Submerged microfiltration membrane performances of microalgal biomass cultivated in secondary effluent

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ABSTRACT

This study was conducted to investigate the feasibility of submerged microfiltration process to separate microalgal biomass in the growth reactor fed by a secondary wastewater effluent. Both nitrogen and phosphorous were utilized by microalgae as nutrient. The results showed that almost 90 % of N and total P content of secondary effluent were consumed by microalgae in the first six days. It was observed that the phosphorus was the limiting nutrient. The growth rate was also a function of pH and optimum pH was determined as 6.0 to 6.5. High growth rate at that pH values was likely due to the fact that CO, was present mostly in soluble form, whereas, at higher pH CO, was in bicarbonate form. Membrane filtration experiments were conducted using polyethersulfone (PES) membranes with 0.1 µm, 1.2 µm and polycarbonate (PC) membranes with 0.1 µm, 1.0 µm pore sizes. The results showed that the use of submerged microfiltration membranes allowed continuous operation for microalgal growth reactor. Membranes effectively separated biomass from the reactor. The steadystate flux values were almost independent of the type and the pore size of the membranes. More than 90% of the total resistance was due to cake formation. Since the cake layer controlled the filtration rate, the average flux values as well as the decline pattern remained largely independent of pore size and the membrane types. The physical surface cleaning experiment conducted for PC 1.0 µm showed that the fouling layer could easily be removed, resulting similar filtration performance to the new membranes

Keywords: Microalgae; Submerged microfiltration; Biofouling; Mixed culture; Secondary effluent

1. Introduction

The last decade has witnessed substantially increased interest on renewable energy, including biofuel from biomass. Since biofuel obtained from food crops was not received well due to concerns about competition with food supply and requirement for extensive amounts of water, fertilizer and pesticides for their growth, research on environmentally friendly biofuels has tended to other feedstocks, such as microalgal biomass.

Earlier research has revealed that the use of microalgae for biofuel production is quite advantageous in many ways, including lower water and land requirement, higher biomass growth rate and higher capture rate of carbon dioxide (CO_2), compared to terrestrial plants [1]. Research on biodiesel production from microalgae has received more emphasis after the discovery that many of the microalgal species can accumulate lipids under stress conditions, such as nutrient deficiency and high illumination [2,3].

In addition to light and CO_2 , microalgae need various nutrients as nitrogen (N), phosphorus (P), minerals and trace elements for their growth. The cost of those constituents is a significant portion of the total cost of biofuel production from microalgae. Yet, it is estimated that both water and nutrient requirements can be reduced by up to 90%, if wastewaters, such as domestic wastewater, is used in production of microalgae [4].

Unlike bacterial cells used for biodegradation of organic substances in wastewaters, most types of algal species tend to remain suspended in water and resist self-flocculation

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and settlement. Therefore, for separation of microalgal biomass from water, coagulation-flocculation may need to be employed. Although coagulants and flocculants are helpful in separation, the process requires additional chemical cost and additional processes, such as settling or a flotation tank, centrifugation and filtration [5–7]. Furthermore, chemicals used in the process accumulate via return streams and may damage and cause reduction in algal growth rate, by being perceived by the cells as impurities.

Membrane filtration was suggested as a novel technique for harvesting and concentrating of microalgae cultures [8-11]. A number of review studies focusing on using membrane filtration emphasized that this process is generally less expensive than using centrifuges for microalgae harvesting. Furthermore, membrane processes require no chemicals, such as coagulants, and thus their accumulation in recycled streams is prevented [12,13]. In most of the studies reported in the literature, micro and ultra-filtration membrane systems were applied in crossflow configuration [14]. However, exposure of microalgal biomass to high shear, especially in intake and pumping systems, may break microalgal cells into smaller particles, which may cause severe membrane fouling by enhancing pore blocking and producing a less porous cake layer on the membrane surface [15,16]. More importantly, the cell breakage will inactivate the cells, and the recycling of the cells to the growth reactor to keep a high concentration of active cells will not be possible. On the other hand, Bilad et al. [17] studied the applicability of submerged microfiltration for harvesting a freshwater green algae species Chlorella vulgaris and a marine diatom Phaeodactylum tricornutum using lab-made membranes, and concluded that submerged microfiltration was a promising low-cost algae harvesting process.

