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Fermentation Factors Optimization To Enhance Yeast Performance In Producing High Yield Of Bioethanol

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Abstract: The decreasing in number by year of fossil fuel has bring attention to the world as it has been identified as future challenge. To avoid depletion of fossil fuel, the fermentation of Oil Palm Trunk extraction by *Saccharomyces cerevisiae* added with sterile mature coconut water can produce a very efficient amount of bioethanol. The purpose of adding mature coconut water (MCW) which act as additive is to magnify the fermentation process in yeast. There are few methods of sterile used such as pasteurization, autoclaving and filtration in order to preserve the natural nutrient content of MCW. On top of that, this review article is an attempt to gather the research findings about the effect of natural nutrient content of MCW after undergoing suitable sterilization methods. With that, the most suitable method chosen is filtration as the process does not introduce any heat supply which will not cause any nutrient denature even as pasteurization and autoclave where those are focusing on heat and steam. As the nutrient preserved adding into fermentation microbe, the fermentation resulted in higher amount of bioethanol production. Hence, this proved that the optimized fermentation performance is by adding the filtered natural nutrient of MCW in *S. cerevisiae* to enhance the production of high yield of bioethanol.

Keywords: Nutrient Addition, Fermentation Performance, Bioethanol Production

1. Depletion of Fossil Fuel

Fossil fuels and minerals are finite and non-renewable and these resources are therefore limited physically and economically [1]. Depletion of fossil fuel can be defined as reduction in the number or quantity of something while fossil fuels is a natural fuel such as coal or gas, formed in the geological past from the remains of living organisms. The depletion of fossil fuels can be defined as extraction of natural gas, oil and coal reserves at a rate higher than nature replenishes them. Those coal, crude oil, and natural gas are all considered as fossil fuels because it has a high carbon content. This is because it

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was formed from the fossilized, buried remains of plants and animals that lived millions of years ago. The example of fossil fuels is crude oil or petroleum (rock oil) which a liquid fossil fuel that are made up mostly of hydrocarbons. It also can be found in underground reservoirs..

The Depletion index of fossil fuel from 1997 year until 2050 year of various fossil fuel such as coal, oil and gas. This depletion of fossil fuels is classified as a new challenging problem in future. Higher energy demands and poor efficiency practices have increased fossil fuel usage. The issue of depletion and over exploitation of natural resources are not recent concerns.

2. Fermentation Process

Fermentation is a process that convert complex sugar into alcohol and acid by organisms and its happens naturally. Any liquid that contain sugar could become medium for organisms to produce other valuable material. In this review, the usage of OPT sap as main carbon sources of fermentation as its contain adequate ready to ferment sugar. The fermentation of OPT sap by microbe will convert sugar into bioethanol. Fermentation process consists a series of bio catalysed reaction by microbes that contain enzyme that can convert sugar into ethanol and carbon dioxide. Various species of *Saccharomyces* have been used for fermentation process as they are very effective for converting complex sugar to ethanol and among them *S. cerevisiae* is experimented as the most efficient [2]. In order to enhance the end product of fermentation, the addition of nutritional supplements is one of the techniques that can be applied. Optimization conditions and parameters of fermentation process are essential to produce high yield of bioethanol. Temperatures ranging from 32°C to 37°C and pH from 4.5 to 5.5 have also been studied for optimizing the preinoculum growth as well as ethanol production in the simultaneous scarification and fermentation [3].

2.1 Bioethanol

Ethyl alcohol is typically known as ethanol. The chemical structure of ethanol is C_2H_5OH. While bioethanol is produce by bioprocess called fermentation. One of the usage of bioethanol is as a gasoline improver or octane enhancer and in bioethanol-diesel blends to reduce the emission of exhaust gasses [4]. There is a growing trend in development countries towards the use of modern technologies and the productive processing of bio-energy using a variety of biofuels that are becoming cost-effectively competitive with fossil fuels [5]. Biofuels are produced from organic materials (biomass) that can be used as fuel for transport, heating and for generating electricity. These are usually mixed with fossil fuels at varying levels from 5% to 85% by volume depending on the engine's suitability. These can also be used in adapted engines pure form [6].

2.2 *Saccharomyces Cerevisiae*

Microorganisms such as yeasts play an essential role in bioethanol production by fermenting a wide range of sugars to ethanol [7]. There are numbers of discovered bacteria also suitable as fermentation microorganisms but the production of bioethanol is not as high as production by yeast. *S. cerevisiae* is one type of yeasts that naturally consumes hexose sugars (e.g. glucose, fructose). *S. cerevisiae* is the most commonly employed yeast in industrial ethanol production as it tolerates a wide range of pH [8] thus making the process less susceptible to infection. *S. cerevisiae* contains maltase enzyme which will encounter a maltose molecule and breaks it in two glucose molecules. Other than that, it also contains the invertase enzyme that can encounter sucrose molecule and break it into glucose and fructose molecule. **Table 1** shows the used of *S. cerevisiae* in production of bioethanol in various research.

