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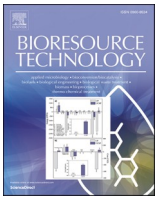


Novel biosurfactants produced from food waste: a variability and validation study

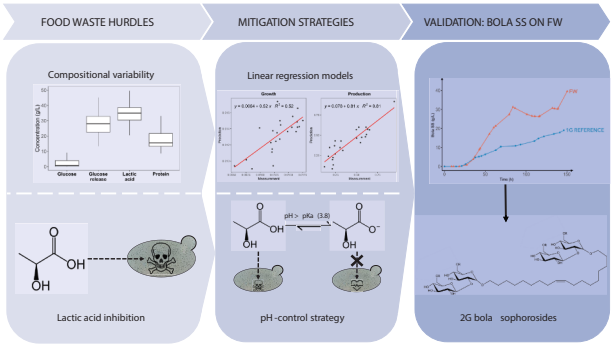
Thibo Van de Craen [a,b], Sophie L.K.W. Roelants [b,c], Karolien Maes [b], Sofie Lodens [b], Sofie L. De Maeseneire [a,*], Wim K. Soetaert [a], Tom Delmulle [a]

[a] Centre for Industrial Biotechnology and Biocatalysis (InBio.be), Department of Biotechnology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Ghent, Belgium
[b] R&D Department, Bio Base Europe Pilot Plant (BBEPP), Rodenhuizekaai 1, 9042 Desteldonk, Belgium
[c] R&D Department, AmphiStar, Suzanne Tassierstraat 1, 9052 Zwijnaarde, Belgium

HIGHLIGHTS

- Food waste supported growth and biosurfactant production despite variability.
- Predictive models accurately forecasted bioprocess results on variable food waste.
- Lactic acid inhibition of *Starmerella bombicola* solved via pH control.
- Bioreactor trials on food waste showed improved growth and production.
- First production of novel bola sophorosides on a second-generation feedstock.

GRAPHICAL ABSTRACT



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ABSTRACT

One-third of global food production is wasted, much of which is composted, gasified or landfilled. This food waste (FW) represents an untapped resource, particularly towards the production of biosurfactants. Genetically engineered *Starmerella bombicola* yeast strains offer a route to produce novel biosurfactants on FW with enhanced structural diversity, such as bola sophorosides. However, FW streams are variable, and often contain inhibitory compounds (e.g. lactic acid (LA), ethanol, etc.), posing bioprocessing challenges. Here we show that hydrolyzed FW consistently supports *S. bombicola* growth and bola sophoroside production, despite FW variability and high LA concentrations. Linear regression models accurately predicted fermentation outcomes across variable FW batches. Furthermore, the inhibitory effect of LA on biosurfactant production by *S. bombicola* was significantly reduced by a dedicated pH-control strategy. A proof-of-concept fermentation confirmed that hydrolyzed FW outperformed conventional media, achieving higher biomass (25.27 ± 4.18 vs. 19.71 ± 2.08 g/L) and productivity (0.28 vs. 0.13 g/L/h). In conclusion, this work demonstrates the viability of (variable) FW as a fermentation feedstock, opening new avenues for its valorization towards sustainable bioproducts.

* Corresponding author at: Coupure Links, 653, Building B, 2nd floor, 9000 Ghent, Belgium.
E-mail addresses: Thibo.vandecraen@ugent.be (T. Van de Craen), Sophie.roelants@amphistar.com (S.L.K.W. Roelants), Sofie.demaeseneire@ugent.be (S.L. De Maeseneire), Wim.soetaert@ugent.be (W.K. Soetaert), Tom.delmulle@ugent.be (T. Delmulle).

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1. Introduction

The mounting pile of food waste (FW) is recognized as a pressing global challenge, with far-reaching environmental and economic consequences. In the European Union alone, each person generates 132 kg of FW on average per year (Eurostat, 2025). While households are the largest contributors, responsible for 54 % of the total, significant waste also stems from manufacturing (19 %), hospitality (11 %), retail (8 %), and agriculture (8 %). At present, the majority of FW is composted, gasified or land-filled. As a result, FW accounts for 8 % of total anthropogenic greenhouse gas emissions, making it comparable to passenger road transport emissions (IEA, 2022; Jaglo et al., 2021). Tackling this issue requires not only reducing food wastage, but also finding innovative solutions to repurpose the waste for sustainable use. Indeed, as a consequence of its abundance and nutrient rich composition, FW possesses a substantial untapped potential as a second-generation (2G) feedstock for value-added bioproducts, including platform chemicals, biopolymers and biosurfactants (Kaur et al., 2019; Ong et al., 2018).

These biosurfactants, including sophorolipids (SLs), are a promising alternative to traditional surfactants due to their mild nature, biodegradability, and sustainable microbial production processes, in comparison to conventional chemical surfactants. SLs are of particular interest due to the high volumetric productivities (up to 3.7 g/L/h) that have been reported with the yeast *Starmerella bombicola* when grown on hydrophilic (e.g. glucose) and hydrophobic (e.g. rapeseed oil) substrates (Gao et al., 2013). In addition to their remarkable productivity, wild-type SLs are distinguished by their structural variations. They are composed of (closed ring) lactonic, (open ring) acidic and minute amounts (<0.1 %) of (di-sophorose-headed) bola SLs with a varying degree of acetylation and aliphatic chain length (Price et al., 2012). This structural variation boasts a wide range of applications, including in the cosmetics (Hillion et al., 1998), personal care (Mager et al., 1987), cleaning (Furuta et al., 2003), pharmaceuticals (Gharaei-Fathabad, 2011) and bioremediation industries (Ducreux et al., 1997).

Yet, despite these advantages, industrial SL production still fully relies on first-generation (1G) feedstocks like glucose and plant oils. The use of these 1G resources raises concerns regarding sustainability, as 1G hydrophilic and hydrophobic substrates contribute respectively 37 % and 42 % of the total end point score in terms of damage to ecosystems and resource (Baccile et al., 2017). Thus, transitioning to 2G feedstocks such as FW presents a more sustainable alternative, a strategy that has already been explored for the production of various surfactants (e.g., rhamnolipids, surfactins, mannosylerythritol lipids, and sophorolipids) (Begum et al., 2023; Domínguez Rivera et al., 2019). Recent studies have successfully valorized FW for the production of wild-type SLs, achieving productivities of up to 2.4 g/L/h (Eras-Muñoz et al., 2024; Kaur et al., 2019; To et al., 2023; Wang et al., 2020; Wongsirichot et al., 2024). These findings underscore the promising potential of FW as an alternative feedstock for SL synthesis. However, the obvious valorization of FW is hindered by several factors, including its scattered availability, transportation costs and impact (environmental and spoilage), seasonal and regional variability and inhibitory compounds such as lactic acid (LA), acetic acid and ethanol (Miao et al., 2024; To et al., 2023). In addition, research on the synthesis of 2G biosurfactants on FW by *S. bombicola* has been limited to wild-type SLs. In order to expand the commercial potential of (2G) biosurfactants, it is essential to evaluate a wider range of compounds. In this regard, research on novel biosurfactants such as bola sophorosides (bola SSs) offers promising opportunities for further innovation and market expansion.

Bola SSs can, among others, be produced by using genetically engineered *S. bombicola* strains fed with fatty alcohols (Roelants et al., 2020). These novel bola SSs demonstrate improved chemical stability at higher pH levels (pH > 6.5), compared to wild-type bola SLs, due to the substitution of the ester bond with a more stable glycosidic bond. This improved structural stability significantly expands their industrial applicability, including in biomedicine, drug delivery and nanotechnology.

This study aimed to develop a sustainable and economically viable production process for 2G bola SSs using FW as a growth medium. To address the scattered nature and compositional variability of FW, centrally collected FW from Belgian supermarkets was evaluated. A detailed variability study assessed the streams variability in terms of physical characteristics, nutrients and inhibitory compounds. Furthermore, this study determined several significant correlations between FW variables, as well as their effect on *S. bombicola* growth and biosurfactant production. These insights will facilitate a more profound comprehension of the relationships between FW composition and bioprocess outcomes. Consequently, assisting in the industrial valorization of variable and complex FW streams into high-value bioproducts.

