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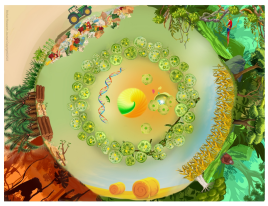

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Original Research Paper

Cascading valorization of defatted rice bran for lactic acid fermentation and biogas production

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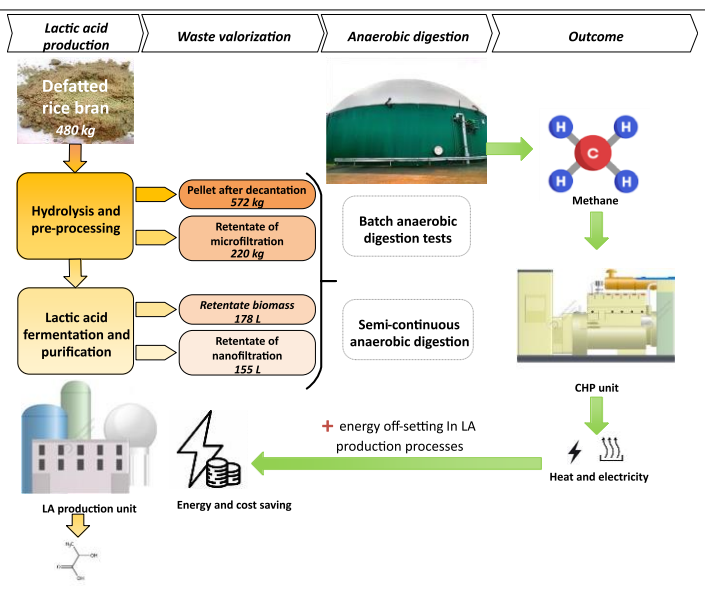
HIGHLIGHTS

- Defatted rice bran was successfully converted into lactic acid and biogas.
- Residues of lactic acid production showed high methane yields of up to 434 L_N kg_{VS}^{-1}.
- Co-digestion of major residues exhibited enhanced tolerance to ammonia inhibition.
- The biogas generated can reduce 50% of the energy costs for lactic acid production.
- A 67% reduction in environmental impact at the midpoint and 71% at the endpoint was achieved.

GRAPHICAL ABSTRACT



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ABSTRACT

This study investigated the integrated valorization of defatted rice bran (DRB) by converting it into lactic acid (LA) and subsequently utilizing the residues from LA production for biomethane generation through anaerobic digestion (AD). Processing 480 kg of DRB resulted in the production of 70 L of pure LA and generated significant waste streams, primarily consisting of 572 kg of decanted hydrolysate pellet (Pellet DEC) and 220 kg of microfiltration retentate (Retentate MF). Exceptionally high methane yields of 374–434 L_N kg_{VS}^{-1} were observed for residues from LA fermentation in biochemical methane potential tests, indicating their high potential for biogas production. During long-term semi-continuous AD, varying organic loading rates (OLRs) from 0.5–2.5 kg_{VS} m^{-3} d^{-1} demonstrated feedstock- and OLR-dependent methane production. Reactor failure at higher OLRs was attributed to the accumulation of total ammoniacal nitrogen (TAN). The co-digestion of Pellet DEC and Retentate MF proved to be more resilient, with OLRs up to 2 kg_{VS} m^{-3} d^{-1}, mitigating TAN inhibition. Methane yields, ranging from 265–334 L_N kg_{VS}^{-1} before reaching inhibitory OLR levels, were higher than those found in the literature. Process integration has emerged as a promising approach because the biogas generated from residues could effectively offset the energy demands of LA production. Supported by life cycle assessment, the integrated processes showed a 67% lower environmental impact at the midpoint and a 71% lower environmental impact at the endpoint, along with an 80% reduction in energy costs compared to the standalone LA production. Results proved a significant enhancement of the sustainability and economic viability of this integrated biorefinery approach.

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Contents

Abbreviations

AD	Anaerobic digestion	MEP	Marine eutrophication potential
ADF	Acid detergent fiber	METP	Marine ecotoxicity potential
ADL	Acid detergent lignin	NDF	Neutral detergent fiber
ALOP	Agricultural land occupation potential	NLTP	Natural land transformation potential
BMBF	Federal Ministry of Education	ODP	Ozone depletion potential
BMP	Biochemical methane potential	OLR	Organic loading rate
C/N	Ratio of carbon to nitrogen	Pellet DEC	Pellet after decantation of hydrolysate
CHP	Combined heat and power	PLA	Polylactic acid
CSTR	Continuously stirred tank reactor	PMFP	Particulate matter formation potential
DRB	Defatted rice bran	POFP	Photochemical oxidant formation potential
EQ	Ecological quality	R	Resources
FDP	Fossil depletion potential	Retentate MF	Retentate after microfiltration
FEP	Freshwater eutrophication potential	Retentate NF	Retentate after nanofiltration
FETP	Freshwater ecotoxicity potential	s.e.m.	Standard error of the mean
GWP100	Greenhouse gas emission potential	TAN	Total ammoniacal nitrogen
HH	Human health	TAP100	Terrestrial acidification potential
HLR	Hydraulic retention time	TETP	Terrestrial ecotoxicity potential
HTP	Human toxicity potential	TS	Total solids
ICP-OES	Inductively coupled plasma optical emission spectroscopy	tVFA	Total volatile fatty acids
IRP	Ionizing radiation potential	ULOP	Urban land occupation potential
LA	Lactic acid	VFA	Volatile fatty acids
LCA	Life cycle assessment	VOA/TIC ratio	Ratio of volatile organic acids to total inorganic carbon
LCI	Life cycle inventory	VS	Volatile solids
L_N	Liters normalized to standard temperature and pressure	WDP	Water depletion potential
MDP	Metal depletion potential		

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Symbols			
e	Euler's number	t_{50}	Half-life
k	First-order decay constant	y	Specific methane yield
R_m	Maximum specific methane production rate	y_m	Maximum specific methane yield at theoretically infinite digestion time
t	Time	λ	Lag phase

1. Introduction

Lactic acid (LA) is an essential organic acid that is widely used in food, pharmaceutical, textile, leather, and chemical industries. Its versatility renders it indispensable for diverse manufacturing processes, fostering innovation and bolstering sustainability efforts. However, the high cost associated with the production of optically pure LA has historically constrained its broader adoption, particularly in fields like polylactic acid (PLA) production, where it serves as a crucial precursor to eco-friendly alternatives to petroleum-based plastics (López-Gómez et al., 2020; Huang et al., 2023). Traditional methods of commercial LA production rely heavily on refined sugars and starch-rich feedstocks, which constitute a significant portion of the overall production costs. Global demand for LA is increasing, with consumption reaching 1.5 million metric tons worldwide in 2022 and projected to soar to 2.8 million metric tons by 2030 amounting to between USD 6.23 billion and USD 7.52 billion. Thus, the exploration of cost-effective production methods has become imperative (SkyQuestt, 2024; Statistica, 2024; Vantage Market Research, 2024).

In response to this demand, the use of residual feedstocks has become a promising strategy to lower production costs and improve sustainability (Alexandri et al., 2019; Costa et al., 2021; Song et al., 2022). Leading biotechnology companies such as Galactic and NatureWorks have pioneered the integration of agricultural and food wastes into their production processes (Costa et al., 2023). Among these renewable feedstocks, defatted rice bran (DRB) has attracted considerable attention as a potential substrate for LA fermentation. Researchers have attempted strides in producing LA from DRB using various microbial strains, demonstrating its viability as a cost-effective alternative to conventional feedstock (Alexandri et al., 2020a and b).

In recent years, there has been a growing emphasis on leveraging agro-industrial waste to replace refined and costly raw materials. DRB, a byproduct of rice milling, has emerged as a particularly promising substrate because of its abundance and potential to reduce substrate costs in LA fermentation (Cristofoli et al., 2023). Notably, various studies have demonstrated impressive productivities, such as 2.7–6.20 g L^{-1} h^{-1} in batch fermentation, underscoring the efficacy of DRB as a substrate for LA production (Wang et al., 2014; Li et al., 2015; Alexandri et al., 2019). However, the downstream processing of LA from agricultural residues, such as DRB, generates additional waste products, posing significant environmental challenges and necessitating efficient waste management strategies. These waste byproducts pose a substantial burden on the environment, requiring sustainable disposal methods to mitigate their impact. In this context, the global importance of valorizing agricultural residues is clear. Converting these residues into valuable resources not only mitigates environmental pollution but also promotes sustainable development. Valorizing agricultural residues aligns with global efforts to address waste management issues, reduce greenhouse gas emissions, and promote renewable energy sources (O'Shea et al., 2021).

Anaerobic digestion (AD) has emerged as a viable solution for managing the waste generated from the downstream processes of LA production (Demichelis et al., 2017; Demichelis et al., 2018). This biological process breaks down organic matter in the absence of oxygen, yielding biogas as a valuable product and energy carrier. The biogas produced can be used as a renewable energy source, reducing reliance on fossil fuels and contributing to energy security. The AD process involves several distinct stages, including hydrolysis, acetogenesis, acidogenesis, and methanogenesis (Hansen, 2023; Wang et al., 2023). Each stage plays a crucial role in breaking down complex organic materials into simpler compounds, ultimately producing methane and carbon dioxide as primary components of biogas.

Implementing AD for the valorization of agricultural residues not only addresses waste management challenges but also enhances the economic viability of agricultural processes. By converting waste into biogas, it creates an additional revenue stream for farmers and industries involved in LA production. Furthermore, the byproducts of AD, such as digestate, can be used to grow microalgae (Ermis et al., 2022) or as nutrient-rich fertilizers, fostering nutrient recycling and supporting sustainable agricultural practices. By addressing both environmental and economic challenges, this approach aligns with global sustainability goals and contributes to a more sustainable and resilient future.

In recent years, few studies have explored the combined production of LA and biogas (**Table 1**). However, these studies have reported promising results, showing significant improvements in revenue from feedstock utilization for LA recovery and subsequent AD compared to traditional methods of producing either LA or biogas alone (Kim et al., 2016; Demichelis et al., 2017; Chopda et al., 2024). Research approaches for the integration of LA and biogas production can be divided into three categories (**Table 1**): (1) LA production in a distinct LA fermentation process using individual bacterial strains or specialized mixed cultures with subsequent valorization of residual biomass feedstocks from upstream and downstream processing for AD; (2) two-stage AD with separation of hydrolysis and acidogenesis from acetogenesis and methanogenesis and adjustment of the first stage for LA accumulation; and (3) ensiling for biomass preservation and storage, subsequent extraction of LA from silage and AD of solid residues. While LA production in two-stage AD plants and during ensiling can benefit from the possibility of using existing infrastructures (Bühlmann et al., 2021), the requirements for downstream purification of LA from complex fermentation broths are increasing. Moreover, improved controllability and optimization of the separate LA fermentation process typically result in higher LA yields and enhanced recovery efficiencies. This approach also provides the opportunity to produce high-quality, optically pure LA.

The primary challenge in process integration lies in optimizing the operation of the individual processes involved in LA fermentation and AD (Bühlmann et al., 2023). The process control and conversion efficiencies mainly depend on the input material. Furthermore, the chosen method of upstream and downstream LA production influences the course and efficiency of the use of residues for subsequent AD. A thorough understanding of the optimal operational conditions and process limitations of the subsequent digestion process is crucial to maintaining stable long-term operation in an integrated system. To date, biogas production from LA fermentation residues has only been considered to a very limited extent, with a focus on LA production from crop materials and food waste (**Table 1**). Apart from the study by Kim et al. (2016), optimization of the process operation in the AD of residues from LA production has not yet been considered.

To the best of our knowledge, this is the first study to consider the cascading utilization of DRB for LA fermentation and subsequent biogas production. Achieving full valorization of agricultural residues, such as DRB, through an integrated approach of LA production and methane generation *via* AD offers significant economic and environmental advantages. Our research aims to investigate the anaerobic digestibility and valorization potential of diverse waste streams generated at various stages of LA production and downstream processes using DRB as a feedstock. Moreover, compliance with EU regulations, which restrict the dumping of agricultural residues in landfills, underscores the importance of this study (Garske et al., 2020). Achieving full valorization of agricultural residues, such as DRB, which encompasses an integrated approach of LA production and methane generation through AD, holds significant economic and environmental advantages. In this regard, our research aimed to investigate

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Table 1.
Comparison of research work on integrated production of lactic acid and biogas and the present study.

Feedstock	Category of Process Integration*	Description of Process Combination	Microorganisms Used for LA Fermentation	Downstream Processing for LA Purification	The Scale of LA Fermentation	Scale of AD	Focus of Research	Ref.
Milled and ensiled grass	(1)	Acid treatment and enzymatic hydrolysis followed by LA fermentation, anaerobic digestion of the solid fraction after hydrolysis separated by centrifugation	*Lactobacillus delbrueckii* and *Pediococcus acidilactici*	Not considered in this study	1 L, Batch	500 mL, BMP test	Pretreatment techniques to enhance the value chain of grass biomass	Chopda et al. (2024)
Ricotta cheese exhausted whey	(1)	Cultivation of LA bacteria, utilization of the liquid fraction after harvesting (centrifugation) of bacteria for anaerobic digestion	*Lactococcus lactis* subsp. *lactis* and *Streptococcus thermophilus*	Not considered in this study, the aim of fermentation of LA bacteria	n.r.	100 mL, BMP test	Mono-digestion of wastewater of lactic acid bacteria fermentation and co-digestion with pig slurry; agronomic use of digestate	Vasmara et al. (2021)
Food waste	(1)	Acid treatment and enzymatic hydrolysis followed by LA fermentation, anaerobic digestion of the solid fraction including an oily phase after LA fermentation separated by centrifugation	*Streptococcus* sp.	Not considered in this study	2 L, Batch	1.5 L, BMP test	Comparison of simultaneous saccharification and fermentation (SSF) and separate enzymatic hydrolysis and fermentation (SHF) for LA production, methane production potentials from solid fermentation residues, mass balance	Dechimelis et al. (2017)
Food waste	(1)	Shredding of food waste followed by LA fermentation, centrifugation of fermentation broth, and utilization of solid residue for anaerobic digestion	Indigenous mixed culture	Centrifugation, LA extraction from the supernatant by the combined process of nanofiltration and water-splitting electrodialysis	3.5 L, Continuous fermentation	5 L, CSTR	Optimization of LA fermentation (temperature, HRT), optimization of anaerobic digestion of the solid residue (OLR)	Kim et al. (2016)
Maize stover	(2)	Ensiling of maize stover for 360 days, pressing after ensiling, determination of lactic acid in press juice, and anaerobic digestion of press cake	Epiphytic lactic acid bacteria	Not considered in this study, only analyses of LA concentration	300 g, Batch	180 mL, BMP test	Optimization of organic acid production during ensiling by adjusting the C/N ratio and buffer capacity using additives, analyses of methane production potentials of solid residues after liquid separation, economic evaluation	Sun et al. (2022)
Grass, rye	(2)	Ensiling of grass/rye, pressing after ensiling, and use of solid residue for anaerobic digestion	Epiphytic lactic acid bacteria; addition of homo- or heterofermentative LAB	Not considered in this study, only analyses of LA concentration	1.5 L, Batch	100 mL, BMP test	Enhancement of LA concentration during ensiling by different silage additives, methane production potentials of solid residues after liquid separation	Haag et al. (2016)
Maize, amaranth	(2)	Ensiling of maize/amaranth, pressing after ensiling, and use of solid residue for anaerobic digestion	Epiphytic lactic acid bacteria; addition of homo- or heterofermentative LAB	Not considered in this study, only analyses of LA concentration	1.5 L, Batch	100 mL, BMP test	Enhancement of LA concentration during ensiling by different silage additives, methane production potentials of solid residues after liquid separation	Haag et al. (2015)
Mixed food waste	(3)	Two-stage anaerobic digestion with LA and organic acid production in the first stage, LA recovery by centrifugation, vacuum filtration, and LA adsorption using a polymeric resin; biomethanation of the liquid and solid residue	Mixed culture	Centrifugation, microfiltration, and adsorption at laboratory scale	290 m^3, Continuous fermentation	400 mL, BMP test	Optimization of LA recovery with a polymeric resin, investigation of the effects of LA recovery on subsequent methane production potential of the solid and liquid residue	Bühlmann et al. (2022); Bühlmann et al. (2021)

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Table 1.
continued.

Feedstock	Category of Process Integration*	Description of Process Combination	Microorganisms Used for LA Fermentation	Downstream Processing for LA Purification	The Scale of LA Fermentation	Scale of AD	Focus of Research	Ref.
Defatted rice bran	(1)	Acid treatment and enzymatic hydrolysis followed by LA fermentation and purification, anaerobic digestion of the residual fractions of hydrolysis, fermentation, and downstream processing for LA purification.	*Bacillus coagulans*	Micro- and nanofiltration, electrodialysis, ion exchange, evaporation at pilot scale	450 L, Continuous fermentation	2 L, BMP test, and 5 L, CSTR	Analysis of methane production potentials of different residues of LA fermentation and purification, optimization of AD of major residues (OLR), LCA, and economic considerations	**Present Study**

* Category of process integration: (1) Separate LA fermentation and AD of residues, (2) Ensiling with LA extraction and AD of solid residues, (3) Two-stage AD with LA accumulation in the first stage and methane production in the second stage.
LA: lactic acid; AD: anaerobic digestion; CSTR: continuously stirred tank reactor; BMP: biochemical methane potential; HRT: hydraulic retention time; OLR: organic loading rate; LCA: life cycle assessment; n.r.: not reported.

the anaerobic digestibility and valorization potential of diverse waste streams generated at various stages of LA production and downstream processes using DRB as a feedstock.

A comprehensive review of the current state-of-the-art reveals several gaps in the literature. While numerous studies have investigated the AD of agricultural residues, there is limited research on the specific waste streams generated from LA production processes. The unique composition of these waste streams necessitates tailored AD strategies to optimize biogas production and ensure process stability. Additionally, the co-digestion of multiple waste streams from LA production, whether in batch or semi-continuous processes, has not been extensively explored despite its potential to enhance methane yields and improve overall process efficiency. Furthermore, long-term semi-continuous studies with optimized OLR have not been investigated. Finally, there is a lack of holistic assessments that integrate LA production with AD, evaluating the environmental and economic impacts of such integrated systems compared to standalone processes. The objectives of this study were as follows:

(1) Assess the methane production potential of DRB raw material and four main waste streams from pilot-scale LA fermentation and purification in batch AD tests, contributing to renewable energy generation and reducing waste.
(2) Determine detailed methane production characteristics and long-term performance of selected waste streams in continuous AD processes, optimizing organic loading rate (OLR) for stable process conditions, thereby enhancing the sustainability and efficiency of biogas production.
(3) Evaluate the effects of co-digestion of two major waste streams from LA fermentation in batch and continuous AD experiments and conduct a life cycle assessment (LCA) of integrating the LA production process with AD compared to the standalone LA production process to highlight the environmental benefits and economic feasibility of a combined approach.

With this cross-disciplinary approach, including biochemical and renewable energy production as well as environmental and economic evaluation, our study seeks to contribute to the development of sustainable waste management strategies. This research fosters a circular economy paradigm by turning waste into valuable resources, thereby addressing the environmental challenges associated with industrial production processes and promoting economic viability.

2. Material and Methods

2.1. Raw materials

Dried, powdered DRB was generously supplied by All Organic Treasures GmbH (Kempten, Germany). A compositional analysis of the DRB was conducted, revealing the following results: starch 35.3 ± 1.5%, protein 17.3 ± 0.2%, cellulose 9.8 ± 0.4%, hemicellulose 20.6 ± 0.7%, lignin 3.9 ± 0.2%, fat 3.0 ± 0.4% and total solids (TS) 89.9 ± 0.9% (Alexandri et al., 2019).

LA fermentation was performed using the thermophilic microbial isolate *Bacillus coagulans* A107. This strain is part of the microbial collection at the Leibniz Institute of Agricultural Engineering and Bioeconomy (Potsdam, Germany). The pre-culture was prepared in MRS broth (Merck, Germany) using Everzit Dol (Evers, Germany) dolomite for buffering for 10-16 h at 52°C.

Inocula for AD experiments consisted of digestate from a farm-scale AD plant (LVAT, Groß Kreutz, Germany) mainly operated with dairy cow manure as feedstock and from previously completed AD tests with crop materials and manures. Prior to use, the inoculum was sieved through a < 2 mm sieve and degassed. The activity of the inoculum was confirmed through reference tests using microcrystalline cellulose as the reference substrate (VDI, 2016). The inoculum used for batch AD tests and startup of the semi-continuous AD experiments was characterized by average chemical properties as follows: pH 7.98 ± 0.12; TS 3.73 ± 0.13 %; volatile solids (VS) 65.27 ± 1.04 $\%_{TS}$; total nitrogen (N) 2.85 ± 0.19 g kg^{-1}; total ammoniacal nitrogen (TAN) 1.65 ± 0.14 g kg^{-1}; organic acids 0.11 ± 0.05 g kg^{-1}.

