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Feasibility of Microalgal-Bacterial Aggregates for Aeration-Free Wastewater Treatment

By

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THESIS

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## **Abstract**

Microalgal-bacterial aggregates (MBAs) have recently attracted significant attention as a potential replacement for conventional, suspended-growth wastewater treatment. Proponents of MBAs often claim several key benefits: 1) elimination or reduction of external aeration requirements, which would greatly reduce energy consumption, 2) improved resource recovery through the production of value-added products from microalgal biomass, and 3) removal of nitrogen, phosphorus, and bioavailable chemical oxygen demand (bCOD) that is competitive with conventional technologies (e.g., activated sludge). This article briefly reviews the development of MBAs and evaluates their feasibility for full-scale implementation. The results suggest that MBAs and similar suspended-growth processes are functionally identical to well-mixed wastewater treatment ponds, which have been used and studied for decades and require a substantial amount of surface area to treat typical municipal wastewater. We estimate that photosynthesis and atmospheric diffusion would provide at most only 2.7% of the oxygen required for bCOD removal in a nitrifying activated sludge process. Thus, MBAs and similar treatment processes are not viable alternatives to conventional wastewater treatment processes when space is limited. However, attached-growth bioreactors designed to maximize atmospheric diffusion of oxygen relative to their footprint are promising for compact, low-energy wastewater treatment with microalgal-bacterial consortia.

# 1 Introduction

The evolution of sanitation and wastewater treatment practices over the last century has dramatically improved the quality of many waterways and has significantly reduced the incidence of waterborne illness. More recently, there has been a shift toward viewing wastewater as a resource from which commodities might be extracted or recovered, including nutrients and energy.<sup>1</sup> Microalgae, sometimes referred to as algae, are promising for simultaneous wastewater treatment and resource recovery due to their ability to remove nitrogen and phosphorus<sup>2-4</sup> through biomass assimilation and produce oxygen through photosynthesis, as well as their suitability for producing value-added products (e.g., biodiesel,<sup>5</sup> ethanol,<sup>6</sup> nutraceuticals,<sup>7</sup> and aquaculture feed<sup>8</sup>). They form a group of more than 25,000 microscopic, photosynthetic eukaryotic and prokaryotic organisms<sup>9</sup> and were first cultivated in the mid-19<sup>th</sup> century by Cohn in Germany and Famintzin in Russia.<sup>10</sup> Shortly before the start of the 20<sup>th</sup> century, algae were discovered in wastewater and investigated for their interactions with bacteria.<sup>11</sup> In 1942, Harden and von Witsch proposed the concept of harvesting fuels that are similar to conventional oil from microalgae.<sup>10</sup> In the years following World War II, microalgae research spread across the globe, with researchers focusing on photobioreactor design and operation with the goal of producing food for a growing population.<sup>10</sup> Research on using microalgae for wastewater treatment and resource recovery has since flourished, with the number of published articles increasing dramatically in recent years (see Figure 1).

Since at least the 1950s, researchers have exploited symbiotic interactions between photoautotrophic microalgae and aerobic, heterotrophic bacteria to treat wastewater.<sup>12</sup> The two microorganisms naturally support each other because microalgae photosynthetically produce oxygen and consume carbon dioxide while the bacteria consume organic molecules and oxygen and produce carbon dioxide,<sup>13</sup> as shown in Figure 2. Many researchers believe that because microalgae provide oxygen and accumulate biomass through photosynthesis, microalgal-bacterial processes will greatly reduce energy requirements and improve resource recovery compared to conventional wastewater treatment technologies (i.e., activated sludge), where intense aeration is required to provide the necessary oxygen and in a typical wastewater treatment plant may account for approximately 55% of the energy used by the facility.<sup>14</sup> Myriad subfields have emerged, including treatment process development, process modeling,<sup>15</sup> biomass valorization,<sup>16</sup> and more. Treatment approaches are generally divided into two categories (see Figure 3). Attached-growth processes use biofilms attached to surfaces to treat wastewater and include algal turf scrubbers,<sup>17</sup> rotating algal biofilms,<sup>18</sup> and rotating biological contactors.<sup>19</sup> Suspended-growth technologies cultivate microorganisms suspended in wastewater and include most photobioreactors,<sup>20</sup> high-rate algal ponds,<sup>21</sup> and microalgal-bacterial aggregates.<sup>22</sup> For a more extensive review of the research on using microalgae for wastewater treatment, see Sections 2 and 3 of the Supplemental Information.

