



Spatial and Temporal Development of Microorganisms and the Effect of Clogging in Up-Flow Sand Filter

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Abstract. Up-flow sand filtration is an excellent alternative to point-of-use treatment for rural water supply. However, the sites and interval of microorganism growth in filter beds and the effect on bio-clogging remain unknown. This study aimed to assess microorganism growth based on biological activity levels using Dissolved Oxygen (DO) consumption and Field Emission Scanning Electron Microscopy (FE-SEM) imaging in various zones of the filter bed. Additionally, pressure drop was monitored to evaluate bio-clogging. The results showed that microorganism activities differed within the 0.50 m up-flow sand filter bed during 8 weeks of acclimatization. Exponential growth started after day 35, and DO levels declined to a minimum of 2 mg/L at 0.10 m bed height (measured from the bottom) after day 46. At 0.30 m and 0.50 m height, the DO decreased to 0.8 mg/L and 0.3 mg/L after days 35 and 46, respectively. FE-SEM images confirmed microorganism growth on samples from 0.1 m after 4 weeks of acclimatization. Substantial growth was seen on samples of 0.10 and 0.30 m height at 8 weeks, while lesser growth was observed on samples of 0.50 m. The pressure drop showed no significant increase, signifying that clogging did not occur during the 135-day operational period. In conclusion, the up-flow configuration prevented bio-clogging in sand filters, reducing maintenance requirements.

Keywords: Acclimatization; Clogging; Microorganism; Sand filter; Up-flow

1. Introduction

Up-flow sand filter is recognized as a highly promising alternative for water remediation, particularly for point-of-use treatment in rural settings. This is due to the ease of material procurement and simplicity of construction, operation, and maintenance (Lahin, Sarbatly, and Chel-Ken, 2021). Up-flow filtration has been used to remove turbidity, suspended solids, phosphorus, nitrogen, heavy metals, bacteria, and algae in multiple water treatment processes (Al-Saedi, Smettem, and Siddique, 2019; Heikal, Wagdy, and Eldidamony, 2017). In this method, feed water entering from the bottom of the filter and initially flowing through the gravel layers improves the treatment process by eliminating the susceptibility to clogging. Additionally, filter cleaning is conducted more rapidly,

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resulting in shorter downtime (Lahin, Sarbatly, and Chel-Ken, 2021).

Up-flow sand filter similar to a down-flow configured sand filter removes pollutants using physical and biological mechanisms. Approximately 70% of suspended particles and pathogenic contents in water are physically separated through straining and adsorption (Shreya *et al.*, 2023; Wu *et al.*, 2020; Polyakov *et al.*, 2019). However, with the aid of biological methods, such as predation, elimination, natural death or inactivation, and microorganism metabolism, a pathogen removal efficiency of up to 99% is achievable (Andreoli and Sabogal-Paz, 2020; Budhijanto *et al.*, 2015; CAWST, 2012). Microorganisms growing in sand filter beds play an essential role in water remediation by consuming pathogens and bacteria in the water passing through the filter beds (Duran-Romero *et al.*, 2020). The establishment of microorganisms in the filter bed is an essential step in commissioning sand filter operations (Bozorg, Gates, and Sen, 2015). Inoculation can either be performed by introducing an existing microorganism from an established sand filter or through inherent inoculation, where the sand filter is exposed to the intended feed water containing microorganisms and nutrients. The microorganisms will then be allowed to grow naturally over time (Ramsay, Breda, and Soborg, 2018). The latter method may require a more extended period but is most feasible for setting up sand filters in isolated areas. Typically, 6-8 weeks of acclimatization is required to establish a newly constructed sand filter, although some studies suggest that ripening might require close to 6 months (Cai *et al.*, 2016).

As microorganisms grow in sand filter beds, Dissolved Oxygen (DO) concentration decreases. This allows for monitoring of the growth of microorganisms using DO concentrations. Studies reported lower concentrations in areas where biological processes occurred in the beds, typically below the top surface of a down-flow sand filter. DO concentrations vary across sand bed levels, reflecting the biological activities (Andreoli and Sabogal-Paz, 2020; Ramsay, Breda, and Soborg, 2018; Young-Rojanschi and Madramootoo, 2015). Therefore, observation of low DO concentrations will signify the establishment of microorganisms as oxygen is consumed during biological treatment processes.