Table 1: Uses of *S. cerevisiae* in fermentation

Employed microorganism	Features	Substrate used	Ethanol yield	References
S. cerevisiae D5a	Improved ethanol yield	Rice hull	•0.58% (w/v) or 100% theoretical yield	[9]
S. cerevisiae 590. E1	Ferment glucose and cellobiose	Whatman paper	•1.09% from 2% glucose •1.16% from 2% cellobiose	[10]
S. cerevisiae 590. E1	Ferment cellulose without additional enzymatic hydrolysis process	Corn stove	•63% theoretical ethanol after 96 h fermentation	[10]
S. cerevisiae RWB 217	Ferment glucose and xylose	2% glucose +2% xylose	•0.43 g/g of sugars	[11]
S. cerevisiae RWB 218	Ferment glucose and xylose	2% glucose +2% xylose	•0.4 g/g of sugars	[11]

3. Oil Palm Trunk as Main Carbon

About 39% of world palm oil production are from Malaysia with 44% of the world exports. There are 5.23 million hectares of oil palm plantation in Malaysia in 2013. Oil palm tree are replanted in every 20-25 years due to decrease of oil production, difficulty in harvesting fruits [12]. Unfortunately, the usage of oil palm trunk is very limited due to the quality of trunk are not suitable for furniture and other application. Interestingly, the inner parts of oil palm trunk contain a large amount of ready to ferment sugar in the sap. This valuable material can be used as main carbon sources for fermentation process to produce bioethanol. Thus this give an added value to oil palm industries which it has a high potential to become main source of bioethanol production in Malaysia.

Unlike other tree trunk, OPT sap contains high moisture and a heterogeneous physical and chemical material composition comprising a huge quantity of short-chain carbohydrates which are great sources of sugar production. OPT sap sugar are inedible and rich in sugar placed OPT sap as one of the potential source for bioethanol production in Malaysia. OPT mostly comprises lignocellulose which is composed of polysaccharides (starch with 39.9% cellulose, 21.2% hemicellulose) as well as 22.6% lignin, 1.9% ash, 3.1% wax and 11.3% other constituents [13]. Naturally glucose is the main sugar component in OPT sap sugar constituents [13]–[15]. Glucose, sucrose and fructose are three main sugar contained in OPT which yield 84.21%, 6.37% and 5.20% respectively [16].

4. Mature Coconut Water

Both green coconut water and mature coconut water contains tremendous properties of nutrients and makes it widely used commercially in beverage industry. Various studies and commercial applications were focusing on using green coconut water but rarely focus on mature coconut water properties and usage.

Usually, only the flesh of mature coconut is used mostly in production of coconut milk and the water of mature coconut water is discarded. The taste of mature coconut water is slightly salty and the volume is smaller than green coconut water [17]. Nevertheless, mature coconut water also contained high in minerals such as potassium, sodium and protein content [17] but rarely used in any application and it is discarded as waste. Mature coconut water has a great potential to be developed as nutrient enhancer in various biological process since it contained minerals, sucrose and protein that are essential for cell metabolic pathways.

Generally, aging of coconut fruit will produce 3 maturity stages of product such as immature, mature and overly mature as stated in **Table 2**. Ever since having different stages, thus the nutrient content might slightly different too. Coconut water contains a complex of vitamins and minerals. Coconut water, green or matured, consists of sugars, vitamins, amino acids and minerals that play different bio-functional roles in the human metabolic system and nutrient sources for microbial growth [18]. Maturity of coconut increase the amount of total reducing sugar [19].

