



Bioconversion of Fiber-Rich Agro-Food Residues into *Yarrowia lipolytica* Biomass: Comparative Evaluation of Acid Hydrolysates for Sustainable Food-Grade Biomass Production

Suheda Ugur

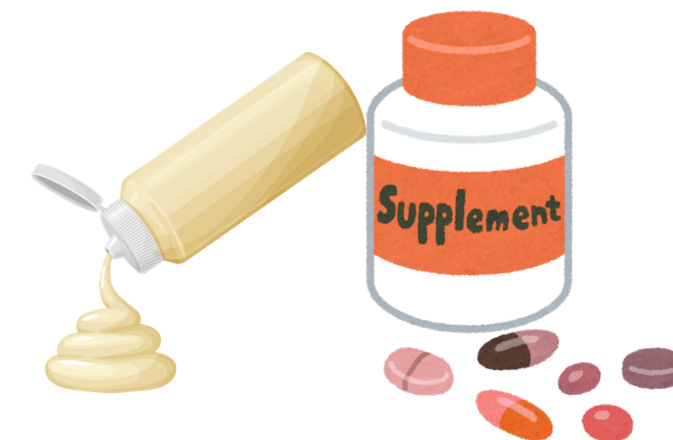
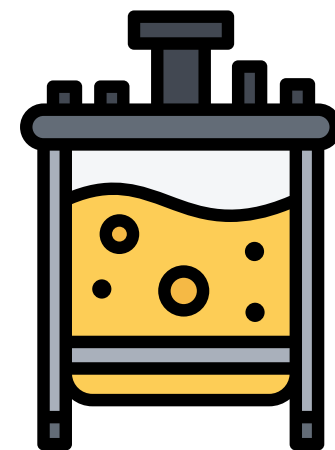
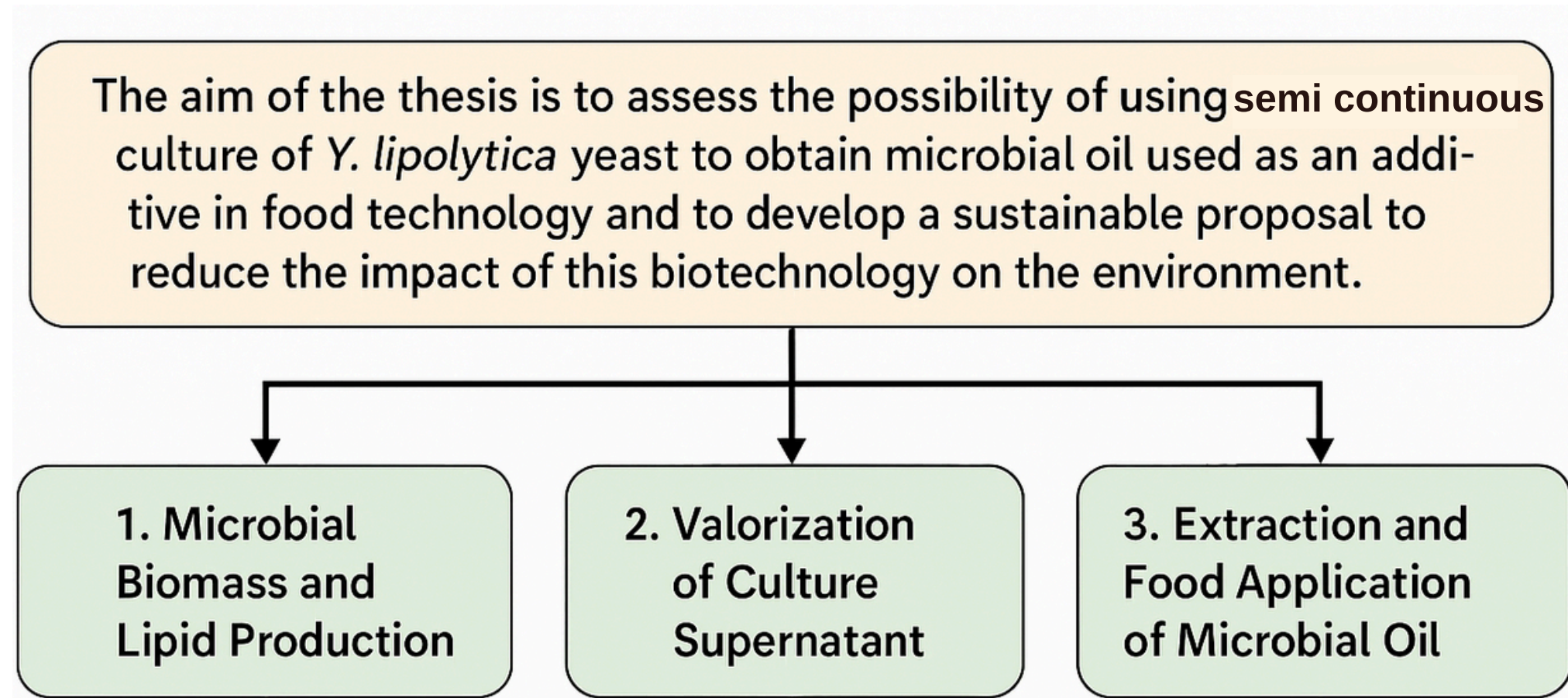


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Research Background

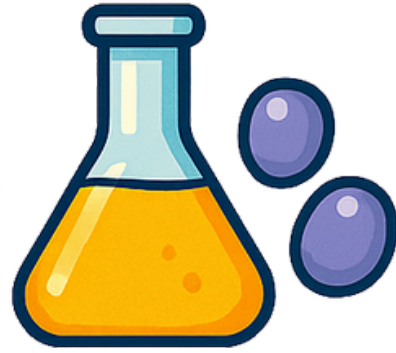
Studies on sustainable microbial oil synthesis in semi-continuous cultures of *Yarrowia lipolytica* yeast and its formulation for food products.



Research Issues Justification

High Oil Yield

Y. lipolytica is a yeast rich in oil. A fed-batch culture was chosen to obtain a high lipid yield



Utilization of Waste Sources

Y. lipolytica can use various nutrient sources, including from waste streams



High Oil Quality

Microbial oil produced by *Y. lipolytica* is rich in unsaturated fatty acids



Applications in the Food Industry

Microbial oils from *Y. lipolytica* can be used in margarine, mayonnaisse, sauces, ice cream, and as food additives or dietary supplements

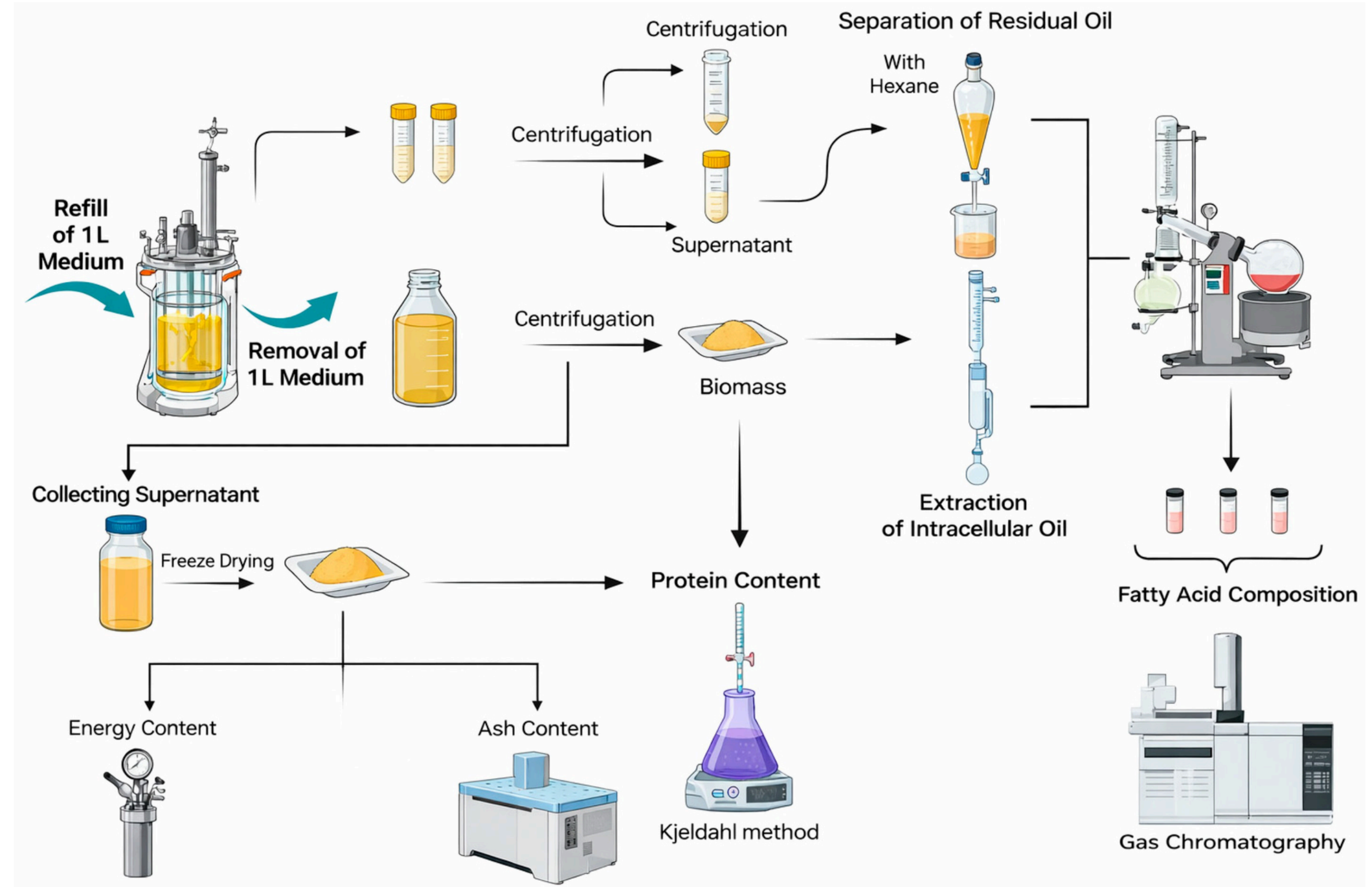


Figure 1. Production of oleaginous yeast biomass rich in storage lipids cultured in waste media with semi-continuous mode.

