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by Setia Budi Sasongko

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Production of *Spirulina platensis* Biomass Using Digested Vinasse as Cultivation Medium

Budiyono, Iqbal Syaichurrozi, Siswo Sumardiono and Setia Budi Sasongko
Department of Chemical Engineering, University of Diponegoro, Semarang, Central Java, Indonesia

Corresponding Author: Budiyono, Department of Chemical Engineering, University of Diponegoro, Semarang, Central Java, Indonesia

ABSTRACT

Spirulina platensis is one of the potential food sources in the future. Many authors have investigated the potential of wastewater as cultivation medium for microalgae, but utilization digested vinasse as cultivation medium has not reported yet. The purpose of this study was to investigate the potential of digested vinasse as cultivation medium for *Spirulina platensis*. Digested vinasse contained 30,250 mg L^{-1} Chemical Oxygen Demand (COD), 11,343.75 mg L^{-1} total carbon, 688.2 mg L^{-1} total N, 219.9 mg L^{-1} PO_4^{-3}-P. Cultivation was done in batch system at room temperature using erlenmeyer and using TL lamp as light source. Optical Density (OD) of culture was measured using spectrophotometry UV-VIS at wave length (λ) 680 nm every day. The results showed that the addition of digested vinasse more than 0.8% v/v (242 mg L^{-1} COD content) caused growth rate of *S. platensis* very slowly. Presence of organic compounds in medium reduced the production of photosynthetic pigment so that it hampered the rate of photosynthesis. Besides that, phenolic compounds contained in digested vinasse could damage the membrane cell of *S. platensis*. The maximum growth rates at digested vinasse addition of 0.0 (without addition); 0.8; 1.6; 2.4; 3.2; 4.0; 4.8 were 0.168; 0.143; 0.073; 0.044; 0.042; 0.030; 0.005 day^{-1}, respectively. Utilization of nutrients in digested vinasse could reduce 50% of the synthetic nutrient need. Medium which had Carbon:Nitrogen:Phosphorus (C:N:P) ratio of 60.5:6.2:1 was the best medium to cultivate *S. platensis* with maximum growth rate of 0.220 day^{-1}.

Key words: Biomass, cultivation, *Spirulina platensis*, digested vinasse

INTRODUCTION

Cultivation of *Spirulina* has many advantages compared to that of other microalgaes. Production of *Spirulina* biomass rate is faster than that of some others. *Spirulina* has so big biomass size that it easy to harvest and *Spirulina* can live in extreme conditions (very alkaline) with pH range of 8-11 (Desmorieux and Decaen, 2005; Richmond, 1988). According to Kozlenko and Henson (1998), *Spirulina* is one of the potential food sources because 1 acre of *Spirulina* can produce 20 times as many proteins as 1 acre soybean or corn. *Spirulina* contains high level of protein in range of 60-71% of dry weight and 65% higher than other natural foods. Fat contents in *Spirulina* have range of 6-7% and the most of that are unsaturated fats (Tietze, 2004; Spolaore *et al*., 2006).

Microalgae can be applied on wider field of technology. It not only produces biomass that can be used as food and energy source but also can be used to reduce COD content, nitrogen-phosphorus, heavy metals of wastewater. COD, N and P contained in wastewater are

utilized by microalgae as nutritional source to grow. From an economic perspective, the culturing microalgae in wastewater can reduce the cost of synthetic nutrient need (Hadiyanto and Hartanto, 2012) cultivated *Spirulina* sp. in medium which contained POME (Palm Oil Mil Effluent). *Spirulina* sp. could thrive with 20% concentration of POME and 50% reduction in synthetic nutrient need. Duangsri and Satirapipathkul (2011) reported that *Spirulina* sp. could grow well in medium brine wastewater. Cheunbarn and Peerapornpisal (2010) reported that medium cultivation that contained 10% swine wastewater treatment effluent, 8 g L^{-1} $NaHCO_3$ and 1.5 g L^{-1} $NaNO_3$ caused the most maximum growth rate of *S. platensis*. Andrade and Costa (2007) conducted cultivation of *S. platensis* using molasses. Concentration of molasses 0.75 g L^{-1} caused the most maximum biomass production of *S. platensis* compared to that of molasses 0.25 and 0.5 g L^{-1}.

