ISSN 2250-3153

Oil Palm Fronds Juice: A Potential Feedstock for Bioethanol Production

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***Abstract*-** The rise in global oil demand along with current environmental crisis; global warming and climate mitigation has been a serious issue. Thus, the need for an alternative fuel from renewable resources that promotes sustainability is vital to replace our dependence on fossil fuel. High sugar content in Oil Palm Frond Juice (OPFJ) indicates its potential as a feedstock in ethanol fermentation. Two strains of *Saccharomyces cerevisiae*; SA79 and HC10 are screened for high fermentation efficiency. Percentage yield bioethanol revealed that *S. cerevisiae* HC10 (55.72%) was proven a better strain than *S. cerevisiae* SA79 (43.19%). A growth profile of *S. cerevisiae* HC10 was performed in 60 h time period with 6 h interval sampling time. Specific growth rate (μ) and doubling time (t_d) of 0.037h^{-1} and 18.7h, respectively shows that *S. cerevisiae* HC10 fermentation of OPFJ is industrially applicable. In OPFJ fermentation, *S. cerevisiae* HC10 shows a much better yield (79.77 %) and thus an optimization using Response Surface Methodology was done. The optimized fermentation parameters were; OPFJ (40%), inoculums size (20%), pH (4.5), and fermentation time (24 h) with ethanol of 6.81 g/L. The batch fermentation was up-scaled to 1.5L working volume in a bioreactor to study relationships between agitation speed (rpm) and initial oxygen concentration (%). The highest ethanol (6.00 g/L) was obatined when the fermentation process was performed at 100 rpm and 30% initial oxygen concentration. In conclusion, OPFJ is a good fermentation feedstock for bioethanol fermentation because it has very high (647.8g/L) sugars content and high yield of ethanol production.

***Index Terms*-** Oil Palm Frond Juice, *Saccharomyces cerevisiae*, bioethanol, Response Surface Methodology

I. INTRODUCTION

Nowadays, energy demand for industrial, commercial and residential purposes, electricity generation and transportation is primarily supplied by fossil fuels. Burning of fossil fuel is known to be one of the main reasons for adverse climate change experienced throughout the centuries. The undesirable phenomenon is strongly linked to the accumulation of greenhouse gases in the atmosphere, and that the human activity especially through the combustion of fossils fuels is the major contributing factor [1].

Furthermore, strong dependence for fossil fuel raise the thought that what will happen when the sources runs out? Thus, the solution is to find the best alternatives before the problem occurs. Brazil has been the leading country in producing renewable fuel using sugarcane as the source [2].

In addition, as reported by Ariffin Fazilah [3], Oil Palm Fronds (OPF) fiber was used as raw material for bioethanol production, whereby the cellulose and hemicellulose component are converted into simple fermentable sugars *via* hydrothermal treatment and enzymatic hydrolysis. However, the pre-treatment process is time consuming and requires high expenses. This obstacle gives rise to the need for finding an alternative approach to obtain renewable sugars from palm oil waste.

Oil palm sap (obtaind by pressing OPF or oil palm trunk) is known to be one of the best sources for producing bioethanol [4]. The main reason is that oil palm frond is considered as waste material in many countries in the world. Using the juice obtained from oil palm frond as sources for fermentation is highly beneficial due to its renewability, low cost, and it is highly available in Malaysia. The juice is believed to contain various simple sugars including monosaccharide such as glucose and fructose as well as disaccharide such as sucrose [5]. Thus, the sugary property of oil palm fronds juice may give us an idea of turning this wasted juice as the alternative feedstock for bioethanol fermentation.

During ethanol fermentation process, enzymes from microorganisms will convert carbon sources, usually sugars, into ethanol anaerobically [6]. Yeasts are among the most frequently used microorganism in ethanol fermentation. *Saccharomyces cerevisiae* are normally chosen for ethanol fermentation due to its safety and high efficiency in fermenting sugars to ethanol [7]. Various parameters should also be taken into account during fermentation. Those parameters include the nutrient supply, oxygen supply, pH of the fermentation environment, fermentation temperature, and also the time for fermentation process.