Fatty Acid	(A) 12h frequency						
	16h	28h	40h	52h	64h	76h	88h
C18:1	64.99	62.90	63.75	64.43	64.64	66.37	68.78
C18:2	20.43	19.67	18.51	18.45	18.22	17.81	17.30

Table 1. Fatty acid composition in the different variants of medium replacement frequencies: (A) 12h frequency, (B) 6h-6h-12h frequency, and (C) 24h frequency

Fatty Acid	(B) 6-6-12h frequency					
	22h	28h	40h	46h	52h	64h
C18:1	62.71	63.30	66.27	65.22	67.61	81.24
C18:2	22.97	18.90	18.02	18.62	17.72	-

Fatty Acid	(C) 24h frequency		
	40h	64h	88h
C18:1	62.96	65.54	68.93
C18:2	23.01	18.67	17.11

Fatty Acid	(A) 12h frequency							(B) 6-6-12h frequency						(C) 24h frequency		
	16h	28h	40h	52h	64h	76h	88h	22h	28h	40h	46h	52h	64h	40h	64h	88h
C14:0	-	0.23	0.13	0.16	0.14	0.15	0.11	-	0.64	0.47	-	0.18	0.11	-	-	-
C16:0	5.00	4.84	4.08	3.74	4.70	4.37	3.66	3.45	6.89	5.53	4.06	3.90	4.75	3.90	5.76	4.75
C16:1	0.28	2.39	4.60	3.51	2.07	0.11	0.61	0.58	0.46	0.44	3.21	1.88	1.00	1.73	2.74	0.92
C17:0	-	-	0.12	-	-	-	-	-	-	-	-	-	-	-	0.29	0.36
C17:1	-	-	0.14	0.12	-	-	-	-	-	-	-	-	-	0.10	-	-
C18:0	1.83	2.28	1.73	2.04	1.93	2.31	0.04	1.59	2.18	0.06	1.80	0.04	2.76	2.15	2.03	2.12
C18:1	64.99	62.90	63.75	64.43	64.64	66.37	68.78	62.71	63.30	66.27	65.22	67.61	81.24	62.96	65.54	68.93
C18:2	20.43	19.67	18.51	18.45	18.22	17.81	17.30	22.97	18.90	18.02	18.62	17.72	-	23.01	18.67	17.11
C20:0	5.44	6.29	5.67	5.77	6.14	5.82	5.99	7.01	5.82	6.09	5.63	5.89	7.61	4.92	5.54	5.67
C20:1	0.88	0.38	0.44	0.52	0.50	0.90	0.93	0.64	0.53	0.96	0.38	0.85	0.64	0.38	0.52	0.69
C21:0	1.16	1.02	0.69	1.12	1.28	1.88	1.93	1.05	1.21	1.96	1.07	1.78	1.88	0.85	0.91	1.45
C22:0	-	-	0.13	0.13	-	-	0.15	-	0.06	0.12	-	0.15	-	-	-	-
C22:1	-	-	-	-	-	-	0.04	-	-	0.02	-	0.02	-	-	-	-
C24:0	-	-	-	-	-	-	0.44	-	-	-	-	-	-	-	-	-
SFA	7.71	7.73	6.63	6.59	7.63	7.73	4.93	5.68	10.30	7.16	6.24	5.14	8.26	6.43	8.60	7.92
UFA	92.30	92.27	93.36	93.40	92.35	91.99	95.05	94.32	89.69	92.78	93.75	94.88	91.73	93.57	91.40	92.08
MUFA	66.43	66.31	69.18	69.18	67.99	68.36	71.76	64.34	64.97	68.67	69.50	71.27	84.12	65.64	69.19	71.30
PUFA	25.87	25.96	24.18	24.22	24.36	23.63	23.29	29.98	24.72	24.11	24.25	23.61	7.61	27.93	22.21	20.78

Oleic Acids (18:1, Omega- 9)
Linoleic Acids (18:2, Omega-6)

AIM OF STUDY:

Possible uses of *Yarrowia lipolytica* biomass:

Single-cell oil production

Single-cell protein production

Animal feed and aquaculture feed supplement

Production of enzymes and organic acids

Source of vitamins and bioactive compounds

Sustainable bioproduct development from agro-food waste

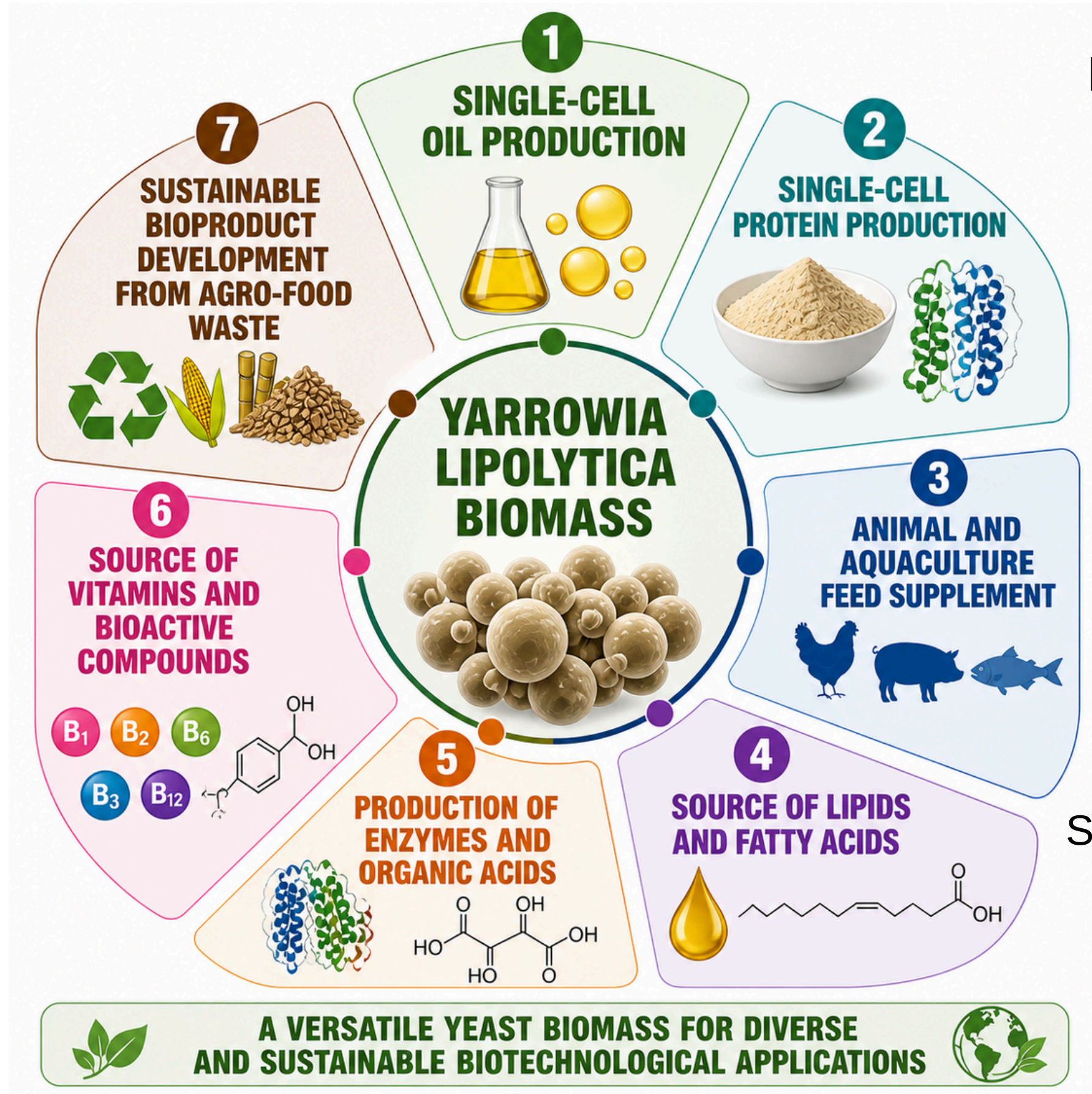


Figure 2. Biotechnological Applications of *Yarrowia lipolytica* Biomass

Wheat Straw



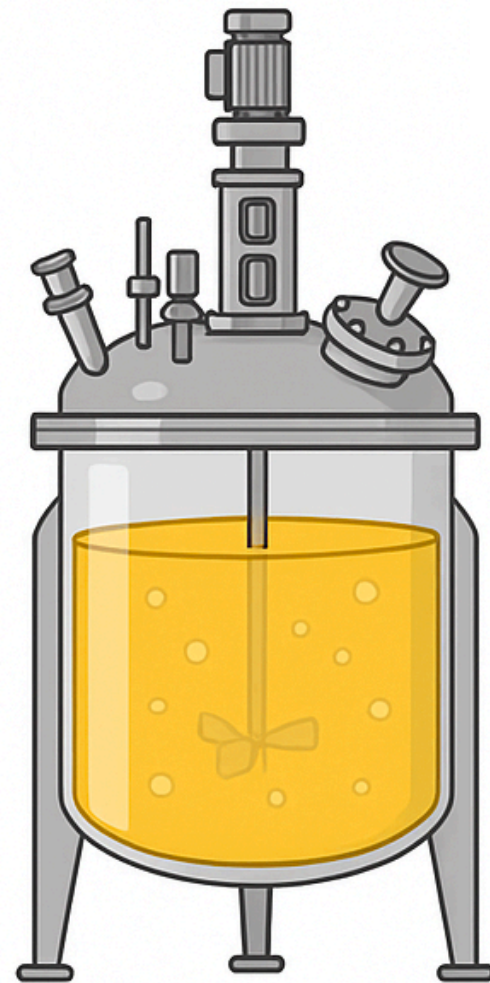
Sugar Beet Pulp



Waste Tea



Orange Peels



**Carbon Source for
Microbial Fermentation**

Studies on using selected
ligno-cellulose waste material
as a carbon source in yeast
semi- continues culture.

Methods:



STEP 1

Prepare Hydrolysates with 1% Acid

Use 1% H_2SO_4 on materials alongside a 4% glucose control group.



