Tomato Leaves Valorization in a Bioreactor for Enhanced Biomass Production

Duygu Mentes[1], Ayse Sultan Akgun[2], Veli Barıs Karatepe[1], Deniz Sevim Cabuk[1], Burcak Hakguden[1], Dilruba Hezer[1], Mecit Halil Oztop [1]*

[1]Middle East Technical University, Food Engineering, Ankara, Turkey
[2]Middle East Technical University, Biotechnology, Ankara, Turkey

Abstract

This research investigates the optimal fermentation conditions for efficiently converting tomato leaves and stems into a valuable biomass source through hydrolysis and submerged fermentation. Tomato leaves and stems were initially subjected to different hydrolysis methods such as acid hydrolysis, alkali pretreated enzyme hydrolysis followed by different treatments such as thermal treatment, titration and activated charcoal. The method yielding the highest concentration of reducing sugar was chosen as a fermentation medium. Reducing sugar content and optical density were measured throughout the fermentation to compare the effects of various parameters, such as agitation speed (100, 200, 300 rpm), inoculum size (1%, 3%, 5% v/v), and fermentation time (24, 72, and 120 hours). Reducing sugar concentrations were determined using the DNS method, while optical density at 600 nm was monitored throughout the fermentation process. The maximum optical density was measured as 1.70 in the fermentation with 300 rpm agitation and 1% inoculum size after 120 hours. Agitation speed and fermentation time significantly effect optical density during fermentation process.

Introduction

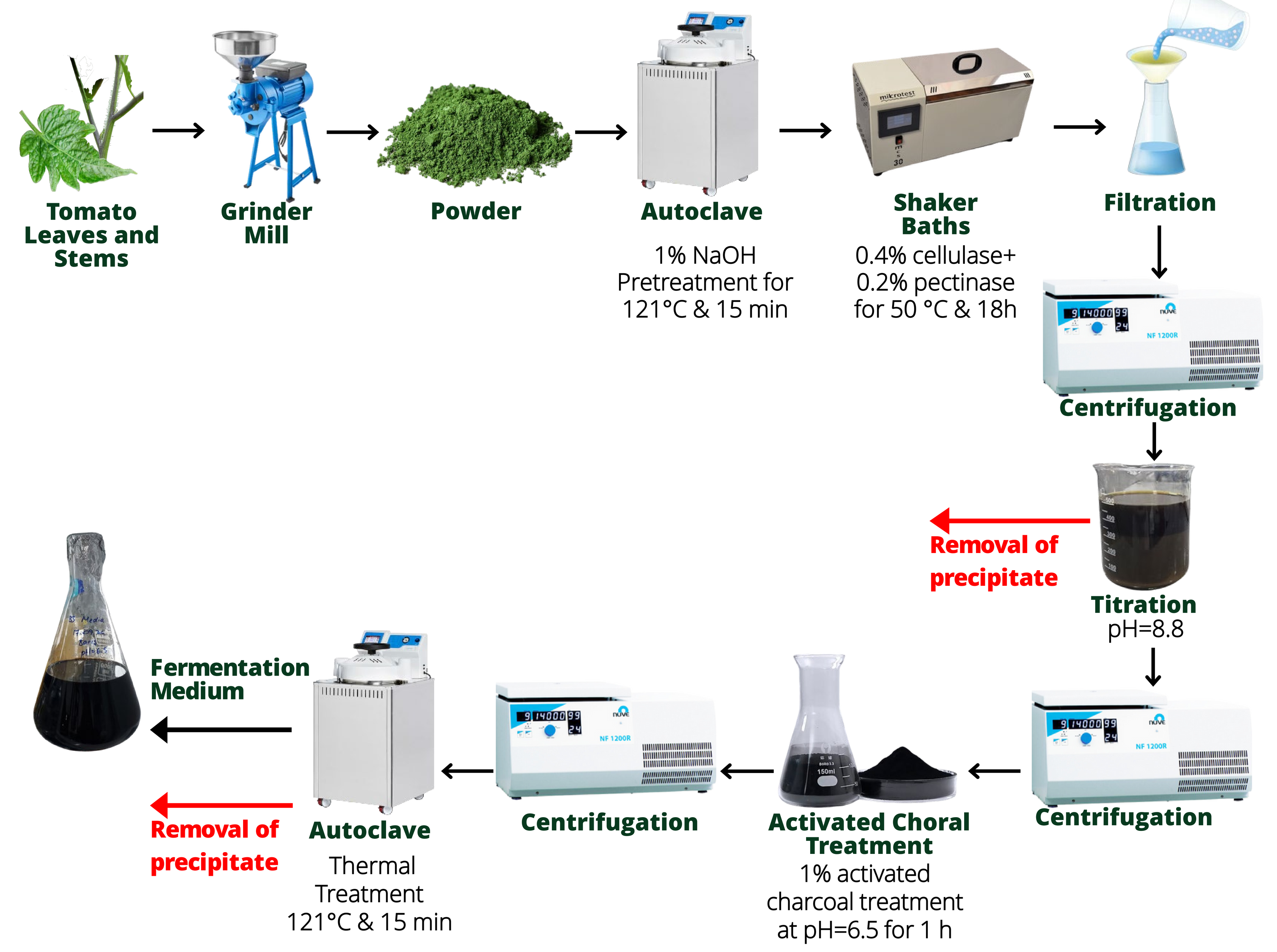
Agricultural biomass residues can be used as a substrate for the production of microbial proteins, as they are cost-effective, nutritious, and sustainable resources (González-Aguilar et al., 2022). The tomato (Lycopersicon esculentum) is the most extensively cultivated vegetable and simultaneously contributes significantly to agricultural waste generation during its production. These by-products, mainly tomato parts, stems, and leaves, are often left in the fields after harvest and constitute one of the largest quantity of agricultural biomass residues.
Tomato stems consist of lignocellulosic materials, including hemicellulose (20–40 wt%), cellulose (30–60 wt%), and lignin (10–25 wt%), as well as pectic substances and polyphenols. Meanwhile, the leaves are primarily composed of carbohydrates, which can serve as a source for microbial protein production (Covino, 2020).