Cultivation of microalgae using digested vinasse has not reported yet. In author's previous research, authors treated vinasse, bottom product of distillation in bioethanol industry, using anaerobic digester. Thus, vinasse that had been processed using anaerobic digester was called digested vinasse. Digested vinasse contained a number of nutrients such as COD, total of nitrogen and PO_4^{-3}P. These contents could be utilized as nutritional source by microalgae.

The objectives of this study were to investigate the effect of addition digested vinasse to growth rate of *S. platensis* and pH culture and to know the best percentage reduction of synthetic nutrients.

MATERIALS AND METHODS

Microalgae and digested vinasse: Microalgae used was *Spirulina platensis* obtained from the collection of C-BIORE (Center of Biomass and Renewable Energy), University of Diponegoro, Indonesia. Culture of *S. platensis* that had OD_{680} value~0.6 was used as inoculum. Digested vinasse was obtained from variable in author's previous research that produced the most of total biogas. Digested vinasse used in this study contained 30,250 mg L^{-1} COD; 11,343.75 mg L^{-1} total Carbon; 688.2 mg L^{-1} total Nitrogen; 219.9 P-PO_4^{-3}. In addition, pH condition of digested vinasse used in this study was 6.7.

Experimental set up: Synthetic medium used was cultivation medium for *S. platensis* developed by Hadiyanto and Hartanto (2012) with nutrients: 1 g L^{-1} $NaHCO_3$ (purity 98%), 0.05 g L^{-1} urea (46% N content), 10 ppm TSP (45% P_2O_5 content). *S. platensis* could grow optimally at room temperature, so cultivation was done at room temperature. Artificial light as light source was obtained from tube light (TL) lamp 18 watt placed with distance of 10-15 cm from culture. Initial pH culture was adjusted 9.0 using HCl 1 M or NaOH 1 M.

Experimental design

Scenario I: Cultivation medium with total volume 1 L was operated in batch system using erlenmeyer. Digested vinasse was added into media with variety in concentration of 0, 0.8, 1.6, 2.4, 3.2, 4.0, 4.8% v/v medium. Inoculum of *S. platensis* 10% v/v was added into medium. In this scenario, all of cultures were added synthetic nutrients 100% (1 g L^{-1} $NaHCO_3$ (purity 98%), 0.05 g L^{-1} urea (46% N content), 10 ppm TSP (45% P_2O_5 content)) (Table 1). Initial pH for all cultures was adjusted 9.0.

Table 1: Composition of digested vinasse, tap water, inoculum and synthetic nutrient in cultivation mediums

Medium	Digested vinasse (mL)	Tap water (mL)	Inoculum (mL)	Synthetic nutrients (%)
Scenario I				
I	0	900		
II	8	892		
III	16	884		
IV	24	876		
V	32	868		100
VI	40	860	100	
VII	48	852		
Scenario II				
A	The best of addition digested vinasse at scenario I			0
B				25
C				50
D				75
E				100

Scenario II: Addition of digested vinasse concentration in medium that had the best growth rate of *S. platensis* from scenario I, was used in this scenario. Then, it was combined with addition of synthetic nutrients of 100, 75, 50, 25, 0% (Table 1). Initial pH for all culture was adjusted 9.0.

Experimental procedures: Cultivation was done using erlenmeyer in 13-20 days. Optical density of all variables was measured two times by using spectrophotometry UV-VIS at λ 680 nm every day. Value of pH medium was measured by using pH meter every day. The results of investigation were used to calculate the growth rate, growth curve and pH profile curve:

$$\mu = \ln (OD_i - OD_0)/(t_i - t_0)$$

Remarks: μ, growth rate (day^{-1}); OD_i, Optical density at t_i; OD_0, Optical density at t_0; t_i, cultivated time I; t_0, cultivated time 0.