2.2. Pilot scale hydrolysis and lactic acid fermentation procedure

Hydrolysis of 480 kg of DRB in 2088 L of demineralized water was conducted in three batches in a 1000 L stirring vessel (Apparate- & Behältertechnik Heldrungen GmbH, Germany) over 24 h for each batch using a two-step process. Alpha-amylase (Termamyl – 324 mL, Termamyl SC, Novozymes, Denmark), glucoamylase (dextrozyme 480 mL, Dextrozyme GA 1.5, Novozymes, Denmark), and protease (Fermgen 240 mL, Fermgen, International Flavors & Fragrances Inc.) enzymes were utilized in conjunction with an acidic medium containing 18 L of 32% HCl (CVB, Berlin, Germany). The hydrolysis involved two steps: Step 1, incubation at 85°C and pH 6.0 for 2 h with Termamyl, and Step 2, incubation at 50°C and pH 4.2 for 21 h with Dextrozyme and Fermgen. Hydrolysis conditions were chosen following the protocols developed by Alexandri et al. (2019).

After hydrolysis, the produced hydrolysate underwent decantation (decanter Z2E-4/401 SP3.08, Flottweg SE, Germany) for 8.5 h, yielding 2065 L of filtrate and 572 kg of residue pellet (Pellet DEC). The filtrate underwent a microfiltration process using a microfiltration device (FUMATECH GmbH, Germany) equipped with a 0.2 µm pore size microfiltration membrane (TAMI Deutschland GmbH, Germany) composed of ZrO_2-TiO_2. This step resulted in 220 kg of residual retentate from microfiltration (Retentate MF). The permeate was then sterilized using 220 L and 400 L stirring vessels (Apparate- & Behältertechnik Heldrungen GmbH, Germany) at 121°C for 15 min. This sterilized permeate served as the substrate for continuous LA fermentation in a 450 L fermenter (Pilotfermenter Typ P, Bioengineering AG, Switzerland), which was conducted for 98 h, under agitation (250 rpm) and at 52°C. The pH was adjusted to 6.0 during the process by the addition of 20% NaOH. Fermentation parameters were based on previous studies carried out in our lab (Azaizeh et al., 2022; Marzo-Gago et al., 2024).

During continuous fermentation, the fermenter's outflow underwent microfiltration using a membrane filter (FUMATECH GmbH, Germany)

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with a 0.2 µm Microza UMP 1047 R membrane (Pall Filtersystems GmbH, Germany). The average LA concentration in the filtrate stream was approximately 31 g L^{-1}, with an average process yield of 0.53 g LA per gram of consumed sugars. The filter system retained the active biomass inside the fermenter, which was available as retentate (Retentate Biomass) after continuous LA fermentation. The permeate from the microfiltration step was subjected to nanofiltration using a nanofiltration membrane (NF2540, Dow, USA) and a nanofiltration device (Umwelt- und Ingenieurtechnik GmbH, Dresden, Germany). The resulting retentate of nanofiltration (Retentate NF) was investigated as another residue stream and potential substrate for AD, whereas the permeate underwent further downstream processing to obtain a final concentrate of 70 L of LA. The process flow, including the occurrence of residual biomass, is shown in **Figure 1**.

2.3. Batch anaerobic digestion tests

Biochemical Methane Potential (BMP) tests for the analysis of residual materials from LA fermentation were conducted using 2 L glass vessels filled with a mixture of inoculum and substrate, maintaining an inoculum-to-substrate ratio of 2 based on VS. DRB and the residual biomass streams from hydrolysis and LA production, including Pellet DEC, Retentate MF, Retentate Biomass, and Retentate NF were analyzed as substrates. In addition, a combination of the major residual biomasses Pellet DEC and Retentate MF was applied as substrate with a mass ratio of the mixture according to their occurrence (ratio Pellet DEC to Retentate MF of 2.6 to 1.0 based on fresh matter). Each experiment was conducted in triplicates. The vessels were incubated in a heated chamber at a constant temperature of 37°C for 30 d. The vessels were manually shaken once daily to prevent scum formation. The biogas generated during the incubation period was collected using wet gas meters and quantified by displacing an acidified saturated NaCl barrier solution (VDI, 2016). Daily, the volume of biogas was determined, adjusted for the volume produced by the inoculum alone, and normalized to standard conditions (dry gas; 0°C; 101,325 Pa). The composition of the biogas, including methane and carbon dioxide content, was analyzed using a portable gas analyzer equipped with infrared and chemical sensors (BIOGAS 5000, Geotechnical Instruments (UK) Ltd, Leamington Spa, UK). The methane concentration of the biogas was corrected to account for its dilution within the headspace of the AD test system in accordance with the VDI (2016) guidelines.

2.4. Semi-continuous long-term anaerobic digestion experiments

2.4.1. Experimental setup of the continuous digestion system

The continuous anaerobic digester setup included a fermenter system with nine cylindrical continuously stirred tank reactors (CSTR) of stainless steel and an automated gas volume-measuring unit. This comprehensive setup allowed for precise monitoring of gas production, ensuring thorough data collection and analysis throughout the experiment. Each fermenter had a total capacity of 4.7 L and a working volume of 3 L. During the experiment, the reactor temperature was maintained at 37°C using a temperature controller unit operating a heating coil at the bottom of each reactor based on temperature measurements within each reactor. The temperature hysteresis, once the set operating temperature was achieved, was maintained within a precise range of ±0.1°C.

The reactor content was stirred with an anchor agitator at a stirring speed of 60 rpm and stirring intervals of 30 s, followed by 30 s breaks and periodic changes in the stirring direction every 15 s. Intermittent stirring at 60 rpm was chosen to balance effective mixing with energy efficiency. It ensures uniform nutrient distribution and prevents dead zones without continuous operation, reducing energy consumption. This approach also minimizes shear stress on microorganisms, maintaining their activity and viability, and prevents solid particle settling, enhancing digestion efficiency and biogas production.

The gas-tight lids of the reactors were equipped with a feeding tube submerged in the liquid reactor content to maintain anaerobic conditions within the reactors and avoid gas loss during feeding. The biogas produced during reactor operation was passed through a gas outlet, which was connected to a glass vessel filled with iron oxide pellets to remove hydrogen sulfide and water from the gas, and a subsequent gas volume measuring device (BlueMethano GmbH, Berlin, Germany). The gas volume was measured according to the displacement principle, in which the produced gas lifted a piston in a guide tube until a magnetic sensor was reached, which caused a digital impulse and the release of the gas at a defined volume. Each impulse corresponds to a volume of 1 mL, enabling precise and accurate gas volume determination. As per the manufacturer's information, the stability of the temperature is maintained with an uncertainty of ±0.1°C, and the gas flow measurement has an uncertainty of ±0.3% over the entire range. Biogas volumes were counted, recorded, and normalized to standard conditions (dry gas, 0°C, 101,325 Pa) using integrated gas temperature and air pressure sensors and a microcontroller. After passing through the gas counter, biogas was collected in gas bags (Tesseraux Spezialverpackungen GmbH, Bürstadt, Germany) for further analysis of gas composition.

Both the batch and continuous AD experiments were conducted at 37°C because this temperature approximates the optimal conditions for the activity of mesophilic microorganisms, which are commonly used in AD processes. Numerous studies have conducted experiments at this temperature (Al Ramahi et al., 2021; Hansen et al., 2021; Fazzino et al., 2023). Mesophilic microorganisms thrive in moderate temperature conditions, and their activity peaks around 37°C. This temperature range is ideal for their metabolic processes, enabling them to break down organic matter more effectively and leading to higher biogas (primarily methane) production. The temperature supports the enzymes involved in the digestion process, facilitating efficient substrate breakdown.

2.4.2. Operation of the continuous digestion system

To start the continuous AD experiments, nine CSTRs were filled with 3 L of inoculum and subsequently operated with residual biomasses from the pilot-scale LA fermentation. Three substrate variants were tested as follows; all variants were realized with three biological replicates in parallel:

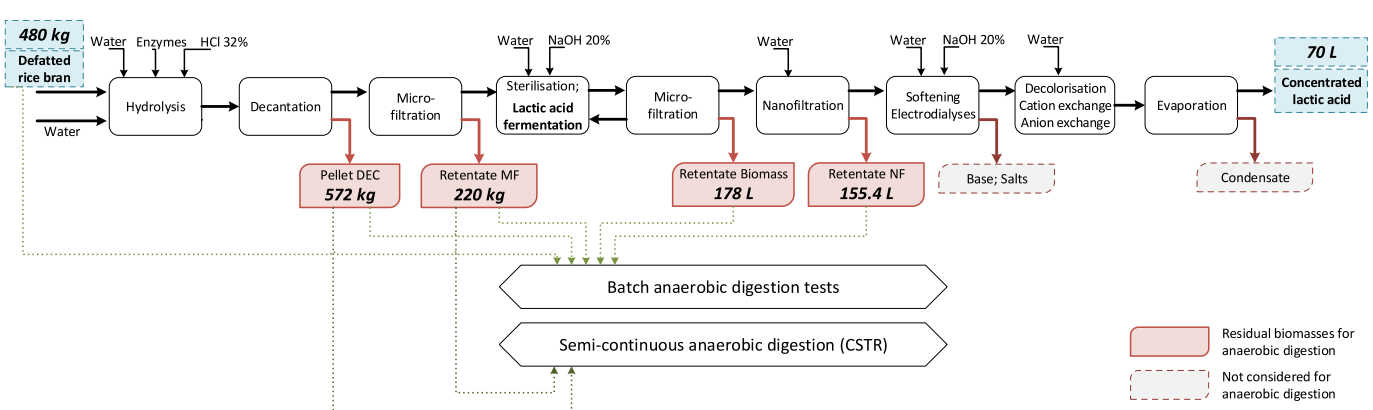

Fig. 1. Schematic representation of the process steps of pilot scale lactic acid fermentation and resulting residual material flows for subsequent utilization by anaerobic digestion.

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(1) Mono-digestion of Pellet DEC (Reactors R10, R20, and R30)
(2) Mono-digestion of Retentate MF (Reactors R40, R50, and R60)
(3) Co-digestion of Pellet DEC and Retentate MF with a ratio of 2.6 to 1.0 (Reactors R70, R80, and R90)

Reactor operation was started at a low OLR of 0.5 kg_{VS} m^{-3} d^{-1} and was increased by 0.5 kg_{VS} m^{-3} d^{-1} steps until reactor failure. Each OLR was kept constant for 56 d, resulting in an overall experimental duration of 231–287 d. The fermenter received semi-continuous feeding once per day at the same time, six days a week. Prior to feeding, substrates were stored frozen at -18°C and gently thawed weekly at 4 °C before feeding. Digestate was removed *via* an outlet at the bottom of the reactors and weighed on each day of feeding to maintain a working volume of 3 L. Reactor samples were collected bi-weekly or weekly for further chemical analyses. Biogas was collected throughout the entire period between feedings (24 h) and analyzed daily using a portable gas analyzer (BIOGAS 5000, Geotechnical Instruments (UK) Ltd, Leamington Spa, UK).

2.5. Analytical methods

After the filtration stage in the downstream processing of LA production, all substrate materials were promptly frozen and stored at -18°C until they underwent chemical composition analysis and methane production. TS and VS contents were determined at 105°C in a hot air oven and at 550°C in a heating furnace, following standard protocols (VDLUFA, 2006). The substrate and digestate pH and conductivity were measured using a Sen Tix 41 measuring electrode (WTW, Weilheim, Germany). The TAN was analyzed by steam distillation and back titration (VDLUFA, 2006). To evaluate process stability during continuous AD, the ratio of volatile organic acids to total inorganic carbon (VOA/TIC ratio) of the reactor content was determined by applying the Nordmann method (Drosg, 2013).

Sugars, including glucose, fructose, sucrose, LA, formic acid, VFAs, and alcohols, were quantified in the cold-water extracts of the substrate. Sugars, LA, and formic acid were analyzed using a high-performance liquid chromatograph (Dionex, Sunnyvale, CA, USA) equipped with a Eurokat H column (Knauer, Berlin, Germany) and a refractive index detector. VFAs (acetic, propionic, n-butyric, iso-butyric, n-valeric, iso-valeric, n-caproic, and iso-caproic acid) and alcohols (methanol, ethanol, propanol, and butanol) were analyzed using gas chromatography (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a flame ionization detector and a PERMABOND® FFAP capillary column (Machery-Nagel GmbH & Co KG, Düren, Germany).

Crude fat, neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were analyzed as previously described (Herrmann et al., 2011). Crude fat was performed using the AnkomXT10-Extractor (Ankom Technology Corp., Macedon, NY, USA), whereas fiber analyses employed the Ankom2000 Fiber Analyser system and filter bag technology (Ankom Technology Corp., Macedon, NY, USA). Elemental carbon, nitrogen, and hydrogen were detected using an elemental analyzer (Vario EL, Elementar Analysensysteme GmbH, Hanau, Germany) by applying the DUMAS combustion method (VDLUFA, 2006). The crude protein content was calculated as 6.25 times the elemental nitrogen content. The C/N ratio was calculated as the elemental carbon content divided by the elemental nitrogen content. The cellulose content was determined as the difference between ADF and ADL, whereas hemicellulose content was determined as the difference between NDF and ADF. Bulk elements, including phosphorus (P), sulfur (S), calcium (Ca), sodium (Na), potassium (K), and magnesium (Mg), were analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES) using an ICP Emission Spectrometer (iCAP 6000 series, Thermo Scientific, Waltham, United States) following microwave digestion (VDLUFA, 2006).

2.6. Kinetic analysis

In the 30-d long batch study of methane production, both first-order kinetics and the modified Gompertz model were employed to analyze the data using MATLAB 2022. The first-order kinetic model, which is a simplistic representation of the methane production process, assumes that the rate of methane production is directly proportional to the concentration of the remaining substrate (**Eq. 1**). This relationship can be mathematically expressed as follows:

$$y\,(t) = y_m * (1 - e^{-k_1 t}) \quad \text{Eq. 1}$$

where y(t) is the cumulative specific CH_4 yield at time t (L_N kg_{VS}^{-1}), y_m is the maximum specific CH_4 yield at a theoretically infinite digestion time (L_N kg_{VS}^{-1}), t is time (d), and k is the first-order decay constant (d^{-1}). The half-life (t_{50}) was deduced from the fitted first-order exponential equations and represented the time at which 50% of the maximum specific methane yield is reached (d).

Conversely, the modified Gompertz model offers a more nuanced understanding of the methane production process by incorporating lag and exponential growth phases (**Eq. 2**).

$$y\,(t) = y_m * \exp\left[-\exp\left\{\frac{R_m * e}{y_m} * (\lambda - 1)\right\}\right] \quad \text{Eq. 2}$$

where y(t) is the cumulative specific CH_4 yield at time t, (L_N kg_{VS}^{-1}), y_m is the maximum specific CH_4 yield at a theoretically infinite digestion time (L_N kg_{VS}^{-1}), R_m is the maximum specific CH_4 production rate (L_N $kg_{VS}^{-1}d^{-1}$), λ is the lag phase (d), t is time (d), and e is Euler's number.

2.7. Statistical analyses

Statistical analyses were performed using the SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA). BMP yields, methane content, and kinetic parameters were subjected to analysis of variance with residual material feedstock as a fixed effect. Significant differences between means were tested by multiple comparisons applying the test procedure SIMULATE with a significance level α of 0.05. For the statistical evaluation of the semi-continuous AD experiments, methane yields and methane production rates were subjected to a linear mixed model analysis with OLR nested within residual material feedstock as a fixed effect, considering repeated measures per fermenter every week with a first-order autoregressive covariance structure. The significance of differences between means of factor level combinations of interest, either different OLR levels in the same material feedstock or different material feedstocks at the same OLR level, were tested by multiple pairwise comparisons applying the option SIMULATE for adjustment of p-values for multiple comparisons to maintain the global significance level α of 0.05.

2.8. Life cycle assessment

This study assessed and compared the environmental impacts of two fermentative LA production pathways in DRB. The first pathway focuses solely on LA production, whereas the second integrates it with AD, utilizing the resulting biogas as an energy source—both heat and electricity—through combined heat and power (CHP) for the LA production process. This study employed a cradle-to-gate approach, with 1 kg of LA as the functional unit, focusing on production processes in Germany, where laboratory experiments were conducted. Given the diverse applications of LA as a chemical intermediate, factors related to its distribution, utilization, and disposal were outside the scope of this assessment.

2.8.1. Life cycle inventory

The LCI for industrial-scale LA production from dry residue biomass assumed a production capacity of 70 kg d^{-1}. The process inventory included the main input/output data for various stages of the LA production process. The background footprint data of the products and processes in the LCI were obtained from the EcoInvent 3.4 database (ecoinvent, Zurich, Switzerland; **Table S1**). All input energies were theoretically calculated, and the calculations are provided in the **Supplementary Material**.

The energy requirements for the LA production process were as follows: hydrolysis: energy or heat required for incubation at 50 and 85°C; microfiltration: energy consumed during microfiltration; sterilization: heat required for autoclaving at 121°C for 15 min; fermentation: heat required for maintaining a constant temperature of 37°C during incubation; microfiltration, nanofiltration, decolorization, cation exchange, and anion exchange: energy consumed in these processes; evaporation: heat input for evaporation water and concentration of the LA to a 99% purity level. The chemical requirements were as follows: ultrapure water, 32% HCl solution for the hydrolysis phase, 20% NaOH solution for neutralization prior to

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fermentation, and NaOH for softening and electrolysis phases. Some energy requirements were calculated, while others were measured. The energy required for hydrolysis and fermentation was calculated, the details of which are provided in the **Supplementary Material**. The energy requirement for running the autoclave was measured using an energy meter, and this value was used directly for the LCA calculation. The energy requirements for microfiltration, nanofiltration, electrodialysis, cation exchange, and anion exchange were calculated. For evaporation, it was assumed that the Mechanical Vapor Recompression technology was used, known for its high energy efficiency in concentrating liquids by evaporating the solvent, typically water, from a solution. This process involves recompressing the vapor generated during evaporation, increasing its temperature and pressure so that it can be reused as the heating medium for further evaporation. This method significantly reduces energy consumption compared to traditional evaporation techniques, requiring only 68.25 kWh m^{-3} (Zhang et al., 2020). As a result, the total energy consumption for concentrating 70 L of LA from 2100 L of dilute LA solution was 135 kWh. The assumptions considered when compiling the LCI were as follows.

- As the experiments were conducted in Germany, all data, including the electricity mix, chemicals, and other secondary data inputs into the LCA, were based on German or European averages available in the database.
- The environmental impacts associated with infrastructure manufacturing, maintenance, and land area usage for the LA production process were ignored, assuming that these factors were identical across all scenarios considered in the study.
- The study excluded the impacts associated with capital goods and infrastructure because of the absence of relevant datasets in the EcoInvent 3.4 database. This assumption is consistent with other LCA studies on biorefineries (Ebrahimian and Mohammadi, 2023; Kiehbadroudinezhad et al., 2023; López-Herrada et al., 2023).
- For simplification, the DRB was assumed to be post-consumer waste. Therefore, all environmental impacts associated with the lifecycle up to the point of waste generation were excluded from the analysis.
- Emissions related to the collection, separation, and transportation of DRB and supplementary chemicals to the biorefinery were assumed to be negligible and, therefore, were not considered in this study. Similar assumptions were made in other studies (Cok et al., 2014; Moussa et al., 2016; Brunklaus et al., 2018; Elginoz et al., 2020; Gadkari et al., 2021).
- Electricity generated from a mixed energy source (fossil and renewable energy) was assumed to supply all the processes.
- Scenario Considerations: Scenario 1: LA production alone, without AD of the waste. The waste generated from LA production (Pellet DEC and Retentate MF) was composted, and the environmental impact of composting was considered. Scenario 2: LA production integrated with AD, where waste (Pellet DEC and Retentate MF) is anaerobically digested in an AD plant. The biogas produced was converted to heat and electricity in a CHP unit, which was used to fuel all energy-intensive processes in LA production. The environmental impacts associated with AD, including emissions of NOx, CO, CO_2, and other gases, were taken into account.
- The production of 70 kg of LA resulted in the generation of 572 kg of pellet DEC and 220 kg of retentate MF, totaling 792 kg of waste byproducts (a mixture of Pellet and Retentate MF). The BMP tests of this mixture suggested a maximum theoretical methane yield of 91.4 L kg^{-1} of fresh matter. However, under the semi-continuous AD conditions applied in this study, with an OLR of 2.0 kg_{VS} m^{-3} d^{-1} and an average efficiency factor of 0.75, the actual methane yield was measured at 67.64 L kg^{-1} of fresh matter. The adjusted yield was used for subsequent calculations for the integration of LA and biogas production. Given the methane yield of 67.64 L kg^{-1} of fresh matter from the mixture of Pellet and Retentate MF, AD of 792 kg of this waste byproduct would produce 53.57 m^3 of methane. This volume of methane was estimated to contain 583.51 kWh of primary energy, based on an energy content of approximately 39.2 MJ m^{-3} of methane. The energy required to operate the AD plant was assumed to be 8% of the energy generated from the biogas, as estimated from literature values (Salter and Banks, 2008; Lantz, 2012; Styles et al., 2016). With typical CHP system efficiencies of 35% for electricity and 50% for heat, 53.57 m^3 of methane would produce 204.23 kWh of electricity and 291.75 kWh of heat. Assuming negligible energy losses, the electricity and heat generated from biogas through AD can offset the electricity and heat requirements of various processes. Specifically, the electricity produced was assumed to offset the electricity demand for autoclaving at 121°C for 15 min, while the heat generated was assumed to offset the thermal requirements for fermentation incubation at 37°C for 98 h, evaporation at 55°C, and the hydrolysis stage (second stage) at 50°C for 21 h.

