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BATCH FERMENTATION STUDY FOR BIOETHANOL PRODUCTION USING PLUM EXTRACT AS SUBSTRATE

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ABSTRACT

Bioethanol production using Batch Fermentation process was investigated in this study. Parts of plum fibre were sundried. The dried samples were crushed into fine particles, weighed and extracted to the batch reactor. Fourteen yeasts were isolated from palm oil mill effluents impacted soil. Out of the 14 isolates, 2 (*Saccharomyces cerevisiae* and *Candida* sp) showed high bioethanol production potentials and were thus selected for further studies. Between these 2 isolates, *Saccharomyces cerevisiae* produced bioethanol more than *Candida* sp. Further optimization using Michaelis-Menten model revealed that pH (4 to 7.0), incubation time (1st week to 10th week), inoculum size (5 to 15%) and carbon: nitrogen ratio (40 to 44 %) affected the following responses (reducing sugar concentration, yeast biomass and ethanol) involved in the batch fermentation of the plum extract to bioethanol. Bioethanol production increased from 0.87mg/l at day 7 to 6.54mg/l at day 63 and remained stable till 70th day with the activity of *Saccharomyces cerevisiae* while that of *Candida* sp increased from day 7 to day 63 to be 4.83mg/l and remained stable till day 70, both at the optimal pH of 6 and yeast inoculum size of 10. Modelling of the biomass as subscript during the batch fermentation over a period of 70 days for the activity of *Saccharomyces cerevisiae* showed that bioethanol production followed a linear model with U_s and K_s for *Saccharomyces cerevisiae* and were evaluated as 0.10423mg/l and 4.695mg/l, R^2 = 0.8471 and that of *Candida* sp fermentation process was limited compared to *saccharomyces cerevisiae*. Findings from the analytical procedures revealed that there was ethanol in the fermentation broth. A comparison between biomass production predicted by the Michaelis-Menten equation decreased from 4.1526 mg/l at 70th day to 2.7852 mg/l on the 7th day and that of *Candida* sp decreased from 0.138mg/l gradually to 0.023 mg/l on the 7th day respectively. The analytical procedures of FTIR, GCMS, and HPLC all aided the characterization of the fermented product as ethanol. The physiochemical components of the bioethanol produced from the analysis are effective to be used. Therefore, the study had demonstrated that ethanol was produced by *Saccharomyces cerevisiae* through batch fermentation of the plum extract.
Keywords: Plum Extract, Batch Fermentation, Substrate, Bioethanol, Kinetic

1.0 INTRODUCTION

Research has risen rapidly over the globe in response to the pressing need to lessen our reliance on fossil fuel products and make all relevant data publicly accessible on viable alternatives to conventional, nonrenewable energy sources. Also, in order to ascertain an effective alternative to non-renewable energy sources and the processes sustainable and environmentally friendly has brought about the study of biofuel (Bilyaminu *et al*., 2016). Renewable energy is expanding rapidly due to environmental sustainability concerns and the depletion of fossil fuel resources. The International Energy Agency reported a steady increase in global renewable energy demand, highlighting the importance of alternative energy sources such as bioethanol (Maity *et al*., 2011). Bioethanol is an oxygenated, renewable liquid fuel capable of reducing greenhouse gas emissions and improving combustion efficiency when blended with gasoline or used alone (Deenanath *et al*., 2013; Tiwari *et al*., 2014). Although first-generation bioethanol feedstocks compete with food resources, second-generation lignocellulosic materials and agricultural wastes provide sustainable and cost-effective alternatives (Bušić *et al*., 2018). Plum (*Prunus salicina*) fibre is an underutilized agricultural resource that does not significantly compete with human food supplies and is widely available in tropical regions (Deenanath *et al*., 2012; Esua *et al*., 2016). Despite its medicinal and nutritional value, its potential for bioethanol production remains largely unexplored. With declining petroleum production and increasing environmental concerns, biomass-derived fuels such as bioethanol have gained attention as viable energy alternatives (Bhaskar *et al*., 2011; Tiwari *et al*., 2014). Consequently, this study aims to produce bioethanol from plum fibre through batch fermentation using *Saccharomyces cerevisiae* and *Candida* species (Bušić *et al*., 2018).

2.0 MATERIALS AND METHOD

2.1 Materials and Equipment

Plum (*Prunus salicina*) fibre was used as the substrate for bioethanol production, with *Saccharomyces cerevisiae* and *Candida* sp. as fermenting yeasts. Laboratory equipment included a pH meter, spectrophotometer, magnetic stirrer, autoclave, incubator,

refrigerator, conical flasks, beakers, Petri dishes, test tubes, centrifuge, batch reactor, grinder, and weighing balance.

2.2 Fermentation of Plum Extract

Batch fermentation was carried out using *S. cerevisiae* under standard laboratory conditions. Parameters were set at pH 6, working volume of 250 mL, inoculum size of 10%, carbon-to-nitrogen ratio of 42, and incubation for 70 days. Bioethanol production was monitored and analyzed using GC–MS. Fermentation kinetics were evaluated through mathematical modeling, and experimental data were statistically analyzed using ANOVA, with graphical representation of results.

2.3 Analytical Methods

Samples were collected every 7 days to assess yeast biomass, reducing sugar concentration, bioethanol production, and carbon dioxide evolution using standard analytical procedures.

