International Journal of Earth, Energy and Environmental Sciences
ISSN: 2517-942X
Vol:12, No:6, 2018

Different Formula of Mixed Bacteria as a Bio-Treatment for Sewage Wastewater

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***Abstract*—**This study aims to investigate the ability of different formula of mixed bacteria as a biological treatments of wastewater after primary treatment as a bio-treatment and bio-removal and bio-adsorbent of different heavy metals in natural circumstances. The wastewater was collected from Sarpium forest site-Ismailia Governorate, Egypt. These treatments were mixture of free cells and mixture of immobilized cells of different bacteria. These different formulas of mixed bacteria were prepared under Lab. condition. The obtained data indicated that, as a result of wastewater bio-treatment, the removal rate was found to be 76.92 and 76.70% for biological oxygen demand, 79.78 and 71.07% for chemical oxygen demand, 32.45 and 36.84 % for ammonia nitrogen as well as 91.67 and 50.0% for phosphate after 24 and 28 hrs with mixed free cells and mixed immobilized cells, respectively. Moreover, the bio-removals of different heavy metals were found to reach 90.0 and 50. 0% for Cu ion, 98.0 and 98.5% for Fe ion, 97.0 and 99.3% for Mn ion, 90.0 and 90.0% Pb, 80.0% and 75.0% for Zn ion after 24 and 28 hrs with mixed free cells and mixed immobilized cells, respectively. The results indicated that 13.86 and 17.43% of removal efficiency and reduction of total dissolved solids were achieved after 24 and 28 hrs with mixed free cells and mixed immobilized cells, respectively.

***Keywords*—**Biological desalination, bio-sorption heavy metals, free cell bacteria, immobilized bacteria, wastewater bio-treatment.

I. Introduction

SEWAGE is a mixture of industrial and domestic wastes. It contains higher than 99% water and the remaining contents are some ions, suspended solids and pathogenic bacteria which must be removed. Domestic and industrial wastes are considered to be harmful pollution sources in the water resources. This harmful pollution in the water environment may negatively affect the ecosystem and human life [1].

Biological wastewater treatment is an essential step of wastewater treatment system and it treats domestic and industrial wastewater, etc. The biological wastewater treatment is known as the secondary treatment process which is used to get rid of any remaining contaminants after primary treatment. Chemical treatment of waste water is carried out by adding chemicals to react with pollutants in the wastewater, where, as in biological treatment microorganisms, are used to degrade wastewater contaminants. This treatment relies on bacteria, algae, fungi, nematodes, protozoa. These microorganisms convert the organic wastes via normal cellular processes to inorganic form. In most new treatment plants, aerobic bacteria are employed in aerated conditions. A mixture of certain bacterial strains producing many enzymes can be used as a powerful biological treatment product [2].

Immobilization of whole cells is of highly importance in the waste water treatment. The immobilization system provides the microbial cells with numerous advantages as compared to the free cells. The immobilized cells exhibit high metabolic activity, high mechanical strength, and resistance to toxic chemicals. This is due to presence of the cells inside the beads and of high biomass concentrations as well as diffusion barriers within the bio-film against the toxic compounds [3]. During continuous operations of laboratory scale bioreactors, immobilized microbial cells are able to maintain the microbial population in carriers without any loss [4]. Traditional biological treatment processes can remove a large fraction of biodegradable organic compounds present in sewage water. Furthermore, the cost of biological treatment is much lower than that of physical and chemical processes [5]. Moreover, immobilized system can be used several times without significant loss of activity [6], [7]. Therefore, the use of immobilized microorganisms is considered promising for wastewater treatment in the past few decades and in the near future [8]. Alginates are polymers consist of different proportions and sequences of mannuronic and guluronic acids derived from brown algae. Alginates are nontoxic to humans and the entrapped microorganisms, easy to handle and cheap. Physiologically, the immobilized cells do not suffer from extreme changes in physicochemical condition during the immobilization process, and the gel is permeable and transparent [9]. However, this substance cannot be maintained for a long time in aqueous condition since the immobilized microorganism can be broken easily during the operation [10]. This technique depends on the physical interaction between the microorganism and the carrier surfaces. Adsorption is based on weak forces, however, still enabling an efficient binding process. Usually in bonds formation, several forces are involved: van der Waals forces, ionic and hydrophobic interactions, and hydrogen bonds. Both electrostatic and hydrophobic interactions govern the cell-support adhesion, which is the key step in controlling the cell immobilization on the support [11], [12].

This study aims to evaluate the efficiency of different bacterial isolates mixture as bio-treatment of wastewater and bio-sorbent of heavy metals from wastewater. Moreover, the

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bacterial mixture in forms of free cells and in alginate immobilized cells was also tested.