With regard to inhibitory compounds, LA was identified as the primary inhibitor in FW, as it repeatedly attained concentrations reported to be inhibitory to both growth and production (±40 g/L). Strategies discussed in literature to mitigate this inhibitory effect, such as FW washing (To et al., 2023), acid hydrolysis (Konishi et al., 2015), the addition of activated charcoal (Zhao et al., 2022), the use of ion-exchange resin (Zhou et al., 2023), liquid–liquid extraction (Roque et al., 2019) or bio-detoxification (Ali et al., 2021), which partially remove the inhibitors and restore cell growth and productivity, all have intrinsic disadvantages considering cost or nutrient removal (e.g., 62 ± 1.72 % free amino nitrogen removal by water washing). In some cases, even other inhibitory compounds are formed (Larsson et al., 1999).

In this study, a lean and comprehensive strategy is employed wherein the pH is maintained above the pKa of LA (~3.8), in an attempt to mitigate most of the inhibitory effect of LA. It was reasoned that at a pH below the pKa of LA, LA is predominantly present in its protonated form, which is more lipophilic. This form can more easily enter the yeast cytoplasm, thereby triggering a series of negative effects (e.g., intracellular acidification, ion disturbance and metabolic imbalance) that ultimately results in cell death (Peetermans et al., 2021). However, by adjusting the pH well above the pKa, the less toxic deprotonated LA form would be more abundant, thereby resolving the issue. As a result, we report the first documented production of bola SSs on FW, demonstrating the feasibility and potency of LA-rich FW as a sustainable substrate for biosurfactant production.

2. Materials and methods

2.1. Strains

A genetically modified *S. bombicola* ATCC22214, knocked out in both the cytochrome P450 monooxygenase (*cyp52M1*) and the fatty alcohol oxidase (*fao1*) gene (Δ*cyp52M1*Δ*fao1*), was used in all growth and production experiments. The strain was modified in-house according to the method described by Roelants et al. (2020) and stored at −80 °C in 2 ml cryovials containing a 35 % glycerol solution.

2.2. Defined culture media

The standard defined (SD) culture medium described by Lang et al. (2000) and an adapted version of the SD medium mimicking FW (mFW) were used in this work. The SD medium contained 132.00 g/L glucose monohydrate (Tereos-Syral), 4.00 g/L yeast extract (Gistex; DSM), 5.00 g/L trisodium citrate dihydrate (Citrique Belge), 1.50 g/L ammonium chloride (VWR chemicals), 1.00 g/L monopotassium phosphate (Buddenheim), 0.16 g/L dipotassium phosphate (Budenheim), 0.70 g/L magnesium sulfate heptahydrate (Sigma Aldrich), 0.50 g/L sodium chloride (Suprasel) and 0.27 g/L calcium chloride dihydrate. The mFW medium was modified by adjusting its glucose and nitrogen content as follows: Specifically, 44.00 g/L glucose monohydrate (Tereos-Syral), 13.93 g/L yeast extract (Gistex; DSM), and 0.42 g/L ammonium chloride (VWR chemicals) were utilized. The glucose was always autoclaved

separately from the yeast extract and the salts.

Prior to autoclaving (Priorclave Tactrol), the pH of the medium was adjusted from ~6.1 to 5.8 using a 2 M H_2SO_4 solution.

2.3. Food waste

The present study examined various batches of centrally collected FW from Belgian supermarkets. Due to its inherent variability and mixed composition, the FW contained a wide variety of materials, including dairy products, cereals, fruits, vegetables, fish, meats, and other food-derived materials. 52 collections (500 mL/batch) were done over a period of 19 months and analyzed as part of the FW variability experiment. Furthermore, a larger batch (~10 L) of FW was used as growth medium for the 2G fermentation after adequate pretreatment.

2.3.1. Food waste variability analysis

Prior to hydrolysis, the FW samples were analyzed in terms of pH, dry matter percentage (DM%), protein content, and ammonium (NH_4^+), glucose, LA, glycerol, acetic acid, propionic acid, ethanol and butyric acid concentration. Furthermore, the outside temperature at the collection site on the day of sampling of each batch was recorded.

To release all of the protein and the glucose from starch in each FW batch, full hydrolysis was carried out in duplicate after pasteurization (1 h at 85 °C). To this end, 1 % [v/v] glucoamylase (CAS: 9032–08–0) and 1 % [m/v] protease (CAS: 9040–76–0) were added to 10 g FW, which was then incubated in thermomixers (1000 rpm, 48 °C) for 17 h in a 15 mL falcon. Subsequently, an analysis of the glucose concentration following hydrolysis was conducted, which is henceforth referred to as the glucose release. All analysis were performed in duplicate.

2.3.2. Food waste hydrolysis

The same protease and glucoamylase as described above were utilized for the enzymatic hydrolysis of a 10-liter FW batch. The enzymes were loaded at 8.00 U/g, equivalent to 0.46 mL of glucoamylase and 0.80 mg of protease per liter of FW.

During the pretreatment, temperature and agitation were controlled at 48 °C and 500 rpm in a 20 L stainless steel Hastelloy® reactor (Büchi AG). The pH was not adjusted. After the enzymatic hydrolysis (4 h), the FW hydrolysate was harvested and centrifuged by a Multifuge X3R Refrigerated Centrifuge (Thermo Scientific) for 10 min at 4816g. The pellet was discarded and the supernatant was sieved at pore sizes ranging from 1000 to 250 μm, using a vibratory sieve shaker (AS 200 Basic, Retsch®). Next, the sieved supernatant was clarified in a EA102 filter press (ErtelAlsop) followed by a cardboard filtration unit with decreasing cut-off from 8, 2, to 0.5 μm (Mini Jet Wine Filter, Buon Vino). Finally, the clarified FW was filter sterilized (Masterfilter Capsule Filter MC-I-PES020-5-C-Hosebarb) with a 0.2 μm cut-off.

2.4. Growth and production experiments

2.4.1. Seed train

A seed train was set-up before every deep well plate (DWP), shake flask and bioreactor experiment by transferring 1 mL of culture from a cryovial to a 250 mL shake flask with 50 mL SD medium. After 28–30 h, microscopy for contamination and an optical density check at 600 nm (OD_{600}) were performed before this culture was transferred to a 500 mL seed shake flask with 100 mL standard growth medium in case of bioreactor experiments, to the main 500 mL shake flask in case of a shake flask experiment or to a DWP. All seed shake flasks were incubated at 30 °C for 28–30 h and shaken at 200 rpm with a 2.5 cm orbit.

2.4.2. Shake flask experiment

The shake flask experiment to assess lactic acid consumption by *S. bombicola* was conducted in 500 mL shake flasks with 100 mL SD medium, containing 40 g/L of glucose and spiked with 25 g/L of LA. After inoculation from the seed train at 5 %, cultures were grown at 30 °C, 200 rpm, 2.5 cm orbit for 164 h. The cultures were performed in duplicate and five 1.5 mL samples were taken for pH, OD_{600}, cell dry weight (CDW) and HPLC analysis.

2.4.3. Food waste validation experiment

24 hydrolyzed FW batches (in duplicate) were selected randomly from the 10 g batches used in the FW variability study for productivity validation in a 24-well DWP (Enzyscreen, CR1224a cover).

Firstly, most solids were removed by centrifugation (10 min; 12,191 g; 4 °C), the resulting supernatant (3.5–5 mL) was then subjected to flocculation. To this end, 0.15 μL polyethyleneimine (~M.N. 60,000, 50 wt% aq. solution, branched, Thermo Scientific Chemicals) was added per milliliter of supernatant, after a 30-minute period of gentle mixing, 100 μL of 30 % NaOH was added to initiate flocculation. The flocculant was then removed by centrifugation (10 min; 12,191 g; 4 °C) and the clarified supernatant was filter sterilized (Minisart sterile NML syringe filter, Sartorius) after pH adjustment to 5.80 ± 0.15 with 50 to 150 μL of 30 % NaOH, i.e. the volume needed per batch to achieve the target pH.

Following the autoclavation of the DWP with 30 g/L oleyl alcohol in each well, 3 mL of the appropriate sterile, hydrolyzed FW was added. After inoculation (4 %), the cultures were incubated at 30 °C, and shaken at 225 rpm and an orbit of 5.1 cm (Van Renterghem et al., 2019). After 3 days of cultivation, 1.3 mL of broth was used for CDW and bola SS analysis.