RESULTS AND DISCUSSION

Effect of addition of digested vinasse (Scenario I): The results showed that the higher the concentration of digested vinasse was added into medium, the slower *S. platensis* grow in medium (Fig. 1). In media I (without addition of digested vinasse), *S. platensis* thrived from beginning of cultivation until 14th day, then decreased until ending of cultivation with the highest OD_{680} 1.126. While in media II, *S. platensis* biomass reached the highest OD_{680} at 16th day with value of 1.070 (Table 2). In the addition of digested vinasse more than 0.8% v/v, *S. platensis* needed a very long time to adapt, 8 days in medium III, 9 days in medium IV, 11 days in medium V, 11 days in medium VI and 14 days in medium VII (Fig. 1). The length of adaptation due to the high concentration of COD added into medium. Travieso *et al.* (1996) stated that microalgae could grow well and set aside maximum of COD 88% when medium contained 250 mg L^{-1} COD concentration of settled piggery wastewater from variety ranges of COD between 250-1,100 mg L^{-1}. The results of this study were consisted with that of Travieso *et al.* (1996), where the addition of digested vinasse 242 mg L^{-1} COD (medium I) caused growth of *S. platensis* better than the addition of digested vinasse more than 242 mg L^{-1} COD (medium III-VII).

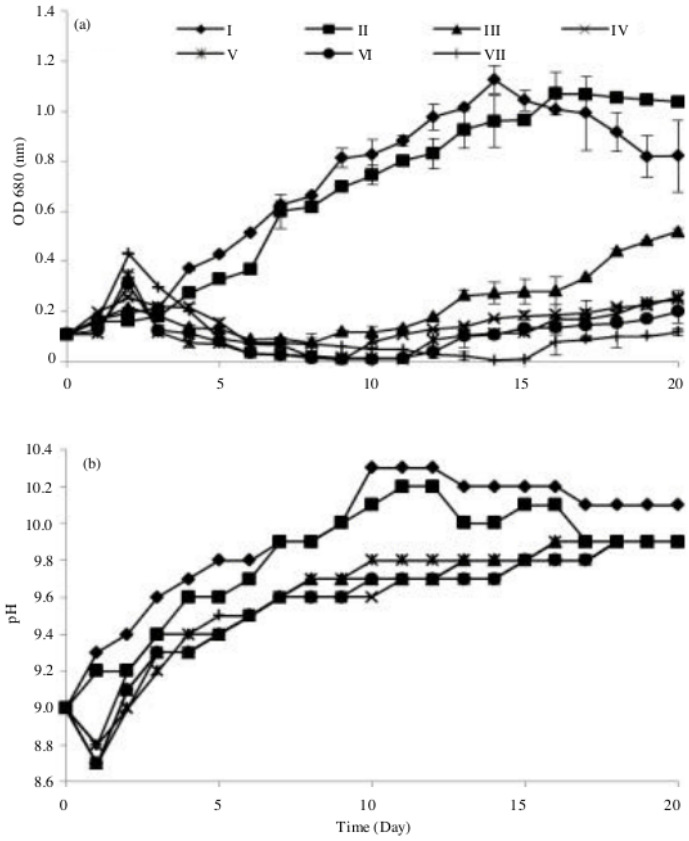

Fig. 1(a-b): The effect of addition of digested vinasse to (a) Growth curve of *S. platensis* and (b) Profile pH medium

Table 2: The effect of addition of digested vinasse to maximum growth rate (μ_{max}), optical density (OD) and maximum time (t_{OD}) (Scenario I)

	Nutrients						
	Digested vinasse						
Medium	% v/v	COD (mg L^{-1})	Synthetic (%)	Ratio of C:N:P in medium	OD 680_{max}	$t_{OD\ 680\ max}$ (day)	μ_{max} (day^{-1})
I	0	0	100	76.3 : 11.7 : 1	1.126	14	0.168
II	0.8	242	100	64.6 : 7.7 : 1	1.070	16	0.143
III	1.6	484	100	60.5 : 6.2 : 1	0.517	20	0.078
IV	2.4	726	100	58.3 : 5.5 : 1	0.256	20	0.044
V	3.2	968	100	57.0 : 5.0 : 1	0.246	20	0.042
VI	4.0	1,210	100	56.1 : 4.7 : 1	0.198	20	0.030
VII	4.8	1,452	100	55.5 : 4.5 : 1	0.116	20	0.005

COD: Chemical Oxygen Demand; C:N:P: Carbon:Nitrogen:Phosphorus; OD 680_{max}: Maximum optical density reached at λ 680 nm; $t_{OD\ 680\ max}$: Time at OD 680_{680} reached; μ_{max}: Maximum growth rate reached at OD 680_{max} and $t_{OD\ 680\ max}$

The addition of high concentration of COD caused dark color and turbidity which were high in medium, so the penetration of light into medium was small. This phenomenon disrupted photosynthetic activity of *S. platensis*. Cheunbarn and Peerapornpisal (2010) stated that *S. platensis* could not thrive in medium which contained high organic substances because that could affect dark color and turbidity. Thus, the photosynthetic rate of *S. platensis* was slowly. Hadiyanto and Hartanto (2012) confirmed that the dark color as an effect of presence of wastewater caused decreasing in the intensity of light into medium so that the process of photosynthesis was impaired.

