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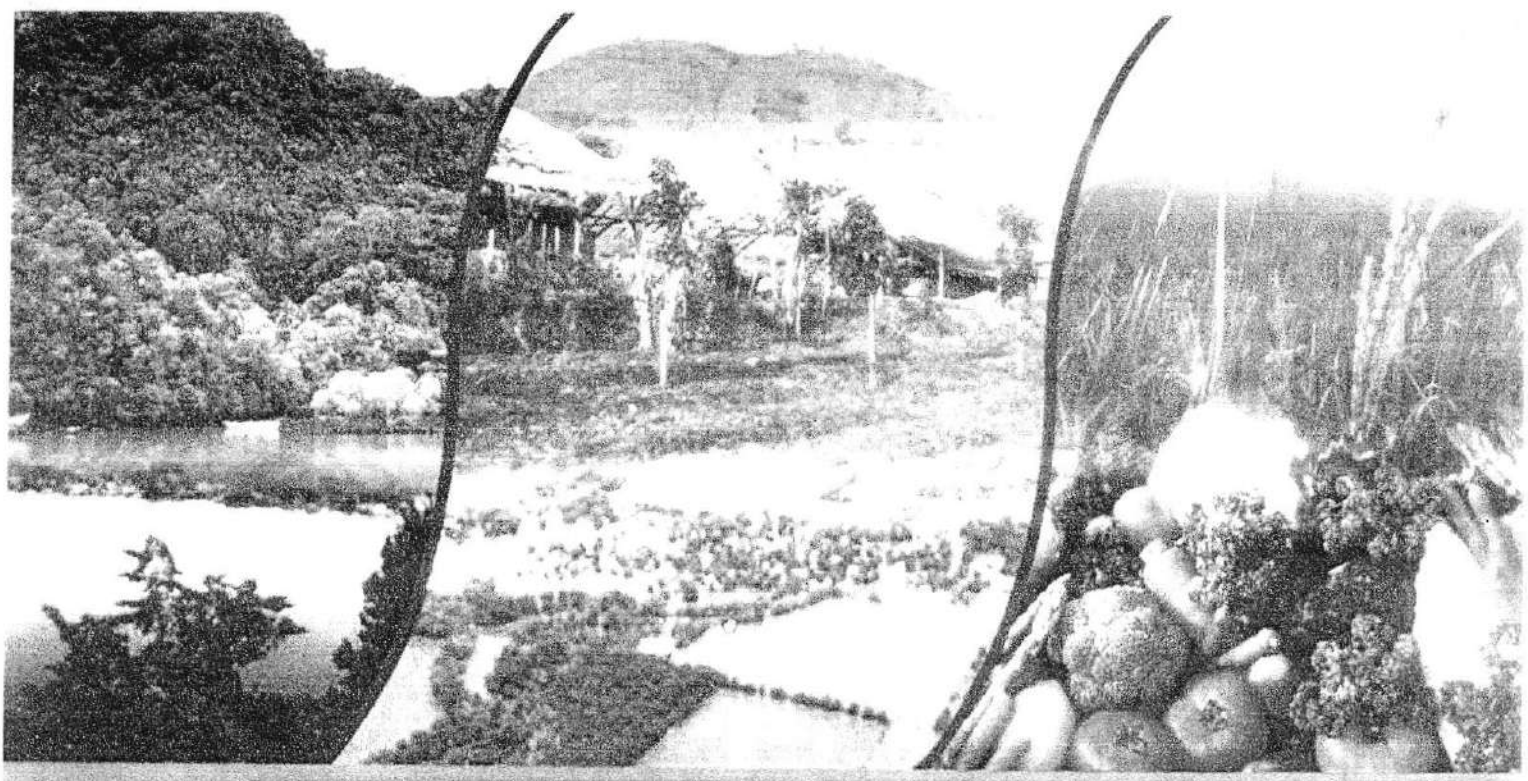
Proceeding



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- *Philippine Society for The Study of Nature (PSSN), Philippine*
- *CAB - University of Kasetsart, Thailand*

Proceedings
International Seminar Natural Resources Climate Change and Food Security in Developing Countries

Honorable Minister of Agriculture
Honorable Mayor of Surabaya
Honorable Speakers and Paticipans
Distinguished Guests, Ladies and Gentlemen.

At this precious moment, let us first express our gratitude to theMerciful God who has granted us with blessings and grace that we coud gather here today to attend the International Seminar on Natural Resources, Climate Change and Food Security in Developing Countries. Let me extend my warmest greetings and welcome to our distinguished speakers and participants, particulary those from abroad, my heartfelt welcome to our beloved city, Surabaya.I would like to express my appreciation to each of you for having attending this International Seminar.

As some of us may probably know, there has been cooperation between Philippine Society forthe Study of Nature (PSSN) and the University of Pembangunan Nasional "Veteran" East Java in the committee of the seminar. Furthermore, we certainly expect more programs to come in the near future as the continuation mutual cooperation.

Distinguished Guests, Ladies and Gentlemen,

In this era of globalization, where there have been rapid changes in climate with various implication as a result of the global activities of technology, we really need to be prepared to meet the challenges driven by the global demands. As we know in the developing countries, climate change cause yield declines for the most important crops. This means crop production to supply sufficent food for 2.5 billion people is really depend on the success of our efforts today. This seminar is an opportune time to discuss problems of mutual interest with delegates from some countries. It is gratifying to note that the agenda of the seminar covering a wide range of very interesting items relatingto the natural resources and food security under threat of climate change.

Distinguished Guests, Ladies and Gentlemen,

On behalf of Rector- UPN "Veteran" East Java, I would like to express my gratefulness to the local government, especially mayor of Surabaya for the support given to the committee. My appreciation also goes to the Steering and Organizing Committee who have made this program possible. May congratulate the commitee for success of conducting this international seminar.

Finaly, I wish all the participans success in a productive discussion throughout the seminar and a very pleasant stay in our beautiful city of Surabaya. Thank you.