As time progresses, the biological layer build-up could cause a considerable yield reduction due to pore clogging (Mohamed *et al.*, 2023; Kurniawan *et al.*, 2022; Mutsvangwa and Matope, 2017). This necessitates subsequent filter cleaning, which disrupts the microorganism population and results in lower filtration efficiency and risk of pathogen breakthrough. Depending on filter size, the recovery process through ripening requires 4–8 weeks (De-Souza *et al.*, 2021; Saravanan and Gobinath, 2015). Bio-clogging occurs more in slow sand filters as microorganism growth is concentrated more on the top layer of the filter bed (schmutzdecke), which is also the site for straining and sedimentation (Donda Paranita, and Simatupang, 2024; Segismundo *et al.*, 2016; Wakelin *et al.*, 2010). However, bio-clogging is expected to be significantly reduced with up-flow configured sand filters that eliminate the schmutzdecke layer and use a deep filtration mechanism (Lahin, Sarbatly, and Chel-Ken, 2022, Zeng, Chen, *et al.* 2020).

The up-flow sand filter is expected to delay clogging but very little is known about the distribution of microorganism growth in the filter bed. This study aims to investigate microbial growth's spatial and temporal profiles at the initiation of the up-flow sand filter and subsequently monitor the clogging effects over time. The experiments were divided into two sections, including monitoring microorganism growth post-commissioning of the up-flow sand filter and examining the clogging effect during the pilot up-flow sand filter operation. The microorganism growth was assessed by monitoring DO concentration and physical microorganism growth on sand particles using Field Emission Scanning Electron

Microscopy (FE-SEM). Furthermore, the clogging effect was monitored through pressure drop variation throughout the pilot up-flow sand filter operation.

2. Methods

2.1. Sand Filter Bed

The up-flow sand filter bed was constructed using 0.10 mm D10 sand media and ranged in size from < 0.075 –2 mm. Sand media was obtained from the Tamparuli River, Sabah, Malaysia, and prepared according to the sand filter manual (CAWST, 2012). Two layers of gravel support were used, each 0.05 m thick. The effective size of each gravel support was 0.49 and 2.10 mm, respectively. The support section comprised a series of small layers that were evenly distributed and compacted to promote uniform water circulation during operation. Subsequently, when the support was even, sand media was poured in small quantities before being leveled and distributed across the filter surface. This process was repeated until sand bed height reached 0.40 m.

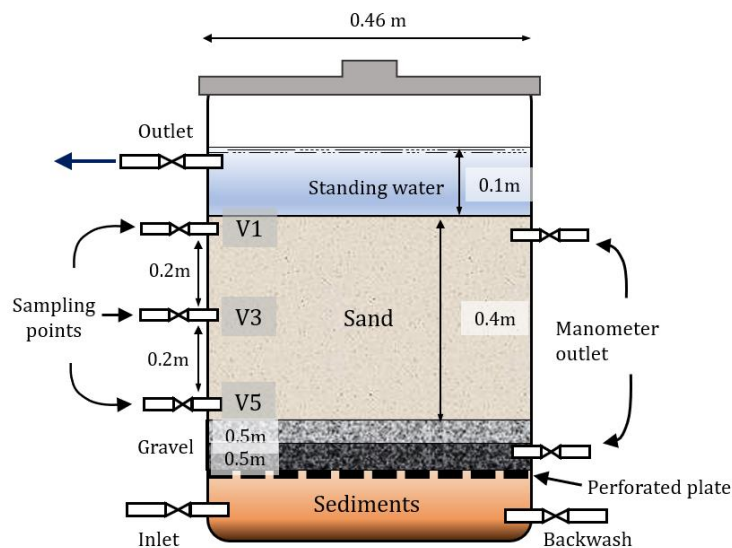


Figure 1 Up-flow Sand Filter Design

The up-flow sand filter was activated by allowing the bed to fluidize for 30 minutes before being left to resettle. Fluidization allowed sand particles to stratify based on size. Larger and denser particles settled at the bottom, while lighter and smaller grains rose to the top of the sand bed.

The filter was monitored for 8 weeks after commissioning to allow for media maturation. During this period, untreated feed water was circulated in the filter for 8 hours daily at 0.072 m/h surface velocity. The sand bed was then submerged in water while the sand filter was not operational to ensure the survival of the developed microorganisms.