2.8.2 Life cycle impact assessment

The LCA was conducted using the Ecoinvent 3.4 database and the ReCiPe 2016 methodology (H, E, and A variants), which are among the most widely adopted approaches for life cycle impact assessment (Vanapalli et al., 2023). This study evaluated the environmental footprint at both midpoint and endpoint levels. The midpoint impact categories considered in this study were the: agricultural land occupation potential (ALOP), greenhouse gas emission potential (GWP100), fossil depletion potential (FDP), freshwater ecotoxicity potential (FETP), freshwater eutrophication potential (FEP), human toxicity potential (HTP), ionizing radiation potential (IRP), marine ecotoxicity potential (METP), marine eutrophication potential (MEP), metal depletion potential (MDP), natural land transformation potential (NLTP), ozone depletion potential (ODP), particulate matter formation potential (PMFP), photochemical oxidant formation potential (POFP), terrestrial acidification potential (TAP100), terrestrial ecotoxicity potential (TETP), urban land occupation potential (ULOP), water depletion potential (WDP). The endpoint impact categories considered in this study were ecological quality (EQ), human health (HH), and resources (R).

2.8.3 Energy cost assessment

The cost associated with the energy requirements in a standalone LA production process was compared with the cost of energy requirements in an LA production process integrated with AD. This comparison considered the energy offset from the energy generated by the AD process. The energy costs were tabulated using the cost of mixed-source energy obtained by Kost et al. (2021). Additionally, the potential cost savings from replacing mixed-source energy with renewable energy sources such as wind, solar, and biogas were analyzed.

3. Results and Discussion

3.1. Chemical characteristics of residues from hydrolysis and lactic acid fermentation

The raw material and residual biomass from LA fermentation used as feedstock in the BMP tests, and semi-continuous study exhibited notable variations in their chemical composition (**Table 2**). DRB was characterized by higher TS and VS contents than the other feedstocks, reaching 899.1 and 816.3 g kg^{-1}, respectively. During each stage of downstream processing, a significant reduction in the TS content of the residual material was observed, primarily due to the addition of water during the hydrolysis and LA fermentation processes, as well as biomass conversion. Following hydrolysis, the majority of solids, predominantly those that are harder to digest and more resistant to hydrolysis, were removed *via* decantation before the start of LA fermentation. Thus, a significant portion of the fiber fractions was concentrated in the Pellet DEC. This observation aligns with the elevated values of NDF, ADF, and ADL in the Pellet DEC compared with those in DRB.

Conversely, the fiber fractions in all other residual biomasses from LA fermentation and downstream processes were very low (**Table 2**). The elevated levels of these components in the Mixture Pellet and Retentate MF can be attributed to the inclusion of a predominant portion of Pellet DEC with high contents of hemicellulose, cellulose, and lignin in the mixture. The fiber fraction of biomass is one of the most crucial characteristics of crop material for biogas production because it encompasses the scarce or indigestible organic part of the cell walls (Herrmann et al., 2016). The lignin content has been reported to be negatively correlated with methane yield because it is more or less non-degradable under AD conditions and can further shield other cell wall components, such as hemicellulose and cellulose, against degradation (Triolo et al., 2011; Khan and Ahring, 2019). Consequently, based on its chemical composition, the Pellet DEC residue was expected to exhibit the lowest degradability and methane yield among the residues analyzed.

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Table 2.
Chemical characterization of residual biomasses from lactic acid fermentation and downstream processing.

Parameter	Unit	Defatted rice bran	Pellet DEC	Retentate MF	Retentate Biomass	Retentate NF	Mixture Pellet & Retentate MF
TS	g kg^{-1}	899.10	312.40	147.40	119.90	142.30	269.80
VS	g kg^{-1}	816.30	297.33	129.94	81.6	93.01	255.15
pH	-	6.79	4.65	3.60	6.29	5.81	4.26
Alcohols	g kg^{-1}	< 0.04	< 0.04	0.09	0.22	0.07	0.08
Lactic acid	g kg^{-1}	< 0.04	< 0.04	7.30	40.34	56.69	2.21
Acetic acid	g kg^{-1}	0.54	0.70	1.19	0.19	0.22	1.04
TAN	mg kg^{-1}	n.a.	44.8	92.8	87.6	86.6	n.a.
Glucose	g kg^{-1}	0.49	3.05	41.18	0.19	0.80	4.33
Sucrose	g kg^{-1}	2.33	0.39	9.13	1.26	3.94	0.58
Fructose	g kg^{-1}	0.14	0.49	< 0.04	0.12	0.22	0.42
Starch	$\%_{TS}$	34.7	n.a.	n.a.	n.a.	n.a.	n.a.
Crude fat	$\%_{TS}$	1.7	2.0	2.7	0.3	0.5	1.8
Crude protein	$\%_{TS}$	18.7	21.9	34.9	25.8	14.1	23.9
NDF	$\%_{TS}$	19.6	59.1	3.4	14.8	0.0	46.2
ADF	$\%_{TS}$	8.0	24.2	0.7	0.0	0.0	19.2
ADL	$\%_{TS}$	2.4	8.8	0.6	0.0	0.0	7.4
Hemicellulose	$\%_{TS}$	11.6	35.0	2.7	0.0	0.0	27.1
Cellulose	$\%_{TS}$	5.5	15.4	0.03	0.0	0.0	11.5
C	$\%_{TS}$	44.5	49.6	48.1	34.0	32.4	49.3
N	$\%_{TS}$	3.0	3.5	5.6	4.1	2.3	3.8
H	$\%_{TS}$	5.4	5.6	6.6	4.9	4.4	5.8
C/N	-	14.9	14.1	8.6	8.2	14.3	12.9
P	g kg^{-1}	17.00	2.35	3.96	5.87	8.09	2.79
S	g kg^{-1}	1.93	0.90	0.57	0.20	0.26	0.80
Ca	g kg^{-1}	0.54	0.20	0.10	0.36	0.11	0.17
K	g kg^{-1}	14.97	2.37	2.56	2.07	2.53	2.51
Na	g kg^{-1}	0.14	0.11	0.12	10.53	14.15	0.10
Mg	g kg^{-1}	6.31	0.99	1.30	2.07	2.84	1.10

TS: total solids; VS: volatile solids; TAN: total ammoniacal nitrogen; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; C/N: ratio of carbon to nitrogen; n.a: not analyzed.

DRB had a close to neutral pH. pH levels declined with the addition of hydrochloric acid for hydrolysis as well as the production of LA, and this was reflected in feedstock Pellet DEC and Retentate MF. Increased pH is noticed in Retentate Biomass and Retentate NF owing to the addition of 20% NaOH for pH control during LA fermentation, as shown in **Figure 1**. This increase resulted in high sodium concentrations in the Retentate Biomass and Retentate NF (**Table 2**), which could potentially hinder AD. Without adaptation, sodium concentrations of 3.5 to 5.5 g L^{-1} have been reported to be moderately strongly inhibitory to methanogens at mesophilic conditions, while sodium concentrations above 8 g L^{-1} have been reported to be strongly inhibitory to methanogens at mesophilic conditions (Chen et al., 2008). However, the inhibition effects of sodium vary widely in the literature (Chen et al., 2008). The concentrations of P, S, Ca, K, and Mg were highest in DRB but declined significantly after fermentation based on fresh matter, mainly due to dilution.

Notable amounts of LA were detected in the Retentate Biomass and Retentate NF at 40.3 and 56.7 g kg^{-1}, respectively. This finding indicated that some of the LA produced was lost during downstream processing and remained in the residual biomass fraction. Alcohols and acetic acid were detected in negligible amounts in all residue feedstocks. A significant concentration of sugar (glucose, sucrose, and fructose) was noted in Retentate MF, with glucose content at 41.2 g kg^{-1} and sucrose content at 9.13 g kg^{-1}. This result indicated that after hydrolysis, some sugar components were removed from the substrate for LA fermentation before conversion into LA.

The crude fat content was high in the decantation Pellet DEC and Retentate MF but negligible in Retentate Biomass and Retentate NF. However, the fat content was generally low across all analyzed biomasses, as the oil, which can constitute 15–22% w/w of rice bran, was extracted as a value-added product beforehand, resulting in DRB as the primary byproduct of the extraction process (Alexandri et al., 2020b). In line with the literature (Alexandri et al., 2020b; Wancura et al., 2023), DRB exhibited high protein concentrations, which were retained in all residual biomass fractions from the LA fermentation and purification processes (**Table 2**). While this is advantageous due to the high methane production potential of proteins during anaerobic degradation, it also presents challenges due to the associated low C/N ratios. A C/N ratio below 20:1 can lead to issues with excessive ammonia formation from the breakdown of nitrogenous matter, potentially resulting in ammonia inhibition of the AD process (Drosg et al., 2013).

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3.2. Biochemical methane potentials

The BMP assay was employed to evaluate the methane yields and biodegradability of diverse residual feedstocks from the LA fermentation and purification processes under standardized AD conditions. As summarized in **Table 3**, all the analyzed feedstocks revealed high methane yields based on VS, indicating the high degradability of the organic fraction. Detailed information on the statistical comparison of the results of the BMP tests can be found in the **Supplementary Material** (**Tables S2** to **S7**). The raw material DRB was easily convertible into biogas, leading to a high methane yield of 395.1 L_N kg_{VS}^{-1} without further pretreatment. This finding can be attributed to the high concentrations of readily degradable substances in this biomass, including high starch and protein contents and low amounts of hardly degradable fiber fractions compared with other crop materials and residues (Ge et al., 2014; Herrmann et al., 2016). The measured methane yields were higher than those reported for rice bran in previous studies (Ali et al., 2018; Jha et al., 2020), which ranged from 232–269 L_N kg_{VS}^{-1}. This higher yield corresponds with the comparatively lower lignin concentrations found in the DRB biomass in the present study compared to those previously documented (Ali et al., 2018; Wancura et al., 2023).

Among the LA fermentation residues, Retentate Biomass yielded the highest methane output (434.4 L_N kg_{VS}^{-1}), which was significantly higher compared with the other residues ($p < 0.01$) and with the raw material DRB ($p < 0.05$). All other residues and the mixture of Pellet DEC and Retentate MF exhibited similar methane yields, ranging from 358.5–388.9 L_N kg_{VS}^{-1} (**Tables 3** and **S3**). As expected, the lowest methane yields were observed for Pellet DEC and the mixture of Pellet & Retentate MF (**Tables 3** and **S3**). The reduced yield is likely attributable to the elevated levels of NDF, ADF, and lignin in these feedstocks, indicating a higher proportion of recalcitrant lignocellulosic material that resists degradation during AD (Paul and Dutta, 2018; Khan and Ahring, 2019).

Although the Retentate Biomass produced the highest methane yield per unit of VS, it exhibited the lowest methane yield per unit of fresh matter, i.e., 35.4 L_N kg^{-1} (**Table S2**). Similarly, low methane yield per unit of fresh matter was observed for Retentate NF and Retentate MF feedstocks (36.2 and 49.7 L_N kg^{-1}) despite exhibiting notable methane yield per unit of VS. In an industrial setting, this translates to higher operational costs due to increased transportation and handling requirements for processing larger volumes to achieve the same energy output. Additionally, these feedstocks require more digester space for a given biogas yield, thereby diminishing the overall plant efficiency. Therefore, despite their promising methane potential per unit of VS, the lower methane yield per unit of fresh matter for the Retentate Biomass and Retentate NF renders them less economically viable for large-scale biogas production compared to Pellet DEC and DRB. The mixture of Pellet DEC and Retentate MF resulted in a higher methane yield per unit of fresh matter than Retentate MF alone ($p < 0.0001$; **Table S2**). Given that Pellet DEC and Retentate MF comprised the majority of the waste generated during feedstock preprocessing for LA production and demonstrated the highest methane yields per unit of fresh matter, these two waste streams, along with their mixture (Mixture of Pellet and Retentate MF), were selected for further investigation in semi-continuous AD experiments.

Notably, the presence of TAN, low pH, and high sodium content did not have a discernible inhibitory effect on methane yield across various feedstocks in the batch experiments. Although high sodium levels are known to elevate both medium conductivity and osmotic pressure, which can hinder AD by causing cellular dehydration, stress, and disruption of electrochemical gradients (Gao et al., 2022), this effect was not observed in the current study.

The methane content in the biogas varied from 53.7% to 61.7% across all feedstocks, with the Retentate Biomass showing the highest methane content. This outcome is likely attributed to the substantial presence of proteins, which decompose into high levels of methane during the AD process (Weiland, 2010). Overall, the methane concentration generally increased with the protein concentration in the feedstocks. However, Retentate MF was an exception; despite having the highest proportion of proteins in TS, it produced relatively low levels of methane in the biogas. (**Tables 3** and **S4**).

The methane formation profiles for all feedstocks demonstrate an immediate onset of methane production without a lag phase, followed by rapid methane formation within the first few days until a plateau is reached, as shown in **Figure 2**. This observation highlights the ease of convertibility and the suitability of DRB and all residual materials from the LA fermentation process for biogas production, as confirmed by the results of kinetic analyses. The k-value and t_{50} obtained from the first-order kinetic equation indicated the rate at which methane was generated during AD. A higher k value and lower t_{50} value indicated more rapid methane production, reflecting efficient degradation of organic matter by the microbial community in the digester. The values of k and t_{50} obtained from the first-order kinetics varied among all the feedstocks, with k-values ranging from 0.121–0.177 d^{-1} and t_{50} values ranging from 4.67–6.93 d. These k-values are in the middle to upper range of values reported in the literature: 0.091–0.15 d^{-1} (Bedoić et al., 2020; López et al., 2021). DRB exhibited a remarkably high k-value of 0.175 d^{-1}, indicating a rapid increase in methane production during the initial days, followed by an early plateau (**Fig. 2**). This pattern may be attributed to the prevalence of easily degradable components, particularly starch, in DRB. Conversely, Pellet DEC and the Mixture Pellet and Retentate MF exhibited lower k-values and higher t_{50} values, with no significant difference ($p > 0.05$; **Tables S5** and **S7**), suggesting slower and potentially incomplete methane production. Similar to the lower methane yield, these shared characteristics could be associated with the presence of challenging-to-degrade lignocellulosic components, as indicated by the

Table 3.
Results of batch anaerobic digestion tests of defatted rice bran and residual biomasses from lactic acid fermentation and downstream processing using defatted rice bran as substrate.

Material	Methane Yield (L_N kg^{-1})	Methane Yield (L_N kg_{VS}^{-1})	Methane Content (%)	k-Value (d^{-1})	BMP_{Gomp} (L_N kg_{VS}^{-1})	R_m (L_N kg_{VS}^{-1} d^{-1})	t_{50} (d)
Defatted rice bran	322.4^{a}	395.1^{b}	54.26^{d}	0.175^{ab}	406.3^{b}	74.21	4.67^{c}
Pellet DEC	111.4^{b}	374.0^{bc}	58.58^{b}	0.121^{d}	393.9^{b}	31.76	6.61^{a}
Retentate MF	49.7^{d}	381.5^{bc}	53.74^{d}	0.177^{a}	394.9^{b}	42.98	5.31^{bc}
Retentate Biomass	35.4^{d}	434.4^{a}	61.72^{a}	0.133^{c}	447.5^{a}	60.42	6.93^{a}
Retentate NF	36.2^{d}	388.9^{bc}	55.82^{c}	0.167^{b}	399.1^{b}	75.56	5.44^{b}
Mixture Pellet & Retentate MF	91.4^{c}	358.5^{c}	56.89^{c}	0.126^{cd}	369.4^{b}	44.50	6.57^{a}
Mean	107.8	388.7	56.83	0.150	401.9	54.91	5.89
Median	70.5	385.2	56.36	0.150	397.0	52.46	6.01
s.e.m.	3.19	7.51	0.31	0.002	7.70	9.58	0.10
Significance	***	***	***	***	***	*	***

s.e.m.: standard error of the mean; *** $P < 0.001$; * $P < 0.05$.
^{abcd} means within a column without a superscript letter in common differ significantly at $P < 0.05$.
BMP_{Gomp}: biochemical methane potential of the modified Gompertz model, R_m: maximum specific methane production rate; t_{50}: half-life; VS: volatile solids.

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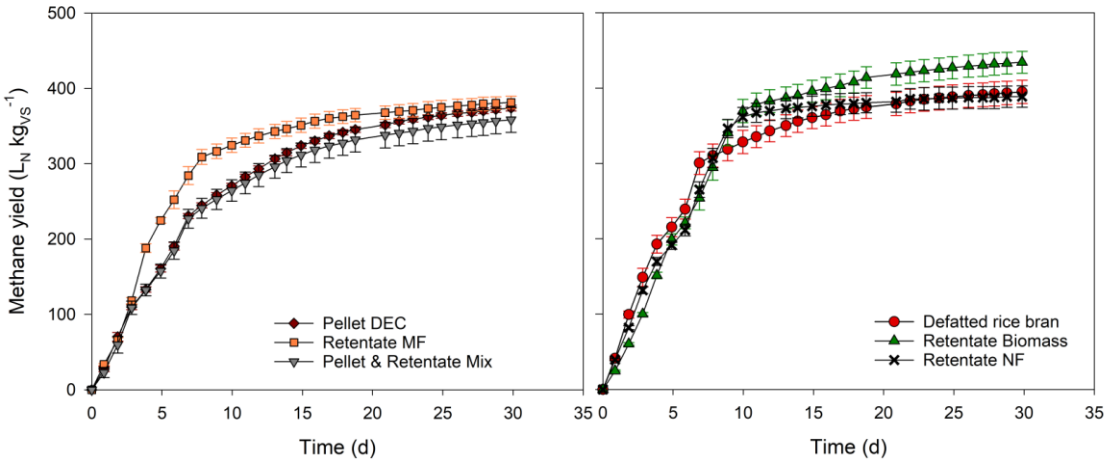

Fig. 2. Cumulative methane yield over time during batch anaerobic digestion of defatted rice bran and residual biomasses of the lactic acid fermentation from defatted rice bran.

elevated levels of NDF, ADF, and lignin.

The predicted methane yield values of all feedstocks from the modified Gompertz model closely matched the experimentally determined values. Thus, the modified Gompertz model effectively corroborates the experimental findings, indicating a strong fit between the experimental data and the model (average adjusted $R^2 = 0.989$ for the first-order exponential model and $R^2 = 0.991$ for the modified Gompertz model). This strong correlation underscores the model's capability to accurately predict BMP test outcomes across various feedstocks. The "R_m" value obtained from the modified Gompertz model represents the maximum methane production rate achievable during the AD process. In this study, DRB and Retentate NF showed the highest R_m values, which was attributed to their substantial concentrations of easily degradable components, such as starch and LA. Conversely, the Pellet DEC and the Mixture Pellet & Retentate MF exhibited the lowest R_m values because of their rich fiber fractions, suggesting a slower rate of substrate degradation in these feedstocks.

