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Application of phycoremediation technology in the treatment of ETP water – An assessment with *Spirulina platensis* (Nordst.) Gomont

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Abstract

Nature suffers from various types of pollution, most of them in water bodies. This adversely affects organisms throughout the food chain and negatively affects the sustainability of the ecosystem. There exist natural resolutions to defeat these issues. Algae are aquatic organisms that can be used for wastewater treatment because of their capacity of absorbing nutrients. *Spirulina platensis* (Nordst.) Gomont. is a microscopic, filamentous cyanobacterium widely used for its biosorbent property and high nutritional value. This study aimed at the cultivation of *Spirulina platensis* in different levels of effluent treatment plant (ETP) water and suggested ETP water as a medium for *Spirulina platensis* culture over a chemically defined medium. The study also aimed to assess the biotransformation of pollutants from sewage water by analyzing the parameters such as pH, COD, BOD, suspended solids, oil and grease, chlorides, sulphates, fluorides, nitrates, ammoniacal nitrogen, and phosphate. The growth pattern of *Spirulina platensis* was studied in different concentrations of ETP water and Zarrouk's medium. The growth was estimated through the measurement of cell concentration using a hemocytometer, determination of turbidity using a spectrophotometer, and specific growth rate evaluation. The results obtained reveal that *Spirulina platensis* has appreciable nutrient scavenging properties and lower concentrations of PT-ETP water and higher concentration of FT-ETP water can be utilized for culturing *Spirulina platensis.* ETP water can be utilized as a nutrient medium for *Spirulina platensis* in controlled concentrations.

Key words: *Spirulina platensis* (Nordst.) Gomont.; phycoremediation; ETP water; nutrient medium; growth estimation.

Introduction

The modern-day world is struggling with numerous ecological and environmental problems among which water pollution is of greater concern that can cause an imbalance in the environment. Water pollution depletes the aquatic ecosystem and causes unfettered phytoplankton expansion in lakes, food chain contamination and other serious issues thus adversely affecting sustainability.

Microalgae or microphytes are a diverse group of prokaryotic and eukaryotic photosynthetic organisms that are inconspicuous to the naked eye. Those are phytoplankton that exists singly or in chains or groups. Microalgae which are capable of photosynthesis, produce about half of the oxygen in the atmosphere, and at the same time, they grow phototrophically by using the greenhouse gas carbon dioxide. Microalgae are particularly attractive for biotreatment because of their ability to photosynthesize, transforming solar energy into useful biomass, and absorbing nutrients like nitrogen and phosphorus, which causes eutrophication in aquatic systems.

Microalgae have commercial uses because they produce a variety of bioproducts that are useful to humans in the production of food, feed, medicine, cosmetics, fertilizers and tools for wastewater treatment. These bioproducts include polysaccharides, lipids, pigments, proteins, vitamins and bioactive compounds. A number of studies have shown that these microalgal chemicals have positive effects on human health, including anti-cancer, anti-inflammatory, antioxidant, antibacterial and anti-obesity properties.

The use of microalgae for wastewater treatment has recently attracted increased interest due to the effective nutrient removal abilities of such microorganisms, and the additional benefits of biofuel production (Cheah *et al.*, 2016). Transesterification of lipids in microalgae allows for the manufacture of biofuels such as biodiesel, bioethanol, biohydrogen, and biomethanol among others. In terms of absorbing solar energy, microalgae are more effective than traditional oleaginous plants. Algae also serve as an effective feedstock. Additionally, microalgae have the capability to bio-sequester carbon dioxide, produce a notable amount of biomass and cellular lipids and have fast growth rates. They can also filter pollutants out of wastewater and make biofuels (Mondal *et al.*, 2017).

Cultivation of microalgae with the phycoremediation technique can provide cost-effective and long-term solutions to these issues. Phycoremediation is the process of application of micro and macro algae to reduce or bio transform contaminants from wastewater, such as nutrients and hazardous compounds (Olguin, 2003; Mulbry *et al.*, 2008). There are several studies that display the possibility of wastewater treatment using different microalgae species. Phycoremediation is a suitable method to eliminate heavy metals, organic substances, phosphates, nitrates, pesticides, andhydrocarbons (Kaloudas *et al.*, 2021). Although several algal species are used for wastewater treatment, species of the genera *Chlorella, Chlamydomonas* and *Scenedesmus* are extensively used (Priyadharshini *et al.*, 2021).

