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Hybrid disinfection of sewage effluent—A comparative study of three secondary treatment plants of Jaipur, India

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ABSTRACT

Chlorination is one of the most widely used methods to disinfect wastewater, despite innumerable objections raised due to the resultant by-products. Any attempt to reduce the dosage of chlorine can be very useful in lowering the concentration of the disinfection by-products, many of which have been reported to be carcinogenic. This study examines the microbiological characterization of secondary treated sewage from three different sewage treatment plants based on different unit processes, namely activated sludge process, moving bed biofilm reactor and rotating biological contractor. The study also assesses the efficacy of using chlorine on major coliform species in order to achieve the objective of bringing the effluent to the desired standard for total coliform count (TCC) of 1,000 per 100 mL. The results indicate that 5 parts per million (ppm) chlorine dose (CD) was able to attain the TCC standard, if the counts for *Serratia/Hafnia* species were ignored. These species offered high resistance to chlorination due to which excessive overall doses were required to confirm to TCC standard. The chlorinated samples were further subjected to ultraviolet (UV-C) disinfection, the results of which can be employed to design a hybrid disinfection strategy with chlorination at a relatively low CD as the first step for removing bulk of the coliform population, followed by another process to which *Serratia/Hafnia* are susceptible. This can not only reduce the CD and thereby the by-products of chlorination but also bring down the overall cost of disinfection.

Keywords: Activated sludge process (ASP); Chlorination; Hybrid disinfection; Moving bed biofilm reactor (MBBR); Rotating biological contactor (RBC); Total coliform count (TCC)

1. Introduction

The volume of sewage effluent is increasing and safe disposal can often prove to be difficult due to the growth in population and urbanization [1,2]. The use of reclaimed wastewater for irrigation and other purposes is the obvious solution to this problem. However, several pioneering studies have provided the technological confidence for the safe reuse of treated effluent [3]. Primary treatment is essential to remove suspended solids and secondary treatment is designed to substantially degrade the soluble organic

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matter. Secondary treatment followed by the disinfection of effluents has been increasingly practised across the globe for water reclamation [4]. A majority of municipal wastewater treatment plants treat the sewage using aerobic biological processes, commonly classified as fixed-film and suspended-growth [5] systems. Fixed-film or attached growth system includes trickling filter and rotating biological contactors where the biomass grows on media and the sewage passes over its surface. In suspended-growth systems, such as ASP, there is continuous mixing of the microorganisms and wastewater in a well aerated tank [6,7].

Untreated wastewater contains a variety of excreted organisms, including pathogens. Pathogens are rarely measured directly in wastewater because their concentration varies and analytical procedures are often difficult or expensive to perform [8]. Instead, indicators of faecal contamination, such as *E. coli* or thermo tolerant coliforms, have been used as proxies for pathogens with similar properties as may be present in wastewater [9,10]. Usually, but not always, their presence in water is proportionately related to the amount of faecal contamination present [11].

Conventional primary and secondary treatment processes have been observed to reduce the concentration of enteric pathogens in raw sewage by 90–99% [12,13], which is inadequate for its reuse because of the presence of pathogens in high number. Therefore, disinfection is mandatory for the removal of these pathogens in order to prevent the spread of waterborne diseases among downstream users and into the environment [14]. The Ministry for Environment (India) constituted a committee in 1999 which recommended the desirable limit of faecal coliform at 1,000 most probable number (MPN) per 100 ml and a maximum permissible limit at 10,000 MPN/100 ml for discharge of effluent into a water body or reuse for agriculture, aquaculture or forestry applications, as also recommended by the United States Environment Protection Agency (USEPA) [5,15]. The three most common methods of disinfection are chlorination, ozonation and UV irradiation [16]. The effectiveness of disinfection depends on the quality of the water being treated (factors that include cloudiness, pH), the type of disinfection process, the dosage of the disinfectant and contact time, besides other environmental factors [17].