Microalgal-bacterial aggregates have been called many different names, including “algal-bacterial flocs,”<sup>23</sup> “granular activated algae,”<sup>24</sup> “oxygenic photogranules,”<sup>25</sup> “microalgal-bacterial granular sludge,”<sup>26</sup> and many more (see Table SI.2 in the Supplemental Information). Gutzeit et al.<sup>22</sup> were among the first to use the term “aggregate” to refer to a suspended, microalgal-bacterial culture. In an article published in 2005, they promoted algae ponds improved by cultivating “stable algae-bacteria aggregates” that ranged in size from 400 to 800  $\mu\text{m}$  and demonstrated good nitrogen and phosphorus removal performance. However, microalgal-bacterial aggregates likely originated with Humenik and Hanna,<sup>23</sup> who in 1970 described a “continuous symbiotic algal-bacterial system” for nutrient removal from municipal wastewater that was capable of producing rapidly settling “algal-bacterial flocs.” The next year, Humenik and Hanna<sup>27</sup> mentioned that an “activated algae” (as opposed to activated sludge) process was under development and that the flocculation ability of the algae was identified as the key to successful operation. While Humenik and Hanna did not call their rapidly settling algal-bacterial flocs “granules” or “aggregates,” today they might describe them as such.

Building on the work of Gutzeit et al.,<sup>22</sup> many researchers have devoted significant resources to investigating microalgal-bacterial aggregates.<sup>24,25,28–47</sup> The primary motivation appears to be the prospect of eliminating external aeration from secondary wastewater treatment through photosynthetic oxygenation while maintaining treatment standards, thus saving energy and potentially reducing greenhouse gas emissions. Some researchers even claim that microalgal-bacterial aggregates can outperform conventional methods<sup>48</sup> and will revolutionize the field of wastewater treatment.<sup>49</sup> Another commonly cited motivation is the improved opportunity for resource recovery through the production of high-value products from microalgal-bacterial aggregates, which settle more quickly and are easier to harvest compared to standard microalgal cultures.<sup>39</sup> While much effort has been devoted to resource recovery and bench and pilot-scale treatment performance, there has been no critical evaluation of claims that the process can provide sufficient oxygen in a reasonable footprint for typical, full-scale secondary municipal wastewater treatment scenarios. This article evaluates the feasibility of microalgal photosynthesis and atmospheric diffusion for aeration-free, suspended-growth, secondary wastewater treatment and recommends research directions that are likely to yield real-world benefits.

## **2 Methods**

### *2.1 Oxygen Demand*

In conventional secondary wastewater treatment, oxygen is supplied to bacteria most commonly through fine bubble diffusers located on the bottom of the process tanks. Oxygen is typically consumed by bacteria through both heterotrophic consumption of organic matter (described here as bioavailable chemical oxygen demand, or bCOD) and autotrophic conversion of ammonia to nitrate, or nitrification.

Nitrification is important for reducing the aquatic toxicity of effluent discharged to receiving waters.<sup>14</sup> Treatment processes relying on microalgal-bacterial symbiosis primarily depend on bacteria to aerobically oxidize the influent organic matter, as shown in Figure 2, but here we assume that the influent nitrogen is removed through either biomass assimilation<sup>50</sup> or ammonia stripping.<sup>51</sup> Assuming the microalgae are strict photoautotrophs, approximately the same amount of oxygen will be required for a symbiotic microalgal-bacterial culture to remove the influent bCOD as would be required with conventional activated sludge. For a conventional activated sludge process operating at 20 °C with a design flow rate of 4.38 m<sup>3</sup> s<sup>-1</sup> (100×10<sup>6</sup> gal d<sup>-1</sup>) and an influent with 250 g bCOD m<sup>-3</sup> and 35 g N m<sup>-3</sup> (total Kjeldahl nitrogen), the required oxygen transfer rates for bCOD removal and nitrification are 59,089 kg O<sub>2</sub> d<sup>-1</sup> and 45,654 kg O<sub>2</sub> d<sup>-1</sup>, respectively. Detailed calculations are available in the Supplemental Information.

