International Journal on Advanced Science Engineering Information Technology

Vol.11 (2021) No. 1
ISSN: 2088-5334

The Linear Model of *Saccharomyces cerevisiae* Turbidity in Liquid Media

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***Abstract*— The study aims to investigate the relationship between the turbidity and density or the total suspended inorganic particles have been obtained many models. The live cells as the homogeneous particle are presumed to cause turbid in liquid media, and that has a linear relationship that can be utilized on the cell counting. The method for the term of clouded liquid form is the measurement based on the reflection and scattered of light, i.e., the turbidimetry. Knowledge attainment of microbial cell counting should be answered how many Nephelometric Turbidity Unit of the one cell. We work to obtain a turbidity model of cells in water-based media for the estimation of cell numbers. This paper aims to construct the computational structure on the turbidity modeling of *Saccharomyces cerevisiae* in pure water and to test a consistent model in liquid nutrients medium. The modeling was performed in systematized stages of the diagnostic-analysis-test; the regression assurances, the simulation of the lowest error, and the coefficient value itself of turbidity factors. We constructed an optimal analysis and diagnosis to create a computational structure of cell turbidity modeling. The measurement and stopping bivariate elimination of the simulation is a subsystem of the algorithm of obtaining and testing models. The first mathematics model is a standard curve on turbidimetry, and the second, turbidity mathematics model of cell growth in liquid nutrients medium. Both models have an equal coefficient of cell turbidity. The turbidity coefficient of cell growth time interval in the carbonyl diamide - potato dextrose broth is significant.**

***Keywords*— Cell; particle; light; computation; turbidimetry.**

Manuscript received 20 Jan. 2020; revised 14 Jun. 2021; accepted 10 Sep. 2020. Date of publication 28 Feb. 2021.

I. INTRODUCTION

The context for computational purposes of cell turbidity focuses on optimization results. It is an adaptation-related process of input, and the selection of equipment characteristics, mathematical processes, and testing [1]. Furthermore, the optimization algorithm is a problem-solving process that aims to discover the most beneficial conditions from a certain point of view, addressing real-world problems and numerical applications to advance the discipline with mathematicians and to understand these phenomena better.

Computation is defined as "to compute" and refers to a process [2]. The "computer" verb version of the term is a machine to process calculations using digits, and the computational behavior depends on the system. The results of the analysis and diagnosis optimization are prepared for the processing phase of machine learning adaptation. This process is an interdisciplinary method that refers to artificial intelligence and statistics for computer applications. Moreover, researchers can develop algorithms based on expertise, e.g., a linear regression analysis [3]. The analysis and diagnosis of linear regression for testing and computation can provide solutions with computer simulations. The model of cells is behavior and has been explored and related to systemized methods to understand [4] the reality.

The focus on *Saccharomyces cerevisiae* turbidity in computational modeling is performed in procedural algorithms. However, the cell turbidity model in the medium and computational efficiency of modeling, estimation method, and using the turbidity data for cell number estimation are not yet available [5]. The correlation between particle, e.g., bacteria and organisms with larger cells, and turbidity, are also not yet completely understood, such as the McFarlan method using photometry for the turbidity. The principles of spectrometric absorbance are at 600 nm for aqueous solution, but the suspended substance occurs, which means the absorbance parameter is not suitable.

Computational models utilize other types of particles whose object differs from this research, such as formulating, modeling, adapting, or determining the results of analysis and diagnosis. In the first year, research on *Saccharomyces cerevisiae* cell turbidity modeling was applied as a standard curve of turbidity method, referred to as turbidimetry, on cell quantity in the medium of purified water. The statistical procedures can be used for simulation of selected models. By utilizing computer simulations, time management can be effective and efficient when facing a complex system [6]. However, its implementation is not only a program execution. The analysis results and the significance of diagnosis can also be simulated to determine a model's suitability. Simulation is a heuristic method used to solve optimization problems [7].

There are no computer programs available for simulating and assessing errors of the model candidates, and computational optimization is a necessity to generate the model [8]. The researches obtain and examine the model of *S cerevisiae* cell turbidity in purified water and liquid medium of cell growth, required two years. Computation in this context is a specific analysis and model diagnosis, which must be optimized. Optimization performs computational procedures and generates test results [9]. A hybrid algorithm allows us to solve complex problems, i.e., the process stages presented by [10]. Performing experiments of cell models in liquid media is a problem-solving system, and that is clear and understood [6]. This paper aims to construct the computational structure in studying the turbidity model of homogeneous cell particles of *Saccharomyces cerevisiae* in pure water and to test a consistent model in liquid nutrients medium.

