 h) to align biogas production with ‘peak demand hours’ (16:00–20:00 h). The power demand curve for Ireland on February 9, 2023 is shown as a reference, with peak demand hours highlighted [data from Single Electricity Market Operator (SEM-O)]. Error bars denote the standard deviations over the operation of 17 days in each scenario.

The analysis considered an AD-based biorefinery with a processing capacity of 35 000 wet tonnes of grass silage per annum with ca. 20% dry matter content, (rationale described in the STAR★Methods) (S.M. O’Keeffe, PhD thesis, Wageningen University, 2010). PHA biopolymer production was considered as an example of a high-value application for the VFA-rich leachate produced in the LBR from grass silage, alongside biogas production in the UASB, which was subsequently used for electricity and heat generation. Residual grass silage in the LBR was assumed to be composted. The key units of the biorefinery included for the economic analysis were: LBR, UASB, PHA unit, CHP system, and composting unit. The analysis draws on findings from experimental trials in this study as well as data adapted from detailed techno-economic assessments of similar processes in the literature [32–34]. Details on how data from the literature was adapted for our model are provided in the STAR★Methods and the supplemental information online. It is important to note that this preliminary economic analysis, which relies on literature data, may introduce a margin of error due to differing conditions and assumptions within this study and the literature sources used for cost estimations. However, such an analysis offers valuable early insights into the economic feasibility of the proposed biorefinery scheme and helps identify key avenues for further improvement.

For the revenue analysis, the PHA selling price was valued at €5 per kg, electricity and heat produced from biogas at €0.7 per kg biogas, and compost at €0.1 per kg (further details in Tables S1 and S11 in the supplemental information online). As demonstrated in the experiments, the LBR–UASB biorefinery can operate in a demand-driven manner by varying the distribution of leachate

towards PHA and biogas outputs as per demand. Therefore, a comparative economic analysis was conducted for the three distribution scenarios as tested in the experiments. Depending on the scenario, the biorefinery produced ca. 150–450 tonnes per annum of PHA from VFAs and an additional 2.2–6.5 GWh of energy in the form of electricity and heat from biogas (Table S2 in the supplemental information online). A summary of the key outcomes of the economic analysis is presented in Table 2 and a breakdown of costs and revenues is presented in Figure 4.

The **capital expenditure (CAPEX)** for the biorefinery, estimated at €11 million, covered direct and indirect costs, as adapted from the literature data (see the STAR★Methods and Tables S3–S10 in the supplemental information online for details). The CAPEX for all three output scenarios was the same, because the PHA production unit and the UASB were designed to process 100% of the leachate extracted each day, regardless of its distribution. This was to allow flexibility so that the PHA and biogas outputs of the biorefinery could be varied (between 0% and 100%) as per the demand. The LBR was the main contributor to the CAPEX (71%), because it required the largest processing capacity among all the units in the biorefinery. The UASB, PHA unit, and composting unit accounted for the remaining 29% of the CAPEX (Figure 4).

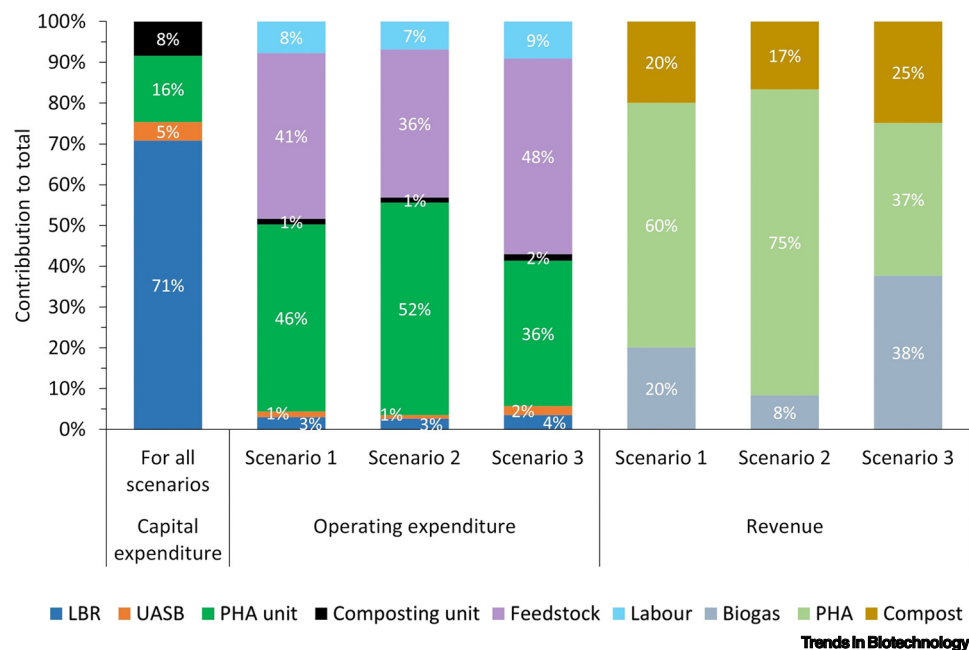
Table 2. Economic analysis of the AD-based biorefinery in three different operational scenarios varying the outputs as per demand

	Scenario 1: equal distribution (50PHA:50Biogas)	Scenario 2: bioproducts-focused distribution (75PHA:25Biogas)	Scenario 3: bioenergy-focused distribution (25PHA:75Biogas)
<b>CAPEX (€)</b>			
LBR	7 789 000		
UASB	501 000		
PHA	1 787 000		
Composting	924 000		
Total CAPEX	11 002 000		
<b>OPEX (€ per year)</b>			
LBR	111 000	111 000	111 000
UASB <sup>a</sup>	53 000	35 000	68 000
PHA <sup>a</sup>	1 695 000	2 162 000	1 118 000
Composting	51 000	51 000	51 000
Feedstock	1 505 000	1 505 000	1 505 000
Labour	285 000	285 000	285 000
Total OPEX	3 700 000	4 149 000	3 138 000
<b>Revenues (€ per year)</b>			
Biogas <sup>b</sup>	495 000	248 000	743 000
PHA	1 474 000	2 211 000	737 000
Compost	490 000	490 000	490 000
Total revenue	2 459 000	2 949 000	1 970 000
<b>Profitability indicators (€)</b>			
NPV	–21 564 000	–21 218 000	–20 945 000

<sup>a</sup>OPEX for UASB and PHA production units vary as per the scenarios because the costs were dependent on the processing capacity based on the leachate distribution pattern.

<sup>b</sup>Revenue from biogas represents the sale of electricity and heat generated by the CHP (details in Tables S1 and S2 in the supplemental information online). Numbers are rounded to the nearest thousand.