3.3. Optimization of residue conversion in semi-continuous anaerobic digestion

3.3.1. Effect of organic loading rate on methane production

The methane production performance of the selected residual feedstocks, Pellet DEC, Retentate MF, and the Mixture Pellet and Retentate MF, was investigated under practically oriented process conditions in semi-continuous AD experiments. The initial startup phase commenced with an OLR of 0.5 kg_{VS} m^{-3} d^{-1} methane yields of all reactors, progressively increased up to week 3 to 4, and stabilized from weeks 5 to 8 at average methane yields of 332, 314, and 306 L_N kg_{VS}^{-1} for Pellet DEC, Retentate MF, and the Mixture Pellet & Retentate MF, respectively. The subsequent stepwise increase in OLR led to a temporary decrease in methane yield in the week after each change in organic loading for all reactors and feedstocks investigated but consistently recovered and stabilized within 1 to 2 weeks after the increase in OLR (**Fig. 3**). The initial decrease in the methane yield likely resulted from the shock response of the microbial community to the sudden increase in organic loading. This shock could have been caused by the accumulation of intermediates, such as VFAs or ammonia, resulting from the rapidly increasing activity of hydrolytic and acid-producing microorganisms after changes in feedstock amounts (Terboven et al., 2017), nutrient imbalances, or a shift in the microbial community composition. However, the subsequent recovery indicated that the microbial community adapted to the new conditions. This adaptation likely involved increased enzyme production, shifts in microbial populations toward more tolerant species, and regulation of metabolic pathways, all of which contributed to the restoration of efficient methane production (Christou et al., 2021; Yu et al., 2021; Nkuna et al., 2022).

With increasing OLR, the methane yields of the reactors operated with Pellet DEC as feedstock (R10-R30) showed a decreasing tendency (**Fig. 3** and **Table 4**) with an average 4.5% lower methane yield at OLR 1.5 compared with OLR 1.0 kg_{VS} m^{-3} d^{-1}. A similar trend was observed for the Mixture Pellet and Retentate MF (R70-R90), with 11% lower methane yields at OLR 2.0 compared with OLR 1.0 kg_{VS} m^{-3} d^{-1}. In contrast, this tendency was not observed for the Retentate MF. The methane yields of the reactors operated with Retentate MF (R40-R60) remained at the same level when organic loading was increased (**Fig. 3** and **Table 4**). However, mean values of methane yields were only significantly lower for Pellet DEC when OLR was raised to 2.0 kg_{VS} m^{-3} d^{-1} ($p = 0.005$; **Tables 4** and **S8**). Detailed information on the statistical comparison of the results of the semi-continuous AD experiments can be found in the **Supplementary Material** (**Tables S8** and **S9**).

Raising the OLR to 2.0 kg_{VS} m^{-3} d^{-1} resulted in a sharp decline in methane yield in the Pellet DEC reactors. Although the initial recovery appeared promising, a gradual decline starting from the 7th week resulted in a 46–64% reduction by the 9th week following the OLR change (range: 105–151 L_N kg_{VS}^{-1}). This decline was attributed to process instability (*Section 3.3.2*), which ultimately led to the decision to halt further increases in OLR in these reactors. Remarkably, the process remained stable for reactors operated with Retentate MF and the Mixture of Pellet and Retentate MF at an OLR of 2.0 kg_{VS} m^{-3} d^{-1}. Further elevating the OLR to 2.5 kg_{VS} m^{-3} d^{-1} caused a notable decrease in the methane yield in the Retentate MF reactors. After stable operation for approximately six weeks, a rapid decline in methane production ensued, dropping by 37–50% (179–239 L_N kg_{VS}^{-1}) by the ninth week.

The Mixture Pellet & Retentate MF reactors exhibited similar behavior at OLR 2.5 kg_{VS} m^{-3} d^{-1}, showing a rapid decline in methane production of 59% and 80% in R70 and R90 by the eighth week (57–109 L_N kg_{VS}^{-1}). Reactor R80 remained stable throughout the experiment. These findings highlight the inherent variability among reactors, with Pellet DEC reactors failing at OLR 2.0 kg_{VS} m^{-3} d^{-1} while Retentate MF and Mixture Pellet & Retentate MF reactors tolerated OLR 2.0 kg_{VS} m^{-3} d^{-1}. Notably, one of the Mixture Pellet and Retentate MF reactors successfully sustained OLR 2.5 kg_{VS} m^{-3} d^{-1} and might have performed well at higher OLRs.

During the stable phases at OLR 1.0 and 1.5 kg_{VS} m^{-3} d^{-1}, the methane yield of Retentate MF exceeded those of Pellet DEC and the Mixture Pellet and Retentate MF. The differences in the methane yield were statistically significant at an OLR of 1.5 kg_{VS} m^{-3} d^{-1} ($p < 0.0001$, **Table S8**). These findings are consistent with the results of the batch AD tests (*Section 3.2*) and can be attributed to the more recalcitrant composition of the Pellet DEC residue. Co-digestion of the two residual materials, Pellet DEC and Retentate MF, in a ratio reflecting their occurrence, did not produce positive synergistic effects on methane yield. However, it did improve process stability compared to the mono-digestion of Pellet DEC.

Methane production rates generally increased with increasing OLR across all feedstocks ($p < 0.0001$; **Tables 4** and **S9**). This increase can be attributed to the greater availability of feedstock for bioconversion as the organic loading increases. Considering the phases of stable reactor

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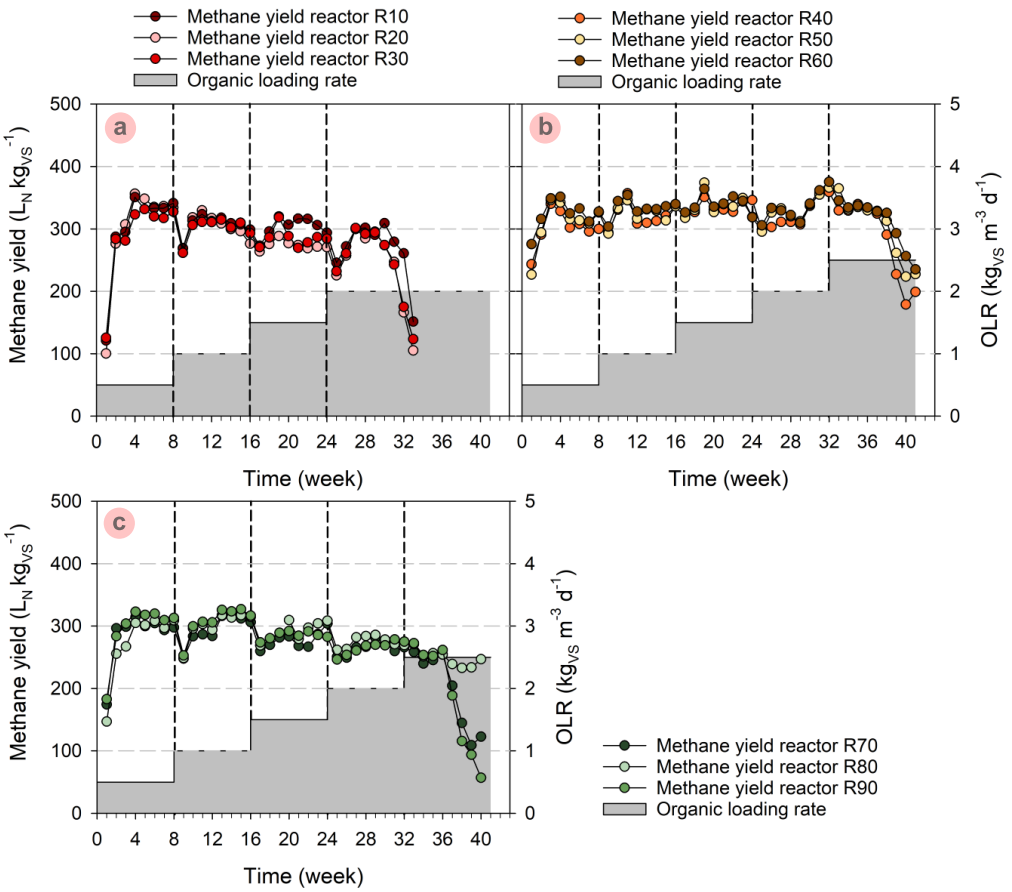

Fig. 3. Specific methane yields at increasing organic loading rates (OLR) during continuous anaerobic digestion of **(a)** R10, R20, R30: 100% Pellet DEC, **(b)** R40, R50, R60: 100% Retentate MF, and **(c)** R70, R80, R90: 72% Pellet DEC and 28% Retentate MF.

Table 4.
Summary of results from semi-continuous anaerobic digestion of residual biomasses from lactic acid fermentation using defatted rice bran as substrate.

Feedstock	OLR (kg_{VS} m^{-3} d^{-1})	Methane Yield (L_N kg_{VS}^{-1})	Efficiency Factor	Methane Production Rate (L_N L_R^{-1} d^{-1})	Average Methane Content (% v/v)	VS Degradation (%)	TAN (mg L^{-1})	pH	VOA/TIC	tVFA (mg L^{-1})
Pellet DEC	0.5	278.0^{abA}	0.74	0.152^{aA}	54.2 ± 1.1	85.5 ± 0.7	1974 ± 107	7.66 ± 0.08	0.13 ± 0.00	0.17 ± 0.03
	1.0	299.2^{aA}	0.80	0.304^{bdA}	53.4 ± 0.6	81.9 ± 0.9	2484 ± 156	7.60 ± 0.06	0.15 ± 0.01	0.45 ± 0.09
	1.5	285.7^{aA}	0.76	0.422^{cA}	53.2 ± 0.6	82.5 ± 1.2	2517 ± 282	7.55 ± 0.12	0.18 ± 0.02	0.87 ± 0.09
	2.0	221.9^{bA}	0.59	0.427^{cdA}	51.8 ± 4.6	73.3 ± 3.6	3825 ± 554	7.52 ± 0.13	0.24 ± 0.16	2.12 ± 3.12
Retentate MF	0.5	304.2^{aA}	0.80	0.171^{adA}	53.5 ± 0.8	72.9 ± 1.7	1791 ± 171	7.64 ± 0.09	0.12 ± 0.01	0.16 ± 0.04
	1.0	325.0^{aA}	0.85	0.325^{bdA}	53.2 ± 0.7	73.9 ± 2.7	2554 ± 298	7.62 ± 0.07	0.11 ± 0.01	0.11 ± 0.07
	1.5	334.8^{aB}	0.88	0.495^{cB}	53.0 ± 0.4	74.0 ± 2.0	3666 ± 333	7.59 ± 0.11	0.09 ± 0.01	0.06 ± 0.05
	2.0	331.2^{aB}	0.87	0.664^{cA}	52.9 ± 0.7	71.2 ± 0.6	4498 ± 302	7.60 ± 0.06	0.14 ± 0.20	0.16 ± 0.08
	2.5	289.0^{aA}	0.76	0.667^{cdA}	50.7 ± 3.3	65.2 ± 6.8	5155 ± 100	7.56 ± 0.17	0.18 ± 0.18	2.90 ± 4.81
Mixture Pellet and Retentate MF	0.5	272.5^{aA}	0.76	0.150^{aA}	53.9 ± 1,3	85.0 ± 0.5	1639 ± 102	7.74 ± 0.10	0.13 ± 0.02	0.15 ± 0.03
	1.0	297.5^{aA}	0.83	0.297^{bA}	53.0 ± 0.5	81.5 ± 1.0	2006 ± 164	7.61 ± 0.07	0.13 ± 0.00	0.11 ± 0.06
	1.5	284.7^{aA}	0.79	0.423^{cA}	52.6 ± 0.4	79.9 ± 1.5	2751 ± 261	7.56 ± 0.12	0.13 ± 0.01	0.07 ± 0.06
	2.0	265.0^{aAB}	0.74	0.520^{cA}	53.2 ± 0.5	77.1 ± 1.2	3494 ± 268	7.60 ± 0.05	0.12 ± 0.01	0.17 ± 0.08
	2.5	203.8^{aA}	0.57	0.470^{dA}	48.6 ± 7.1	70.7 ± 5.0	4290 ± 309	7.45 ± 0.27	0.32 ± 0.34	3.76 ± 6.11

^{abcd} means of the same feedstock at different OLRs without a superscript letter in common differ significantly at $P < 0.05$.
^{AB} means between different feedstock at the same OLR without a superscript letter in common differ significantly at $P < 0.05$.
OLR: organic loading rate; VS: volatile solids; TAN: total ammoniacal nitrogen; VOA/TIC: ratio of volatile organic acids to total inorganic carbon; tVFA: total volatile fatty acids.

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operation, Retentate MF (R40-R60) exhibited the highest methane production rates at OLR 2.0 kg_{VS} m^{-3} d^{-1}, followed by the Mixture Pellet and Retentate MF (R70-R90) at OLR 2.0 kg_{VS} m^{-3} d^{-1} (**Table 4**). The methane content in the biogas remained relatively stable across different OLRs for all feedstocks, with slight variations. Pellet DEC (R10-R30) and the Mixture Pellet and Retentate MF (R70-R90) maintained a slightly higher average methane content than Retentate MF. The methane content of the biogas was decreased when reactor instability occurred (**Table 4**). Overall, the anaerobic co-digestion of a Mixture of Pellet & Retentate MF at an OLR of 2.0 kg_{VS} m^{-3} d^{-1} could be regarded as optimal, leading to high waste utilization and methane production performance.

3.3.2. Process stability

Process stability in semi-continuous AD experiments was assessed by monitoring key parameters, such as TAN, pH, VOA/TIC ratio, and tVFA concentration. The TAN concentrations increased progressively with increasing OLR for all feedstocks (**Fig. 4** and **Table 4**). For reactors operated with Pellet DEC, the highest TAN concentrations were observed at an OLR of 2.0 kg VS m^{-3} d^{-1}, exceeding 3000 mg L^{-1}. This increase coincided with a decline in methane yield, suggesting that elevated TAN levels inhibit methanogenic activity, leading to reduced methane production. TAN, which consists of free ammonia (NH_3) and ammonium ion (NH_4^+), is a well-known inhibitor in AD processes. Free ammonia, in particular, is known to be toxic to methanogenic archaea, which are the microorganisms responsible for methane production. High TAN concentrations can disrupt microbial cell membranes, inhibit enzyme activity, and interfere with cellular metabolism, leading to a decreased methane yield and process instability (Li et al., 2023). Threshold levels for ammonia inhibition vary widely in the literature.

Chen et al. (2008) summarized that TAN concentrations ranging from 1.7 to 14 g L^{-1} have been reported to cause a 50% inhibition in methane production. Similar to the present study, a strong inhibitory effect and decrease in methane production at TAN concentrations above 3000 mg L^{-1} were reported by Ward et al. (2014). TAN inhibition depends on pH due to a shift in the free ammonia-to-ammonium ratio toward toxic ammonia with increasing pH values (Chen et al., 2008). However, the pH remained stable throughout the experiment, ruling out pH-related inhibition as a contributing factor. For Pellet DEC, VOA/TIC and tVFA remained low and stable across OLRs of 0.5, 1.0, and 1.5 kg_{VS} m^{-3} d^{-1}. However, a sharp increase in these parameters was observed at OLR 2.0 kg_{VS} m^{-3} d^{-1}, close to reactor failure. This abrupt rise in the VOA/TIC ratio and tVFA concentration, reaching up to 0.5 and 7.2 g L^{-1}, respectively, was likely triggered by the elevated TAN concentration. This increase suggests a disruption in the AD process (Li et al., 2023). Elevated TAN levels may have partially or significantly inhibited methanogens, resulting in decreased methanogenic activity, acid accumulation, and subsequent methane yield reduction. Similar trends were observed for Retentate MF and Mixture Pellet and Retentate MF at an OLR of 2.5 kg_{VS} m^{-3} d^{-1}. The VOA/TIC ratio and tVFA levels remained stable across OLRs of 0.5, 1.0, 1.5, and 2.0 kg_{VS} m^{-3} d^{-1} but were abruptly increased at an OLR of 2.5 kg_{VS} m^{-3} d^{-1}, reaching up to 0.8 and 12.0 g L^{-1}, respectively. The rapid accumulation of large amounts of acetic and propionic acid accompanied this sudden rise (**Fig. 5**). An exception is reactor R80 with Mixture Pellet & Retentate MF, which did not experience methane inhibition despite elevated TAN levels.

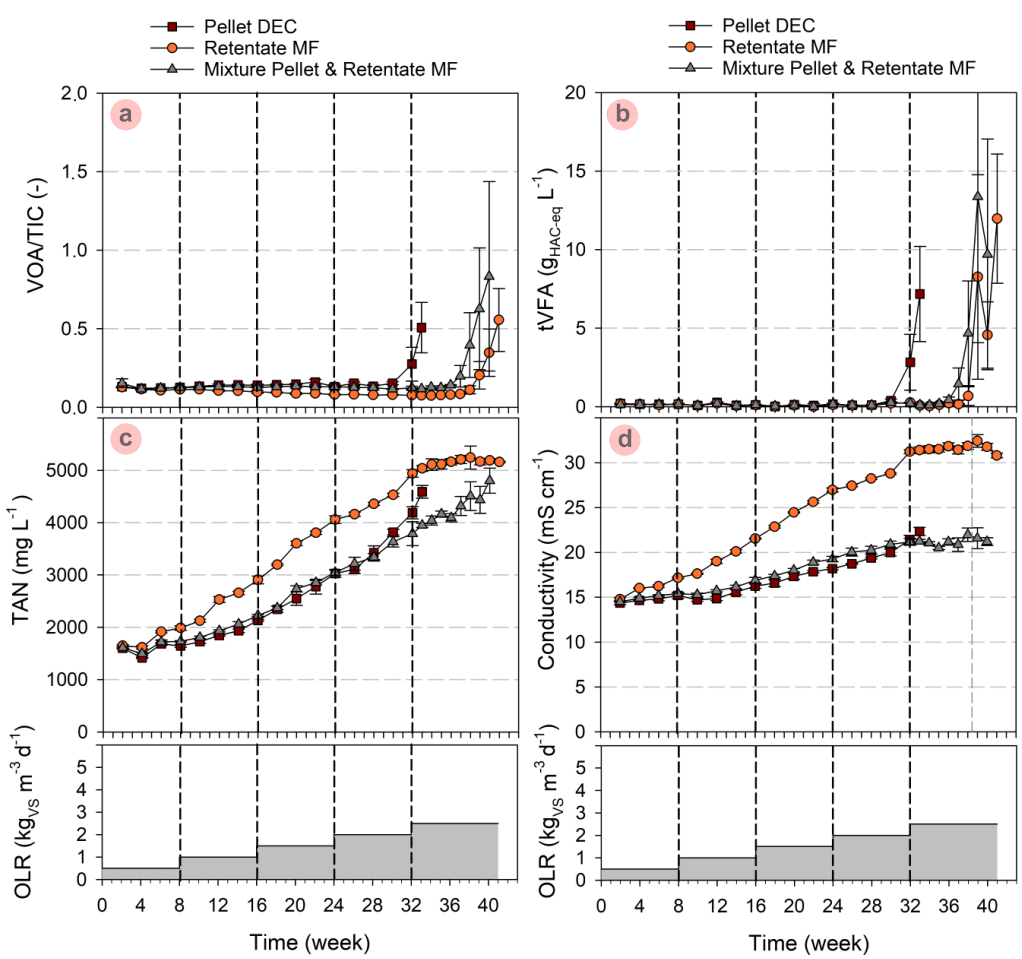

Fig. 4. Course of **(a)** the ratio of volatile organic acids to total inorganic carbon (VOA/TIC) **(b)** total volatile fatty acids (tVFA), **(c)** total ammonical nitrogen (TAN), and **(d)** electrical conductivity during anaerobic digestion of Pellet DEC, Retentate MF and the Mixture Pellet & Retentate MF at increasing organic loading rates (OLR).