Thus, the aims of this study was to produce ethanol though fermentation process using OPF juice as substrate. Parameters which affect the ethanol fermentation processes were examined and optimized. With this, it is possible to reduce the overall cost for bioethanol production since the feedstock (OPF juice) used was obtained from agriculrural residual (OPF) and is free.

II. MATERIALS AND METHODS

2.1 Raw Material and sample preparation

The OPF was harvested from a local oil palm plantation located at Bukit Minyak, Penang, Malaysia. The leaves of the fronds were cut off and discarded and the OPF was cut into 3 to 4 shorter pieces for easy transport. Hard outer skin layer of OPF were peeled off to avoid mechanical failure in the next step. The peeled OPF were pressed using a sugarcane pressing machine [5] within 24 hours after they were harvested from the tree [8]. OPF

juice produced was collected and was centrifuged at 10 000 rpm for 15 minutes (U-1900, Hitachi). A clear yellowish-coloured supernatant obtained was stored at -20°C [5] for further use.

2.2 Determination Sugars Composition in OPF Juice

Three types of fermentable sugars which are present in OPFJ such as glucose, fructose and sucrose were determined by High Performance Liquid Chromatography (HPLC) (Shimadzu LC, Japan) equipped with Refractive Index (RI) detector using APS-Hypersil column (diameter of 250mm × 46mm). The mobile phase used was 70% Acetonitrile and 30% of de-ionized water. Oven temperature was set at 40°C whereas pump flow rate was set at 0.6 mL/min.

2.3 Pure Yeast Culture Establishment

Two strains of yeast, *S. cerevisiae* SA79 and *S. cerevisiae* HC10 which were previously determined as potential ethanol producer were used in this study. The strains were subculture in 100 mL nutrient broth supplemented with g/L; Yeast Extract, 5; Peptone, 10 and 15% of glucose solution, 50 [8]. Glucose solution was prepared and autoclave separately from the nutrient broth. The strains were cultured in a 250 mL conical flask and were incubated at 30°C using incubator shaker for 24 hours and agitated at 150 rpm. Agar slant were prepared using universal bottles and the medium composition used was as described above but with addition of agar powder (20g/L) [8]. After incubation, the culture was streaked onto agar slant and incubated for 2-3 days at 30°C. The grown colonies in the agar slant were sealed carefully using parafilm and were stored in refrigerator at 4°C prior to use.

2.4 Yeast Screening

Inoculums for both strains were prepared in 100 mL medium broth using 250 mL conical flask incubated at 30°C for 24 hours and agitated at 150 rpm [8]. Optical Density (OD) for each inoculums were measured using spectrophotometer (U-1900, Hitachi, Japan) and were standardized to an approximate value of OD 0.8. Nutrient broth (98 mL) was prepared for both strains. The pH of the medium was adjusted to 4.5 [9] prior to autoclave at 121°C for 15 minutes. Standardized inoculums (2 mL) was then transferred into the sterilized medium and the flasks were incubated at 30°C [10] for 24 hours [9] at 150 rpm [10]. After fermentation, yeast biomass, glucose consumption and ethanol production were analyzed in order to determine the best yeast strain.

2.4.1 Growth Profile of Yeast

The inoculums of the selected strain was prepared according to the method described above. The inoculums (2% v/v) was then transferred into 200 mL sterilized nutrient broth containing 30 mL OPFJ as the carbon source. The flasks were incubated at 30°C for 60 hours and shake at 150 rpm. Sample (10mL) was taken every 6 hours interval for 60 hours. Analysis of yeast biomass, sugar utilization and ethanol production were performed. Samples were stored at -20°C if no analysis was carried out immediately. Kinetic parameters were determined to gain deeper understanding about the strain.

2.5 Bioethanol Production Process

The OPFJ was thaw to ambient temperature and autoclave at 121°C with a retention time of 15 minutes. Nutrient broth medium without glucose solution was prepared with an initial pH adjusted to pH 4.5 prior to autoclave at 121°C for 15 minutes.