Chlorination is the most widely used method for disinfecting sewage due to its low cost and a reasonably good record against the majority of microrganisms [18,19]. It is a powerful oxidizing agent and has been used as an effective disinfectant for about a century. Chlorination can help to destroy microbes by disrupting metabolism and protein synthesis or by modifying purine and pyrimidine bases [20]. The process of chlorination involves bubbling of chlorine gas or dissolving of chlorine compounds and their subsequent dosing. Chlorine in any form hydrolyses in the presence of water and forms hypochlorous acid (HOCl) as follows [9,10]:

In the case of calcium hypochlorite:

$$Ca(OCl)_2 + 2H_2O = Ca^{++} + 2OH^- + 2HOCl \quad (1)$$

A recent study indicated that a dose of 17.5 ppm in the form of calcium hypochlorite was required to bring the total coliform count (TCC) to less than 1,000 per 100 mL [15] for secondary treated sewage from sewage treatment plants (STPs) Delawas, Jaipur [21]. Based on a microbiological analysis of the effluent samples, six genera of bacteria, namely *Escherichia*, *Klebsiella*, *Enterobacter*, *Serratia/Hafnia* and *Citrobacter* were found to be dominant of the total coliform population [21]. A detailed analysis of the effect of chlorine on these individual species indicated that the counts of *Escherichia*, *Klebsiella* and *Citrobacter* could be brought down to below 1,000 per 100 ml with only 7.5 ppm of chlorine; *Enterobacter* offered some resistance at a chlorine dose (CD) of 10 ppm. However, for *Serratia/Hafnia*, which was more resistant to chlorination, a much higher dose of 17.5 ppm was required to meet the TCC norms. This brought forth the importance of a hybrid disinfection strategy to avoid high doses of chlorine by adopting a serial step of another disinfectant that had the potential to remove chlorine resistant species in order to optimize the overall disinfection process [22].

There were published reports [23,24] from laboratory tests of synergistic benefits for using two or more disinfectants in drinking water treatment which gives the bases that the overall inactivation of microrganisms is greater than the sum of the inactivation achieved for each disinfectant individually [25,26] but only few studies are available in case of wastewater treatment. A combination of ozonation followed by chlorination was considered an effective way to reduce trihalomethanes (THMs) and other halogenated disinfection by-products (DBPs) [27,28] and [12] reported that the sequential combination of free chlorination followed by monochloramination produced superior inactivation power compared to the sum of both disinfectants examined separately. Similar synergies have been seen for ozone and chloamines. Some other synergistic study includes combinations such as UV/O_3, O_3/H_2O_2 [27]. The combined performance of UV light followed by chlorine during disinfection of reclaimed water was experimentally assessed by some authors [29,30].

Sequential disinfection was proposed, to eliminate the inactivation lag phase [28]. Thus, a substitutive disinfectant technology that can supplement the insufficient Ct value rate and simultaneously optimize the removal of microorganisms during the conventional treatment processes must be used [31]. Such technology has a low risk of DBPs while having a strong enough Ct value to inactivate the microorganism. It was reported that in a sequential disinfection scheme, a strong primary disinfectant is first applied to achieve a portion of the target inactivation level followed by the secondary disinfectant to attain further inactivation and to provide residual disinfection for water distribution [31,32]. But in this study, a reverse sequence was adopted using a different combination of disinfectant i.e. chlorine followed by UV light as in the case of wastewater, presence of any residual was not compulsory so UV or ozone could be used as a secondary disinfectant. A combination of disinfectants such as chlorine and UV-C light was known to lead to greater inactivation when the disinfectants are added in a series rather than individual [26]. Novelty of this study was to use a hybrid disinfection technology for disinfection purpose of secondary treated wastewater of three STPs. The hybrid technique focuses on the removal of major coliform species by chlorination and then targeting chlorine resistant species by UV radiation. This study also compares the microflora present in the effluent of three STPs, evaluates the effect of chlorination as a single technology and as hybrid technology with UV light on total coliform removal.

2. Materials and methods

2.1. Effluent sample collection

A detailed microbial analysis was carried out before and after disinfection in batch process of the samples taken from three STPs based on different technologies. Secondary treated effluent was collected from three different STPs of 62.5 million litres per Day (MLD), Jaipur (North), India, based on the conventional activated sludge process (ASP); 0.2 MLD at Malaviya National Institute of Technology (MNIT), Jaipur, using RBC; and 1 MLD at Jawahar Circle, Jaipur, India, using MBBR.