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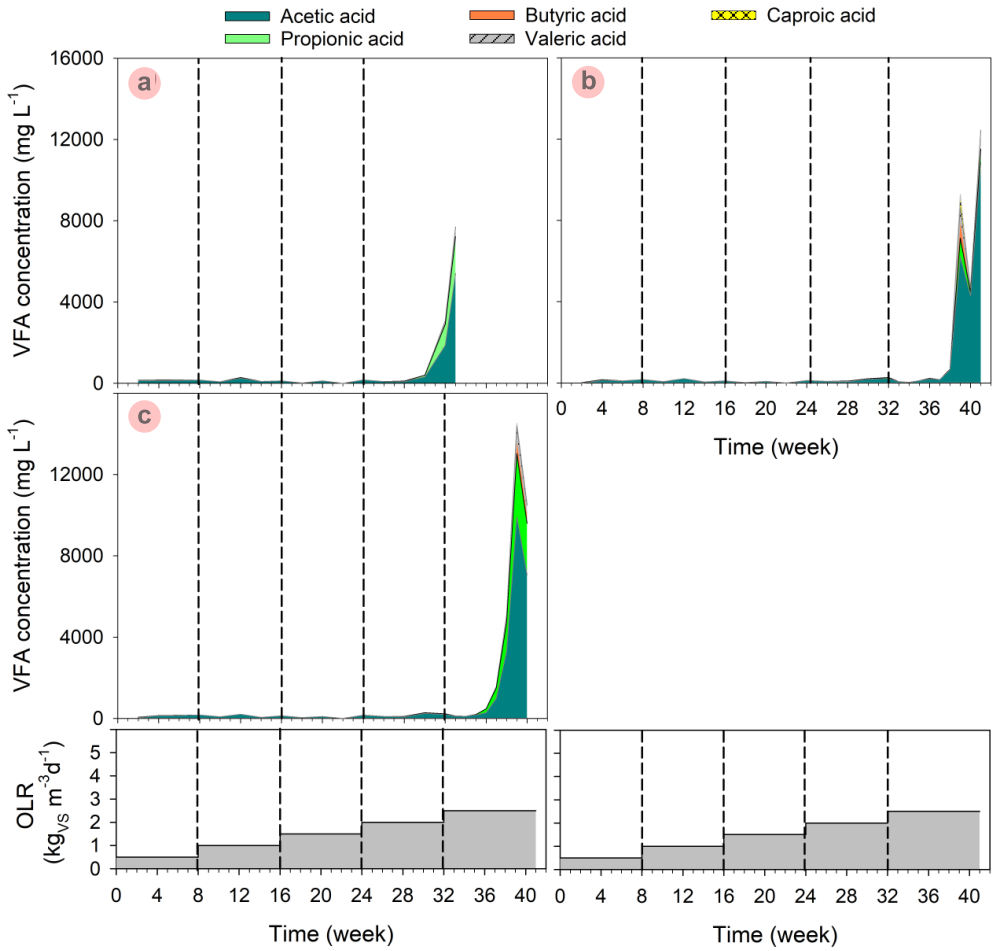

Fig. 5. Concentrations of volatile fatty acids (VFA) at increasing organic loading rates (OLR) during continuous anaerobic digestion of **(a)** 100% Pellet DEC, **(b)** 100% Retentate MF, and **(c)** 72% Pellet DEC and 28% Retentate MF.

Notably, the TAN levels in the reactors operated with Retentate MF exceeded the TAN levels of the Pellet DEC (**Fig. 4** and **Table 4**). These results suggest that the inhibitory threshold for TAN may differ among feedstocks, with Retentate MF demonstrating greater tolerance than Pellet DEC. Pellet DEC, characterized by the highest VS content, was the most susceptible to TAN inhibition, leading to reactor failure at TAN levels above 3000 mg L^{-1}. In contrast, Retentate MF, characterized by its lower VS content, demonstrated notable resistance to total TAN inhibition, maintaining stable operation up to TAN concentrations of 5000 mg L^{-1}. This resilience may be attributed to the higher water content in the Retentate MF, leading to an increased hydraulic loading rate (HLR) during daily feeding, possibly mitigating inhibitory effects of TAN by diluting TAN concentrations, facilitating the removal of accumulated TAN, and promoting the growth of ammonia-tolerant microorganisms. This observation aligns with the findings of Zhang et al. (2017), who reported a similar dilution-based TAN inhibition mitigation.

Interestingly, the Mixture of Pellet & Retentate MF, despite having a C/N ratio closer to that of the Pellet DEC, inherited the TAN resistance of Retentate MF. This resistance indicates that adding Retentate MF to Pellet DEC helped reduce TAN accumulation and the resultant VS content, which in turn increased the HLR and enhanced resistance to TAN inhibition. These findings highlight the complex relationship between C/N ratio, VS content, and TAN inhibition during AD. It is plausible that additional factors beyond the scope of this study may have contributed to the resilience of Retentate MF to TAN inhibition, either independently or synergistically, with increased HLR. These results indicate that co-digestion of feedstocks with diverse characteristics can be a strategic approach to optimize AD performance, enhance tolerance to TAN, and achieve higher OLRs, as reported by Kabir et al. (2015). In this scenario, AD using Pellet DEC alone proves less profitable due to reactor failure at OLRs exceeding 1.5 kg_{VS} m^{-3} d^{-1}, limiting its operational capacity under higher organic loads. Conversely, while Retentate MF can withstand higher OLRs, its lower methane yield per unit of fresh matter makes it economically less viable. However, co-digesting Pellet DEC and Retentate MF offers a more promising solution. This combined approach not only yields higher methane per unit of fresh matter but also enables operation at elevated OLRs (up to 2.0 kg_{VS} m^{-3} d^{-1}), making it the most profitable option for industrial biogas production.

3.3.3. Volatile solid removal efficiency and methane yield efficiency factor

The relationship between increasing the OLR, methane production performance, and VS degradation varied among the feedstocks, highlighting the importance of tailoring the operational parameters to specific substrate characteristics. For Pellet DEC, a suitable OLR range of 0.5 to 1.5 kg_{VS} m^{-3} d^{-1} was observed, where methane yields (278-299 L_N kg_{VS}^{-1}) and VS degradation (82.5-85.5%) were maximized. Beyond this range, a sharp decline in both parameters at 2.0 kg_{VS} m^{-3} d^{-1} occurred due to process inhibition. The efficiency factor, a measure of methane yield in continuous versus batch reactors, provided further insights into the performance of the Pellet DEC system under varying OLRs. Within a suitable OLR range, 74% to 80% of the methane production potential analyzed in batch AD tests was reached under semi-continuous process conditions. However, a steep decline to 59% was observed at OLR 2.0 kg_{VS} m^{-3} d^{-1}, where inhibitory

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effects adversely affected the performance of the continuous reactor (Table 4).

Retentate MF exhibited a different response to OLR, with VS degradation (71.2–74.0%) and efficiency factor (0.8–0.88), increasing from 0.5 to 1.5 kg_{VS} m^{-3} d^{-1} and remaining stable around OLR 2.0 kg_{VS} m^{-3} d^{-1}. However, a further increase in OLR led to a notable decline in all parameters. The Mixture of Pellet & Retentate MF followed a trend similar to Pellet DEC but with a wider suitable OLR range (0.5–2.0 kg_{VS} m^{-3} d^{-1}), likely due to the combined characteristics of the two feedstocks. VS degradation and efficiency factor peaked within this range (77.1–85.0% and 0.74–0.83, respectively), indicating a synergistic effect of the mixed feedstock on overall process performance. However, similar to the individual feedstocks, a sharp decline in all the parameters was observed at 2.5 kg_{VS} m^{-3} d^{-1}. Overall, the efficiency factor analysis revealed that while continuous reactors could achieve methane yields comparable to batch reactors under optimal OLRs, their performance might be more sensitive to fluctuations in the OLR, particularly at higher loading rates. This issue underscores the importance of tailoring OLRs to specific feedstock and reactor configurations to maximize the methane yield and process efficiency in industrial biogas production.

3.4. Environmental and economic benefits of process integration

3.4.1. Life cycle impact assessment

The relative environmental performance of LA production from the DRB, both standalone and integrated with AD (which offsets the energy requirements of the LA production process through the energy generated from AD, was analyzed at both the midpoint and endpoint levels (Fig. 6) using inventory data provided in Table 5. Detailed midpoint and endpoint characterization data for all technologies, including the individual contributions of each sub-process, are provided in the Supplementary Material (Tables S10–13). Additionally, this section identifies and critically discusses hotspot subprocesses and pathways that are expected to be of particular interest to LCA practitioners and policymakers, highlighting their environmental implications. Notably, normalized scores presented in both the midpoint and endpoint characterization analyses reflected the environmental impacts associated with the production of 1 kg of LA from the DRB.

The midpoint normalization results indicated that climate change and human toxicity were the major environmental impact categories for standalone LA production, contributing 43% and 33% of the total environmental impact score, respectively (6.37 kg CO_2-eq and 4.85 kg 1,4-DCB-eq out of 14.92). In contrast, for the LA production process integrated with AD, climate change contributed 29% (1.23 kg CO_2-eq out of a total environmental impact of 4.22), agricultural land occupation contributed 23% (0.97 m^2a), and human toxicity contributed 28% (1.16 kg 1,4-DCB-eq). Overall, integrating the LA production process with AD resulted in an approximately 67% reduction in the total midpoint environmental impact compared to the standalone LA production process. By integrating AD with the LA production process, environmental impact values significantly declined: climate change impact reduced from 6.37 to 1.23 kg CO_2-eq, fossil depletion from 1.66 to 0.45 kg oil-eq, freshwater ecotoxicity from 0.13 to 0.03 kg 1,4-DCB-eq, human toxicity from 4.85 to 1.16 kg 1,4-DCB-eq, ionizing radiation from 1.09 to 0.24 kg U235-eq, marine ecotoxicity from 0.12 to 0.03 kg 1,4-DCB-eq, and metal depletion from 0.07 to 0.03 kg Fe-eq. However, the environmental impact of agricultural land occupation increased from 0.46 to 0.97 m^2a.

For the standalone LA production process, the energy required for hydrolysis, autoclaving, evaporation, and running the fermenter (stirring and incubating at 37°C) contributed about 90% of the total CO_2 emissions impacting climate change, with the remaining 10% from composting Pellet

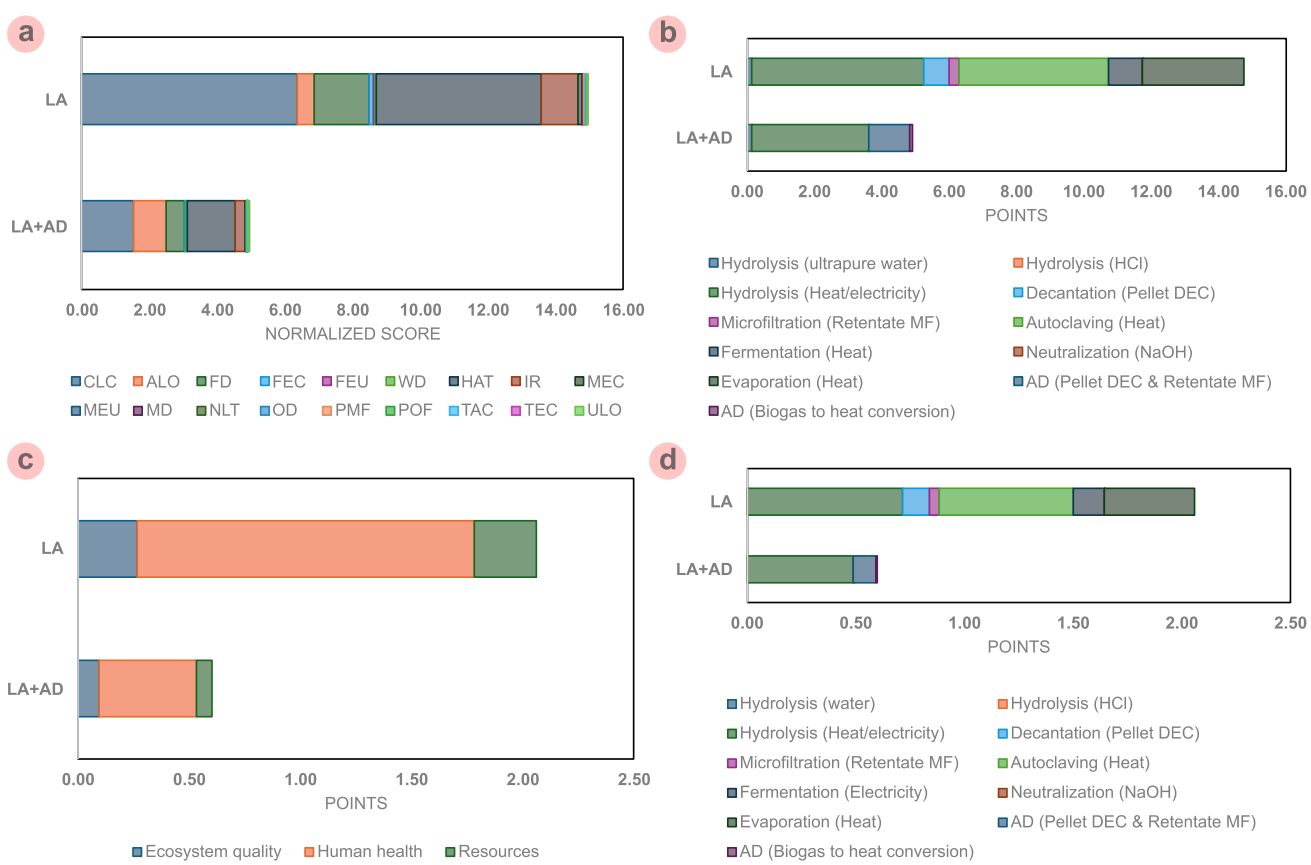

Fig. 6. Comparative midpoint and endpoint normalization data for each scenario, **(a)** classification based on midpoint impact categories; **(b)** classification of midpoint impacts based on individual processes; **(c)** classification based on endpoint damage categories; and **(d)** classification of endpoint impacts based on individual processes.

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Table 5.
Inventory data for standalone LA production process (Scenario 1) and LA production process integrated with AD (Scenario 2).

Processes	Input/Output	Type	Units	For Producing 70 L		For Producing 1 L	
				LA	LA+AD	LA	LA+AD
Hydrolysis	DRB	Input	kg	480.00	480.00	6.86	6.86
	Water	Input	m^3	2088.00	2088.00	29.83	29.83
	Enzymes (Termamyl)	Input	kg	0.32	0.32	0.00	0.00
	Enzymes (Dextrozyme)	Input	kg	0.48	0.48	0.01	0.01
	Enzymes (Protease)	Input	kg	0.24	0.24	0.00	0.00
	HCl (32%)	Input	kg	0.01	0.01	0.00	0.00
	Electricity (85°C for 2 h)	Input	kWh	158.00	158.00	2.26	2.26
	Heat (50°C for 21 h)	Input	kWh	73.00	0.00 *	1.04	0.00 *
Decantation	Biowaste (Pellet DEC)	Output	kg	572.00	0.00	8.17	0.00
Microfiltration	Electricity	Input	kWh	0.24	0.24	0.00	0.00
	Biowaste (Retentate MF)	Output	kg	220.00	0.00	3.14	0.00
Autoclaving	Electricity (121°C for 15 min)	Input	kWh	200.00	0.00 **	2.86	0.00 **
Neutralization	NaOH (20%)	Input	kg	0.01	0.01	0.00	0.00
Fermentation	Heat	Input	kWh	45.15	0.00 *	0.65	0.00 *
Microfiltration	Electricity	Input	kWh	0.24	0.24	0.00	0.00
Nanofiltration	Electricity	Input	kWh	12.33	12.33	0.18	0.18
	Water	Input	m^3	100.00	100.00	1.43	1.43
Softening electrodialysis	Electricity	Input	kWh	1.13	1.13	0.02	0.02
	Water	Input	m^3	100.00	100.00	1.43	1.43
Decolorization, cation, and anion exchange	Electricity	Input	kWh	2.27	0.00 *	0.03	0.00 *
	Water	Input	m^3	100.00	100.00	1.43	1.43
Evaporation (assuming MVR evaporator)	Heat (68.25 kWh m^{-3} at 70°C)	Input	kWh	135.00	0.00 *	1.93	0.00 *
	Lactic acid	Output	L	70.00	70.00	1.00	1.00
Anaerobic digestion of biowaste	Biowaste	Input	kg	-	792	-	11.31
	Methane generated	Output	m^3	-	53.57	-	0.77
	Primary energy	Output	kWh	-	582.50	-	8.32
	Parasitic load (8% of PE)	Input	kWh	-	46.60	-	0.67
	Electrical energy (from PE)	Output	kWh	-	204.23	-	2.92
	Heat energy (from PE)	Output	kWh	-	291.75	-	4.17

* Energy offset from AD in the form of heat.
** Energy offset from AD in the form of electricity.

DEC and Retentate MF residues. In the LA production process integrated with AD, these energy requirements account for about 95% of total CO_2 emissions, with negligible contributions from AD of the residues. In terms of human toxicity impacts, the energy required for hydrolysis, autoclaving, and running the fermenter contributed over 97% to the standalone LA production process, with negligible contributions from composting waste residues. For the integrated LA production process, these energy requirements account for approximately 83% of the total human toxicity impact, with the remainder from AD of the residues. Energy requirements contributed 70% of the total fossil depletion impact for the standalone LA production process and over 90% for the integrated process. Freshwater ecotoxicity is entirely due to the energy requirements of the standalone process, whereas it is entirely due to the AD of waste residues in the integrated process. For the standalone process, agricultural land occupation impact is 77% from energy requirements and the rest from composting waste residues. In contrast, in the integrated process, the impact of agricultural land occupation is entirely due to the AD of waste residues.

In the endpoint damage categories, human health (specifically human toxicity) was the most significant, contributing approximately 73–74% for both scenarios: the standalone LA production process (1.53 out of 2.08) and LA production process integrated with AD (0.37 out of 0.51). Overall, integrating AD into the LA production process resulted in an approximately 71% reduction in endpoint damage impacts. For the standalone LA production process, approximately 90% of the endpoint impact was due to energy requirements (mainly from hydrolysis, autoclaving, and evaporation), and approximately 8% was from composting waste residues. In the integrated LA production process, approximately 78% of the endpoint impact was due to energy requirements for LA production (after accounting for the energy offset by the AD process), with the remaining impact from AD of the waste residues.

Although the use of chemicals (NaOH and HCl) and enzymes in the carbohydrate hydrolysis process during LA fermentation was an important contributor to both the standalone LA production process and the LA production process integrated with AD, the overall environmental impact on LA production was insignificant. These contributions amounted to less than 1% of the total environmental impact in both midpoint and endpoint categories.

3.4.2. Recommendations for reducing the environmental footprint

Given that autoclaving for sterilization is one of the most energy-intensive processes in LA production, accounting for 32% of the total energy requirement, it is recommended to implement a heat recovery system to utilize the waste heat from the autoclave. This system should capture and reuse waste heat to achieve significant energy savings and enhance process efficiency. Specifically, a plate heat exchanger system can recover heat from the autoclave's exhaust water, which can then be used to maintain the required temperatures for subsequent stages in the process, such as 50°C for 21 h during enzymatic hydrolysis and 37°C for 98 h during bacterial fermentation. Maintaining 50°C for hydrolysis accounted for 37% of the

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total energy requirement, while keeping 37°C for fermentation accounted for 7%. Utilizing waste heat from the autoclave would lead to substantial energy savings, thereby reducing overall operational costs and environmental footprints. The integration of heat recovery systems not only improves energy efficiency but also supports sustainable production practices in LA fermentation.

Various studies have recommended ways to reduce energy costs, overall operational expenses, and environmental footprint. High water consumption has been observed in fermentative LA production processes from different feedstocks, where water is typically used in acid, alkali, or solvent solutions, washing feedstock for decontamination, and steam condensation water (Liu et al., 2015; Wang et al., 2021; Ma et al., 2022). To reduce overall water consumption, wastewater recycling after treatment has been proposed as a sustainable practice. For instance, in a study on neutral and low-pH fermentation for LA production, Li et al. (2021) integrated AD for wastewater treatment with sludge dewatering, a membrane bioreactor, and reverse osmosis for water recovery. The recycled water supplemented the water requirements of the process. Similarly, Mussatto et al. (2013), in their techno-economic analysis, proposed integrating reverse osmosis technology to treat wastewater in their biorefinery. Vanapalli et al. (2023) suggested using closed-loop wastewater recirculation as cooling water after advanced water treatment in the LA production process. However, for the present study, a detailed analysis of the techno-economic feasibility and environmental impacts associated with integrating these additional processes is necessary before recommending them as potential solutions.

Further, the current study found that using a mixed energy source consisting of 60% fossil fuels and 40% renewables resulted in higher environmental impact. Therefore, replacing the energy requirements for LA production entirely with renewable energy sources such as wind, solar, or biogas is recommended. Replacing mixed-source electricity with renewable energy can reduce midpoint environmental impacts by 73–79% for standalone LA production and endpoint environmental impacts by 75–82% (**Fig. 7**). For the LA production process integrated with AD, this change can reduce midpoint environmental impacts by 53–57% and endpoint environmental impacts by 64–70% (**Fig. 7**).

3.4.3. Energy cost assessment

The total energy required to produce 1 L of LA was estimated to be 8.96 kWh for the standalone process. Li et al. (2021) used techno-economic analysis and LCA to predict an energy requirement of 8.80 kWh per kilogram of LA produced. As discussed previously in the LCA, the major energy consumption is mainly for sterilization by autoclave (32% of the total energy), hydrolysis (37% of the total energy, with 85°C for 2 h and then 50°C for 21 h), evaporation (22% of the total energy) and constant temperature fermentation (37°C for 98 h, consuming 7% of the total energy). Other processes, such as evaporation, electrodialysis, nanofiltration, and microfiltration, contributed to the remaining 2% of the energy usage. The cost of electricity generated from mixed sources was noted to be €0.4 per kWh. Therefore, the total energy cost for producing 1 L of LA was €3.63 for the standalone production process (**Fig. 8**). Conversely, for the LA production process integrated with AD, the energy from biogas offsets various energy requirements, reducing the cost to €0.732 per 1 L of LA, representing an approximately 80% reduction in energy cost.