Table 2: Physicochemical properties of 3 stages of coconut water

Physicochemical properties	Coconut maturity stage		
	Immature	**Mature**	**Overly-mature**
Volume of water (mL)	684 ± 27.0^{a}	518 ± 14.2^{b}	332 ± 19.9^{c}
TSS (°Brix)	5.6 ± 0.14^{b}	6.15 ± 0.21^{a}	4.85 ± 0.17^{c}
TA[d] (%)	0.089 ± 0.004^{a}	0.076 ± 0.008^{b}	0.061 ± 0.003^{c}
Ph	4.78 ± 0.13^{c}	5.34 ± 0.12^{b}	5.17 ± 0.10^{a}
Turbidity	0.031 ± 0.0013^{c}	0.337 ± 0.108^{b}	4.051 ± 0.323^{a}
Sugar Content			
Fructose (mg/mL)	39.04 ± 0.824^{a}	32.52 ± 0.227^{b}	21.48 ± 0.21^{c}
Glucose (mg/mL)	35.43 ± 0.510^{a}	29.96 ± 0.243^{b}	19.06 ± 0.19^{c}
Sucrose (mg/mL)	0.85 ± 0.010^{c}	6.36 ± 0.06^{b}	14.37 ± 0.25^{a}
Minerals			
Potassium (mg/100 mL)	220.94 ± 0.320^{c}	274.32 ± 0.139^{b}	35.11 ± 0.133^{a}
Sodium (mg/100 mL)	7.61 ± 0.041^{b}	5.60 ± 0.016^{b}	36.51 ± 0.020^{a}
Magnesium (mg/100 mL)	22.03 ± 0.069^{b}	20.87 ± 0.023^{b}	31.65 ± 0.038^{a}
Calcium (mg/100 mL)	8.75 ± 0.045^{c}	15.19 ± 0.028^{b}	23.98 ± 0.054^{a}
Iron (mg/L)	0.294 ± 0.082^{b}	0.308 ± 0.011^{b}	0.322 ± 0.049^{a}
Protein (mg/mL)	0.041 ± 0.007^{b}	0.042 ± 0.002^{b}	0.217 ± 0.018^{a}
TPC[e] (mg/L)	54.00 ± 3.135^{a}	42.59 ± 0.834^{b}	25.70 ± 1.756^{c}

Approximately 25% of coconut fruit weight is contributed by coconut water which composed of 95.5% of water, 4% carbohydrates, 0.1% fat, 0.02% calcium, 0.01% phosphorous, 0.5% iron, in addition to amino acids, vitamin C, B complex vitamins and mineral salts [20]. Coconut water also rich of various amino acid such as lysine, histidine, tyrosine and tryptophan, fatty acid, glucose, fructose, cellulose, sucrose, and organic acids such as tartaric, citric and malic acids [21]. **Table 3** shows the amino acid compositions in mature coconut water.

Table 3: Amino Acids Contain in MCW.

GABA/Amino Acids	Amount (mg / 100ml)		Mg/kg body weight
	MCW	SCW	
GABA	12.80	10.10	na
Aspartic acid	7.21	7.33	na
Threonine	1.45	1.47	15
Serine	3.81	3.73	na
Glutamic acid	280.52	324.41	na
Proline	8.56	8.63	na
Glycine	4.10	4.25	na
Alanine	26.46	26.61	na
Cystine	nd	nd	na
Valine	1.26	1.27	26
Methionine	2.53	2.52	10.4
Isoleucine	0.94	1.08	20
Leucine	1.49	1.53	39
Tyrosine	8.88	9.83	20
Phenylalanine	65.18	64.19	25
Histidine	2.66	2.90	10
Lysine	4.35	4.33	30
Arginine	3.00	2.97	na
Tryptophan	0.32	0.32	4

5. Nutrient Effect in Fermentation Performance of *S. cerevisiae*

S. cerevisiae is the main microorganism employed in fermentation, mainly in wine making and also in bioethanol industry. Effectiveness of *S. cerevisiae* growth affected by few factors which are pH, temperature, time and size of inoculum and addition of additive. Most *S. cerevisiae* strains grow at pH values between 2.50 and 8.50, but they are acidophilic organisms which grow better under acidic conditions [22]. It ferments all kind of hexoses with yields close to the maximum and it is very tolerant to high ethanol concentrations [23]. *S. cerevisiae* is often chosen for the ethanol production mainly due to its excellent fermenting capacity, capacity to grow rapidly under anaerobic conditions and high tolerance to ethanol [12]. *S. cerevisiae* is robust and the fermentation process and product of fermentation is highly influenced by medium composition. Nitrogen is essential for cell proliferation in anaerobic condition which resulted high yield of bioethanol production and yeast growing activity. Therefore, the fermentation performance can be enhanced by the presence of nutrients and especially good nitrogen sources and metals. Bioethanol yield from sugar fermentation can be enhanced by adding micronutrients such as N, P, K and Mg salt as these micronutrients are necessary for yeast growth and metabolisms. Those essential inorganic nutrients are exclusively obtained from coconut water which can be added as additive in fermentation process. Thus, coconut water may play an important role as fermentation enhancer which provides essential nutrients for cell such as *S. cerevisiae* to produce high yield of bioethanol.