2.4.4. Bioreactor experiments

A two liter bioreactor (DASGIP® Parallel Bioreactor System, Eppendorf SE, Eppendorf, Hamburg), with 1 L working volume, was inoculated from the seed train at 5 %. Cultures were grown at 30 °C, pH was adjusted to 5.8 before autoclavation and controlled at 4 by automatic base control with NaOH (2 M). Dissolved oxygen was controlled at 30 % by stirrer and aeration cascade (400–1200 rpm; 0.5–1 vvm). If a SD or mFW medium was used, the medium salts were autoclaved separately from the glucose (121 °C, 60 min). For FW fermentations, the vessel was filled with 20 mL dH_2O before autoclaving and the filter-sterilized FW hydrolysate was added after autoclavation. Media sterility was verified by plating 100 mL onto brilliance urinary tract infections clarity (Thermo Scientific) and plate count agar plates (VWR chemicals) (24 h, 30 °C).

Silicone peroxide tubing (VWR) was used to connect the sterile addition flasks and Norprene chemical tubing (Saint-Gobin Performance Plastics) was used to connect the hydrophobic substrate flasks. To allow for sterile gas exchange with the environment, PTFE filters (13 mm, 0.2 μm cut-off, VWR) were fitted to the addition bottles and an air filter was fitted to the incoming air (Midisart 2000, 0.2 μm PTFE). A combination of wheat processing byproducts was used as 2G glucose feed (324.4 g/L glucose) to the FW medium, whereas the SD medium received a 1G glucose feed (600 g/L glucose) to serve as a conventional benchmark and maintain glucose levels above 0 g/L, thereby averting starvation. Oleyl alcohol (Caldic) was supplied at 0.5 g/L/h as hydrophobic substrate for bola SS production.

A 10 mL sample was taken from the bioreactor approximately every 8 h and analyzed to determine pH, CDW, OD_{600}, bola SS titers, and glucose consumption.

2.5. Analytical methods

2.5.1. Dry matter analysis

The DM% of FW was determined with a thermogravimetric moisture analyzer (MA 50/1.X2.IC.A.WH, Radwag) at 105 °C.

2.5.2. BCA assay

The protein content of FW was analyzed with a Pierce BCA protein assay kit (Thermo Scientific).

2.5.3. YSI analysis

Ammonium levels in FW were measured using a YSI 2900 Biochemistry analyzer (YSI Inc. Life Sciences) equipped with a YSI 2974 ISE ammonium electrode.

2.5.4. Cell dry weight measurements

The CDW of bioreactor experiments was determined by first subjecting a 5 mL sample to centrifugation at 20,598 g for 5 min. The pellet was then resuspended in 5 mL of physiological solution [0.9 % (w/v) NaCl] and subjected to a second centrifugation of 5 min at 20,598 g. Thereafter, the resulting pellet was resuspended in 5 ml of milli-Q water. The DM% was then determined at 105 °C using an infrared moisture analyzer (Sartorius). For shake flask and DWP experiments, given the limitation on sample volume, 1 mL of sample was taken and the resultant pellets were washed with physiological solution and dried at 60 °C until they had a constant weight.

2.5.5. Optical density measurements

The optical density (OD) at 600 nm was employed as a rapid metric for quantifying the biomass present in the fermentation broth. Measurements were conducted using a Cary 60 UV–VIS G6860A spectrophotometer (Agilent Technologies).

2.5.6. pH measurements

Online pH monitoring was conducted in real-time during fermenter experiments using pH Sensor InPro 3253 325/PT1000 (Mettler Toledo), while offline pH verification of fermentation broth and FW samples was performed with mobile pH meters (Hanna HI 99121/99141/991003).

2.5.7. HPLC-RID quantification

Hydrophilic compounds such as carbohydrates, organic acids and glycerol were analyzed on a Metacarb 67H column (300 x 6.5 mm, 35 °C, Agilent Technologies) for 22.5 min with an aqueous solution of 2.5 mM sulfuric acid at a flow rate of 0.8 mL/min (1260 Infinity, Agilent Technologies) with RID detection (G1362A, Agilent Technologies). All samples were filtered through 0.2 μm polyethersulfone (PES) filters (25 mm, VWR) after centrifugation (5 min, 14,100 g). Samples were typically injected at 10 μL; if further dilution was required, lower injection volumes (up to 0.2 μL) were used. Chromatograms were analyzed in OpenLAB CDS Chemstation (edition C.01.05).

2.5.8. HPLC-ELSD quantification

For bola SS quantification, samples were diluted 4 times in 70 % EtOH aq. (VWR), vortexed for 5 min (Vortex-Genie 2, Scientific Instruments) and centrifuged (5 min, 14,100 g). After filtration (0.2 μm PES 25 mm, VWR), the samples were analyzed on a C18 zorbax Eclipse Plus column (4.6 mm x 150 mm, 40 °C, Agilent Technologies) with an ELSD detector (1260 Infinity ELSD, Agilent Technologies) at an evaporation temperature of 90 °C, a nebulization temperature of 70 °C and a gas flow rate of 1.60 standard liters per minute. 100 % acetonitrile (VWR HPLC grade) and milli-Q water with 0.05 % (v/v) acetic acid (Merck) were used as the mobile phase in gradient elution at a constant flow rate of 1 mL/min. The gradient started at 80 % milli-Q, decreased to 5 % over 22.2 min, held constant for 2.3 min, increased to 100 % in 3 min, held constant for 1 min, and returned to 80 % milli-Q in 1.5 min. Depending on the concentration, 10 to 0.2 μL of sample was injected. Chromatograms were analyzed in OpenLAB CDS Chemstation (release C.01.05).

To quantify the C18:1 bola SSs, a dilution series of the purified product was prepared from a previously purified batch. The structure of the pure C18:1 bola SS in-house standard was verified using NMR analysis, as described by Roelants et al. (2020).

2.5.9. Statistical analyses

Descriptive statistics, including the mean, median, standard deviation, range, and coefficient of variation (CV), were calculated. To account for confounding variables, partial correlations between all possible variable pairs were computed using the ppcor package in R (Kim, 2015). Multiple testing corrections were applied using the Holm-Bonferroni method for each variable. Linear regression models were developed separately for growth and production, with each initial model including only first-order terms. Model optimization was then carried out using two approaches: (1) stepwise backward elimination based on statistical significance (sequentially removing the least significant parameter with $p \geq 0.05$), and (2) minimization of the Akaike Information Criterion (AIC), allowing retention of some nonsignificant terms if justified by improved model fit. To ensure selection of the most generalizable and robust models, both optimization approaches were validated and compared using k-fold cross-validation ($k = 6$).

Statistical significance was set at $p < 0.05$, experiments were performed in duplicate and all statistical analyses were performed in RStudio (Version 2024.12.1, Build 563).

Table 1
Food waste composition and variability (n = 52, in duplicate). (SD: Standard deviation; CV: coefficient of variation; DM: dry matter).

Variable	Mean	SD	CV (%)	Min	Max
pH	3.95	0.19	4.71	3.50 ± 0.06	4.58 ± 0.00
DM%	19.71	2.85	14.46	15.77 ± 0.35	31.09 ± 1.18
Protein (g/L)	22.43	16.64	74.21	8.69 ± 0.92	82.59 ± 2.76
Ammonium (g/L)	1.23	0.31	24.89	0.40 ± 0.14	1.83 ± 0.91
Initial glucose (g/L)	2.58	4.27	165.38	0.03 ± 0.00	25.56 ± 0.87
Lactic acid (g/L)	35.04	7.86	22.44	12.30 ± 0.20	52.34 ± 1.57
Glycerol (g/L)	0.64	1.94	301.21	0.00 ± 0.00	13.49 ± 0.18
Acetic acid (g/L)	6.49	1.94	29.87	2.83 ± 0.01	10.99 ± 0.35
Propionic acid (g/L)	0.54	0.32	59.83	0.16 ± 0.01	1.58 ± 0.04
Ethanol (g/L)	3.77	1.48	39.26	0.89 ± 0.03	9.24 ± 0.13
Butyric acid (g/L)	0.25	0.14	57.76	0.09 ± 0.01	0.83 ± 0.01
Glucose release (g/L)	28.55	8.79	30.77	13.41 ± 0.57	57.19 ± 1.48

3. Results & discussion

3.1. Food waste variability study

3.1.1. Food waste compositional variability

As mentioned previously, this study focused on centrally collected food waste (FW) from Belgian supermarkets. The mean, median, standard deviation, range, and coefficients of variation (CV) of all analyzed parameters are provided in Table 1 and illustrated in Supplementary Material.