2.2. The Pilot Up-flow Sand Filter Setup

The pilot plant used to monitor filter clogging was based on the design described by Lahin, Sarbatly, and Chel-Ken (2022). During the pilot up-flow sand filter operation, three surface velocities were used, 0.072, 0.1805, and 0.4813 m/h, denoted by Q1, Q2, and Q3, respectively. The total monitoring period was 135 days.

2.3. Feed Water

The feed water used during the study contained natural pollutants found in surface water. The purpose was to promote microorganism growth organically by introducing

polluted feed water. Lahin, Sarbatly, and Chel-Ken (2022) provided details on the feed water content.

2.4. DO Concentration

DO concentration in the up-flow sand filter bed was measured as a reference for microorganism growth. It was documented using sampling valves installed at 0.20 m intervals across the filter. The concentration was assessed onsite with a HI98193 Hanna Portable DO Meter. DO sampling was conducted in the morning, 1 hour after the daily start-up and operation of the filter.

2.5. Physical Microorganism Growth

Microorganism growths on sand grains were examined using FE-SEM. Samples from different filter bed heights were carefully washed with distilled water and air-dried without being fixed, dehydrated, or frozen. These samples were mounted on a metal stub with carbon conductive paint before platinum coating was sputtered on the sample with a JEOL JEC-3000FC Auto Fine Coater. FE-SEM images were then captured at a 5 kV accelerating voltage using JEOL JSM-7900F FE-SEM.

2.6. Pressure Drop Monitoring

Pressure drops across the filter bed was measured using manometer tubes connected to manometer outlets installed lateral to the up-flow sand filter column (Figure 1). The bottom manometer channel was located at the base of the sand filter bed, while the top indicator was 0.05 m from the surface. The height differences between the two manometers were documented after the readings stabilized.

3. Results and Discussion

3.1. Microorganism Activity

DO concentration across the filter bed was monitored at 3 – 4 day intervals to assess the microbial activity. The microorganisms were established during the 8-week observation period following the activation of the up-flow sand filter. Filter bed depth was divided into three sections, lower (0.10 m from filter bottom), middle (0.30 m from filter bottom), and upper part (0.50 m from filter bottom), denoted by V5, V3, and V1, respectively (Figure 1). The influent and effluent water DO concentrations were also recorded as a reference, with labels In and Effl., respectively.

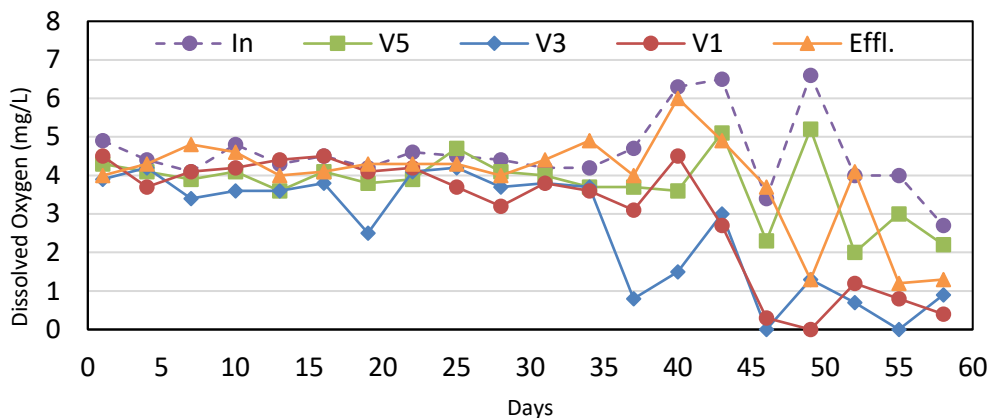


Figure 2 DO Concentration across Filter Bed at 8 Weeks during Acclimatization Period

Figure 2 shows DO concentration over the 8-week acclimatization period. Initial observations showed a varying decline in concentration between sand bed sections. An average of 8.5%, 17.48%, and 4.02% reduction was observed in V5, V3, and V1,

respectively. This pattern persisted until day 34 when the reduction changed significantly. From day 35, a further reduction in DO concentration was observed in V5, with an average decrease of 27.2% and a concentration of 3.4 mg/L compared to 4.0 mg/L during the first 34 days. Subsequently, the value dropped from day 46 to 58, falling in the 2 – 3.3 mg/L range. In V3, DO concentration dramatically decreased to 0.8 mg/L after day 35, remaining below 1 mg/L until after the commissioning. The lowest V3 reading was 0 mg/L on days 46 and 55. V1 decreased to 0.3 mg/L on day 46 and was maintained at a lower concentration until day 58.