2.3.1 Yeast Biomass Estimation

Batch fermentation of plum extract using *Saccharomyces cerevisiae* was conducted at pH 6, with a 250 mL working volume, 10% inoculum, C/N ratio of 42, and 70 days of incubation. Bioethanol production was analyzed by GC–MS, fermentation kinetics were modeled mathematically, and data were statistically evaluated using ANOVA. Alcohol content was determined through titration, with blank (VB) and sample (VA) titres recorded and calculated using the standard formula.

$$\text{Ethanol(\% vol/vol)} = 25 - 25 \times \frac{\text{VA}}{\text{VB}} \quad (2.1)$$

2.4 First order fermentation rate kinetics

The first-order kinetic model is widely used to describe batch fermentation processes, though literature often presents the model in its final form without detailing the derivation (Agarry *et al*., 2013; Agarry *et al*., 2015; Nwankwegu *et al*., 2016; Aghalibe *et al*., 2017; Asgari *et al*., 2017; Amagbo & Ere, 2019). The derivation of the fermentation rate model is presented to emphasize its development. Based on the mass balance principle, the bio-kinetic model predicts bioethanol production at any time during batch fermentation, as expressed in Equation (2.2). Inflow of mass into system $= Q_o C_{EH(o)}$(2.3)

Outflow of mass from system $= QC_{EH}$ (2.4)

Rate of EH fermentation $= -r_{EH}V$ (2.5)

Rate of accumulation = $\frac{d(C_{EH}V)}{dt}$ (2.6) where: Q_o = Inlet volumetric flow rate (kg/day), Q = Outlet volumetric flow rate (kg/day), $C_{TPH(o)}$ = Initial concentration of Pollutant (TPH) (mg/kg), C_{EH} =Instantaneous concentration of fermentation (EH) (mg/l), V = Volume of reactor (m^3), r_{EH} = Rate of EH fermentation (mg/l.day), k_E = EH fermentation rate constant (day^{-1}) t = Time of EH fermentation (day), Now, substituting Equation (2.3), (2.4), (2.5) and (2.6), gives the equation;

$$Q_o C_{EH(o)} = QC_{EH} - r_{EH}V + \frac{d(C_{EH}V)}{dt} \quad (2.7)$$

The system in which the fermentation takes place is considered as batch reactor, and in batch reactor, there is no inflow and outflow of mass into and out of the reactor. Therefore, the flow terms in the mass balance equation are neglected.

Thus, $Q_o C_{EH(o)} = QC_{EH} = 0$ (2.8)

Also, for a batch process, the volume of reactor (the vessel used for the fermentation process) is constant. Hence, the accumulation term can be expressed as:

$$\frac{d(C_{EH}V)}{dt} = V\frac{dC_{EH}}{dt} \quad (2.9)$$

Therefore, equation (2.16) reduces to

$$-r_{EH}V = -V\frac{dC_{EH}}{dt}$$

Or $$-r_{EH} = -\frac{dC_{EH}}{dt} \quad (2.10)$$

But for first order kinetics, the rate of fermentation can be expressed according to Equation (2.10).

$$-r_{EH} = -\frac{dC_{EH}}{dt} = k_d C_{EH} \quad (2.11)$$

By integrating Equation (2.11) using the separation of variable method, we obtain as follows.

$$\int_{C_{EH(o)}}^{C_{EH(t)}} \frac{dC_{EH}}{C_{EH}} = -k_E \int_0^t dt \quad (2.12)$$

$$ln\left(\frac{C_{EH(t)}}{C_{EH(o)}}\right) = -k_E t \quad (2.13)$$

$$ln\, C_{EH(t)} - ln\, C_{EH(o)} = -k_E t$$

$$ln\, C_{EH(t)} = ln\, C_{EH(o)} - k_E t \quad (2.14)$$

A plot of $ln\, C_{EH(t)}$ against t, gives a linear (straight line) graph with slope equivalent to "k_E" and intercept equivalent to $ln\, C_{EH(o)}$.

However, to obtain the instantaneous EH concentration, exponential of both sides of equation (2.14) is taken to give:

$$C_{EH(t)} = C_{EH(o)} e^{-k_E t} \quad (2.15)$$

Equation (2.15) is used to predict the concentration of EH remaining in the fermentation at any time.

2.5 Michaelis-Menten Equation

The fermentation rate of EH was also studied using Michaelis-Menten Equation, which is expressed as:

$$-r_{EH} = -\frac{dC_{EH(t)}}{dt} = \frac{\mu EH(t)_{max}}{K_E + C_{EH(t)}} \quad (2.16)$$

Equation (2.27) is linearized to obtain the maximum specific rate constant and the Michaelis-Menten constant according to Equation (2.16).

$$-\frac{1}{r_{EH}} = \frac{1}{\mu_{max}\frac{K_E}{\mu_{max}\left(\frac{1}{C_{EH(t)}}\right)}} \quad (2.17)$$

where: $\mu_{\max}$ is the maximum specific fermentation bioethanol rate (mg/l.day), $C_{EH(t)}$ is EH concentration (mg/l) with time *t*, (day) K_E is Michaelis-Menten constant relating to fermentation rate (mg/l).

A plot of $-\frac{1}{r_{EH}}$ against $\frac{1}{C_{EH(t)}}$ gives the slope as $\frac{K_E}{\mu_{max}}$ and $\frac{1}{\mu_{\max}}$ as intercept.

3.1 RESULTS AND DISCUSSION

3.1.1 Identification of yeasts isolated from palm oil polluted soil

The characteristics of yeast isolates from palm oil mill effluent are summarized in Table 3.1. All isolates fermented galactose and glucose, while none tested positive in the germ tube assay. Urea fermentation was observed only in isolates S4, S6, and S13, which were identified as *Saccharomyces* spp. Overall, two genera were identified: *Candida* spp. (S3, S12, S14) and *Saccharomyces* spp. (S4, S6, S13) (Table 3.1).

Table 3.1: Sensitivity of fungal isolates from palm oil polluted soil

Isolate	Galact	Sucros	Germ	Lactos	Glucos	Urease	Probable genera
S_3	+	+	_	_	+	_	*Candida* sp.
S_4	+	_	_	+	+	+	*Saccharomyces* sp.
S_6	+	_	_	+	+	+	*Saccharomyces* sp.
S_{12}	+	+	_	_	+	_	*Candida* sp.
S_{13}	+	_	_	+	+	+	*Saccharomyces* sp.
S_{14}	+	+	_	_	+	_	*Candida* sp.