**Figure 4.** Breakdown of capital expenditures (CAPEX), operating expenditures (OPEX), and revenues for the proposed anaerobic digestion (AD)-biorefinery. The AD-based biorefinery using grass silage feedstock included leach bed reactor (LBR), upflow anaerobic sludge blanket (UASB), polyhydroxyalkanoate (PHA) production, and composting units. It generated revenue by selling electricity and heat from biogas, PHA biopolymer from volatile fatty acids (VFAs), and compost from residual grass silage. The three scenarios correspond to different distribution ratios of leachate towards PHA and biogas production, tested for demand-driven operation of the biorefinery: Scenario 1: equal distribution (50PHA:50Biogas); Scenario 2: bioproducts-focused distribution (75PHA:25Biogas); and Scenario 3: bioenergy-focused distribution (25PHA:75Biogas). CAPEX remained constant because the designed capacity was the same across all scenarios, while the OPEX and Revenues varied as per the scenarios (also see Tables S1–S11 in the supplemental information online).

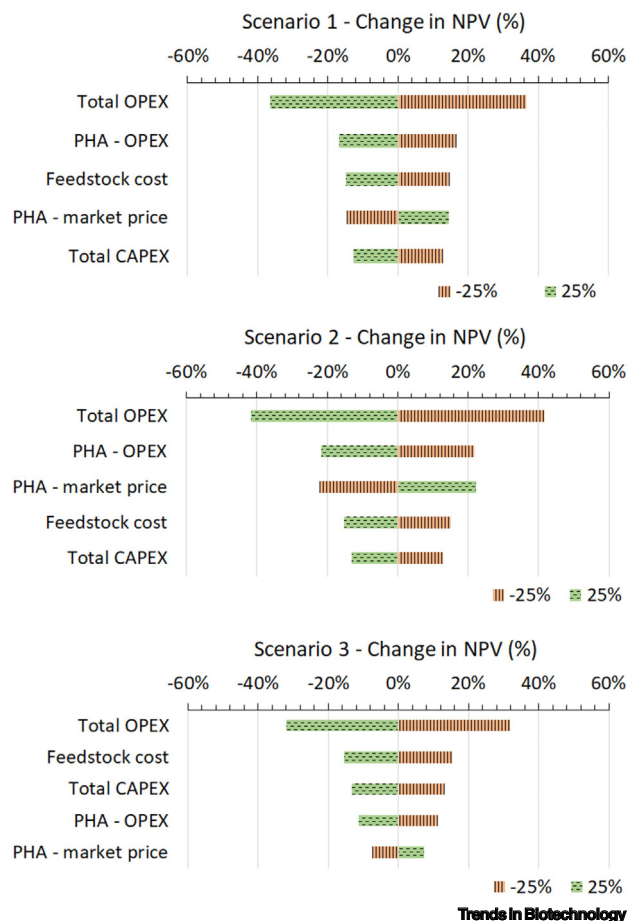
The **operating expenditure (OPEX)** included costs for feedstock, maintenance, depreciation, raw materials, utilities, labour, and waste disposal, as adapted from the literature data (see the STAR★Methods and Tables S3–S10 for details). The PHA production unit accounted for most of the operating costs in all three scenarios (36–52%), as a result of the high utility costs involved in PHA production and downstream purification [33]. This led to Scenario 2, with bioproducts-focused distribution having the highest OPEX (€4.1 million) among the three scenarios, because most of the leachate extracted daily (75%) was used for PHA production in this scenario. Grass silage feedstock cost (priced at €43 per tonne wet weight) was the second highest contributor to the OPEX (36–48%), indicating the importance of feedstock availability and pricing in determining profitability. This was based on the cost of pit grass silage (ca. 20% dry matter) in Ireland and could vary depending on the feedstock used and the location [7].

The revenue structure depended on the distribution of PHA and biogas outputs from the biorefinery as per the three scenarios considered (Figure 4). Understandably, PHA contributed the most (75%) to the revenue in Scenario 2 with bioproducts-focused distribution. However, that was not the case in Scenario 3 with bioenergy-focused distribution, where biogas revenue nearly matched the PHA revenue (38% versus 37%), despite most of the leachate being used for the biogas production. Notably, in Scenario 1 with equal distribution, the contribution of PHA revenue was three times higher than biogas revenue (60% versus 20%). This highlighted the advantage of using VFAs to produce a higher-value product, such as PHA, rather than just for energy (biogas).



None of the three distribution scenarios for the proposed biorefinery model were profitable in the present analysis, with OPEX higher than the revenues, resulting in financial losses and a negative **net present value (NPV)** after 20 years. The bioenergy-focused scenario (Scenario 3) performed marginally better than the other two scenarios, showing lower losses and having the least negative NPV. This could be attributed to higher operating costs in the PHA production process. Although the bioproducts-focused scenario generated almost 50% higher revenues, it incurred greater losses due to its 32% higher operating costs compared with the bioenergy-focused scenario (Table 2). This suggests that biogas production from UASB, despite its lower market value compared with PHA, offers a sustainable and cost-effective revenue stream, because it requires comparatively lower capital and operating costs. Similarly, composting, while contributing the least to the total revenue (17–25%), may still provide a valuable revenue stream with relatively low associated costs. However, its contribution to overall profitability may be limited.

A sensitivity analysis revealed the key parameters influencing the profitability of the proposed AD-based biorefinery system (as indicated by the NPV) across the three output distribution scenarios. The top five parameters influencing the NPV in each scenario are presented in Figure 5. The NPV for all three scenarios was most sensitive to the total OPEX, with a  $\pm 25\%$  variation in OPEX causing a corresponding change in the NPV  $\pm 32\text{--}42\%$ . The OPEX for the PHA unit, primarily driven by the energy costs (Table S9), was the major component in the total OPEX that influenced the NPV,



**Figure 5. Sensitivity analysis.** A sensitivity analysis was performed to account for uncertainty and evaluate the influence of key parameters on the profitability of the biorefinery, as indicated through net present value (NPV), across the three output distribution scenarios: Scenario 1: equal distribution (50PHA:50Biogas); Scenario 2: bioproducts-focused distribution (75PHA:25Biogas); and Scenario 3: bioenergy-focused distribution (25PHA:75Biogas). The bars represent percentage changes in NPV when key cost parameters are varied by  $\pm 25\%$ , illustrating the sensitivity of profitability to these parameters and identifying potential areas for improvement. Only the top five most influential parameters are presented (also see Table S12 in the supplemental information online). Abbreviations: CAPEX, capital expenditure; OPEX, operating expenditure; PHA, polyhydroxyalkanoate.

causing a  $\pm$  17–22% change in the NPV, particularly in Scenario 1 with equal distribution and in Scenario 2 with bioproducts-focused distribution. Apart from the OPEX, the PHA market price and the feedstock cost had the most influence on the profitability of these two scenarios ( $\pm$  15–22% change in NPV), while total CAPEX featured last in the top five parameters. Only in Scenario 3 with bioenergy-focused distribution did total CAPEX feature among the top three parameters influencing the NPV, largely due to the lower OPEX related to the PHA unit in this scenario. This highlights the trade-offs between prioritising bioproducts or bioenergy production in such a dynamic biorefinery system, where optimising one output can affect the sensitivity of the system to certain economic parameters. It is also apparent that, overall, the profitability of Scenario 2 with a focus on PHA is more sensitive to the parameters tested, which is expected because the PHA technology and market are yet to mature.