Objectives

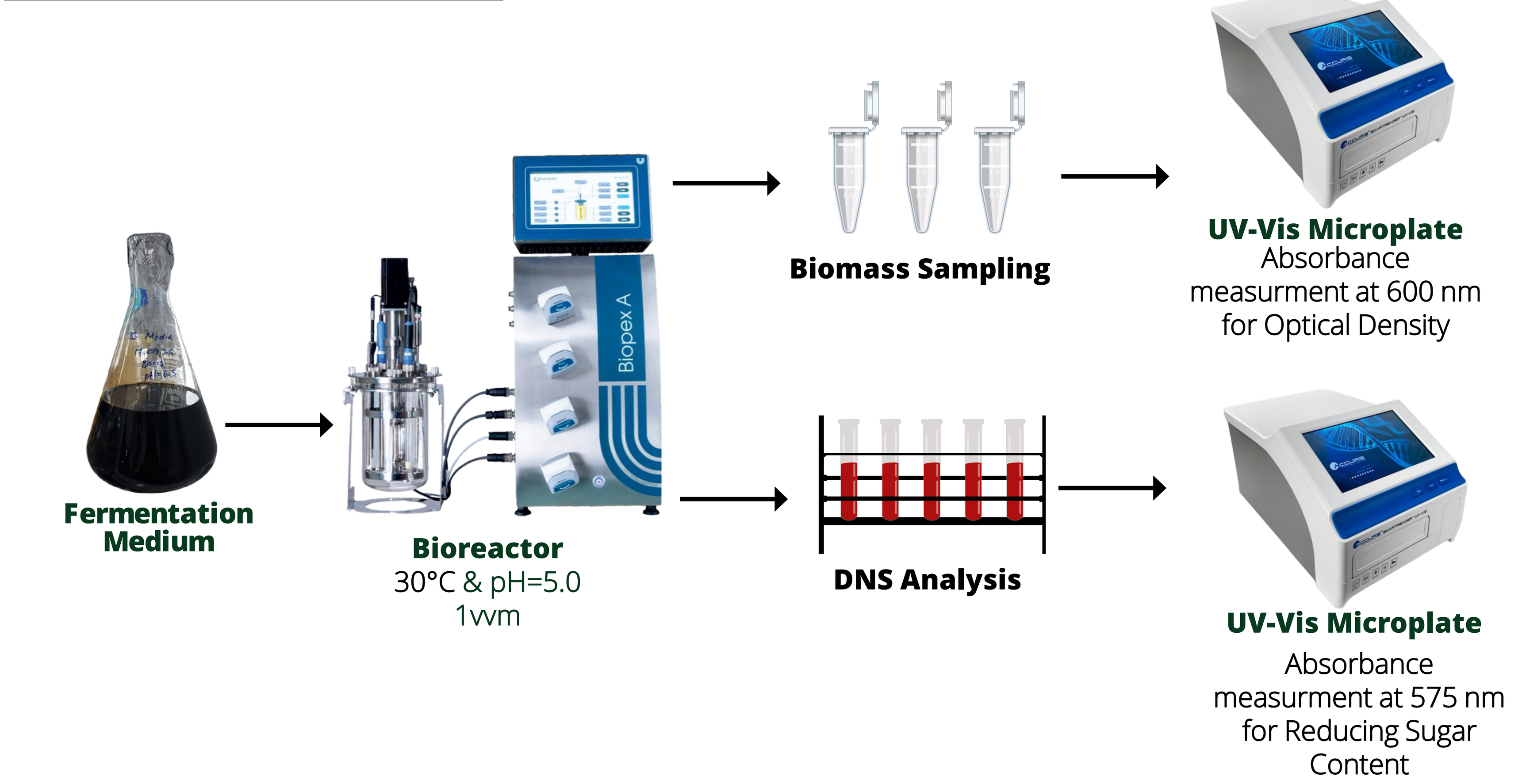
- Converting one of the most largest agricultural wastes from tomato production into a hydrolysate which has high amount of fermentable sugars to be use as fermentation medium.
- Examining the effectiveness of method of hydrolysis, pretreatments types on converting polysaccharides in tomato leaves and stems into reducing sugars
- Examining the effectiveness of titration, activated charcoal treatment, and thermal treatment on the amount of fermentable sugars in the fermentation medium.
- Production of a fermentation medium using different hydrolyzation methods to obtain a toxin-free, clear medium with a high amount of reducing sugars.
- Revealing the optimum fermentation conditions in a lab-scale bioreactor to produce the maximum biomass concentration using hydrolysate derived from tomato leaves and stems.