'*Spirulina*' is the commercial name attributed to the biomass of the blue-green algae, *Spirulina platensis* (Nordst.) Gomont.(*Arthrospira platensis*) *S.platensis* is a microscopic, trichomatous cyanobacterium that can be utilized by humans and discrete animals (Rippka *et al.*, 1979). They are characterized by circular, multicellular trichomes which are open left-handed helices that gained their name from this feature. *Spirulina* is known as a superfood because of its richness in iron, magnesium, vitamins minerals and also its 60–70% protein content. As an eco-friendly tool, *Spirulina* is being investigated for food security, malnutrition, and dietary support in long-term spacecraft or Mars missions. The cultivation and production of *Spirulina* are mostly commercial. But *Spirulina* possessed an appreciable capacity for growth, as a means for nutritional intensification, attaining necessities of life, and pollution moderation. One of the main barriers to the small-scale culture of *Spirulina* is the cost and availability of inorganic nutrients.

Materials and method

Test organism and culture conditions

Pure culture of the test organism, *Spirulina platensis* (Nordst.) Gomont. was purchased from Microalgal Culture Lab, Central Marine Fisheries Research Institute (CMFRI), Kochi, Kerala. The culture was obtained in Zarrouk's medium (1966) and is grown in the culture lab for optimization for one month. The culture in Zarrouk's Medium was selected as control.

All glass vessels were sterilized by washing with liquid detergent and kept in a hot air oven (100°C) for 30 min. Conical flasks for maintaining culture are washed with detergent and autoclaved (121°C) for 15 min. Tap water was collected, filtered, and sterilized by boiling it for 20 min in sterile glass tumblers. The sterilized water is then cooled to room temperature and used for medium preparation.

Inoculation was accomplished in a laminar air flow chamber. The cultures were maintained in the culture room at 27°C under alkaline conditions (pH 10) with the illumination of 3000 lux from two cool white fluorescent lamps programmed for 24 h/day. The cultures were shaken manually to give 3–5 rotations every now and then for aeration and to keep them in uniform suspension and to prevent sticking of the cyanobacteria to the wall of the container.

The volume of the medium was maintained at less than half of the culture vessel volume in order to provide an adequate air supply and eliminate contamination due to slashing during shaking.

Effluent treatment plant water

Effluent Treatment Plant (ETP) water was collected from Modern Hospital, Kodungallur, Thrissur, Kerala. Two types of ETP water samples – partially treated water and fully treated water samples – were taken and used for growing the test organism. ETP water samples were analyzed for their physicochemical parameters before and after treatment with *S. platensis*. After the treatment with *S. platensis* for 20 days, the samples were filtered through a GFC filter paper using Millipore apparatus and analyzed for physicochemical parameters. The parameters analyzed are pH, suspended solids, oil and grease, COD, BOD, chloride, sulfate, fluoride, nitrate, ammoniacal nitrogen, and phosphate.

Experimental design

The experiment was set up in two types of ETP water samples – partially treated water and fully treated water samples. Different concentrations of experimental growth media are prepared by mixing ETP water and Zarrouk's medium (Table 1). 50 ml pure *S. platensis* culture was inoculated into each prepared culture medium. Pure *S. platensis* in Zarrouk's medium was taken as control. All samples were prepared in triplicates and average values were taken. The cultures were maintained in the culture room at 27°C under alkaline conditions (pH 10) with the illumination of 3000 lux from two cool white fluorescent lamps programmed for 24 h/day. Growth was analyzed on 5, 10, 15, and 20th days after treatment.

Estimation of growth

Spirulina platensis growth under different concentrations of ETP water was analyzed by the estimation of the cell concentration, turbidity, and specific growth rate in 5 days intervals for 20 days.

Table 1: Composition of sample (volume of Zarrouk's medium and ETP water).