3.1.2 Screening characteristics of the isolates for bioethanol production

The yeast isolates were screened for their capacity to produce bioethanol by carrying out plate hydrolysis. Screening characteristics of the isolates are given in Table 3.2. From the table, isolates S_3, S_4, S_6, S_{12}, S_{13}, and S_{14} gave positive result to the screening test. Isolates S_4, S_6 and S_{13} were later identified to belong to the same genus whereas S_3, S_{12} and S_{14} belonged to a separate genus. Represented in Table 3.2 below;

Table 3.2: Screening characteristics of the isolates for bioethanol production

Isolate	Test
S_1	-
S_2	-
S_3	+
S_4	+
S_5	-
S_6	+
S_7	-
S_8	-
S_9	-
S_{10}	-
S_{11}	-
S_{12}	+
S_{13}	+
S_{14}	+

Legend: S_3: *Candida* sp.; S_{13}: *Saccharomyces cerevisiae*

3.1.3 Substrate selection for biomass and bioethanol production

The best combinations for plum substrate and corn steep liquor substrates for bioethanol and yeast biomass production were examined. Plum extract concentration of 20% w/v in combinations with 0.1, 0.2, 0.3, 0.4, 0.5, 1, 2, and 3 % w/v concentrations of corn steep liquor were investigated. The result showed that 20% concentration of plum extract and 2% of corn steep liquor gave the best bioethanol and yeast biomass production for both *Candida* sp and *Saccharomyces cerevisiae* with *Saccharomyces cerevisiae* in mineral salt medium (MSM) having higher bioethanol and biomass production. This is seen in Figures 3.1 and 3.2.

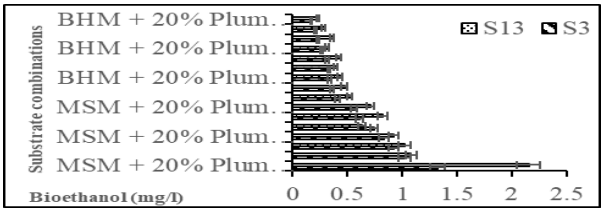


Figure 3.1: Effect of different substrate combinations on the production of bioethanol

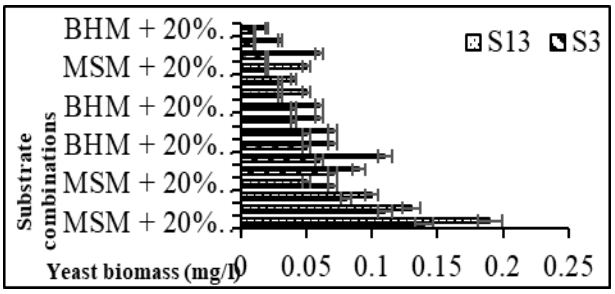


Figure 3.2: Effect of different substrate combinations on the production of biomass

3.2 Proximate analysis (chemical characteristics) of the plum substrate

The chemical characteristics of the plum extract as substrate used for the production of the bioethanol in the study were determined. The carbohydrate content was 64.2% while the moisture content was 26.52% as represented in Table 4.3 below shows the proximate composition of the plum, including protein, fibre, lipid and ash contents.

Table 3.3: Proximate composition of the plum substrate used in the study

Sample	Plum (%)
Ash	2.48
Moisture Content	26.52
Crude Protein	0.36
Crude Fiber	4.71
Crude Lipid	1.73
Carbohydrate	64.2

3.3 Physiochemical result

The physicochemical properties of the produced bioethanol were determined and compared to absolute ethanol (Table 3.3). The bioethanol had a slightly higher density (0.9 g/mL) than absolute ethanol (0.78 g/mL) but a lower API gravity. Its flash point (17.3 °C) was higher than that of absolute ethanol.

Table 3.4: Physiochemical characteristics of the bioethanol produced in the study against commercially sold absolute ethanol

Sample	Bioethanol	Commercial Absolute Ethanol
Density (g/ml)	0.90	0.78
API Gravity	25.17	49.91
Viscosity at 40°C ($mPa.s^{-1}$)	0.68	0.74
Flash Point (°C)	17.3	12.8
Colour	Clear	Clear
pH	5.1	6.4
Calorific Value (MJ/kg)	23.2	26.4

cx3.4Characterization of the product obtained from batch fermentation of the plum extract using *Saccharomyces cerevisiae* distillate (Figure 4.3).

3.4.1 FTIR analysis of the fermentation distillate

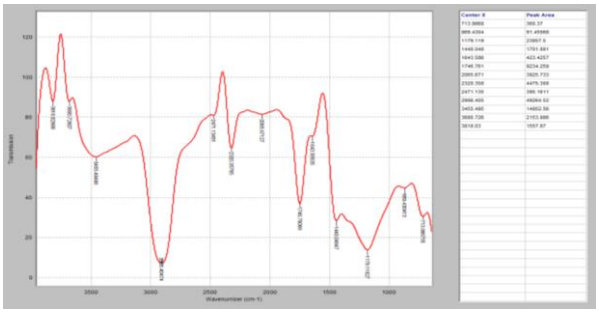


Figure 3.3: FTIR spectrum of the product from the fermentation broth by *Saccharomyces cerevisiae*.

3.4.2 GC-MS analysis of the fermented product

GC–MS analysis of the distillate revealed the presence of 12 fatty acid–related compounds. GC–MS analysis identified 12 compounds in the distillate, with 9,12-octadecadienoic acid (Z, Z-) as the predominant component (40.39%, retention time 19.338 min). Other compounds included linoelaidic acid (9.84%), 9,19-eicosadiene (2.47%), and 3Hpyrazol-3-one, 4-benzyl-2,4-dihydro-5-methyl-2-phenyl- (1.15%), while dimethyl sulfoxide was present at the lowest concentration (0.69%, retention time 7.087 min). The GC–MS chromatogram (Figure 3.4) shows the highest peak at 19.338 min, and mass spectra for the identified components are presented in Figures 3.5–3.10.