Secondary treated effluent samples were collected from the outlet of the secondary clarifier of three different STPs for the evaluation of TCC as well as the counts of different dominant coliform species. Care was taken to obtain a sample that truly represented existing conditions in such a way that it did not deteriorate or become contaminated. Samples were collected in air-tight bottles and transported to the Environmental Laboratory of MNIT Jaipur, in an icebox, where a detailed experimental analysis was carried out within four hours of collection. The characteristics of secondary treated effluent samples obtained from three STP's are shown in Table 1.

2.2. Determination of bacterial counts

In secondary treated effluent of STPs, large number of species was present; so instead of conducing biochemical test, only simple morphological study was carried out for sensitive and resistant species against chlorination. In this study also, six bacterial genera *Escherichia*, *Klebsiella*, *Enterobacter*, *Serratia/Hafnia* and *Citrobacter* were considered as they were the most dominant gram-negative coliform population in the initial screening. Spread plate technique was used for obtaining the counts of individual species through tenfold serial dilution of the original sample and dispersing it on their selective media plates [33]. A 250 μl of diluted sample was transferred to the centre of an agar plate and spread evenly over the surface with a sterile bent glass rod or spreader [34]. For this study, EMB agar media (eosin methylene blue agar) and XLD agar media (xylose lysine deoxycholate agar) were used. XLD agar was used for the identification and enumeration of *Citrobacter*, while EMB agar was used for gram-negative coliforms such as *E. coli*, *Serratia/Hafnia*, *Enterobacter* and *Klebsiella*. Diluted samples were put in duplicate to confirm the reproducibility [5]. Petri plates were incubated at 37˚C and observed between 24 and 48 h as described by Buck and Cleverdon [34].

Colonies were counted with the help of a microprocessor colony counter (Labtronics), and the number of bacteria in the original samples was calculated. Colony morphology was used for the classification of these species after obtaining segregated colonies in the range of 30 to 300 cells per plate [6]. The TCC was obtained by adding all these individual counts. Colilert 18 based on IDEXX's patented defined substrate technology was also used for obtaining the TCCs as this represents an acceptable international procedure [35]. Hundred millilitres of volume of the diluted sample was poured into a bottle and the dehydrated powdered reagent was added to it, as prescribed in the manual. After the powder was dissolved, the sample containing the medium was dispensed into a Quanti-Tray avoiding air bubbles. The tray was then heat-sealed by the help of colilert 18 instrument [36]. Following incubation at 37˚C for 18–22 h and the number of yellow wells (small and large) were counted. The MPN counts of total coliform bacteria were then read from the MPN Table supplied with the instrument.

Table 1
Qualitative analysis of the secondary treated effluent of three STP's

Parameter	ASP secondary treated effluent	MBBR secondary treated effluent	RBC secondary treated effluent
BOD	18–20 mg/l	20–30 mg/l	25–32 mg/l
COD	250–300 mg/l	120–140 mg/l	105–110 mg/l
pH	7.2–7.9	7.4–7.9	7.5–7.8
Turbidity	50–52 NTU	38–40 NTU	41–45 NTU
TSS	30–35 mg/l	25–28 mg/l	28–30 mg/l

2.3. Chlorination

Chlorination was carried out using calcium hypochlorite solution in a batch process to study the effect of contact time and applied dose on coliform counts [5]. CDs of 2.5 and 5 ppm were added to the samples and the contents were mixed thoroughly using a magnetic stirrer (Remi) for 20 min [16]. Subsequently, dechlorination of the samples was carried out by 10% sodium thiosulphate solution to freeze the further disinfection action of residuals before putting them to the bacteriological analysis [21].

2.4. UV disinfection unit

A closed vessel vertical UV reactor unit having eight-watt UV lamp enclosed in quartz tube was used for disinfection. For the batch process of disinfection, 800 ml sewage sample was passed through the 8-watt UV unit using a peristaltic pump (160 rpm) for a contact time of 94 s to find out the disinfection efficiency on the microbiological population of the chlorinated effluent.

3. Results and discussion

The following sections show the quantitative analysis of major coliform species present in secondary treated sewage and the efficiency of chlorine as a disinfectant on samples obtained from different STPs based on suspended growth (ASP) combined growth process (MBBR) and fixed film (RBC).