Name of Sample	Composition of Sample (Zarrouk's medium + ETP water)
Control	100% Zarrouk's medium
P20	20% Partially treated ETP water + 80% Zarrouk's medium
P40	40% Partially treated ETP water + 60% Zarrouk's medium
P60	60% Partially treated ETP water + 40% Zarrouk's medium
P80	80% Partially treated ETP water + 20% Zarrouk's medium
P100	100% Partially treated ETP water
F20	20% Fully treated ETP water + 80% Zarrouk's medium
F40	40% Fully treated ETP water + 60% Zarrouk's medium
F60	60% Fully treated ETP water + 40% Zarrouk's medium
F80	80% Fully treated ETP water + 20% Zarrouk's medium
F100	100% Fully treated ETP water

(1) Measurement of cell concentration: For the quantitative estimation of growth, 1 ml of culture was taken after thorough mixing and transferred onto a calibrated hemocytometer. The cells were counted and represented as cells/ml.

(2) Determination of turbidity: The samples were collected aseptically and the optical density of cell suspension (Turbidity) was measured using a double-beam UV-VIS spectrophotometer (Systronics) at 750 nm.

(3) Determination of specific growth rate: The specific growth rate was calculated by using the formula of Guillard (1973).

$$\text{Specific growth rate } (\mu) = \frac{\ln N_t - \ln N_0}{t_t - t_0}.$$

Here, N_0 and N_t are the values of absorbance at 750 nm during the exponential phase at time t_0 and time t_t respectively.

Results and discussion

The present study is focused on the evaluation of the growth rate of *Spirulina platensis* in different

Table 2: Growth estimation of *Spirulina platensis* in PT-ETP water.

	Cell concentration – Cells/ml (10*4)						Turbidity at 750 nm						Specific growth rate					
Days	**C**	**P20**	**P40**	**P60**	**P80**	**P100**	**C**	**P20**	**P40**	**P60**	**P80**	**P100**	**C**	**P20**	**P40**	**P60**	**P80**	**P100**
1	22	17	15	10	5	3	0.39	0.27	0.3	0.25	0.21	0.1	0	0	0	0	0	0
5	23	20	22	13	9	5	0.43	0.3	0.3	0.28	0.25	0.12	0.008	0.009	0.005	0.009	0.015	0.015
10	27	22	24	15	12	7	0.47	0.35	0.4	0.33	0.3	0.14	0.007	0.013	0.007	0.014	0.015	0.013
15	35	38	33	18	15	8	0.51	0.42	0.4	0.38	0.32	0.17	0.007	0.015	0.006	0.012	0.005	0.016
20	55	44	37	33	29	12	0.55	0.49	0.5	0.42	0.38	0.21	0.006	0.013	0.012	0.008	0.014	0.018

Table 3: Growth estimation of *Spirulina platensis* in FT-ETP water.

	Cell concentration – Cells/ml (10*4)						Turbidity at 750 nm						Specific growth rate					
Days	**C**	**P20**	**P40**	**P60**	**P80**	**P100**	**C**	**P20**	**P40**	**P60**	**P80**	**P100**	**C**	**P20**	**P40**	**P60**	**P80**	**P100**
1	16	13	11	12	4	6	0.23	0.21	0.21	0.21	0.21	0.22	0	0	0	0	0	0
5	21	16	14	19	7	10	0.26	0.24	0.24	0.25	0.26	0.27	0.01	0.011	0.011	0.015	0.018	0.017
10	30	28	21	24	22	32	0.32	0.29	0.28	0.31	0.32	0.35	0.018	0.016	0.013	0.018	0.018	0.022
15	39	38	36	32	38	58	0.41	0.35	0.35	0.4	0.43	0.48	0.021	0.016	0.019	0.022	0.025	0.027
20	58	42	40	39	54	69	0.59	0.43	0.46	0.54	0.56	0.68	0.031	0.017	0.023	0.026	0.022	0.03

volumes of ETP water to suggest it as the growth medium and to assess the bioremediation potential of the organism. Observations were made on the growth rate by the determination of cell concentration, turbidity, and specific growth rate in 5 days intervals for 20 days.

Effect of partially treated ETP water

Control culture showed an exponential phase from the 1st day to the 20th day and showed a peak value of growth on the 20th day of culture. The cell concentration and turbidity increased gradually with a uniform rate of specific growth (Table 2). In PT-ETP treatment, a gradual decline of growth was observed in higher concentrations of PT-ETP water samples. The maximum concentration of *S. platensis* was observed in the lower ETP-water concentrations viz. P20 and P40. Cell concentration decreases in higher concentrations of PT-ETP water, viz. P60, P80, and P100. The control culture exhibited maximum growth with respect to cell concentration and turbidity. The specific growth rate of *Spirulina platensis* in PT-ETP water was observed as random values along the different days' intervals from the 1st to 20th day of culture. It manifests a higher level of growth rate in the lower concentrations and a lower growth rate athigher concentrations of PT-ETP water samples.

