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LIGNOCELLULOSIC WASTE AS A SUSTAINABLE SOURCE OF FERMENTABLE SUGARS FOR THE PRODUCTION OF BIOACTIVE COMPOUNDS WITH APPLICATIONS IN FOOD SAFETY

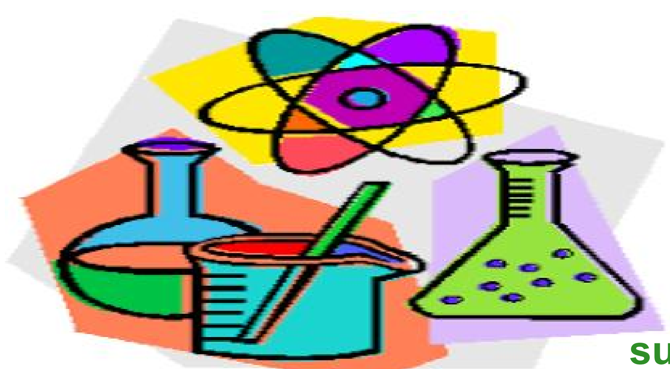
Greta Naydenova, Dragomir Yankov

Institute of Chemical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria

Introduction

Building trust in food systems requires science-based solutions that integrate safety, sustainability, and high added value. In line with the motto "Trust through science", this study explores the valorization of agro-industrial residues as alternative carbon sources for the production of bioactive compounds relevant to food safety.

Lignocellulosic and organic by-products, including DDGS, coffee grounds, and sunflower husks, were investigated through pretreatment and hydrolysis, with evaluation of reducing sugar release.



Objective

The aim of this study is to evaluate the efficiency of different lignocellulosic substrates for reducing sugar production and their application as carbon sources for *Lactobacillus spp.* fermentation for lactic acid and bioactive metabolite production, with potential to enhance food microbiological safety.

Materials and Methods

❖ **Raw materials:**

Lignocellulosic substrates: DDGS (distillers grains), sunflower hulls (SH) and spent coffee grounds (SCG).