Further cost reductions can be achieved by replacing the energy requirement with renewable sources such as wind, solar, or biogas (**Fig. 8**). Citing cost data from the study by Kost et al. (2021), the cost of electricity from wind energy (offshore) ranged from €0.122 to €0.616 per kWh, solar energy from €0.111 to €0.408 per kWh, and biogas energy from €0.1447 to €0.1977 per kWh, compared to €0.4 per kWh from mixed sources. These figures highlight the falling prices of renewable energy, making it increasingly competitive with traditional energy sources. Using the highest prices for simplicity, replacing mixed-source electricity with wind energy could reduce energy costs by approximately 79% for both standalone and AD-integrated LA production. Similarly, switching to solar energy could lower costs by approximately 72% for both processes, while using biogas-derived energy could reduce costs by approximately 52.5%.

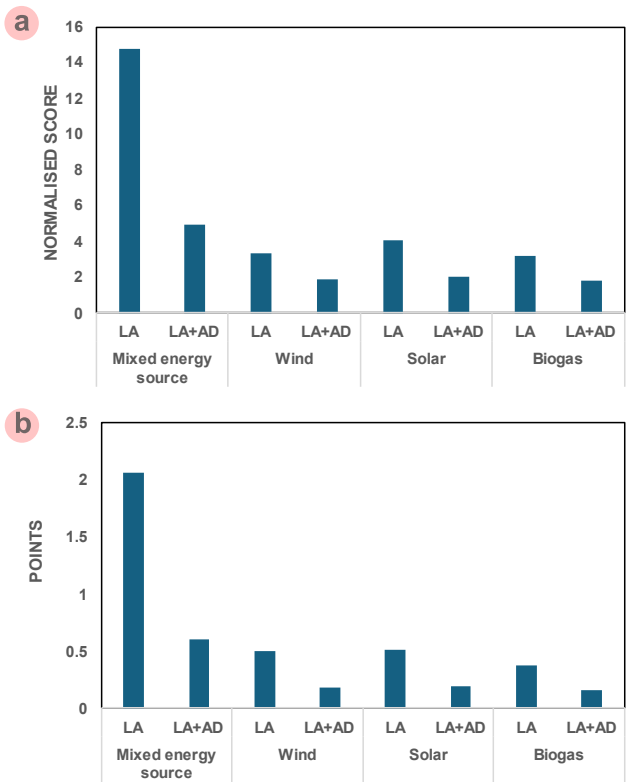

Fig. 7. Scenario analysis with respect to change in the source of energy used; **(a)** midpoint impacts and **(b)** endpoint impacts.

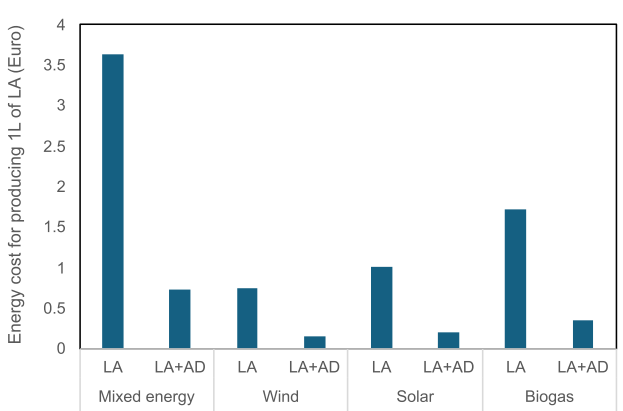

Fig. 8. Comparison of energy cost (in euro) for producing 1 L of lactic acid using energy from different sources.

3.5. Significance of the study

The biotechnological production of LA has already been industrialized, largely due to the high optical purities achieved (Olszewska-Widdrat et al., 2019). Utilizing renewable resources is essential not only for minimizing costs but also for ensuring the environmental sustainability of the process (López-Gómez et al., 2019). DRB is an abundant residue that holds the potential for exploitation and valorization in LA production. Our previous study demonstrated the feasibility of using DRB as a substrate for LA fermentation, yielding high product levels and productivity (Alexandri et al., 2019). While batch processes are commonly preferred in industrial applications, continuous mode production could offer greater advantages for

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LA production. This approach may result in higher final titers and reduced operating costs by requiring the preparation of inoculum only once (López-Gómez et al., 2019).

Furthermore, cell recirculation has been shown to increase cell densities, thereby enhancing LA yields and productivities (López-Gómez et al., 2019; Alexandri et al., 2020a; Olszewska-Widdrat et al., 2020). There are few publications on continuous LA fermentations at pilot scales, with most studies conducted in batch mode at technical scales of 50 L working volume, reporting LA yields of over 70% (Olszewska-Widdrat et al., 2020; Azaizeh et al., 2022; Marzo-Gago et al., 2024). While this work did not focus on optimizing LA fermentation parameters and processes, the results were promising, with an average product yield of 53% throughout the process. Further research into mass transfer phenomena at pilot scales could improve strain performance and final product titers.

In this study, enzymatically hydrolyzed DRB serves as the substrate, with the fermentable sugars primarily converted into LA through homolactic fermentation. During this process, glucose derived from the hydrolyzed DRB undergoes glycolysis, breaking down into two molecules of pyruvate. The enzyme lactate dehydrogenase then catalyzes the reduction of each pyruvate molecule into LA, with NAD+ being regenerated to sustain the glycolytic pathway. The final product of this process is LA (Wang et al., 2021).

Meanwhile, the unhydrolyzed DRB (Pellet DEC and Retentate MF), along with the non-fermentable fraction of the hydrolyzed DRB composed of sugars, amino acids, fatty acids, and the residual cell biomass of the LA bacteria (Retentate Biomass and Retentate MF), are subjected to anaerobic digestion. This multi-step process begins with hydrolysis, where complex organic materials are broken down into simpler monomers. These monomers are subsequently converted into VFAs and other intermediates during acidogenesis. In the next stage, acetogenesis transforms the VFAs into acetic acid, hydrogen, and carbon dioxide. Finally, during methanogenesis, methanogenic archaea convert acetic acid, hydrogen, and carbon dioxide into methane and water, resulting in biogas production. This process efficiently recycles the fermentation byproducts into valuable energy resources. A schematic representation of both processes is provided in **Figure 9** (Feng et al., 2023).

The primary objectives of this study were to assess the methane production potential of DRB raw material and four main waste streams from pilot-scale LA fermentation and purification, to detail the methane production characteristics and long-term performance of selected waste streams in continuous AD processes, to evaluate the effects of co-digestion of two major waste streams from LA fermentation, and to conduct a LCA of integrating the LA production process with AD compared to the standalone LA production process. The batch AD tests confirmed that waste streams derived from LA production, including DRB raw material, possess significant methane production potential comparable to existing literature (Haag et al., 2015; Haag et al., 2016; Vasmara et al., 2021; Bühlmann et al., 2021; Bühlmann et al., 2022; Chopda et al., 2024). However, the co-digestion of Pellet DEC and Retentate MF exhibited lower methane yields compared to the monodigestion of DRB, Pellet DEC, and Retentate MF. These results highlight the viability of these materials for renewable energy generation through AD, with the novelty of our work lying in the long-term continuous study and the determination of optimal OLR for maximum methane yield and ammonia inhibition resilience.

In continuous AD processes, this study focused on methane production characteristics and the long-term performance of selected waste streams. According to Kim et al. (2016), their study was the first to systematically recover both LA and biogas from food waste, reporting the best methane yield of 250 L kg_{VS}^{-1}. In comparison, our study exhibited higher yields: monodigestion of Pellet DEC at an optimum OLR of 1.5 produced 285.7 L kg^{-1}, monodigestion of Retentate MF at an OLR of 2.5 yielded 289.0 L kg^{-1}, and co-digestion of Pellet DEC and Retentate MF at an OLR of 2 achieved 265.0 L kg^{-1}. Optimizing OLR proved essential for maintaining stable process conditions and enhancing biogas production efficiency. The monodigestion of Pellet DEC exhibited tolerance to OLR up to 1.5 kg_{VS} m^{-3} d^{-1}, with a decline beyond this point, while the co-digestion of Pellet DEC with Retentate MF tolerated OLR up to 2 kg_{VS} m^{-3} d^{-1}. Evaluating the effects of co-digestion of two major waste streams from LA fermentation in both batch and continuous AD experiments revealed that co-digestion can enhance methane yields compared to the digestion of individual waste streams, although batch experiments showed lower methane yields for co-digestion than monodigestion. These findings support the hypothesis that optimal OLR is crucial for preventing process inhibition and ensuring consistent methane yields over extended periods and that combined processing of waste streams improves overall process efficiency and biogas production.

The LCA underscored the environmental and economic advantages of integrating the LA production process with AD. This integrated approach resulted in a reduced environmental footprint and enhanced economic viability compared to the standalone LA production process. These findings validate the potential of a combined system to effectively address both environmental and economic challenges associated with industrial waste management. To the best of our knowledge, no other study has provided a detailed LCA for the combined LA production and AD process.

Overall, the findings align with the initial hypotheses and objectives, demonstrating the feasibility and benefits of valorizing agricultural residues and industrial byproducts through AD. By optimizing methane production, ensuring stable process conditions, and evaluating the synergistic effects of co-digestion, this research advances our understanding of sustainable waste management strategies. The LCA further reinforces the advantages of integrating LA production with AD, promoting a circular economy paradigm. These contributions are crucial for developing environmentally sustainable and economically viable solutions for managing agricultural and industrial wastes.

3.6. Practical implications of the study

The integration of AD with LA production from DRB has significant industrial applications and presents a compelling strategy for sustainable resource management and bio-based product manufacturing. The hydrolysis

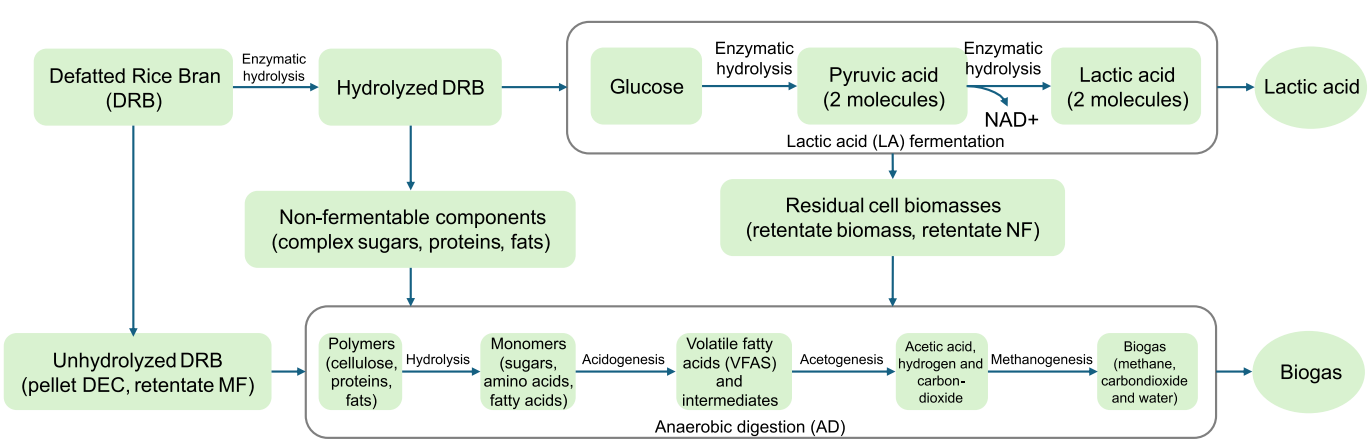

Fig. 9. Schematic representation of the lactic acid and anaerobic digestion processes, their mechanisms and integration.

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of DRB for LA fermentation produces considerable solid waste fractions, which have traditionally been considered low-value byproducts. Incorporating AD transforms these wastes into valuable resources such as methane and organic fertilizers. Methane is a renewable energy source that can be used to generate electricity and heat *via* direct combustion in a CHP unit. This energy can support energy-intensive operations, such as hydrolysis, sterilization, fermentation, electrodialysis, and evaporation, reducing reliance on external energy sources and lowering operational costs. Alternatively, upgraded biogas can be injected into the grid to generate revenue and offset energy costs.

This approach not only facilitates the valorization of waste residues, diverts them from landfills, and minimizes their environmental impact but also aligns with circular economy principles by reintegrating waste streams into the production cycle. By reducing reliance on fossil fuels and decreasing greenhouse gas emissions, the process promotes sustainability within an industrial framework. Overall, integrating the LA production process with AD resulted in an approximately 67% reduction in the total midpoint environmental impact compared to the standalone LA production process and a 71% reduction in endpoint damage impacts. Furthermore, the total energy cost for producing 1 L of LA was €3.63 for the standalone production process. Conversely, for the LA production process integrated with AD, the energy from biogas offset various energy requirements, reducing the cost to €0.732 per 1 L of LA, an approximately 80% reduction in energy cost.

Cost reductions achieved through energy savings and waste valorization enhance the economic viability of LA production. Consequently, the production of lactic-acid-derived products, such as PLA bioplastics, has become more affordable, increasing their market competitiveness and fostering broader adoption. Integrating biogas as an energy vector into industrial processes substantially enhances both economic and environmental sustainability, highlighting its potential for widespread industrial applications.

3.7 Limitations of the study and scope for future research

This study highlights the substantial environmental and economic benefits of integrating anaerobic digestion with LA production, significantly reducing both endpoint damage impacts and energy costs. However, despite these positive outcomes, there are opportunities for further exploration to expand their applicability and enhance their economic and operational feasibility. This study did not include scalability studies, which are essential for understanding how the process is performed when scaled from pilot to industrial levels. Additionally, it lacked a detailed techno-economic assessment, which is crucial for a comprehensive evaluation of the financial viability of the process. Furthermore, a thorough cost-benefit analysis that encompasses all aspects of capital investment, including construction and operational costs for both LA and biogas production, as well as potential revenues from byproducts, such as electricity and heat, was not conducted.

To build on the current findings, future research will focus on scalability studies to understand the dynamics and cost implications of expanding the process from pilot to industrial scale. Comprehensive techno-economic analyses, including detailed assessments of capital investments, operational costs, and potential revenues from both LA and biogas production facilities, are crucial for uncovering economic trade-offs and identifying cost-effective strategies. Further investigation should also encompass a complete cost-benefit analysis that accounts for all expenses and benefits, including capital investment and operational costs for LA and biogas production, as well as the revenues generated from byproducts, such as electricity and heat. Additionally, the impact of plant size and technology on the overall economics of the process warrants thorough exploration, particularly in terms of economies of scale and the efficiencies provided by different technologies.

Moreover, there is a need to meticulously evaluate the complexity of digester design and ongoing operational expenses, such as labor, maintenance, and energy consumption. Additionally, region-specific factors should be considered, including land and construction costs, regulatory and permitting expenses, and other localized conditions that can influence the feasibility and cost-effectiveness of the project. By addressing these areas, future research can provide robust data and insights, facilitate informed decision-making, and potentially lead to more sustainable and economically viable industrial practices in bio-based product manufacturing.

4. Conclusions

The present study demonstrated the potential for valorizing waste streams from DRB preprocessing for LA production through AD. All waste streams from LA fermentation and purification were effectively converted into biogas, with Pellet DEC and Retentate MF showing the highest methane yields. Long-term semi-continuous AD confirmed their suitability but highlighted susceptibility to TAN inhibition at higher OLRs due to protein content and low C/N ratios. However, co-digestion of Pellet DEC and Retentate MF offered high methane yields and resistance to TAN inhibition at an optimal OLR of 2.0 kg_{VS} m^{-3} d^{-1}.

Integrating AD with LA production from DRB provides an effective waste management solution and a sustainable approach to energy recovery. Utilizing biogas to offset energy demands enhances the economic viability of LA production, contributing to a more sustainable and circular bioeconomy. This approach could be extended to similar waste streams from other agricultural and industrial processes, reducing environmental pollution and increasing renewable energy production. The methodologies and optimizations developed in this study can be scaled to large operations, facilitating the adoption of AD systems that manage substantial waste volumes. This integrated approach offers environmental and financial benefits, encouraging investment in AD technologies and reducing reliance on fossil fuels. Policy support and incentives for renewable energy and sustainable waste management could further accelerate the adoption of AD technologies.

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Supplementary Material

Table S1.
Background datasets adopted from EcoInvent 3.4 for representing the material and energy parameters considered during the life cycle assessment of lactic acid production from defatted rice bran – Standalone process *vs.* process integrated with anaerobic digestion.

Field	Adopted datasets
Electricity	Electricity, high voltage, production mix {Europe without Switzerland}| Transformation | Cut-off, U
Water	Water, ultrapure {Germany}| water production, ultrapure | Cut-off, U
HCl	Hydrochloric acid, without water, {Europe without Switzerland}| Market for | Cut-off, U
NaOH	Sodium hydroxide {Europe without Switzerland}| market for | Cut-off, U
Enzyme	Enzymes {Europe without Switzerland}| enzymes production | Cut-off, U
Treatment of biowaste	Industrial composting {Europe without Switzerland}| Transformation | Cut-off, U
Treatment of biowaste	Anaerobic digestion {Europe without Switzerland}| Transformation | Cut-off, U
Biogas to energy	Heat and power co-generation, biogas, gas engine {Europe without Switzerland}| Trasformation | Cut-off, U
Electricity	Electricity production, wind, >3MW turbine {Europe without Switzerland}| Transformation | Cut-off, U
Electricity	Electricity production, photovoltaic, 570kWp open ground installation {Europe without Switzerland}| Transformation | Cut-off, U
Electricity	Heat and power co-generation, biogas, gas engine {Europe without Switzerland}| Transformation | Cut-off, U

Table S2.
Statistical results for multiple comparisons of feedstock effects on means of methane yields (in $L_N\,kg^{-1}$) in BMP tests.

Comparison of effects	Estimate (Difference)	*P*-value[1]	Significance	95% Confidence limits[1]	
				Lower	Upper
DRB – Pellet DEC	211.06	<.0001	***	195.88	226.24
DRB – Retentate MF	272.73	<.0001	***	257.55	287.91
DRB – Retentate Biomass	287.02	<.0001	***	271.85	302.20
DRB – Retentate NF	286.20	<.0001	***	271.03	301.38
DRB – Mix. Pellet & Retentate MF	231.06	<.0001	***	215.88	246.23
Pellet DEC – Retentate MF	61.66	<.0001	***	46.48	76.84
Pellet DEC – Retentate Biomass	75.96	<.0001	***	60.78	91.13
Pellet DEC – Retentate NF	75.14	<.0001	***	59.96	90.31
Pellet DEC – Mix. Pellet & Retentate MF	19.99	0.0088	**	4.82	35.17
Retentate MF – Retentate Biomass	14.29	0.0708	n.s.	-0.88	29.47
Retentate MF – Retentate NF	13.47	0.0947	n.s.	-1.70	28.65
Retentate MF – Mix. Pellet & Retentate MF	-41.67	<.0001	***	-56.85	-26.49
Retentate Biomass – Retentate NF	-0.82	1.0000	n.s.	-16.00	14.36
Retentate Biomass – Mix. Pellet & Retentate MF	-55.96	<.0001	***	-71.14	-40.79
Retentate NF – Mix. Pellet & Retentate MF	-55.14	<.0001	***	-70.32	-39.97

[1] adjusted for multiplicity, adjustment = SIMULATE
DRB: Defatted rice bran; Pellet DEC: pellet after decantation; Retentate MF: retentate after microfiltration; Retentate Biomass: biomass retentate after microfiltration of fermentation broth; Retentate NF: Retentate after nanofiltration; Mix. Pellet & Retentate MF: mixture of pellet after decantation and retentate after microfiltration; n.s.: not significant.

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Table S3.
Statistical results for multiple comparisons of feedstock effects on means of methane yields (in $L_N\,kg_{VS}^{-1}$) in BMP tests.