A model can be seen as a point-view to parameterized mathematical equations and a physical point-view for consistency [11]. Researchers work on the microbe's discipline and looking beyond the role of the model, which is useful in the preparation of the fermentation process of biofuels; a part of the renewable energy and environmental preservation.

A. Characteristics of Spectrum and Particle in Liquid

The turbidity quantity is applied, but the estimated cell concentration per unit volume remains far from accurate [12]. Therefore, an experiment on the relation between cell turbidity in suspension with purified water and a cell growth medium is required. Similarly, the photometric method's precision in measuring turbidity caused by bacteria cell can be tested using the McFarland method [13], [14], in addition to cell turbidity experiments using turbidimetry.

It is essential to understand that light reflects at a higher angle than the light diffraction by its solvent. There are two factors to consider: wavelength and particle size [15]. The angle of the reflected light is not formed by particles when the wavelength is greater than the size of the particle. The light travels parallel to the direction of light, which means that there is no expected light scattering. This occurs on absorbed light by the substance that is diluted.

The particle interacts with the light on bigger molecule sizes using the same unit, such as light wavelength. The Static Light Scattering (SLS) measures particle size based on light. Meanwhile, Dynamic Light Scattering (DLS) shows the characteristics of a polymer molecule and particle science such as complex fluids, gel, and crystalized liquids. In physics and science in general, this is called optical design. The particle size information on an application-level can be used to obtain higher precision. Furthermore, the angular-based SLS method occurs in reflected light, which appears in light scatter. The particle has a size of >50 Angstrom (A), and it is reflected against dust.

The particle sizes of 5–20.000 A or 0.005–20 nm are used for time-based DLS or light scatter frequency. Regarding the problem of particle size interacting with light, equipment such as a microscope can be used for particles sized >300 A or >0.3 nm. The size of *Saccharomyces cerevisiae* particle is around 7–10 μm or 0.007–0.010 mm. However, the interaction of light and yeast cells was examined for observing the count of particles in a suspension. The measurement aspect of light needs a device that detects the reflected light or scattering.

DLS involves a time measurement that is related to macromolecule's motion in a suspension. The smaller macromolecule size is slower in contrast to the larger ones. However, the amplitude of the scattered light wavelength highly depends on the particle's size, contrast, and concentration. Examples from a study on blood as suspension showed that the "particle" of hemoglobin was the main cause of the light scatter, in addition to the absorption of UV, blue, and green spectrums [16]. It was compared to the light scatter in sickle cells. Furthermore, the amplitude of light scatter was observed using the SLS, and DLS approaches.

Another study on the relationship between total dissolved solids and turbidity using turbidity free medium as the control variable was performed by [17]. This study aims to monitor the count of dissolved solids in water. This research is intended to find total suspended solids (TSS) within a certain volume unit, with TSS as the variable; while possessing cell unit (particle) per mL. Independent and dependent variables for both relations can be determined based on the utility of the parameter. Both linear regression relations can be analyzed parametrically and non-parametrically [18].

The scattered spectrum is attenuation (T), as it is blocked by suspended particles. An assumption is that incoming light frequency is equal to its scattering by particles. Moreover, T and TSS are linear for homogeneous particles [19]. The TSS is in an mg unit of cell mass, and cell concentration as the cell number per mL [11], and heterogeneous cell particles were used for the cell density because they can represent measurement [6] for more than one species. That unit was chosen with the consideration that a cell is a particle, and a proposition was needed as the point-view of the physics toward the cell body. The highly complex interaction between the light waves and particles involves atoms and molecules contained in the particles. However, this focuses more on the absorption of photons. Such a phenomenon occurs in spectrophotometry. Meanwhile, light reflection is caused by particles blocking the incident spectrum and is known as turbidimetry.

The study on water suspension in the air (aerosol) is related to Mie's theory, explains that the diameter of a spherical droplet is highly precise, and the size can be determined with an accuracy of ±2nm [20]. Many problems based on Mie's theory use the algorithms as detailed. As the scattering of a plane electromagnetic wave by a dielectric sphere is considered a canonical problem, Mie's theory remains to be

widely used as a standard reference to validate methods intended for more complex scattering problems.

The explanation above means that scattering occurs to visible light, i.e., the wavelength of 400–700 nm. Homogeneous particles that have a diameter larger than 700 nm show the reason why light scattering occurs. This is the reason why the spectrophotometric method is unsuitable for the previous study of cell quantity prediction tests per mL [12]. This is based only on the light absorbance and should be considered the light scattering. A problem also arises from the estimated cell count between McFarlan's method of turbidity with the photometric method, and the result from [13], [14].