## 2.2 Photosynthetic Oxygenation

Photosynthesis is the process by which energy carried by electromagnetic radiation is captured by light-sensitive pigments (e.g., chlorophyll) and converted to chemical energy.<sup>52</sup> Specifically, photons derived from photosynthetically active radiation (PAR) are absorbed by pigments and the resulting captured energy is used to generate nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine triphosphate (ATP), both of which are used to synthesize biomass.<sup>53</sup> In oxygenic photosynthesis, the electrons used to reduce NADP<sup>+</sup> to NADPH are donated by water, resulting in the production of O<sub>2</sub>.<sup>54</sup> Theoretically, the absorption of eight PAR photons is sufficient to produce one molecule of oxygen, but experience has shown that approximately ten photons are required.<sup>54</sup> Thus, the photosynthetic oxygen production potential may be determined based on the amount of available PAR, which depends highly on the latitude, season, and climate.

One method is to use oxygen production rates based on biomass synthesis per unit of energy absorbed (e.g. 1.55 kg O<sub>2</sub> (kg algae)<sup>-1</sup> × 4.17×10<sup>-5</sup> kg algae (kJ total radiation)<sup>-1</sup> = 6.46×10<sup>-5</sup> kg O<sub>2</sub> (kJ total radiation)<sup>-1</sup>.<sup>55</sup> When using this method, it is important to consider which wavebands are included in the measurement of radiant energy, as solar radiation is comprised of more than just PAR (most often defined as the waveband between 400 and 700 nm, which happens to overlap with the visible region nearly perfectly).<sup>56</sup> At the outer edge of the Earth's atmosphere, solar radiant energy is comprised of approximately 5% ultraviolet (UV, below 400 nm), 28% visible, and 67% infrared (above 740 nm) radiation.<sup>53</sup> The ozone layer and water vapor in the atmosphere absorb most of the UV and much of the infrared radiation, respectively, which increases the fraction of radiation in the visible region at the Earth's surface to approximately 50%.<sup>53</sup> When using the energy flux approach, Oakley<sup>55</sup> recommends assuming that only 3% of the total incident solar radiant energy is utilized for photosynthesis.

While considering energy flux may be satisfactory for designing pond systems, photosynthetic oxygen production potential is more realistically described by the number of available photosynthetically active photons.<sup>56</sup> The most accurate way to accomplish this would be to estimate the incident photon flux density weighed by the photosynthetic action spectrum.<sup>56</sup> However, this approach is generally not used unless the additional technical and computational effort is worth the minor improvement compared to other methods.<sup>56</sup> Alternatively, a value known as the photosynthetic photon flux density (PPFD,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) describes the photosynthetic potential of incident solar radiation and is considered sufficient for most conditions.<sup>56</sup> One way to estimate the PPFD is to convert estimates of PAR irradiance ( $\text{W m}^{-2}$ ) using experimentally determined conversion factors.

For this analysis, we assume an average solar irradiance (global horizontal radiation) of  $251 \text{ W m}^{-2}$  based on data available from the National Renewable Energy Laboratory's National Solar Radiation Data Base<sup>57</sup> from 1998 to 2020 for Yuma, Arizona, in the United States. According to data curated by the World Meteorological Organization,<sup>58</sup> Yuma receives more sunlight than any other location in the world for which data are available, with over 4,000 hours of sunshine each year on average. Assuming that 45.8% of the total incident radiation is PAR (a conservative overestimate)<sup>59</sup> and a PPFD to PAR irradiance ratio of  $4.57 \mu\text{mol W}^{-1} \text{s}^{-1}$ ,<sup>56</sup> we estimate that  $525 \mu\text{mol PAR photons m}^{-2} \text{s}^{-1}$  are available on average for photosynthetic oxygen production. We assume that all PAR photons are absorbed by light-sensitive pigments and that 30% of absorbed photons are utilized for photosynthesis.<sup>59</sup> Thus, if 10 photons are required per molecule of oxygen, we estimate that the photosynthetic oxygen production potential of sunlight under these conditions is approximately  $0.044 \text{ kg O}_2 \text{ m}^{-2} \text{d}^{-1}$ . Applying the energy flux approach to this scenario yields a similar value of  $0.042 \text{ kg O}_2 \text{ m}^{-2} \text{d}^{-1}$ .

