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Supplementary Materials

Ethanol Production from a Mixture of Waste Tissue Paper and Food Waste through Saccharification and Mixed-Culture Fermentation

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Citation: Ma, H.; Wang, Y.; Lv, P.; Zhou, J.; Gao, M.; Qian, D.; Song, B.; Wang, Q. Ethanol Production from a Mixture of Waste Tissue Paper and Food Waste through Saccharification and Mixed-Culture Fermentation. *Fermentation* **2024**, *10*, 194. https://doi.org/10.3390/fermentation10040194

Academic Editor: Konstantinos G. Kalogiannis

Received: 2 February 2024
Revised: 18 March 2024
Accepted: 19 March 2024
Published: 2 April 2024

1. Methods

1.1. Calculation Formula

The utilization rate of sugars and the yield of ethanol are calculated using the following formulas:

The sugar utilization rate is calculated as:

$$\text{Sugar Utilization Rate} = \frac{\rho(\text{consumed glucose}) + \rho(\text{consumed xylose})}{\rho(\text{initial glucose}) + \rho(\text{initial xylose})} \times 100\% \quad \text{(Equation 1)}$$

The ethanol conversion rate is determined by:

$$\text{Ethanol Conversion Rate} = \frac{\rho(\text{ethanol in the fermentation liquid})}{\rho(\text{consumed glucose}) + \rho(\text{consumed xylose})} \times 100\% \quad \text{(Equation 2)}$$

In these equations, ϱ denotes the concentration of the respective compounds in the fermentation medium. Equation 1 represents the efficiency with which the fermentative organism utilizes the available sugars (glucose and xylose), while Equation 2 quantifies the efficiency of converting these consumed sugars into ethanol.

1.2. Statistical Analysis

The experimental data were analyzed using SPSS 27 for Least Significant Difference (LSD) analysis and Analysis of Variance (ANOVA).

2. Results

The ethanol yields from four different types of fermentation substrates were analyzed using SPSS 27, with the results displayed in Table S1. It was observed that both the urea-added group and the mixed substrate fermentation group showed significant intergroup differences compared to the other two groups. This indicates that adding urea and FW during the waste paper fermentation process can enhance ethanol yield. The significance level between the urea-added group and the mixed substrate fermentation group was 0.012, signifying that the difference is statistically significant, though not as pronounced as in other comparisons. This suggests that adding urea and FW during the waste paper fermentation process can achieve similar effects, suggesting that FW can serve as a high-quality nitrogen source for fermentation.

Letting A represent *Saccharomyces cerevisiae* and B represent *Candida shehatae*, a two-way ANOVA was conducted using SPSS 27 to examine the effect of pH on ethanol production. The results are presented in Table S2. From Table S2, it can be observed that pH variation significantly impacts the ethanol yield of both *Saccharomyces cerevisiae* and

Candida shehata. Furthermore, by comparing the F-values and III sum of squares between groups A and B, it can be inferred that the effect of pH variation is more pronounced on *Candida shehata*. This finding corroborates the analysis results presented earlier regarding Figures 5.

The experimental results concerning temperature variations were analyzed using SPSS 27 through LSD analysis and ANOVA, with the findings presented in Table S3 and S4.

According to Table S3, the ANOVA reveals significant differences in yield among groups at different temperatures, indicating that temperature has a very significant impact on ethanol yield. From Table S4, it is evident that during the isothermal fermentation process, at a temperature of 33°C, the yield increased by 3.87% ($p < 0.01$) and 0.98% ($p = 0.011$) compared to the other two groups, indicating the highest ethanol yield at this temperature. Compared to all given isothermal conditions, the differences under non-isothermal fermentation conditions were significant, suggesting that non-isothermal fermentation can significantly enhance ethanol yield.

Table S1. Least Significant Difference Test for the results of the different substrates

(I) Group	(J) Group	Mean value difference (I-J)	Standard Error	Sig.	95% Confidence Interval	
					Lower Limit	Upper Limit
WTP	WTP with urea	-17.50000%*	0.44860%	<0.001	-18.5345%	-16.4655%
	WTP with FW	-18.95333%*	0.44860%	<0.001	-19.9878%	-17.9189%
	FW	-11.53333%*	0.44860%	<0.001	-12.5678%	-10.4989%
WTP with urea	WTP	17.50000%*	0.44860%	<0.001	16.4655%	18.5345%
	WTP with FW	-1.45333%*	0.44860%	.012	-2.4878%	-0.4189%
	FW	5.96667%*	0.44860%	<0.001	4.9322%	7.0011%
WTP with FW	WTP	18.95333%*	0.44860%	<0.001	17.9189%	19.9878%
	WTP with urea	1.45333%*	0.44860%	.012	0.4189%	2.4878%
	FW	7.42000%*	0.44860%	<0.001	6.3855%	8.4545%
FW	WTP	11.53333%*	0.44860%	<0.001	10.4989%	12.5678%
	WTP with urea	-5.96667%*	0.44860%	<0.001	-7.0011%	-4.9322%
	WTP with FW	-7.42000%*	0.44860%	<0.001	-8.4545%	-6.3855%

***. The significance level of the difference in mean values is 0.05.**

Table S2. Analysis of variance for the results of the pH

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Source
Corrected Model	A	34.098a	3	11.366	66.296	＜0.001
	B	109.168b	3	36.389	168.074	＜0.001
Intercept	A	14819.944	1	14819.944	86443.068	＜0.001
	B	16030.830	1	16030.830	74042.554	＜0.001
pH	A	34.098	3	11.366	66.296	＜0.001
	B	109.168	3	36.389	168.074	＜0.001
Error	A	1.372	8	.171		
	B	1.732	8	.217		
Total	A	14855.413	12			
	B	16141.730	12			
Corrected Total	A	35.469	11			
	B	110.900	11			

a R Squared = 0.961

b R Squared = 0.984

Table S3. Analysis of Variance for the results of the different Temperature

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Between Groups	124.767	3	41.589	309.404	＜0.001
Within Groups	1.075	8	0.134		
Total	125.843	11			

Table S4. Least Significant Difference Test for the results of the different Temperature

(I) Group	(J) Group	Mean value difference (I-J)	Standard Error	Sig.	95% Confidence Interval	
					Lower Limit	Upper Limit
30℃	33℃	-3.87667%*	0.29935%	＜0.001	-4.5670%	-3.1864%
	35℃	-2.89333%*	0.29935%	＜0.001	-3.5836%	-2.2030%
	Non-Isothermal	-8.93667%*	0.29935%	＜0.001	-9.6270%	-8.2464%
33℃	30℃	3.87667%*	0.29935%	＜0.001	3.1864%	4.5670%
	35℃	0.98333%*	0.29935%	.011	0.2930%	1.6736%
	Non-Isothermal	-5.06000%*	0.29935%	＜0.001	-5.7503%	-4.3697%
35℃	30℃	2.89333%*	0.29935%	＜0.001	2.2030%	3.5836%
	33℃	-0.98333%*	0.29935%	.011	-1.6736%	-0.2930%
	Non-Isothermal	-6.04333%*	0.29935%	＜0.001	-6.7336%	-5.3530%
Non-Isothermal	30℃	8.93667%*	0.29935%	＜0.001	8.2464%	9.6270%
	33℃	5.06000%*	0.29935%	＜0.001	4.3697%	5.7503%
	35℃	6.04333%*	0.29935%	＜0.001	5.3530%	6.7336%

*. The significance level of the difference in mean values is 0.05.