Effect of fully treated ETP water

In fully treated ETP water treatment, there occurred a gradual increase in the growth of *Spirulina platensis* in higher volumes of ETP water (Table 3). Maximum growth was displayed in higher concentrations (F60, F80, and F100) of FT-ETP water as compared to the lower concentrations (control, F20). A moderate expansion of growth of *Spirulina* was observed in higher volumes of fully treated ETP water from the 1st day to the 20th day.

In FT-ETP treatment, maximum cell concentration was noticed in the higher concentrations mainly in the F100 culture. The cell counts of F20 and F40 appear to be lesser as compared to the cell count of higher concentration FT-ETP samples. There was a gradual increase in cell concentration on each day of the assessment from the 1st to the 20th day. The control culture showed a slightly higher rate of cell count as compared to that of other low-concentration cultures.

Low-concentration cultures exhibited a gradual increase in from 1st to the 20th day of culture in turbidity. The control had a higher rate of turbidity when compared to that of low concentrations. Specific growth rate showed the growth rate of *Spirulina platensis* in each interval. It was observed as random values in each interval as the rate of growth was different in each interval.

Physico-chemical analysis of ETP water

The laboratory analysis of partially treated ETP water and fully treated ETP water before and after the treatment of *Spirulina platensis* (Nordst.) Gomont. has manifested the capacity of microalgae to remove several nutrients from sewage water.

In PT-ETP, the water parameters have changed considerably after the treatment of microalgae. The pH becomes increased and the suspended solid contents, oil, and grease value were reduced with the microalgal treatment. The chemical oxygen demand (COD) value of PT-ETP was 100 mg/l before the treatment which lowered to 54 mg/l after the treatment. The biological oxygen demand (BOD) was minimized to 7.3 mg/l from 12.67 mg/l. Besides, several water nutrients such as chlorides, sulfates, fluorides, nitrates, ammoniacal nitrogen, and phosphates have been reduced in a 20 days laboratory culture of *Spirulina platensis* (Table 4).

The test result of FT-ETP water showed a similar pattern to results obtained from PT-ETP water. ie., the water parameters such as COD, BOD, suspended solid contents, oil, and grease, chlorides, sulfates, fluorides, nitrates, ammoniacal nitrogen, and phosphates were reduced when treated with *Spirulina platensis* (Table 5).

Phycoremediation is a promising treatment to remove or recycle the contamination in wastewater which was completely environment friendly and sustainable for the future generation. The increased population and the urbanized society bring on the cumulation of sewage dumping to the environment. Microalgal phycoremediation was adopted as a mechanism to effectively purify the wastewater and generate O_2 during the process. The findings of the present study evidently point out that sewage water was a worthwhile medium for culturing *Spirulina platensis*. Moreover, it revealed the power of microalgae to absorb nutrients like carbon, nitrogen, sulfur, phosphorous, etc., and followed the elimination of pollutants from the wastewater.

Table 4: Physico-chemical analysis of PT- ETP water (before & after algal treatment).

S. No.	Parameters	Unit	Results (Before treatment)	Results (After treatment)	Percentage of reduction (%)
1.	pH@ 25°C		7.48	7.65	+2.27
2.	Suspended solids	Mg/l	15	11	–26.66
3.	Oil and grease	Mg/l	1.64	1.2	–26.82
4.	COD	Mg/l	100	54	–46
5.	BOD for 3 days at 27°C	Mg/l	12.67	7.3	–42.38
6.	Chloride as Cl^-	Mg/l	739.13	712.21	–3.64
7.	Sulphate as SO_4	Mg/l	30.81	30.63	–0.58
8.	Flourideas F	Mg/l	1.42	1.33	–6.33
9.	Nitrate as NO_3	Mg/l	8.05	3.32	–58.75
10.	Ammoniacal nitrogen	Mg/l	30.09	20.01	–33.49
11.	Phosphate as PO_4	Mg/l	8.48	3.05	–64.03

Table 5: Physico-chemical analysis of FT-ETP water (before & after algal treatment).