STEP 2

Measure Initial Reducing Sugars

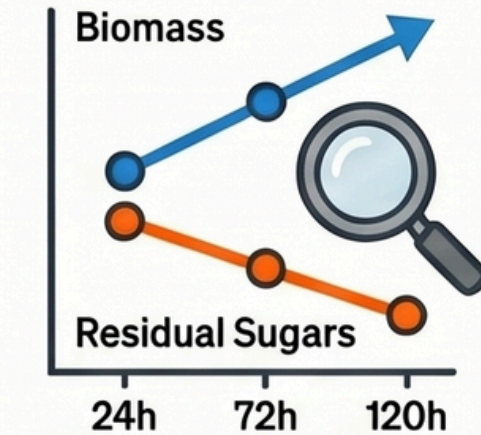
Establish a baseline sugar concentration before culturing begins.



STEP 3

Conduct Shake-Flask Cultivation

Initiate the fermentation process using standardized shake-flask cultures.



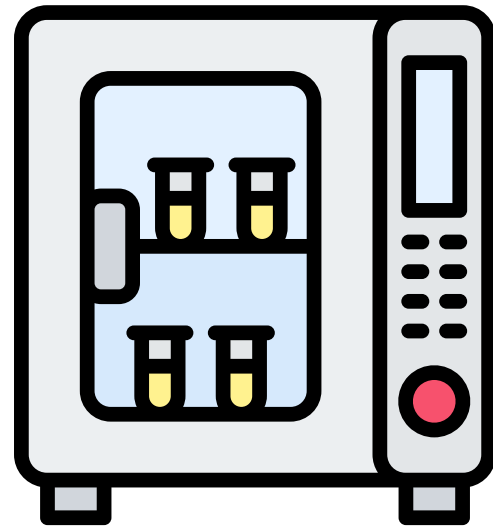
STEP 4

Monitor Biomass and Residual Sugars

Collect samples at 24h, 72h, and 120h to evaluate production efficiency.

Supplemented by
N limited medium

Not supplemented



Incubation
180 rpm, 28 °C

Culturing
24h, 72h, 120h.

DNS method for reducing sugar determination -
Spectrophotometer
540 nm



SUPPLEMENTED MEANS :



Control medium:

Glucose-based nitrogen-limited medium

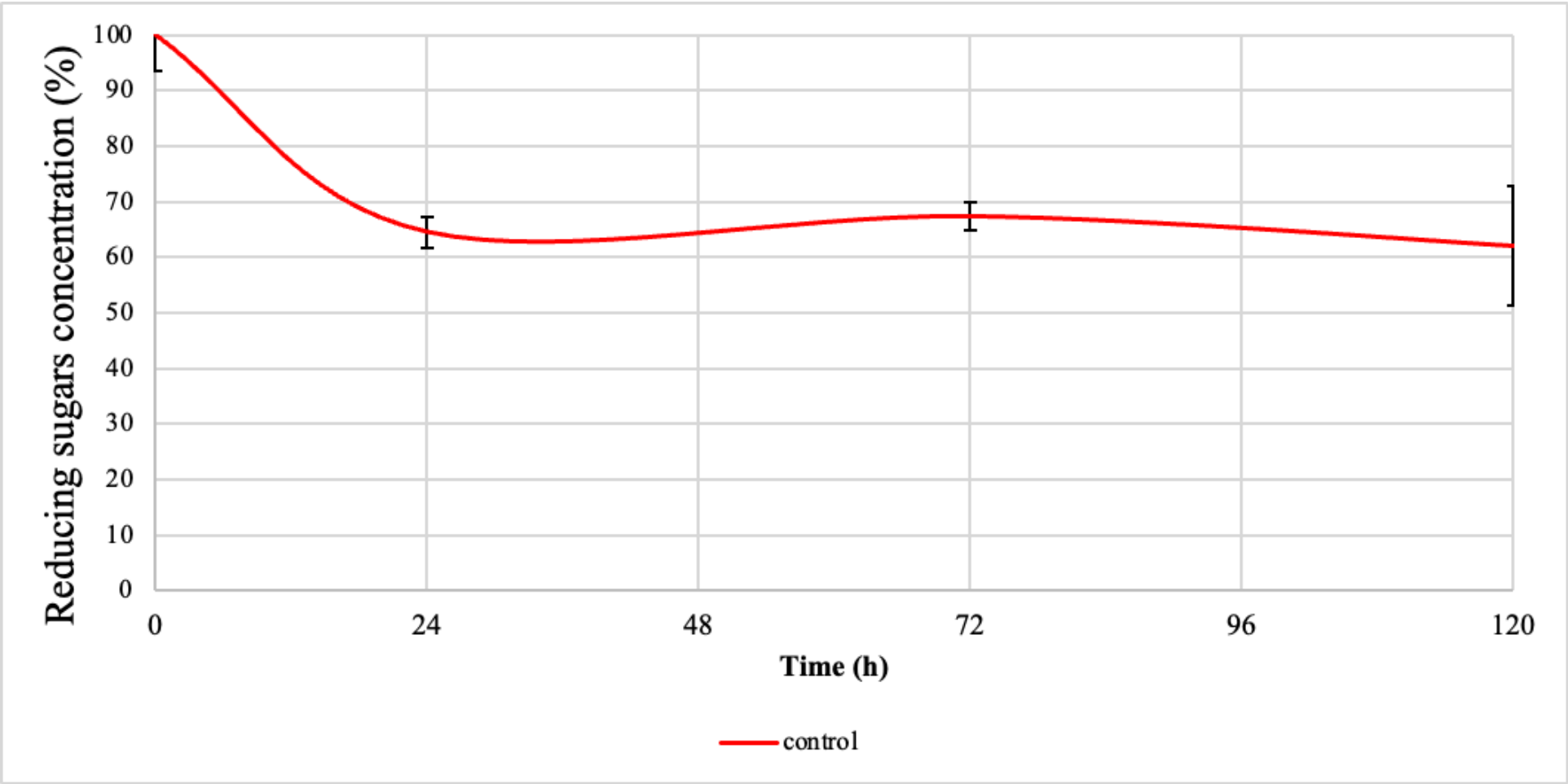
The control medium contained 4% glucose as the main carbon source and was prepared under nitrogen-limited conditions to promote lipid accumulation by *Yarrowia lipolytica*.

Tea waste, wheat straw, orange peel, and beetroot pulp extracts were used as carbon substrates.

The extracts were supplemented with mineral salts from the nitrogen-limited medium, including KH_2PO_4 , MgSO_4 , CaCl_2 , FeSO_4 , ZnSO_4 , and MnSO_4 . In the supplemented conditions, yeast extract and peptone were additionally added to the lignocellulosic extract solutions to support *Yarrowia lipolytica* growth.

RESULTS:

Control

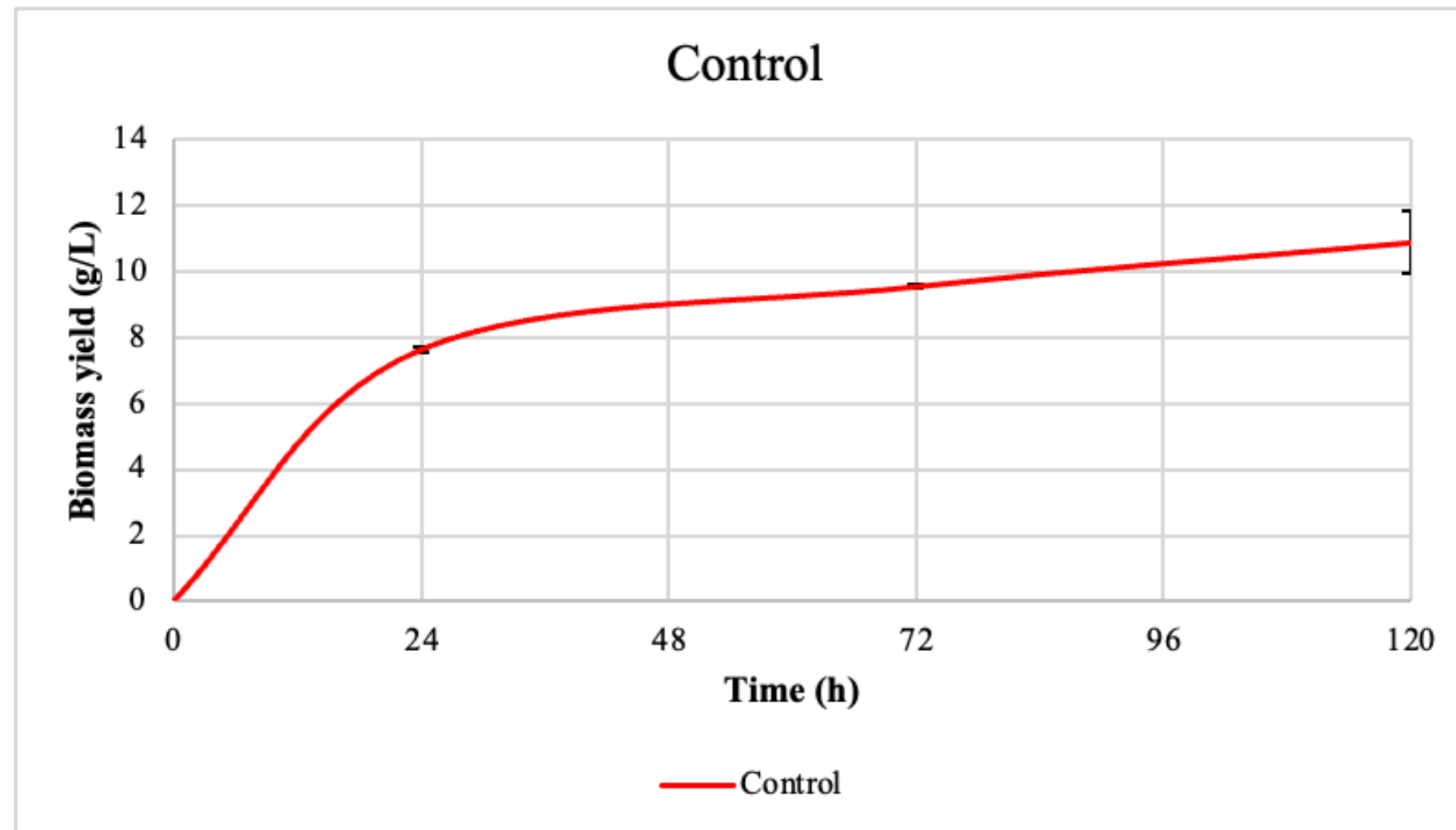


4% of Glucose N-limited Medium

Reducing sugar concentration decreased rapidly during the first 24 h, indicating initial glucose utilization by *Yarrowia lipolytica*. After 24 h, sugar consumption slowed and remained relatively stable, likely due to nitrogen limitation restricting biomass formation.