Submerged microfiltration is commonly applied for wastewater treatment in activated sludge reactors known as submerged membrane bioreactors (MBRs), and it is preferred over external cross-flow filtration as it is cheaper due to lower energy consumption and the absence of pressure resistant membrane housings [18,19]. In addition, in immersed system, the shear is generally provided by coarse air bubbles, thus limiting the exposure of microalgal cell to shear stress.

In this paper, submerged membrane filtration was studied for the separation of a mixed microalgal culture cultivated in a biologically pre-treated domestic wastewater (secondary effluent). The growth reactor was operated in continuous-flow mode where the wastewater was continuously fed to the reactor, the filtered water effluent was continuously withdrawn and discharged, while the algal biomass was retained in the reactor to maintain a high microalgal concentration. High biomass concentration within the reactor is desirable because the rate of growth is directly proportional to the concentration of algal biomass. Without concentrating the biomass within the reactor, a typical pretreated domestic wastewater with low levels of nutrients may support only a few hundred milligrams of biomass per liter of water. This is not a sufficiently high concentration to constitute an economically feasible process.

Singh and Thomas [19] recently reported that coupled nutrient removal and microalgae cultivation processes in a membrane-equipped-photobioreactor using domestic wastewater and four wild species of microalgae. It was shown that the reactor was able to remove on average 50% of ammonia (NH₄⁺), 75% of nitrite (NO₂⁻), 35% of nitrate (NO₃⁻) and 60% of phosphate (PO₄³⁻) consistently from an MBR effluent under the conditions tested. However, the study did not include a comprehensive analysis of the membrane performance for biomass filtration.

Bilad et al. [20] and Discard et al. [21], both used photobioreactors equipped with membranes for growth of pure Chlorella vulgaris cultures. These cultures were cultivated in synthetic medium prepared from pure chemicals dissolved in demineralized water. The former study showed the advantages of using membranes for separation and the return of biomass to the reactor without any loss and for allowing the reuse of the growth medium through recycling. However, the latter study revealed the limitations of recycling of nutrients in the growth medium, including accumulation of exopolymeric particles, algogenic organic matter, counter ions and non-limiting nutrients that might negatively affect the growth of the microalgal cells. Marbelia et al. [22] reported on treatment feasibility of a synthetic wastewater subjected to a membrane bioreactor (MBR) for removal of organic matter, followed by a microalgae (Chlorella vulgaris) membrane photobioreactor (MPBR) for removal of nutrients.

As opposed to these studies, the present study is focused on a mixed microalgal culture cultivated in a real wastewater effluent. Furthermore, the performance of the submerged microfiltration equipment was tested with different membrane types and pore sizes; fouling mechanisms of the membranes were identified, and the recovery of flow flux following physical cleaning of the membranes was observed. The cell growth parameters, recycling growth medium and concentrating factors were focused on while assessing the membrane performance.

2. Materials and methods

2.1. Characteristics of wastewater

The secondary effluent obtained from Omerli Domestic Wastewater Treatment Plant, İstanbul, Turkey was used as the algal growth culture medium in the experiments. The secondary effluent is a term used to indicate that the domestic wastewater has been subjected to pretreatment for removal of parts of particulates and dissolved organic substances by a biological treatment process. The average characteristics of the secondary effluent used in the study are presented in Table 1.

2.2. Preparation of stock microalgal suspension

A mixture of several spontaneously reproducing indigenous species in secondary effluent of domestic wastewater was used as the inoculums for the initial growth of microalgal cells. The inoculums were collected from rain water ponds. This mixture was cultivated in a 20 L reactor. The wastewater effluent was first fed to the reactor. Pure CO_2 was supplied to the reactor by bubbling pressurized gas through glass diffusers. No additional mixing was provided in the reactor.