With respect to physicochemical properties, the pH and DM% exhibited low CVs (4.71 and 14.46 %, respectively), confining them to a range of 3.5–4.58 and 15.77–31.09 %, and a mean of 3.95 ± 0.19 and 19.71 ± 2.85 %, respectively. Conversely, protein, initial glucose, and glycerol demonstrated the highest CVs (74.21, 165.38, and 301.21 %, respectively), with glucose and glycerol exhibiting high variability mainly due to their low concentrations, where small deviations result in disproportionately high CVs. In contrast, the protein range exhibited a greater extent (8.69 g/L to 82.95 g/L), although it remained considerably lower than the findings reported by de Abreu et al. (2023), who observed protein concentrations ranging from 22.0 to 422.5 g/L across various types of FW, and from 55.0 to 255.1 g/L, with a CV of 33.2 % for supermarket FW, more specifically.

The high CV for protein concentrations observed in this study suggests significant inconsistency in a crucial factor for stable microbial growth and product formation. However, the relatively low concentrations of protein are not inherently problematic. When converted to yeast extract equivalents, even the lowest measured protein concentration in

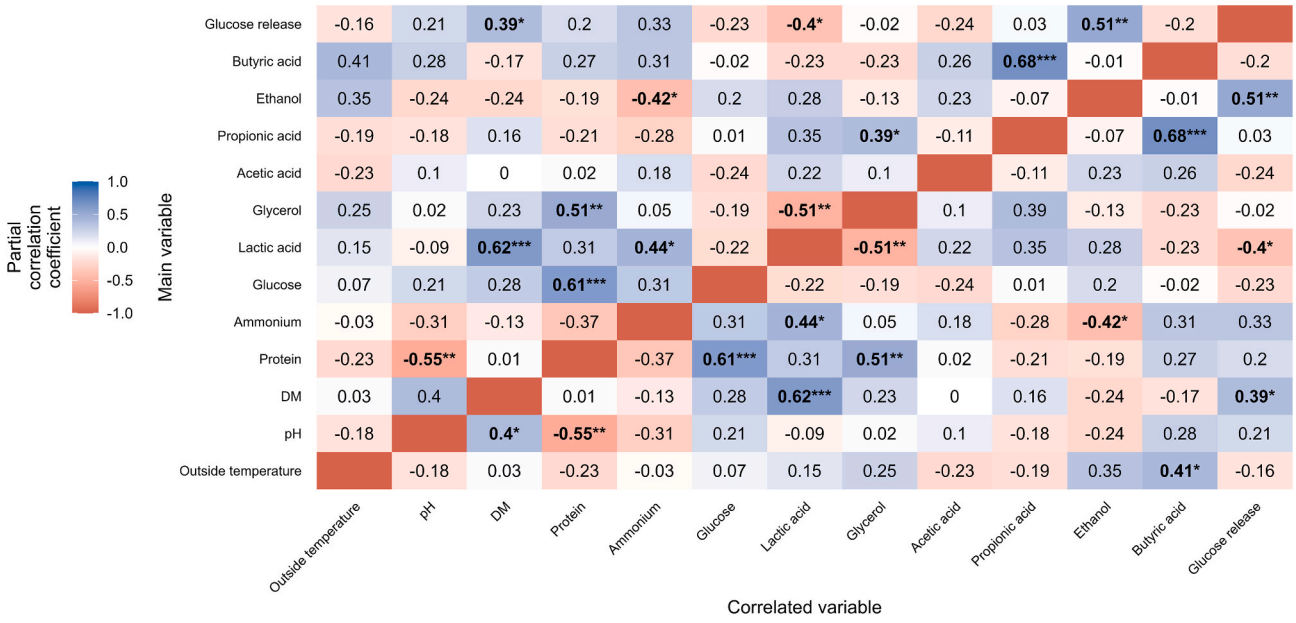

Fig. 1. Partial correlation heatmap with Holm-Bonferroni corrected significance. With main parameters on the y-axis and the corresponding correlated variables on the x-axis. All significant correlations are indicated in bold (*: 0.05 > p > 0.01; **: 0.01 > p > 0.001; ***: 0.001 > p).

FW corresponds to more than three times the typical yeast extract concentration used in standard media. This finding indicates that, despite its variability, the protein levels in FW are sufficient to support microbial growth.

The glucose release from FW ranges from 13.41 g/L to 57.19 g/L, with a CV of 30.77 %. Given that glucose release was achieved through exhaustive starch hydrolysis using an excess of amylase, this approach enabled a comprehensive assessment of the total hydrolysable starch content in each FW batch, allowing for comparison with values reported in previous studies. A study by de Abreu et al. (2023) revealed a substantial range of starch contents, from 0.0 to 651.0 g/kg, and a range of 0.0 to 252.0 g/kg starch with a CV of 106.7 % was observed for supermarket FW. Consequently, the glucose release observed in this study (mean of 28.55 ± 8.79 g/L) reflects the relatively low starch content and variability of the FW compared to what was observed for supermarket FW by de Abreu et al. (2023).

These relatively low glucose concentrations that result from starch hydrolysis present a significant challenge to the efficiency of fermentation, as *S. bombicola* exhibits a high glucose consumption rate, typically requiring 100–150 g/L glucose in the culture medium (Casas and García-Ochoa, 1999; Van Renterghem et al., 2018). To address this, the FW must be supplemented with additional hydrophilic substrates. Ideally, these would consist of 2G streams, which are abundant in readily accessible carbohydrates, such as molasses, bakery waste, and starch processing side streams.

The quantification of other fermentable sugars (e.g., fructose, sucrose, and mannose) was primarily impeded by their low abundance in the FW samples, likely resulting from microbial spoilage occurring prior to analysis, and minimizing their contribution to the fermentation. Quantifying these low sugar concentrations was further complicated by peak overlapping with other complex compounds present in the FW matrix. Finally, according to Rosa and Lachance (1998), *S. bombicola* is incapable of metabolizing several other sugars (e.g., rhamnose, xylose, arabinose, lactose, maltose, and cellobiose), which were therefore excluded from the analysis.

With respect to the potential inhibitors, the highest concentrations were observed for LA, ranging from 12.30 ± 0.20 g/L to 52.34 ± 1.57 g/L, with an average of 35.04 ± 7.86 g/L. The prevalence of LA and lactic acid bacteria (LAB) in foodstuffs such as yogurt and fermented vegetables within the FW is likely the underlying cause of these elevated levels. Furthermore, the introduction of carbohydrate- and nutrient-rich streams to these LAB by mixing different FW streams could lead to the sustained production of LA within the FW (Stiles and Holzapfel, 1997; To et al., 2023).

Other potential inhibitors, such as ethanol and organic acids, were rarely detected at inhibitory concentrations. Acetic acid, in particular, was identified as a notable exception, achieving concentrations greater than the inhibitory concentration (10 g/L) in two out of 52 batches, with a maximum recorded concentration of 10.99 ± 0.35 g/L (To et al. 2023). However, the average acetic acid concentration was found to be 6.49 ± 1.94 g/L, exhibiting low to medium variability (CV = 29.87 %), which was below the inhibitory concentration on *S. bombicola* growth and production. According to the same study, the mean LA concentration of 35.04 ± 7.86 g/L is predicted to be highly inhibitory to *S. bombicola* growth and production if no countermeasures are implemented. Consequently, LA can be regarded as the primary inhibitor, and addressing its inhibitory effect through targeted interventions will be imperative in optimizing fermentation performance.

