ISBN: 978-602-61830-0-2
June 2017

THE EFFECT OF VARIATIONS IN PH MEDIUM FOR DRY MASS AND PROTEIN PRODUCTION ON INDUSTRIAL WASTE MEDIA BY *Saccharomyces cerevisiae*

Endah Rita Sulistya Dewi[1,*], Anang M. Legowo[2], Munifatul Izzati[3]
[1]Doctoral Program on Environmental Science, Diponegoro University, Semarang, Indonesia
[2]Department of Agricultural Sciences, Faculty of Animal and Agricultural Science, Diponegoro University, Semarang, Indonesia
[3]Department of Biology, Faculty of Sciences and Mathematics, Diponegoro University, Semarang, Indonesia
endahrita@yahoo.co.id

ABSTRACT - Including Ascomycetes *Saccharomyces cerevisiae* that contains a lot of protein , carbohydrates and fat so it can be consumed by humans and animals in order to supplement nutrient needs. Industrial wastewater non dairy creamer (NDC) has potential as a medium for the growth of microorganisms . The purpose of this study was to evaluate the effect of variation in pH medium for the dry mass and protein production on industrial waste by *Saccharomces cerevisiae*.. This study uses a completely randomized design. This treatment includes , P1 (NDC 25 %) , P2 (NDC 50 %) , P3 (NDC 75 %) and P4 (NDC 100 %) , which inoculated with *Saccharomyces cerevisiae* a number of 10^6 cells / ml , in 3,4,5 and 6 pH medium. Variance analysis showed that the variations of pH medium give affect for the dry mass, but does not significantly influence the protein content of the products.

Keywords **:** *Saccharomyces cerevisiae*, protein product, non dairy creamer.

INTRODUCTION

Increased industrial sector is currently expected to realize the achievement of public welfare. Various industries are growing rapidly; however, these conditions will also be accompanied by the increasing waste generated.

Industrial non dairy creamer, PT. Kievit Indonesia is located in the town of Salatiga precisely on Jl. Pigeon 1, Village Mangunsari Sidomukti District of Salatiga is an Industrial Food Ingredient made from raw coconut oil.

Waste liquid non dairy creamer which flowed along the flow of waste disposal can lead to contamination when not treated optimally. In addition to causing the quality of the water body is lowered, the odor generated can disturb the environment. Odor generated can be caused by excessive content of organic compounds contained in wastewater. Associated with these conditions need to be a good treatment by utilizing waste into useful raw material.

Research on various waste as a growing medium for microorganisms has been carried out single cell protein production, in the hope of getting low cost growth media, and in doing so has not been in use for waste non dairy creamer. The use of Saccharomyces *cerevisiae* in wastewater treatment non dairy creamer is expected to have a high enough prospects in the production of single cell protein. Single Cell Protein (PST) is a dry cell or a biomass of microorganisms such as yeast, bacteria and algae that can be used as a protein source for food and feed. Single cell protein can be an alternative for protein needs in the future, because in addition to containing a specific protein, it also contains carbohydrates, fats, minerals and other nutrients needed humans and animals (Nasseri et al., 2011; Prakash et al. 2013; Jaganmohan et al ,. 2013). The use of *Saccharomyces cerevisiae* in wastewater treatment non dairy creamer is expected to have a high enough prospects in the production of single cell protein.

Based on laboratory analysis of non dairy creamer wastewater containing organic materials, especially carbon source in the form of carbohydrates, proteins and fats so that potential as a medium for the growth of microorganisms. Liquid industrial waste management efforts of non dairy creamer can be done by changing into raw materials. The raw material in this case is a microorganism fermentation media which was developed for the production of single cell protein, with the expectation that the growth of low cost media, and in doing so has not been in use for waste non dairy creamer.