Figure 2. Reducing sugar utilization by *Yarrowia lipolytica* in control medium

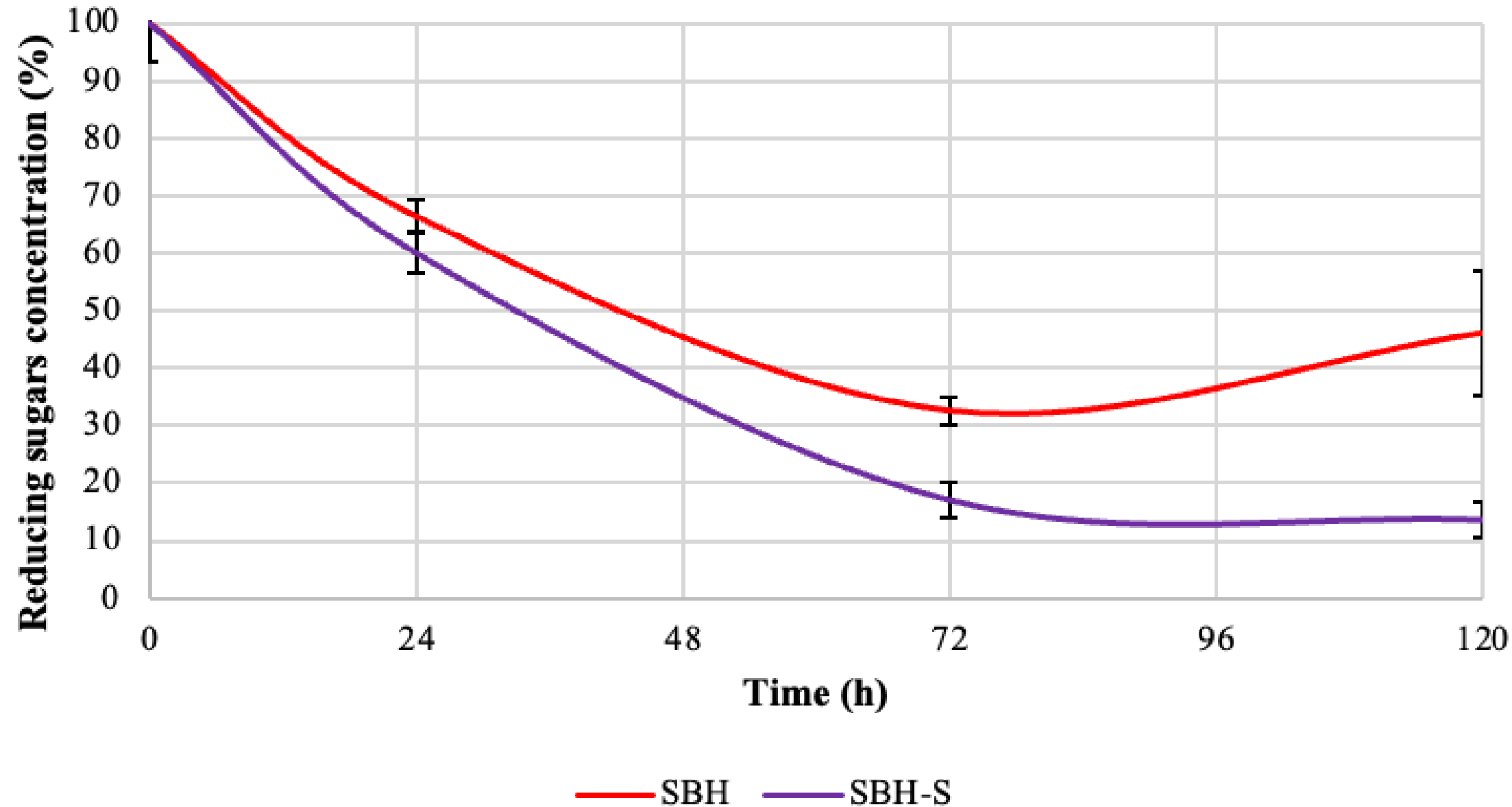
Control



Biomass increased rapidly during the first 24 h, indicating active cell growth. After this phase, growth continued at a slower rate, likely due to nitrogen limitation. Although high C/N conditions favor lipid accumulation in *Yarrowia lipolytica*, they may limit further biomass production over time.

Figure 2. Biomass production during fermentation of utilization by *Yarrowia lipolytica* in control medium

Sugar Beet Pulp



Reducing sugar concentration decreased during fermentation, indicating sugar utilization by *Yarrowia lipolytica*.

The supplemented medium (SBH-S) showed faster and greater sugar consumption than the non-supplemented medium (SBH).

The supplementation improved microbial activity and enhanced substrate utilization.

Figure 3. Changes in reducing sugar concentration during fermentation of sugar beet pulp with and without supplementation

Sugar Beet Pulp

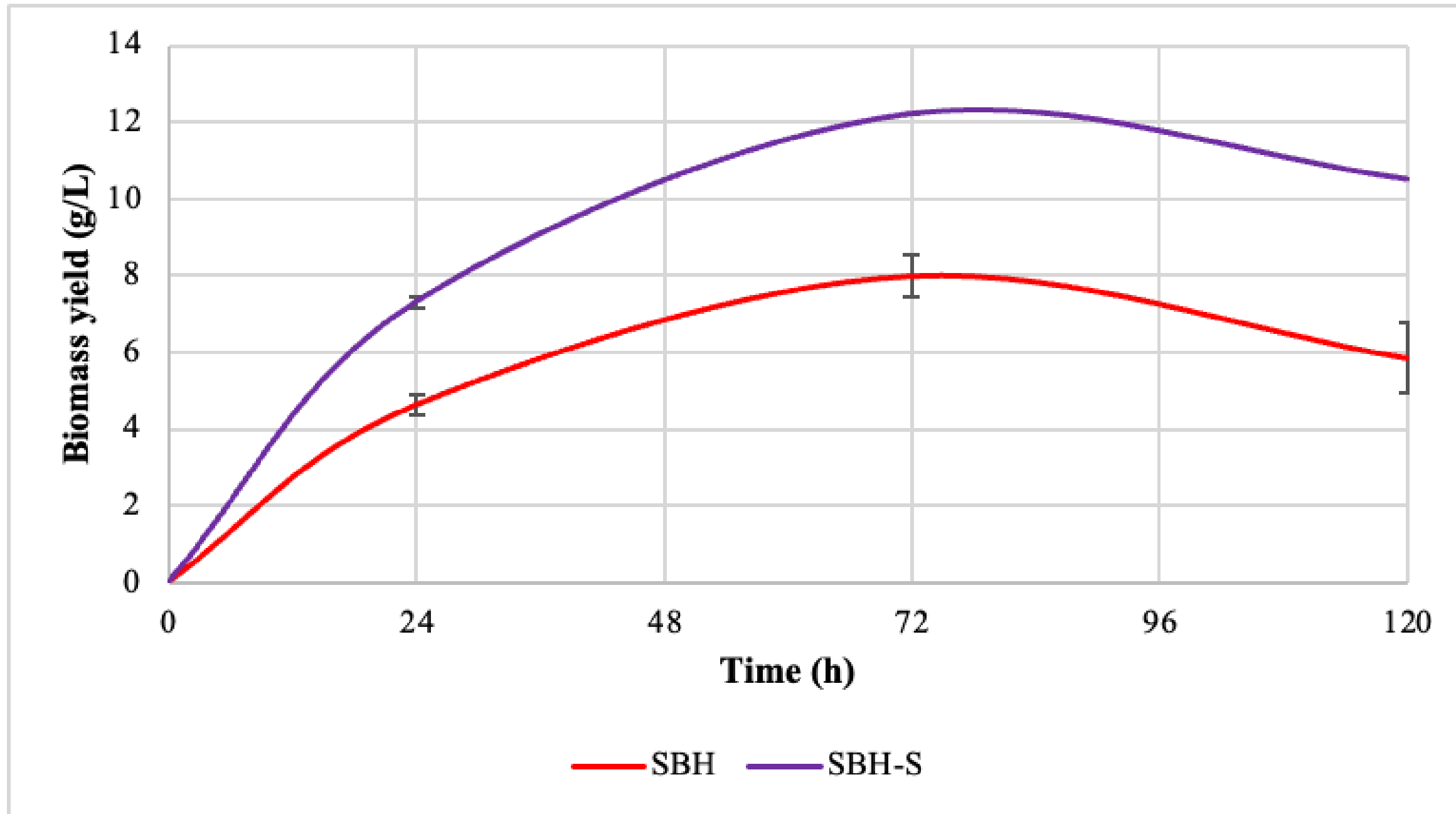


Figure 4. Biomass production during fermentation of hydrolysed sugar beet pulp with and without supplementation

Biomass yield increased over time in both conditions, with higher production observed in the supplemented medium (SBH-S).

The maximum biomass yield was reached around 72 h, likely due to maximum substrate utilization and the transition from active growth to the stationary phase.

After this point, nutrient depletion and metabolic stress may have limited further biomass accumulation.