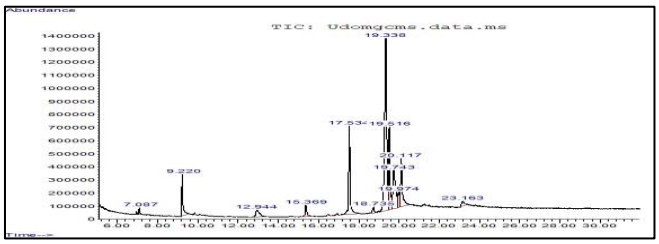
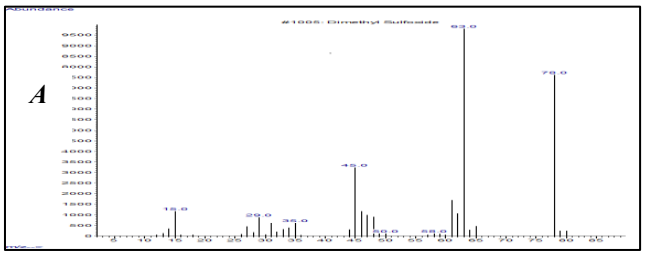


Figure 3.4: GC-MS chromatogram of the fermented product in the study



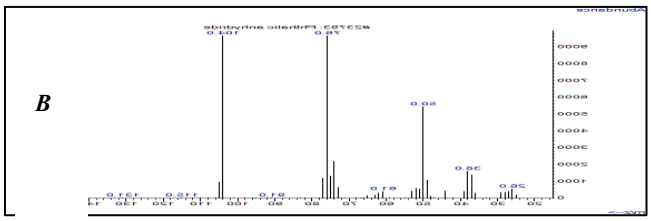


Figure 3.5: Mass spectrum of batch production of bioethanol from the plum extract by *Saccharomyces cerevisiae* for the resolution of (*A*) hit 1 (dimethyl sufoxide) and (*B*) hit 2 (Phthalic anhydride)

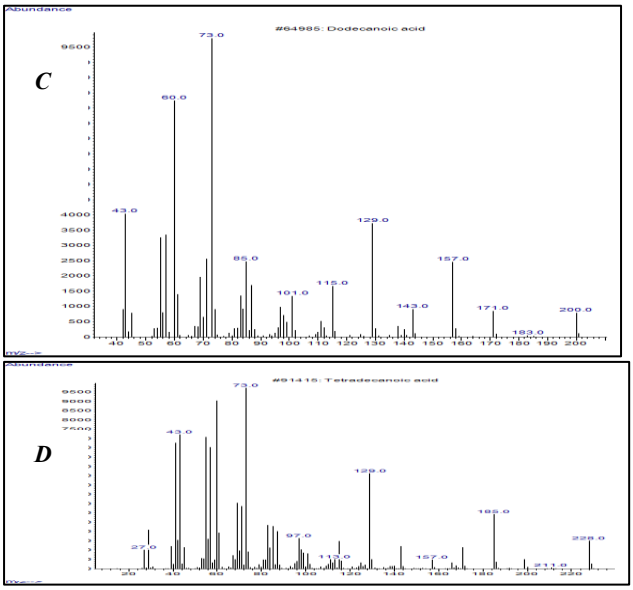


Figure 3.6: Mass spectrum of batch production of bioethanol by *Saccharomyces cerevisiae* for the resolution of (*C*) hit 3 (Dodecanoic acid) and (*D*) hit 4 (Tetradecanoic acid)

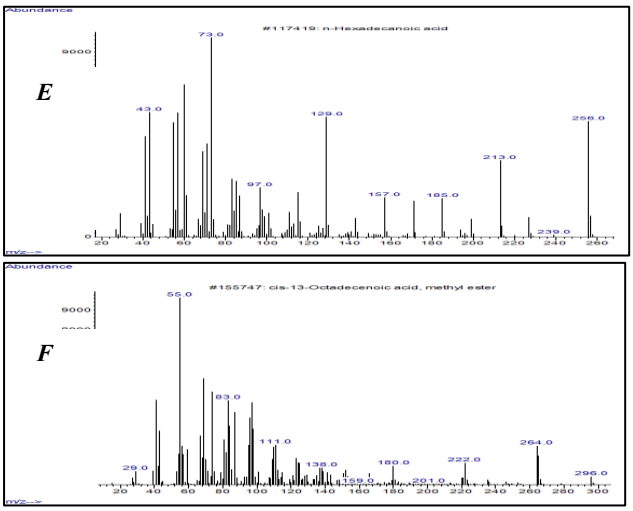
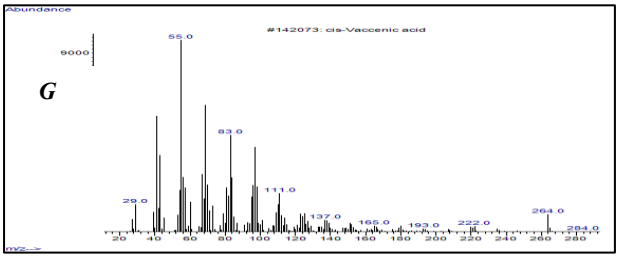


Figure 3.7: Mass spectrum of batch production of bioethanol by *Saccharomyces cerevisiae* for the resolution of (*E*) hit 5 (n-Hexadecanoic acid) and (*F*) hit 6 (cis-13-Octadecanoic acid, methyl ester)