Overall, the compositional variability observed in FW (CV-range of 4.71–301.21 %) is higher than that of conventional 2G feedstocks such as bagasse (typical CV-range of 2.38–42.96 %) (Rocha et al., 2015). However, certain variables (i.e. pH, DM%, ammonium, LA, acetic acid and glucose release) exhibited a significantly lower degree of variability compared to other complex supermarket FW streams, with a CV range of 33.2–106.7 %. This reduced variability can be attributed to the centralized collection of FW at an industrial scale, which helps limit fluctuations in composition. Therefore, centralized collection of complex waste streams at an industrial scale could be a promising strategy to mitigate the adverse effects of feedstock variability on bioprocesses.

3.1.2. *Food waste compositional correlation analysis*

Following the characterization of the compositional variation between the collected FW batches, the correlations between the FW variables were determined. A total of 25 significant correlations for 12 separate main variables (shown on the y-axis) were found (see Fig. 1).

The findings indicate a positive correlation between butyric acid levels and propionic acid concentrations. This observation can be attributed to the proliferation of spoilage microorganisms, such as *Clostridium tyrobutyricum*, which are capable of producing organic acids, including propionic acid and butyric acid (Brändle et al., 2016).

Notably, the correlation between external temperature and butyric acid levels was found to be non-reciprocal. The positive correlation was only significant when the outside temperature was considered as the primary parameter. This could be attributed to the increased growth of butyric acid producing microorganisms at elevated temperatures. Concurrently, propionic acid demonstrated a positive correlation not only with butyric acid, but also with glycerol levels. This phenomenon could be attributed to the utilization of glycerol as a substrate for propionic acid production, as well as the acidogenic microorganisms' demonstrated preference for glucose and protein (Clomburg and Gonzalez, 2013; Yin et al., 2016). Consequently, due to its comparatively diminished degradation rate, glycerol appears to accumulate as lipids undergo hydrolysis.

In turn, LA was positively correlated with both the ammonium concentration and DM%, whereas it was negatively correlated with the glycerol concentration and glucose release. The positive correlation between LA and ammonium is likely attributable to the fact that both LA and ammonium are products of microbial degradation processes that occur during FW decomposition (Ramsay and Pullammanappallil, 2001; Song et al., 2022). Conversely, the positive correlation with DM%, and the negative correlation with glycerol and glucose release, could be explained by the fact that a higher DM% indicates a greater presence of solid substrates (including glucose as starch and glycerol) that serve as substrates for LA production by LAB.

The correlation between the LA concentration and the DM% was found to be reciprocal as the DM% is positively correlated with LA and glucose release. The positive correlation to glucose release is evident, as starch constitutes a significant component of the dry matter, prior to its hydrolysis to glucose by amylase enzymes.

On the other hand, the observation that the pH level exhibited a positive correlation with the DM% was found to be asymmetrical, as it was only significant when the pH was the main parameter. This effect suggests a potential mechanism in which higher moisture content (i.e., lower DM%) fosters microbial growth and organic acid production, leading to a decrease in pH (Xu et al., 2022). Conversely, a higher DM% may signify a substrate that is less accessible to microorganisms, thereby leading to a higher pH. Furthermore, a negative correlation between pH and protein concentrations was identified. This might be attributed to microbial activity, which, as the fermentation progresses, secrete proteolytic enzymes that catalyze the breakdown of intact, insoluble proteins into soluble peptides and amino acids. Simultaneously, the synthesis of organic acids by these microorganisms contributes to a decrease in pH (Mayta-Apaza et al., 2022).

Regarding the protein concentration, a positive correlations with initial glucose and glycerol concentrations suggest that FW with more readily available protein is also richer in initial glucose and glycerol. Furthermore, the previously documented negative correlation between pH and protein concentrations was identified as bidirectional.

The correlations with initial glucose and glycerol concentrations were also found to be bidirectional, as they both correlate positively with the protein concentration. In addition, a bidirectional negative correlation was found between glycerol and LA. Notably, the positive correlation between propionic acid and glycerol was found to be non-symmetrical.

Finally, the glucose release (a good measure of the starch content in the FW) exhibited a negative correlation with the LA concentration and a positive correlation with both the DM% and the ethanol concentration. The negative correlation between glucose release and LA is intuitive, given that LAB can convert starch to LA (Gänzle and Follador, 2012). The positive correlation between glucose release and ethanol concentrations, which is bidirectional, is less intuitive. A potential explanation for this phenomenon is a change in microbial activity during food spoilage. Given the complexity of FW, the initial microbial load consists of both heterofermentative and homofermentative microorganisms. As fermentation progresses and acids accumulate, LAB (often homofermentative) tend to predominate due to their acid tolerance, thereby shifting the balance (Ge et al., 2025; Zúñiga et al., 1993). This phenomenon might also provide a rationale for the bidirectional negative correlation between ethanol concentrations and ammonium concentrations, as elevated ammonium levels reflect more extensive microbial breakdown and a consequent lower pH, which might be unfavorable to ethanol fermentation (Ramsay and Pullammanappallil, 2001). This hypothesis is further substantiated by the positive correlation between ammonium concentrations and LA concentrations.

Table 2
Overview of growth in cell dry weight (CDW) and production in bola sophorosides (SS) on a selection of food waste batches after 72 h in a 24-deep well plate (n = 24, in duplicate).

Variable	Mean	SD	CV (%)	Min	Max
CDW (g/g)	0.0133	0.0031	23.4458	0.0055	0.0175
Bola SS (g/L)	0.40	0.21	53.02	0.12	0.95

3.1.3. *Modelling S. bombicola growth and production on food waste*

As can be seen in Table 2, all FW samples allowed growth and bola SS production by *S. bombicola* with a range of 0.0055–0.0175 g/g CDW and 0.12–0.95 g/L bola SSs, respectively. On average, 0.0133 ± 0.0031 g/g CDW (CV = 23.4458 %) and 0.40 ± 0.21 g/L bola SS (CV = 53.02 %) were produced. The wide ranges and considerable variability observed in these data can be attributed to the inherent compositional variability of the FW, as discussed in section 3.1.1.

Ensuring consistent growth and optimal bola SS production during fermentation is imperative for bioprocess optimization and ensuring efficient downstream processes. Nevertheless, substantial variations in *S. bombicola* growth and bola SS concentrations resulting from FW fermentation may pose challenges when processes are scaled up to an industrial level. This study proposes a strategy to address this challenge by enhancing our understanding of how FW composition affects fermentation characteristics and developing a method to more accurately predict the fermentation outcome based on the composition of incoming FW.

To this end, first-order linear regression models were constructed for both growth and production. An overview of the linear regression model performance statistics and k-fold validation metrics on growth and production can be found in Table 3. The selection of the AIC refinement model for bola SS production and the p-value approach for *S. bombicola* growth modeling was based on these metrics. The primary focus was on optimizing the k-fold validation metrics, as these metrics are effective in limiting overfitting and indicating enhanced model performance on new data.

The resulting model parameters can be found in Table 4. The model shows that *S. bombicola* growth is negatively correlated with ammonium and protein concentrations, but positively correlated with glucose release. Conversely, bola SS production is positively correlated with pH, LA, glycerol and propionic acid, whereas it is negatively correlated with acetic acid and butyric acid.

Given that all FW batches permitted growth and production, the results of this study demonstrated, for the first time, that, despite its inherent compositional variability, hydrolyzed FW can consistently support microbial growth and biosurfactant production. Moreover, the constructed models exhibited a reasonable capacity to predict *S. bombicola* growth in terms of CDW and production in terms of bola SS concentrations, with R^2 values of 0.5168 and 0.8055, respectively (see Table 4). These models have the potential to assist in the development of new strategies that might allow for increased consistency and predictability of FW fermentations. A promising strategy to mitigate the variability in composition of FW and enhance the consistency of fermentation could be implemented through mixing different FW batches. Alternatively, the FW could be supplemented with specific components to address any deficiencies. This approach will further reduce the inherent variability in FW composition, thereby leading to improved consistency in fermentation. The integration of these

Table 3
An overview of the linear regression model performance statistics (RSE: residual standard error; MRS: mean residual sum; AIC: Akaike Information Criterion) and k-fold (k = 6) validation statistics (RMSE: root mean squared error; R^2: R-squared or coefficient of determination; MAE: mean absolute error) for growth in cell dry weight (CDW) and production in bola sophorosides (SSs).