### Discussion

Discussions with industrial operators in The Netherlands (who requested to remain anonymous) highlighted that instability in COD-VFA concentration in the leachate was a major bottleneck in implementing the LBR-UASB in a biorefinery approach. This paper demonstrates how we operated a continuous laboratory-scale LBR-UASB reactor over 136 days, and how throughput management lends itself to long-term stability in the process. The throughput rate for the LBR-UASB system (the rate at which the VFA-rich leachate can be extracted) is dependent on the capacity of the LBRs to produce COD and VFA. In our case, leaching 1 kg wet weight of grass silage produced 0.08 kg COD in the leachate (8% yield). Of this COD, ca. 60% was contributed by VFAs (acidification, Table 1) and, generally, ca. 35% of this VFA-COD can be converted to PHA [35]. Therefore, the overall mass yield of the process to produce PHA was ca. 0.02 kg PHA per 1 kg of wet grass silage ( $1 \times 0.08 \times 0.6 \times 0.35 = 2\%$  yield), rendering COD and VFA yields in the LBR a limiting factor. Other research using grass in a similar approach to produce PHA has obtained comparable yields [36]. Acidification levels of up to 90% have been reported in the literature for food waste [37]. However, lignocellulosic feedstock, such as grass silage, is more difficult to degrade, and no pretreatment was used in our experiments to improve the degradation. A larger size or greater number of LBRs and/or pretreatment of the feedstock to improve COD and VFA yields could facilitate increasing the throughput rate.

Furthermore, our work demonstrated effluent reuse within the system to reduce water use and enhance circularity, while also addressing the challenges of VFA consumption and inefficient biogas generation in the LBR- issues that have been reported in the literature and by industrial operators [16]. The pH adjustment strategy using HCl acid in this study reduced the water usage for leaching in the LBR and the potential need for downstream effluent treatment. However, it is important to note that using HCl may lead to a gradual accumulation of chloride ions, potentially increasing the salinity over time. In our system, chloride ion concentrations increased from  $0.3 \text{ g l}^{-1}$  to  $1.8 \text{ g l}^{-1}$  over the course of reactor operation. Although this was within the safe operational limits for AD, further increases may risk inhibiting microbial activity [38,39]. To mitigate this, future research should explore alternative strategies for pH adjustment. For instance, using part of the VFA-rich leachate instead of HCl could potentially reduce both costs and salinity build-up. Additionally, periodic dilution of the effluent with fresh water could be used to manage chloride concentrations within safe operational limits. A thorough environmental and cost-benefit analysis is necessary before implementing these strategies.

Unlike variable renewable electricity sources, such as wind and solar, biogas production is not dependent on weather conditions. Moreover, biogas is less susceptible to the volatilities of international energy markets, such as those for natural gas or oil, making it a reliable source of renewable energy [40]. Our previous work highlighted the role of AD as a source of dispatchable



renewable energy by producing biogas on demand using the LBR–UASB reactor [21]. Expanding on this, our present study demonstrated additional flexibility by tuning the system for either bioproducts-focused (VFA/PHA) or bioenergy-focused (biogas) output scenarios. Such AD-based biorefineries can dynamically adapt their operation and offer multiple benefits: maximising revenue by aligning outputs with market demand, reducing the carbon footprint of biobased products through renewable energy use [41], supporting the decarbonisation of electricity grids, and enhancing energy security [40].

The preliminary economic analysis conducted in this study suggested that the proposed biorefinery process is not currently economically viable, as revenues cover only 60–70% of the OPEX, resulting in a loss across all scenarios. However, our analysis did not account for potential subsidies or incentives for renewable energy or biobased products, which could improve the economic performance. Literature reports PHA selling prices ranging from €1.2 to €10.4 per kg PHA (in US\$ 2020), with €5 per kg being the most commonly reported by both researchers and industrial operators [35]. For comparison, the market price of conventional petroleum-based polypropylene plastic is ca. €1.3 per kg (as of March 2024, Plastic Portal<sup>TM</sup>). With technology advancements and scale-up in PHA production, operating costs and market prices for PHA are likely to reduce, potentially bringing PHA closer in price to conventional plastics. The effects of these alternative scenarios have not been investigated in our analysis. Interestingly, despite the differences in OPEX and revenue across the three scenarios tested, the 20-year NPV was similar ( $-\text{€}21.2 \pm 0.3$  million) (Table 2). This suggests that, regardless of whether PHA or biogas output was prioritised, the overall economic performance of the biorefinery is similar. While none of the scenarios tested are currently profitable, the similar NPV values highlight the dynamic capability of this biorefinery model, which could be adapted to align future directions of technological developments and policy shifts to optimise its economic viability.

To make this biorefinery model viable in its early stages, subsidies, incentives, and further technological developments will be essential. The sensitivity analysis indicated where policy interventions and technological advances might be most effective in improving financial viability. The NPV was found to be most sensitive to the total OPEX of the system, particularly the OPEX of the PHA unit. The literature suggests that utility costs constitute ca. 75% of the OPEX for PHA production [32]. Therefore, efforts directed at improving the process efficiency with regards to utility consumption or government subsidies for utility costs could be highly effective in improving the economic viability. In addition, feedstock cost stands out as a key driver of profitability, emphasising the importance of strategic procurement and location of the biorefinery. The simplified sensitivity analysis undertaken here may not fully capture the potential interactions between multiple parameters. A more detailed techno-economic assessment involving equipment scaling, mass and energy balances, and detailed cost analysis is needed for more accurate insights. Furthermore, the financial underperformance of the proposed biorefinery model can largely be attributed to the choice of feedstock and end-products. Grass silage feedstock and the OPEX for PHA production together accounted for ca. 86% of the total OPEX. Reducing these costs, either by using alternative feedstock available at little to no cost or producing less cost-intensive products than PHA, could substantially change the economic performance. For instance, using a feedstock with a gate fee of €25 per tonne (a typical fee paid to a facility for treating food waste) [32,42] and a reduction in the total OPEX by 25%, would yield a positive NPV for all scenarios, ranging from €0.9 to €2.8 million, indicating that the model could potentially be profitable.