Table 1 Chemical characteristics of secondary effluent

Parameters	Values
pH	7.42
DO (mg/L)	8.39
Alkalinity (mg/L as $CaCO_3$)	110
COD (mg/L)	34.1
$BOD_5 (mg/L)$	6
$NH_3-N (mg/L)$	0.24
$NO_3^N (mg/L)$	12.5
$PO_4^{3-}P(mg/L)$	2.7
TN (mg/L)	17
TC (mg/L)	50.5
IC (mg/L)	37.8
TOC (mg/L)	12.7

A custom-made plexiglass submerged membrane module with 100 cm² active membrane area was placed in the reactor. Microalgal suspension was grown under approximately 150 µmol photon m⁻² s⁻¹ continuous illumination at $25^{\circ}C \pm 2^{\circ}C$. The biomass concentration in the reactor was allowed to increase from 20-30 mg/L initial concentration to about 3-3.5 g/L, and the concentration was maintained at that level by random batch feeding with secondarily treated wastewater. It took about six weeks to reach the maximum concentration and it was kept on desired concentrations by harvesting the biomass once a week. The CO₂ flow was adjusted by an automatic control system (WTW pH 296) to maintain the pH in between 6.0 and 6.5, where the highest growth rate was obtained (Section 2.3). This culture was also used as inoculants in experiments for biomass production and nutrient removal. The particle size distribution of stock microalgal suspension ranged from 2 to 20 µm, with the mean particle size of 10 µm as shown in Fig. 1.

The identification of microalgal species was performed morphologically by using a Carl Zeiss, Axio Scope Trinocular Phase Contrast Microscope with the help of books on microalgae systematics [23–27]. It was observed that the microalgal stock suspension mainly contained *Scenedesmus sp.* species. Other particulate matters except microalgae was not observed by basic microscopy.

2.3. CO, Addition to the growth reactors

A set of experiments were conducted in order to determine the effect of the addition of CO_2 to wastewater in the reactor on biomass growth. CO_2 addition drops the pH of the wastewater; however, CO_2 consumption due to photosynthetic activity increases the pH. Therefore, the experiments were conducted at steady-state with respect to pH by bubbling pure CO_2 gas into the 20 L glass reactors, and by continuously monitoring and maintaining the pH of wastewater at the desired values. In three of the reactors, the pH was kept in the range of 6.0–6.5, 7.0–7.5 and 7.5–8.0, by adding different amounts of CO_2 . Microalgal biomass concentration in the



Fig. 1. The particle size distribution of microalgal suspension in secondary effluent.

reactors was monitored for 15 d and maximum biomass concentration in each reactor was determined.

2.4. Batch experiments for biomass production and nutrient removal

The secondary effluent was used as the culture medium after sterilizing it in an autoclave at 121°C and then filtering through 0.45 µm glass fiber membrane filter. Then 5 mL of stock mixed microalgae culture was inoculated into the 500 mL of secondary effluent in 750 mL flasks. The contents of the flasks were mixed at 100 rpm by magnetic stirrers under approximately 150 µmol photon m⁻² s⁻¹ continuous illumination at 20 ± 2°C for 10 d. During the experiments, the pH was kept at the prescribed value for both cultures by bubbling CO₂ gas through glass diffusers into the flasks. Samples were collected to determine the biomass and nutrient concentrations.

2.5. Submerged filtration experiments

A schematic diagram of the experimental setup used in filtration experiments is presented in Fig. 2. The reactor was a 40 L stirred glass tank reactor with working volume of 20 L of stock microalgal suspension and fed with secondary effluent continuously by a peristaltic pump. Water head was kept constant by adding secondary effluent manually after during filtration experiment. The reactor was positioned on a platform to create the desired pressure difference (0.15 bar) needed for microfiltration of the suspension. The filtrate was delivered to a collection flask below the platform. All filtration experiments were carried out at room temperature ($25 \pm 2^{\circ}$ C) and at 1.6 m. of water-head trans-membrane pressure.

A custom-made plexiglass submerged membrane modules used in the reactor for these experiments had 25 cm² active membrane area. Two different membrane types, polyethersulfone (PES) and polycarbonate (PC), were used in the modules. PES flat sheet membranes with 1.2 and 0.1 μ m pore sizes and PC flat sheet membranes with 1.0 and 0.1 μ m pore sizes were supplied from Sterlitech Corporation. According to the manufacturer, BSA protein binding capacities of PES and PC membranes are less than 20 µg/cm² and 5 µg/cm², respectively.