### *2.3 Artificial Lighting*

Instead of relying on sunlight, artificial lights (e.g., light-emitting diodes, or LEDs) can drive photosynthetic oxygen production. While this approach is typically not recommended for microalgae cultivation unless high-value products are produced,<sup>54,60</sup> in the context of wastewater treatment the cost and required footprint need only be reasonable compared to conventional external aeration. We assume that the average PAR efficiency for a compact LED is  $3.6 \mu\text{mol s}^{-1} \text{W}^{-1}$  and that photons are utilized with 100% efficiency.<sup>60</sup> Detailed calculations and methods are available in the Supplemental Information.

### *2.4 Atmospheric Diffusion*

Diffusion of oxygen from the atmosphere through the surface of the process volume must also be considered. We use the two-film theory of interphase gas-liquid mass transfer<sup>61,62</sup> in combination with mass transfer coefficients estimated at  $17 \text{ }^\circ\text{C}$  for rotating biological contactors (RBCs), a generous

assumption when considering a suspended-growth scenario without significant mechanical mixing.<sup>63</sup> The flux of a substrate from the gas phase to the liquid phase is given by Equation 1.

$$\frac{dC}{A \cdot dt} = K_L(C^* - C_L) \quad (1)$$

Where  $C$  ( $\text{g m}^{-3}$ ) is the concentration of the substrate (oxygen in this case),  $A$  ( $\text{m}^2$ ) is the area through which the substrate diffuses,  $K_L$  ( $\text{m s}^{-1}$ ) is the oxygen transfer coefficient,  $C^*$  is the equilibrium concentration in the liquid as given by Henry's law under standard conditions,<sup>64</sup> and  $C_L$  is the concentration in the bulk liquid.  $C^*$  is calculated as shown in Equation 2 and  $C_L$  is assumed to be  $0.5 \text{ g m}^{-3}$ .

$$C^* = \frac{P}{H} = 0.21 \times 101325 \text{ Pa} \times \left(1.2 \times 10^{-5} \frac{\text{mol } 32 \text{ g } O_2}{\text{m}^3 \text{ Pa mol}}\right)^{-1} = 8.17 \left[\frac{\text{g } O_2}{\text{m}^3}\right] \quad (2)$$

The values of  $K_L$  are assumed to be between the minimum and maximum values given by Bintanja et al.,<sup>63</sup> or  $5.6 \times 10^{-6} \text{ m s}^{-1}$  and  $43.3 \times 10^{-6} \text{ m s}^{-1}$ . Thus, Equation 1 predicts that the oxygen contributed from diffusion will be between  $3.7 \times 10^{-3} \text{ kg d}^{-1} \text{ m}^{-2}$  and  $2.9 \times 10^{-2} \text{ kg d}^{-1} \text{ m}^{-2}$ .

### 3 Results

For the full-scale wastewater treatment scenario described above, photosynthesis would require approximately 136 ha (335 acres), atmospheric diffusion and photosynthesis would require between 81.8 and 125 ha (202 and 309 acres), and conventional activated sludge with external aeration would require 2.2 ha (5.5 acres). Using artificial lighting to produce one kilogram of oxygen would require 24.1 kWh, which translates to 1.42 GWh per day for the full-scale scenario. Alternatively, external aeration for conventional activated sludge would require 0.50 kWh for each kilogram of oxygen or 0.052 GWh per day.

### 4 Discussion

The alluring promise of controlled photosynthesis that inspired wastewater treatment researchers in the late 1950s continues to exert its influence today, as demonstrated by the flood of publications in this area in recent years (see Figure 1). With many modern researchers devoting significant time and resources to harnessing microalgae for simultaneous wastewater treatment and resource recovery, it is important that efforts be productive and worthwhile. While the goals and motivations driving the development of microalgal-bacterial aggregates are noble, the approach is fundamentally flawed. Authors often claim that photosynthesis will greatly reduce or eliminate the need for external aeration in wastewater treatment, and



