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KINETICS PARAMETERS ANALYSIS OF BIOFLOCCULANT PRODUCTION FROM *Alcaligenes latus* ON THE SUBSTRATE OF HYDROLIZATE OF SOLID WASTE FROM PULP AND PAPER INDUSTRY AND ON GLUCOSE

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ABSTRACT

There are two kinds of waste being discharged in the pulp and paper industry, namely wastewater and solid waste which contains lignocellulosic materials. Lignocellulosic materials are potential to be used as substrate for microbial bioconversion. During the study *Alcaligenes latus* used the hydrolizate of solid waste from pulp and paper industry to produce bioflocculant, which could flocculate wastewater that contained suspended solids. For all the experiments *A. latus* grew well on solid waste hydrolizate, giving about 2.21 g product/g substrate and 1.09 g biomass/g substrate. Cultivation on glucose yielded about 4.42 g product/g substrate and 2.84 g biomass/g substrate. The specific growth rates of bacteria on the substrate of hydrolizate of solid waste from pulp and paper industry and glucose were 0.0527/h and 0.0237/h, respectively.

Keywords: Alcaligenes latus, bioflocculant, solidwaste, wastewater

Pulp and paper industry has been one of the industrial activities that attract a lot of environmental concerns, due to the pollution that might be generated. During the process, solid and liquid wastes are discharged, which need further treatment to avoid environmental pollution. One of the way to treat wastewater from pulp and paper industry is flocculation. Flocculation could reduce suspended solids in wastewater and perform suspended solids and colloid to become big particles which are easy to separate with precipitation.

However, studies indicated that the monomers of acrylamide being used to flocculate wastewater is both neuro-toxic and strongly carcinogenic in human body (Kurane & Nohata, 1994). The use of these flocculating agents is therefore harmful to the environment and may be a dangerous source of pollution that can adversely affect the future generations. Thus, a safe biodegradable flocculant that is produced on substrate of hydrolizate of solid waste from pulp and paper industry by microorganisms is expected to minimize environmental and health risks. This study observed the kinetics process during the production of bioflocculant by *Alcaligenes latus* grown on the hydrolizate of solid waste of pulp and paper industry.

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MATERIALS AND METHOD

Microorganism

A. latus which is capable of producing a new bioflocculant was used. Solid waste was obtained from PT. Kertas Bekasi Teguh (KBT), Bekasi-Indonesia. The material was dried to 10% moisture content, grounded and then sieved to pass a 40-60 mesh sieve.

Hydrolysis with Diluted Acid

Alkali treatment was applied to the solid particles of waste using sodium hydroxide 1 N (1 : 20 (b/v)). The solution was mixed for 2 hours then autoclaved at 121°C for 15 minutes (Anis *et al.*, 1994). Hydrolysis of the solid waste was performed using sulfuric acid 0.3 M (256 g/l) at 96°C for 5 hours. The mixture was then filtered, washed and neutralized.

Cultivation of the Microorganism

Composition of the medium was as follows: 15 g glucose or 10 g/l hydrolizate of solid waste, 6.75 g K_2HPO_4, 2.25 g KH_2PO_4, 0.3 g $MgSO_4.7H_2O$, 0.15 g NaCl, 1.5 g urea, 0.75 g yeast extract, and 1.5 destiled water, adjusted to pH 7.2. Cultivation was carried out in a bioreactor (Mini Jarfermentor M-100) at 30°C for 7 days with 6 blade turbines operated at 150 rpm (Kurane & Nohata, 1994). Samples were analyzed every 12 hours for biomass, product (bioflocculant), reducing sugar, viscosity and pH values.

Purification of Bioflocculant

The culture broth was diluted with ten volumes of distilled water, and NaOH (0,02-1%) was added to dissolve the bioflocculant. The broth was then heated and maintained at 121°C for 10 min, following which the cells were removed by centrifugation (40 000 g x 30 min). The liquid sample was concentrated with a membrane filter (0,2 µm pores) after removing the cells.

Kinetic Parameters

Kinetics parameters of bioflocculant production was estimated from logistic equation for cells growth,

$$dx/dt = \mu x(1.0-x/x_{max}) \quad \ldots\ldots\ldots (1),$$

Luedeking-Piret equation for bioflocculant production,

$$dP/dt = nx + mdx/dt \quad \ldots\ldots\ldots\ldots(2),$$

and modification of Luedeking-Piret for substrate reduction (Weiss & Ollis, 1980),

$$ds/dt = \alpha(dx/dt) - \beta(x) \quad \ldots\ldots(3).$$

Equations (1)-(3) provided the basis for describing the time course for xanthan and other extracelluler polysaccharides production by microorganism. The major convenience of equations (2) and (3) was that the non-growth-associated parameters, β and n, could be evaluated from stationary phase data ($dx/dt = 0$) leaving only the single growth-associated parameters α and m, respectively, to be evaluated from the growth-phase data (Weiss & Ollis, 1980).