Fermentation of OPT profile by *S. cerevisiae* without and with nutrients addition are shown in **Figure 1** (A) and (B). In both settings, it shows the fermentation profile for OPT without and with nutrient were similar except for ethanol production was significantly higher. Ethanol production in fermentation of OPT by *S. cerevisiae* increased up to 26.5% by addition of nutrition in the fermentation medium which bioethanol yield in fermentation setting without nutrition mark as 55.39%, meanwhile with nutrition addition marks as 81.89%. This can be clearly shown in **Table 4** whereby the production of bioethanol gradually increased due to the nutrients received by the yeast from MCW to grow healthily. Therefore, the addition of nutrients is one of the reliable methods to increase ethanol yield during fermentation of OPT.

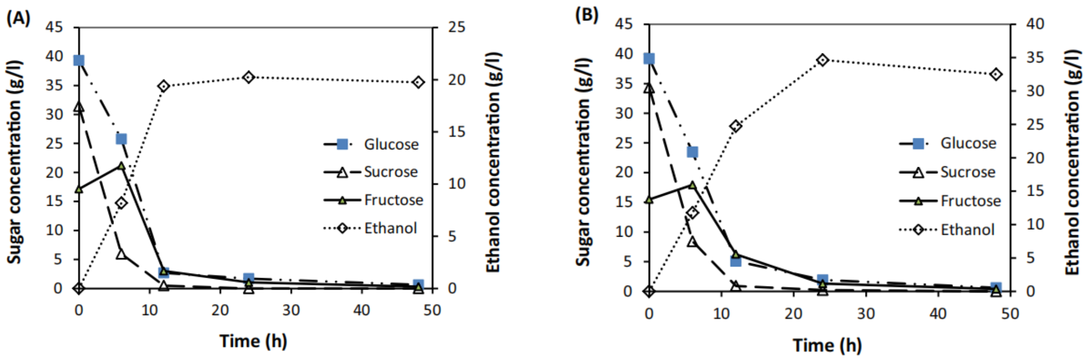

Figure 1: Fermentation profile for OPT fermentation by *S. cerevisiae* (A) without nutrient addition (B) with nutrient addition

Table 4: Comparison of bioethanol production with and without nutrient additives in fermentation by *S. cerevisiae*

Additives Sources	Nutrients Content	Yield of ethanol	References
Mature Coconut Water	Amino acid - lysine, histidine, tyrosine and tryptophan	81.89%	[20]
No additives	-	55.39%	[12]
E. *foetida* flour (EF) and hydrolyzed chicken feathers (HF)	Nitrogen, acid hydrolysis, amino acid and peptides	50% with control	[24]
No additives	-	71%	[25]
1g Alanine amino acid and 1g Epsom salt	Alanine amino acid and Epsom salt	58.43%	[26][26]

6. Sterilization Technique

Sterilization is any process that kills and eliminate transmissible agents such as microbes. In practice, sterility is achieved by exposure of the object to be sterilized to chemical or physical agent for a specified time [27]. Pasteurization is a process that combined of time and temperature, which using a relatively low temperature for a longer time interval of using a relatively high temperature for a short time interval [28]. Some studies have been made to evaluate the effect of pasteurization conditions on the inactivation of oxidative enzymes, peroxidase and polyphenol oxidase in coconut water as it has already been verified the activity of thermal resistant enzymes in such product [28].

Secondly, the technique used is autoclave. Generally, an autoclave is a pressure chamber used to carry out industrial and scientific processes requiring elevated temperature and pressure different from ambient air pressure. Autoclave is one of the moist heat sterilizations because it is used to achieve microbial inactivation. Moist heat sterilization involves the use of steam range in between of 121-1340 °C. Steam under pressure is used to generate high temperature needed for sterilization. Pressured steam in autoclaves used to kill bacteria and other living cells.

Sterilization by filtration method exclude living organisms based on the size of the organisms. The bigger particles compare to membrane pore size of the membrane were trapped by passing the membrane during filtration process. For the purpose of sterilization, selection of membrane that are smaller pore size than contaminants should be made to achieve the objective of the process.

7. Conclusion

A large amount of ethanol must be produced in order to fulfil the increasing worldwide demand**.** To conclude, the nutrient content in mature coconut water can be used to enhance the fermentation performance by *S. cerevisiae* . This is due to the yeast that accept higher concentration of sugar resulted in increase in fermentation rate. The chosen sterilization method to preserve the natural nutrient content before added into the yeast is filtration. Sterilization by filtration technique does not require any heat which clearly will not denature any of the nutrient contained MCW. By adding nutrient as additive will improve the fermentation performance and producing a higher yield of bioethanol. As the concentration of total sugar decrease, the bioethanol concentration increasing showed that fermentation rate is stable. The outcome stated of having additive is 81.89% while with the absence of additive is 55.39%. This shows that addition of nutrients is a devoted choice in fermenting the OPT in order to obtain higher yield of bioethanol.

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