Material and Methods

Production of Fermentation Medium



Bioreactor Fermentation



Results and Discussion

Table 1. Reducing sugar content of fermentation mediums produced with different hydrolyzation and pretreatments

10% Tomato Leave	Hydrolyzation Time (min)	Enzymatic Hydrolyzation	Reducing Sugar Content (g/L)
Hydrothermal Pretreatment	15	Cellulase	8,67
1% Alkaline Pretreatment	15	Cellulase	19,87
1% Alkaline Pretreatment	15	Pectinase +Cellulase	23,91
1% Acid Hydrolysis	15	-	3,42
1% Alkaline Pretreatment + Titration + Activated Charcoal Treatment	15+15	Pectinase +Cellulase	30.28

- The highest amount of reducing sugar, 30.28 g/L, was obtained by combining 1% alkaline pretreatment, titration, and activated charcoal treatment. Titration was applied to remove polysaccharides that were not broken down during hydrolysis, while activated charcoal treatment eliminated any toxins present due to plant defense mechanisms or formed during alkaline pretreatment.

- According to the comparison results ($p < 0.05$), **agitation speed and time** significantly affect **optical density**, while **inoculum size and time** significantly affect the **reduction of sugar content**.
- Inoculum size did not show a significant influence on biomass production, while significantly effects the reducing sugar usage in the medium.
- The highest optical density was measured at **1.70** with 300 rpm, 1% inoculum size, and 120 hours of fermentation. When response optimization was applied to maximize optical density, the optimal parameters were also determined to be **300 rpm, 1% inoculum size, with a fermentation time of 120 hours.**

Table 2. Optical density (OD600) and reducing sugar concentration (g/L) for different parameters during fermentation

Fermentation Parameters		Optical Density (OD600)	Reducing Sugar Content (g/L)
Agitation Speed (rpm)	100	1.22^{b}	0.59^{a}
	200	1.24^{b}	0.70^{a}
	300	1.48^{a}	0.62^{a}
Inoculum Size (v/v)	1	1.32^{a}	0.65^{a,b}
	3	1.29^{a}	0.57^{b}
	5	1.32^{a}	0.69^{a}
Fermentation Time (hours)	24	1.16^{b}	0.75^{a}
	72	1.33^{a}	0.62^{b}
	120	1.44^{a}	0.54^{b}

Note: Values are expressed as mean ± SE (n = 3). The levels of each factor have been compared among themselves. In each column, different letters represent significant differences ($p < 0.05$).