In media I and II, pH increased from the beginning of cultivation until 12th day with the highest pH were 10.3 and 10.2, respectively (Fig. 1). The increasing of pH was caused by presence of sodium bicarbonate ($NaHCO_3$) in medium. Sodium bicarbonate was dissolved into Na^+ and HCO_3^- (bicarbonate ion). Na^+ was used as micro nutrient by *S. platensis* while HCO_3^- was converted in form CO_2 and OH^- with help of enzyme carbonic anhydrase (Reuter and Muller, 1993; Jaiswal *et al*., 2005; Badger *et al*., 1994). CO_2 formed was utilized as carbon source for photosynthesis. Ion OH^- accumulated in medium caused alkaline in pH (Richmond and Grobbelaar, 1986; Grobbelaar, 2004; De Morais and Costa, 2007).

The increasing in pH was related with photosynthetic activity, where the higher the rate of photosynthetic activity, the higher the pH medium (Andrade and Costa, 2007). The maximum pH value indicated that chlorophyll a concentration of *S. platensis* was maximum and growth phase of *S. platensis* no longer sustained and reached death phase (Kim *et al*., 2007). In Fig. 1, *S. platensis* reached the point of death on the 14th day and 16th day respectively in medium I and II after it reached the highest pH at 12th day.

While in medium III-VII, pH decreased at first time of cultivation and then went up. This phenomenon might be caused by activity of bacteria in medium. The more concentration of digested vinasse were added, the more amount of bacteria was contained in medium. Based on principle that was proposed by Oswald *et al*. (1957), at beginning of cultivation oxidation bacteria in medium converted organic compounds of wastewater into CO_2 via respiration. CO_2 formed reacted with water to form carbonate, so medium to be acidic. Then, carbonate was used by *S. platensis* for photosynthetic process and released OH^-, so pH medium gradually increased.

Besides activity of bacterial oxidation might participate in the system, *S. platensis* also utilized organic carbon as source of carbon to produce CO_2 through respiration. This growth was called heterotrophic growth, whereby energy and carbon source derived from organic carbon such as glucose. Carbon dioxide formed caused acidic in pH medium. Furthermore, *S. platensis* used CO_2 for photosynthesis. This growth was called photoautotrophic growth which utilized light as primary energy source and carbon dioxide as primary carbon source. Because of photoautotrophic growth, pH medium was gradually increased. Photoautotrophic and heterotrophic processes that took place simultaneously in cell were called mixotrophic growth (Marquez *et al*., 1993).

Ogbonna and Tanaka (1998) stated that addition of organic carbon would reduce the production of photosynthetic pigments, so rate of photosynthesis was slowly. Carbon dioxide was the main of carbon source needed in photosynthesis. In this study, carbon dioxide was obtained from $NaHCO_3$. The more digested vinasse was added into medium, the more slowly rate of photosynthesis went on in system (Fig. 1).

Production of biomassa in media I and II declined after 14th dan 16th, respectively. This declined phase was called death phase because a lot of algal cells were death. This phase was occurred due to the age old culture and the limitation of light and energy supply. Membran cell of

S. platensis broke (lysis), so organic materials in cell were out and dissolved into medium (Fogg and Thake, 1987). This phenomenon might cause decreasing in pH medium. This could be shown in Fig. 1, growth of *S. platensis* decreased and pH of medium decreased too.

The maximum growth rate (μ_{max}) of *S. platensis* on each medium could be seen in Table 2. The greater the concentration of digested vinasse, the smaller the maximum growth rate. Media II, with the addition of the smallest concentration of digested vinasse (0.8% v/v or 242 mg L^{-1} COD), had the highest μ_{max} of the others (medium III-VII). However, medium II had less μ_{max} than media I (without digested vinasse addition) although medium II had more time of exponential phase than medium I. That was caused by dark color and turbidity medium that reduced penetration of light into medium. Richmond (1988) confirmed that organic carbon and light were two important factors that influenced the growth of mixotrophic microalgae.