The AD-based biorefinery proposed in our economic analysis exemplifies the cascading use of biomass in a circular bioeconomy. Considering the example of the proposed



bioproducts-focused output scenario (75PHA:25Biogas), a biorefinery could produce ca. 23 kg of VFAs (yielding ca. 13 kg of PHA biopolymer) and an additional 75 kWh of biogas, generating a gross revenue of ~€84 per tonne wet weight of grass silage (for reference, grass silage was priced at €43 per tonne in the analysis year). However, in its current state, the proposed model incurs operating costs of €118 per tonne, resulting in a loss of €34 per tonne of grass silage. With future optimisations and policy interventions, this model could provide farmers with an alternative enterprise whereby they sell feedstock to a biorefinery or set up cooperatives to run such biorefineries. By creating an alternative revenue stream, farmers could reduce their labour and regulatory burdens associated with livestock farming. Diversification of farm activities is widely recognised as an effective way of mitigating financial and climate risks for farmers [43]. A recent study in an Irish beef farm context found that reallocating 15% of the farm area to produce grass silage for AD instead of livestock, with a proportional reduction in livestock numbers, decreased farm-level GHG emissions by up to 24% [44]. Moreover, the LBR-UASB system presented in this study could treat other lower-quality, high-solids feedstocks. Previous studies demonstrated the use of LBR systems for producing VFAs from various feedstocks, such as food waste [45,46], Napier grass [47], and distillery by-products [17]. This unlocks opportunities to use other lower-quality agricultural residues in such systems. However, further investigation is needed to assess the VFA and biogas yields of such feedstocks in the LBR-UASB system.

Furthermore, to capture more value from the grass feedstock, fibre can be extracted post digestion for the production of biocomposite construction or insulation materials. For example, the EU project 'Grassification' explored the use of roadside grass cuttings in a biorefinery approach to produce fibres alongside biogas. Grass fibres from a dry AD reactor, similar to the LBR in our study, could be used to replace 50% of wood fibres in biocomposites without compromising quality [48]. The European Bioeconomy strategy identifies reducing dependence on non-renewable resources, strengthening the biobased sector, and deploying local rural circular bioeconomies as key pillars to deliver the EU's decarbonisation targets [49]. Therefore, despite the initial economic challenges in profitability of biorefineries indicated by our economic analysis, the urgent need to transition to low-carbon resources and decarbonise a hard-to-abate sector, such as agriculture, supports further exploration of these pathways.

### Concluding remarks

Conducting larger-scale continuous laboratory trials, such as in our study, is crucial for identifying operational challenges and advancing the technology readiness level of novel systems. Our research showcases the potential of a two-phase AD reactor as a dynamic biorefinery capable of simultaneously producing both bioproducts and bioenergy, supporting the transition away from fossil-based resources. The operational strategy we developed demonstrates how careful throughput management lends itself to long-term stability of the process, addressing previously reported issues in the industry. By incorporating a closed-loop operational strategy for reutilising reactor effluent within the process, we demonstrate a way to enhance system circularity, reduce waste, and improve resource efficiency without compromising the system performance. Furthermore, this work addresses the need for adaptable and bespoke biotechnologies capable of meeting the challenges of evolving market conditions through a dynamic, demand-driven approach to production. Such an approach allows flexibility in aligning the bioproduct and bioenergy outputs to changing market conditions, thereby providing economic resilience. Going beyond technical investigation, this work evaluates the economic viability of the proposed biorefinery model. Although our preliminary analysis indicates the biorefinery model is not economically viable in its current state, through a sensitivity analysis we identified key areas for improvement, offering a

### Outstanding questions

How does the LBR-UASB system perform in producing VFAs and biogas when using lower-quality biomass, such as roadside grass cuttings or the organic fraction of municipal solid waste?

How can the LBR operation be optimised to obtain a specific VFA profile or to enhance chain elongation to obtain longer-chain VFAs?

What are the long-term impacts of using the acid-dosing strategy to adjust the pH of the UASB effluent before reutilising it for leaching in the LBR and how can they be overcome?

What alternative strategies can be used to effectively inhibit methanogens in the UASB effluent before its reutilisation for leaching in the LBRs?

What is the economic performance of the proposed biorefinery model based on a detailed techno-economic assessment involving precise equipment sizing, mass and energy balances, and other critical parameters?

How can system nonprofitability be addressed?

How are the outcomes of the sensitivity analysis impacted if two or more parameters are varied together?



roadmap for scaling up and optimising such systems for future implementation (see [Outstanding questions](#)). Moreover, this technology has broader implications for agricultural systems, offering opportunities for diversification of farmers' revenues and creating alternative uses for surplus and residual produce. While this study focuses on grass feedstock, the proposed biorefinery model has the potential to transform various other organic residues into renewable resources. Overall, our research highlights AD as a centrepiece of the circular bioeconomy and offers pathways to support climate mitigation efforts.

## STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
  - Characterisation of the feedstock
  - Characterisation of the inoculum
- METHOD DETAILS
  - LBR-UASB reactor setup and operation
  - Experimental design
    - Commissioning stage: Determining the optimum throughput
    - Scenario 1: Closed-loop operation with equal distribution of leachate
    - Scenario 2 and 3: Varying biorefinery outputs on demand
  - Economic analysis methodology
    - Description of the biorefinery model and process units
    - Leach bed reactor (LBR)
    - Composting unit
    - PHA production unit
    - Upflow anaerobic sludge blanket (UASB) reactor
    - Combined Heat and Power (CHP) system
    - Economic assessment assumptions and calculations
  - Analytical methods
- QUANTIFICATION AND STATISTICAL ANALYSIS

## Resource availability

### Lead contact

Requests for further information and resources should be directed to and will be fulfilled by the lead contact, David Wall ([david.wall@ucc.ie](mailto:david.wall@ucc.ie)).

### Materials availability

This study did not generate any new or unique materials.

### Data and code availability

All data reported in this paper will be shared by the lead contact upon request. This paper does not report original code. Any additional information required to reanalyse the data reported in this paper is available from the lead contact upon request.

## Author contributions

Conceptualisation, R.S., R.O., D.M.W., and J.D.M.; methodology, R.S., R.O., D.M.W., and J.D.M.; formal analysis, R.S.; investigation, R.S., A.H., and M.M.; Writing – original draft, R.S.; writing – review & editing, R.S., A.H., M.M., A.B., R.O., D.M.W., S.B., and J.D.M.; visualisation, R.S. and A.H., supervision, A.B., R.O., D.W., J.D.M.; project administration, D.W. and S.B.; funding acquisition, D.W. and S.B.

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### Declaration of interests

The authors declare no competing interests.