Orange Peels

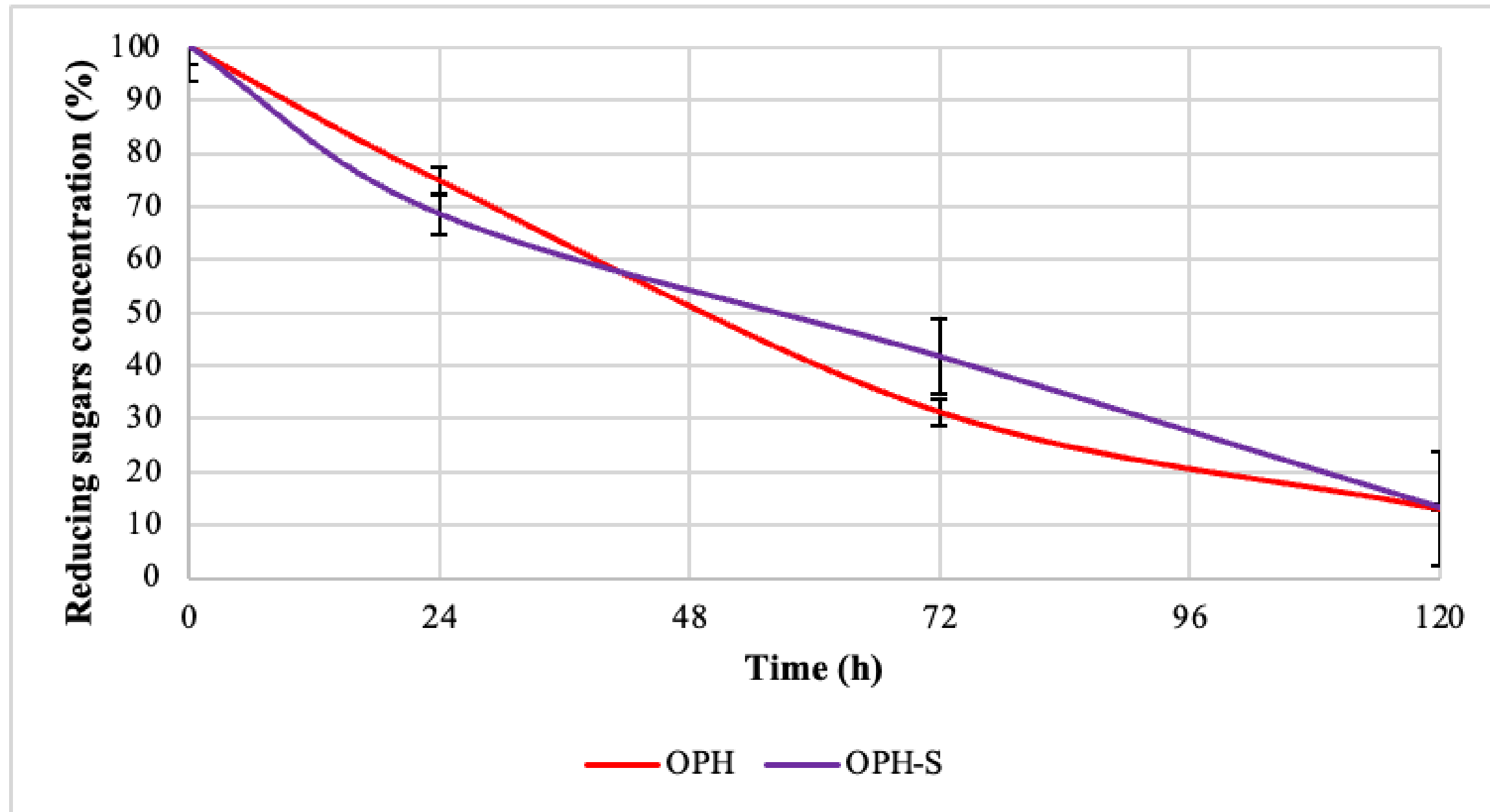
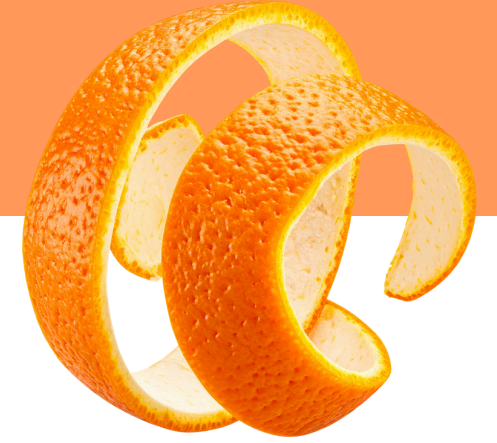


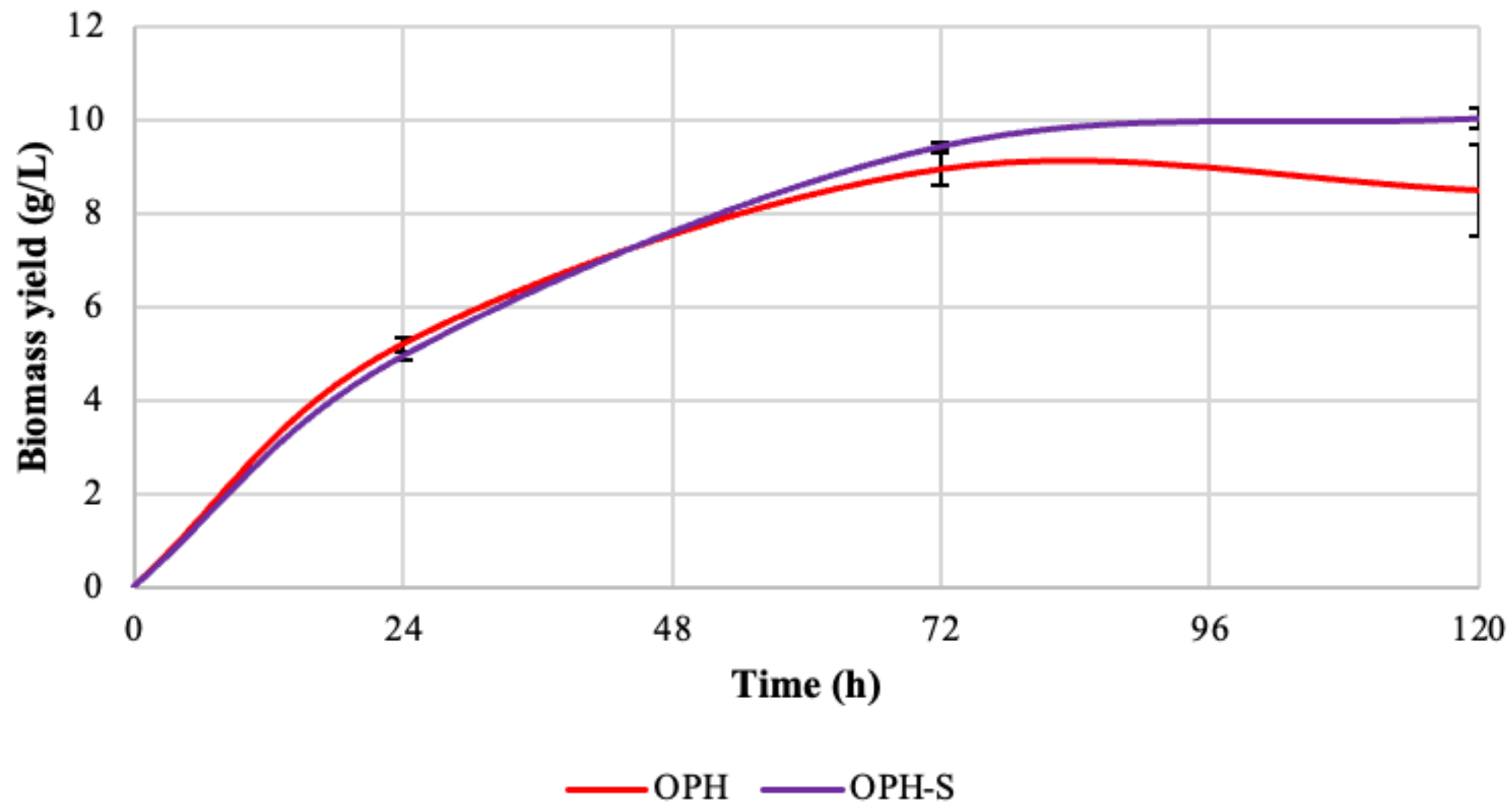
Figure 5. Changes in reducing sugar concentration during fermentation of orange peel hydrolysate with and without supplementation

Reducing sugar concentration decreased steadily over time in both conditions, indicating substrate utilization by *Yarrowia lipolytica*.

Sugar consumption was slightly faster in the non-supplemented medium (OPH), especially at later stages.

! This highlights that supplementation did not significantly enhance sugar uptake in this substrate.

Orange Peels



Biomass yield increased over time in both conditions, with slightly higher production observed in the supplemented medium (OPH-S). The maximum biomass was reached around 72–96 h, followed by a slight decline or stabilization.

This indicates that supplementation had a moderate positive effect on microbial growth in this substrate.

Figure 6. Biomass production during fermentation of orange peel hydrolysate with and without supplementation

Tea Waste

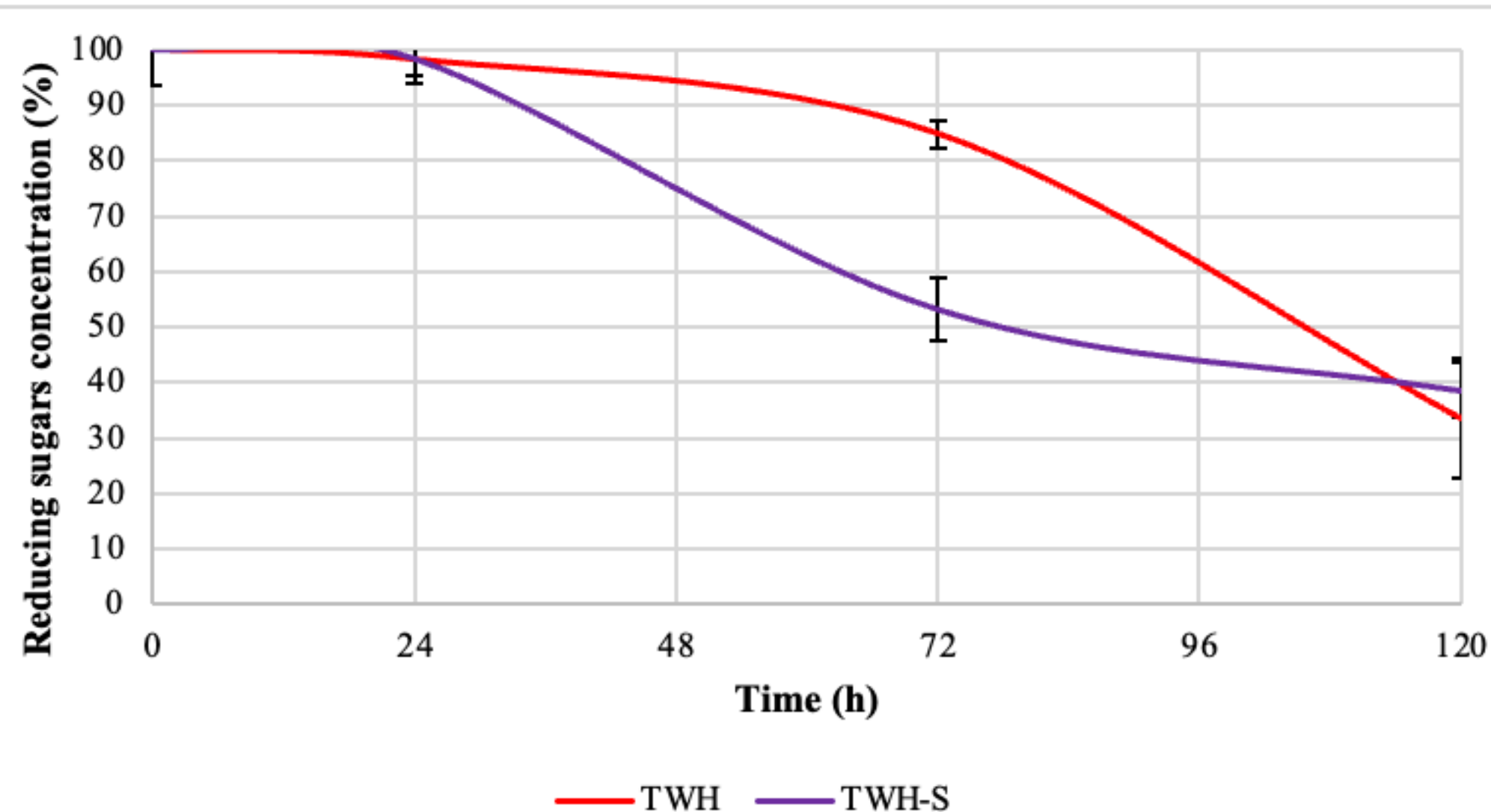
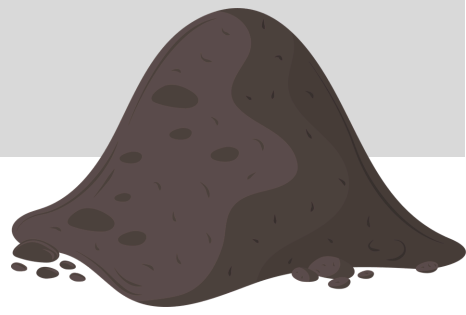