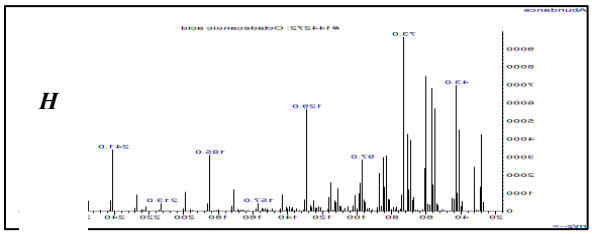


Figure 3.8: Mass spectrum of batch production of bioethanol by *Saccharomyces cerevisiae* for the resolution of (*G*) hit 7 (cis-Vaccenic acid) and (*H*) hit 8 (Octadecanoic acid)

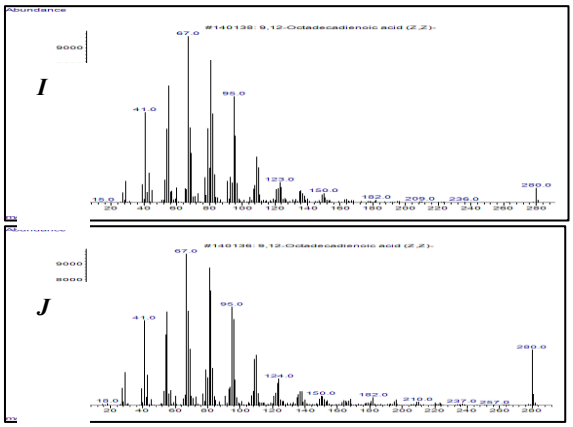


Figure 3.9: Mass spectrum of batch production of bioethanol by *Saccharomyces cerevisiae* for the resolution of (*I*) hit 9 (9, 12-Octadecanoic acid (z.z)-) and (*J*) hit 10 (9, 12-Octadecanoic acid (z.z)-)

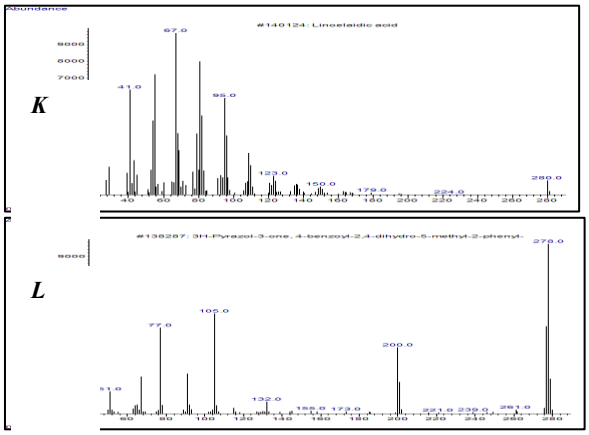


Figure 3.10: Mass spectrum of batch production of bioethanol by *Saccharomyces cerevisiae* for the resolution of (*K*) hit 11 (Linoelaidic acid) and (*L*) hit 12 (3H-pyrazol-3-one, 4-benzyl-2,4-dihydro-5-methyl-2-phenyl-)

3.4.3 HPLC analysis of the fermentation distillate

HPLC analysis of the fermentation distillate showed ethanol as the major product (20.96 mg/L, retention time 5.120 min), along with secondary metabolites including acetic acid (5.36 mg/L) and benzaldehyde (5.35 mg/L). The full compound profile, including retention times and concentrations, is shown in Figure 3.11.

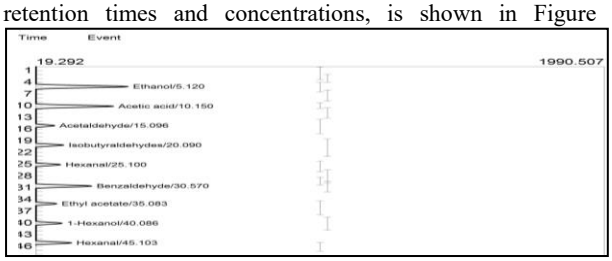


Figure 3.11: HPLC chromatogram of the fermented product by *Saccharomyces cerevisiae* analyzed

3.5 Effect of Conditions on the Production

3.5.1 Effect of pH on the production of reducing sugar, yeast biomass and bioethanol by *Saccharomyces cerevisiae* and *Candida* sp.

Combined effects of pH-incubation time, pH-inoculum size and pH-carbon/nitrogen ratio on CO_2, reducing sugar, yeast biomass and bioethanol by *Saccharomyces cerevisiae* and *Candida* sp and pH plot is presented in Figure 3.12. The pH ranges that had the maximum effect on reducing sugar, yeast biomass and bioethanol were pH 4.0 to 4.5, pH 4.5 to 5.0, 5.5 to 6 and 6.5 to 7.0, respectively.

3.5.2 Effect of inoculum size on the production of reducing sugar, yeast biomass and bioethanol by *Saccharomyces cerevisiae* and *Candida* sp.

Combined effects of inoculum size-incubation time, inoculum size-pH and inoculum size-carbon/nitrogen ratio on CO_2, reducing sugar, yeast biomass and bioethanol by *Saccharomyces cerevisiae* and *Candida* sp.

Table 3.5A: Production (Fermentation) using *Saccharomyces cerevisiae*

Days	Bioethanol (mg/l)	Biomass (mg/l)	Reducing Sugar (mg/l)
0	0	0	1.532
7	0.87	0.21	0.798
14	1.56	0.36	0.713
21	2.35	0.48	0.672
28	2.91	0.55	0.504
35	3.63	0.62	0.472
42	4.24	0.68	0.423
49	4.73	0.75	0.405
56	5.62	0.81	0.314
63	6.54	0.98	0.152
70	6.54	0.98	0.152

Table 3.5B: Production (Fermentation) using *Candida* sp

Days	Bioethanol (mg/l)	Biomass (mg/l)	Reducing Sugar (mg/l)
0	0	0	0.687
7	0.87	0.20	0.571
14	0.95	0.31	0.489
21	1.34	0.45	0.401
28	1.95	0.50	0.343
35	2.43	0.59	0.302
42	2.87	0.64	0.263
49	3.54	0.71	0.201
56	4.07	0.79	0.127
63	4.83	0.85	0.052
70	4.83	0.85	0.052

3.5.3 Effect of pH on specific time of production using *Saccharomyces cerevisiae* and *Candida* sp

Fig 3.12 shows the effects of incubation parameters namely: pH and substrate concentration and these were studied. From the study the optimum pH for the production of bioethanol and yeast biomass by both *Saccharomyces cerevisiae* and *Candida* was pH 6.0. When pH was set at 4, bioethanol and biomass produced reduced significantly. *Saccharomyces cerevisiae* did better than that of *Candida* sp.