### Resources

<sup>i</sup>[www.theguardian.com/environment/2024/feb/02/why-are-farmers-protesting-across-the-eu-and-what-can-the-bloc-do-about-it](http://www.theguardian.com/environment/2024/feb/02/why-are-farmers-protesting-across-the-eu-and-what-can-the-bloc-do-about-it)

<sup>ii</sup>[www.cso.ie/en/releasesandpublications/ep/p-coa/censusofagriculture2020-preliminaryresults/landutilisation](http://www.cso.ie/en/releasesandpublications/ep/p-coa/censusofagriculture2020-preliminaryresults/landutilisation)

<sup>iii</sup>[www.semopx.com/market-data/document-library/index.xml?keyword=](http://www.semopx.com/market-data/document-library/index.xml?keyword=)

<sup>iv</sup>[www.plasticportal.eu/en/cenove-reports?year=2024&week=12](http://www.plasticportal.eu/en/cenove-reports?year=2024&week=12)

<sup>v</sup>[www.sem-o.com/market-data/dynamic-reports/#BM-010](http://www.sem-o.com/market-data/dynamic-reports/#BM-010)

<sup>vi</sup>[www.osti.gov/etdeweb/biblio/22138958](http://www.osti.gov/etdeweb/biblio/22138958)

<sup>vii</sup><https://data.oecd.org/conversion/purchasing-power-parities-ppp.htm#indicator-chart>

<sup>viii</sup>[www.payscale.com/research/IE/Job=Quality\\_Assurance\\_\(QA\)\\_%2F\\_Quality\\_Control\\_\(QC\)\\_Engineer/Salary](http://www.payscale.com/research/IE/Job=Quality_Assurance_(QA)_%2F_Quality_Control_(QC)_Engineer/Salary)

<sup>ix</sup>[www.training.itservices.manchester.ac.uk/public/gced/CEPCI.html?reactors/CEPCI/index.html](http://www.training.itservices.manchester.ac.uk/public/gced/CEPCI.html?reactors/CEPCI/index.html)

<sup>x</sup>[www.grantthornton.ie/globalassets/1.-member-firms/ireland/insights/publications/grant-thornton-renewable-energy.pdf](http://www.grantthornton.ie/globalassets/1.-member-firms/ireland/insights/publications/grant-thornton-renewable-energy.pdf)

<sup>xi</sup>[www.semopx.com/documents/general-publications/MONTHLY-mkt-report-December2023.pdf](http://www.semopx.com/documents/general-publications/MONTHLY-mkt-report-December2023.pdf)

<sup>xii</sup>[www.seai.ie/sites/default/files/publications/Electricity-Gas-Prices-2023-H1.pdf#page=11.11](http://www.seai.ie/sites/default/files/publications/Electricity-Gas-Prices-2023-H1.pdf#page=11.11)

### Supplemental information

Supplemental information to this article can be found online at <https://doi.org/10.1016/j.tibtech.2025.01.005>.

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## STAR★Methods

## KEY RESOURCES TABLE

Reagent or resource	Source	Identifier
Biological samples		
Grass silage	Grassland Research Centre of Teagasc, the Irish Food and Agriculture Development Authority, Moorepark, Ireland	N/A
Granular sludge	Carbery UASB reactor treating cheese processing effluent, originally sourced for Paques, The Netherlands	<a href="https://www.paquesglobal.com/services/biocatalyst">https://www.paquesglobal.com/services/biocatalyst</a>
Chemicals, peptides, and recombinant proteins		
Nitrogen gas (99.99%)	BOC Gases Ireland Ltd.	CAS no.7727-37-9
Hydrochloric acid (37% diluted to 1M)	Merck (Sigma Aldrich)	Cat#100317
Volatile free acid standard mix	Merck (Sigma Aldrich)	Cat#CRM46975
Critical commercial assays		
Chemical oxygen demand (COD) test kit	Reagecon, Ireland	Cat#420721
Software and algorithms		
Apex Fusion aquarium controller	Neptune systems, USA	<a href="https://www.neptunesystems.com/apex-fusion/">https://www.neptunesystems.com/apex-fusion/</a>
SPSS® Statistics	IBM®	<a href="https://www.ibm.com/products/spss-statistics">https://www.ibm.com/products/spss-statistics</a>
Other		
Agilent Gas Chromatographer 7890B	Agilent Technologies, USA	<a href="https://www.agilent.com/en/product/gas-chromatography/gc-systems/7890b-gc-system">https://www.agilent.com/en/product/gas-chromatography/gc-systems/7890b-gc-system</a>
MRU Optima 7 Biogas analyser	MRU, Germany	<a href="https://www.mru.eu/en/products/detail/optima-biogas/">https://www.mru.eu/en/products/detail/optima-biogas/</a>
Mettler Toledo F20 benchtop pH probe	Mettler Toledo	<a href="https://www.mt.com/my/en/home/products/Laboratory_Analytics_Browse/pH-meter/pH-meters/F20-Meter.html">https://www.mt.com/my/en/home/products/Laboratory_Analytics_Browse/pH-meter/pH-meters/F20-Meter.html</a>
Apex A3 aquarium controller system	Neptune Systems, USA	<a href="https://www.neptunesystems.com/apex-a3-series/">https://www.neptunesystems.com/apex-a3-series/</a>
Hach DR3900 Spectrophotometer	Hach	<a href="https://ie.hach.com/dr3900-spectrophotometer-with-rfid-technology/product?id=24821473448">https://ie.hach.com/dr3900-spectrophotometer-with-rfid-technology/product?id=24821473448</a>

## EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

## Characterisation of the feedstock

Pit silage made from perennial ryegrass (*Lolium perenne*) was sourced from the Grassland Research Centre of Teagasc, the Irish Food and Agriculture Development Authority, located at Moorepark, Co. Cork, Ireland. The grass silage was packed in vacuum bags and stored at -20°C until use over one year of laboratory trials. On average, the grass silage had a dry matter content of 193 g kg<sup>-1</sup> wet weight and a volatile solids content of 905 g kg<sup>-1</sup> dry matter, as measured immediately before loading in the reactor each week.

## Characterisation of the inoculum

The inoculum for the UASB experiments consisted of granular sludge acquired from an industrial-scale UASB in Co. Cork, Ireland, treating the effluent from cheese manufacturing to produce biogas under mesophilic conditions (ca. 35°C). The





granular sludge had a total solids content of  $118 \text{ g kg}^{-1}$  wet weight and a volatile solids content of  $760 \text{ g kg}^{-1}$  total solids. Granules, ranging in size from 2 to 5 mm, were selected by sieving before seeding the UASB. Subsequently, the granular sludge was degassed for one month and acclimatised to the leachate produced from grass silage by gradually increasing the loading rate to the UASB until reaching the set organic loading rate for the experiments. The LBRs were not inoculated.

## METHOD DETAILS

### LBR-UASB reactor setup and operation

The LBR-UASB reactor used for the experiments comprised of three LBRs, one UASB, a leachate tank, an effluent tank, and a VFA collection tank as shown in Figure 2. The LBRs were fitted with a sprinkler on the lid, an outlet at the bottom, and a feedstock holding cage suspended at the centre. The feedstock holding cage had a perforated base with a muslin cloth draped around it to prevent washout of grass silage with the leachate. The three LBRs were loaded with 1 kg wet weight of grass silage each fed sequentially at 7-day intervals. For example, the first LBR was fed on day 0 and emptied/reloaded on day 21; the second LBR was fed on day 7 and emptied/reloaded on day 28, and so on. This effected a total solids retention time of 21 days per LBR. The temperature of the LBRs was maintained at ca.  $35^\circ\text{C}$  using heating jackets. A common leachate tank collected the leachate discharged by the three LBRs. The temperature of the leachate tank was maintained at  $35^\circ\text{C} \pm 2^\circ\text{C}$  by placing it on a hot plate.