Figure 7. Changes in reducing sugar concentration during fermentation of tea waste hydrolysate with and without supplementation

Reducing sugar concentration decreased over time in both conditions, indicating substrate utilization by *Yarrowia lipolytica*. Sugar consumption was faster in the supplemented medium (TWH-S) during the early stages, while the non-supplemented medium (TWH) showed greater overall sugar depletion at later stages.

This suggests that supplementation enhanced initial uptake but did not improve long-term substrate utilization.

Tea Waste

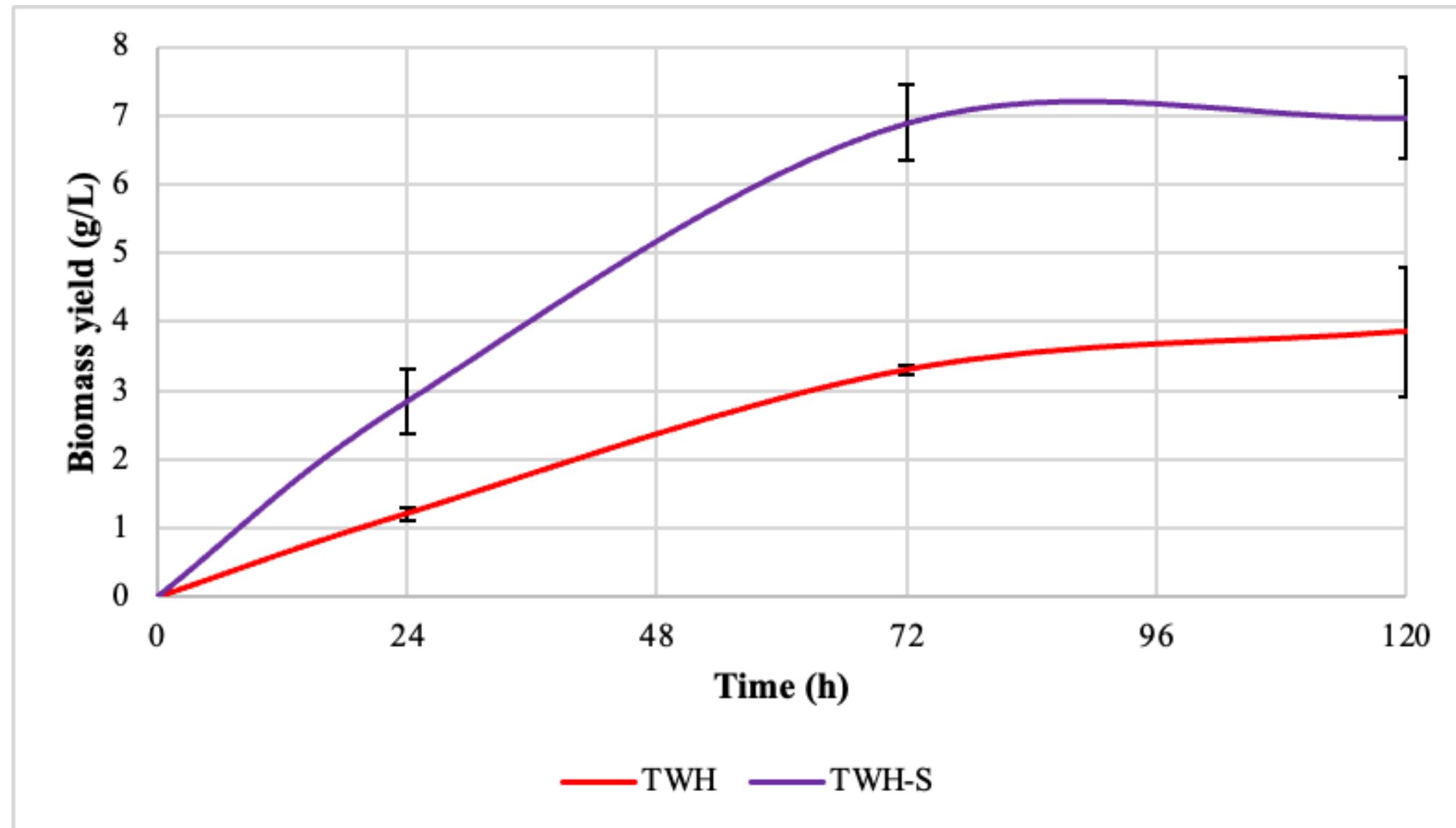
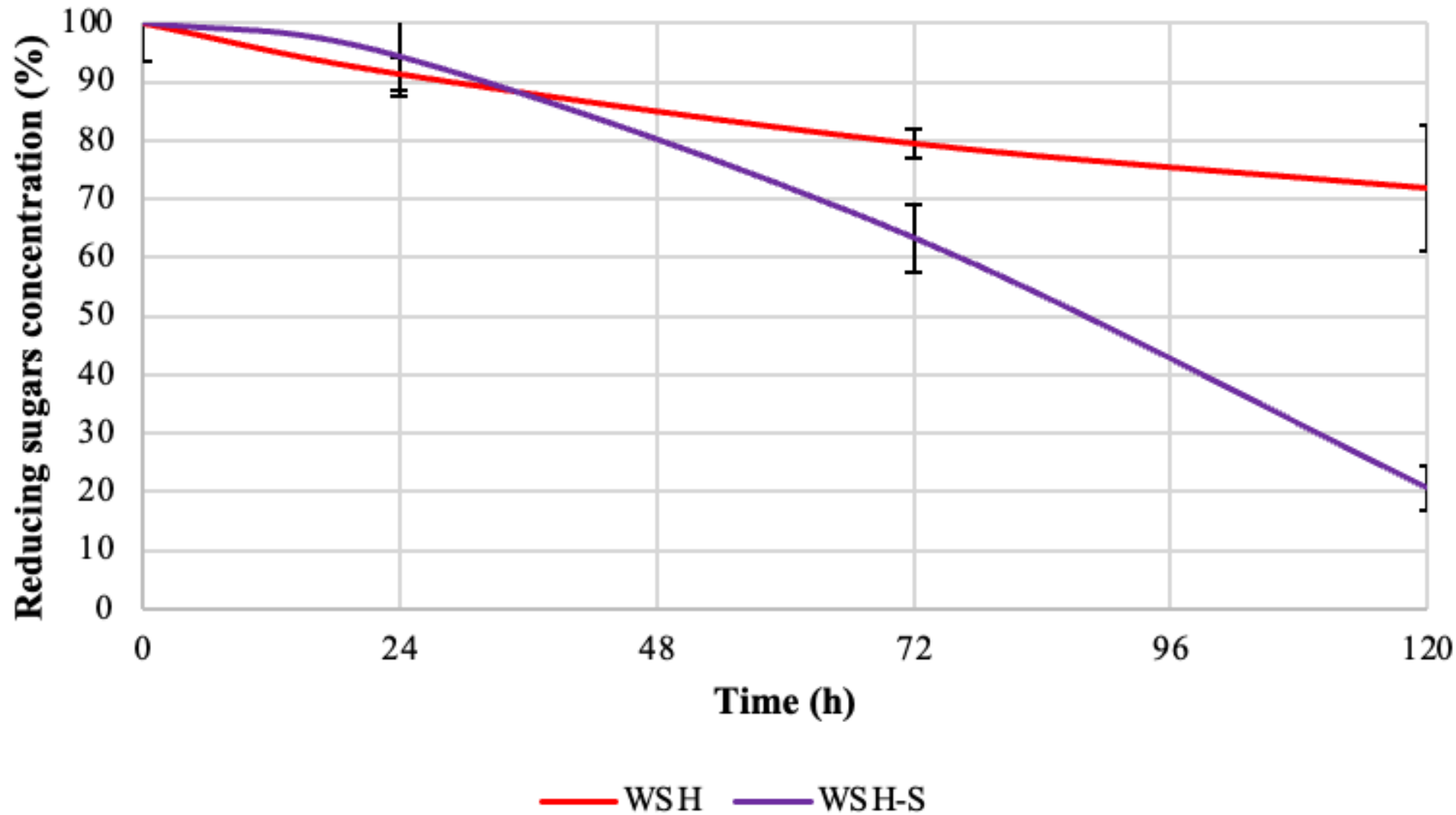


Figure 8. Biomass production during fermentation of tea waste hydrolysate with and without supplementation

Biomass yield increased over time in both conditions, with significantly higher production in the supplemented medium (TWH-S). The maximum biomass was reached around 72–96 h, followed by a slight stabilization. This indicates that supplementation strongly enhanced microbial growth and biomass formation in tea waste hydrolysate.