Prof. Dr. Teguh Soedarto, Ir., MP.
(Rector of Univeristy of Pembangunan Nasional "Veteran" East Java, Surabaya-Indonesia)

KEYNOTE SPEECH

THE MINISTRY OF AGRICULTURE - REPUBLIC OF INDONESIA

SUSWONO

Future agriculture development, particularly increasing production to attain and maintain food security facing four main constraints *i.e.* (a) decreasing and degradation of land and water resources, (b) more frequent climate anomaly and climate change, (c) shrinking and conversion of fertile agricultural lands, and (d) fragmentation of present agricultural lands and limited availability of potential lands for expansion.

Increasing intensity of climate anomaly and climate change are threatening national food security and farmers welfare. Climate change with increasing atmospheric temperature will (a) change the rainfall pattern, (b) increasing the frequency of extreme climate such as El Nino and La Nina, and (c) rising the sea level.
Attaining soybean, sugar, meat and maintaining rice and maize self sufficiency is one of four goals in national food security. Climate variability and climate change are the main challenge in the achievement particularly for rice, maize and soybean that are vulnerable to climate change.

In coping with climate change the strategy are mitigation to harness the adverse effect of climate change by reducing GHG emission particularly in developed countries. The effects have been observed and the negative impacts already felt by the poor in the developing countries with limited ability and resources the need adaptation to increase resilience. Without proper anticipation, adaptation and mitigation efforts national food security certainly will be threatened.

Agriculture Complex Linkages to Climate Change Issues

Agriculture development is one of national agenda because of its multiple objectives both for economic and social purposes. It provides devisa and substatially contributes to gross domestic product. Socially, through agriculture rural development and poverty alleviation can be attained. Agriculture is also important politically by enhanciing national integrity by promoting inter-regional trade and environmently through development of sustainable agriculture
Agriculture has strong interlinkage with climate change. Agriculture has strategic role as the biggest employer and staple food provider but also as one of the most vulnerable to climate change. Despite small contribution presently, agriculture development can be directed to become sink by sequestering CO_2 in mitigation efforts.

Climate change is believed by increasing GHG emitted by human activities. Agriculture is one of the GHG sources through development af agricultural lland on peat soils as well as emission from paddy cultivation and livestoccks. GHG emission from agriculture excluding peat and forest fire is only 6%. Forest fire is the biggest contributor (65%), while paddy cultivation and livestock, respetively 24% and 9.3%.

Plants have the ability to absorb carbon in the form of CO2 is very large, some of which are utilized in process plants metabilisme, and most other stored in various form of organic material. At the annual crop of organic material are stacked in

With proper anticipation and intervention climate change can bring benefit to increase food production during La Nina with longer growing period. Rainfall during La Nina in 2010 cropped area became 109.3% of the target although because of poor anticipation due to pest and disease and post harvest handling productivity became 90.5% and production was only 98.9% of the target.

In the coming years threat of climate change to food security will become more apparent both directly due to pest and disease, shortage of available lands foe expansion due to the new forest and land management policy.

General Strategy and Government Policy

Many evidences showed that global warming and climate change is occurring. Therefore efforts to mitigate climate change through reduction of GHG emission and to increase the resilience of agriculture system to climate change should not be delayed. Delay action may result in more loss and high cost for adaptation. Kemal Dervis, Head of UNDP stated that" *If there is no mitigation....then the impact on developing countries 20-30 years from now will become much more severe and the adaptation needs,climate proofing, building dams against floods, changing crops...will become huge and impossible to handle*".

General policy in coping with climate change in agriculture is priotizing adaptation action program to maintain food security. Action program in mitigation is to support national commitment in reducing GHG emission by 26-41% through environmental fliendly and lo emission technology and farming system development in plantation, food crops and animal husbandry. Although the mitigation efforts are in the context of the four agriculture development goals particularly food security.

Direction and general strategy of agriculture in coping with climate change are stipulated in Agriculture Road Map Strategy in Coping with Climate Change that was launched in early 2010. Technically, the implementation of the strategy is carried out through: (1) optimizing the management of existing land, water and irrigation resources, (2) adjusting cropping patter and soil management particularly for food crops and crop diversification, (3) development and application of adaptive technologies with guideline and tools, and (4) application of environment friendly adaptive technologies.

To support the strategy, the Ministry of Agriculture has indetified and produced as well as developing many innovative adaptive technology either for mitigation and adaptation. Several strategic programs as communication, dissemination and information system can delivered through climate field schools, integrated resource and crop managemant (PTT) and system for rice intensification (SRI), along with support for field school for integrated resource and crop managemant (SLPTT) in all the provinces with rice production centres.

Adaptation strategy is based on several studies, among others are: (a) identification of impacts and vulnerability of agricultural resource and production, (b) identification and chracterization of landl and water resources potential, (c) identification of available adaptive technologies and farming system models. The application of adaptation program is through several programs as:

(a) Development of communication system as agro-meteorology information network (SJII) and field schools (climate, integrated resource and crop management)
(b) Development of farming institution,protection, support, insurance, capital, PUAP etc.

(a) increasing productivity by development and application of breakthrough farming technology, including new adaptive technology, harvest and post harvest technology
(b) Increasing cropping indices
(c) Soil and water conservation to maintain fertility and land productivity and improving the environment
(d) Protection of agricultural land from conversion through enforcement of Agricultural Land Protection Act
(e) Acceleration of the implementation of sustainable agricultural land protection Act No.41/2009, and the implementation of Ministry of Agriculture Regulation No. 53/2007 on Guideline for Mountinous Land Agriculture

For agricultural lands expansion to cater requirement of agricultural products is directed by policy as follow:

(1) Expansion of new agricultural lands priotizes in fulfilling land sfor food crops farming particularly rice through development of new lang by 100,00 ha annualy
(2) New areas priotize the utilization of idle lands or abandoned/dedraded lands particularly the APL areas aspecially mineral soil either in the dry or swampy lands.
(3) New areas for plantation priotize the concecionary land or lands with licence or arleady cleared lands still unutilized
(4) Encourage businesses/concecionary land owners to speed the utilization of idle (abandoned) lands, those have been cleared with penalty of withdrawing the licence or transfering the right
(5) Pushing the implemetation Agririan Reform that been launched 3 year earlier

During the enforcement of Presidential Decree No. 10/2011 Government will evaluate with due dilligent the implication and impacts of the policy both to mitigation aspects through MRV and the impacts on economic growth particularly agricultural sector.