The UASB had a working volume of 2 L. The UASB design and its operation have been described in detail in our previous work [21]. The UASB was inoculated with 1 L of sieved granular sludge, and the remaining volume was filled with deionised water. The temperature of the UASB was kept at  $35 \pm 1^\circ\text{C}$  (mesophilic range) using a heated water bath. The UASB was fed leachate collected in the leachate tank using a peristaltic pump (Masterflex® Ismatec® Reglo ICC, USA). Throughout all the experiments, the UASB was fed between 14:00 and 16:00 h every day as the goal was to produce most of the biogas during peak electricity demand hours of 16:00–20:00 h (as per Single Electricity Market Operator, Ireland<sup>v</sup>). This demand-driven operation of the UASB has been demonstrated previously by the authors [21].

The process started with 10 l of deionised water in the leachate tank. The water/leachate was recirculated over the grass in the LBRs using a peristaltic pump at a rate of  $15 \text{ l day}^{-1} \text{ kg}^{-1}$  wet weight of grass silage. The leachate percolated through the grass silage and discharged into the leachate tank. In effect, leaching extracted the organic matter from the grass silage through the hydrolysis process that occurred in the LBRs. The organic matter was quantified in terms of the COD concentration of the leachate. VFAs form a portion of this COD in the leachate. At the start of the process, the three LBRs were sequentially loaded with 1 kg of grass silage each over three consecutive weeks. This resulted in a gradual build-up of COD in the leachate, reaching approximately  $20 \text{ g l}^{-1}$  by the end of the third week, with VFAs contributing about 50% of the COD. This COD/VFA concentration was deemed sufficient for further downstream processing or applications such as PHA production [29,50], and hence it was maintained for further operation. Subsequently, leachate extraction began, with a portion of the leachate sent to the UASB to produce biogas, and another portion of the leachate collected in a 'VFA tank' for (assumed) utilisation of the VFAs. The downstream processing of the VFAs, such as for extracting specific VFAs or producing PHA biopolymer, was outside the scope of our experiments as this work focused on VFA production rather than processing. The effluent from the UASB was collected in an 'effluent tank'. When operated in an 'open-loop' manner during the commissioning stage (Baseline operation), the leachate was replenished with deionised water. Whereas in the 'closed-loop' operation of Scenarios 1–3, water use was partially replaced by the recirculated UASB effluent. Furthermore, in closed-loop mode, the pH of the effluent was lowered to  $5.0 \pm 0.2$  by dosing 1 M HCl before recirculating it back to the leachate tank. It was expected that a pH of ca. 5 would inhibit methanogenic archaea in the UASB effluent, without disturbing the natural pH of the leachate resulting from the hydrolysis and acidogenesis processes in the LBR [51]. The pH adjustment and the effluent recirculation were automated using a Neptune Apex aquarium controller system coupled with a peristaltic pump (DOS, Neptune system, USA).

A gas bag containing nitrogen gas was connected to the leachate, the effluent and the VFA tanks. These gas bags acted as expansion vessels to balance the pressure differences that occurred due to the emptying and filling of the tanks, ensuring an anoxic process. Prior to the start of experiments and after each LBR was reloaded, the system was flushed with nitrogen gas to ensure an anoxic environment.

### Experimental design

To address the specific objectives of this study, the LBR-UASB reactor setup was first commissioned and then operated in three different scenarios:

*Commissioning stage: Determining the optimum throughput.* Maintaining a stable level of COD-VFA concentration in the leachate over long-term operation is critical for the LBR-UASB biorefinery. Preliminary trials indicated that sustained production of both VFAs and biogas in the LBR-UASB system depended on the COD balance within the system such that the rate of COD consumption for VFA and biogas production should not exceed the rate of COD production in the LBRs. This balancing of the COD is essentially a function of the throughput of the system, and therefore, the rate at which leachate is extracted for further downstream processing. In the commissioning stage, this throughput of the system was determined through trials targeting a sustained COD level of ca.  $20 \text{ g l}^{-1}$  in the leachate. The trials aimed to achieve the highest possible throughput while maintaining the desired COD level in the leachate. The leachate extracted daily was distributed evenly (50:50 volumetric distribution) towards (assumed) PHA and biogas production. As the literature and the preliminary trials indicated, reutilising the UASB effluent for leaching was detrimental to long-term performance [16]. Thus, it was decided to initially determine the throughput of the system without effluent recirculation, that is, in an open-loop operation. The amount of leachate extracted daily was replenished with deionised water. The system performance was monitored in terms of COD-VFA yield ( $\text{g l}^{-1}$ ), acidification, and methane yield ( $\text{l CH}_4$  per kg COD fed to the UASB). The last stable HRT in the commissioning stage was considered as the 'Baseline' for comparison with the other operational scenarios.

*Scenario 1: Closed-loop operation with equal distribution of leachate.* Once a stable operation was achieved in the commissioning stage, Scenario 1 aimed to achieve zero discharge of effluent and minimise water use in the LBR-UASB biorefinery system by reutilising the effluent. A strategy for inactivating methanogenic archaea contained in the effluent by reducing the effluent pH was tested in Scenario 1. The pH of the effluent was lowered to  $5.0 \pm 0.2$  using 1 M HCl before recirculation back to the leachate tank, thus operating the system in a closed loop. The system was operated at the optimum throughput determined in the commissioning stage and with a 50:50 distribution of the leachate for (assumed) PHA and biogas production. Only the leachate used towards PHA production was replenished with water; therefore, the requirement of water in the system was reduced by half. The system performance was compared to the Baseline operation in terms of COD-VFA yield, acidification, and biogas output.

*Scenario 2 and 3: Varying biorefinery outputs on demand.* A biorefinery based on LBR-UASB can potentially vary the product outputs as per demand to maximise revenues. This can be achieved by varying the distribution of the leachate towards VFA processing or biogas production while keeping the overall throughput of the system constant. Two different leachate distribution ratios (and consequently, product output ratios) were experimentally tested in Scenarios 2 and 3. In Scenario 2, the leachate distribution for (assumed) PHA and biogas production was set at 75:25 respectively, representing a 'Bioproducts-focused' scenario; this was followed by a distribution ratio of 25:75 for Scenario 3, representing a 'Bioenergy-focused' scenario. The amount of leachate used for (assumed) PHA production was replenished with water, while the leachate used for biogas production was recirculated back after exiting the UASB and undergoing pH adjustment. The system performance was compared with that of the other operational modes of the experiment.

Each operational stage was run for two HRTs of the leachate tank of 17 days each (determined during the commissioning stage). The results are presented as an average over the second HRT, as the first HRT was considered a stabilisation period. When effluent recirculation was employed in the closed-loop operation of Scenarios 1–3, a 60-minute period after 16:00 was allocated for the pH adjustment of the collected effluent. Following this, the leachate tank was replenished with appropriate quantities of pH-adjusted effluent and water as per the operational scenario. The overall experimental design is illustrated in Figure 1 and operational parameters in each scenario are presented in Table 1.