2.5.1 Shake Flask System and Optimization

Optimization of fermentation process for ethanol production was performed using Central Composite Design from Design-Expert® version 7 program. The conditions for 30 combinations of experimental run with 4 parameters such as amount of OPFJ (%, v/v), size of inoculums (%, v/v), initial pH and incubation time are shown in Table I. Total working volume for each flask was kept constant at 100 mL for every run. Each run were done in duplicates. After fermentation, 15 mL of sample were taken to analyze for biomass, sugar utilization and ethanol production. The best combination condition suggested by the design program was validated by performing fermentation according to the suggested parameters.

Table I: Thirty Combinations of Experimental Runs using Response Surface Method; Central Composite Design

Std	Run	Block	OPFJ (%)	Inoculum (mL)	Initial pH	Incubation Time (h)
18	1	Block 1	60	15	6.0	72
7	2	Block 1	40	20	7.0	48
1	3	Block 1	40	10	5.0	48
16	4	Block 1	80	20	7.0	96
3	5	Block 1	40	20	5.0	48
4	6	Block 1	80	20	5.0	48
19	7	Block 1	60	15	6.0	72
10	8	Block 1	80	10	5.0	96
14	9	Block 1	80	10	7.0	96
13	10	Block 1	40	10	7.0	96
17	11	Block 1	60	15	6.0	72
6	12	Block 1	80	10	7.0	48
15	13	Block 1	40	20	7.0	96
8	14	Block 1	80	20	7.0	48
5	15	Block 1	40	10	7.0	48
2	16	Block 1	80	10	5.0	48
12	17	Block 1	80	20	5.0	96
20	18	Block 1	60	15	6.0	72
11	19	Block 1	40	20	5.0	96
9	20	Block 1	40	10	5.0	96
26	21	Block 1	60	15	8.0	72
23	22	Block 1	60	5.0	6.0	72
25	23	Block 1	60	15	4.0	72
27	24	Block 1	60	15	6.0	24
28	25	Block 1	60	15	6.0	120
24	26	Block 1	60	25	6.0	72
22	27	Block 1	100	15	6.0	72
30	28	Block 1	60	15	6.0	72
21	29	Block 1	20	15	6.0	72
29	30	Block 1	60	15	6.0	72

2.5.2 Bioethanol Production Using Bioreactor System

Bioethanol production process was further performed using 2.5 L bench-top Minifors Bioreactor. Factors which affect the ethanol fermentation process such as Agitation (rpm) speed and initial oxygen concentration (%) were investigated. The optimized conditions [such as amount of OPFJ (%, v/v), size of inoculums (%), initial pH and incubation time] obtained from shake flask system were used as initial conditions in the bioreactor system. Total working volume of the fermentation process were kept constant at 1.5L and the process was carried out at 30°C with an air flow rate of 1vvm [11]. Each run were performed in duplicates according to the conditions shown in Table II. After fermentation, 15 mL of sample were taken for analysis of biomass, sugar utilization and ethanol production.

Table II: Agitation and Initial Oxygen Concentration used in Bioreactor System for Bioethanol Production

Batch	Sample name	Agitation (rpm)	Initial O_2 concentration (%)
1st	1	200	10
	2	200	20
	3	200	30
2nd	4	100	30
	5	200	30
	6	300	30

2.6 Analysis

2.6.1 Determination of Yeast Biomass

The yeast biomass was determined by measuring the dry cell weight. Cells suspensions were vacuum-filtered through 0.45µm filter paper and were rinsed 2 times using distilled water. The filtered papers were dried in an oven at 70°C for more than 24 hours until constant weight was obtained. The dried filter papers were weighed on an analytical balance to obtained the dry cell weight.

2.6.2 Determination of Sugars Residual

The remaining sugars in OPFJ after fermentation was determined according to the method described in Section 2.2.

2.6.3 Determination of Ethanol using GC System

Ethanol produced were analyzed using Gas Chromatography (GC) (Shimadzu, Japan) equipped with Flame Ionization Detector. Helium (He) was used as carrier gas and RT-Q-BOND column (inner diameter of 0.32 mm) was used and the oven temperature was set at 200°C. The flow rate used was 21.9 mL/min and the operating pressure was 71.1 kPa. Five min holding time was used for each sample.

