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Physical Factors Optimization of *Saccharomyces cerevisiae* Fermentation to Enhance The Production of Bioethanol: A Review

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Abstract: The high demand for fossil fuels will reduce the world fuel reserve and may lead to the shortage and price increase of the fuel. To overcome this scenario, alternative energy such as renewable energy can reduce the reliance on limited fossil fuels. This review paper attempts to gather the research findings for biofuel production using biomass through fermentation process that dependant on various physical factors. The *Saccharomyces cerevisiae* (*S. cerevisiae*) is the favored choice for ethanol fermentation due to its ability to ferment a wide range of sugars. It is also discovered that great sources of sugar can be acquired from the waste of oil palm. Oil palm trunk (OPT) is rich in moisture content and sugar component. Therefore, this review focused on optimizing the fermentation physical factors such as pH, temperature, incubation period, concentration of inoculum and agigation speed to produce high yield of bioethanol from OPT through fermentation process.

Keywords: Oil Palm Trunk (OPT), Bioethanol, *Saccharomyces Cerevisiae*

1. Introduction

At present, fossil fuels are the main sources of fuel used but this energy cannot be renewed and will soon be depleted. The depletion of fossil fuel reserves will cause the unstable petrol prices and increase the environmental and political pressures [1]. The increasing demand for fossil fuels will likely cause the decline of the world fuel reserve, which may lead to the shortage of this fossil fuel and also cause the price to increase dramatically [2]. The use of fossil fuels as primary energy resources has resulted the global environmental problems [3]. The release of carbon dioxide (CO_2) from the vehicle and other industries is one of the largest potential contributors.

Alternative energy such as renewable energy reduces the reliance on limited fossil fuels. Nowadays, many researchers have been trying to discover the search for alternative energy originated from biomass as renewable sources to replace the use of fossil fuels instead. The renewable energy from the production of bioethanol through the fermentation process is the best option as reviewed by Oh et al.

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[4]. The need to meet energy demand with environmental impact and non-renewable fuel stocks generated from fossil fuels triggered research into renewable and environmental friendly energy sources, of which bioethanol is one of them [5]. Bioethanol is green energy, less hazardous, decomposable, and produces less airborne contaminants, as compared to petroleum fuel [6]. **Table 1** showed that bioethanol is considered low-emission renewable energy production compared to fossil fuels [7].

Table 1: Gas emission from renewable energy production compared to gasoline [7]

Sources	Gas emission (CO_2 e/MJ)
Fossil (Gasoline)	94
Corn	76
Sugarcane	45
Switchgrass	43
Corn Stover	43
Miscanthus	43

2. Fermentation

Recently, increasing attention has been focused on converting biomass into fuel ethanol [8]. The use of biomass energy has been recognized for the pupose of providing energy to the world [9]. Ethanol and biodiesels have been industrially produced from biomass by fermentation and chemical trans-esterification of plant oils, respectively [10]. In ethanol fermentation, sugars are converted by microorganisms to produce ethanol and carbon dioxide (CO_2) [11]. Fermentation essentially takes place in anaerobic conditions [12]. Microorganism that is most commonly used in fermentation process is *S. cerevisiae* [13]. *S. cerevisiae* consumes significant amounts of substrate in adverse conditions, has a high resistance to ethanol, and inhibitors present in the medium [14].

Malaysia oil palm industry generates excessive oil palm wastes. Out of the total nation's agricultural industry, 85.5 % of waste comes from oil palm industry [15]. Among the wastes were empty fruit bunches, palm oil mill effluent, palm kernel shell, oil palm trunk, oil palm leaves, oil palm fronds and mesocarp fiber [16]. The waste generated from oil palm trunk (OPT) has been chosen for bioethanol production because it contains high moisture and a heterogeneous physical and chemical material composition including a huge quantity of short-chain carbohydrates which are great sources of sugar production as in **Table 2** [17].