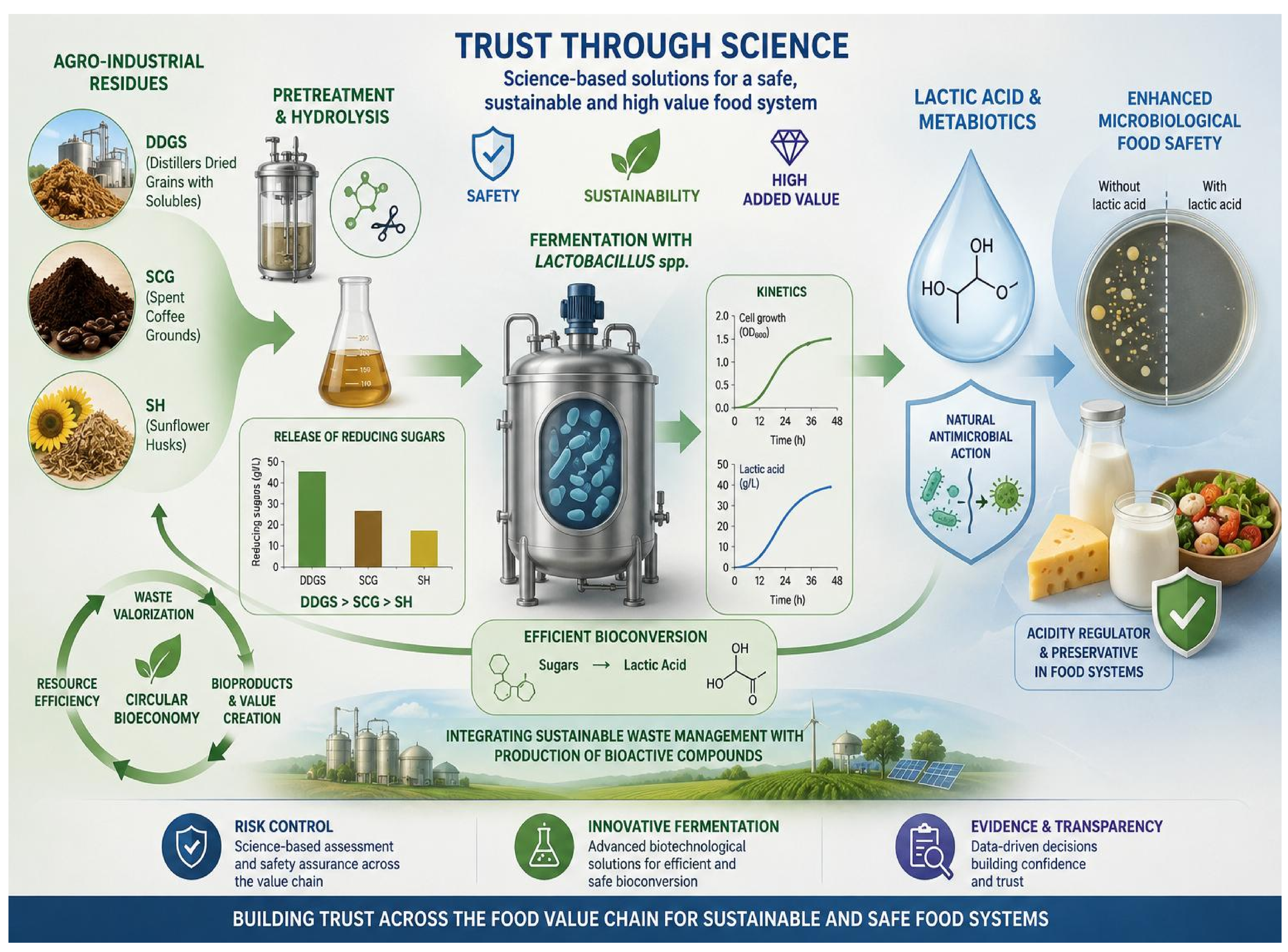
❖ **Two-stage hydrolysis process:**

Stage I – Pretreatment conditions

- Solid loading: 10% (w/v)
- Volume: 60 mL
- Two approaches:
  - **Hydrothermal treatment:** distilled water, 1 atm, 2 h (autoclave)
  - **Acid hydrolysis:** 1% H_2SO_4, same conditions
- Liquid fractions collected for HPLC analysis
- Solid residues washed (~500 mL distilled water), washings also analyzed

Stage II – Enzymatic hydrolysis

- Enzyme: cellulase (1 g/L)
- Conditions: pH 4.5, 1 atm, 1 h
- Solid loading: 10% (w/v)



Materials and Methods

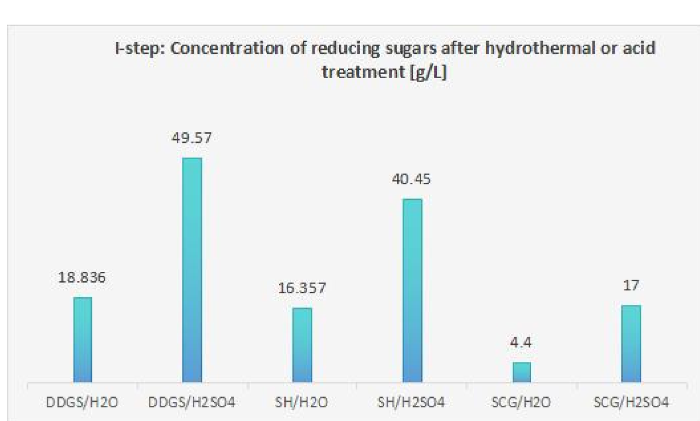
Analytical methods:

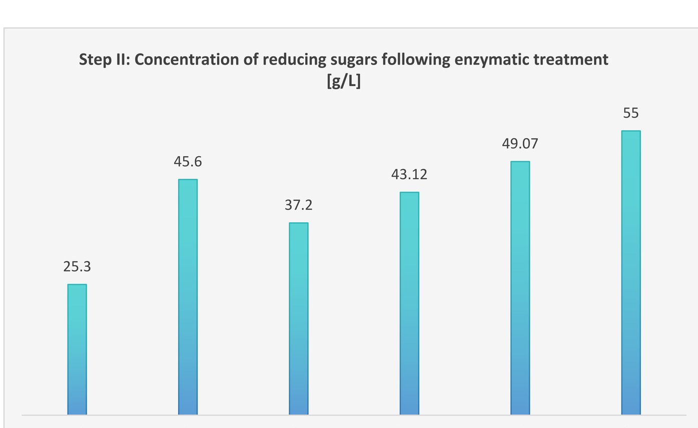
- Reducing sugars determined by Bertrand method
- Sugars profile analyzed by HPLC

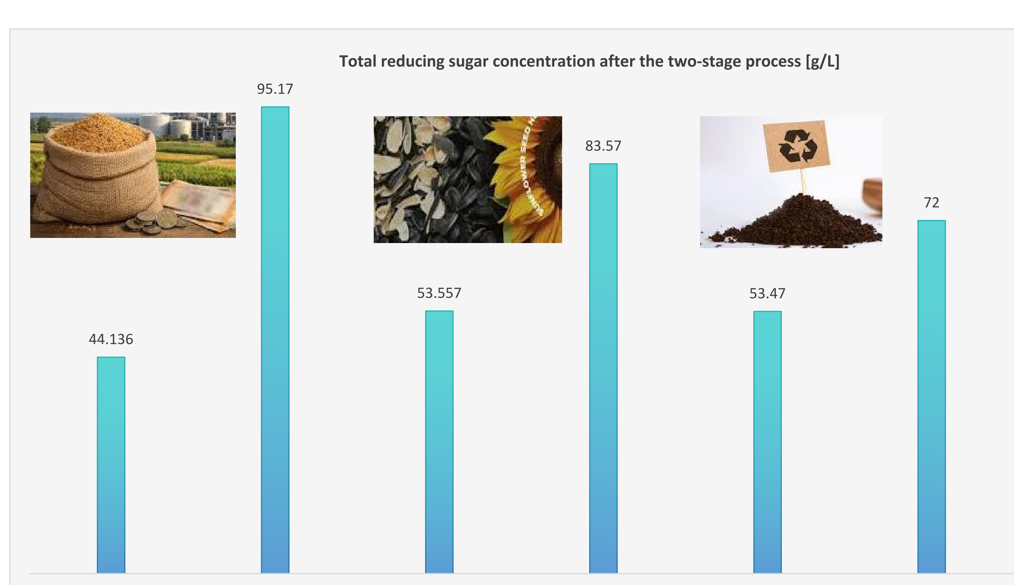
Fermentation experiment:

- Microorganisms: *Lactobacillus spp.*
- Medium: MRS (without lactose in experimental variants, and with 11 g/l lactose for control)
- Temperature: 37° C, static cultivation
- Volume: 125 mL