S. No.	Parameters test	Unit	Results (Before treatment)	Results (After treatment)	Percentage of reduction (%)
1.	pH@ 25°C		7.63	7.7	+0.91
2.	Suspended solids	Mg/l	10	8	–20
3.	Oil and grease	Mg/l	1.02	0.98	–3.92
4.	COD	Mg/l	70	32	–54.28
5.	BOD for 3 days at 27°C	Mg/l	7.33	5.35	–27.01
6.	Chloride as Cl^-	Mg/l	714.5	707.2	–1.02
7.	Sulphate as SO_4	Mg/l	35.86	34.54	–3.68
8.	Flourideas F	Mg/l	1.33	1.31	–1.5
9.	Nitrate as NO_3	Mg/l	6.99	2.32	–66.8
10.	Ammoniacal nitrogen	Mg/l	20.62	12.71	–38.36
11.	Phosphate as PO_4	Mg/l	5.53	3.63	–34.35

Mahapatra *et al.* (2013) evaluated algal efficiency in sewage treatment plants of 67.65 million liters per day. They assessed the growth of microalgae based on several criteria such as cell count, cell shape, cell volume, pack volume, cell weight, cell density, and cell index. The parameters such as DO, pH, electrical conductivity, and turbidity were calculated in the scene and samples were examined for the parameters like salinity, COD, BOD, nitrites, nitrate, and ammonia. The result was evident for the remediation of nutrients by the physicochemical analysis. Pathak *et al.* (2014) studied the phycoremediation of textile effluent by utilizing the microalgae *Chlorella pyrenoidosa*. To show the ability of algae to refine the wastewater, two 15-day batch experiments using autoclaved and un-autoclaved textile wastewater were conducted and that brought about the remediation of pollutant load in both autoclaved and unautoclaved water which causes eutrophication. Jais *et al.* (2017) examined the ability of microalgae to phycoremediate the wastewater from fresh edibles and meat shops comprising intense levels of nutrients, BOD, COD, TSS, and the subsequent production of biomass.

Rawat *et al.* (2011) discussed the dual behavior of microalgae in the phycoremediation process using residential wastewater and biomass productivity for sustainable biofuel production. This research examined the microalgal biomass generation methods employing wastewater streams and wastewater treatment in high-rate algal ponds (HRAP) and also discussed the manufacture of biodiesel using the transesterification process of lipids and additional biofuels like biomethane and bioethanol. Tang *et al.* (2011) aimed to display the phycoremediation capacity of *Chlorella minutissima* on primary and tertiary treated effluent for the deletion of minerals and biodiesel synthesis. They analyzed the chemical and physical parameters of polluted water such as nitrate, phosphate, EC, TDS, BOD, COD, etc., and found out that *Chlorella minutissima* can be utilized for the decontamination of contaminant load as well as used as a powerful source for biodiesel production.

The present work analyses the growth rate of *Spirulina platensis* under varying concentrations of ETP water. Cell count, turbidity, and specific growth rate were analyzed to assess the growth of algae. Higher concentrations of FT-ETP water and lower concentrations of PT-ETP water have shown growth greater than the control. Dry cell concentration, protein content estimation, lipid profiling, and nutrient analysis can be further performed for assessing the microalgal growth for future studies.

This study depicted the effect of algae to remove pollutants from sewage water and suggested ETP water as a growth medium for *Spirulina platensis*. The physicochemical analysis of sewage water before and after the treatment of microalgae showed a considerable reduction of nutrients in the test results. The 20 days experiment showed an increased amount of *Spirulina* in the cultures of low concentrations of partially treated ETP water and higher concentrations of fully treated ETP water. The physicochemical analysis showed a reduction value of pollutants in the sewage water after the treatment of *Spirulina platensis*.

The pH of the water increased to 2.27% in PT-ETP water and 0.91% in FT-ETP water. The microalgae reduce dissolved CO_2 concentrations through photosynthesis which, in turn, raises the pH level. Other parameters such as suspended solids showed a 26.6% reduction in PT-ETP and a 20% reduction in FT-ETP water. The reduction in suspended solids offers an efficient method to reduce the solid wastes in ETP and other related waste treatment plants. Sludge formation is a key feature of waste treatment plants. Reduction in suspended solids points out the chance of utilizing microalgae for the removal of sludge. Effective waste treatment with minimal sludge can be achieved along the mass culture of *S.platensis* and subsequent treatment. Further studies are required for confirmation.