The experiments were carried out at three stages. First, pure water was used in the filtration experiments. Then, the filtration of secondary effluent was performed. Finally, the filtration of microalgal suspension in secondary effluent was done. During the experiments, water flux as a function of time was monitored by measuring the weight of collected filtrate by a balance. Volume was kept constant during the experiments by feeding the secondary effluent by a peri-



Fig. 2. A schematic diagram of the continuous-flow experimental setup.

staltic pump and the feeding rate was adjusted according to the filtration flux. The data were collected and noted at one-minute interval using a computer. The steady-state flux values observed during the experiments were used in data analysis to determine various resistance values for secondary effluent and microalgal suspension.

Membrane fouling due to microalgal biomass or wastewater was studied by measuring resistances at a constant trans-membrane pressure (ΔP). The water flux (J) is a function of the ΔP , water viscosity (μ) and the total resistance to the flux (R_i) according to the following equation:

$$J = \frac{\Delta P}{\mu R_t} \tag{1}$$

The total resistance, R_t (m⁻¹), is composed of three components: the intrinsic resistance of the membrane (R_m), the resistance attributable to cake layer on the membrane surface (R_c), and the fouling resistance caused by pore blocking (R_n).

$$R_{t} = R_{u} + R_{c} + R_{u} \tag{2}$$

 R_m was determined by measuring the steady-state pure water flux, while R_t was determined by measuring the steady-state flux of either the secondary effluent or the microalgal biomass suspension. After the filtration experiments were completed, the membrane surface was flushed with deionized water and cleaned with a sponge to remove the cake layer. Then, the deionized water flux was measured to obtain $R_m + R_p$. The pore blocking resistance was then calculated by subtracting R_m values from $R_m + R_p$ values. R_c was calculated by subtracting $R_m + R_p$ from R_t [28].

2.6. Flux recovery

To determine the physical cleaning efficiency of the selected membrane, dead-end filtration experiments were performed using a stainless steel stirred cell (Sterlitech HP4750) under 1 bar constant pressure. For these experiments, 1.0 μ m pore sized PC membrane filters were used. Approximately 100 mL microalgal suspension was filtered for 30 min periods. The membrane was washed

with deionized water thoroughly and its surface was gently cleaned with a sponge at the end of each filtration cycle. The flux was monitored as a function of time for each cycle.

2.7. Analytical methods

The pH and dissolved oxygen concentration (DO) were measured with a Mettler Toledo pH Meter and a HACH LDO101 oxygen probe, respectively. Photosynthetically active radiation (PAR) was measured with LI-193 quantum sensors and recorded by an LI-1400 Data Logger. Alkalinity, chemical oxygen demand (COD), biochemical oxygen demand (BOD) and NH₃–N analysis were performed based on the methods presented in the standard methods [29]. Inorganic nitrogen, NO₃⁻ and inorganic phosphorus, PO₄^{3–} were analyzed by using ion chromatography (Shimadzu HIC 20A). Total nitrogen (TN), total carbon (TC), inorganic carbon (IC) and total organic carbon (TOC) analysis were performed by using a TOC analyzer (HACH IL 550 TOC-TN).

Total suspended solids (TSS) was used as a method to measure the microalgal biomass concentration, X (mg-biomass/L). TSS was measured gravimetrically, according to the Standard Methods. The absorbance of TSS at 680 nm (OD₆₈₀) was used for rapid determination of the algal biomass concentration in suspensions. A Genesys 10S UV-VIS spectrometer was used for optical density measurements. TSS for the calibration curve was determined gravimetrically according to the Standard Methods. The correlation established between X (mg/L), measured by TSS, and OD₆₈₀ is as follows:

 $X = 382.7 \times OD_{680nm} + 0.411 \ (R^2 = 0.998)$

3. Results and discussion

3.1. Effect of pH on microalgal growth

The results of the experiments shown in Fig. 3 indicate that higher CO₂ addition that reduced the pH to 6.0–6.5 was



Fig. 3. Biomass growth with respect to different pH ranges.

able to produce higher growth rate and biomass concentration, compared to lower CO_2 additions. However, further increase in CO_2 addition that caused the pH to drop to 5.5 has impaired the growth of biomass, likely by making the medium too acidic for the culture (not shown in the figure). Therefore, the pH of wastewater was maintained within the range of 6.0–6.5 in the subsequent experiments. High growth rate at pH of 6 to 6.5 is likely due to the fact that CO_2 present mostly in soluble form; whereas, at higher pH CO_2 is in bicarbonate form.