III. RESULTS AND DISCUSSIONS

3.1 Sugar Composition of OPF Juice

Table III showed the major types of sugars (Glucose, sucrose and fructose) founded in OPFJ with their respective concentration (g/L). Glucose shows the highest percentage (77.96%) followed by sucrose (16.22%) and fructose (5.82%). These sugars are among some of the common sugars favorable for yeast consumption.

Previous study shows the same pattern whereby glucose is the dominant sugar followed by sucrose and fructose [5]. However, the percentages of individual sugars composition obtained by the study were different from this study. This may due to several factors such as the way of handling the juice during and after pressing and the difference in individual oil palm tree itself. In this study, 16.22% of sucrose was recorded, which shows 10.67% less than sucrose recorded by Zahari et al. [5]. However, glucose and fructose obtained from this study were 7.06% and 3.61% higher than the one recorded by Zahari et al., [5], respectively. This result was an indication of the breakdown of sucrose into its monomer; glucose and fructose. Furthermore, the difference in the age of oil palm tree that was harvested also gives different amount of sugar whereby older tree contains lesser sugar [12].

Table III: Sugar Composition in Oil Palm Fronds Juice (OPFJ)

Sugar	Amount of sugar (g/L)	Percentage (%)
Fructose	37.72	5.82
Glucose	504.99	77.96
Sucrose	105.04	16.22
Total	647.76	100

3.2 Selection of Potential Yeast for Ethanol Production

As shown in the Figure 1, *S. cerevisiae* HC10 was found to be well grown throughout the fermentation when compared to *S. cerevisiae* SA97 indicated by the biomass yield of 0.04g cell/g glucose and 0.03g cell/g glucose, respectively. This shows that *S. cerevisiae* HC10 is more suitable to be used in present study. Previous study stated that apart from glucose, other component in fermentation medium can affect the effectiveness of fermentation since yeasts has a complex nutritional requirements to achieve optimum fermentation and the requirements would vary from one strain to another [9].

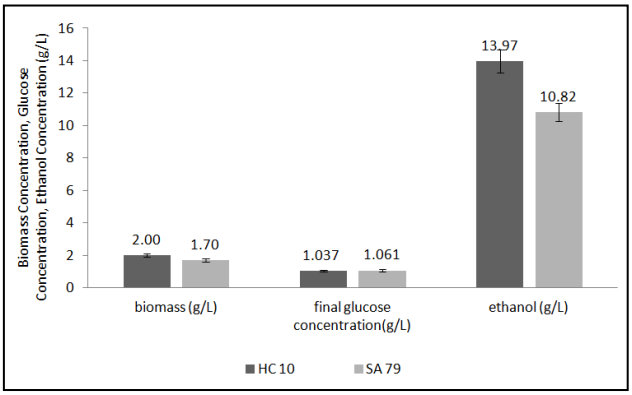

Figure 1: Biomass, glucose residual and ethanol obtained from *S. cerevisiae* HC 10 and *S. cerevisiae* SA 79.

On the other hand, only small differences (0.0237 g/L) in glucose residual were detected after the fermentation process. However, the ethanol yield was significant different in *S. cerevisiae* HC10 (0.29g ethanol/g glucose) and *S. cerevisiae* SA79 (0.22g ethanol/g glucose). On top of that, ethanol yield

indicated that *S. cerevisiae* HC10 (55.72%) was more efficient than *S. cerevisiae* SA79 (43.19%). The ethanol yield obtained from this study was higher than the ethanol yield (6.5 g/L) recorded by Maurice (2011). Higher ethanol production suggested that *S. cerevisiae* SA79 and *S. cerevisiae* HC10 are able to produce more ethanol than the conventional Baker's Yeast. *S. cerevisiae* HC10 was chosen as the best yeast to be used further for the experiment due to its ability to grow well in the fermentation media and to produce higher yield of ethanol.