Table 2 : Sugar present in oil palm trunk sap [17]

Sugar Component	Contents (%w/w)
Sucrose	6.37
Glucose	84.21
Fructose	5.20
Xylose	1.62
Galactose	1.51
Rhamnose	0.07
Others	1.02

2.1 Microbes for Fermentation

At present, yeasts are used by many researchers to produce fuel ethanol from renewable energy sources through fermentation process [18]. In a fermentation process, yeasts convert sugar into alcohol (ethanol) and carbon dioxide. There are numbers of discovered bacteria suitable to be used in fermentation process as shown in **Table 3**. Engineered *Pichia stipitis* (BCC15191) was used in the

fermentation of hydrolyzed sugarcane bagasse and resulted in 8.4 g/L of ethanol after 24 hours [19]. *Zymomonas mobilis* was also used in bioethanol production as the fermentation agent, and the result was 3.54g/L/h of ethanol yield [20]. Meanwhile, few research works were carried out using yeast from *S. cerevisiae* as fermentation agent in bioethanol production [21,22]. As shown in **Table 3**, microorganisms yeasts play a vital role in bioethanol production by fermenting a wide range of sugars to ethanol. The most fermentation agent used by many researchers is *S. cerevisiae* as it tolerates a wide range of pH [9]. Mohd Azhar et al had proven that yeasts especially *S. cerevisiae* is the common microbes employed in ethanol production due to its high ethanol productivity, high ethanol tolerance, ability of fermenting wide range of sugars compared to other types of microorganisms, low cost and its availability [18].

Table 3 : List of microorganisms used in ethanol production with different feedstock

Microorganism	Feedstock	Ethanol yield	References
Bacterium			
Pichia stipitis BCC15191	Sugarcane bagasse	8.4 g/L	[19]
Zymomonas mobilis AX101	Wheat straw and corn stover	3.54g/L/h	[20]
Yeast			
Candida shehatae NCL-3501	Rice straw	0.45 g/g of sugar	[21]
S. cerevisiae 590. E1	Corn stover	63%	[23]
S. cerevisiae ATCC 24680	OPT sap	0.50 g/g	[22]

3. Optimization of Physical Factors

The efficiency of the fermentation process and *S. cerevisiae* growth affected by a few factors which are pH, temperature, time, size of the inoculum, and agitation rate. Various process parameters such as incubation temperature, incubation period, initial pH, and nitrogen sources were studied to achieve the maximum yield of ethanol [24]. These findings indicate that the optimization of process parameters is necessary to make the fermentation process economical, and the medium designed in the present study could be exploited on a commercial scale after suitable processing [24]. According to the research of Mohd Azhar et al [18], cultural conditions play an important role in microbial growth and the production of ethanol.

3.1 Temperature

Bioethanol production during fermentation depends on several factors such as pH, temperature, incubation time, agitation rate, and inoculum size. Temperature influenced the growth rate of the microorganisms [25]. High temperature is a stress factor for microorganisms and is unfavorable for cell development,. The optimal fermentation temperature range is between 20 °C and 35 °C [25]. Free cells of *S. cerevisiae* have an optimum temperature of about 30 °C, whereas immobilized cells have a significantly higher optimum temperature owing to their capacity to pass heat from the surface of the particles to the inside of the cells [26]. Phisalaphong et al. stated that enzymes that regulate microbial activity and fermentation processes are sensitive to high temperatures that can deform its tertiary structure and inactivate enzymes. The temperature is therefore closely controlled during the fermentation cycle [27].

Most of the fermentation process performed using *S. cerevisiae* had been conducted at 30 °C while fermentation carried out using *Kluyveromyces marxianus* has been tested at 42°C as shown in **Table 4**. The ideal temperature for the production of bioethanol depends on the ideal temperature of the yeast. It was proven that a suitable value of temperature will help the effectiveness of ethanol fermentation. Research by Cazetta et al., in 2007 concluded that ethanol yield may be significantly affected by fermentation temperatures, which increase to some degree with the increase in temperature [28]. However, several earlier studies have also shown that temperatures above 37 °C are harmful to the

growth of bioethanol production. On the other hand, much lower temperatures during fermentation cause lower specific cell growth rates and lower ethanol tolerance. Thus, it can be concluded that the optimum temperature for the production of bioethanol is between 30 to 33 °C.