Technically, the TSS value can still be quantified with a multiplication factor over the dilution. However, this is rather unsuitable for standard curves. The application of yeast cell particles depends on the ability of NTU value from suitable suspended solids. The gravimetry of cell acquisition is carried out by filtering, where the filter is capable of filtering around a 2.5 μm cell size. However, this concept is used as an application for independent variables in medium for turbidity and for multiple regression models.

B. Gravimetry Traditions in Turbidity Measurement

Gravimetry is generally used for turbidity based on total particle weight in the unit volume of the suspension fluid from samples in water. For example, the unit used is $mg.L^{-1}$. The particles are not defined as one or several types, but rather as total weight. This relation can be arranged in a curve, and the relationship has a linear line as in reality and can be stated as a linear equation.

The problem is that the factors that influence light scattering come from individual particle sizes and the count of particles reflecting light. Therefore, a question asked is to what extent do the various particle sizes influence? The particles in question may come from rivers, ponds, or other open areas. Until recently, turbidity is measured based on the relation between total particle weights in every suspended volume unit.

Live and dead yeast cells differ in size, particularly in micrometers. The sizing is important because even the smallest angle of light reflection connects to wavelengths in nano- and micrometers. This study aims to reveal the changes or differences in light scattering effects between live and dead cells. Similarly, light scatters as the impact of the differences in cell age (time, hour) at initial growth (cell multiplication) is also observed, due to the assumption that growing cells have different sizes.

C. Turbidity as Experimental Variable

The case is considered on the application of turbidity measurements caused by the presence of particles exposed to light. TSS is an independent variable. However, in certain cases, TSS may become a dependent variable, with river water turbidity in two certain seasons as the independent variable. Determining the type of variables in an experiment, and definite answers on why the dependent variable changes must be provided.

Experiments are conducted to assess the relationship between independent and dependent variables, where both provide highly accurate answers. Variables have a parameter that can be changed. The independent variable in this study is cell weight ($mg.L^{-1}$), and can be stated by its cells count. It has different values and causes turbidity as a measurement result. An independent variable is also chosen since cells caused light scatter, and the measurement observed is the turbidity value of the count of cells. The relation between both is stated as a regression equation, as reflected in the coefficient which belongs to the particles. The regression equation has the characteristics of a variable relation and can be used to predict its independent variables with relatively high accuracy.

Accuracy of the regression equation is shown by the prediction results, as well as its uncertainty value, which is expressed in the interval unit value ($X \pm x$). The experiment in this research is performed in order to accept or reject the hypothesis of the equation as a model with the smallest standard error value [21]. A statistician means that the word "model" is a parameterized mathematical equation, or is not the equation itself [11].

D. Turbidity Factors

The influential factors when cells are considered as particles are the form of yeast colony and yeast cells' growth. In turbidity measurement, it should be noted that cells can multiply from one to two cells. In microbiology, doubling time means generation time, and *S cerevisiae*, the generation time is approximately 90 minutes [22]. Yeast grows buds to multiply, and there will be changes such as cell multiplication that may cause light scatter during turbidity measurement.

There is a phenomenon where a group of cells covalently bonds by 30–50 cells after the buds are detached from their parent's body [23]. Furthermore, this group of cells can be dispersed mechanically without re-aggregation. A group of cells in the form of a solid medium is expressed by a layer of film on the surface of the medium. Oxygen is a vital factor in cell activity. The outer layer of the cells contains lectin, which is affected by sugar, temperature, ethanol, cation, pH, and mechanical stirring. Cell aggregation in the form of suspension, which interacts with light, manly perform scatter measurements. This phenomenon potentially becomes a spectrum scattering factor. The size particle grows very large, multiplying 30–50 times and influencing turbidity value as a parameter. It can be speculated that there is a potential error in the predictive application from the use of turbidity, in order to determine the population of *Saccharomyces cerevisiae* cells. This problem has been answered in preliminary experiments [24].

The agitation factor has certain limits, and on the contrary, the possibility of agitation with low rpm triggers aggregation in the form of flocks [23]. This also occurs with 1% ethanol, whereas 10% of ethanol becomes toxic for cells. The source of 0–2% carbon as a nutrient in the fermenter can induce cell flocculation. Flocculation is generally formed by extreme pH of 2.5, while optimum fermentation can be achieved at pH 3–5. Thus, cell aggregate can be reduced by adding pH, mechanical stirring, or ethanol. At 50–60 °C, reversible dispersion may occur, while optimum results can be achieved at 35–37 °C. The descriptions above are the problem found by the researcher, which are sought to be solved using a liquid that is called ES4.9PB; Ethanol Saline pH 4.9 Phosphate Buffer [24].