Comparison of effects	Estimate (Difference)	*P*-value[1]	Significance	95% Confidence limits[1]	
				Lower	Upper
DRB – Pellet DEC	21.04	0.3961	n.s.	-14.48	56.56
DRB – Retentate MF	13.58	0.7895	n.s.	-21.95	49.11
DRB – Retentate Biomass	-39.31	0.0274	*	-74.84	-3.79
DRB – Retentate NF	6.23	0.9896	n.s.	-29.30	41.76
DRB – Mix. Pellet & Retentate MF	36.59	0.0420	*	1.06	72.11
Pellet DEC – Retentate MF	-7.47	0.9766	n.s.	-42.97	28.04
Pellet DEC – Retentate Biomass	-60.36	0.0010	**	-95.87	-24.85
Pellet DEC – Retentate NF	14.82	0.7271	n.s.	-50.32	20.69
Pellet DEC – Mix. Pellet & Retentate MF	15.54	0.6875	n.s.	-19.96	51.05
Retentate MF – Retentate Biomass	-52.89	0.0039	**	-88.40	17.39
Retentate MF – Retentate NF	-7.35	0.9783	n.s.	-42.86	28.16
Retentate MF – Mix. Pellet & Retentate MF	23.01	0.3113	n.s.	-12.50	58.52
Retentate Biomass – Retentate NF	45.54	0.0104	*	10.04	81.05
Retentate Biomass – Mix. Pellet & Retentate MF	75.90	0.0002	***	40.40	111.41
Retentate NF – Mix. Pellet & Retentate MF	30.36	0.1106	n.s.	-5.15	65.87

[1] adjusted for multiplicity, adjustment = SIMULATE
DRB: Defatted rice bran; Pellet DEC: pellet after decantation; Retentate MF: retentate after microfiltration; Retentate Biomass: biomass retentate after microfiltration of fermentation broth; Retentate NF: Retentate after nanofiltration; Mix. Pellet & Retentate MF: mixture of pellet after decantation and retentate after microfiltration; n.s.: not significant.

Table S4.
Statistical results for multiple comparisons of feedstock effects on means of average methane content (in %) in BMP tests.

Comparison of effects	Estimate (Difference)	*P*-value[1]	Significance	95% Confidence Limits[1]	
				Lower	Upper
DRB – Pellet DEC	4.32	<.0001	***	-5.78	-2.87
DRB – Retentate MF	0.51	0.8369	n.s.	-0.94	1.97
DRB – Retentate Biomass	-7.45	<.0001	***	-8.91	-6.00
DRB – Retentate NF	1.57	0.0330	*	-3.03	-0.12
DRB – Mix. Pellet & Retentate MF	-2.63	0.0006	***	-4.08	-1.17
Pellet DEC – Retentate MF	4.84	<.0001	***	3.38	6.30
Pellet DEC – Retentate Biomass	-3.13	0.0002	***	-4.58	-1.67
Pellet DEC – Retentate NF	2.75	0.0005	***	1.30	4.21
Pellet DEC – Mix. Pellet & Retentate MF	1.70	0.0196	*	0.24	3.16
Retentate MF – Retentate Biomass	-7.97	<.0001	***	-9.42	-6.51
Retentate MF – Retentate NF	-2.09	0.0045	**	-3.54	-0.63
Retentate MF – Mix. Pellet & Retentate MF	-3.14	0.0002	***	-4.60	-1.68
Retentate Biomass – Retentate NF	5.88	<.0001	***	4.42	7.33
Retentate Biomass – Mix. Pellet & Retentate MF	4.83	<.0001	***	3.37	6.28
Retentate NF – Mix. Pellet & Retentate MF	-1.05	0.2152	n.s.	-2.50	0.40

[1] adjusted for multiplicity, adjustment = SIMULATE
DRB: Defatted rice bran; Pellet DEC: pellet after decantation; Retentate MF: retentate after microfiltration; Retentate Biomass: biomass retentate after microfiltration of fermentation broth; Retentate NF: Retentate after nanofiltration; Mix. Pellet & Retentate MF: mixture of pellet after decantation and retentate after microfiltration; n.s.: not significant.

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Table S5.
Statistical results for multiple comparisons of feedstock effects on means of average first-order decay constant k (in d^{-1}) in BMP tests.

Comparison of effects	Estimate (Difference)	*P*-value[1]	Significance	95% Confidence Limits[1]	
				Lower	Upper
DRB – Pellet DEC	0.053	<.0001	***	0.045	0.062
DRB – Retentate MF	-0.002	0.9794	n.s.	-0.010	0.007
DRB – Retentate Biomass	0.042	<.0001	***	0.034	0.051
DRB – Retentate NF	0.008	0.0697	n.s.	-0.001	0.017
DRB – Mix. Pellet & Retentate MF	0.049	<.0001	***	0.041	0.057
Pellet DEC – Retentate MF	-0.055	<.0001	***	-0.064	-0.047
Pellet DEC – Retentate Biomass	-0.011	0.0085	**	-0.020	-0.003
Pellet DEC – Retentate NF	-0.045	<.0001	***	-0.054	-0.037
Pellet DEC – Mix. Pellet & Retentate MF	-0.004	0.5630	n.s.	-0.013	0.004
Retentate MF – Retentate Biomass	0.044	<.0001	***	0.036	0.053
Retentate MF – Retentate NF	0.010	0.0209	*	0.001	0.018
Retentate MF – Mix. Pellet & Retentate MF	0.051	<.0001	***	0.043	0.060
Retentate Biomass – Retentate NF	-0.034	<.0001	***	-0.043	-0.026
Retentate Biomass – Mix. Pellet & Retentate MF	0.007	0.1319	n.s.	-0.002	0.015
Retentate NF – Mix. Pellet & Retentate MF	0.041	<.0001	***	0.032	0.050

[1] adjusted for multiplicity, adjustment = SIMULATE
DRB: Defatted rice bran; Pellet DEC: pellet after decantation; Retentate MF: retentate after microfiltration; Retentate Biomass: biomass retentate after microfiltration of fermentation broth; Retentate NF: Retentate after nanofiltration; Mix. Pellet & Retentate MF: mixture of pellet after decantation and retentate after microfiltration; n.s.: not significant.

Table S6.
Statistical results for multiple comparisons of feedstock effects on means of the maximum specific methane yield of the modified Gompertz model (in L_N kg_{VS}^{-1}).

Comparison of effects	Estimate (Difference)	*P*-value[1]	Significance	95%Confidence limits[1]	
				Lower	Upper
DRB – Pellet DEC	12.43	0.8573	n.s.	-24.25	49.11
DRB – Retentate MF	11.34	0.8948	n.s.	-25.34	48.02
DRB – Retentate Biomass	-41.19	0.0251	*	-77.87	-4.51
DRB – Retentate NF	7.15	0.9843	n.s.	-29.53	43.83
DRB – Mix. Pellet & Retentate MF	36.86	0.0483	*	0.18	73.54
Pellet DEC – Retentate MF	-1.09	1.0000	n.s.	-37.77	35.59
Pellet DEC – Retentate Biomass	-53.62	0.0033	**	-90.30	-16.94
Pellet DEC – Retentate NF	5.28	0.9962	n.s.	-41.96	31.40
Pellet DEC – Mix. Pellet & Retentate MF	24.43	0.2856	n.s.	-12.25	61.11
Retentate MF – Retentate Biomass	-52.53	0.0039	**	-89.21	-15.85
Retentate MF – Retentate NF	-4.19	0.9983	n.s.	-40.87	32.49
Retentate MF – Mix. Pellet & Retentate MF	25.52	0.2470	n.s.	-11.16	62.20
Retentate Biomass – Retentate NF	48.34	0.0077	**	11.66	85.02
Retentate Biomass – Mix. Pellet & Retentate MF	78.05	<.0001	***	41.37	114.73
Retentate NF – Mix. Pellet & Retentate MF	29.71	0.1393	n.s.	-6.97	66.39

[1)] adjusted for multiplicity, adjustment = SIMULATE
DRB: Defatted rice bran; Pellet DEC: pellet after decantation; Retentate MF: retentate after microfiltration; Retentate Biomass: biomass retentate after microfiltration of fermentation broth; Retentate NF: Retentate after nanofiltration; Mix. Pellet & Retentate MF: mixture of pellet after decantation and retentate after microfiltration; n.s.: not significant.

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Table S7.
Statistical results for multiple comparisons of feedstock effects on means of the half-life t_{50} (in d).

Comparison of effects	Estimate (Difference)	*P*-value[1]	Significance	95%Confidence Limits[1]	
				Lower	Upper
DRB – Pellet DEC	-1.94	<.0001	***	-2.44	-1.44
DRB – Retentate MF	-0.46	0.0744	n.s.	-0.96	0.04
DRB – Retentate Biomass	-2.26	<.0001	***	-2.76	-1.76
DRB – Retentate NF	-0.77	0.0028	**	-1.27	-0.27
DRB – Mix. Pellet & Retentate MF	-1.90	<.0001	***	-2.40	-1.40
Pellet DEC – Retentate MF	1.48	<.0001	***	0.98	1.98
Pellet DEC – Retentate Biomass	-0.31	0.3357	n.s.	-0.81	0.19
Pellet DEC – Retentate NF	1.17	<.0001	***	0.67	1.67
Pellet DEC – Mix. Pellet & Retentate MF	0.04	0.9998	n.s.	-0.46	0.54
Retentate MF – Retentate Biomass	-1.80	<.0001	***	-2.30	-1.30
Retentate MF – Retentate NF	-0.31	0.3468	n.s.	-0.81	0.19
Retentate MF – Mix. Pellet & Retentate MF	-1.44	<.0001	***	-1.94	-0.94
Retentate Biomass – Retentate NF	1.49	<.0001	***	0.99	1.99
Retentate Biomass – Mix. Pellet & Retentate MF	0.35	0.2340	n.s.	-0.15	0.85
Retentate NF – Mix. Pellet & Retentate MF	-1.13	<.0001	***	-1.63	-0.63

[1] adjusted for multiplicity, adjustment = SIMULATE
DRB: Defatted rice bran; Pellet DEC: pellet after decantation; Retentate MF: retentate after microfiltration; Retentate Biomass: biomass retentate after microfiltration of fermentation broth; Retentate NF: Retentate after nanofiltration; Mix. Pellet & Retentate MF: mixture of pellet after decantation and retentate after microfiltration; n.s.: not significant.

Table S8.
Statistical data for multiple comparisons of means of methane yields in semi-continuous anaerobic digestion experiments, comparison of feedstock and organic loading rate effects (only relevant comparisons are listed).

Comparison of effects	Estimate (Difference)	*p*-value[1]	Significance	Confidence limits[1]	
				Lower	Upper
Pellet DEC, OLR 0.5 – Retentate MF, OLR 0.5	-26.21	0.994	n.s.	-117.80	65.38
Pellet DEC, OLR 0.5 – Mix. Pellet&Retentate, OLR 0.5	5.512	1.000	n.s.	-86.08	97.10
Retentate MF, OLR 0.5 - Mix. Pellet&Retentate, OLR 0.5	31.72	0.974	n.s.	-59.87	123.31
Pellet DEC, OLR 1.0 – Retentate MF, OLR 1.0	-25.82	0.096	n.s.	-54.39	2.74
Pellet DEC, OLR 1.0 – Mix. Pellet&Retentate, OLR 1.0	1.69	1.000	n.s.	26.88	30.25
Retentate MF, OLR 1.0 - Mix. Pellet&Retentate, OLR 1.0	27.51	0.066	n.s.	-1.05	56.07
Pellet DEC, OLR 1.5 – Retentate MF, OLR 1.5	-49.11	<.0001	***	-72.06	26.17
Pellet DEC, OLR 1.5 – Mix. Pellet&Retentate, OLR 1.5	1.04	0.873	n.s.	-22.12	24.21
Retentate MF, OLR 1.5 - Mix. Pellet&Retentate, OLR 1.5	50.16	<.0001	***	27.00	73.32
Pellet DEC, OLR 2.0 – Retentate MF, OLR 2.0	-109.31	0.0032	**	-187.02	-31.61
Pellet DEC, OLR 2.0 – Mix. Pellet&Retentate, OLR 2.0	-43.14	0.639	n.s.	-120.84	34.57
Retentate MF, OLR 2.0 - Mix. Pellet&Retentate, OLR 2.0	66.18	0.1494	n.s.	-12.87	145.23

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Table S8.
continued.

Comparison of effects	Estimate (Difference)	*p*-value[1]	Significance	Confidence limits[1]	
				Lower	Upper
Retentate MF, OLR 2.5 - Mix. Pellet&Retentate, OLR 2.5	82.27	0.594	n.s.	-61.11	225.65
Pellet DEC, OLR 0.5 – Pellet DEC, OLR 1.0	-21.20	0.988	n.s.	-89.04	46.64
Pellet DEC, OLR 0.5 – Pellet DEC, OLR 1.5	-7.72	1.000	n.s.	-74.49	59.04
Pellet DEC, OLR 0.5 – Pellet DEC, OLR 2.0	56.13	0.396	n.s.	-28.18	140.44
Pellet DEC, OLR 1.0 – Pellet DEC, OLR 1.5	13.48	0.718	n.s.	-12.43	39.38
Pellet DEC, OLR 1.0 – Pellet DEC, OLR 2.0	77.33	0.005	**	19.69	134.97
Pellet DEC, OLR 1.5 – Pellet DEC, OLR 2.0	63.85	0.019	*	7.49	120.22
Retentate MF, OLR 0.5 – Retentate MF, OLR 1.0	-20.81	0.990	n.s.	-88.66	47.02
Retentate MF, OLR 0.5 – Retentate MF, OLR 1.5	-30.63	0.838	n.s.	-97.39	36.14
Retentate MF, OLR 0.5 – Retentate MF, OLR 2.0	-26.98	0.987	n.s.	-112.53	58.57
Retentate MF, OLR 0.5 – Retentate MF, OLR 2.5	15.17	1.000	n.s.	-104.15	134.50
Retentate MF, OLR 1.0 – Retentate MF, OLR 1.5	-9.81	0.948	n.s.	-35.72	16.10
Retentate MF, OLR 1.0 – Retentate MF, OLR 2.0	-6.16	1.000	n.s.	-65.59	53.27
Retentate MF, OLR 1.0 – Retentate MF, OLR 2.5	35.99	0.971	n.s.	-66.24	138.23
Retentate MF, OLR 1.5 – Retentate MF, OLR 2.0	3.65	1.000	n.s.	-54.55	61.86
Retentate MF, OLR 1.5 – Retentate MF, OLR 2.5	45.80	0.851	n.s.	-55.72	147.33
Retentate MF, OLR 2.0 – Retentate MF, OLR 2.5	42.15	0.958	n.s.	-72.60	156.90
Mix. Pellet&Retentate, OLR 0.5 – Mix. Pellet&Retentate, OLR 1.0	-25.02	0.957	n.s.	-92.87	42.82
Mix. Pellet&Retentate, OLR 0.5 – Mix. Pellet&Retentate, OLR 1.5	-12.19	0.999	n.s.	-79.03	54.65
Mix. Pellet&Retentate, OLR 0.5 – Mix. Pellet&Retentate, OLR 2.0	7.48	1.000	n.s.	-78.07	93.03
Mix. Pellet&Retentate, OLR 0.5 – Mix. Pellet&Retentate, OLR 2.5	65.73	0.671	n.s.	-55.55	187.00
Mix. Pellet&Retentate, OLR 1.0 – Mix. Pellet&Retentate, OLR 1.5	12.83	0.776	n.s.	-13.26	38.93
Mix. Pellet&Retentate, OLR 1.0 – Mix. Pellet&Retentate, OLR 2.0	32.51	0.661	n.s.	-26.93	91.94
Mix. Pellet&Retentate, OLR 1.0 – Mix. Pellet&Retentate, OLR 2.5	90.75	0.123	n.s.	-13.76	195.26
Mix. Pellet&Retentate, OLR 1.5 – Mix. Pellet&Retentate, OLR 2.0	19.67	0.978	n.s.	-38.62	77.96
Mix. Pellet&Retentate, OLR 1.5 – Mix. Pellet&Retentate, OLR 2.5	77.92	0.250	n.s.	-25.94	181.78
Mix. Pellet&Retentate, OLR 2.0 – Mix. Pellet&Retentate, OLR 2.5	58.24	0.763	n.s.	-58.54	175.03

[1] adjusted for multiplicity, adjustment = SIMULATE
Pellet DEC: pellet after decantation; Retentate MF: retentate after microfiltration; Mix. Pellet&Retentate: a mixture of pellet after decantation and retentate after microfiltration; OLR: organic loading rate; n.s.: not significant.

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Table S9.
Statistical data for multiple comparisons of means of methane production rates in semi-continuous anaerobic digestion experiments, comparison of feedstock and organic loading rate effects (only relevant comparisons are listed).

Comparison of effects	Estimate (Difference)	p-value[1]	Significance	Confidence limits[1]	
				Lower	Upper
Pellet DEC, OLR 0.5 – Retentate MF, OLR 0.5	-0.0193	0.419	n.s.	-0.0533	0.0145
Pellet DEC, OLR 0.5 – Mix. Pellet&Retentate, OLR 0.5	0.0016	1.000	n.s.	-0.0323	0.0356
Retentate MF, OLR 0.5 - Mix. Pellet&Retentate, OLR 0.5	0.0210	0.333	n.s.	-0.0130	0.0549
Pellet DEC, OLR 1.0 – Retentate MF, OLR 1.0	-0.0218	0.369	n.s.	-0.0583	0.0148
Pellet DEC, OLR 1.0 – Mix. Pellet&Retentate, OLR 1.0	0.0062	0.999	n.s.	-0.0303	0.0428
Retentate MF, OLR 1.0 - Mix. Pellet&Retentate, OLR 1.0	0.0280	0.153	n.s.	-0.0086	0.0646
Pellet DEC, OLR 1.5 – Retentate MF, OLR 1.5	-0.0728	0.004	**	-0.1175	-0.0281
Pellet DEC, OLR 1.5 – Mix. Pellet&Retentate, OLR 1.5	-0.0007	1.000	n.s.	-0.0456	0.0442
Retentate MF, OLR 1.5 - Mix. Pellet&Retentate, OLR 1.5	0.0721	0.005	**	0.0272	0.1169
Pellet DEC, OLR 2.0 – Retentate MF, OLR 2.0	-0.2369	0.063	n.s.	-0.4869	0.0130
Pellet DEC, OLR 2.0 – Mix. Pellet&Retentate, OLR 2.0	-0.0936	0.851	n.s.	-0.3436	0.1563
Retentate MF, OLR 2.0 - Mix. Pellet&Retentate, OLR 2.0	0.1433	0.428	n.s.	-0.1105	0.3972
Retentate MF, OLR 2.5 - Mix. Pellet&Retentate, OLR 2.5	0.1967	0.635	n.s.	-0.2198	0.6132
Pellet DEC, OLR 0.5 – Pellet DEC, OLR 1.0	-0.1519	<.0001	***	-0.1872	-0.1166
Pellet DEC, OLR 0.5 – Pellet DEC, OLR 1.5	-0.2706	<.0001	***	-0.3103	-0.2309
Pellet DEC, OLR 0.5 – Pellet DEC, OLR 2.0	-0.2752	0.005	**	-0.4508	-0.0997
Pellet DEC, OLR 1.0 – Pellet DEC, OLR 1.5	-0.1187	0.0002	***	0.1595	-0.0779
Pellet DEC, OLR 1.0 – Pellet DEC, OLR 2.0	-0.1233	0.217	n.s.	-0.2991	0.0525
Pellet DEC, OLR 1.5 – Pellet DEC, OLR 2.0	-0.0046	1.000	n.s.	-0.1814	0.1721
Retentate MF, OLR 0.5 – Retentate MF, OLR 1.0	-0.1543	<.0001	***	-0.1896	-0.1190
Retentate MF, OLR 0.5 – Retentate MF, OLR 1.5	-0.3241	<.0001	***	-0.3638	-0.2844
Retentate MF, OLR 0.5 – Retentate MF, OLR 2.0	-0.4929	0.0003	***	-0.6740	-0.3118
Retentate MF, OLR 0.5 – Retentate MF, OLR 2.5	-0.2989	0.0510	n.s.	-0.5988	0.0009
Retentate MF, OLR 1.0 – Retentate MF, OLR 1.5	-0.1697	<.0001	***	-0.2106	-0.1289
Retentate MF, OLR 1.0 – Retentate MF, OLR 2.0	-0.3385	0.0024	**	-0.5199	-0.1572
Retentate MF, OLR 1.0 – Retentate MF, OLR 2.5	-0.1446	0.6128	n.s.	-0.4446	0.1554
Retentate MF, OLR 1.5 – Retentate MF, OLR 2.0	-0.1688	0.0697	n.s.	-0.3511	0.01345
Retentate MF, OLR 1.5 – Retentate MF, OLR 2.5	0.0251	1.000	n.s.	-0.2754	0.3257
Retentate MF, OLR 2.0 – Retentate MF, OLR 2.5	0.1939	0.445	n.s.	-0.1547	0.5426

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Table S9.
continued.