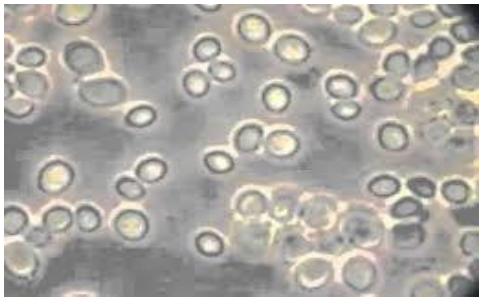



Figure 1. Colony of *Saccharomyces cerevisiae* (a) Cell of *Saccharomyces cerevisiae* (b)(Okafor, 2007)

MATERIALS AND METHODS

Research Variabel

The independent variable in this study is a variation of the concentration of waste liquid non-dairy creamer which comprises a solution of liquid wastes with a concentration of 25%, 50%, 75% and 100% is used as a growth medium and pH medium (pH 3,4,5 and 6). The dependent variable in this study is the number of dry mass and protein products.

Research Design

The design of this study using a completely randomized design. Preparation of inoculum according Mondal research, et al., (2012) which states Saccharomyces *cerevisiae* cultured in medium YPDA for 4 days and incubated for 28^{o} C, then washed in distilled water and 25 ml of sterile distilled resulting final concentrations were 10^{6} cells / ml for use. Furthermore *Saccharomyces cerevisiae* were inoculated in appropriate medium concentrations and different nd d, each treatment was repeated 3 times. The treatment used is:

Data collection research done at the 24th hour, 48th hour, 72th hour and 96th hour covering the number of cells, protein products, the measurement of pH and temperature, each repeated three times.

Procedures

Preparing tools and materials are pure culture of *Saccharomyces cerevisiae*, liquid waste non dairy creamer, yeast extract, peptone, dextrose, distilled water, 0,5N NaOH, H2O, Na2CO3, CuSO4, K-Na-Tartrate, Folin-ciocalteau and Bovine Serum Albumine (BSA).

Making the YEPD medium YEPD medium made of a mixture of yeast extract, peptone, dextrose, and distilled water. All materials included in the glass beaker and stirred until homogeneous. The mixture is then put in a jam jar erlemeyer or until the volume of 150 mL. Jar covered with foam and aluminum foil, and then sterilized by autoclaving at 121^{o}C for 15 minutes. Furthermore, the medium is cooled and after cold ready inoculated.

Making the effluent medium non dairy creamer. Medium is made from liquid waste non dairy creamer artificially with a concentration of 25%, 50%, 75% and 100%. Furthermore, the material is stirred until homogeneous, filtered, and put in a jam jar. Jam jar and covered with foam and sterilized by autoclaving at 121^{o}C for 15 minutes, then cooled and after a cold medium ready for inoculation.

Growth of *Saccharomyces cerevisiae* in YEPD medium and medium effluent non dairy creamer. Growth of *Saccharomyces cerevisiae* in YEPD medium and liquid waste based on the method of Amaria, et al., (2001). In the medium and medium YEPD liquid waste non dairy creamer, *Saccharomyces cerevisiae* suspended in sterile distilled water with a density of 10^{6} cells / mL. Each medium in the bottle hours inoculated with 1 mL of the suspension. Furthermore, both medium and medium YEPD liquid waste non-dairy creamer incubated in an incubator at 30 ° C for 20 hours. Then the pH of the medium is measured and calculated *Saccharomyces cerevisiae* cell population, every 24 hours for 4 days.

Measurement of pH of the medium. PH measurement and wastewater medium YEPD non dairy creamer done at the 24th hour, 48thhour, 72thhour, and 96th hour by using a pH meter.

Counting the number of cells of *Saccharomyces cerevisiae*. Counting the number of cells / mL done every 24 hours based on the method of Hadioetomo (1993) in (Purwitasari, et al., 2004) by means of hemocytometer on a light microscope or through analysis of optical density/OD (Optical Density) using a photometer at a wavelength of 550 nm.

Pulverizing the cells of *Saccharomyces cerevisiae*. Pulverizing the cells of *Saccharomyces cerevisiae* by the method of Amaria et al., (2001). *Saccharomyces cerevisiae* cells were harvested when optimal growth by centrifugation at 3000 rpm 2 times each for 10 minutes. The precipitate was washed with distilled water and filtered the way given the No.40 Whatman filter paper. Furthermore, cells dried at 50-60^{o}C for 3 days. After drying, grinding done cells, then the resulting powder is weighed and analyzed cell dry weight protein content with Micro Kjeldahl method