E. The Computational Definition

Computation draws on the ways of mathematicians and science to advance the discipline, to envision new problem-solving strategies, to test new real-world solutions, and to understand different phenomena [2]. Furthermore, a model of computation is a mathematical abstraction of computing systems, and the computation refers to a process in definite terms [3] in computational biology and physical modeling that is a set of propositions (assumptions), the equation, and data [6],[11].

This context is cell turbidity research in liquid media and requires complex problem-solving. The underlying is a method to discover the way to the targets by process analysis and diagnosis [12],[23]. The models finding and testing were performed "drawing on the ways" or systematized exploring, i.e., stages of diagnostic-analysis-test [5]. A heuristic approach was used for the development of a numerical technique application that was based on algorithms and simulations. The algorithms involve computing analytical solutions to result in models with exploiting statistical procedures of International Business Machine (IBM) applications.

II. Material and Method

A. Materials

The materials for experiments in the laboratory were as follow; *Saccharomyces cerevisiae* using the product from Sigma Aldrich, the pure water - Otsuka, standard turbidity - Sigma Aldrich, potato dextrose broth (PDB) - Difco, starch - Merck, dextrose -Merck, dextrose - Difco, potato infusion, carbonyl diamide - Merck, potato dextrose agar – Merck. The preparation for cell enumeration uses the centrifuge that has the rotor of 25 cm and the K-centrifuge PLC series type. The hemocytometer is Neubauer (*Improve/Tuerk*) type. The unit of turbidimeter has a tungsten lamp and Orbeco-Hellige model of 965-10A with the stabilizer voltage, the aerated 35 ^{o}C of the incubator with heater-blower, the rotary shaker VRN-200 and thermometer for yeast growth, the binocular microscope, laminar airflow, autoclave 17L type, plastic petri dish, micropipette, pH meter from Hanna Instruments and HI 8424 type, the magnetic stirrer Labinco L32 and Lab Companion HP 3000, the analytical balance from Denver Instrument Company and, A-160 type. The Computational software using MS Windows 10 OS; the DOSBox 0.74, Excel MS Office 2010, SPSS v.16.0 (IBM), and the Printfil 5.22 Personal Edition

B. Methods

The experiment was performed with the assumption that the cell is a particle that receives spectrums and scattering. Two stages of modeling processes of cell turbidity are involved using the fixed computation term and context. The first experiment, obtaining the bivariate data that consists cell number of *Saccharomyces cerevisiae* and was suspended in pure water. The cells were homogenized to avoid aggregates using ES 4.9PB and physical effects by centrifuge [24]. The cell number per mL was a quantity after avoiding cell aggregate, and it was calculated with the hemocytometer technique of Neubauer type under a binocular microscope. A turbidimeter measured the turbidity unit.

Suitable data transformation was discovered with the consideration a cell has NTU value, i.e., the simple linear regression coefficient, β1, and the accuracy statement. Model 1 is the category of the parameterized equation [11]. It has the function and utility for cell number estimation per ml; y = f (x1). The statistical procedures toward the dataset of the ten bivariate and five replications were implemented for discovering model 1 [25]; $Yi = \beta o + \beta 1Xi + \epsilon, i=1,2,....n,$ where $\epsilon i \sim N(0,\sigma 2)$

The variance of β1 is formed (1), where statistics of b is normal and given the confidence interval (CI) at α=0.05 and the critical value of tα/2,(n-2) :

$$\text{var } b = \sigma^2 \left[\frac{1}{\sum(x_i - \bar{x})^2}\right] \quad (1)$$

The relationship between turbidity and cell number per mL in pure water uses the null hypotheses Ho: β1 = β2 = 0 and the alternative hypothesis Ha: β1 ≠ β2 ≠ 0. Tests were performed at the F statistic-one tail significance level (QF), and a continuing diagnosis by the Cook's distance, Leverage, and standardized residual for the elimination of the bivariate [8].

The second is the computation of relative accuracy from the turbidity predictor of one NTU value, which used the equation of cell number estimation variance and relative accuracy:

$$V_x = \frac{V_y}{b^2}\left\{1 + \frac{1}{N} + \left[\frac{(Y-\bar{y})^2}{b^2}\right].\frac{1}{\sum(x_i - \bar{x})^2}\right\} \quad (2)$$

$$\pm 100\, b\, \{[t_{\alpha/2,}\, (Vx)^{1/2}]/(Y - a)\} \quad (3)$$

The numeric calculation was performed by the DOSBox 0.74, Windows 10 OS., and Printfil 5.22 Personal Edition.