Variable	Model type	Linear model statistics		K-fold validation statistics			
		RSE	MRS	AIC	RMSE	R^2	MAE
Production	P-value optimization model	0.1164	0.7681	−28.04	0.1383	0.5706	0.1182
	AIC-refined model	0.1097	0.8055	−30.24	0.1223	0.6840	0.1068
Growth	P-value optimization model	0.002317	0.5168	−217.50	0.002468	0.6220	0.0020
	AIC-refined model	0.002278	0.5562	−217.55	0.002563	0.6031	0.0021

Table 4
Optimized linear regression models for the prediction of production in bola sophoroside (SS) and growth in cell dry weight (CDW) on food waste.

Variable	Model parameters	Regression coefficient	P-value	R^2
Bola SS (g/L)	Intercept	−3.0406	0.0025	0.8055
	pH	0.8540	0.0009	
	Lactic acid	0.0140	0.0088	
	Glycerol	0.0238	0.0883	
	Acetic acid	−0.0622	0.0047	
	Propionic acid	0.2368	0.0538	
	Butyric acid	−0.6177	0.0041	
CDW (g/g)	Intercept	0.0193	0.0001	0.5168
	Protein	−0.0001	0.0119	
	Ammonium	−0.0071	0.0093	
	Glucose release	−0.0002	0.0013	

modeling strategies could establish more predictable and stable bioprocesses, which in turn could facilitate the scale-up of FW fermentation to many high-value-added bioproducts.

3.2. Impact and mitigation of lactic acid inhibition

While it has been demonstrated that lactic acid (LA) is the primary inhibitor in FW of growth and production of SLs by *S. bombicola*, and preliminary findings regarding the underlying mechanisms have been reported, the reason for the decreased SL production remains uncertain (To et al., 2023).

Consequently, it is impossible to discern whether the reduced SL production is the result of increased lactate concentrations at pH 5.6 or a gradual decrease in pH. Based on the observation by Qin et al. (2015) that high concentrations of lactate can downregulate the expression of genes involved in glycolysis and gluconeogenesis, To et al. (2023) hypothesize that increased lactate concentrations at pH 5.6 may precipitate a sequence of metabolic interactions, culminating in diminished uridine diphosphate glucose synthesis and, consequently, constrained SL production. Conversely, a gradual decrease in pH would over time result in an increased prevalence of the more toxic protonated form of LA and an additional inhibitory effect would occur due to the pH eventually becoming too low for *S. bombicola*. Given that this pH decline occurs

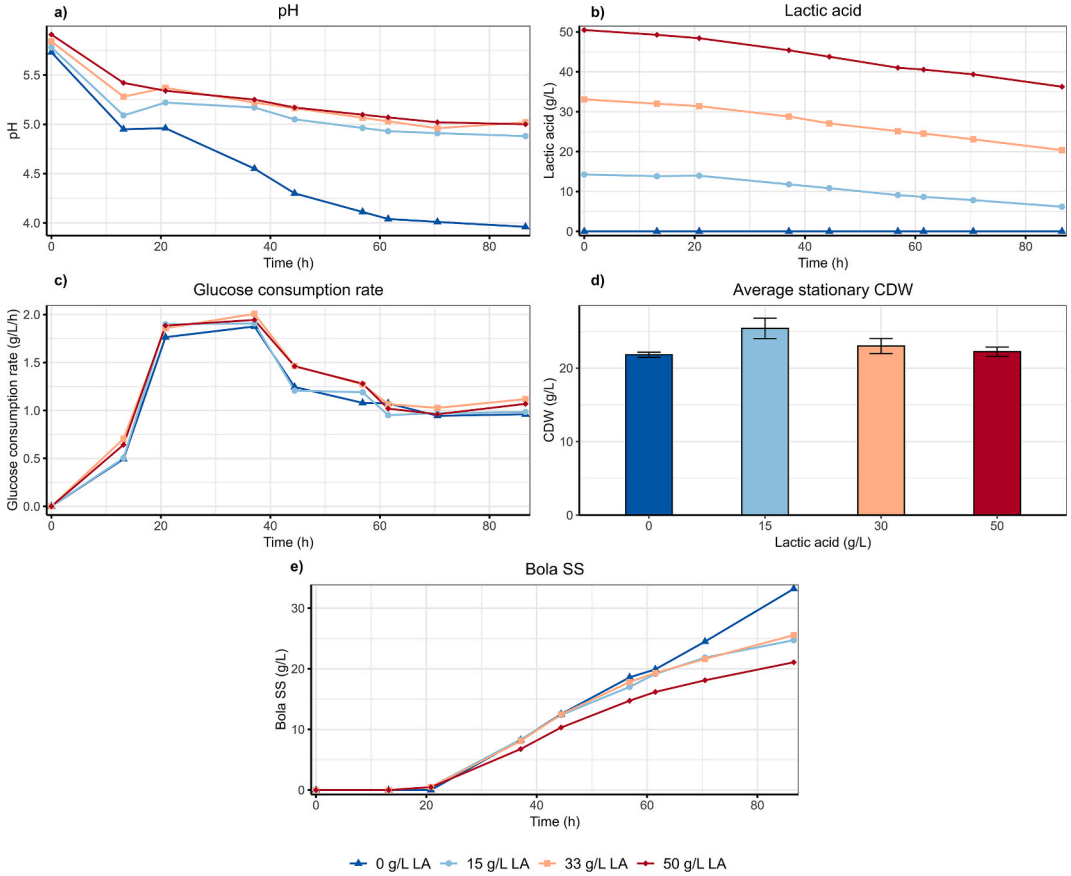

Fig. 2. Performance of *S. bombicola* on a medium mimicking food waste spiked with 0, 15, 33 and 50 g/L lactic acid (LA). (a) pH (b) lactic acid (c) glucose (d) average stationary (40–90 h) cell dry weight (CDW) (e) Bola sophoroside (SS) production.

over time, its impact would be amplified during the production phase, while the growth phase would experience a comparatively weaker effect. This observation aligns with the authors' findings of an insignificant inhibitory effect on growth as opposed to production. To further study the interaction between LA and pH and limit the inhibitory effect of LA, in our experiments, the pH was adjusted to 5.8 before inoculation and controlled above pKa of LA (3.8) throughout the fermentation.

In a first experiment, the strategy was evaluated in a defined medium mimicking the nitrogen and glucose content of a FW medium (mFW). As demonstrated in previous studies, the inhibitory effect of high LA concentrations on SL production is less pronounced in media with high yeast extract concentrations (To et al., 2023). Consequently, to emphasize any potential inhibitory effects of LA, the mFW used in this experiment mimicked the minimal concentrations of ammonium (0.40 g/L) and protein (8.69 g/L) previously observed in the FW batches. In accordance, 13.93 g/L yeast extract, containing predominantly protein (62.38 %) and a small amount of ammonium, and an additional 0.42 g/L NH_4Cl (i.e. 0.14 g/L ammonium) were incorporated into the mFW medium. This medium was spiked with 15, 33, and 50 g/L LA, to approximate the minimum (12.30 g/L), average (35.04 g/L), and maximum (52.34 g/L) concentrations of LA measured in the FW batches (see Table 1). A control bioreactor with mFW medium devoid of LA was run along with these spiked media. All conditions were run in parallel and fed with 600 g/L of glucose as hydrophilic carbon source and 0.5 g/L/h of oleyl alcohol which functioned both as hydrophobic carbon source and as a precautionary antifoaming agent.

As illustrated in Fig. 2a), in contrast to the mFW medium without LA, the pH is not controlled at pH 4 in any of the conditions where LA is supplied. Instead, it stabilizes around pH 5. The cause of this is yet to be elucidated, as the buffering components used in the mFW medium (citrate, K_2HPO_4/KH_2PO_4, and LA) do not exhibit a specific buffering capacity at pH 5. Notwithstanding this, according to the Henderson-Hasselbalch equation a significantly lower amount of protonated LA (2.97 g/L vs 26.65 g/L) is present in 50 g/L LA at pH 5 compared to 40 g/L LA at pH 3.5, as used by To et al. (2023), so the intended goal of converting the toxic protonated LA to lactate by pH control is still in effect (Po and Senozan, 2001).