Technology Preparedness to Land Resources Optimation

Direction of adaptive farming system development is aimed to: (a) development of green economy i agriculture as *Indonesia Carbon Efficient Farming* (ICEF), integrated crop livestocks systems (SIPT/SITT), (b) integrated farming system on dry climate (SPTL-KIK), (c) Development of innivative adaptive farming as , SRI, Ekofarming, IP 200-400, (d) land and water resources optimization, increasing productivity and existing agricultural land protection (PLPPB), and (e) expansion of agricultural lands without deforestation, utilization of idle, abandoned and degraded lands.

To support adaptation efforts, direction of adative technology development among other are: (a) development of high yield low emission varieties, drought and inundation tolerant, early and solinity tolerant varieties, (b) innovation of soil and water management, soil tillage, intermitent irrigation, peat soil management technology, compasting technology, (c) zero waste technology through agricultural waste utilization for organic fertilizer, feed and fuel (biogas) development.
Innovative technology for rice crop are (a) soil and water management for water use efficiency, (b) drough and inundation tolerant variety, salinity tolerant, pest and disease resistant and early maturity, (c) integrated pest management and harvest and post harvest handling.

factors in the efficacy of climate information system. However, the effictivity of coordiantion system and working relation along with span of control between Central-Province-District particularly in the implementation and span of control of program.

Remarks

There are close linkages between climate change and agriculture, because this sector is most affected and vulnerable but also contribute to the process of climate change, although small.

Climate change is happening and exacerbated by human activities, but also has opportunity to slow down and minimizing the adverse effects. There is strong and close relation between climate change and agriculture, because it is the most vulnerable but also potentially conributing although among the least.

Climate change among others causing more frequent climate anomaly will seriously affect national food security, particularly rice production system. Although agriculture potentially contributing in mitigating but the main priority is in adaptation efforts particularly is securing national rice production.

Beside to adjustment of approaches and technologies and farming patterns, various rescue strategies in food production and national food security is usage optimization of existing farmland and reorient the direction of the development and expansion of new agricultural areas. Optimization efforts are directed at intensification to increase produktivktas crops and land, while the expansion of agriculture is more aimed at potential productivity and land safely (allowed), especially the land abandoned, degraded in the APL.

Climate information systems and technology is one key to the success of the strategy and policy management and development resources to maintain food security in the face of climate change.

Sub Theme : <u>Bioproduct and Post Harvest</u>
Poster Presentation

Sub Theme : Social and Economic Issues
Oral Presentation

Sub Theme : Strategy and Food Security Policy
Oral Presentation

IMPROVEMENT OF ETHANOL PRODUCTION USING POLYPLOID *SACCHAROMYCES CEREVISIAE*

Muhammad Thamrin Hidayat and Isnawati

ABSTRACT

Polyploid organisms represent the ones containing more of chromosomes than their parental cells. In some respects, they possess several advantages. In order to render organisms polyploid, colchicine is used. The purpose of the present research was to render *Saccharomyces cerevisiae* cells polyploid by exposing it to colchicine solution of various concentrations. An indicator of *S. cerevisiae* polyploidy was its level of DNA. Concentrations and durations of exposure used in the research were 0, 1, 2, and 3 ppm and 48, 72, and 96 hours, respectively. In addition, the present research investigated whether the polyploid *S. cerevisiae* were capable of fermentative process by producing higher levels of ethanol. In doing so, fermentative process was carried out twice with durations of 48 and 96 h. Previously, *S. cerevisiae* cells were immobilized twice by means of Na-Alginate and $CaCl_2$. Results indicated that exposure of colchicine solutions of 0, 1, 2, and 3 ppm to the yeast cells affected population density of the yeast cell suspensions. A concentration of 2 ppm and duration of 48 hours were effective in producing a higher level of DNA (polyploid) relative to other concentrations. The highest level of ethanol was derived from *S. cerevisiae* cells exposed to 2 ppm of colchicine solution both at the first and second fermentations.

Keywords: Polyploid, S. cerevisiae, ethanol, immobilization.

INTRODUCTION

Leavens or yeasts represent single-celled organisms of 4-8 □m in width and 5-16 □m in length. These microorganisms are widely used by people in food processing of *tapai*, soy sauces, cheese, and yogurt due to their safety and effectiveness. It has been known that yeasts have a wide range of purposes from such highly important commercial sectors as processed biofuels, chemicals, enzyme industries, pharmaceuticals, and agriculture, to improvement of environmental sectors. During the energy crisis of increasingly depleted fossil fuels, yeasts hold significant potentials in the production of ethanol as an environmentally friendly biofuel (Farrell *et al.*, 2006).

The current practice of ethanol production was considered as in the need for improvement. This was reflected in the emergence of studies leading to a variety of straightforward methods of ethanol production with increased output.

In an experiment by Radoi (2002), it has been described that *S. cerevisiae* generated mutants of *S. cerevisiae* capable of enhancing fermentation output and that these mutants could tolerate highly low-acidic media, SO_2, and malic acid. Efforts by the abovementioned researchers to

The present research was expected to generate some polyploid *S. cerevisiae* and provide a method for speeding up fermentative process and contribute to a high level of ethanol in a short time.

MATERIALS AND METHOD

Materials used in this study were pure cell cultures of *S. cerevisiae*, colchicine solution, Yeast Extract Peptone Dextrose (YPD) medium (Stansfield, 2007), Peptone Patato Glucosa of Marck, Patato Dextrose Agar (PDA), glucose, alcohol, spirits, matches, aluminum foils, non-absorbent cottons, methanol, sterile aquabides, ethanol, $CaCl_2$ and Na Alginate, and Whitman filter paper no. 40 with millipore of 0.22 □m. Material originated from the kit produced by MACHERY-NAGEL (MN) of February 2008/Rev.2.