The second experiment has independent variables of the factor which influence turbidity. The experiment assesses the turbidity model of the cell that was growing in the medium of potato dextrose broth, with adding carbonyl diamide of 0.5%. The data was found with arranging a matrix of the independent and dependent variables on turbidity.

Using the matrix (Table 1), the turbidity cell in the medium of the PDB is a function of predictors; y = f (x1, x2, x3, x4), where x1 = number of cell.ml^{-1}, x2 = growth time's interval, and x3 = weight of cell granule, and x4 = volume PDB medium. The granule of yeast and medium volume fulfils the variable of density [6], and the standard turbidity (Sigma Aldrich) used 3 values. The statistical procedures toward dataset were implemented for the 10 bivariate and 5 replications for discovering the model 2; $Yi = \beta o + \beta 1xi1 + \beta 2xi2 + \beta 3xi3 + \beta 4xi4 + \epsilon$, i=1,2,....n where $\epsilon i \sim N(0,\sigma 2)$.

The estimation of cell quantity by a standard curve was verified to cell enumeration by drop method-plate count. The relationship between turbidity and cell number per mL of PDB was found using the null hypotheses Ho: β1 = β2 = β3 = β4 = 0 was not significant, i.e., P<0.05; which means receiving the alternative hypothesis Ha: β1 ≠ β2 ≠ β3 ≠ β4 ≠ 0 and the independent variable is significant to influence the turbidity. The test was performed at the QF, and the conclusion was to continue diagnosis using the Cook's distance, Leverage, and standardized residual.

A statistical test used was the "enter" and "backward" method using t distribution [26]. The results at a significance value of P < 0.05 or P > 0.05 belong to the coefficient of multiple regressions, respectively.

TABLE I
THE DATA MATRIX FOR FULFILLING THE RANDOM PRINCIPLE; GROWTH TIME'S INTERVAL, WEIGHT OF CELL GRANULE

Saccharomyces cerevisiae	The volume of medium (mL)	Cell quantity in the medium				Turbidity (NTU) of cell in the medium – hours**)			
Granule (g)		The plate method*)		Turbidity Method		4	..	..	<16
		Total	cell. mL^{-1}	Total	cell. mL^{-1}				

*) Verifications of colony number used Potato Dextrose Agar (Merck)

**) The dependent variable was chosen randomly on a time interval.

The result of model 2 is multiple regressions, and the test of the regression coefficient of the cell number factor was compared to the standard curve above. The determination of cell quantity per mL in the PDB using model 2 was a snapshot cell population type [5] of experimental measurement. The turbidity value was obtained from rotated agitation of PDB medium at 75 rpm (Rotary shaker VRN-200), aerated 35 oC, and 13 hours interval cell doubling.

The variance of βcell is the form (1), and the CI of PDB has tα/2,(n-p-1) value of distribution for this context [26], where p represents the number of significant independent variables. Comparing two values was used CI at α =0.05, an implementation of the mathematical equation of uncertainty that has percentage form.

III. RESULTS AND DISCUSSION

A. Data Transformation

Data transformation is an adaptation of computational aspects in this research. Although simple, it shows the overall accuracy. The transformation of data on the count of particles (cell) in a medium is carried out by numerical simplification through division. For the computational aspect, it means that it can be formulated in simple regression equations. Furthermore, deciding the choice or determining the results of the regression diagnosis is a crucial aspect of computation that must be considered.

Transformation is a matter of suitability and functionality, where the data as an outlier stays unchanged; this is for the fulfillment of regression assumptions. The measurements that are used in this study are normality and independent variables in order to meet linear regression and variance similarity. Those requirements are randomly-picked available data. Suitability is obtained by performing a trial, and this is a suitable transformation by simplifying numeric, dividing by 10^5 for every five data sets [8], and the applicable utility of the computer program shows the standard error value of regression coefficient. The result of data transformation shows heteroscedasticity [27]. Log transformation to eliminate heteroscedasticity can be more interpretable and able to perform statistical inference. However, the assessment of the determination coefficient (r^2) is lower than the data transformation with a division of 10^5. Normality, as a data pair test, uses the standardized residual of bivariate analysis.

B. Discovering Turbidity Model of S. cerevisiae in Media.

This research is intended to measure turbidity and to study the relations in the turbidity-causing particles in water. The soil particles using total suspended solids (TSS) as the concept [28]. The concept of particle concentration is based on Mie or Lorentz-Mie's theory, where light scatters depends on the shape of the particles [29]. The concepts for cell particle and the utility cell counting used the concept that predictor is the population form of one species.