3.2. Determination of biomass production and nutrient removal rates

The results of the triplicated batch experiments conducted under the conditions presented in Section 2.3 and in the pH range of 6.0-6.5 are presented in Fig. 4. It was shown that within the first six days, NO₃-N and PO₄³⁻-P concentrations dropped rapidly while the algal biomass concentration increased exponentially. The results also showed that almost 90% of N and total amount of P content of wastewater were consumed by microalgae in the first six days. The biomass concentration stabilized afterward when it reached about 600 mg L⁻¹ and the P content of the secondary effluent dropped below the detection limit. At this point, the NO₃-N concentration was still relatively high (about 2 mg L⁻¹), which indicated that P was the element that limited the growth of the biomass under the experimental conditions. After nine days, the nutrient concentration decreased substantially. As a result, the biomass concentration decreased due to death phase. The released nutrients were likely used for the growth of the new cells that was seen as an increase in biomass concentration.

3.3. Flux and resistance analysis of submerged microfiltration results

The flux decline curves of microfiltration of secondary effluent for four different membranes are presented in Fig. 5. The initial flux (J_o), the average steady state fluxes (J_{avo}) and estimated resistance values are presented in Table 2. The percent contributions of each resistance type to the total resistance were calculated and also presented in the Table in parenthesis. Both the initial and the average final flux values of larger pore sized membranes were observed

to be higher than those of smaller pore sized membranes. In other words, the smaller pore sized membranes offered much higher total resistance to the flux. Although the contribution of cake resistance to the total resistance was



Fig. 4. Nutrient removal and biomass production of mixed microalgae culture.



Fig. 5. Time-dependent fluxes of four different membranes during filtration of secondary effluent.

Table 2

Flux and resistance values obtained from microfiltration of secondary effluent

	J_o	J _{avg}	Resistance			
	$(L m^{-2} h^{-1})$	$(L m^{-2} h^{-1})$	R_{t} (m ⁻¹)	$R_m(m^{-1})$	$R_{p} (m^{-1})$	R_{c} (m ⁻¹)
PES 1.2	1849.0	401.3	1.35×10^{11}	3.60×10^{10}	5.58×10^{10}	4.27×10^{10}
			(100%)	(26.8%)	(41.5%)	(31.8%)
PES 0.1	709.7	61.2	8.83×10^{11}	1.02×10^{11}	1.06×10^{11}	6.75×10^{11}
			(100%)	(11.5%)	(12.0%)	(76.5%)
PC 1.0	2148.3	267.5	2.02×10^{11}	3.71×10^{10}	5.61×10^{10}	1.09×10^{11}
			(100%)	(18.4%)	(27.8%)	(53.9%)
PC 0.1	918.2	160.2	3.37×10^{11}	7.71×10^{10}	1.54×1^{09}	2.58×10^{11}
			(100%)	(22.9%)	(0.5%)	(76.7%)

the same for PES 0.1 and PC 0.1 with about 77%, the magnitude of the cake resistance for PES 0.1 was about 2.5 times greater than that of PC 0.1. The contribution of pore resistance to the total resistance was low for membranes with small pores (0.5 and 12%), but it was high for membranes with larger pores (28 and 42%). The pore resistance of PC 0.1 membrane was about 10 times lower than that of PES 0.1. PES membranes have spongelike structures with tortous path; whereas, PC membranes were cylindrical straight holes. However, similar behavior was not observed at larger pore sized membranes. These results clearly indicated that particles in secondary effluent mostly accumulated on the surface of smaller pore sized membrane; whereas, more particles were able to penetrate and deposit in the pores of larger pore sized membranes, as would be expected. The contribution of the cake resistance for larger pore sized membranes was not as significant as the smaller pore sized membranes. The percentage of the total resistance of the filtration for PES 1.2 and PC 1.0 were 32% and 54%, respectively. When the types of the membranes compared, PES membranes with small pore size showed much smaller total resistance, whereas, it was the lowest for large pore size had. Difference in PC membranes with different pore size was not as significant as PES membranes.

Among the membrane types tested, PES 1.2 µm yielded the highest and PES 0.1 µm the lowest average flux values. The flux values for PC membranes remained in between the two PES membranes. It is known that while PES has more interconnected pore structure, PC has more straight cylindrical pores. Due to the tortuous structure of the pores, the smaller PES presented significantly more membrane resistance and higher pore fouling compared to the PC, resulting the highest flux decline among the membranes.