3.1. Quantitative analysis of coliforms in secondary treated effluent of the three STPs

The primary treated sewage from ASP, MBBR and RBC had a TCC of 191×10^7, 198×10^6, 176×10^6, respectively. Secondary treated effluent from Delawas ASP contained 176×10^4 TCC per 100 ml. In the ASP effluent among the six species observed, the most dominant species were *Enterobacter* and *Klebsiella*, *Citrobacter* and *Serratia/Hafnia* were the dominant species in effluent of MBBR, Jawahar Circle, which contained 168×10^4 TCC per 100 ml. In effluent of RBC (MNIT), TCC was 316×10^4 per 100 ml and *Enterobacter* and *Serratia/Hafnia* were the dominant species. The TCC values obtained by the Colilert method were comparable to the sum of the six species monitored in the samples through spread plate method and hence they represented the dominant coliform groups in the effluents despite having a different relative presence. Fig. 1 presents the population distribution of different dominant coliform bacteria isolated from the three STPs.

It was evident that though the decrease observed in the microbial load after secondary treatment was substantial, being more than 90%, the presence of a large number of coliforms after the secondary treatment was still a matter of concern as the samples do not conform to the norms of reuse for irrigation set by the World Health Organization [8].

3.2. Effect of chlorine disinfection on different effluents

Disinfection was carried out at 2.5 ppm of CD in a batch process for 20 min of contact time after which the minimum and maximum counts among different coliform species were observed for *E. coli* and *Serratia/Hafnia*, respectively. *Serratia/Hafnia* seemed to be the most resistant among the above species against

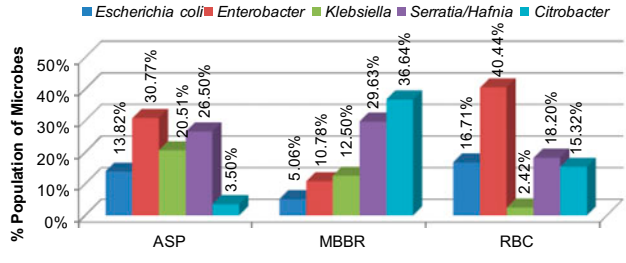

Fig. 1. Bacterial distributions in the isolates from secondary treated effluent of three STPs. Results are presented as the average of twelve independent experiments (each experiment contained samples in duplicates).

chlorination. At 5 ppm of CD, all the coliforms were reduced to zero except for *Serratia/Hafnia*, which still maintained counts of more than 1,000 per 100 mL required as per USEPA standards for disposal in streams [5,15]. The different bacterial counts obtained before and after disinfection at pre-determined CDs in secondary treated effluent of 3 STPs are shown in Figs. 2a–2c.

From Figs. 2a–2c, it was clear that *Serratia/Hafnia* was the most resistant species against chlorination due to which high doses of chlorine were required for bringing the TCC within the standard. Excessive dose of chlorine was not only expensive but it could also give rise to a series of DBPs such as THMs and haloacetic acids (HAAs), many of which were proven carcinogens [26]. Therefore, an attempt was made to design a hybrid disinfection strategy where a combination of CD (2.5 ppm) for 20 min and UV (8 watt) for 94 s was employed for disinfection of secondary treated sewage of three STP's of Jaipur, namely ASP, MBBR and RBC.

The results of this hybrid treatment for effluents of three STPs are shown in Figs. 3a–3c which evidently support our hypothesis that hybrid disinfection was more effective for the reduction of TCC than the single disinfection method. This may be due to the fact that chlorine disinfection was based on its reaction with the cell constituents and *Serratia/Hafnia* had higher lipid content than other coliform species. In addition, the Lipopolysaccharide (LPS) layer was attached to the outer membrane of these gram-negative bacteria which makes them difficult to obliterate, thus possibly resulting in the consumption of a high amount of chlorine. It was also reported that *Serratia/Hafnia* is resistant to many antimicrobial agents due to certain characteristics such as its ability to survive in aerobic and anaerobic conditions (unique membrane), and its motility, as it had 100–1,000 flagella per swimmer cell and also secretes acylated homoserine lactones (AHL's) which are involved in swarming motility [26,37]. From Figs. 3a–3c, it can be concluded that though the *Serratia/Hafnia* counts were lower or