The present study was of a factorial design aiming at determining effect of colchicine solution concentration on population density of *S. cerevisiae*. Early population density of *S. cerevisiae* was observed and the final one at the end of colchicine treatment was observed again. An indicator for the effect of colchicine solution on the non-formation of spindle was the presence or absence of an increased level of DNA. Thus, there should be an analysis of DNA level in *S. cerevisiae* population exposed to colchicine solution of various concentrations as well as those unexposed to colchicine solution. The present research was additionally aimed at determining levels of ethanol produced by respective fermentative groups. *S. cerevisiae* population groups were exposed to colchicine solutions of 0, 1, 2, and 3 ppm with exposure durations of 48, 72, and 96 h. These were compared to length of the fermentative process with 10% glucose solution. Durations of fermentative process with glucose solution were 48 h (2 days) and 96 h (4 days) and the fermentative process was conducted twice for the same durations. Cells employed were those that have been immobilized. Ethanol produced was analyzed by means of gas chromatography. Furthermore, ethanol levels produced in each treatment were analyzed in terms of duration of exposure, colchicine solution concentration, length of fermentation, and *S. cerevisiae* population for difference in DNA levels. Additionally, in order to determine whether the immobilized cells of *S. cerevisiae* were capable of re-fermentation subsequent to the first fermentation treatment of 48- and 96-h durations, the second fermentative process was necessary to be performed with the same durations. In doing so, cells of *S. cerevisiae* were immobilized in

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On the basis of Table 1, results of statistical test for the order of effect of colchicine solution concentrations on the population density of *S. cerevisiae* suspensions were obtained (Table 2). Similarly, the order of effect of exposure durations on the population density of *S. cerevisiae* suspension was obtained, as well (Table 3).

Subsequent to isolation of *S. cerevisiae* suspension population, levels of DNA were then quantified by means of spectrophotometer, the results of which were shown in Table 4.

In order to determine the possible difference in DNA levels of *S. cerevisiae* suspensions among treatments with colchicine solution concentrations, statistical tests were conducted. Results were shown in Table 5.

In order to determine the possible difference in DNA levels of *S. cerevisiae* suspensions among treatments with different durations of exposure to colchicine solution, statistical tests were conducted. Results were shown in Table 6. Similarly, in order to determine the possible interactional effects of exposure durations with colchicine solution exposures among groups, ANOVA was performed and the results were shown in Table 7.

Analysis by the use of Duncan^{ab} for treatment interactions of colchicine solution exposures and durations of colchicine solution exposure with regard to levels of DNA indicated that the combination of 3-ppm treatment and 96-h immersion produced the lowest DNA level of 265 500 □/ml and that the the combination of 2-ppm and 48-h immersion produced the highest DNA level of 432 500 □/ml.

Results of correlation analysis of population density of *S. cerevisiae* suspensions and DNA levels were shown in Table 8.

Table 1. Results of analysis of variance for population density among concentrations and exposure durations.

Sources of Variance	Total Amount of Type III Squares	df	Avarage Squares	F	Significance
Conc.	7028.333	3	2342.778	3.499	0.050
Duration	9529.333	2	4764.667	7.117	0.009
Conc.*Duration	3007.667	6	501.278	0.749	0.622
Error	8034.000	12	699.500		
Total		23			

Table 6. The order of DNA levels due to effect of exposure durations to colchicine solutions

Exposure Durations	N	Subset 1	2	3
96 h	8	316.000		
72 h	8		343.250	
48 h	8			405.750
Significance		1.000	1.000	1.000

Note: Unit of DNA levels = µ/ml

Table 7. ANOVA of DNA levels due to interactional effect of exposure durations and colchicine solution concentrations.

	Total Amount of Squares	df	Avarage Squares	F	Significance
Inter-group	68277.750	11	6207.068	1664.465	0.000
Intra-group	44.750	12	3.729		
Total	68322.500	23			

Note: Unit of DNA levels = µ/ml

Table 8. Results of correlation analysis of population density of *S. cerevisiae* suspensions and DNA levels

		Population Density/0.025mm^3	DNA levels
Population Density/0,025mm^3	Pearson Correlations	1	–477
	Sig.		.018
	N	24	24
DNA Levels	Pearson Correlations	–477	1
	Sig.	.018	
	N	24	24

Note: Unit of DNA levels = µ/ml

Subsequent to exposure of *S. cerevisiae* to colchicine for 48, 72, and 96 h, suspensions of *S. cerevisiae* were then immobilized with $CaCl_2$ and Na Alginate. Cells of *S. cerevisiae* contained the beads were placed into 40 ml of 10% glucose solution. Cells of *S. cerevisiae* were expected to undergo the first fermentative process. The first fermentation took place for 48 and 96 h. Ethanol produced was measured by means of GC, the results of which were shown in Tables 9 and 10 below.

Table 9 showed ethanol produced from colchicine exposures in fermentative processes for durations of 48 and 96 h. At end of the first fermentative process (48 and 96 h), the beads were separated by glucose solution and then washed with sterile aquabides. In the second treatment of

Tabel 12 Ethanol produced in the first and second 96-h fermentative processes

Colchicine Concentrations	96/I			96/II		
	48 h	72 h	96 h	48 h	72 h	96 h
0 ppm	3.60%	0.15%	1.62%	4.99%	0.65%	0.80%
1 ppm	5.27%	0.62%	6.46%	3.04%	0.85%	1.24%
2 ppm	7.00%	5.82%	8.45%	9.00%	1.20%	2.34%
3 ppm	3.96%	0.22%	3.08%	5.80%	3.88%	0.00%
Total	19.83%	6.81%	19.61%	22.83%	6.58%	4.38%