The analysis and diagnosis based on the assessment of distance Cook (Cook's D) and Leverage value are carried out by correlating the data [30]. The problems found during the discussion of computational aspects are how to measure the results of turbidity data elimination and when (at which value) it will be applied. The difficulty in determining optimum value occurs when Cook's D is used in statistical analysis applications, but it was not a tool for measuring the result of a regression equation as a model.

In intended optimization, the measurement is carried out by assessing relative error for a predictor of turbidity value in *S cerevisiae* cell count. By using a turbidity predictor, the simulation program in a computational aspect is applied and can provide the prediction of the cell count [8]. The differential value of variance (Vx), Eq. (2), over one of turbidity predictor value is defined as a relative error value. Model determination and utilization for implementation in growth medium become the consideration when the smallest Vx (2) value is obtained from the simulation results. The optimization type is categorized as the dimension of one variable, independent against time, continuum (continuous variable x value), and constrained, i.e., the turbidity value on the interval (intra-extrapolation limit) of measurement does not result in regression heteroscedasticity [1]. Simulation is a heuristic method that intends to solve the problem of optimization at a sufficient and rational level.

Statistical experiment at 95% of CI with an accepted hypothesis is considered correct according to science. The performance and results have been compared with Excel computing and presented in Table 2. For utilization, the preferred model output for turbidimetry and model testing is one that can estimate the count of cells per volume, and a viable method with agar plate technique is applied in this experiment. The above description structurally becomes a simulation algorithm, as shown in Fig. 1.

Assumptions in data analysis play a role in making conclusions from previous research. The first assumption is based on research variables that replication is a subset taken for a combination of one bivariate set in order to perform variant analysis. Each replication is considered independent; i.e., the count of cells is selected randomly every time.

TABLE II
THE STATISTIC TEN BIVARIATE ON EXCEL APPLICATION SHEET IN MS WINDOWS SYSTEM.

X2	Y2	XY	(ΣXΣY)/n	(ΣX2-[(ΣX)2/n]	b	a
39.0625	169	81.25				
225	441	315				
650.25	1600	1020				
1296	3600	2160	38072.15	14307.99	1.470	2.955
2025	4900	3150				
7482.25	16384	11072				
10302.25	23409	15529.5				
17292.25	38416	25774				
39312.0625	88919	59101.8				

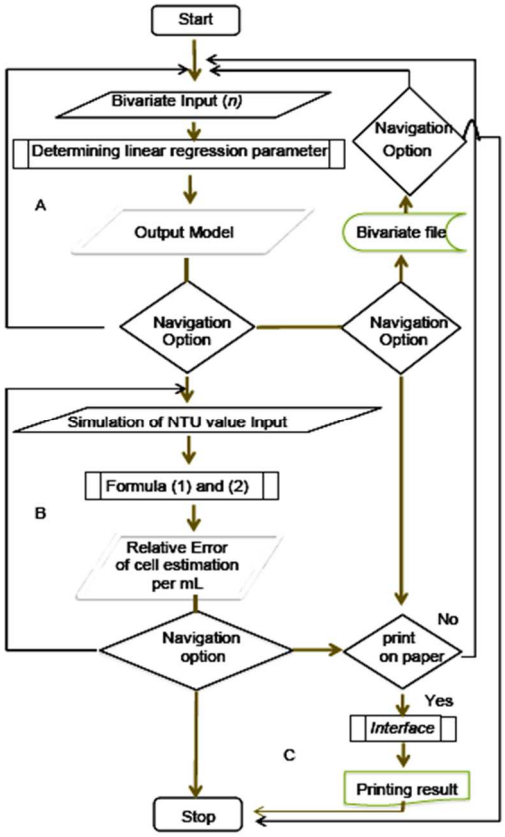

Fig. 1 Algorithm of Measurement and stopping bivariate elimination consist of a subsystem (aspect) A: statistics, B: Predictor Simulation value, and C: Printed.

Second, replication is implemented when bivariate data is taken as the data set known as reproducibility. Both assumptions are tested using a procedure of homogeneous slope homogeneity, i.e., regression coefficient, which uses the analysis of covariance assumptions.

The procedure utilization of the covariance assumption test (Fig. 2) means to ensure that each data set consists of 10 bivariate in every five datasets has a homogenous or non-homogenous regression coefficient. The homogeneous coefficient provides the information that all 50 bivariate data can be combined and vice versa. It is mentioned that in the analysis assumption, the requirement is fulfilled if the coefficient of regression direction is not equal to zero. The bivariate regression properties occur. The F distribution test toward the homogeneous regression coefficients for each data follows the ANCOVA formula and its practical action model finding. Therefore, the structure of computational model findings can be arranged and seen in Fig. 2.