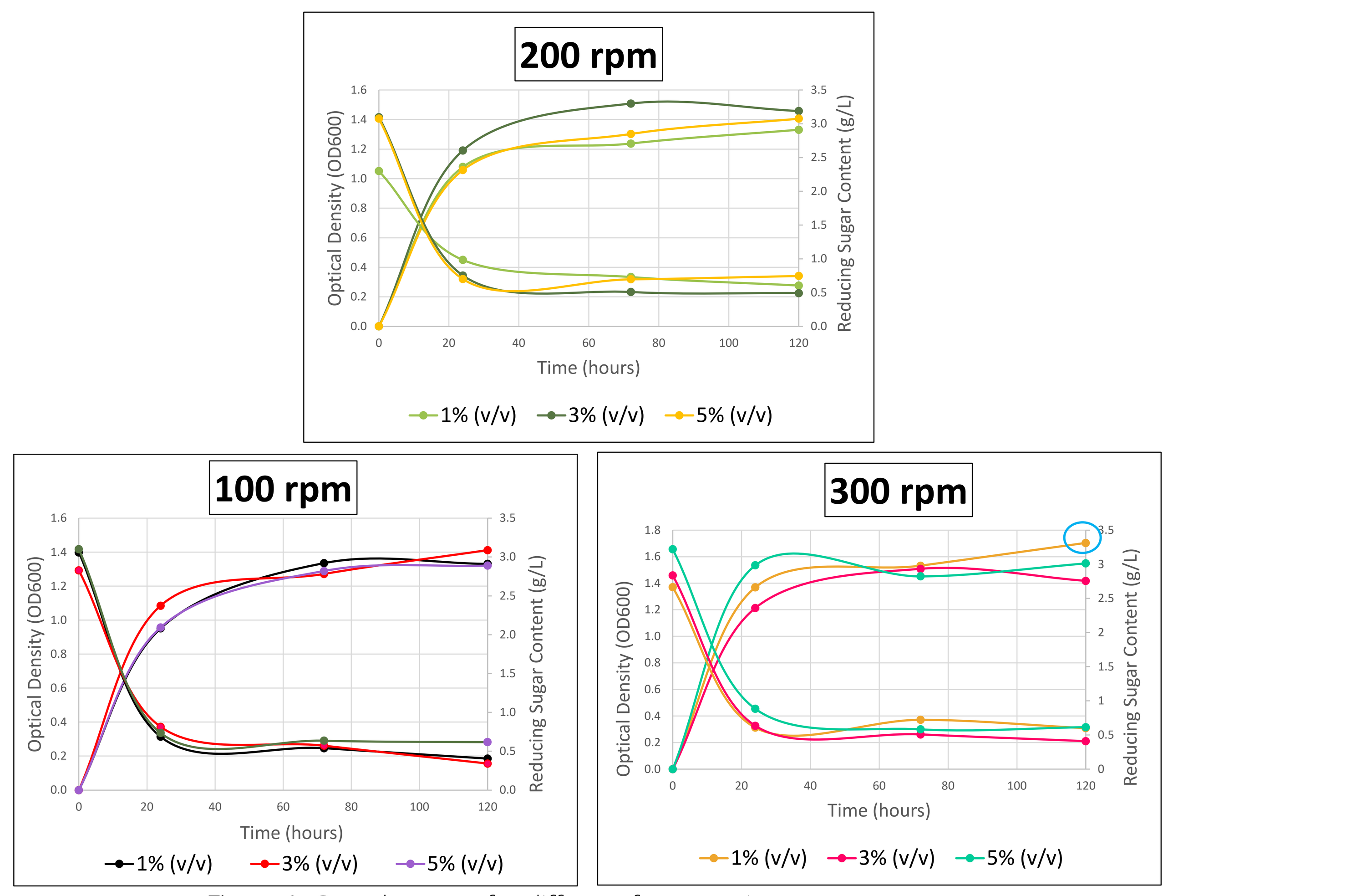


Figure 1. Growth curves for different fermentation parameters

- According to the growth curves, the optical density results show a drastic increase as the agitation speed increases for the first 24 hours. For both curves, unused fermentable sugars remain at the end of 120 hours with a value of around 0.5 g/L. Without changing the agitation speed, increasing or decreasing the inoculum size does not significantly affect the optical density results. The reason for this could be the contribution of agitation to more effective aeration and, consequently, to the dissolved oxygen levels within the bioreactor.

Conclusion

To conclude, utilizing one of agricultural waste, tomato leaves, as a sustainable carbon source for fermentation hydrolysate through different hydrolyzation methods gives considerably high amount of reducing sugar content. The fermentation hydrolysate also used for the biomass production through optimized fermentation parameters. The maximum amount of biomass produced via 300 rpm, 1% inoculum size and 120 hours as 1.70. Maximizing biomass yield offers valuable opportunities for various downstream applications, including production of microbial proteins.

References

Covino, C., Sorrentino, A., Di Pierro, P., Roscigno, G., Vece, A. P., & Masi, P. (2020). Lignocellulosic fibres from enzyme-treated tomato plants: Characterisation and application in paperboard manufacturing. International Journal of Biological Macromolecules, 161, 787-796

Salazar-López, N. J., Barco-Mendoza, G. A., Zuñiga-Martínez, B. S., Domínguez-Avila, J. A., Robles-Sánchez, R. M., Ochoa, M. A. V., & González-Aguilar, G. A. (2022). Single-Cell Protein Production as a Strategy to Reincorporate Food Waste and Agro By-Products Back into the Processing Chain. Bioengineering (Basel, Switzerland), 9(11), 623. https://doi.org/10.3390/bioengineering9110623

Acknowledgements

This study has received funding from the European Union's Horizon 2020-PRIMA Section I Program under grant agreement #2232 (ProxIMed).

The PRIMA programme is supported under Horizon 2020, the European Union's Framework Programme for Research and Innovation