Data Analysis

The data observed density *Saccharomyces cerevisiae* and protein production on culture media and media environment maintenance performe d by analysis of variance (ANOVA), after the distribution normality test and homogeneity of variance error. If you acquired a significant difference between each treatment is then followed by a double range test of Duncan / DMRT (Duncan's New Multiple Range Test) to determine differences between treatments and treatments which give the best effect (Gomez, 1995)

RESULTS AND DISCUSSION

Performed analysis of variance which states there is significant in the level of 5%, in pH medium for dry mass, but this there is no significant for medium protein content of products. pH changes in the microbial environment may affect the microbial growth process. At the time of the growth of a microbe , the hydrogen ion concentration (pH) in the media affect the growth of protein (both enzyme and its transportation system) contained in the cell membrane. Protein structure will change when the pH of the media is also changing.

Microbes have an enzyme that works perfectly in a certain pH range , that if there are deviations pH of the growth and metabolism can be stopped(Garcia, et al., 2015).

The results showed that the highest dry mass in the range of pH 4.0-5.0. At the pH of the cells to grow exponentially. Appropriate research Yaicin and Yesim (2008) which states that the highest values of dry mass and specific growth rate were obtained at pH 4.0 for both of the strains *Saccharomyces cerevisiae* from Turkey.

According Okafor (2007) , Saccharomyces cerevisiae has a tolerant nature of the acidic environment, with a pH range between 3.5 and 4.5 . As a result , the yeast can take place in a clean medium without sterile , at pH 4.0 to 4.5.

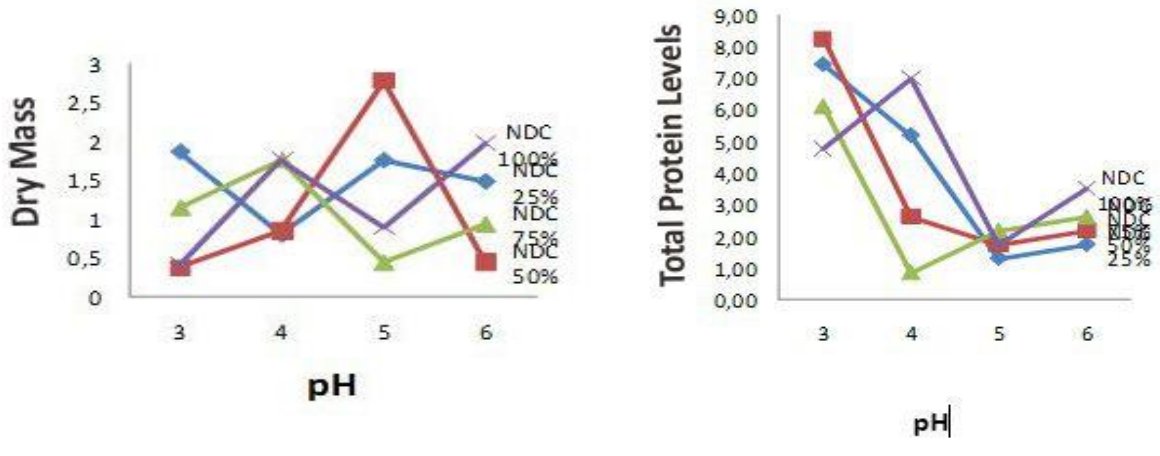
The growth of *Saccharomyces cerevisiae* is best achieved on further media concentration of 50 % to 100%, 75 % and 25 % are converted at the dry mass.

Total protein content, when viewed from the substrate concentration, the highest yields are in P4 (konsnsentrasi 100%), it may be possible because the nutrient content in a concentration of 100%, still relatively adequate for growth, so the impact on the results prroduk protein. Dhanasekaran, et al., (2011), stating that the nutrient-containing sugar will provide energy for metabolic processes *Saccharomyces cerevisiae*, while in the manufacture of non dairy creamer products are sugar content.

Non dairy creamer is milk or cream substitute product that is a product of lipid emulsion in water, made from vegetable oils such as coconut oil is hydrogenated with the addition of permitted food additives, namely glucose syrup, sodium caseinate, emulsifiers and stabilizers, salt and water (SNI Agency , 2012).