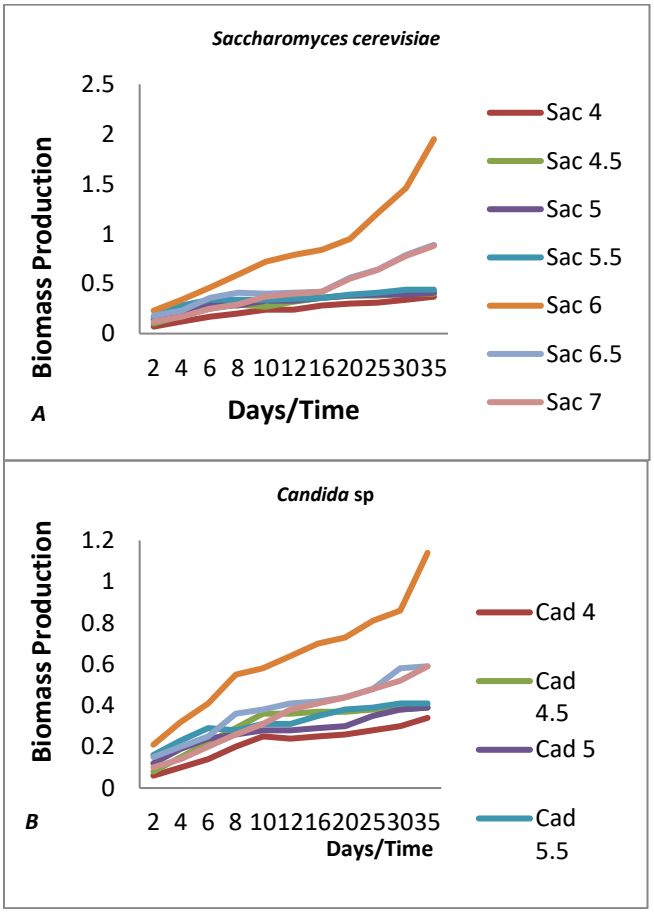
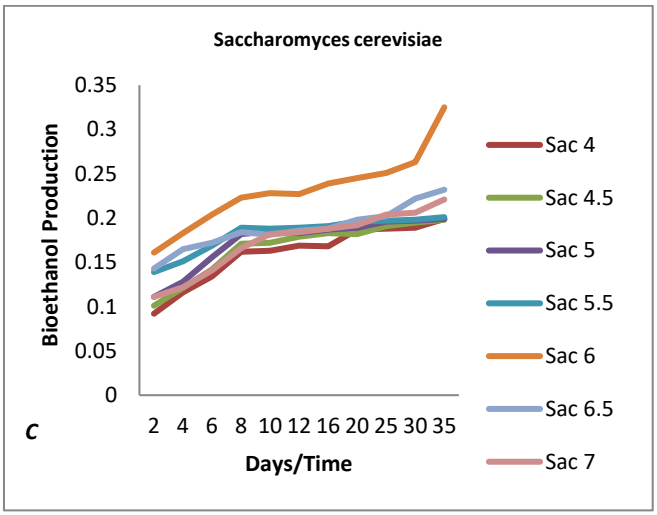


Fig 3.12a: A plot of maximal growth rate for biomass of *candida* sp and *saccharomyces cerevisiae* as a function of pH



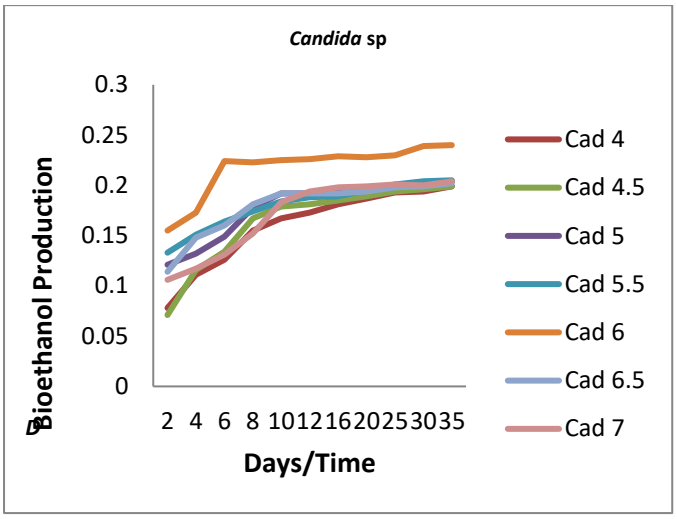
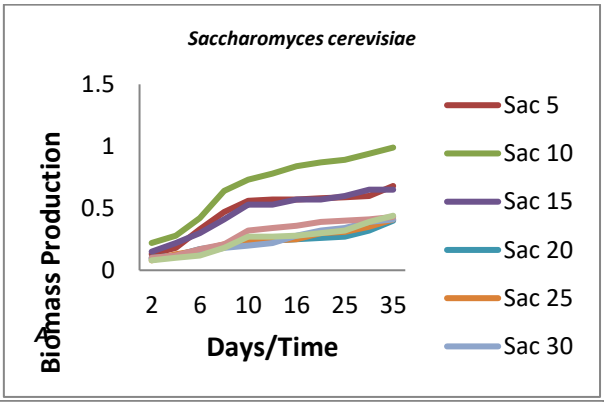


Fig 3.12b: A plot of maximal growth rate for bioethanol of *candida* sp and *saccharomyces cerevisiae* as a function of pH

3.5.4Effect of inoculum size on specific time of production using *Saccharomyces cerevisiae* and *Candida* sp

Fig 3.13 shows the effects of incubation parameters namely: inoclum size and substrate concentration and these were studied. From the study the optimum inoculums size for the production of bioethanol and biomass by both *Saccharomyces cerevisiae* and *Candida* was inoculum size of 10. The activity of *Saccharomyces cerevisiae* did better than that of *Candida* sp for the production.