Effect of variation of synthetic nutrient addition (Scenario II): After cultivation in 13 days, maximum growth rate on each medium was shown in Table 3. Maximum growth rate in media A, B, D, E had almost the same value, there was 0.163-0.169 day^{-1}, while medium C had maximum growth rate of 0.220 day^{-1}. This result was caused by availability of optimum number of nutrients in medium. Phang and Ong (1988) reported that the ideal C:N:P ratio for microalgal cultivation was 56:9:1. Medium C with C:N:P ratio of 60.5:6.2:1 gave the best results compared to C:N:P ratio of the other mediums. From Table 3, C:N:P ratio of medium C was the closest to the ideal C:N:P ratio proposed by Phang and Ong (1988). However, after 9th day of cultivation, the growth rate *S. platensis* in medium C decreased. Phenol in vinasse that was not degraded during the anaerobic processing might cause decreasing in growth rate of *S. platensis*.

$OD\ 680_{max}$ on media A and B were too low (below 0.6). OD value that can be used as inoculum was at least 0.5-0.6. Besides that, biomass in media A and B were yet to be harvested because it was just little. Meanwhile, media C, D, E had $OD\ 680_{max}$ that was eligible as inoculum. The higher synthetic nutrients were added in medium, the higher $OD\ 680_{max}$ and $t_{OD\ 680\ max}$ (Table 3). These results showed that the utilization of digested vinasse could not replace all of synthetic nutrient need. Medium A (without synthetic nutrients) had the smallest $OD\ 680_{max}$ and μ_{max} because availability ratio of carbon, nitrogen and phosphorus was not optimal.

Medium C had the highest μ_{max} that was achieved at 9th day of cultivation. *S. platensis* could grow quickly in medium C until 9th day of cultivation then decreased till the end of cultivation. This phenomenon was caused by nutrient that was needed by *S. platensis*, that already exhausted absorbed in the first 8 days. While in medium D and E, *S. platensis* could grow up to

Table 3: The effect of addition of digested vinasse to maximum growth rate (μ_{max}), optical density (OD) and maximum time (t_{OD}) (Scenario II)

	Nutrients					
Medium	Synthetic nutrients (%)	Digested vinasse (%v/v)	Ratio of C:N:P in medium	$OD\ 680_{max}$	$t_{OD\ 680\ max}$ (day)	μ_{max} (day^{-1})
A	0	0.8	51.6 : 3.1 : 1	0.465	9	0.163
B	25	0.8	57.0 : 5.0 : 1	0.498	9	0.169
C	50	0.8	60.5 : 6.2 : 1	0.780	9	0.220
D	75	0.8	62.9 : 7.0 : 1	0.756	12	0.163
E	100	0.8	64.6 : 7.7 : 1	0.919	13	0.165

COD: Chemical Oxygen Demand; C:N:P: Carbon:Nitrogen:Phosphorus; $OD\ 680_{max}$: Maximum optical density reached at λ 680 nm; $t_{OD\ 680\ max}$: Time at $OD\ 680_{680}$ reached; μ_{max}: Maximum growth rate reached at $OD\ 680_{max}$ and $t_{OD\ 680\ max}$