Microbial population growth started immediately after commissioning, as observed by DO consumption in the filter bed. Based on the general microbial growth curve, the lag phase lasted until day 34, when a minimal increase in DO consumption was observed. The exponential phase began on day 35, marked by a rapid reduction in DO concentration. Meanwhile, the stationary phase began around days 46 to 49, when DO concentration reduction appeared to stabilize. This phase continued until the end of the experiment. Table 1 summarizes the average oxygen consumption observations during non-rapid (1-34 days) and rapid (35 – 58 days) DO reduction.

Table 1 The Average DO Concentration in Different Sand Filter Sections \pm Standard Deviation

| Time (Day) | Average DO Concentration (mg/L) \pm S.dev | | | | |
|------------|---|-----------------|-----------------|-----------------|-----------------|
| | In | V5 | V3 | V1 | Out |
| 1 - 34 | 4.43 \pm 0.24 | 4.05 \pm 0.27 | 3.71 \pm 0.45 | 4.04 \pm 0.38 | 4.28 \pm 0.24 |
| 35 - 58 | 4.71 \pm 1.35 | 3.42 \pm 1.11 | 1.32 \pm 1.19 | 1.84 \pm 1.56 | 3.49 \pm 1.69 |

The observed oxygen consumption patterns suggested that the microbial population was primarily concentrated in the middle layer of the sand bed, supported by the lowest level of DO in V3. Since the water flowed from the bottom, microbes and nutrients were introduced to the lowest layer of the filter first. The larger pores in the bottom layer allowed most pollutants and particulate matter to stream through before entering the sand bed. Additionally, rapid growth at the bottom section was also hindered by the elevated shearing caused by the increased flow rate and pressure in this section. Straining occurred in the deeper layer of the filter due to smaller pore sizes, resulting in the retention of most microorganisms and attachment taking place in the deeper section of the filter bed (V3). This led to a larger population of microorganisms. Lower nutrients and DO availability in the top layer of the filter prevented the rapid growth of microorganisms at this site.

3.2. Physical Growth of Microorganisms

FE-SEM images (Figure 3 – 5) show that the physical growth of microorganisms on the sand surface started from the bottom and progressively moved upwards. Figure 4 shows that at week 4, growth was observed in V5, with less growth in V3, and no significant growth in V1. The images from week 8 show significant microorganism growth in V5 and V3. Meanwhile, in V1, a more negligible growth was observed, consistent with DO consumption results.

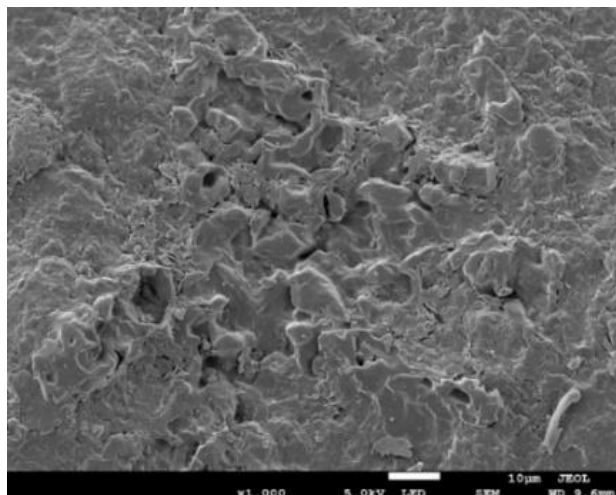
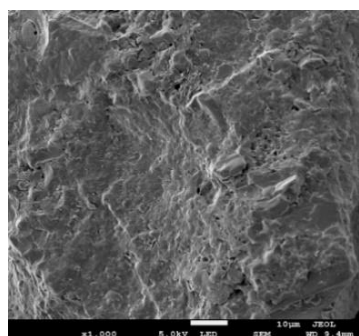
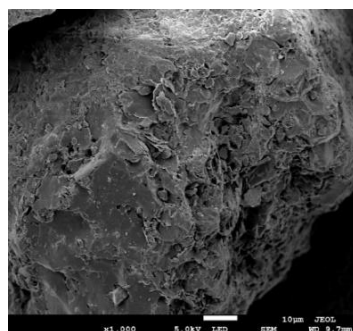