The COD of water diminished to 46% in PT-ETP and 54.28% in FT-ETP water. The BOD of water diminished to 42.38% in PT-ETP and 27.01% in FT-ETP water. COD (Chemical Oxygen Demand) and BOD (Biological Oxygen Demand), are the two main characteristic attributes that designate the strength of sewage water. Both parameters are used to test the oxygen-demanding strength of aquatic systems. A similar study conducted by Kotteswari *et al.* (2012) using the blue-green algae *Nostoc* sp. to treat the dairy effluent compared the microalgal growth by analyzing the physicochemical parameters using standard methods. Along with COD and BOD, the dairy effluents parameters such as total dissolved solids (TDS) TSS, pH, alkalinity, and phosphate were manifested to be reduced and showed not only the capacity of *Nostoc sp.* to minimize the levels of TDS, TSS, BOD, and COD but also the potential of microalgae to remediate pollutants from several sites. In the present study also, all such parameters showed a reduction in both PT-ETP and FT-ETP water after *Spirulina platensis* treatment.

Nitrate, ammoniacal nitrogen, and phosphate are other important parameters analyzed to assess the nutrient absorption capacity of *S.platensis.* All these are crucial parameters in wastewater treatment as they have a significant role when discharged into the environment. Nitrogen exists in different forms in the environment and is absorbed by various organisms in different forms. Phosphate is also an important parameter for the flourishing of microalgae. The results of the study have shown that the three parameters have reduced considerably with the algal treatment. There was a reduction of 66.8, 38.36 and 34.35% in the nitrate, ammoniacal nitrogen, and phosphate respectively in 100% fully-treated ETP water.

A growth higher than that of control was observed with 100% fully-treated ETP, which indicates that FT-ETP can be used as a growth medium for *S.platensis.* Thus phycoremediation can be done with *S.platensis* and utilized to remove the excess nitrogen and phosphate. Similar results are reported by Sharma *et al.* (2021) who analyzed the phycoremediation property of microalgae *Chlorella minutissima* as biofertilizer for the production of crops. They tested the wastewater parameters such as biological oxygen demand (BOD), dissolved oxygen (DO), chemical oxygen demand (COD), electrical conductivity (EC), potassium (K), phosphorous (P), and nitrate (N), and found to be diminished when treated with the microalgae and calculated the rate of plant growth, yield, and chemical character of soil by the introduction of algal biomass into the plots of spinach and baby corn.

Water contamination by inorganic and organic pollutants is a serious issue in the current scenario. The contaminants are not easy to destroy biologically but can be transformed from highly toxic to low-toxic forms (Jiang *et al.*, 2018). The present study is in concurrence with the previously stated studies and *S.platensis* can be considered as a potential organism for phycoremediation of ETP water. The percentage of reduction of the nutrients in ETP water after *S.platensis* treatment suggests the utilization of ETP water as a low-cost medium for biomass production.

Conclusion

The present study depicted the growth of *Spirulina platensis* (Nordst.) Gomont. in different volumes of ETP water to suggest it as the growth medium. The study also aimed to suggest *S.platensis* as a tool for phycoremediation.

Monoculture of *S.platensis* was inoculated in Zarrouk's medium as control and varying concentrations of fully-treated and partially-treated ETP water were used as test samples. Observations were made on the growth rate by the determination of cell concentration, turbidity, and specific growth rate in 5 days intervals for 20 days. The laboratory analysis results of physicochemical analysis of partially treated ETP water and fully treated ETP water before and after the treatment of *S. platensis* (Nordst.) Gomont. has manifested the capacity of microalgae to remove several nutrients from sewage water. The results showed the effect of algae to remove pollutants from the sewage water and suggested ETP water as a growth medium for *S. platensis.* The study needs to be conducted *in vivo* for accomplishing its usage in open systems. Dry cell concentration, protein content estimation, lipid profiling, nutrient analysis, etc can be further performed for assessing the growth of microalgae for future studies.

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