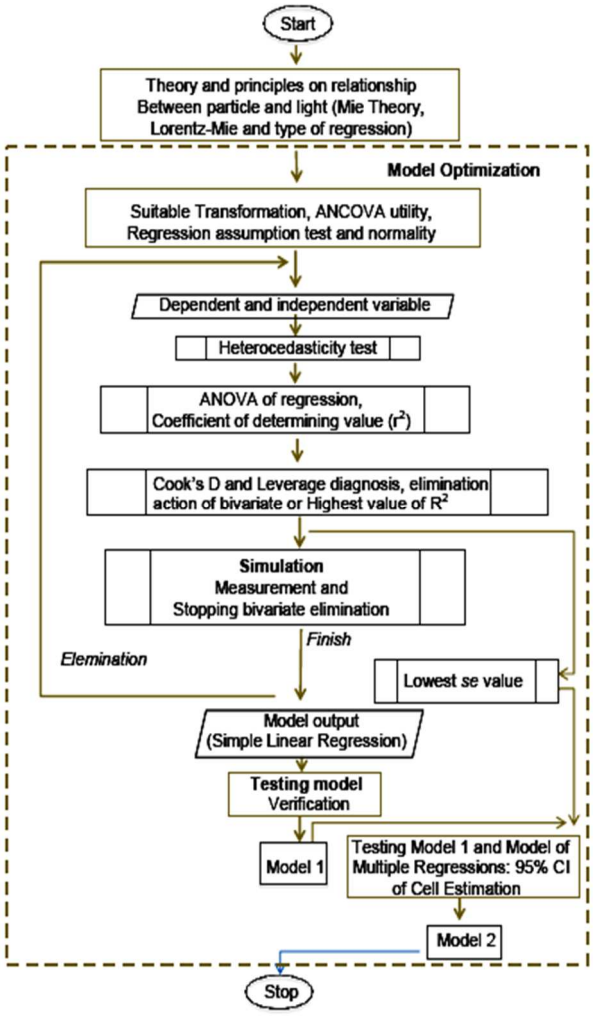

Fig. 2 Algorithm of obtaining and testing models; model 1 is a turbidity standard curve; model 2 is multiple regressions for the medium of multiplied cell. The optimal workflow has been structured

The results of Cook's D diagnosis process show a set of data and turbidity simulation, which has the smallest relative error for the estimated number of cells. mL^{-1}. The computational system was made to adapt to machine work (the computer requires machine language). To have a clear flow in data processing after input becomes the characteristic of a system. The process flow is necessary to know the

applicable requirements when deciding to choose the model [31].

The scheme of a simulation algorithm in Fig. 1 is part of a whole computing process in Fig. 2. The experiment was performed for the selection of model candidates, testing model verification [21], and the results of the simulation printout of turbidity values (NTU) were similar compare using the other program for model 1 (Table II). Simulation product is applied in a modeling process of growing cells in a liquid nutrient medium. Turbidity was caused by cell, medium volume, and time as an increase in cell number. The count of cells rises at a certain period and is determined by model 1.

TABLE III
THE DETERMINATION COEFFICIENT, RELATIVE SE OF MULTIPLE REGRESSIONS, SACCHAROMYCES CEREVISIAE CELL TURBIDITY BY FACTORS OF THE MULTIPLICATION CELL PERIOD, YEAST GRANULE, AND CELL NUMBER PER ML IN PDB BY COOK'S D DIAGNOSIS IMPLEMENTATION

		Y = -16.609 + 4.079X1 + 12.257.10-6 X2		
Adjusted R^2	Turbidity Factors	Coef.	%se*) *n=50*	%se*) *df=44*
0.877	Cell	$12.257.10^{-6}$	23.96	7.70
	Time period**)	4.079	20.93	22.03
		Y = -15,792 + 4,122X1 + 11,862.10-6 X2		
				df=43
0.882	Cell	$11.862.10^{-6}$		7.67
	TMN**	4.122		20.66
		Y = -30.365 + 8.790X1 + 9.679.10-6 X2		
			n=50	
0.523	Cell	$9.679.10^{-6}$	22.54	
	TMN	8.790	33.42	
		Y = -43,351 + 443,566X1 + 7,198X2 + 8,684.10-6 X3		
0.756	Cell	$8.684.10^{-6}$	16.22	
	TMN	7.198		20.08
	Yeast granule	443.566	32.79	

*) based by references [30]
**) Time period of multiple cell number

The Cook's D diagnosis, bivariate eliminations [8], and analysis result can assess the best multiple regressions equation (Table III). The smallest relative value was considered for the chosen model of multiple regression. The CI of the coefficient on the number of cells per mL is an interval of $(1.171 – 1.201).10^{-5}$ NTU in the growth medium. This value is smaller compared to cells in purified water, the interval of $(1.433–1.507).10^{-5}$ NTU. The addition of one *Saccharomyces cerevisiae* cell in purified water contributes to additional light scattering that NTU value, whereas the population of growing cells in the medium is $(1.171 – 1.201).10^{-5}$ NTU. The cell scatters value in the medium is influenced by the time of cell population growth, in which every hour contributes to an additional value of (3.27 – 4.974) NTU.