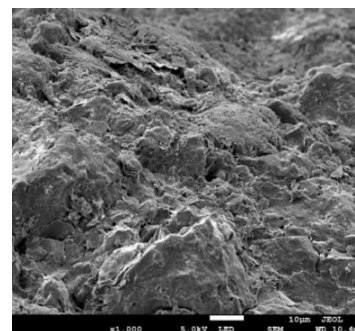
Figure 3 FE-SEM Images of Sand Media at Start-up



V1

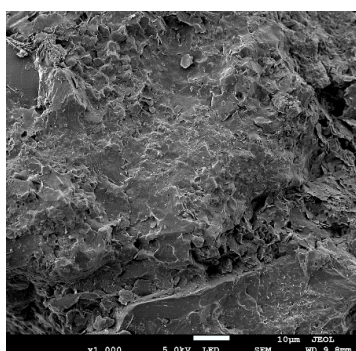


V3

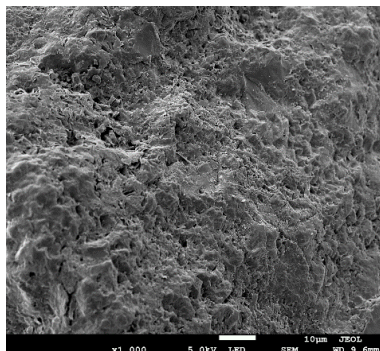


V5

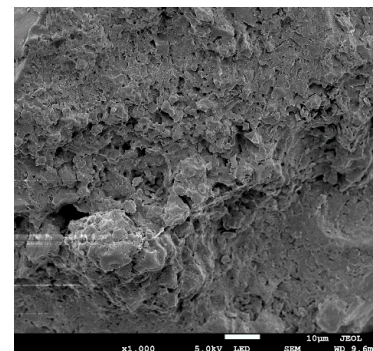
Figure 4 FE-SEM Images of Sand Media from Different Layers at Week 4



V1



V3



V5

Figure 5 FE-SEM Images of Sand Media from Different Layers at Week 8

Based on DO consumption and microorganism growth images, this study found that the microorganisms in the up-flow sand filter were established 7–8 weeks post-commissioning. The sand filter bed was established simply by inoculating the up-flow sand filter with natural microorganisms and exposing it to the intended feed water for acclimatization. However, other studies found that using the same inoculation technique might require over 90 days to achieve stable microorganism growth in down-flow granulated media filters ([Duran Romero *et al.*, 2020](#); [Gibert *et al.*, 2013](#)). The results also show that the up-flow sand filter required a shorter acclimatization period due to the up-flow configuration and sand media stratification ([Salkar and Tembhurkar, 2016](#)).

Aside from flow configuration, the type of filter media could influence the structure of the microorganism community in granular filters. For instance, [Wakelin et al. \(2010\)](#) reported no significant variation between bacteria and archaea compositions at different depths for anthracite and Granulated Activated Carbon (GAC). Meanwhile, sand media exhibited a high level of bacterial richness at 0.40 m deep in the filter bed. In another study, nitrifiers were identified in the zone of the filter bed prone to clogging, confirming that heterotrophs were the primary contributors to organic removal and biofilm development ([Freitas et al., 2021](#); [Bassin et al., 2012](#)).

This study provided good insight into spatial and temporal information on microorganism growth in up-flow sand filters but lacked a detailed analysis of the morphological diversity of the microorganisms. The complexity of microorganism species in filter beds is a crucial indicator for sand filter performance as different types of microorganisms, such as heterotrophs, nitrifiers, and oxidizers, play different roles in pollutant removal ([Chan et al., 2018](#)).