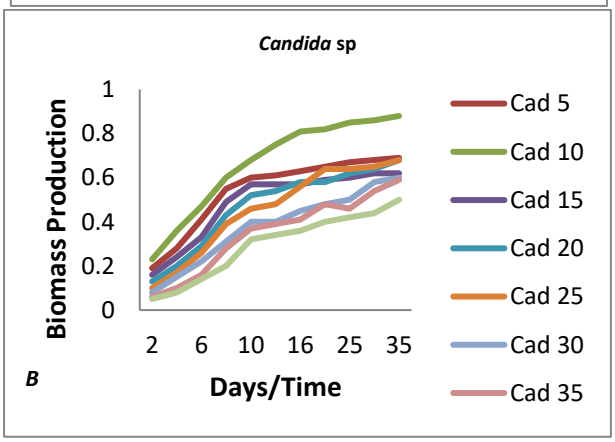


Fig 3.13a: A plot of maximal growth rate for biomass of *candida* sp and *saccharomyces cerevisiae* as a function of inoculum size

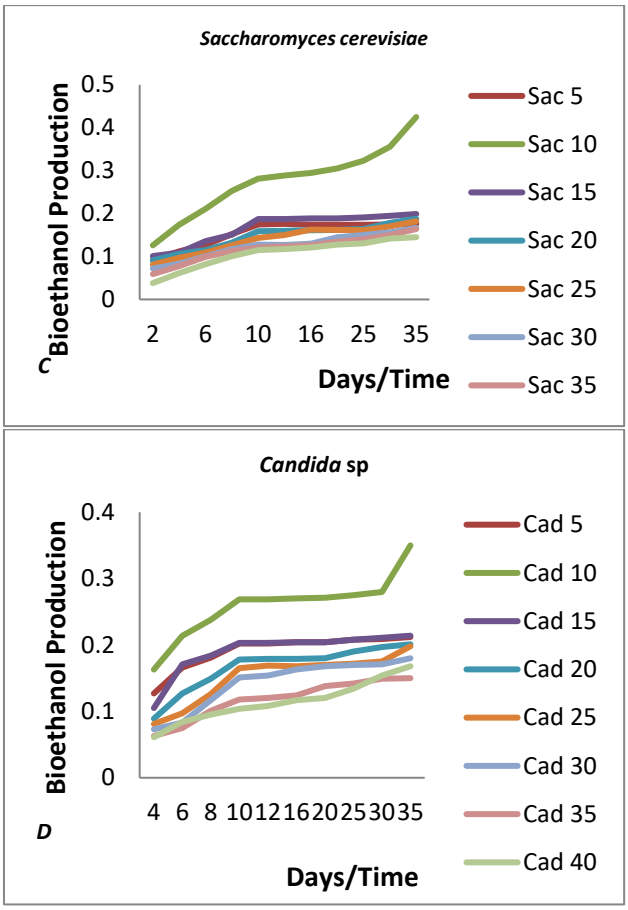


Fig 3.13b: A plot of maximal growth rate for bioethanol of *candida* sp and *saccharomyces cerevisiae* as a function of inoclum size

3.5.5 Effect on production with *Saccharomyces cerevisiae* and *Candida* sp

3.5.5.1 Effect on production with *Saccharomyces cerevisiae*

Figure 3.14 shows that bioethanol production by *Saccharomyces cerevisiae* increased from day 0 to day 63 and stabilized until day 70 at optimal pH and inoculum size. The fermentation showed significant improvement compared to other strains, with increasing biomass and a corresponding decrease in sugar concentration.

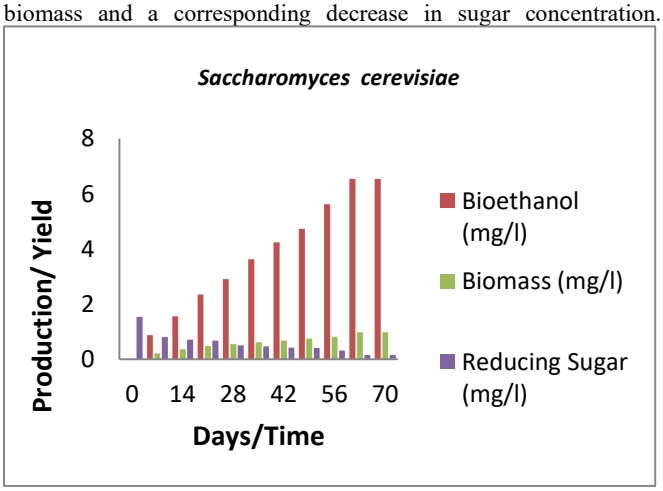


Fig 3.14: A plot of production with *Saccharomyces cerevisiae*

3.5.5.2 Effect on production with *Candida* sp.

Figure 3.15 shows Candida sp. reduced sugar levels from day 1 to 63, stabilizing until day 70, with a corresponding increase in biomass.