Flux decline curves for filtration of microalgal suspension for four different membranes are presented in Fig. 6. A rapid membrane fouling occurred during the filtration of microalgal suspension for all the membranes.

The initial and the average fluxes as well as the resistance values presented in Table 3. Compared to the filtration of secondary effluent alone, the total resistances have increased by at least an order of magnitude for filtration of microalgal suspension. More than 90% of the total resistance was estimated to be due to cake formation for all the membrane types, showing that the cake resistance was the dominant



Fig. 6. Time-dependent fluxes of four different membranes during filtration of microalgal suspension.

fouling mechanism. The average particle size of suspension was about 8–10 μ m, therefore the filtration occurred through sieving, causing cake layer on the membrane surface. The average flux for four membranes was not significantly different from each other; in other words, the average flux values as well as the decline pattern remained largely independent of pore size and the membrane types because the formed cake layer controlled the filtration rate in all the membranes tested. A similar result was reported in the literature for cross-flow microfiltration of an algal suspension, where the cake formation was observed to be rapid and the steady-state flux values independent of pore sizes [8].

In this study, it was observed that the pore resistance values for microalgal suspension had insignificant contribution to the total resistance; in fact, the pore resistances were even lower than those obtained for filtration of secondary effluent. This further confirmed that the cake layer covered the surface and most likely protected the pores from heavy fouling.

3.4. Effect of physical cleaning on flux recovery

The effect of physical cleaning of PC 1.0 membrane on flux is depicted in Fig. 7. After the first and the second cleaning, the initial flux recovery obtained was 78% and 85%, while no

Table 3

Flux and resistance values obtained from microfiltration of microalgal suspension

	J _o	J _{avg}	Resistance			
	$(L m^{-2} h^{-1})$	(L m ⁻² h ⁻¹)	R_{t} (m ⁻¹)	$R_m(m^{-1})$	$R_{p} (m^{-1})$	$R_{c} (m^{-1})$
PES 1.2	1191.84	39.7	1.36×10^{12}	3.60×10^{10}	3.72×10^{10}	1.29×10^{12}
			(100%)	(% 2.6)	(% 2.7)	(% 94.6)
PES 0.1	372.72	30.3	1.78×10^{12}	1.02×10^{11}	6.64×10^{09}	1.67×10^{12}
			(100%)	(% 5.7)	(% 0.4)	(% 93.9)
PC 1.0	1510.32	39.5	1.37×10^{12}	3.71×10^{10}	1.19×10^{10}	1.32×10^{12}
			(100%)	(% 2.7)	(% 0.9)	(% 96.4)
PC 0.1	776.40	33.6	1.61×10^{12}	7.71×10^{10}	8.20×10^{10}	$1.45 imes 10^{12}$
			(100%)	(% 4.8)	(% 5.1)	(% 90.1)



Fig. 7. Effect of physical cleaning on flux decline for 1.0.

changes were observed on the steady-state fluxes after cleaning compared to the unused membrane. These observations indicated that not much irreversible fouling occurred on the membrane during filtration. Similar results were reported in the literature. Castaing et al. [30] reported that the cake layer was easily removed from the surface by physical cleaning.

4. Conclusions

The results showed that the use of submerged microfiltration membranes allowed continuous operation for microalgal growth reactor. Membranes effectively separated biomass from the microalgal reactor fed by secondary effluent.

In this study, phosphorus was the limiting nutrient. The results showed that nutrient from the secondary effluent could effectively be used to grow algae until P was consumed. It was observed that the growth rate was a function of pH, and the optimum pH was found as 6.0–6.5.

Filtration of microalgal suspension through PES and PC type membranes revealed that the cake formation was the dominant fouling mechanism for both 0.1 μ m and 1.2 μ m pore size membranes. The average particle size of suspension was about 8–10 μ m, therefore the filtration occurred through sieving, causing cake layer on the membrane surface. The steady state flux values were observed to be almost independent of the type and the size of the membranes. The physical surface cleaning easily removed the fouling layer from PC 1.0 μ m membranes, resulting similar filtration performance of the new and cleaned membranes.

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