The present research provided an evidence that 2-ppm colchicine solution with 48-h immersion was capable of forming a high level of *S. cerevisiae* DNA and, in fact, it could also form a high level of ethanol, on average. Thus, the three factors of solution concentration, exposure duration, and DNA level represented the ones influencing the formation of a high level of ethanol. Variable of DNA level constituted the largest contributor to a higher level of ethanol production. Thus, chromosomes possessed by the cells of *S. cerevisiae* served as metabolic controllers. Additionally, it could be argued that the 2-ppm concentration capable of rendering cells of *S. cerevisiae* polyploid. Furthermore, the 2-ppm concentration did not lead to chromosomal damages. This was indicated by the observation that the cells remained capable of proper metabolisms. Several studies have reported that duration of fermentative process represented a determinant of ethanol production. Vucurovic (2008) reported a relationship of a longer duration of fermentative process and a higher glucose concentration in producing a higher level of ethanol. In addition, he added that immobilized cells produced a higher level of ethanol than their non-immobilized counterparts. In the present study, the first and second 48-h fermentative processes with exposures to 0-, 1-, and 3-ppm colchicine solutions provided increases in ethanol level, on average. In the first fermentative process, the level of ethanol produced was 0.505%, while the second produced 3.225% at exposure to 0 ppm of colchicine solution. At exposure to 1 ppm of colchicine solution, the first fermentative process produced an ethanol level of 0.414%, while the second produced 2.412%. And at exposure to 3 ppm of colchicine solution, the first fermentative process produced an ethanol level of 0.327%, while the second 4.057%. This possibly occurred due to the adaptation of the existing cells to novel environment. In addition, many of those cells possessed diploid or haploid chromosomes, leading to extremely low levels of metabolism;

would be less effective; in other words, continuation of fermentative process beyond 96 h did not produce a higher level of ethanol.

CONCLUSION

Results of this study could be summarized as follows:

1. The 2-ppm colchicine concentration with exposure duration of 48 hours most effectively increased levels of *S. cerevisiae* DNA up to 432.500 μg/ml relative to other concentrations and exposure durations.
2. The highest level of ethanol was produced by cells of *S. cerevisiae* exposed to 2-ppm colchicine solution with exposure duration of 48 hours in 96-h fermentative process, both in the first and second fermentative processes.
3. Suspensions of *S. cerevisiae* with higher levels of DNA produced larger amount of ethanol content than those with lower levels of DNA.
4. The 48- and 96-h durations of exposure to colchicine solution caused *S. cerevisiae* to produce higher levels of ethanol than the 72-h duration of exposure.
5. The 2 X 48 h durations of fermentative processes produced higher levels of ethanol than the 96-h duration of fermentative process.

The present research recommended the need for further research to examine methods for producing higher levels of ethanol by: (a) using different strains for exposure to colchicine solution and (b) combining various strains of *Saccharomyces* exposed to colchicine solution by way of immobilization.

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B.b.1.

Improvement of Ethanol Production Using Polyploid *Saccharomyces cerevisiae*

Muhammad Thamrin Hidayat
Isnawati

ABSTRACT

Polyploid organisms represent the ones containing more of chromosomes than their parental cells. In some respects, they possess several advantages. In order to render organisms polyploid, colchicine is used. The purpose of the present research was to render *Saccharomyces cerevisiae* cells polyploid by exposing it to colchicine solution of various concentrations. An indicator of *S. cerevisiae* polyploidy was its level of DNA. Concentrations and durations of exposure used in the research were 0, 1, 2, and 3 ppm and 48, 72, and 96 hours, respectively. In addition, the present research investigated whether the polyploid *S. cerevisiae* were capable of fermentative process by producing higher levels of ethanol. In doing so, fermentative process was carried out twice with durations of 48 and 96 h. Previously, *S. cerevisiae* cells were immobilized twice by means of Na-Alginate and $CaCl_2$. Results indicated that exposure of colchicine solutions of 0, 1, 2, and 3 ppm to the yeast cells affected population density of the yeast cell suspensions. A concentration of 2 ppm and duration of 48 hours were effective in producing a higher level of DNA (polyploid) relative to other concentrations. The highest level of ethanol was derived from *S. cerevisiae* cells exposed to 2 ppm of colchicine solution both at the first and second fermentations.

Keywords: *Polyploid, S. cerevisiae, ethanol, immobilization.*

A. Introduction

Leavens or yeasts represent single-celled organisms of 4-8 μm in width and 5-16 μm in length. These microorganisms are widely used by people in food processing of *tapai*, soy sauces, cheese, and yogurt due to their safety and effectiveness. It has been known that yeasts have a wide range of purposes from such highly important commercial sectors as processed biofuels, chemicals, enzyme industries, pharmaceuticals, and agriculture, to improvement of environmental sectors. During the energy crisis of increasingly depleted fossil fuels, yeasts hold significant potentials in the production of ethanol as an environmentally friendly biofuel (Farrell *et al.*, 2006).

The current practice of ethanol production was considered as in the need for improvement. This was reflected in the emergence of studies leading to a variety of straightforward methods of ethanol production with increased output.

In an experiment by Radoi (2002), it has been described that *S. cerevisiae* generated mutants of *S. cerevisiae* capable of enhancing fermentation output and that these mutants could tolerate highly low-acidic media, SO_2, and malic acid. Efforts by the abovementioned researchers to increase ethanol production using a variety of techniques required prohibitive costs and complicated research procedures.

Microorganisms frequently used in ethanol production were the yeasts *S. cerevisiae*. Nevertheless, some breakthroughs were indispensable to an improved production of ethanol from *S. cerevisiae*. Research on *S. cerevisiae* was underway that aimed at looking for organisms or microorganisms genetically superior so as to produce a larger amount of ethanol.

The search for these superior organisms was intended to obtain microorganisms with high levels of productivity, resistance to adverse conditions, and resistance to pests or competitors. An effort that has been accomplished was to generate superior mutants. In doing so, chemical agents were used to generate polyploid organisms. One way to render cells polyploid was by the use of colchicine (Eigsti and Dustin, 1957). Several studies have shown the most effective results by using colchicine (Ajijah, 2003).

Colchicine represents a chemical substance with a tricyclic structure and a tropolon ring. The chemical agent can halt the mitotic rate in anaphase by means of tubulin binding or breakdown of the spindle, where tubulin being a spindle-forming element. An increase in the number of cells' chromosomes results in an increase in the level of DNA as a constituent of chromosomes as well. These cells represent the so-called polyploid ones. Treatment with colchicine within a specified period can result in such a geometrical increase in genome as 4n, 8n, 16n, and so on (Brewbaker, 1983, in Ajijah, 2003).