Wheat Straw



Reducing sugar concentration decreased in both conditions, with a markedly faster and greater reduction in the supplemented medium (WSH-S). This indicates enhanced substrate utilization by *Yarrowia lipolytica* in the presence of supplementation. The non-supplemented medium (WSH) showed limited sugar consumption over time.

Figure 9. Changes in reducing sugar concentration during fermentation of wheat straw hydrolysate with and without supplementation

Supplementation greatly improved reducing sugar utilization in wheat straw hydrolysate, suggesting enhanced metabolic activity of *Yarrowia lipolytica*.

Wheat Straw

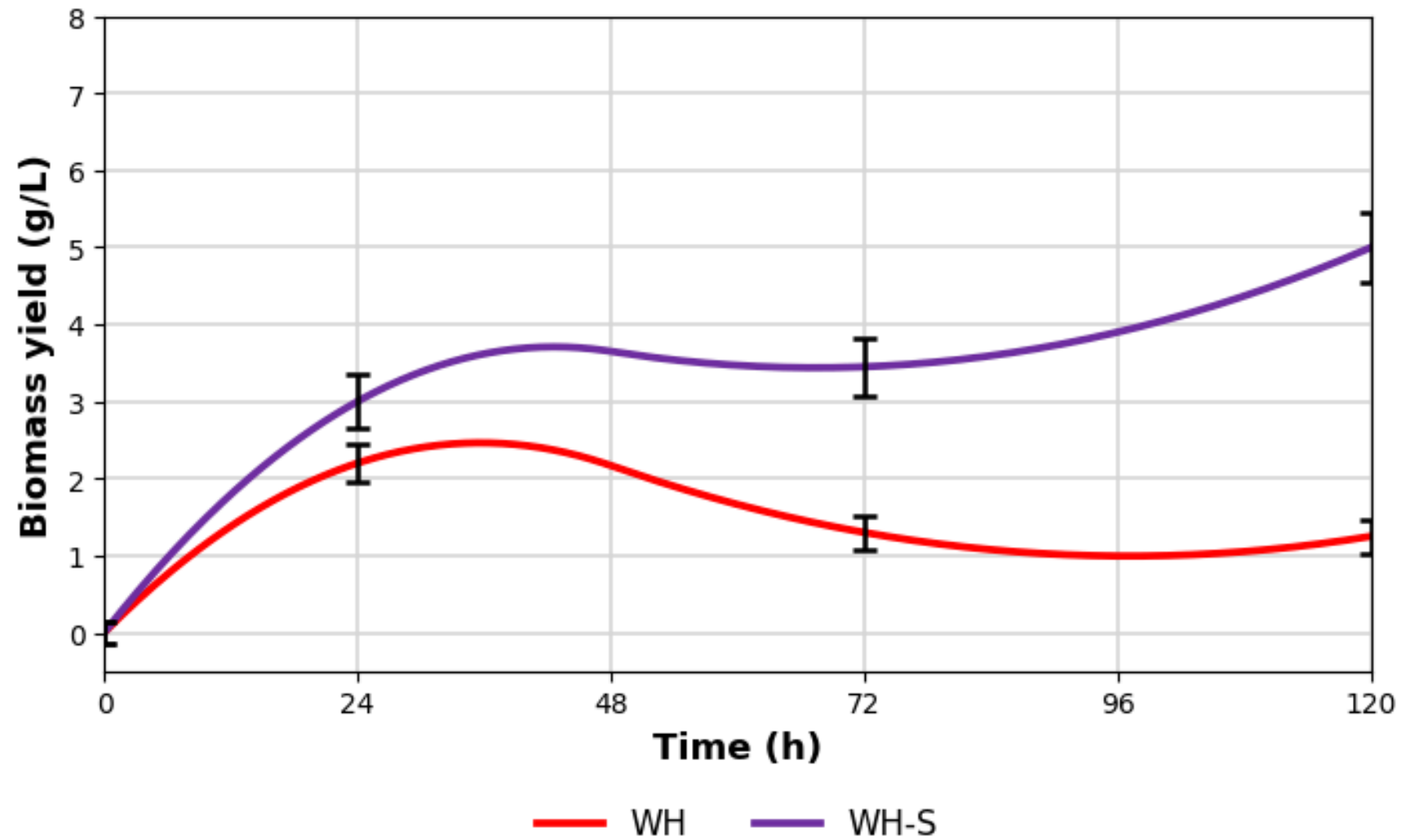


Figure 10. Biomass production during fermentation of wheat hydrolysate with and without supplementation

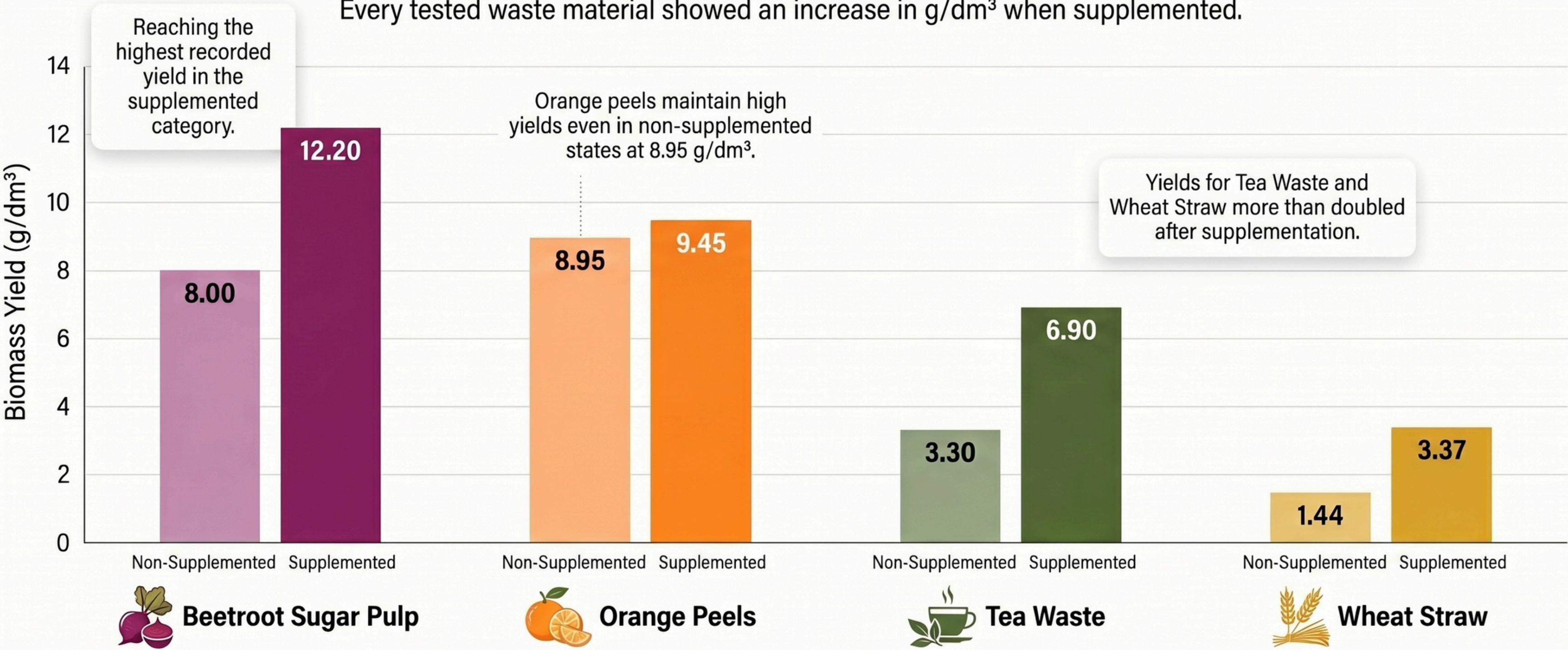
Biomass yield increased over time in both conditions, with higher production observed in the supplemented medium (WH-S). The supplemented culture showed a continuous increase in biomass, whereas the non-supplemented condition (WH) reached a low plateau after 24 h. These results suggest that supplementation improved microbial growth and biomass formation in wheat hydrolysate.

Boosting Biomass: Supplementation Effects on Lignocellulosic Waste

Demonstrating significant biomass yield increase (g/dm³) across various waste types when supplementation is applied.

Supplementation Drives Higher Yields

Every tested waste material showed an increase in g/dm³ when supplemented.



Lignocellulosic Waste Types

Application for the Food Product:

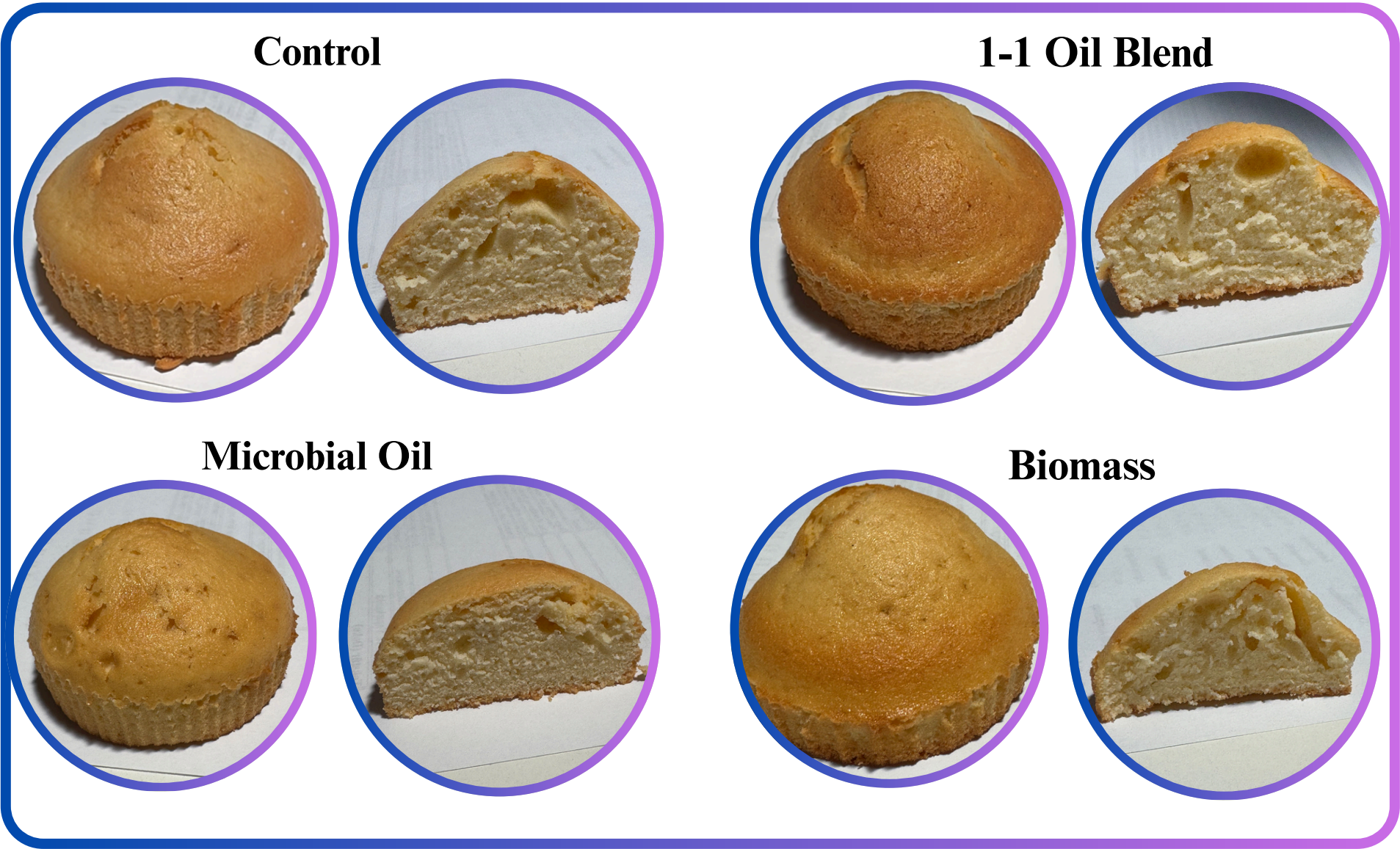
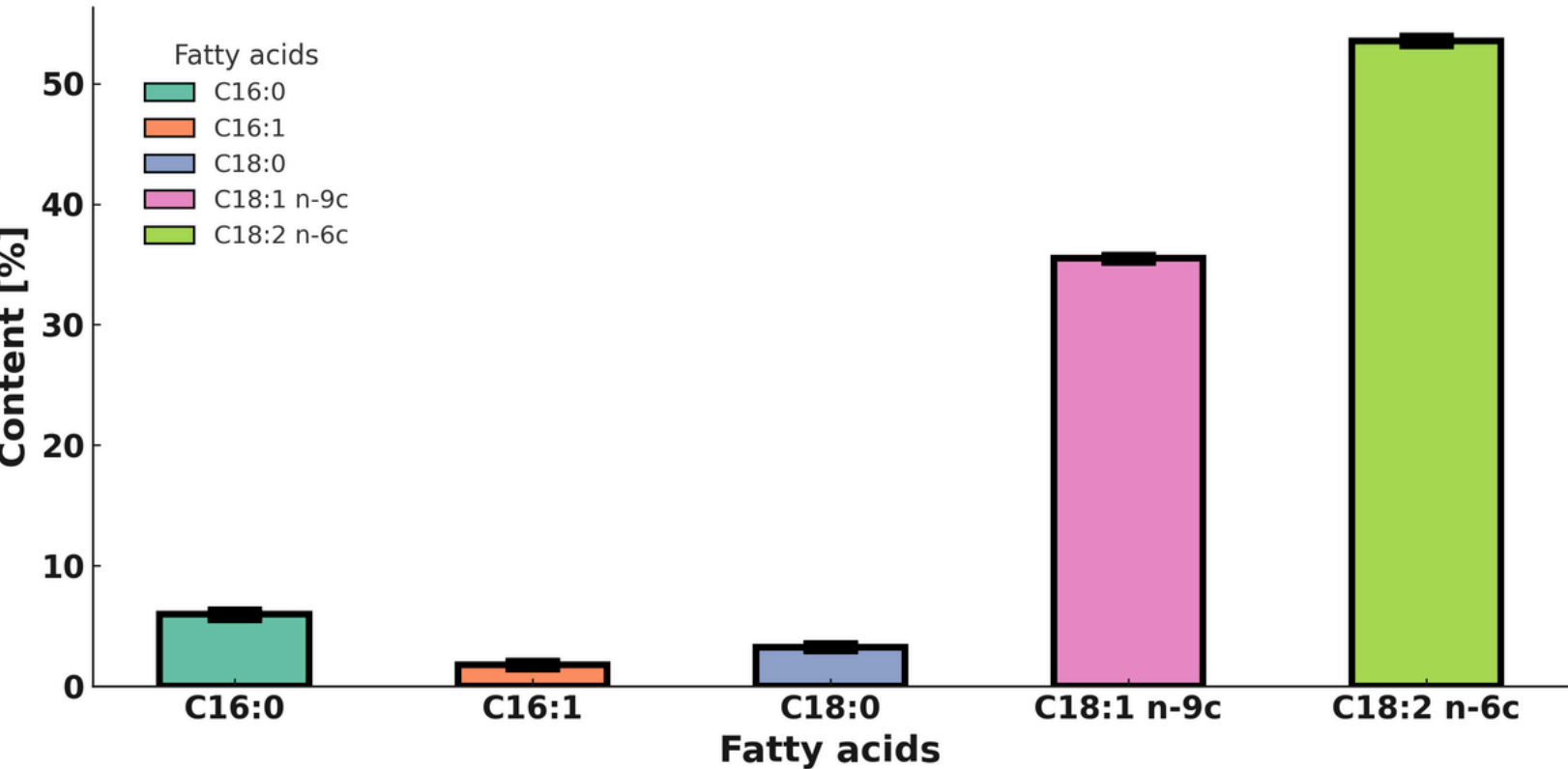
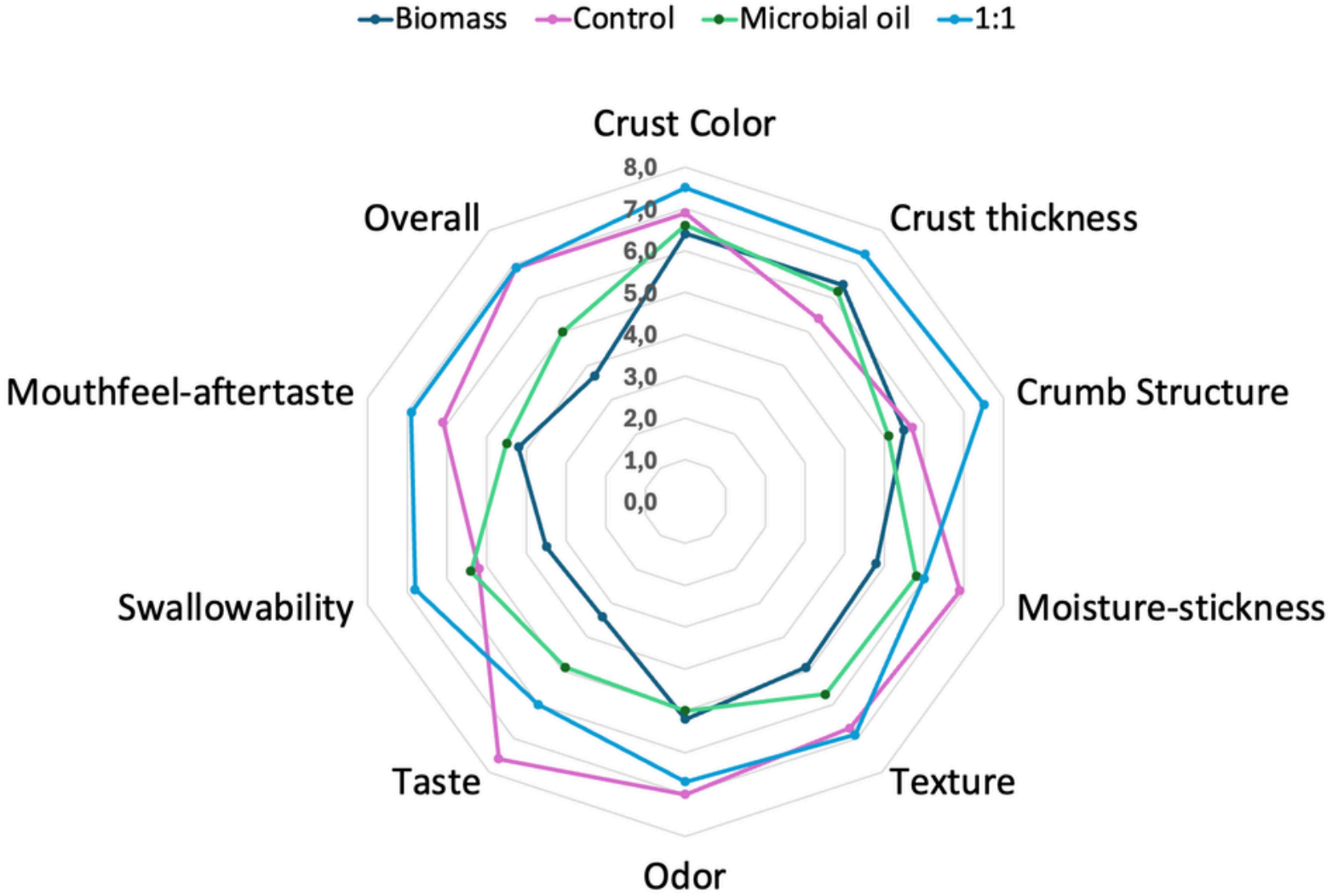


Figure 12. Effect of microbial oil and biomass incorporation on muffin quality, sensory properties, and fatty acid composition

Muffins formulated with oil-containing microbial biomass showed acceptable visual and sensory characteristics. The biomass may contribute to muffin structure due to its lipid content and could be considered as a partial fat-replacing ingredient. These results highlight the potential use of microbial biomass in sustainable bakery formulations.



Conclusion:



Yarrowia lipolytica was able to utilize different lignocellulosic hydrolysates as alternative carbon sources for biomass production. Among the tested substrates, beetroot pulp showed strong potential, likely due to its naturally higher nitrogen content, which may support microbial growth and protein formation.



Supplementation generally improved substrate utilization and biomass yield, especially in beetroot pulp. The increase in protein content of beetroot-derived biomass also suggests that beetroot pulp can be a promising substrate for producing nutritionally valuable microbial biomass.



thank you!



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