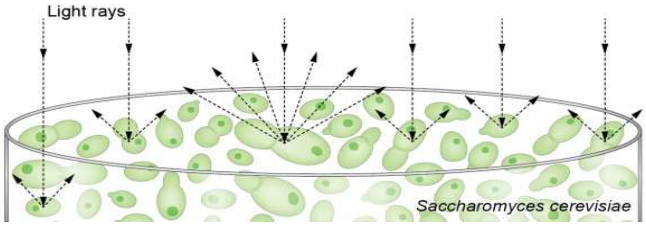

Fig. 3 Larger particles result in higher scatter as compared to smaller ones and the differences between growing cells and cells generated from buds (Illustration was based by the principle of spectrum interaction in reference [15])

The model achievements for standard curve and its application, and it can be stated that this study provides a logical knowledge; a standard cell that is ready to scale up can generate higher turbidity than growing cells. Similarly, larger particles result in higher scatter as compared to smaller ones. This image is shown in Figure 3, and the refraction properties like scattering light can be correlated with cell size and morphology [6]. The values of both NTU mean that multiple regressions show many smaller sized cells as compared to cells grown from a parent.

TABLE IV
THE IMPLEMENTING THE TWO BEST TURBIDITY MODELS FOR CELL NUMBER ESTIMATION PER ML MEDIA

Model*	Adjusted R^2	Inoculation**	Intervals*** Standard curve	Interval Growth Medium	Conclusion (P=0.05)
A	0.882	1	$(5.04–5.70).10^8$	$(5.52–7.54).10^8$	*equal*
		2	$(2.96–3.27).10^9$	$(5.52–7.54).10^8$	*equal*
B	0.877	1	$(5.04–5.70).10^8$	$(5.39–7.37).10^8$	*equal*
		2	$(2.96–3.27).10^9$	$(2.72–3.72).10^9$	*equal*

*) A: $Y = -15.792 + 4.122X_1 + 11.862.10^{-6}X_2$ and B: $Y = -16.609 + 4.079X_1 + 12.257.10^{-6}X_2$
**) 1. Granules. 2. Inoculum
***) $y= 1.47.10^{-5}x + 2.955$

Time is a factor that affects turbidity due to the increase in the count of smaller cells. Therefore, this answer to the question of why the increase in time (hour), instead of particle property, can increase turbidity values (NTU). Turbidity is a phenomenon where the sum of light reflection encounters cells at 90° of the incoming light, known as light scatter. Larger cells will result in a higher light scatter. Therefore, the addition of a small cell will increase light scatter when compared to the addition of one cell during the population growth. Implementing the best model for the standard curve was performed on the observation data of scale-up from the granule yeast in a standard laboratory medium for 6.5 hours. The scale-up by volume continued for 13 hours. The total was $3.12x10^9$ cells, and the best model on scale-up resulted in an estimation value of $3.3x10^9$ cells with *se* value of 7.67%. Both intervals have equal values (Table IV).

IV. CONCLUSION

The turbidity value of each particle yeast cell and its multiples in the nutrient medium is based on the regression coefficient on the model. The computational turbidity modeling for the aspect of cell number per mL in media

consisted first. A standard curve for the turbidity model in pure water, and the second, a significant turbidity factor at multiple regressions was the cell particles and time interval of multiplied cells in the medium.

Saccharomyces cerevisiae cell was considered a suspended particle in purified water or cell growth medium. The only one cell particle in purified water contributes to the additional light scattering $(1.433 - 1.507).10^{-5}$ NTU, whereas the population of growing cells in the medium is $(1.171 - 1.201).10^{-5}$ NTU. The time of cell population growth, which every hour contributes to an additional value of (3.27 – 4.974) NTU. Moreover, random bivariate data in each data set is considered reproducible and required bivariate transformation suitable with the count of particles.

ACKNOWLEDGMENT

The researchers would like to express their gratitude to the Directorate General of Higher Education of the Indonesian Ministry of Research, Technology, and Higher Education for providing funds for this research; No.: 3/E/KPT/2018 and No.:7/E/KPT/2019.

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