### Economic analysis methodology

*Description of the biorefinery model and process units.* The economic analysis considered a case of an LBR-UASB biorefinery processing grass silage, with downstream processing to produce PHA biopolymer from VFAs, electricity and heat from biogas, and compost from residual grass silage, as illustrated in Figure 1. The biorefinery was assumed to process 35 000 tonnes per annum

(tpa) wet weight of grass silage based on the scale for grass biorefineries suggested by O'Keefe (2009), considering the existing grass biorefinery models in Europe and assessing logistical and financial feasibility in an Irish context (S.M. O'Keefe, PhD thesis, Wageningen University, 2010). A capacity of 35,000 tpa requires a catchment area of approximately 1000 ha, with a transportation radius under 2 km, and involves approximately 25 farms - a scale which could be practical to form a cooperative. Pit grass silage at ca. 20% dry matter content was assumed based on the grass silage used in our experiments. The modelled biorefinery consisted of the following main units: LBR, UASB, PHA production, CHP, and composting. The LBR-UASB operation was assumed to be similar to that of the laboratory-scale reactor in our study. As in the experiments, the process was assumed to operate at a stable leachate COD concentration of 20 g l<sup>-1</sup>. The VFA-rich leachate is assumed to be distributed between PHA and biogas production in the UASB as per the three output distribution scenarios (see Table 1 and Experimental design). However, the PHA production unit and the UASB were designed for maximum capacity, as if all the leachate was used entirely for either PHA or biogas production, respectively, to allow for demand-driven operation. Therefore, the CAPEX for the PHA production and UASB units accounts for this maximum capacity, but the OPEX is as per the leachate distribution in each operational scenario.

A brief description of how the unit processes in our biorefinery model were adapted from the literature is provided below:

**Leach bed reactor (LBR).** The LBRs considered in the proposed biorefinery resemble the garage-type solid-state anaerobic digesters with a leachate collection and recirculation system, similar to the laboratory-scale LBR-leachate tank setup used in our experiments (Figure 2). An example of a supplier for such reactors is Bekon GmbH, Germany. Process economics for the LBRs were adapted from the TEA of similar solid-state anaerobic digesters (SS-AD) by Lin and colleagues, who modelled SS-AD processing yard trimmings and liquid effluent from conventional anaerobic digesters using SuperPro Designer [32]. Equipment for processing liquid AD effluent was excluded to align with the proposed biorefinery. The specifications and process economics of the plant modelled by Lin and colleagues are provided in Tables S4 and S5 in the supplemental information online.

**Composting unit.** After the leaching process in the LBR ca. 30% of solids in the grass silage are destroyed (as per experiments in this study) and the residual quantity (70%) is assumed to be composted. Of this, 20% is assumed to end up as finished compost [32], giving an overall compost output of 14% of the original grass silage input to the biorefinery. The composting unit was also modelled based on the TEA conducted by Lin and colleagues in SuperPro Designer for composting yard trimmings and liquid AD effluent [32]. The specifications and process economics for the composting unit are presented in Tables S6 and S7 in the supplemental information online. Since the substrate for composting in our process (leached grass silage) differs from the yard trimmings in the study by Lin and colleagues, we validated our assumptions based on the AIKAN© process developed by Solum A/S<sup>vi</sup>. This process, which is similar to our LBR-UASB, achieves ca. 20% compost output from the LBR residues, closely aligning with our assumption of 14% and supporting its validity.

**PHA production unit.** PHA process economics were adapted from the TEA of an industrial-scale PHA production facility by Wang and colleagues using SuperPro Designer [33]. Their process involved enzymatic hydrolysis of cheese by-products to produce a feedstock suitable for PHA production under sterile conditions with pure microbial strains. In contrast, in our biorefinery model, the feedstock for PHA production is readily available in the form of VFA-rich leachate, eliminating the need for enzymatic hydrolysis. Also, we assume a process using a mixed microbial culture, which does not require sterile conditions, thereby significantly reducing costs [36]. Therefore, the costs from the study of Wang and colleagues were adapted by appropriately excluding those that were not relevant. The study by Wang and colleagues also included downstream processing including centrifugation, washing, and spray drying to produce a dry PHA powder with less than 5% moisture content. Therefore, costs to produce a high-quality PHA are included in our analysis, justifying a slightly higher selling price. Detailed specifications and process economics for the PHA production unit in the proposed biorefinery are provided in Tables S8 and S9 in the supplemental information online.

**Upflow anaerobic sludge blanket (UASB) reactor.** To the best of our knowledge, a TEA of a UASB reactor using SuperPro Designer is not available in the literature. Sato and colleagues developed equations for estimating the CAPEX and OPEX for UASBs based on daily processing capacity (m<sup>3</sup> day<sup>-1</sup>) by studying the costs for 14 UASB sewage treatment plants [34]. Therefore, the CAPEX and OPEX of the UASB were estimated using these equations presented below (Equations 1 and 2). The costs were based on an analysis



in 2003 within an Indian scenario; therefore, they were converted from INR to EUR using purchasing power parity (PPP) from the OECD<sup>vii</sup> before adapting to our process (also see Table S1 and S10 in the supplemental information online).

$$\text{For CAPEX, } CC = 494x^{-0.20} \quad [1]$$

$$\text{For OPEX, } OC = 457x^{-0.49} \quad [2]$$

Where,  $CC$  is the annual capital cost per unit volume ( $\$ \text{m}^{-3} \text{day}^{-1}$ ),  $OC$  is the annual operating cost per unit volume ( $\$ \text{m}^{-3} \text{day}^{-1}$ ) and  $x$  is the treatment volume ( $\text{m}^3 \text{day}^{-1}$ ).

**Combined Heat and Power (CHP) system.** The biogas production in the UASB is considered to be aligned with the peak electricity demand hours to enable on-demand use in a CHP during these periods. A CHP system for electricity and heat production from biogas was included in the cost of the LBR unit in the study by Lin and colleagues, and therefore, was not considered separately [32]. Upon scaling up the CHP system in the study by Lin and colleagues to match the size of our biorefinery, the resulting CHP capacity was approximately 1000 KWe. This exceeds what would typically be required in our process, as only part of the leachate is used for biogas production, while the rest is used for VFA-PHA production. However, the excess CHP capacity is essential for demand-driven electricity generation, as in this approach, the entire electricity output is generated during a few hours of the day, rather than being evenly spread throughout the day [31]. Specific costs related to grid connections or heat mains were not accounted for separately in this preliminary analysis, assuming they are covered under the 'Auxiliary facilities' costs in our CAPEX calculations (see Table S3 in the supplemental information online).