According to Okafor, N (2007), the better nutrients in the substrate where growth, more rapid cell growth that will increase the levels of cell proteins. In addition, levels of cell proteins is influenced by the time of breeding. Breeding time is too short will result in lower amounts of protein as substrate component biokonfersi not optimal. While the breeding time is too long will cause deterioration due autobiodegradasi protein to meet its energy needs with regard to the availability of nutrients in the medium that is increasingly inadequate.

Levels of protein products may be obtained on the fermentation time to 48 hours (H2), the substrate concentration liquid waste non dairy creamer concentration of 100%, considering the hours to 72 (H3) has been declining, which can be explained that at the time of cell growth already experienced phase stationary phase even death.



(a) (b)

Fig. Effects of pH medium For Dry Mass *Saccharomyces .cerevisiae* (a); Effect of pH Medium For Total Protein Levels *Saccharomyces cerevisiae* (b).

CONCLUSION

The effect of liquid waste non dairy creamer can be affect for dry mass *Saccharomyces cerevisiae*, but does not significantly influence the protein content of the products. So has potential to be used as a growth medium, with the addition of nutritional recommendations on the media and that it will be obtained more optimal results.

ACKNOWLEDGEMENTS

The authors wish to thank Management of PT.Kievit for their agreements and supporting and for supplying the waste liquid non dairy creamer to analysis.

REFERENCES

Amaria, Agustini, R., Cahyaningrum, S.E., Santosa, S.J dan Narsito. 2007. Adsorsi Seng (II) Menggunakan Biomassa Saccharomyces cereviceae yang Diimobilisasi pada Silika Secara Sol Gel. Akta Kimindo.2(2): 63-74

Dhanasekaran, D., Lawanya, S., Saha, S., Thajuddin, N., and A. Panneerselvam. 2011. Production of Single Cell Protein From Pineapple Waste Using Yeast. Innovative Romanian Food Biotecnology. 8. 26-32.Issue of March.

Gomez, A.A. dan Gomez, K.A. 1995. Prosedur Statistik Untuk Penelitian Pertanian. Jakarta: UI-Press.

Garcia Jaime Moreno, Teresa Garcia Martinez, M. Carmen Mill, Juan Carlos Mauricio, Juan Moreno. 2015. Proteins involved in wine aroma compounds metabolism by a Saccharomyces cerevisiae flor-velum yeast strain grown in two conditions. Journal Food Microbiology 51 (2015) 1-9

Jaganmohan, P. B. Purushottam Daas and S.V. Prasad. 2013. Production of Single Cell Protein (SCP) with Aspergillus terreus Using Solid state Fermentation. European Journal of Biological Sciences 5(2): 38-43. ISSN 2079-2085

Mondal, A.K., Sengupta, S., Bhowal, J and D.K. Bhattacharya. 2012. Utilization of Fruit Wastes in Producing Single Cell Protein. International Journal of Science Environment and Technology. 1(5) : 430-438

Nasseri, A.T, S. Rasoul Amini, M. H. Morowvat and Y. Ghasemi. 2011. Single Cell Protein : Production and Process. American Journal of Food Technology 6 (2) : 103 – 116

Okafor. 2007. Modern Industrial Microbiology and Biotechnology. Science Publisher, Enfieid, NH, USA. South Carolina

Prakash, B. P. Porumal, P. Tamilmani and Lini.P. Mathew. 2013. Economic Utilization of Horn Residues for Microbial Biomass & SCP Production by Solid State Fermentation. Asian Journal of Biochemical and Pharmaceutical Research Issue. 3(1):104-110. ISSN 2231-2560

Purwitasari, E., Pangastuti, A., dan R. Setyaningsih. 2004. Pengaruh Media Tumbuh terhadap Kadar Protein Saccharomyces cereviceae dalam Pembuatan Protein Sel Tunggal. Bioteknologi 1 (2); 37-42 ISSN: 0216-6887.

SNI Agency, 2012. Krimer Nabati Bubuk. Penerbit Badan Standarisasi Nasional. Jakarta.

Yaicin Seda Karasu and Z. Yesim Ozbas. 2008. Effect of pH and Temperature on Growth and Glycerol Production Kinetics of Two Indigenous Wine Strains of Saccharomyces cerevisiae from Turkey. Brazilian Journal of microbiology. 39(2). ISSN 1678-4405.