The values of certain parameters of these models were calculated from the linear relationship. These models were assumed on unlimited condition for substrate hydrolizate of solid waste of pulp and paper industry and glucose.

Flocculation Activity

Kaolin clay was chosen as a test standard material for flocculation. In a 100 ml graduated cylinder, 80 ml of kaolin clay suspension (5 000 ppm), 10 ml of 1% $CaCl_2$ solution, and 0.5 ml of the culture was mixed, and adjusted to pH 7.0 with NaOH (or HCl). The test cylinder was mixed gently at room temperature and then kept standing for 5 min. The formation of visible aggregates was observed by measuring the decrease in turbidity of the upper phase using a spectrophotometer at 550 nm. The flocculating activity was calculated by the following equation (Kurane & Nohata, 1994).

$$\text{Flocculating activity} = 1/A - 1/B \quad \ldots\ldots\ldots(4)$$

A: optical density at 550 nm of sample,

B: optical density at 550 nm of reference,

RESULTS AND DISCUSSION

Cultivation

The decrease of pH value during cultivation was likely due to the production of acid. Some strains of Alcaligenes produced acid from D-glucose and D-xylose and used those acids as carbon source for growth and produce bioflocculant (Breed *et al.*, 1974). The lowest pH value (6.93) was observed 36 hours after inoculation (Figure 1).

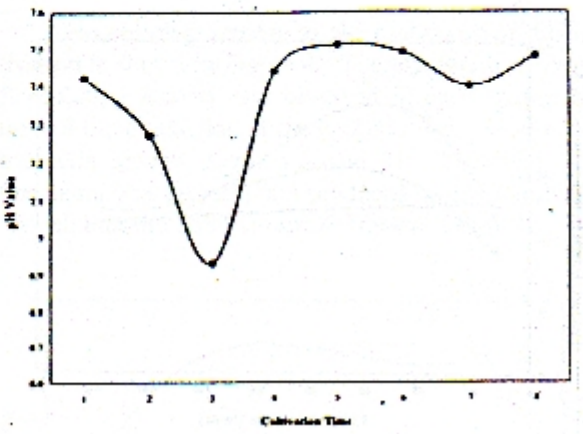

Figure 1. Effect of cultivation time on pH value

Curve of Cells Growth

A representative course of flocculant formation and a cells growth curve when hydrolizate of solid waste and glucose were used as the carbon source are presented in Figures 2 and 3. The maximum rate of flocculant formation was observed in the early stationary phase, and no rapid decrease of the flocculating activity was observed in the late stationary phase. As shown in Figure 2 the production of bioflocculant was parallel to the growth curve.

According to Figure 2, there was no adaptation growth phase. It means that bacteria grew at exponential growth phase directly, since the cells which were inoculated into the bioreactor came from propagation process for 2 days. This process increased the efficient use of substrate by *A. latus* and reduced the cultivation time. After exponential phase, cells grew at stationary and death phases. Stationary phase occurred at 84 hours and then bacterial growth curve continued to death phase. Death phase occurred due to the depletion of the substrate, and on the other hand, cells might have produced secondary metabolites, which inhibited cells growth.

The highest product (bioflocculant) yielded was observed at 60 hours and then decreased. It happened because cell did not produce bioflocculant in the death phase and some parts of the product was converted into other products by enzyme which was produced by *A. latus* (Kurane & Nohata, 1994). Cells in the stationary phase degraded bioflocculant which contained sugar, resulting in the decreas of bioflocculant concentration in the medium from 13.01 g/l to 11.51 g/l (Figure 2).