thus significantly reduce energy consumption compared to conventional practices. However, because photosynthesis and atmospheric diffusion require vast amounts of surface area to deliver sufficient oxygen, microalgal-bacterial aggregates and all other suspended-growth treatment approaches without external aeration are only practical if land use is not a consideration. For example, based on the oxygen production rates estimated above, a typical facility treating  $4.38 \text{ m}^3 \text{ s}^{-1}$  ( $100 \times 10^6 \text{ gal d}^{-1}$ ) would require between approximately 81.8 and 125 ha (202 and 309 acres) of suspended-growth, microalgal-bacterial bioreactors, or 37 to 56 times more surface area than conventional activated sludge, even with generous assumptions (including ignoring microalgal oxygen consumption<sup>65</sup> under lightless conditions). Conversely, assuming that the conventional activated sludge process produces photosynthetic oxygen at the maximal rate over its entire footprint, only 1.8% to 2.7% of the oxygen required for bCOD removal would be supplied by photosynthesis and passive diffusion from the atmosphere. Artificial lights could be used to drive photosynthesis in a more compact footprint, but even the most efficient LEDs would consume approximately 48 times more energy to provide the necessary oxygen than would be required by conventional activated sludge aerated with fine bubble diffusers.

The review of the literature performed for this study revealed three articles that quantify the photosynthetic oxygen production capacity of microalgae in microalgal-bacterial consortia.<sup>66–68</sup> Gikonyo et al.<sup>67</sup> used photosynthetic irradiance (PI) and rapid light curves (RLCs) to experimentally quantify net and gross photosynthetic activity, respectively, and estimated the “ideal” oxygen production potential per unit of biomass (measured as volatile suspended solids, or VSS) of “oxygenic photogranules” to be  $284.4 \text{ mg O}_2 (\text{g VSS})^{-1} \text{ h}^{-1}$ . They claim this rate is sufficient to treat most domestic wastewaters at “modest retention times,” but do not provide detailed calculations. In earlier research, Flores-Salgado et al.<sup>68</sup> estimated the maximum specific oxygen production rate of non-granular microalgal-bacterial consortia per unit biomass to be  $13.76 \pm 1.48 \text{ mg O}_2 (\text{g VSS})^{-1} \text{ h}^{-1}$ , which is nearly 21 times lower than the value found by Gikonyo et al.,<sup>67</sup> and claim that bacterial respiration required only 15% of the oxygen produced by microalgae. Holmes et al.<sup>66</sup> modeled the behavior of a simplistic algal-bacterial system and found that algae enhanced COD removal and provided approximately one-third of the bacterial oxygen demand. Assuming that aeration accounts for approximately 55% of the energy used at a wastewater treatment plant, the work of Flores-Salgado et al. and Holmes et al. suggests that oxygen produced by microalgae could reduce energy requirements for wastewater treatment by 55% and 18%, respectively.

The results reported by Gikonyo et al.,<sup>67</sup> Flores-Salgado et al.,<sup>68</sup> and Holmes et al.<sup>66</sup> appear to provide evidence of the practicality of photosynthetic aeration for wastewater treatment. However, to extrapolate their results to full-scale systems, one must assume that oxygen production rates measured in the laboratory and given in terms of mass of oxygen produced per unit of photosynthetic biomass per unit of time (e.g.,  $\text{mg O}_2 (\text{g VSS})^{-1} \text{ h}^{-1}$ ) adequately describe photosynthetic oxygen production within a full-scale,

suspended-growth wastewater treatment processes. This assumption is flawed for two reasons. First, it assumes that the biomass located in the lightless regions of a bioreactor will produce oxygen through photosynthesis, a physical impossibility. Even if the reactor is considered well-mixed, it is unreasonable to assume that all photosynthetically active microorganisms throughout the entirety of the bioreactor simultaneously receive photons and produce oxygen. Second, photosynthetic oxygen production utilizing sunlight is fundamentally limited by the number of photons incident on the Earth's surface. For example, if all available photons are already utilized for photosynthesis, then increasing the biomass concentration will not affect oxygen production. Thus, for suspended-growth treatment processes relying on photosynthesis and atmospheric diffusion, it is more appropriate to quantify oxygen production potential in terms of the required footprint. For example, Grobbelaar et al.<sup>69</sup> estimated the contributions of both atmospheric diffusion ( $7.3 \times 10^{-2}$  to  $1.5 \text{ kg } O_2 \text{ d}^{-1} \text{ m}^{-2}$ , depending on flow velocity) and photosynthesis ( $2.2 \times 10^{-2}$  to  $5.5 \times 10^{-2} \text{ kg } O_2 \text{ d}^{-1} \text{ m}^{-2}$ , depending on latitude) for high-rate algal ponds, yielding results that are similar to those presented here.