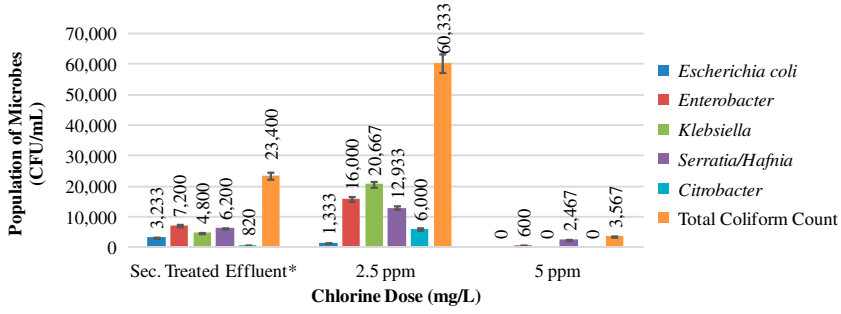

Fig. 2a. Effect of chlorination on secondary treated effluent of ASP at 2.5 and 5 ppm. Results are presented as the average of twelve independent experiments (each experiment contained samples in duplicates), and error bars represent the standard error.
Note: The secondary treated effluent values are in thousands.

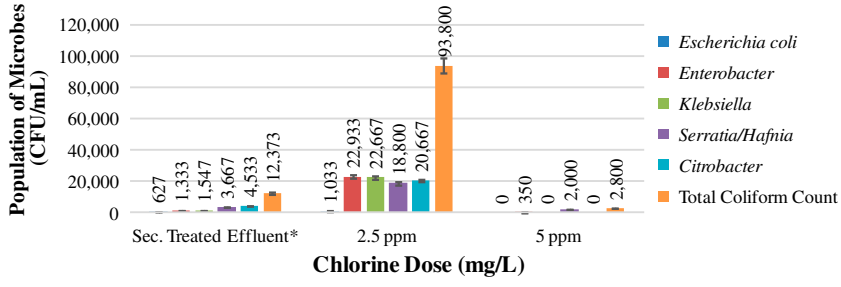

Fig. 2b. Effect of chlorination on secondary treated effluent of MBBR at 2.5 and 5 ppm. Results are presented as the average of twelve independent experiments (each experiment contained samples in duplicates), and error bars represent the standard error.
Note: The secondary treated effluent values are in thousands.

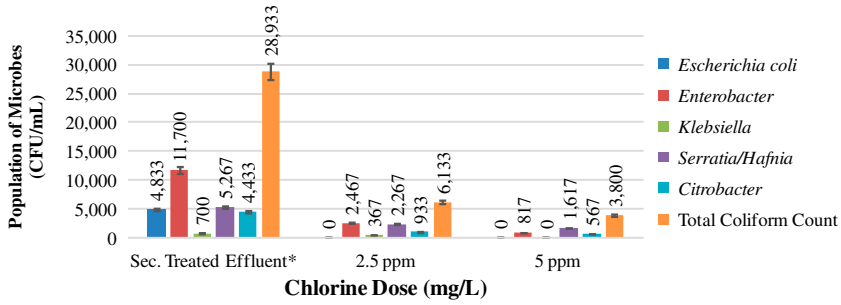

Fig. 2c. Effect of chlorination on secondary treated effluent of RBC at 2.5 and 5 ppm. Results are presented as the average of twelve independent experiments (each experiment contained samples in duplicates), and error bars represent the standard error.
Note: The secondary treated effluent values are in thousands.

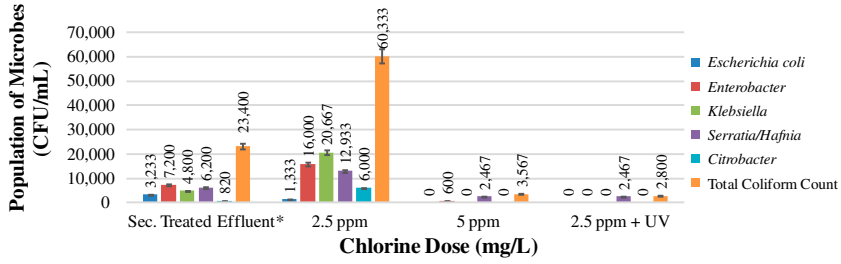

Fig. 3a. Hybrid disinfection of secondary treated effluent of ASP using 2.5, 5 and 2.5 ppm for 20 min plus UV for 94 s. Results are presented as the average of twelve independent experiments (each experiment contained samples in duplicates), and error bars represent the standard error
Note: The secondary treated effluent values are in thousands.