The average CDW during the stationary phase (40–90 h) at 50 g/L LA (21.44 ± 2.02 g/L) was only 9.42 % lower (not significantly; $p = 0.16$) than at 0 g/L LA (23.67 ± 2.91 g/L). Furthermore, average stationary CDWs of 24.40 ± 2.11 g/L and 23.00 ± 1.03 g/L were observed for 15 and 33 g/L LA, respectively. Thus, no significant inhibitory effect of LA was found on *S. bombicola* growth at pH 5. Production of bola SSs was observed across all concentrations of LA. A notable decline in product titer and volumetric productivity is evident as LA concentrations increase. Specifically, the titers went from 33.19 g/L to 24.72 g/L, 25.55 g/L, and 21.07 g/L for 0, 15, 33 and 50 g/L LA, respectively. Consequently, the observed decline in volumetric productivity compared to 0 g/L LA was 23.68 % for 15 g/L LA, 21.05 % for 30 g/L LA, and 36.84 % for 50 g/L LA. The glucose consumption rate of all scenarios is very similar, reaching approximately 1.8–2 g/L/h after 17 h and dropping steadily to approximately 1 g/L/h from 29 h to 66 h. The average overall glucose consumption rates (0 g/L LA = 1.18, 15 g/L LA = 1.21, 33 g/L LA = 1.33, 50 g/L LA = 1.29 g/L/h) do not indicate an inhibitory effect on glucose consumption.

In summary, *S. bombicola* exhibited no significant growth inhibition; however, bola SS production was reduced by up to 36.84 % for 50 g/L LA. This inhibition is notably less pronounced in comparison to the findings reported in previous studies, where washed FW without LA was compared to washed FW spiked with 40 g/L LA at a constant pH of 3.5, resulting in a more substantial decrease of 24.84 % in CDW and 45.25 % in SL production (To et al., 2023). The hypothesis that this inhibition was exclusively caused by the fact that high concentrations of lactate downregulate the synthesis of uridine diphosphate glucose, eventually leading to a reduced SL titer is no longer valid (Qin et al., 2015; To et al., 2023). This is because while, according to the Henderson-Hasselbalch equation, a substantial increase in lactate levels (47.03 vs. 13.35 g/L) is observed at 50 g/L LA at pH 5 in comparison to 40 g/L LA at pH 3.5, the inhibitory effect on growth is eliminated and the effect on SL production is reduced by 18.67 % (Po and Senozan, 2001). Furthermore, it must be emphasized that bola SSs are generated in this process, which necessitate a twofold increase in UDP-glucose compared to wild-type SLs. Consequently, the inhibitory effect of lactate on SL production is lower than initially presumed. These findings underscore the importance of controlling pH above the pKa of LA to mitigate its inhibitory effect on growth and production by limiting the formation of the more toxic protonated LA. To further investigate this, additional experiments are warranted. A particularly relevant approach is that employed by Qin et al. (2015), who used comparative transcriptomics based on high-throughput RNA sequencing to analyze gene expression changes in *Bacillus coagulans* under non-stress, sodium lactate stress, and calcium lactate stress conditions. Applying a similar strategy to *S. bombicola* could yield valuable insights into how lactate stress influences SL synthesis.

Surprisingly, the LA concentrations decreased over the course of fermentation, from 15 g/L to 6.19 g/L, 33 g/L to 20.37 g/L, and 50 g/L to 36.26 g/L (see Fig. 2b). However, the observed rate of LA decrease could not be attributed conclusively to medium dilution by feeding glucose and oleyl alcohol. This is because in a typical bioreactor configuration, losses such as evaporation and sampling are difficult to account for.

Consequently, despite the absence of substantiating evidence supporting LA consumption by *S. bombicola*, the possibility cannot be discounted (Rosa and Lachance, 1998). Indeed, it is known that other oleaginous yeasts, such as *Yarrowia lipolytica* can exhibit LA consumption rates of up to 0.125 g/L/h (Mansour et al., 2008). Here, the genes *jen1*, *cyb2-1*, and *cyb2-2*, associated with lactate transport and lactate dehydrogenases, respectively, have been identified as crucial in the catabolism of lactate to pyruvate. In the context of this research, a putative D-lactate dehydrogenase has been identified in *S. bombicola* through a BLAST analysis (GenBank accession number: PV748046). Furthermore, according to Claus et al. (2022), *S. bombicola* is predicted to possess multiple monocarboxylate transporters, which may facilitate LA transport. Consequently, a batch shake flask experiment was performed to thoroughly evaluate LA consumption by *S. bombicola*.

In this experiment, the SD culture medium was utilized, containing 40 g/L of glucose and spiked with 25 g/L of LA. Given that no fed-batch feed was supplied and evaporation or sampling losses were measured by periodically weighing the shake flasks, it can be concluded that the depletion of LA in this instance could solely be attributed to the consumption of LA by *S. bombicola*. Yet, no significant drop in LA was observed (see Supplementary Material), even when glucose was depleted during the last 72 h. Consequently, *S. bombicola* is likely incapable of metabolizing LA, and the drop in LA during the bioreactor experiment can be fully attributed to bioreactor dilution.

The results of this study demonstrate that pH control is a crucial factor in limiting the inhibitory effects of LA on *S. bombicola*. Specifically, the growth inhibition was rendered insignificant and the inhibitory effect on SL production was reduced by 18.67 % at 50 g/L LA compared to the previously reported conditions at 40 g/L LA. It is concluded that the inhibitory impact of LA can be significantly mitigated up to a concentration of 50 g/L when pH is maintained above the pKa of LA. This allows for the utilization of LA-rich FW without the necessity for extensive pretreatment steps, thereby simplifying the FW pretreatment process and preserving valuable carbon and nitrogen sources.

Although FW rarely contains LA concentrations above 50 g/L (only 3.85 % of batches based on the results shown in Table 1), it is advisable to aim for LA levels below 33 g/L for bioprocessing purposes in order to maximize process efficiency. Higher concentrations are still tolerated, but they exhibit more pronounced inhibitory effects. These findings provided a foundation for a subsequent experiment, wherein the pH control strategy was validated on FW.

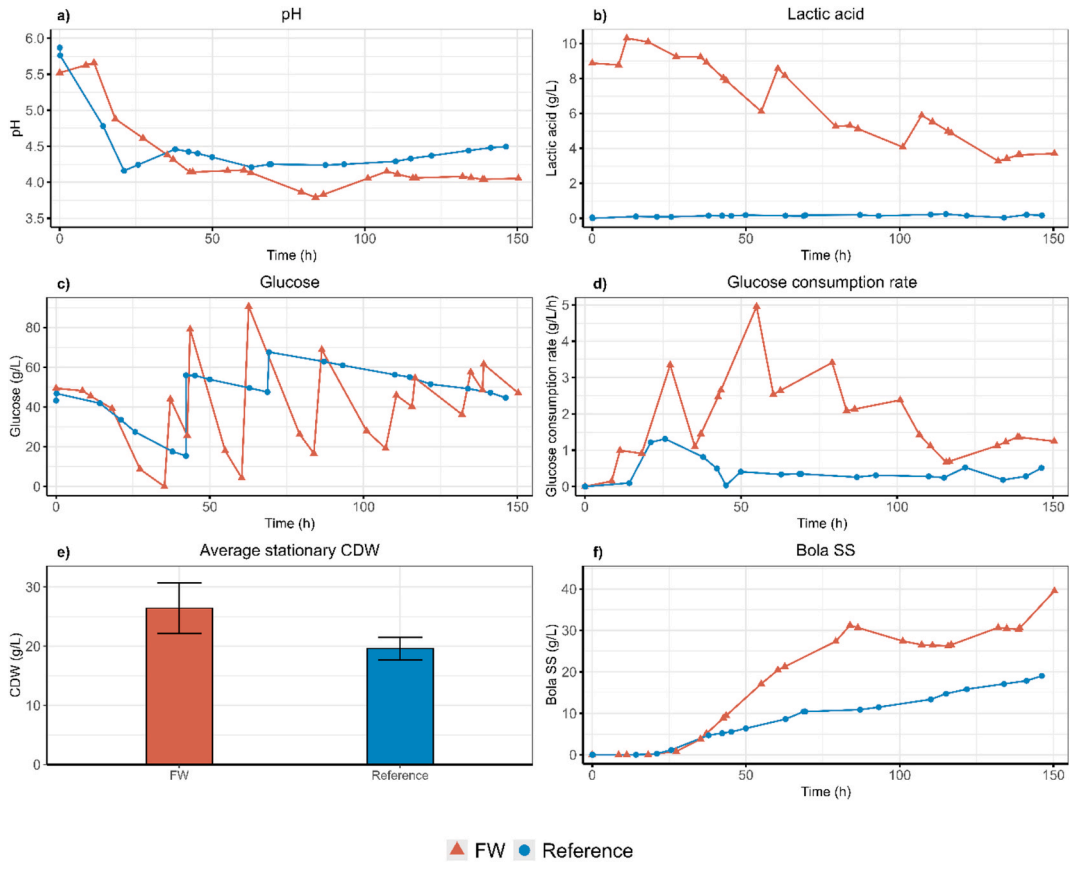

Fig. 3. Performance of *S. bombicola* on SD medium and hydrolyzed food waste (FW) medium. (a) pH (b) lactic acid (c) glucose (d) glucose consumption rate (e) average stationary (after 30 h) cell dry weight (CDW) (f) bola sophoroside (SS) production.