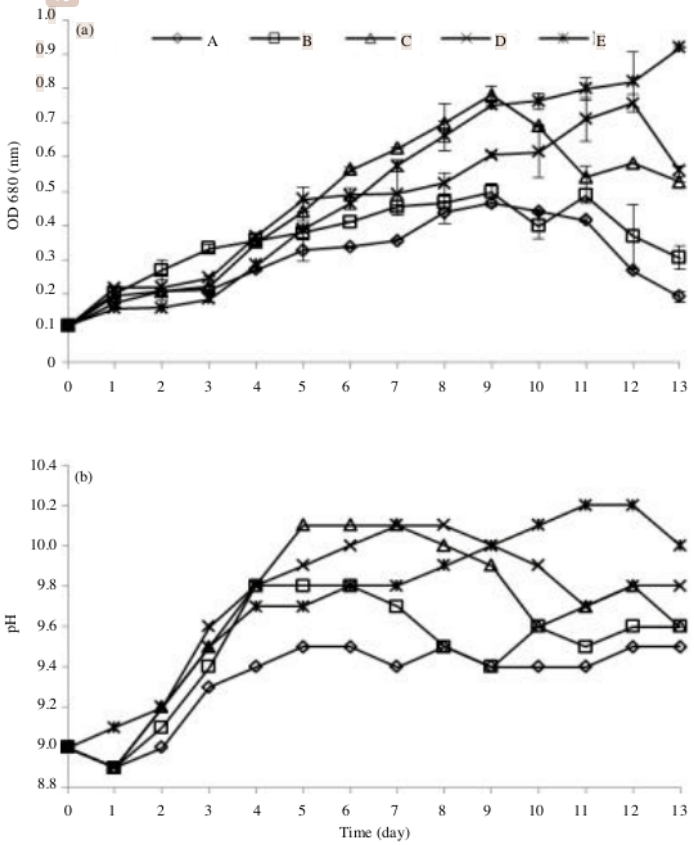

Fig. 2(a-b): The effect of variation of synthetic nutrient addition to (a) Growth curve of *S. platensis* and (b) Profile pH medium

2th and 13th day. Synthetic nutrient was very important as source of nutrition beside nutrient from digested vinasse. These indicated that autotrophic character of *S. platensis* was more than heterotrophic character if there were a number of inorganic carbon source ($NaHCO_3$) in medium.

pH of media A, B, C and D decreased at beginning of cultivation. While medium E, pH went up from beginning to end cultivation (Fig. 2). Decreasing in pH was caused by the lower availability of bicarbonate in medium A, B, C, D than that in medium E. Bicarbonate in medium was dissolved into CO_2 and OH^- during cultivation. Accumulation of OH^- caused increasing in pH (Richmond and Grobbelaar, 1986; Grobbelaar, 2004; De Morais and Costa, 2007). Besides that, decreasing in pH could be caused by mixotrophic growth which utilized organic carbon (such as organic acid, acetic acid, sugar and glycerol) as carbon source via respiration (Borowitzka, 1998; Kawaguchi, 1980; Fogg, 1975; Ogbonna *et al.*, 2000; Wood *et al.*, 1999). Respiratory activity produced CO_2 that caused pH medium low. In medium E, pH rose at the early cultivation because photosynthesis process was more dominant than respiration process.

The results of this study were much better than the results of study reported by Hadiyanto and Hartanto (2012) whereby medium of cultivation that contained 20% v/v POME and 50% synthetic

nutrients gave the best growth rate of *Spirulina platensis* (0.142 day^{-1}). Meanwhile in this study, medium C, with addition of the same synthetic nutrients (50%) and 0.8% v/v digested vinasse had growth rate of 0.220 day^{-1}. In addition, the results of this study were better than the results of study conducted by Andrade and Costa (2007) whereby maximum growth rate of *S. platensis* was 0.147 day^{-1} in medium which contained 0.25 g L^{-1} molasses.

Microalgal growth could be hampered by the presence of phenol in medium. Some authors reported that *Chlorella* sp., *S. obliquus* and *Spirulina maxima* could not grow by using phenol as carbon source (Semple and Cain, 1997; Klekner and Kosaric, 1992; Scragg, 2006) added that Chlorella vulgaris and Chlorella VT-1 also could not thrive in medium containing phenol compounds. Phenol in medium damaged the structure of membrane cell (Leonard and Lindley, 1999). Digested vinasse used in this study might contain phenolic compounds.

CONCLUSION

Medium that contained 0.8% v/v digested vinasse (242 mg L^{-1} COD) was the best medium to grow *S. platensis*. Inhibition of growth was caused by presence of organic compound in medium so medium became dark and turbidity that caused slowly penetration of light into medium. Besides that, organic compounds in medium reduced production of photosynthetic pigments so rate of photosynthesis was very slowly. Combination of 0.8% v/v digested vinasse and 50% synthetic nutrient (medium C) gave the most satisfactory result which was the largest growth rate value of 0.220 day^{-1}. Medium C had ideal C:N:P ratio which was 60.5:6.2:1.

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