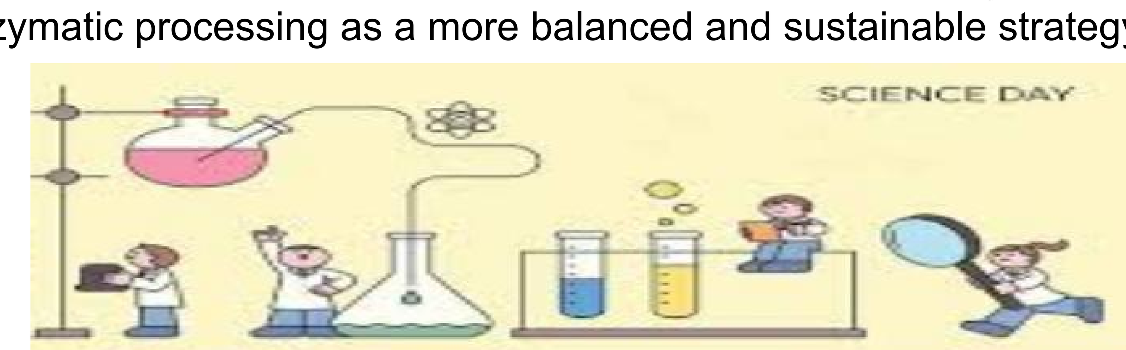
RESULTS AND DISCUSSIONS

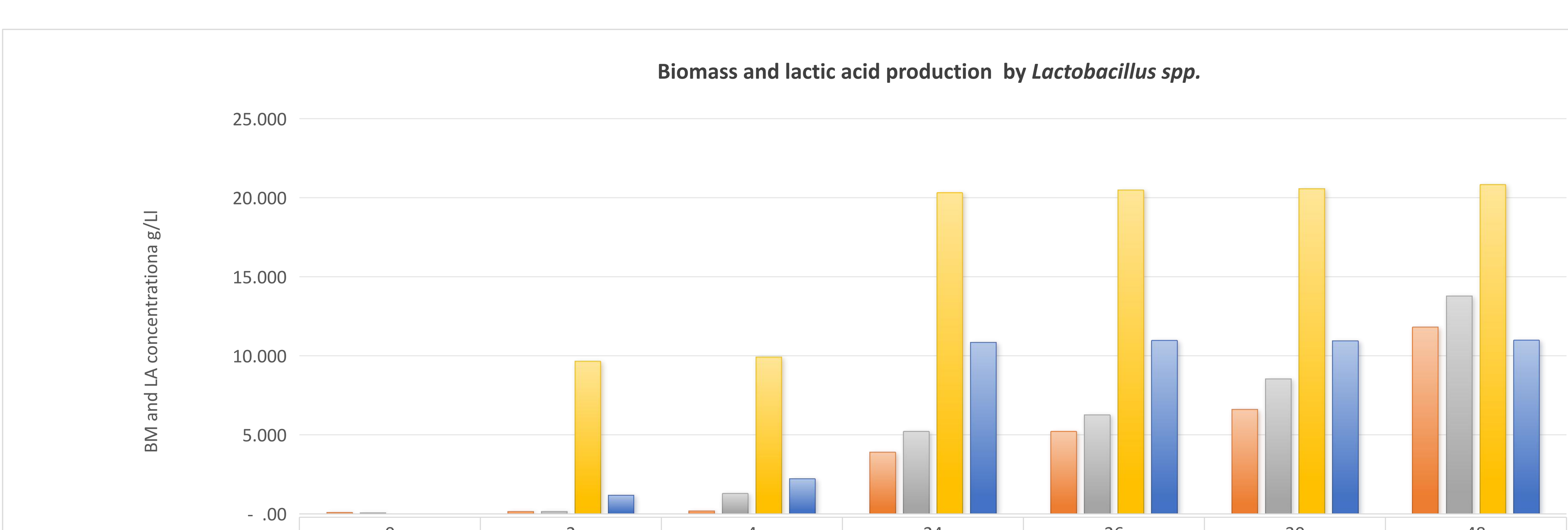






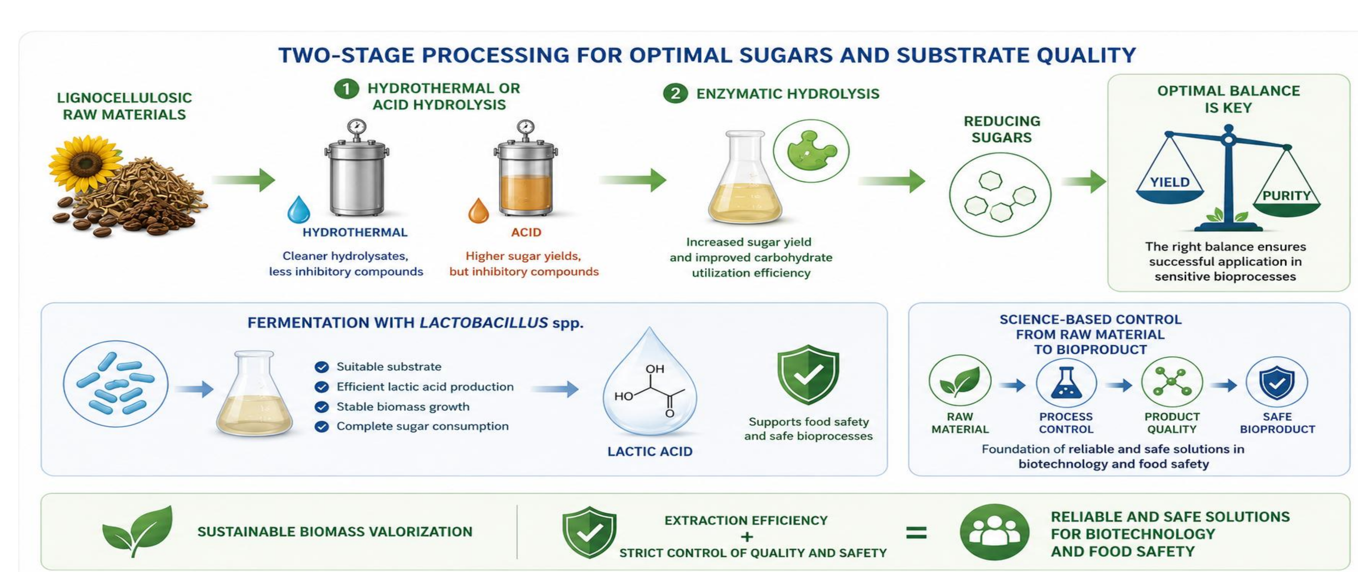
- Acid hydrolysis yields higher reducing sugar concentrations (up to 95.17 g/L for DDGS), but generates inhibitory compounds that may limit fermentation efficiency.
- Hydrothermal extraction results in lower sugar yields, yet produces cleaner hydrolysates with improved suitability for biotechnological applications.
- Enzymatic hydrolysis significantly enhances sugar release, confirming the effectiveness of the two-stage process.
- Although the highest-yield hydrolysate (DDGS, acid + enzymatic) was used for fermentation, future research should focus on hydrothermal + enzymatic processing as a more balanced and sustainable strategy.





	0	2	4	24	26	30	48
BM in DDGS hydrolysate g/L	0.091	0.148	0.195	3.890	5.177	6.601	11.810
BM in MRS control g/L	0.0804	0.154	1.292	5.177	6.247	8.535	13.769
LA in DDGS hydrolysate g/L	- .00	9.653	9.913	20.284	20.482	20.566	20.832
LA in MRS control g/L	- .00	1.175	2.22	10.8316	10.9314	10.94	10.96

- *Lactobacillus spp.* efficiently utilized the DDGS hydrolysate (acid + enzymatic), with complete glucose consumption within 24 h.
- Lactic acid production reached ~20.8 g/L, accompanied by a pH decrease from 5.34 to ~3.5, indicating active homolactic metabolism.
- A steady increase in biomass (up to ~11.8 g/L) confirmed good compatibility of the detoxified hydrolysate.
- Despite the high performance, the need for detoxification highlights limitations of acid hydrolysis and supports the future implementation of cleaner hydrolysates in biotechnological applications.



CONCLUSIONS

The two-stage processing of lignocellulosic raw materials through hydrothermal or acid hydrolysis followed by enzymatic hydrolysis is an effective strategy for producing reducing sugars with distinct technological profiles. Acid hydrolysis provides higher yields but generates inhibitory compounds, whereas hydrothermal treatment produces cleaner hydrolysates that are suitable for biotechnological applications. Enzymatic hydrolysis further enhances sugar yield and improves carbohydrate utilization efficiency.

Fermentation experiments with *Lactobacillus spp.* confirm that the obtained hydrolysates are suitable substrates for efficient lactic acid production, supporting stable biomass growth and complete sugar consumption. The results clearly demonstrate that the optimal balance between yield and substrate purity is crucial for successful application in sensitive bioprocesses.

Overall, the study highlights that sustainable biomass valorization depends not only on extraction efficiency but also on strict control of product quality and safety. This science-based control from raw material to final bioproduct forms the foundation of reliable and safe solutions in biotechnology and food safety.

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