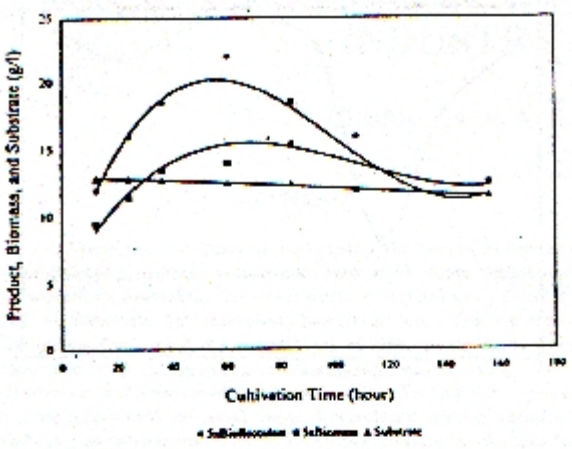

Figure 2. Effect of cultivation on product, biomass, and substrate by *A. latus* on *glucose*

Kinetics Parameters of Cultivation

Kinetics of cultivation was analyzed from biomass growth (dx/dt), bioflocculant production (dP/dt) and reducing substrate (dS/dt). On the other hand, parameters to estimate kinetic variables (μ, m, n , α, β, Yp, Yx) were used.

Biomass growth was calculated from equation (1), where μ the specific growth rate and x_{max} is maximum concentration of biomass in the stationary phase. The integrated form of eq. (1) together with Xo = X (t=0) are given as

$$X(t) = (Xoe_{\mu}{}^{t})/(1.0-(Xo/X_{max})(1.0-e_{\mu}{}^{t}) \quad \ldots\ldots\ldots(5)$$

or rearranged to

$$Ln\ X/(Xmax-X) = t-ln[Xmax/(Xo-1.0)] \quad \ldots\ldots(6)$$

Integrated form of eq. (2) and X(t) from eq. (5), give equation with two initial conditions (Xo,Po), final condition (Xmax) and three parameters (μ, n, and m),

$$P(t)=Po+mXo[e_{\mu}{}^{t}/(1-(Xo/X_{max})(1-e_{\mu}{}^{t})]-1+n(X_{max}/\mu)$$
$$ln[1-Xo/X_{max}(1-e_{\mu}{}^{t})] \quad \ldots\ldots\ldots\ldots\ldots(7)$$

Where P(t) is product concentration at t hours, and Po is product concentration at t=0 (g/l). The value of n calculated from the equation is as follows

$$n = [dP/dt)stationary]/Xmax \quad \ldots\ldots\ldots(8)$$

Equation (3) was used to estimate substrate consumption. The integrated form of eq. (3) is given as

$$So-St = \alpha(X-Xo) - \beta(X_{max}/\mu)ln[1-(Xo/X_{max})\cdot$$
$$(1-e_{\mu}{}^{t})] \quad \ldots\ldots\ldots\ldots\ldots\ldots(9)$$

Where So is the initial substrate concentration and S(t) is substrate concentration at t hours. The β value was calculated from the equation as follows

$$\beta = [(ds/dt)stationary]/Xmax \quad \ldots\ldots(10)$$

Yp (yield product) value was calculated from the linear regression between So-S and P-Po, and Yx (Yield biomass) was calculated from the linear regression between So-S and X-Xo.

Table 1. Values of certain kinetics parameters calculated from linear regression of growth of *A. latus* on substrate of hydrolizate of solid waste and glucose

Kinetic Parameters	Hydrolizate of solid waste	Glucose
μ (/hour)	0.0227	0.0237
Xo (g/l)	1.2869	1.6172
n (gp/gx h)	0.010584	0.00330
m (gp/gx)	2.73	6.20
α (gs/gx)	0.599	0.623
β (gs/gx h)	0.000712	0.00330
Yp (gp/gs)	2.21	4.42
Yx (gx/gs)	1.09	2.84

The kinetic parameters (Table 1) show that specific growth for both substrates were very small, and there was relationship between bioflocculant production and cells growth (m>n). Substrate was used for cells growth and only a little part of substrate consumed was used for non-cells growth (α>β). On the other hand, cultivation of *A. latus* on glucose had more yield of product (4.42 g product/g substrate) and biomass (2.84 g biomass/g substrate) than that of the cultivation on substrate of hydrolizate of solid waste (2.21 g product/g substrate and 1.09 g biomass/g substrate, respectively). It shows that glucose was a better substrate for the growth of *A. latus* than the hydrolizate of solid waste of pulp and paper industry.

Substrate Effect

A. latus which was inoculated on substrate of hydrolizate of solid waste had a smaller yield than that of on glucose, because there was likely other materials that inhibited cells growth in the media. These materials were produced during the degradation of hexose and pentose into other materials such as furfural and hydroxil methyl furfural.

Cells growth curve on substrate of hydrolizate of solid waste of pulp and paper industry is presented in Figure 3. There was diauxic growth effect in cells growth which was likely due to the presence of many kinds of carbon sources in the hydrolizate of solid waste from pulp and paper industry (for example xylose and arabinose).