External aeration-free bioreactors that rely on photosynthesis and atmospheric diffusion for oxygen, including many of the microalgal-bacterial aggregate processes reported in the literature,<sup>24–26,28,29,34,42,43,49</sup> operate using nearly the same principles as facultative ponds. The only apparent difference is that the former is often considered well-mixed, while facultative ponds develop both aerobic and anaerobic zones. To ensure that photosynthetic oxygen production is not overwhelmed by the influent biological oxygen demand (BOD), facultative ponds are designed using equations that explicitly account for the available solar radiation.<sup>55</sup> Although it is likely that atmospheric diffusion of oxygen is more significant in well-mixed bioreactors than in ponds, our analysis more than adequately accounts for this contribution by assuming generous oxygen transfer coefficients observed in rotating biological contactors. Using the surface area estimated for the scenario accounting for both photosynthesis and atmospheric diffusion, the organic loading rate for the hypothetical microalgal-bacterial aggregate bioreactor is between 473 and 724  $\text{kg BOD d}^{-1} \text{ ha}^{-1}$ . These estimates are greater than the design organic loading rates for facultative ponds given by Mara et al.,<sup>70</sup> which range from 100 to 350  $\text{kg BOD d}^{-1} \text{ ha}^{-1}$  depending on the temperature, and are similar to the maximum BOD loading rate of 421  $\text{kg BOD d}^{-1} \text{ ha}^{-1}$  calculated using the energy flux method given by Oakley.<sup>55</sup> The differences are likely due to the generous assumptions used here regarding photosynthetic efficiency and atmospheric diffusion. Contrastingly, the equivalent value for conventional activated sludge is estimated to be 26,660  $\text{kg BOD d}^{-1} \text{ ha}^{-1}$ . It is unclear how the bioreactors utilizing microalgal-bacterial aggregates and other similar processes reported in the literature are different from well-mixed ponds given that they do not provide any form of external aeration and rely solely on photosynthesis and atmospheric diffusion for oxygen.

As shown above, photosynthesis and passive atmospheric diffusion are not practical sources of oxygen for suspended-growth wastewater treatment when space is limited. The morphology of the photosynthetic biomass (i.e., granules, aggregates, flocs, etc.) cannot overcome the fundamental, physical limits imposed by photosynthesis and gas-liquid mass transfer. However, while photosynthesis is limited by the PAR photon flux, and thus primarily by the footprint of the system, atmospheric diffusion is dependent only on the gas-liquid mass transfer rate and the amount of liquid surface area exposed to the atmosphere. Fortunately, attached-growth bioreactors can be designed to take advantage of atmospheric diffusion in ways that suspended-growth bioreactors cannot. For example, a recently developed bioreactor known as a rotating algal biofilm (RAB)<sup>18</sup> cultivates algae on long conveyer belts that are submerged in liquid before traversing vertical towers and descending back into the liquid on the other side. There are two characteristics of this system that address key limitations: 1) the vertical towers enable significant intensification of surface area exposed to the atmosphere relative to the footprint and 2) the liquid layer flowing vertically across the surface of the biofilm is thin and turbulent, which may increase the gas-liquid mass transfer rate of oxygen. Thus, researchers seeking to replace energy-intensive, conventional secondary wastewater treatment with a low-energy, microalgal-bacterial process should focus on attached-growth systems if space is limited. However, if land use is not a concern, MBAs and other suspended-growth wastewater treatment processes that rely on photosynthesis and atmospheric diffusion for oxygen may be appropriate if designed accordingly as well-mixed pond systems.

## **5 Supporting Information**

Methods for literature review, literature review of research on microalgal-bacterial consortia for wastewater treatment, specific review of literature on microalgal-bacterial aggregates, energy calculations, supplementary tables and a figure, Excel workbook with calculations.

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