Cell engineering of the yeast *S. cerevisiae* in order to render it polyploid by means of the chemical agent colchicine was expected to confer the yeast a greater ability of fermentation with the resulting increase in ethanol production. This expectation was based on the fact that several polyploid plants exhibited superior properties.

Based on the earlier studies, it has been expected that the presence of polyploid *S. cerevisiae* would produce a higher level of ethanol. However, several questions remained to be addressed: was there any effect of colchicine solution concentration on the density of *S. cerevisiae* population? What colchicine solution concentration and duration of colchicine exposure that rendered *S. cerevisiae* polyploid? Was population density of poliploid *S. cerevisiae* capable of contributing an optimal fermentation process so as to produce a larger amount of ethanol with a shorter fermentative duration?

The present research was expected to generate some polyploid *S. cerevisiae* and provide a method for speeding up fermentative process and contribute to a high level of ethanol in a short time.

B. Materials and Methods

Materials used in this study were pure cell cultures of *S. cerevisiae*, colchicine solution, Yeast Extract Peptone Dextrose (YPD) medium (Stansfield, 2007), Peptone Patato Glucosa of Marck, Patato Dextrose Agar (PDA), glucose, alcohol, spirits, matches, aluminum foils, non-absorbent cottons, methanol, sterile aquabides, ethanol, $CaCl_2$ and Na Alginate, and Whitman filter paper no. 40 with millipore of 0.22 μm. Material originated from the kit produced by MACHERY-NAGEL (MN) of February 2008/Rev.2.

The present study was of a factorial design aiming at determining effect of colchicine solution concentration on population density of *S. cerevisiae*. Early population density of *S. cerevisiae* was observed and the final one at the end of colchicine treatment was observed again. An indicator for the effect of colchicine solution on the non-formation of spindle was the presence or absence of an increased level of DNA. Thus, there should be an analysis of

DNA level in *S. cerevisiae* population exposed to colchicine solution of various concentrations as well as those unexposed to colchicine solution. The present research was additionally aimed at determining levels of ethanol produced by respective fermentative groups. *S. cerevisiae* population groups were exposed to colchicine solutions of 0, 1, 2, and 3 ppm with exposure durations of 48, 72, and 96 h. These were compared to length of the fermentative process with 10% glucose solution. Durations of fermentative process with glucose solution were 48 h (2 days) and 96 h (4 days) and the fermentative process was conducted twice for the same durations. Cells employed were those that have been immobilized. Ethanol produced was analyzed by means of gas chromatography. Furthermore, ethanol levels produced in each treatment were analyzed in terms of duration of exposure, colchicine solution concentration, length of fermentation, and *S. cerevisiae* population for difference in DNA levels. Additionally, in order to determine whether the immobilized cells of *S. cerevisiae* were capable of re-fermentation subsequent to the first fermentation treatment of 48- and 96-h durations, the second fermentative process was necessary to be performed with the same durations. In doing so, cells of *S. cerevisiae* were immobilized in beads, washed with sterile aquabides, then retested for ability to perform re-fermentation in a 10% glucose solution for the same durations of 48 and 96 h.

Procedure used in the present research consisted of two stages. One stage was to expose suspensions of *S. cerevisiae* to colchicine solution concentrations of 0, 1, 2, and 3 ppm with exposure durations of 48, 72, and 96 h. At the end of the predetermined durations (48, 72, and 96 h), suspensions of *S. cerevisiae* were centrifuged, supernatant discarded to remove the existing colchicine solution, and the produced pellets were immobilized with $CaCl_2$ and Na Alginate to form beads of compounds of CaCl2 and Na Alginate. Those beads contained a pool of *S. cerevisiae* cells. The second stage was to perform fermentative process. In doing so, 10% glucose solution was prepared and 40 tightly sealed bottles were provided to be filled with 40 ml of the glucose solution. Then, the immobilized beads

were placed into those bottles containing 40 ml 10% of glucose solution. Fermentative process took place for durations of 48 and 96 h. At the end of 48 and 96 h, products were separated by beads. Products were placed into the tightly sealed bottles and then ethanol produced was measured by GC.

After the first fermentation by the beads, they were washed with sterile aquabides. At the end of the second fermentative process (48 and 96 h), products were separated by beads. It was then tested for ethanol content by GC.

To ascertain whether or not *S. cerevisiae* had undergone changes in DNA levels. DNA analysis was required. The purpose of the DNA analysis was to determine the number of chromosomes in the suspensions of *S. cerevisiae* due to effect of exposure to colchicine solution of various concentrations and durations. DNA levels of *S. cerevisiae* were determined by isolating DNA by means of materials from the kit produced by MACHERY-NAGEL (MN) of February 2008/Rev.2. The MN kit protocol was used for yeast DNA. Upon completion of DNA isolation, it was followed by OD measurements by means of spectrophotometer in order to determine levels of DNA.

C. Results

Exposure to colchicine solutions of 0, 1, 2 and 3 ppm with durations of 48 and 96 h had different effects on the population growth of *S. cerevisiae* suspensions, the statistics of which was shown in Table 1.

Table 1: Results of analysis of variance for population density among concentrations and exposure durations

Sources of Variance	Total Amount of Type III Squares	df	Avarage Squares	F	Significance
Conc.	7028.333	3	2342.778	3.499	0.050
Duration	9529.333	2	4764.667	7.117	0.009
Conc.*Duration	3007.667	6	501.278	0.749	0.622
Error	8034.000	12	699.500		
Total		23			

On the basis of Table 1, results of statistical test for the order of effect of colchicine solution concentrations on the population density of *S.*

cerevisiae suspensions were obtained (Table 2). Similarly, the order of effect of exposure durations on the population density of *S. cerevisiae* suspension was obtained, as well (Table 3).

Table 2 — The order of population density due to effect of colchicine solution concentration/0.025 mm^3

Colchicine Concentrations	*n*	Subset	
		1	2
2 ppm	6	266.17	
3 ppm	6	284.33	284.33
1 ppm	6	290.83	290.83
0 ppm	6		314.00
Significance		.142	.082

Table 3 — The order of population density due to effect of exposure duration/0.025 mm^3

Exposure Durations	*n*	Subset	
		1	2
72 h	8	274.00	
48 h	8	275.50	
96 h	8		317.00
Significance		.910	1.000

Subsequent to isolation of *S. cerevisiae* suspension population, levels of DNA were then quantified by means of spectrophotometer, the results of which were shown in Table 4.