3.3 Growth Profile of S. cerevisiae HC10

S. cerevisiae HC10 enter exponential phase from 0 h to 18 h, indicated by a sharp increased in biomass (Figure 2). Lag phase was not shown in the growth curve since the yeast culture has already been acclimatized during inoculums preparation. During the exponential phase, the yeast rapidly consumes the most preferable carbon source which is commonly identified as glucose [13].

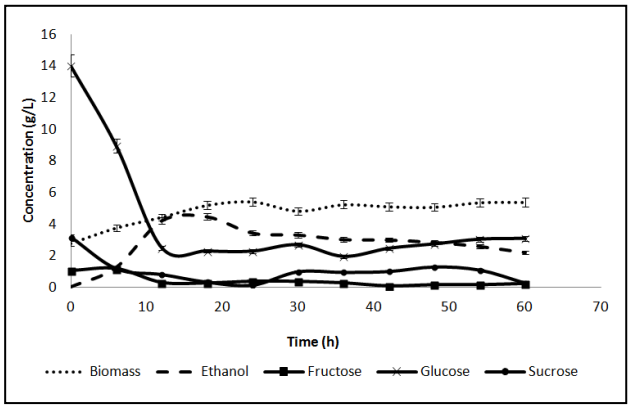

Figure 2: Concentration of biomass, ethanol and sugars residual during the fermentation process.

From 24 h to 30 h, the growth declined but starting to rise up again after 36 h. This phenomenon suggested that the culture is undergone diauxic growth. It is a process whereby a shift of metabolism happens from fermentation to respiration when glucose becomes limiting [13]. Ethanol and other product such as acetate that are produced during fermentation will be consumed as the replacement for the previously consumed carbon sources [13]. The yeast strain enters the stationary phase starting from 36 h to 60 h and no net increased in cell population are shown in the Figure.

On the other hand, during 0 h to 18, all the sugars concentration shows decreased in pattern with marginally decreased in glucose concentration. The result suggests that all of the sugar were intensively consumed during exponential phase for growth. During 0 h to 6 h of fermentation, sucrose concentration decreased while fructose concentration shows a small increased. This phenomenon was due to the breakdown of sucrose into fructose and glucose [14]. However, reduction in glucose concentration was rapidly due its consumption twice as fast as fructose when both of the sugar are present in a fermentation medium [15]. All sugars were not fully utilized at the end of the fermentation, suggesting a longer fermentation period should be performed.

Also shown in the Figure, ethanol production was the highest during the exponential phase. However, 18 h onwards ethanol production was reduced due to microbial stress factor such as high ethanol concentration in the fermentation environment that starts to take place [16]. In addition, decreased in ethanol concentration until end of the fermentation may due to ethanol being used up as an alternative carbon source as well as ethanol evaporation due to the continuous agitation action during the fermentation.

Kinetic values of the fermentation process are determined and shown in Table IV. Specific growth rate (μ) in the range of 0.03 h^{-1} and 0.40 h^{-1} is considered to be relevant to many industrial application [17]. A study by Estela-Escalante [18] shows that apple juice fermentation by *S. cerevisiae* has a μ value of 0.13 h^{-1} which is slightly higher than μ obtained in this study. In natural environments, a low μ indicates that the cell growth was constrained by limited amount of growth-limiting nutrients [19].

Table IV: Kinetic values obtained from fermentation of OPFJ by *S.cerevisiae* HC10

Kinetic Parameter	Value
Specific growth rate (μ)	0.037 h^{-1}
Doubling Time (td)	18.73 h
Glucose consumption	10.9232 g/L
Biomass yield coefficient ($Y_{x/s}$)	0.2066 g cell/g glucose
Ethanol yield coefficient ($Y_{p/s}$)	0.4005 g ethanol/g glucose
Maximum productivity of biomass	0.0895 g L^{-1} h^{-1}
Final productivity of biomass	0.0895 g L^{-1} h^{-1}
Maximum productivity of ethanol	0.1514 g L^{-1} h^{-1}
Final productivity of ethanol	0.0363 g L^{-1} h^{-1}
Percent yield of ethanol	79.77 %

Although the doubling time obtained from this present study was 18.73 h but it still represent a fast growth in the natural environment [19]. At near 0 growth rate, the cells are likely to have longer life span due to increasing level of stress response [20]. In this study, glucose was identified as the primary substrate and thus was referred for further kinetics parameter calculation. An amount of 0.2g of cell was produced for every grams of glucose consumed shows 0.1g higher than the studied reported by Estela-Escalante (2012). Meanwhile, 0.4g of ethanol was produced by consumption of 1 grams of glucose.