Table 4 : Optimized temperature used in bioethanol production

Yeast strain	Feedstock	Temp (°C)	Ethanol yield (g/L/h)	References
S. cerevisiae	Cassava starch	32	2.10	[29]
S. cerevisiae	Corn stover	30	0.57	[30]
S. cerevisiae	Instant noodle waste	30	1.72	[31]
S. cerevisiae	Wood	30	1.16	[32]
S. cerevisiae	Reed	38	0.57	[33]
S. cerevisiae	Sweet potato	30	4.76	[34]
Kluyveromyces marxianus	Water hyacinth	42	0.31	[35]
Kluyveromyces marxianus	Wheat straw	42	0.50	[36]
S. cerevisiae	Paper sludge	33	0.59	[37]
S. cerevisiae	Cassava mash	33	2.41	[38]

3.2 pH

pH is one of the main factors that affects ethanol fermentation. The growth and fermentation rate of yeast are affected by pH as well as the fermentation products. The research done by Zabed et al. found that ethanol production was influenced by the pH of the broth as it affects bacterial contamination, yeast growth, fermentation rate, and by-product formation [39]. The permeability of some essential nutrients into the cells is influenced by the concentration of H^+ in the fermentation broth [39]. **Table 5** shows the optimized pH for different microbes. The optimum pH for a bacterium is in the range of 5.0 to 7.0 meanwhile for yeast, is in the range between pH 5.0 to 5.5.

Table 5 : Optimized pH used in bioethanol production

Microorganism	pH	References
Bacterium		
Pichia stipitis BCC15191	5.5	[19]
Zymomonas Mobilis	5.0	[20]
Thermoanaerobacterium saccharolyticum ALK2	7.0	[40]
Yeast		
Candida shehatae NCL-3501	5.5	[21]
S. cerevisiae	5.3	[34]
S. cerevisiae	5.5	[32]
S. cerevisiae	5.5	[41]
S. cerevisiae	5.5	[42]

Table 5 also shows the usage of yeast in most of the fermentation process, and *S. cerevisiae* was used by most researchers in bioethanol production. *S. cerevisiae* species, are stable microorganisms in food and beverage fermentation and may be employed in many forms under different fermentation conditions [43]. Coculturing *S. cerevisiae* with other yeasts or microbes is aimed to optimize ethanol yield, shorten fermentation time, and reduce process cost [44]. **Table 6** shows that *S. cerevisiae* had been used as a fermentation agent at the optimized pH range between 5.0 to 5.5.

Table 6 : Optimized pH used in bioethanol production using various samples

Sample	pH	References
Sugarcane	5.5	[24]
Sweet potato	5.0	[45]
Corn stover	5.5	[30]
Sweet potato	5.3	[34]
Wood	5.5	[32]
Reed	5.0	[33]
Oil Palm Trunk	5.5	[41]
Oil Palm Trunk	5.5	[42]

3.3 Incubation time

Fermentation time affects the growth of microorganisms [39]. Complete fermentation can be achieved at a lower temperature by using longer fermentation time which results in the lowest ethanol yield [39]. **Table 7** shows the optimized incubation time in *S. cerevisiae* fermentation in various studies.

From **Table 7**, it can be seen that high ethanol production of 89.1 g/l was produced in 66 hours by Choi et al. in 2010 [29]. Swain et al. found out that combining bacteria (*Trichoderma sp*.) and *S. cerevisiae* had shown an incredible value within 72 hours with 172 g/kg of ethanol production [46].