Table 4: Results of analysis of variance for DNA levels among concentrations and exposure durations

Sources of Variance	Total Amount of Type III Squares	df	Avarage Squares	F	Significance
Conc.	17307.417	3	5769.139	1547.032	0.000
Duration	33877.000	2	16938.500	4542.168	0.000
Conc.*Duration	17093.333	6	2848.889	763.948	0.000
Error	44.750	12	3.729		
Total		23			

Note: Unit of DNA levels = μ/ml

In order to determine the possible difference in DNA levels of *S. cerevisiae* suspensions among treatments with colchicine solution concentrations, statistical tests were conducted. Results were shown in Table 5.

Table 5 — The order of DNA levels due to effect of colchicine solutions concentrations

Colchicine Concentrations	*n*	Subset			
		1	2	3	4
3 ppm	6	315.167			
0 ppm	6		345.833		
2 ppm	6			376.917	
1 ppm	6				382.083
		1.000	1.000	1.000	1.000

Note: Unit of DNA levels = μ/ml

In order to determine the possible difference in DNA levels of *S. cerevisiae* suspensions among treatments with different durations of exposure to colchicine solution, statistical tests were conducted. Results were shown in Table 6. Similarly, in order to determine the possible interactional effects of exposure durations with colchicine solution exposures among groups, ANOVA was performed and the results were shown in Table 7.

Table 6 — The order of DNA levels due to effect of exposure durations to colchicine solutions

Exposure Durations	*N*	Subset		
		1	2	3
96 h	8	316.000		
72 h	8		343.250	
48 h	8			405.750
Significance		1.000	1.000	1.000

Note: Unit of DNA levels = μ/ml

Table 7: ANOVA of DNA levels due to interactional effect of exposure durations and colchicine solution concentrations

	Total Amount of Squares	df	Avarage Squares	F	Significance
Inter-group	68277.750	11	6207.068	1664.465	0.000
Intra-group	44.750	12	3.729		
Total	68322.500	23			

Note: Unit of DNA levels = μ/ml

Analysis by the use of Duncan[ab] for treatment interactions of colchicine solution exposures and durations of colchicine solution exposure with regard to levels of DNA indicated that the combination of 3-ppm treatment and 96-h immersion produced the lowest DNA level of 265 500 μ/ml and that the the combination of 2-ppm and 48-h immersion produced the highest DNA level of 432 500 μ/ml.

Results of correlation analysis of population density of *S. cerevisiae* suspensions and DNA levels were shown in Table 8.

Table 8: Results of correlation analysis of population density of *S. cerevisiae* suspensions and DNA levels

		Population Density/0.025mm^3	DNA levels
Population Density/0,025mm^3	Pearson Correlations	1	–477
	Sig.		.018
	N	24	24
DNA Levels	Pearson Correlations	–477	1
	Sig.	.018	
	N	24	24

Note: Unit of DNA levels = μ/ml

Subsequent to exposure of *S. cerevisiae* to colchicine for 48, 72, and 96 h, suspensions of *S. cerevisiae* were then immobilized with $CaCl_2$ and Na Alginate. Cells of *S. cerevisiae* contained the beads were placed into 40 ml of 10% glucose solution. Cells of *S. cerevisiae* were expected to undergo the first fermentative process. The first fermentation took place for 48 and 96 h. Ethanol produced was measured by means of GC, the results of which were shown in Tables 9 and 10 below.

Table 9 – Ethanol produced in the first fermentative process

Durations of colchicine exposure (h)	0 ppm Duration		1 ppm Duration		2 ppm Duration		2 ppm Duration	
	48 h	96 h	48 h	96 h	48 h	96 h	48 h	96 h
	Ethanol product (%)		Ethanol product (%)		Ethanol product (%)		Ethanol product (%)	
48	0.525	3.600	0.203	5.270	3.850	6.690	0.370	3.960
72	0.190	0.150	1.170	0.620	4.260	5.820	0.790	0.220
96	0.800	1.620	0.870	6.460	7.320	8.450	0.610	3.080
Average	**0.505**	**1.810**	**0.414**	**4.116**	**5.143**	**6.987**	**0.327**	**2.420**

Table 9 showed ethanol produced from colchicine exposures in fermentative processes for durations of 48 and 96 h. At end of the first fermentative process (48 and 96 h), the beads were separated by glucose solution and then washed with sterile aquabides. In the second treatment of fermentative process, the beads were put into 40 ml of 10% glucose solution. This also required the same durations of 48 and 96 h. Ethanol produced was tested by the use of GC and results were shown in Table 10.

Table 10 – Ethanol produced in the second fermentative process

Durations of colchicine exposure (h)	0 ppm Duration		1 ppm Duration		2 ppm Duration		2 ppm Duration	
	48 h	96 h	48 h	96 h	48 h	96 h	48 h	96 h
	Ethanol product (%)		Ethanol product (%)		Ethanol product (%)		Ethanol product (%)	
48	1.025	4.990	3.036	3.040	1.400	8.860	5.270	5.800
72	1.570	0.650	0.000	0.850	5.550	1.200	0.730	3.880
96	7.080	0.800	4.200	1.240	6.430	2.340	6.170	0.000
Average	**3.225**	**2.147**	**2.412**	**1.710**	**4.460**	**4.133**	**4.057**	**3.227**

Table 10 indicated an effect of colchicine solution concentrations and exposure durations on the ethanol obtained. Data of the first and second 96-h fermentative processes from Table 9 and 10 were combined in order to determine correlations of the two fermentative processes. Results were shown in Table 11 and 12.