There is a difference of 0.1g L^{-1} h^{-1} between maximum and final ethanol productivity, indicated that ethanol production reaches its maximum value within the course of 60 h fermentation, which can also be seen in Figure 2. This is a usual pattern found in batch mode fermentation whereby substrates are a limiting factor. The percentage yield of ethanol obtained was around 80% which is considerably high with reference to a study on *S. cerevisiae* ethanol fermentation of tropical maize syrup that has ethanol yield of around 90% [14]. One of the reason behind high percentage yield gained from Chen et al. [14] was due to the used of concentrated tropical maize syrup.

3.4 Optimization of Bioethanol Production Process Using Shake Flask System

Optimization using CCD design revealed that the result obtained was suitable for quadratic model. But there is a

considerable probability of having a significant lack of fit. Based on Model Summary Statistic (Table Va), the standard deviation (1.03) was low enough and acceptable. R-squared (0.71) shows that the model is having a considerable regression but acceptable and PRESS value (86.48) was low (Table Vb). ANOVA report for Response Surface Quadratic Model implies that the model was significant (F value: 2.69) (Table VI). In addition, only pH and time factors bring significant effect ($P<0.05$) in ethanol production. The normal plot of residuals shows approximately linear thus no transformation correction is needed.

Table Va: Fit Summary Analysis (Sequential Model Sum of Squares [Type I])

Source	Sum of Square	df	Mean Square	F value	p-value prob>F	
Mean	511.51	1	511.51			Suggested
Linear	13.52	4	3.38	1.99	0.1265	
2FI	11.45	6	1.91	1.17	0.3616	
Quadratic	15.00	4	3.75	3.53	0.0321	Suggested
Cubic	12.04	8	1.51	2.70	0.1040	Aliased
Residual	3.90	7	0.56			
Total	567.42	30	18.91			

Table Vb: Fit Summary Analysis (Model Summary Statistics)

Source	Std. Dev.	R-squared	Adjusted R-squared	Predicted R-squared	PRESS	
Linear	1.30	0.2418	0.1204	-0.1629	65.02	
2FI	1.28	0.4467	0.1554	-0.4484	80.98	
Quadratic	1.03	0.7149	0.4487	-0.5469	86.48	Suggested
Cubic	0.75	0.9302	0.7110	-5.8940	385.43	Aliased

Table VI: Analysis of Variance

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	39.97	14	2.85	2.69	0.0337	significant
A-vol OPFJ	1.12	1	1.12	1.05	0.3219	
B-vol inoculum	0.21	1	0.21	0.2	0.6646	
C-pH	5.42	1	5.42	5.1	0.0393	
D-time	6.77	1	6.77	6.37	0.0233	
AB	0.54	1	0.54	0.51	0.4872	
AC	3.96	1	3.96	3.73	0.0727	
AD	0.66	1	0.66	0.62	0.4429	
BC	2.56	1	2.56	2.41	0.1416	
BD	3.26	1	3.26	3.07	0.1003	
CD	0.48	1	0.48	0.45	0.5131	
A^2	1.03	1	1.03	0.97	0.3394	
B^2	3.41	1	3.41	3.21	0.0933	
C^2	4.4	1	4.4	4.14	0.06	
D^2	4.89	1	4.89	4.6	0.0487	
Residual	15.94	15	1.06			
Lack of Fit	14.71	10	1.47	5.95	0.0313	significant
Pure Error	1.24	5	0.25			
Cor Total	55.91	29				