Table 7 : Optimized incubation time in bioethanol production

Microbe	Incubation time (h)	Concentration of bioethanol	References
S. cerevisiae	48	24.6 g/L	[48]
S. cerevisiae CHY1011	66	89.1 g /L	[29]
S. cerevisiae ZU-10	72	41.2 g/L	[30]
S. cerevisiae	24	914 g/L	[34]
S. cerevisiae CHFY0321	42	86.1 g/L	[38]
S. cerevisiae BY4743	24	31.06 g/L	[49]
S. cerevisiae	72	172 g/kg	[46]
S. cerevisiae CICC 1308	48	32.91g/L	[50]
S. cerevisiae	96	584.3 g/kg	[51]
S. cerevisiae	96	13.3 g/L	[52]
S. cerevisiae K3	72	767 g/kg	[53]
S. cerevisiae	50	818 g/kg	[54]
Kluyveromyces marxianus CECT 10875	72	36.2 g/L	[36]
Kluyveromyces marxianus	72	64.3 g/L	[55]
Pichia kudriavzevii DMKU 3-ET15	48	78.6 g/L	[56]
Escherichia coli KO11 and *Klebsiella oxytoca* P2	24	37.5 g/L	[47]
Kluyveromyces marxianus TISTR5925	72	45.4 g/L	[57]

Other researchers were using other types of yeast like *Kluyveromyces marxianus*, *Pichia kudriavzevii* and *Klebsiella oxytoca* at a time range of 24 to 72 hours of fermentation. High ethanol production was produced within 48 hours by Limtong et al. [56]. The combination of bacteria (*Escherichia coli* KO11) and yeast (*Klebsiella oxytoca* P2) as a microbe for fermentation showed that in 24 hours, they can reach the maximum value of ethanol production of 37.5 g/l [47]. Table 7 shows that the incubation time were constructed from 48 to 72 hours.

From **Table 8**, there was various biomass that had been used as a feedstock for the fermentation. Several research works were using oil palm trunk (OPT) as the feedstock while the others were fruits, waste, and juice. By using the OPT as the feedstock, it is observed that the maximum ethanol production reached as high as 81.8% within 50 hours of fermentation in a research carried out by Shahirah et al. [58]. The time range that can be concluded from the researcher that using OPT is in the range of 48 to 72 hours. Other researcher that were using various sources, the time range used were around 24 to 72 hours. These various feedstocks showed a promising maximum amount of bioethanol production. Also, feedstock like sugar cane juice is a promising biomass for maximum ethanol production of 64.3 g/l in 72 hours of fermentation. From **Table 8,** it can be concluded that promising ethanol production with maximum value is around 48 to 72 hours of fermentation.

Table 8: Incubation time in the fermentation of various sample

Sample/medium	Time range (hours)	Results	References
Oil palm trunk	72	45.4	[57]
Oil palm trunk	96	58.43% (w/w)	[51]
Oil palm trunks	96	13.3 g/L	[52]
Oil palm trunk	72	76.7%	[53]
Oil palm trunk	50	81.8%	[54]
Industrial Potato Waste	48	24.6 g/L	[48]
Cassava starch	66	89.1 g /1	[29]
Corn stover	72	41.2 g/L	[30]
Sweet potato	24	4.76 g/L/h and 91.4%	[34]
10875 Wheat straw	72	36.2 g/L	[36]
Cassava mash	42	86.1 g/L	[38]
Sugarcane leaf waste	24	31.06 g/L	[49]

3.4 Inoculum Size

A suitable and optimal inoculums size is critical to achieving more efficient bioethanol production from OPT sap [22]. However, a study from Zabet et al., in 2014 found that inoculum concentration does not give significant effects on the final ethanol concentration but it affects the consumption rate of sugar and ethanol production. [39]. Besides, inoculum size also affects yeast growth, and the course of fermentation as stated in the research of Erten et al. in 2006 [59]. The researchers also concluded that yeast inoculum level significantly affected wine fermentation. It shortened the fermentation time. The non-*Saccharomyces* yeasts disappeared quickly with increasing inoculum size [59]. From **Table 9**, it can be clearly seen that most bacterium has 10% v/v of its microbes as optimum inoculum size meanwhile optimum of inoculum size in yeast is in a range of 3 to 10% v/v.