Tabel 11 — Ethanol produced in the first and second 48-h fermentative processes

Conchicine Concentrations	48/I 48 h	48/I 72 h	48/I 96 h	48/II 48 h	48/II 72 h	48/II 96 h
0 ppm	0.525%	0.19%	0.80%	1.025%	1.57%	7.08%
1 ppm	0.203%	1.15%	0.87%	3.036%	0.00%	4.20%
2 ppm	4.000%	4.26%	7.32%	1.400%	5.55%	6.43%
3 ppm	0.370%	0.79%	0.61%	5.270%	0.73%	6.17%
Total	**5.090%**	**6.390%**	**15.00%**	**10.730%**	**7.85%**	**23.88%**

Tabel 12 — Ethanol produced in the first and second 96-h fermentative processes

Colchicine Concentrations	96/I 48 h	96/I 72 h	96/I 96 h	96/II 48 h	96/II 72 h	96/II 96 h
0 ppm	3.60%	0.15%	1.62%	4.99%	0.65%	0.80%
1 ppm	5.27%	0.62%	6.46%	3.04%	0.85%	1.24%
2 ppm	7.00%	5.82%	8.45%	9.00%	1.20%	2.34%
3 ppm	3.96%	0.22%	3.08%	5.80%	3.88%	0.00%
Total	**19.83%**	**6.81%**	**19.61%**	**22.83%**	**6.58%**	**4.38%**

D. Discussion

The present research provided an evidence that 2-ppm colchicine solution with 48-h immersion was capable of forming a high level of *S. cerevisiae* DNA and, in fact, it could also form a high level of ethanol, on average. Thus, the three factors of solution concentration, exposure duration, and DNA level represented the ones influencing the formation of a high level of ethanol. Variable of DNA level constituted the largest contributor to a higher level of ethanol production. Thus, chromosomes possessed by the cells of *S. cerevisiae* served as metabolic controllers. Additionally, it could be argued that the 2-ppm concentration capable of rendering cells of *S. cerevisiae* polyploid. Furthermore, the 2-ppm concentration did not lead to chromosomal damages. This was indicated by the observation that the cells remained capable of proper metabolisms. Several studies have reported that duration of fermentative process represented a determinant of ethanol production. Vucurovic (2008) reported a relationship of a longer duration of fermentative process and a higher glucose concentration in producing a higher level of ethanol. In addition, he added that immobilized cells produced a higher level of ethanol than their non-immobilized counterparts. In the present study, the

first and second 48-h fermentative processes with exposures to 0-, 1-, and 3-ppm colchicine solutions provided increases in ethanol level, on average. In the first fermentative process, the level of ethanol produced was 0.505%, while the second produced 3.225% at exposure to 0 ppm of colchicine solution. At exposure to 1 ppm of colchicine solution, the first fermentative process produced an ethanol level of 0.414%, while the second produced 2.412%. And at exposure to 3 ppm of colchicine solution, the first fermentative process produced an ethanol level of 0.327%, while the second 4.057%. This possibly occurred due to the adaptation of the existing cells to novel environment. In addition, many of those cells possessed diploid or haploid chromosomes, leading to extremely low levels of metabolism; whereas population densities as the results of exposures to 1-, 2-, and 3-ppm colchicine solutions were higher than that of 2-ppm colchicine solution.

First exposure to 2-ppm colchicine solution with 48-h duration of fermentative process produced a total of 5.143% ethanol, on average, while the second produced 4.46%. This process occurred due to cells' ability to perform highly rapid metabolisms. The first and second 96-h fermentative processes exposures to colchicine solutions of 0 and 3 ppm showed an increase from the first to the second fermentative process. At exposure to colchicine solution of 0 ppm in the first fermentation process, ethanol produced was 1.81%, on average, while the second produced only 2.147%. Similarly, at exposure to 3-ppm colchicine solution in first fermentation process, ethanol produced was 2.42% and the second produced 3.227 %. This possibly occurred due to a less decrease in nutrients so that the cells remained capable of maintaining the same rate of growth as previously, leading to an ability of the second fermentative process to perform metabolism properly. In contrast to exposures to 1- and 2-ppm colchicine solutions, initial fermentation processes proceeded in a highly fast rate so that the growth medium started to run out, leading to cells' tendency to form spores. In case of this, polyploidy would turn into haploidy. Thus, fermentative or metabolic processes were inhibited and resulted in a

decreased ethanol output. However, dual fermentative processes could be performed despite a somewhat decreased output.

It should be addressed whether the continuous 96-h fermentative process would produce a higher level of ethanol than the halved 96-h fermentative process (performed in two 48-h durations). It was observed the halved 96-h fermentative process produced a higher level of ethanol than the continuous 96-h one. The total amount of ethanol produced in the first and second 48-h fermentative processes was 20.543%, while it was only 15.333% for the continuous 96-h fermentative process. Thus, the halved 96-h fermentative process produced a higher level of ethanol than the continuous 96-h one. This was not due to a better fermentative medium since the formed ethanol did not represent a barrier to growth process. In addition, fresh medium was rich in nutrients for cells' lives. Widjaja (2008) and Vucurovic (2008) suggested that the use of immobilized cells of *S. cerevisiae* was advantageous since it could be used repeatedly in accordance with the purposes of the fermentative process. Therefore, continuation of 96-h fermentative process beyond 96 h would be less effective; in other words, continuation of fermentative process beyond 96 h did not produce a higher level of ethanol.

E. Conclusion and Suggestion

Results of this study could be summarized as follows:

1. The 2-ppm colchicine concentration with exposure duration of 48 hours most effectively increased levels of *S. cerevisiae* DNA up to 432.500 μg/ml relative to other concentrations and exposure durations.
2. The highest level of ethanol was produced by cells of *S. cerevisiae* exposed to 2-ppm colchicine solution with exposure duration of 48 hours in 96-h fermentative process, both in the first and second fermentative processes.
3. Suspensions of *S. cerevisiae* with higher levels of DNA produced larger amount of ethanol content than those with lower levels of DNA.

4. The 48- and 96-h durations of exposure to colchicine solution caused *S. cerevisiae* to produce higher levels of ethanol than the 72-h duration of exposure.
5. The 2 X 48 h durations of fermentative processes produced higher levels of ethanol than the 96-h duration of fermentative process.

The present research recommended the need for further research to examine methods for producing higher levels of ethanol by: (a) using different strains for exposure to colchicine solution and (b) combining various strains of *Saccharomyces* exposed to colchicine solution by way of immobilization.

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