3.3. Clogging

Post-acclimatization of the up-flow sand filter, a pilot system was used to monitor the clogging effect for 135 days through pressure drop measurements. Figure 6 shows that a higher pressure drop was recorded at increased surface velocity. In the operation, the pressure drop ranged from 0.08 to 0.20 kPa in Q1, 0.23–0.45 kPa in Q2, and 0.61–1.05 kPa in Q3. During Q3, the pressure drop increased initially due to an algae bloom.

Following backwashing, the pressure drops stabilized at 0.61–0.76 kPa. Although slight increments of pressure drops were observed in Q3 compared to Q1 and Q2, the increase was insignificant, with R2 of 0.476, 0.0339, and 0.4273, respectively. This shows that the length of the operational period did not considerably affect the pressure drop of the up-flow sand filter bed.

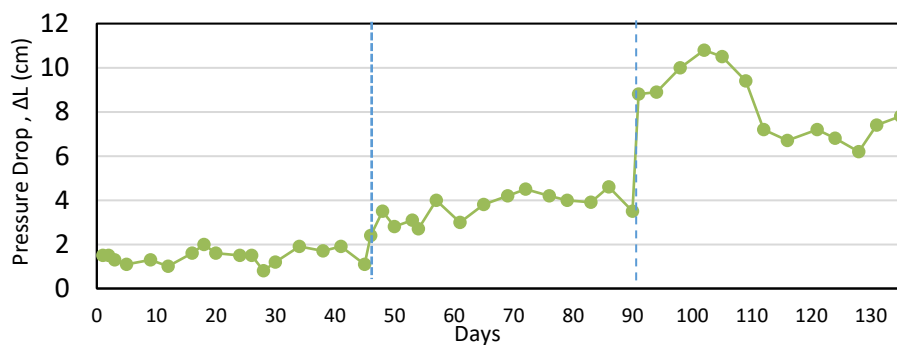


Figure 6 Pressure Drop for 135 Days of Operation in Varied Flowrate

Various factors contribute to clogging in sand filters. These include sand particle and pore size, particulate concentration, and nutrient contents in the feed water ([Al-Saedi, Smettem, and Siddique, 2019](#)). In addition, microorganism growth and death rate affect biomass accumulation ([Wang et al., 2023](#); [Abukhanafer et al., 2021](#)) in the filter bed, other than the time elapsed. These factors influence the amount of pore size reduction caused by accumulated attached biomass and strained particles in the sand filter. Clogging primarily occurs in the first few centimeters of downflow configured sand filters ([Chen et al., 2021](#); [Altmann et al., 2016](#)), where sand particles are typically smaller, and the schmutzdecke layer develops. The treatment mechanisms in downflow sand filters also rely heavily on the top layer, where most of the straining and biological treatment occurs ([Chen et al., 2021](#)).

Based on the results, the direction of water flow and sand grain stratification were the most essential factors in reducing clogging in the up-flow sand filter. In addition to the

distribution of microorganisms in the filter bed, the water flow corresponded with the stratification of sand filter grains, which ranged from coarse to fine, enabling deeper penetration and distribution of the particles. The results were similar to studies by [Suryawan *et al.* \(2021\)](#) and [Altmann *et al.* \(2016\)](#). Furthermore, the support gravel at the bottom caused some particles to become trapped before entering the sand filter bed. The up-flow sand filter in this study was designed with a buffer zone at the bottom to promote sedimentation of larger particles before the water entered the support gravel layer, acting as a particulate pre-removal mechanism.

4. Conclusions

In conclusion, after 8 weeks of monitoring post-commissioning of the up-flow sand filter, microorganism growth started at the bottom and progressed upwards into the deeper sites of the filter bed. FE-SEM imaging confirmed that consistent DO levels below 1 mg/L resulted in exponential levels of microorganism activities at 0.3 m from the base of the filter. Furthermore, the development of microorganisms deeper in the filter bed was influenced by the increased availability of nutrients and oxygen due to the flow direction of the feed water. Higher shearing at the bottom of the filter bed and insufficient nutrients and oxygen supplies at the top layer prevented microorganism growth. This study successfully showed that up-flow sand filters prevented clogging through the pre-removal of higher-density sediments by its bottom buffer zone, pre-filtration by layers of gravel support, and the distribution of filtration sites deeper in the bed. Although clogging was not significant during the 135 days of monitoring, the effects in the up-flow sand filter over a long-term period could be further explored.

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