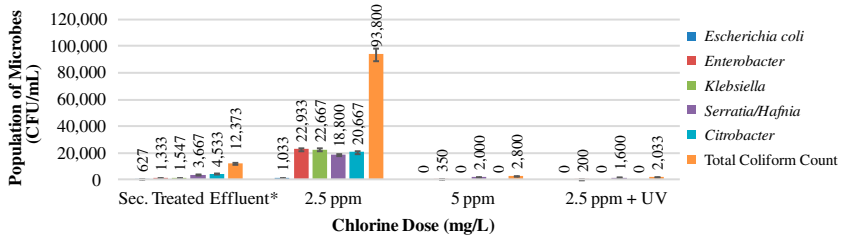

Fig. 3b. Hybrid disinfection of secondary treated effluent of MBBR using 2.5, 5 and 2.5 ppm for 20 min plus UV for 94 s. Results are presented as the average of twelve independent experiments (each experiment contained samples in duplicates), and error bars represent the standard error.
Note: The secondary treated effluent values are in thousands.

equivalent in the ASP effluent as compared to those of MBBR or RBC, its removal efficiency due to equivalent doses of chlorination was low, which may be attributed to much higher TSS and BOD in ASP effluent compared to that in the other two processes as the conventional ASP has lower treatment efficiency. The residual organics are thus apparently consuming a large amount of chlorine and may further give rise to THMs and HAAs [38].

On the contrary, UV disinfection was based on the deactivation of DNA and hence the susceptibility of these species against UV radiation was expected to be high, this was also supported by the results of Sobsey et al. [12]. Thus, designing a hybrid system can not only help to optimize the overall cost of disinfection, but it can also result in significantly lower doses of chlorine and, consequently, reduction in DBPs associated with the chlorination process [22].

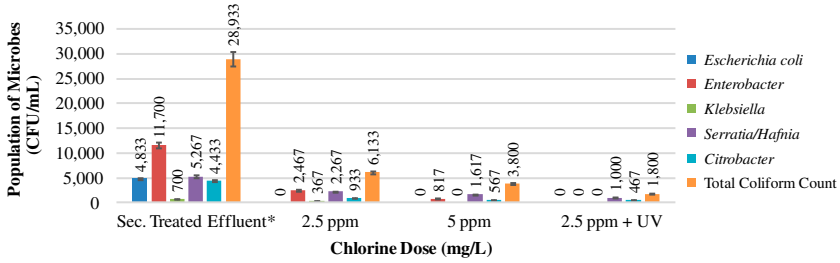

Fig. 3c. Hybrid disinfection of secondary treated effluent of RBC using 2.5, 5 and 2.5 ppm for 20 min plus UV for 94 s. Results are presented as the average of twelve independent experiments (each experiment contained samples in duplicates), and error bars represent the standard error.
Note: The secondary Treated Effluent values are in thousands.

4. Conclusion

The variation in microbiological dominance in different secondary treated STP effluents showed that the ASP had an abundance principally of *Enterobacter* and *Klebsiella*; the MBBR effluent had chiefly *Serratia/Hafnia* and *Citrobacter*; and the effluent from RBC had more of *Enterobacter* and *Serratia/Hafnia*. The effect of chlorination on individual coliform species was found to be widely different with *Serratia/Hafnia* being the most resistant species, which governs the overall CD for achieving the desired TCC.

The new hybrid disinfection strategy with chlorination followed by UV light (2.5 ppm + UV) resulted in effective removal of most of the species (when compared to the TCC for 5 ppm) and can bring down the overall cost of disinfection process. So we can conclude that the combination of these two disinfectant agents was effective in protecting public health, as each agent acts to a different degree against the different microbial species studied. Reduction in CD resulted as a consequence of hybrid process can bring down the DBPs substantially in tertiary treated sewage and, hence, can go a long way in mitigating serious environmental consequences associated with the current practices of chlorination of sewage. Detailed study on DBPs and cost analysis will be included in the sequential paper of this research.

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