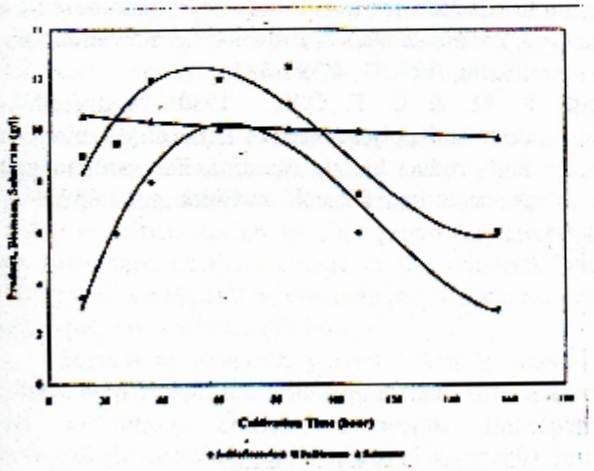

Figure 3. Effect of cultivation on product, biomass, and substrate by *A. latus* on substrate of hydrolizate of solid waste from pulp and paper industry

Productivity of Cultivation

Productivity of cultivation was estimated following the equation of

$$t = [(1/\mu m)\ln(Xf/Xo)] + tT + tL + tD \quad \ldots\ldots\ldots(11)$$

$$P = Xf/t \quad \ldots\ldots\ldots(12)$$

where

t = Cultivation time
μm = Maximum specific growth rate
Xo = Cells concentration (at the beginning of cultivation)
Xf = Cell concentration (at the en of cultivation)
tT = Cyclus culture time
tL = Lag time
tD = Delay time

By assuming propagation time as the time before cultivation (cyclus culture time, delay time and lag time), the equation (11) and (12) can be used to determine the cultivation time and the productivity of cultivation of *A. latus* on both a media (glucose and hydrolizate of solid waste of pulp and paper industry). The results show that productivity of cultivation of *A. latus* on glucose (0.0446) was higher than that of the productivity on substrate of hydrolizate of solid waste of pulp and paper industy (0.0412).

Flocculating Activity of Bioflocculant

Flocculating activity of the bioflocculant during cultivation is shown in Figure 4. The maximum increase of flocculating activity was observed in early stationary phase and the production of the bioflocculant was parallel to the cells growth (Figures 2 and 3). Therefore, the bioflocculant was probably not produced by cell autolysis, but by cell biosynthesis (Kurane & Nohata, 1994).

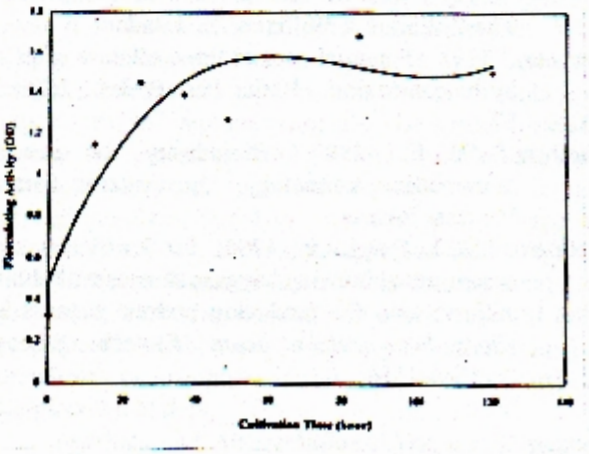

Figure 4. Effect of cultivation time on flocculating activity

CONCLUSIONS

Solid waste of pulp and paper industry which contained lignocellulosic materials could be used as a substrate for *A. latus* growth to produce bioflocculant. Cultivation of *A. latus* on substrate of hydrolizate of solid waste from pulp and paper industry (yielded about 2.21 g product/g substrate and 1.09 g biomass/g substrate) was lower than that of cultivation on glucose (yielded 4.42 g product/g substrate and 2.84 g biomass/g substrate).

NOMENCLATURE

g(p) = Gram of product
g(x) = Gram of biomass
g(s) = Gram of substrate
μ = Specific growth rate (h^{-1})
m = Product growth-associated parameter [g(p)/g(x)]
n = Product non-growth-associated parameter [g(p)/g(x).h]
α = Substrate growth-associated parameter [g(s)/g(x)]
β = Substrate non-growth-associated parameter [g(s)/g(x).h]
Yp = Yield of product [g(p)/g(s)]
Yx = Yield of biomass [g(x)/g(s)]

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