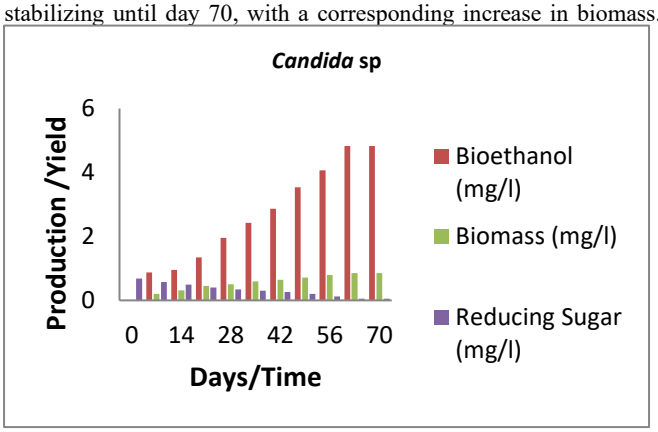


Fig 3.15: A plot of production with *Candida* sp

3.6 Model Performance Based on Predicted Production

3.6.1 Comparison of Experimental Measurement and Predicted Production.

The experimentally measured residual EPH concentrations were compared with predictions from batch degradation rate and Michaelis–Menten kinetic models (Figure 3.16). Predicted biomass for *Saccharomyces cerevisiae* decreased from 4.15 mg/L at 70 days to 2.79 mg/L at 7 days, while *Candida* sp. decreased from 0.138 mg/L to 0.023 mg/L. Pearson correlation analysis indicated moderate agreement between measured and predicted biomass, with correlation coefficients of 0.3201 and 0.585 yeast respective.

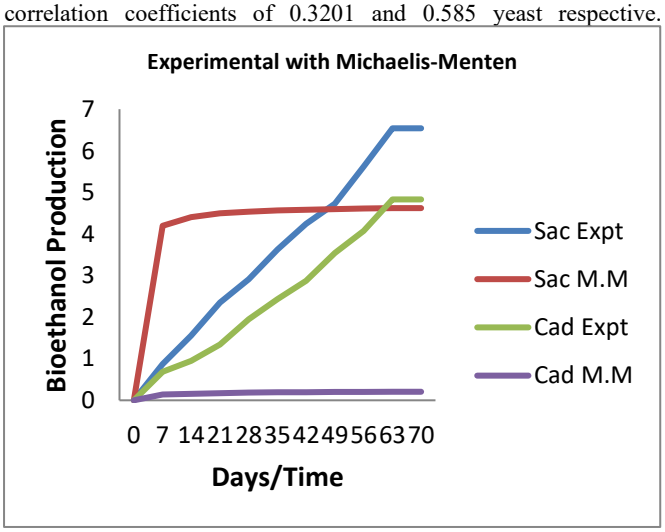


Figure 3.16: Comparison of bioethanol production from experiment with Michaelis-Menten model for *Saccharomyces cerevisiae* and *Candida* sp.

3.7 Evaluation of Michaelis-Menten constants for bioethanol production with *Saccharomyces cerevisiae* and *Candida* sp

3.7.1 Evaluation of Michaelis-Menten constants for bioethanol production with *Saccharomyces cerevisiae*

Figure 4.17 shows the plots for evaluation of u_s and K_s for *Saccharomyces cerevisiae* samples. Again, by comparing the regression line equations with equation (2.25), U_s and K_s for *Saccharomyces cerevisiae* were evaluated as 0.10423mg/l.day and

4.695mg/l, R^2 = 0.8471 respectively. Also for *Saccharomyces cerevisiae*, upon substitution of the evaluated U_s and K_s in equation (2.27), the Michaelis-Menten equation was obtained as $r_{EFP} = \frac{4.695\ EFP}{0.10423 + EFP}$ For *Saccharomyces cerevisiae*, biomass modeling during batch fermentation showed bioethanol production with a 0.2% error, outperforming *Candida* sp.

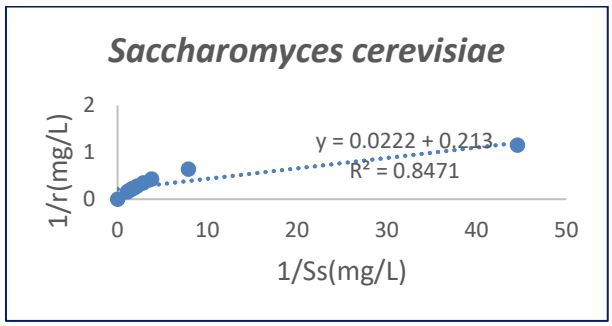


Figure 3.17: Linewaver-Burke plot for evaluation of Michaelis-Menten constants for *Saccharomyces cerevisiae* samples

3.7.2 Evaluation of Michaelis-Menten constants for bioethanol production with *Candida* sp

Figure 3.18 shows the plots for evaluation of u_c and K_c for *Candida* sp samples. Again, by comparing the regression line equations with equation (2.16), U_c and K_c for *Candida* sp were evaluated as 0.2235mg/l.day and 0.4268mg/l, R^2 = 0.185 respectively. Also, for Candida sp, upon substitution of the evaluated U_C and K_C in equation (2.16), the Michaelis-Menten equation for *Candida* sp samples was obtained as $r_{EPH} = \frac{0.2235EFP}{0.4268 + EFP}$. *Candida* sp. produced less bioethanol than *S. cerevisiae*, as shown by biomass modeling during batch fermentation.

4. CONCLUSION

This study evaluated bioethanol production from plum (*Prunus salicina*) fibre using yeast isolates from palm oil mill effluent–impacted soil. Fourteen isolates were screened, with *Saccharomyces cerevisiae* and *Candida* sp. showing the highest potential. *S. cerevisiae* produced a maximum bioethanol yield of 6.54 mg/L under optimized batch conditions. Plum fibre combined with corn steep liquor is an effective substrate for bioethanol production. *Saccharomyces cerevisiae* outperformed *Candida* sp., showing linear ethanol production over 70 days. Key fermentation parameters (pH, incubation time, inoculum size, C:N ratio) significantly influenced yield. FTIR, GC–MS, and HPLC confirmed ethanol as the main product, meeting fuel standards, highlighting plum fibre as a sustainable feedstock for large-scale bioethanol production.

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