Table 9 : Optimized inoculum size of microbes

Employed Microorganism	Inoculum size (% v/v)	References
Bacterium		
Zymomonas Mobilis	10	[20]
Thermoanaerobacterium Saccharolyticum ALK2	10	[40]
Pichis stipitis	10	[32]
Yeast		
S. cerevisiae	5	[29]
S. cerevisiae	10	[60]
Kluveromyces marxianus	8	[61]
S. cerevisiae	3	[62]
S. cerevisiae	3	[59]
Candida shehatea	5	[63]

Table 10 shows the optimum inoculum size of yeast in *S. cerevisiae* with the different samples used by each research. Thus, it can be concluded that at average, the fermenting medium used in the production of bioethanol has an inoculum size of 10% v/v.

Table 10 : Optimized inoculum size in *S. cerevisiae* with various sample

Sample	Inoculum size (% v/v)	References
Sugarcane bagasse	3	[62]
Waste potato mash	3	[64]
Oil Palm Trunk	10	[22]
Oil Palm Trunk	10	[65]
Oil Palm Trunk	5	[66]
Cassava starch	5	[29]
Corn stover	5	[30]
Sweet potato	7	[34]
Wood	10	[32]
Reed	10	[33]
Oil Palm Fronds	20	[67]
Sweet potato	10	[45]
Sugarcane	3	[24]

3.5 Agitation Rate

An increase in agitation speed results in a better-dissolved oxygen concentration in the fermentation medium, thus yeast is supplied with an adequate amount of oxygen, making them favor respiration than fermentation [67]. A study by Rodmui et al [68] in 2008 found that agitation to have a major impact on growth rate and ethanol production since substantial changes in the formation of biomass were observed in the experimental studies. The concentration of dissolved oxygen from agitation was thought to have been involved in the processing of cell density and ethanol. By changing agitation in the batch fermenter, yeast growth and ethanol production could be improved [68]. **Table 11** shows the comparison of agitation optimization and microbes between bacterium and yeast. The optimum agitation rate for a bacterium is in a range between 120 to 200 rpm meanwhile 110 to 200 rpm is for yeast.

Table 11: Agitation optimization

Microorganism	Agitation rate (rpm)	Reference
Bacterium		
Bacillus subtilis	121	[69]
Pichia stipitis	200	[70]
Escherichia coli SJL25	150	[71]
Pichia stipitis	150	[72]
Yeast		
Candida tropicalis	200	[68]
Kluveromyces marxianus	156	[61]
Candida glabrata	150	[73]
S. cerevisiae	150	[32]
S. cerevisiae	150	[34]
S. cerevisiae	110	[41]
S. cerevisiae	120	[30]

Table 12 shows that most of the fermenting medium used for bioethanol production has an agitation rate of 150 rpm with various ethanol concentrations.

Table 12 : Optimized agitation rate in bioethanol production with various sample

Sample	Agitation rate (rpm)	Reference
Oil Palm Fronds	100	[67]
Reed	150	[33]
Wood	150	[32]
Sweet Potato	150	[34]
Oil Palm Trunk	150	[58]
Corn stover	120	[30]
Cassava starch	120	[29]
Oil Palm Trunk	110	[41]

4. Conclusion

This review was carried out to find the optimum value of different parameters to get the highest production of bioethanol. Yeast is the most common microorganism in the production of bioethanol which plays an important role in the fermentation of ethanol sugars. This review paper addressed the optimum value of different parameters during the fermentation cycle including temperature, pH, incubation time, inoculum size, and agitation rate using *Saccharomyces cerevisiae*. Overall, the findings indicate that the optimum value of temperature, pH, incubation period, inoculum size and agitation rate for fermentation process using *S. cerevisiae* are in the range of 30 to 33 °C, 5 to 5.5 pH, 48 to 72 hours, 10% v/v, and 150 rpm, respectively. Therefore, better yield and concentration of bioethanol depends on the selection of microorganisms and fermentation mode and techniques as well as the influence of several physical factors.

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