Table VII: Criteria used in numerical optimization

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
OPFJ Volume	in range	40	80	1	1	3
Inoculum Volume	maximize	10	20	1	1	3
pH	targeted = 4.50	4	8	1	1	3
Time	in range	24	96	1	1	3
Ethanol	maximize	0.3629	7.5973	1	1	3

Table VIII: Suggested experimental conditions (solution) based on the preset criteria

Volume OPFJ (%)	Volume Inoculum (ml)	pH	Time (h)	Ethanol (g/L)	Desirability	
40.96	20	4.5	24.06	7.61	1	Selected
40.74	20	4.5	24.07	7.62	0.99	
40.00	20	4.5	24.00	7.66	0.99	
42.02	20	4.5	24.00	7.58	0.99	
42.81	20	4.5	24.07	7.54	0.99	

Numerical optimization was carried out in order to determine the conditions which gave the highest ethanol production. The criteria for each factor used in optimization are shown in Table VII and the suggested experimental conditions based on the preset criteria are shown in Table VIII. An experiment was conducted to validate the suggested combination and the ethanol obtained (6.81g/L) was closer to the suggested value (7.61g/L).

3.5 Ethanol Production Using Bioreactor System

S. cerevisiae is facultative anaerobes and required oxygen for growing but ethanol fermentation is an anaerobic process. Thus, initial oxygen concentration should be high enough to enable biomass growth but not too high until disable the fermentation process because in oxygen rich environment, respiration will occur instead of fermentation. The results obtained demonstrated that during 24 hour of fermentation, the yeast has experiences a longer respiration time (Table IX). Increased in biomass produced from 10% to 30% initial oxygen concentration was the factor that causes the ethanol production to increase. A decrease in ethanol for sample number 2 was due to evaporation of alcohol as a result of contamination.

Due to a positive outcome from initial oxygen concentration of 30%, this value was used in second phase of the experiment whereby agitation factor was further investigated. Results obtained revealed that increasing agitation rate give a higher biomass concentration but a much lower ethanol concentration. Agitation plays an important role in homogenising the fermentation medium as well as aiding in oxygen transfer rate in system. Increase in agitation speed results in a better dissolve oxygen concentration in the fermentation medium, thus yeast are supplied with an adequate amount of oxygen, making them to favour respiration than fermentation.

Table IX: Effect of agitation (rpm) and initial oxygen concentration (%) on biomass and ethanol production

Sample Name	Agitation	Initial O_2 Concentration (%)	Biomass (g/L)	Ethanol (g/L)
1	200	10	6.9150 ± 0.97	3.6250 ± 0.81
2	200	20	6.8550 ± 1.09	2.5314 ± 1.12
3	200	30	7.7300 ± 0.33	4.7580 ± 0.54
4	100	30	6.9450 ± 0.53	6.0010 ± 0.44
5	200	30	7.3230 ± 0.25	4.8085 ± 0.29
6	300	30	8.8000 ± 0.48	1.5147 ± 0.16

IV. CONCLUSION

OPFJ was proven to contain a high amount (647.76 g/L) of fermentable sugar; glucose, fructose, and sucrose. Conversion of fermentable sugar by *S. cerevisiae* HC10 from OPFJ to bioethanol was high (79.77%) thus proving that OPFJ was a good fermentation feedstock. Optimized fermentation conditions for bioethanol production were OPFJ (40%), inoculums size (20%), pH (4.5), and fermentation time (24h). Ethanol production using bioreactor system indicated that initial concentration of oxygen is important for biomass growth but must be controlled to prevent prolong of respiration time. Higher agitation results in better oxygen transfer rate but needed to be regulated to ensure a high production of ethanol.

ACKNOWLEDGMENT

The authors would like to thank Universiti Sains Malaysia for providing short term grant (304/PTEKIND/6312113). We would like to acknowledge the contribution from the School of Industrial Technology staffs, especially the laboratory assistant namely Mr. Azmaizan Yaakob and Mrs. Najmah Hamid. They have been very helpful in giving equipmental assistance and providing for all my laboratory needs.

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