Comparison of effects	Estimate (Difference)	p-value[1]	Significance	Confidence limits[1]	
				Lower	Upper
Mix. Pellet&Retentate, OLR 0.5 – Mix. Pellet&Retentate, OLR 1.0	-0.1473	<.0001	***	-0.1826	-0.1120
Mix. Pellet&Retentate, OLR 0.5 – Mix. Pellet&Retentate, OLR 1.5	-0.2729	<.0001	***	-0.3128	-0.2330
Mix. Pellet&Retentate, OLR 0.5 – Mix. Pellet&Retentate, OLR 2.0	-0.3705	0.002	**	-0.5516	-0.1894
Mix. Pellet&Retentate, OLR 0.5 – Mix. Pellet&Retentate, OLR 2.5	0.1499	<.0001	***	0.1259	0.1739
Mix. Pellet&Retentate, OLR 1.0 – Mix. Pellet&Retentate, OLR 1.5	-0.1256	0.0002	***	-0.1667	-0.0846
Mix. Pellet&Retentate, OLR 1.0 – Mix. Pellet&Retentate, OLR 2.0	-0.2232	0.019	*	-0.4046	-0.0419
Mix. Pellet&Retentate, OLR 1.0 – Mix. Pellet&Retentate, OLR 2.5	0.2972	<.0001	***	0.2713	0.3231
Mix. Pellet&Retentate, OLR 1.5 – Mix. Pellet&Retentate, OLR 2.0	-0.0976	0.4911	n.s.	-0.2799	0.0847
Mix. Pellet&Retentate, OLR 1.5 – Mix. Pellet&Retentate, OLR 2.5	0.4228	<.0001	***	0.3910	0.4547
Mix. Pellet&Retentate, OLR 2.0 – Mix. Pellet&Retentate, OLR 2.5	0.5204	0.0003	***	0.3409	0.6999

[1] adjusted for multiplicity, adjustment = SIMULATE
Pellet DEC: pellet after decantation; Retentate MF: retentate after microfiltration; Mix. Pellet&Retentate: a mixture of pellet after decantation and retentate after microfiltration; OLR: organic loading rate; n.s.: not significant.

Table S10.
Midpoint characterization data for LA production process - standalone; Agricultural land occupation potential (ALOP), greenhouse gas emission potential (GWP100), fossil depletion potential (FDP), freshwater ecotoxicity potential (FETP), freshwater eutrophication potential (FEP), human toxicity potential (HTP), ionizing radiation potential (IRP), marine ecotoxicity potential (METP), marine eutrophication potential (MEP), metal depletion potential (MDP), natural land transformation potential (NLTP), ozone depletion potential (ODP), particulate matter formation potential (PMFP), photochemical oxidant formation potential (POFP), terrestrial acidification potential (TAP100), terrestrial ecotoxicity potential (TETP), urban land occupation potential (ULOP), water depletion potential (WDP).

Impact category	Unit	Hydrolysis			Decantation	Microfiltration	Autoclaving	Fermentation	Electrodialysis	Evaporation
		Water ultrapure	HCl (32%)	Heat/electricity	Biowaste (Pellet DEC)	Biowaste (Retentate MF)	Heat/electricity	Heat/electricity	NaOH (20%)	Heat/electricity
GWP100	kg $CO_{2\text{-eq}}$	2.87E-02	1.34E-04	2.13E+00	4.57E-01	1.76E-01	1.85E+00	4.19E-01	1.94E-04	1.25E+00
ALOP	m^2a	2.33E-03	1.45E-05	1.33E-01	7.47E-02	2.87E-02	1.15E-01	2.61E-02	7.28E-06	7.75E-02
FDP	kg $oil_{\text{-eq}}$	8.33E-03	4.05E-05	5.75E-01	7.57E-02	2.91E-02	4.99E-01	1.13E-01	5.21E-05	3.36E-01
FETP	kg $1,4\text{-DCB}_{\text{-eq}}$	5.58E-04	3.16E-06	4.53E-02	3.68E-03	1.41E-03	3.93E-02	8.93E-03	2.85E-06	2.65E-02
FEP	kg $P_{\text{-eq}}$	2.16E-05	1.22E-07	2.90E-03	6.88E-05	2.64E-05	2.51E-03	5.71E-04	1.05E-07	1.69E-03
WDP	m^3	3.88E-02	6.63E-07	1.10E-02	5.17E-04	1.99E-04	9.51E-03	2.16E-03	6.92E-07	6.42E-03
HTP	kg $1,4\text{-DCB}_{\text{-eq}}$	2.23E-02	1.86E-04	1.76E+00	7.32E-02	2.82E-02	1.53E+00	3.47E-01	1.09E-04	1.03E+00
IRP	kg $U235_{\text{-eq}}$	9.28E-03	5.14E-05	3.90E-01	2.23E-02	8.56E-03	3.38E-01	7.68E-02	2.58E-05	2.28E-01
METP	kg $1,4\text{-DCB}_{\text{-eq}}$	5.57E-04	3.87E-06	4.32E-02	3.36E-03	1.29E-03	3.75E-02	8.52E-03	2.71E-06	2.53E-02
MEP	kg $N_{\text{-eq}}$	2.97E-05	1.40E-07	1.44E-03	8.30E-04	3.19E-04	1.24E-03	2.83E-04	2.07E-07	8.40E-04
MDP	kg $Fe_{\text{-eq}}$	3.17E-03	1.40E-05	1.53E-02	1.88E-02	7.24E-03	1.33E-02	3.02E-03	1.03E-05	8.95E-03
NLTP	m^2	3.52E-06	1.51E-08	8.54E-05	4.41E-05	1.70E-05	7.40E-05	1.68E-05	2.31E-08	4.99E-05
ODP	kg $CFC^{-11}_{\text{-eq}}$	1.21E-08	8.78E-11	8.74E-08	2.75E-08	1.06E-08	7.57E-08	1.72E-08	1.06E-10	5.11E-08
PMFP	kg $PM10_{\text{-eq}}$	6.37E-05	2.64E-07	1.56E-03	2.33E-03	8.94E-04	1.35E-03	3.07E-04	5.01E-07	9.11E-04
POFP	kg NMVOC	8.13E-05	3.36E-07	1.93E-03	9.30E-04	3.58E-04	1.68E-03	3.81E-04	5.42E-07	1.13E-03
TAP100	kg $SO_{2\text{-eq}}$	1.48E-04	7.11E-07	7.81E-03	1.49E-02	5.73E-03	6.77E-03	1.54E-03	8.86E-07	4.57E-03
TETP	kg $1,4\text{-DCB}_{\text{-eq}}$	4.98E-06	1.13E-07	1.96E-05	1.79E-05	6.90E-06	1.70E-05	3.85E-06	1.36E-08	1.14E-05
ULOP	m^2a	3.14E-04	1.44E-06	8.00E-03	6.06E-03	2.33E-03	6.93E-03	1.57E-03	2.16E-06	4.68E-03

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Table S11.
Midpoint characterization data for LA production process – integrated with AD; Agricultural land occupation potential (ALOP), greenhouse gas emission potential (GWP100), fossil depletion potential (FDP), freshwater ecotoxicity potential (FETP), freshwater eutrophication potential (FEP), human toxicity potential (HTP), ionizing radiation potential (IRP), marine ecotoxicity potential (METP), marine eutrophication potential (MEP), metal depletion potential (MDP), natural land transformation potential (NLTP), ozone depletion potential (ODP), particulate matter formation potential (PMFP), photochemical oxidant formation potential (POFP), terrestrial acidification potential (TAP100), terrestrial ecotoxicity potential (TETP), urban land occupation potential (ULOP), water depletion potential (WDP).

Impact Category	Unit	Hydrolysis			Decantation	Microfiltration	Autoclaving	Fermentation	Electrodialysis	Evaporation	Anaerobic digestion	
		Water Ultrapure	HCl (32%)	Heat/ Electricity	Biowaste (Pellet DEC)	Biowaste (Retentate MF)	Heat/ Electricity	Heat/electricity	NaOH (20%)	Heat/ Electricity	Biowaste	Biogas to Heat Conversion
GWP100	kg $CO_{2\text{-eq}}$	2.87E-02	1.34E-04	1.46E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	1.94E-04	0.00E+00	2.23E-02	1.82E-03
ALOP	m^2a	2.33E-03	1.45E-05	9.07E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00	7.28E-06	0.00E+00	8.65E-01	2.98E-02
FDP	kg $oil_{\text{-eq}}$	8.33E-03	4.05E-05	3.94E-01	0.00E+00	0.00E+00	0.00E+00	0.00E+00	5.21E-05	0.00E+00	1.20E-01	3.22E-03
FETP	kg 1,4-$DCB_{\text{-eq}}$	5.58E-04	3.16E-06	3.10E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00	2.85E-06	0.00E+00	3.48E-03	2.46E-04
FEP	kg $P_{\text{-eq}}$	2.16E-05	1.22E-07	1.98E-03	0.00E+00	0.00E+00	0.00E+00	0.00E+00	1.05E-07	0.00E+00	1.52E-04	7.74E-06
WDP	m^3	3.88E-02	6.63E-07	7.52E-03	0.00E+00	0.00E+00	0.00E+00	0.00E+00	6.92E-07	0.00E+00	2.64E-03	4.56E-05
HTP	kg 1,4-$DCB_{\text{-eq}}$	2.23E-02	1.86E-04	1.21E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	1.09E-04	0.00E+00	1.57E-01	8.88E-03
IRP	kg $U235_{\text{-eq}}$	9.28E-03	5.14E-05	2.67E-01	0.00E+00	0.00E+00	0.00E+00	0.00E+00	2.58E-05	0.00E+00	1.38E-02	2.69E-03
METP	kg 1,4-$DCB_{\text{-eq}}$	5.57E-04	3.87E-06	2.96E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00	2.71E-06	0.00E+00	3.32E-03	2.37E-04
MEP	kg $N_{\text{-eq}}$	2.97E-05	1.40E-07	9.83E-04	0.00E+00	0.00E+00	0.00E+00	0.00E+00	2.07E-07	0.00E+00	3.48E-04	8.13E-05
MDP	kg $Fe_{\text{-eq}}$	3.17E-03	1.40E-05	1.05E-02	0.00E+00	0.00E+00	0.00E+00	0.00E+00	1.03E-05	0.00E+00	1.55E-02	1.53E-03
NLTP	m^2	3.52E-06	1.51E-08	5.85E-05	0.00E+00	0.00E+00	0.00E+00	0.00E+00	2.31E-08	0.00E+00	9.35E-05	2.62E-06
ODP	kg $CFC^{-11}{}_{\text{-eq}}$	1.21E-08	8.78E-11	5.98E-08	0.00E+00	0.00E+00	0.00E+00	0.00E+00	1.06E-10	0.00E+00	2.68E-08	7.40E-10
PMFP	kg $PM10_{\text{-eq}}$	6.37E-05	2.64E-07	1.07E-03	0.00E+00	0.00E+00	0.00E+00	0.00E+00	5.01E-07	0.00E+00	7.44E-04	2.70E-04
POFP	kg NMVOC	8.13E-05	3.36E-07	1.32E-03	0.00E+00	0.00E+00	0.00E+00	0.00E+00	5.42E-07	0.00E+00	2.31E-03	5.28E-05
TAP100	kg $SO_{2\text{-eq}}$	1.48E-04	7.11E-07	5.35E-03	0.00E+00	0.00E+00	0.00E+00	0.00E+00	8.86E-07	0.00E+00	2.04E-03	1.88E-03
TETP	kg 1,4-$DCB_{\text{-eq}}$	4.98E-06	1.13E-07	1.34E-05	0.00E+00	0.00E+00	0.00E+00	0.00E+00	1.36E-08	0.00E+00	2.28E-05	9.47E-07
ULOP	m^2a	3.14E-04	1.44E-06	5.48E-03	0.00E+00	0.00E+00	0.00E+00	0.00E+00	2.16E-06	0.00E+00	5.38E-03	1.51E-04

Table S12.
Endpoint characterization data for LA production process – standalone.

Impact category	Unit	Hydrolysis			Decantation	Microfiltration	Autoclaving	Fermentation	Softening Rlectrodialysis	Evaporation
		Water Ultrapure	HCl (32%)	Heat/ Electricity	Biowaste (Pellet DEC)	Biowaste (Retentate MF)	Heat/ Electricity	Heat/ Electricity	NaOH (20%)	Heat/ Electricity
Ecosystem quality	Points	0.00	0.00	0.09	0.02	0.01	0.08	0.02	0.00	0.05
Human health	Points	0.00	0.00	0.55	0.03	0.01	0.48	0.11	0.00	0.32
Resources	Points	0.00	0.00	0.07	0.07	0.03	0.06	0.01	0.00	0.04

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Table S13.
Endpoint characterization data for LA production process – integrated with AD.

Impact Category	Unit	Hydrolysis			Decantation	Micro-filtration	Autoclaving	Fermentation	Softening Electrodialysis	Evaporation	Anaerobic digestion	
		Water Ultrapure	HCl (32%)	Heat/ Electricity	Biowaste (Pellet DEC)	Biowaste (Retentate MF)	Heat/ Electricity	Heat/ Electricity	NaOH (20%)	Heat/ Electricity	Biowaste	Biogas to Heat Conversion
Ecosystem quality	Points	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00
Human health	Points	0.00	0.00	0.38	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00
Resources	Points	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00

Table S14.
Midpoint characterization data of all products and processes from Ecoinvent 3.4 database.

Impact Category (midpoint)	Unit	Water (ultrapure)	HCl	Enzymes	Energy (mixed source)	Composting (biowaste)	NaOH	Anaerobic digestion (biowaste)	Energy (biogas)
Agricultural land occupation	m^2a	7.82E-05	1.21E-01	5.75E+00	4.02E-02	9.14E-03	5.05E-02	1.97E-03	2.38E-03
Climate change	kg $CO_{2\text{-eq}}$	9.61E-04	1.12E+00	6.63E+00	6.45E-01	5.60E-02	1.35E+00	7.64E-02	3.90E-02
Fossil depletion	kg $oil_{\text{-eq}}$	2.79E-04	3.39E-01	1.79E+00	1.74E-01	9.26E-03	3.62E-01	1.07E-02	4.21E-03
Freshwater ecotoxicity	kg 1,4-$DCB_{\text{-eq}}$	1.87E-05	2.65E-02	1.14E-01	1.37E-02	4.50E-04	1.98E-02	3.07E-04	3.22E-04
Freshwater eutrophication	kg $P_{\text{-eq}}$	7.25E-07	1.02E-03	4.14E-03	8.78E-04	8.41E-06	7.31E-04	1.34E-05	1.01E-05
Human toxicity	kg 1,4-$DCB_{\text{-eq}}$	7.48E-04	1.56E+00	3.69E+00	5.34E-01	8.96E-03	7.57E-01	1.39E-02	1.16E-02
Ionising radiation	kg $U235_{\text{-eq}}$	3.11E-04	4.30E-01	1.77E+00	1.18E-01	2.72E-03	1.79E-01	1.22E-03	3.52E-03
Marine ecotoxicity	kg 1,4-$DCB_{\text{-eq}}$	1.87E-05	3.24E-02	1.05E-01	1.31E-02	4.11E-04	1.88E-02	2.93E-04	3.09E-04
Marine eutrophication	kg $N_{\text{-eq}}$	9.94E-07	1.17E-03	3.38E-02	4.35E-04	1.02E-04	1.44E-03	3.08E-05	1.06E-04
Metal depletion	kg $Fe_{\text{-eq}}$	1.06E-04	1.17E-01	4.71E-01	4.64E-03	2.30E-03	7.15E-02	1.37E-03	2.00E-03
Natural land transformation	m^2	1.18E-07	1.27E-04	9.19E-04	2.59E-05	5.39E-06	1.60E-04	8.26E-06	3.42E-06
Ozone depletion	kg CFC-$11_{\text{-eq}}$	4.07E-10	7.35E-07	6.37E-07	2.65E-08	3.37E-09	7.37E-07	2.37E-09	9.67E-10
Particulate matter formation	kg $PM_{10\text{-eq}}$	2.14E-06	2.21E-03	1.42E-02	4.72E-04	2.85E-04	3.48E-03	6.57E-05	3.53E-04
Photochemical oxidant formation	kg NMVOC	2.72E-06	2.81E-03	1.91E-02	5.86E-04	1.14E-04	3.76E-03	2.04E-04	6.91E-05
Terrestrial acidification	kg $SO_{2\text{-eq}}$	4.95E-06	5.95E-03	5.04E-02	2.37E-03	1.82E-03	6.15E-03	1.81E-04	2.46E-03
Terrestrial ecotoxicity	kg 1,4-$DCB_{\text{-eq}}$	1.67E-07	9.45E-04	6.98E-03	5.93E-06	2.20E-06	9.46E-05	2.01E-06	1.24E-06
Urban land occupation	m^2a	1.05E-05	1.21E-02	7.83E-02	2.42E-03	7.42E-04	1.50E-02	4.75E-04	1.97E-04
Water depletion	m^3	1.30E-03	5.55E-03	2.13E-01	3.33E-03	6.33E-05	4.80E-03	2.33E-04	5.96E-05

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Table S15.
Endpoint characterization data of all products and processes from Ecoinvent 3.4 database.

Impact category (endpoint)		Unit	Water (ultrapure)	HCl	Enzymes	Energy (mixed source)	Composting (biowaste)	NaOH	Anaerobic digestion (biowaste)	Energy (biogas)
Ecosystem quality	Agricultural land occupation	Points	1.91E-06	2.94E-03	1.72E-01	9.54E-04	2.14E-04	1.25E-03	4.70E-05	5.67E-05
	Climate change, ecosystems	Points	2.47E-05	2.89E-02	1.64E-01	1.67E-02	9.20E-04	3.44E-02	1.39E-03	5.77E-04
	Freshwater ecotoxicity	Points	1.60E-08	2.18E-05	9.88E-05	1.48E-05	2.22E-07	1.67E-05	2.93E-07	2.53E-07
	Freshwater eutrophication	Points	4.76E-08	6.72E-05	2.72E-04	5.76E-05	5.52E-07	4.80E-05	8.83E-07	6.64E-07
	Marine ecotoxicity	Points	5.04E-06	7.11E-03	2.64E-02	4.57E-03	6.90E-05	5.41E-03	9.20E-05	8.27E-05
	Natural land transformation	Points	1.37E-05	1.57E-02	6.15E-02	4.00E-03	1.62E-03	1.76E-02	1.10E-03	2.67E-04
	Terrestrial acidification	Points	1.11E-07	1.33E-04	1.18E-03	5.63E-05	4.43E-05	1.38E-04	3.93E-06	5.97E-05
	Terrestrial ecotoxicity	Points	2.57E-07	5.51E-04	3.22E-03	2.06E-05	5.73E-06	2.69E-04	4.48E-06	3.47E-06
	Urban land occupation	Points	3.17E-07	3.63E-04	2.36E-03	7.30E-05	2.24E-05	4.51E-04	1.43E-05	5.94E-06
	Total	Points	4.62E-05	5.58E-02	4.31E-01	2.64E-02	2.90E-03	5.96E-02	2.65E-03	1.05E-03
Human health	Climate change, human health	Points	3.11E-05	3.63E-02	2.06E-01	2.10E-02	1.16E-03	4.32E-02	1.75E-03	7.26E-04
	Human toxicity	Points	1.66E-04	2.66E-01	9.39E-01	1.45E-01	2.26E-03	1.93E-01	3.04E-03	3.07E-03
	Ionising radiation	Points	4.97E-08	6.87E-05	2.82E-04	1.89E-05	4.35E-07	2.86E-05	1.95E-07	5.62E-07
	Ozone depletion	Points	1.21E-08	2.25E-05	1.44E-05	5.28E-07	8.51E-08	2.31E-05	6.04E-08	2.06E-08
	Particulate matter formation	Points	5.42E-06	5.61E-03	3.60E-02	1.20E-03	7.22E-04	8.83E-03	1.67E-04	8.94E-04
	Photochemical oxidant formation	Points	2.48E-07	2.02E-04	1.22E-03	9.01E-06	4.72E-06	2.68E-04	4.21E-05	6.11E-06
	Total	Points	2.02E-04	3.09E-01	1.18E+00	1.68E-01	4.15E-03	2.46E-01	5.00E-03	4.70E-03
Resources	Fossil depletion	Points	3.35E-05	4.07E-02	2.15E-01	2.09E-02	1.11E-03	4.34E-02	1.28E-03	5.05E-04
	Metal depletion	Points	4.93E-06	5.41E-03	2.17E-02	2.13E-04	1.07E-04	3.31E-03	6.37E-05	9.27E-05
	Total	Points	3.84E-05	4.61E-02	2.37E-01	2.11E-02	1.22E-03	4.67E-02	1.34E-03	5.97E-04
Total	Total	Points	2.87E-04	4.10E-01	1.85E+00	2.15E-01	8.26E-03	3.52E-01	8.99E-03	6.35E-03

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