**Economic assessment assumptions and calculations.** The process specifications and economics for each unit, developed based on similar processes identified in the literature, are provided in the supplemental information (Tables S1-S10). The analysis was conducted in Microsoft Excel, focusing on the major capital expenditures, operating expenditures and revenues to determine profitability using net present value (NPV) as the indicator. Since none of the scenarios evaluated were profitable, other indicators such as pay-back period and internal rate of return are not presented. The CAPEX was estimated by adding direct and indirect costs in developing the biorefinery plant on a green field and included equipment purchase and installation, auxiliary facilities, electrical, piping, instrumentation, buildings, engineering, construction, yard development, and contractor fees (details in the supplemental information online). Cost estimation factors used by Lin and colleagues [32], based on the default values in SuperPro Designer, were used for estimating these costs (Table S3 in the supplemental information online). The OPEX for each unit included costs for annual maintenance, depreciation, utilities, raw materials, waste disposal, and other miscellaneous expenses. Feedstock cost was added as a common operational expenditure for the entire biorefinery. Labour costs were excluded from the OPEX data obtained from the literature to avoid double-counting and were calculated separately for the entire biorefinery plant. It is assumed that four operators, one quality control manager, and one plant manager are required to operate the biorefinery plant [52]. The salaries were estimated from the median salaries for these roles in Ireland<sup>viii</sup>. Revenue was generated through the sale of PHA, electricity and heat produced from biogas, and compost (Table 2). All energy produced from biogas as electricity and heat was assumed to be sold based on 2023 market prices in Ireland (see Table S11 in the supplemental information online). As such, the analysis did not account for biogas use on-site for self-consumption (accruing savings in OPEX) and the sale of surplus energy (for revenue), and therefore, did not consider a differential pricing for the energy produced. For simplicity, the acid-dosing strategy with HCl used for testing UASB effluent recirculation in experimental work was excluded from the economic analysis, as this was tested as a proof of concept. Actual implementation would have implications on utilities (e.g., process water use) and waste disposal (effluent treatment) costs, which were beyond the scope of this analysis. Similarly, costs associated with transportation, interest on capital, and taxes were not considered in this preliminary economic analysis.

Where the cost data available was for a different year and operational scale, the **Chemical Engineering Plant Cost Index (CEPCI)** and the six-tenth rule for scaling, were used to adjust the data, as per Equation 3 [53].

$$CP = CR \left( \frac{SP}{SR} \right)^n \left( \frac{IP}{IR} \right) \quad [3]$$



where, CP is the cost for the present project; CR is the cost for the reference project; SP is the scale for the present project; SR is the scale for the reference project;  $n$  is the scaling factor 0.6; IP is the CEPCI for present project analysis year (2023), and IR is the CEPCI for reference project analysis year (2023)<sup>ix</sup>.

The project feasibility was analysed based on the NPV of the biorefinery with a 20-year lifetime as per Equation 4 [54]. A discount rate of 10%, which is typical for biomass facilities, was assumed<sup>x</sup>.

$$NPV = CC + C_i \frac{(1 + d)^L - 1}{d(1 + d)^L} \quad [4]$$

where, CC: Capital cost in year 0;  $C_i$ : Net cashflow in each year;  $d$ : Discount Rate (10%),  $L$ : project lifetime (20 years).

Electricity and heat prices for revenue calculations were determined as follows:

The price of electricity generated during peak demand hours was determined using the following steps–

- i) 2023 Day-ahead market (DAM) prices of electricity traded on the Single Electricity Market Operator (SEM-O) (market for Republic of Ireland and Northern Ireland) were used as the reference price
- ii) Ex-Ante Market look back dataset published by SEM-O was obtained<sup>iii</sup>
- iii) Hourly DAM prices were filtered from the dataset to obtain daily prices between 16:00 and 20:00 h in 2023 (typical peak demand hours)
- iv) The average DAM price so obtained was 151.6 €/MWh. This price was 25% higher than the average DAM prices of 121.91 €/MWh for 2023<sup>xi</sup>, representing a premium for selling electricity during peak demand hours, and was used in the analysis.

The price of natural gas for heat revenue calculation was determined as follows–

For simplicity, it is assumed that there is demand for the heat generated in the vicinity and it replaces natural gas use. Therefore, natural gas price is used as a proxy for the revenue generated by selling the heat. Gas prices for business customers published biannually by the Sustainable Energy Authority of Ireland (SEAI) based on Eurostat were used as the reference. An average price of 76 €/MWh for Ireland in 2023 across all consumption bands was used in the analysis (calculated from 80 €/MWh for Jan-Jun and 72 €/MWh for Jul-Dec)<sup>xii</sup>.

Cost data were compared against multiple sources in literature and through personal communication with industrial operators (requested to remain anonymous) to ensure quality and reliability. A sensitivity analysis was conducted to account for uncertainty and identify key parameters that impact the profitability (as indicated by the NPV) of the project. The values for several parameters including the total CAPEX, total OPEX, OPEX specific to PHA unit, feedstock cost, PHA market price, electricity and gas market prices, grass silage-to-COD yield, and VFA-to-PHA yield were varied by  $\pm 25\%$  from the base case values [55]. Only the top five parameters having the most influence on the NPV in terms of percentage change are presented (see Table S12 in the supplemental information online).

#### Analytical methods

Standard methods 2540 G were used to analyse the dry matter and volatile solid content of the grass silage and the inoculum [56]. The leachate and UASB effluent samples were centrifuged at 12,000 RPM for 10 minutes before the COD and VFA measurements. The supernatant was analysed for measuring the soluble component of COD using Reagecon COD vials (Range 0-1500 mg l<sup>-1</sup>, Code: 420721) and a Hach Lange DR3900 Spectrophotometer at 620 nm wavelength. For the sake of simplicity, this soluble COD is referred to as COD. The VFA analysis was conducted using gas chromatography (Agilent 7890B, USA) with a flame ionisation detector [57]. The analysis accounted for acetic, propionic, isobutyric, butyric, isovaleric, valeric, and caproic acid using a volatile free acid mix (Sigma Aldrich) as a standard. The sum of all measured VFAs is referred to as the total VFA concentration. The pH was analysed using a Mettler Toledo F20 benchtop pH meter. For determining the composition, biogas was collected in a gas bag once a week and analysed using MRU Optima 7 BioGas Analyser.



The COD removal efficiency in the UASB was calculated as follows:

$$CODrem_{eff} = ((COD_{in} - COD_{out})/COD_{in}) \times 100\% \quad [5]$$

where:  $COD_{in}$  is the COD of the leachate fed to the UASB and  $COD_{out}$  is the COD of the effluent exiting the UASB.

Similarly, VFA removal efficiency for the UASB was calculated based on the total VFA present in the influent leachate and the effluent, respectively.

Acidification was defined as the fraction of COD in the leachate contributed by the VFAs. This was calculated by converting individual VFAs to their COD equivalents and dividing the sum of total VFA-COD by the COD of the leachate [18,27].

### QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis was conducted using IBM® SPSS® to assess the differences in system performance across the operational scenarios. A two-sample t-test was used to compare two groups, while one-way ANOVA with Tukey's HSD post-hoc analysis was used for multiple group comparisons. The significance level ( $\alpha$ ) was set as 0.05 for all tests.