3.3. Validation of bola sophoroside production on food waste

In order to validate the pH control strategy, hydrolyzed LA-rich FW was compared to the SD medium in a fed-batch fermentation. A 2G glucose feed (324.4 g/L glucose) was supplied to the FW medium, and a 600 g/L pure glucose feed was used with the SD medium to maintain hydrophilic carbon levels during the fermentation. Oleyl alcohol was fed continuously at 0.5 g/L/h as both a hydrophobic substrate and as a precautionary antifoaming agent.

A hydrolyzed FW batch with a highly favorable composition (see Supplementary Material), such as a LA concentration of 11.70 g/L and a glucose release of 65.04 g/L, was identified. When applying the models designed in section 3.1.3, this FW batch would achieve a CDW higher than the maximum CDW achieved before (0.0233 g/g vs.0.0175 g/g) and a bola SS concentration very close to the previously obtained maximum (0.82 g/L vs. 0.95 g/L) on DWP scale. Consequently, this promising batch was selected for the validation of bola SS production on FW at bioreactor scale. To reflect industrially relevant conditions, this large FW batch underwent hydrolysis using reduced enzyme loading and a shorter incubation time compared to the FW variability analysis batches, ensuring a more cost-effective and scalable process.

As observed in the LA-spiking experiment, LA concentrations decreased over time from 8.91 g/L to 3.74 g/L, attributed to the continuous fed-batch oleyl alcohol feed and intermittent 2G glucose shots (Fig. 3b). In contrast to the LA-spiking bioreactor experiment, sharp increases in LA concentration were observed during fermentation (around 55 and 100 h). The cause of these increases remains to be elucidated through further investigation. As can be seen, the LA concentration after inoculation of the FW was below the 11.70 g/L observed after hydrolysis, owing to dilution by filtration steps during pretreatment.

The results obtained under these conditions are discussed with respect to pH and LA dynamics, glucose utilization, growth, and bola SS production, shedding light on the bioprocessing performance of FW in comparison to the SD medium.

As demonstrated in Fig. 3a), a notable difference in pH dynamics was observed, with the pH of the SD medium not dropping below 4, whereas the pH of the FW was controlled at 4 after 83.83 h. This lower pH in FW, compared to all conditions with LA in the spiking experiment (pH~5), indicates a higher proportion of protonated LA. Specifically, according to the Henderson-Hasselbalch equation, 11.70 g/L of LA at pH 4 yields a greater concentration of protonated LA than 50 g/L LA does at pH 5 (4.53 vs. 2.97 g/L) (Po and Senozan, 2001). Notwithstanding its higher protonated LA concentration, the ensuing experimental results demonstrate the superior performance of FW compared to SD medium across all parameters.

In the FW condition, glucose depletion was observed after 35 h due to a higher-than-expected glucose consumption by *S. bombicola* (Fig. 3c). However, carbon starvation is implausible due to the short duration and the presence of other unquantified sugars (e.g. sucrose and fructose) and glycerol that help *S. bombicola* bridge the gap until the first glucose shot (Poe et al., 2020; To et al., 2023). As demonstrated in Table 5, the overall glucose consumption rate was found to be more than twice as high in FW (1.26 g/L/h) compared to the SD medium (0.62 g/L/h), with FW exhibiting a rate comparable to that observed during the LA-spiking experiment.

FW outperformed the SD medium in terms of biomass formation, producing an average stationary phase (after 30 h) CDW of 25.27 ± 4.18 g/L, significantly higher than the SD medium (19.71 ± 2.08 g/L; $p = 0.003$) (Fig. 3e). However, FW-induced growth was only significantly greater than the 0 g/L ($p = 0.047$) and 50 g/L ($p = 0.015$) LA spike conditions, while no significant difference was observed compared to

Table 5
Summary of bioprocess performance for different bioreactor experiments.

Bioreactor experiment	Condition	Glucose consumption rate (g/L/h)	Stationary CDW (g/L)	Volumetric productivity (g/L/h)	Volume-adjusted productivity (g/L/h)
LA-spiking	0 g/L	1.18	23.67 ± 2.91	0.38	0.53
	15 g/L	1.21	24.40 ± 2.11	0.29	0.39
	33 g/L	1.33	23.00 ± 1.03	0.30	0.41
	50 g/L	1.29	21.44 ± 2.02	0.24	0.34
FW validation	SD	0.62	19.71 ± 2.08	0.13	0.10
	FW	1.26	25.27 ± 4.18	0.28	0.52

15–33 g/L LA on mFW medium (Table 5).

Productivity measurements further demonstrated the merits of FW, as it boasted a volumetric productivity of 0.28 g/L/h, 2.15 times higher than that of the SD medium (0.13 g/L/h). The productivity of the SD medium merely represents one-third of the productivity attained with the mFW medium at 0 g/L LA. When accounting for dilution effects and a 500 mL FW sample taken after 107.17 h to avoid bioreactor overflow, the volume-adjusted productivity in FW reached 0.52 g/L/h, 5.2 times higher than that of the SD medium (0.10 g/L/h) (Table 5).

In summary, FW demonstrated comparable performance to the 0 g/L LA mFW medium in terms of glucose consumption, growth, and volume-adjusted productivity. It outperformed (1.28 times better growth and 5.2 times better production) the SD medium typically used for SL production by *S. bombicola*. Interestingly, even the mFW medium with 50 g/L LA demonstrated superior performance in terms of growth and bola SS production when compared to the SD medium. This suggests that whilst FW with low LA concentrations ($\leq$ 33 g/L) is preferred, FW with LA concentrations up to 50 g/L also has potential. These findings underscore the efficacy of the pH control strategy in mitigating LA inhibition of *S. bombicola* growth and productivity in FW.

As such a foundational proof-of-concept for the production of 2G bola SS on FW by *S. bombicola* is provided, paving the way for the production of numerous novel bioproducts on FW.

4. Conclusion

This study demonstrates that hydrolyzed FW is a viable feedstock for the production of novel biosurfactants by *S. bombicola*, despite its compositional variability and high LA levels. Through the implementation of predictive modeling and a dedicated pH-control strategy, the key fermentation challenges, particularly FW variability and LA inhibition, were effectively mitigated. A proof-of-concept study demonstrated the superior performance of FW over conventional media, establishing FW as a sustainable substrate. These findings pave the way for scalable bioprocesses using FW, enabling the development of 2G bioproducts and contributing to the advancement of a circular bioeconomy.

CRediT authorship contribution statement

Thibo Van de Craen: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Sophie L.K. W. Roelants:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Karolien Maes:** Supervision, Methodology, Conceptualization. **Sofie Lodens:** Supervision, Funding acquisition, Conceptualization. **Sofie L. De Maeseneire:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. **Wim K. Soetaert:** Supervision, Resources, Project administration, Funding acquisition. **Tom Delmulle:** Writing – review & editing, Validation, Supervision, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Authors Sophie L.K.W Roelants and Wim K. Soetaert are a named inventor on a licensed patent relevant to this work ("Improved production of symmetrical bolaform sophorosides", WO/2020/104582). Although the patent is licensed, no royalties have been received at the time of publication. All other authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2025.133043.

Data availability

Data will be made available on reasonable request.

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