II. Materials and Methods

A. Source of Sample

Sewage wastewater sample was collected after primary treatment from Sarpium forest site -Ismailia Governorate, Cairo, Egypt. It is the site where the sustainable forestry in desert lands of Egypt using treated sewage water project (Implemented by Ain Shams Univ. and funded by Science & Technology Development Fund - STDF) is conducted. The sample was placed in sterilized bottles and kept at 4 ºC for further studies. The sewage wastewater sample was transferred to the central Lab. for wastewater and industrial wastes, Water and wastewater company canal provinces. Characteristics of the collected wastewater sample are shown in Table I.

TABLE I
The Bio-Chemical Characters of the Sewage Wastewater Sample

Parameters	Unit (mg/L. wastewater sample)
BOD	273
COD	356
Ammonia Nitrogen	26.5
Phosphate	3.60
Turbidity (NTU)	23.2
TDS	700
pH	7.5
Ca	85.77
Mg	17.02
Na	146.2
K	16.89
SO_4	149.5
Cl	191.3
HCO_3	152.7
Total alkaline	250.7
Al	0.00
B	0.00
Cd	0.00
Co	0.00
Cr	0.00
Cu	0.01
Fe	0.10
Mn	0.30
Mo	0.003
Ni	0.001
Pb	0.01
V	0.0
Zn	0.02

B. Preparation of Different Forms of Bacterial Mixture

1. Free Cells Mixture

Different species of bacteria and yeasts were kindly provided by Department of Microbiology & Virology Lab., Ain Shams University. Each bacterial species and yeasts was grown separately on liquid media and the prepared liquid cultures (10^8 cfu/ml) were mixed [13].

2. Alginate-Immobilized Cells Inoculum

One hundred ml of liquid cultures mixture was added to an equal volume of a sterile solution of sodium alginate (2% w/v). The mixture was added drop-wise into 200 ml of 2% $CaCl_2$ sterile solution using a sterilized Pasteur pipette. Beads of approximately 2 mm in diameter were obtained and hardened in 2% $CaCl_2$ solution for 2 hrs. The beads were then rinsed with sterilized water and maintained at 4 °C. All steps were conducted under aseptic conditions [14].

C. Reactor Design

Sequencing batch reactor of 60 L volume capacity was operated in this study. The reactor was supplied by a Separix column with a thermostat and an air diffuser. The temperature of the reactor was adjusted at 30±1 °C. In each treatment, 30 L of wastewater was fed to the reactor.

1. First Batch Reactor Cycle

Fixed volume of free bacterial cells mixture was added to the sewage wastewater in the reactor. The cycle duration was 48 hrs. Samples were collected at intervals of 4 hrs. up to the end of the cycle. Bacterial cells were removed from the samples by filtration throw filter membrane (45 µm). The filtrated samples were kept at 4 ºC. Biochemical analysis of the samples was carried out at the Central Lab. for Wastewater and Industrial Wastes, Water and Wastewater Company Canal Provinces.

2. Second Batch Reactor Cycle

In the second cycle of this study fixed weight of immobilized mixed bacterial cells beads was added to the sewage wastewater in the reactor. The duration for the cycle was 48 hrs. Samples were collected at intervals of 4 hrs. up to the end of the cycle. Bacterial cells were removed from the samples by filtration throw filter membrane (45 µm). The filtrated samples were kept at 4 ºC. Biochemical analysis of the samples was carried out at the Central Lab. for Wastewater and Industrial Wastes, Water and Wastewater Company Canal Provinces.

D. Statistical Analysis

Data were statistically analyzed according to [15] using H.S.D parameter at 5%.

III. Results and Discussion

Different Bio-Treatment of Sewage Wastewater

Mixture of different bacteria and yeasts was used in forms of free cells and alginate immobilized cells as bio-removal agents for heavy metals, e.g. copper, iron, manganese, lead and zinc and to reduce COD, BOD, ammonia nitrogen, phosphate, turbidity, TDS in sewage water sample. The treated sewage water was incubated at 30 ºC, and samples were taken for analyses after different incubation periods.

Data presented in Tables II and III, Figs. 1 and 2 indicated that the highest removal percents of COD, BOD, ammonia nitrogen, phosphate, turbidity, TDS, copper, iron, manganese, lead and zinc were recorded after 24 in case of free cells treatment and after 28 hrs in case of immobilized cells treatment. The percentage of removal rate was found to be

76.92 and 76.70% for BOD; 79.78 and 79.70% for COD. In addition, the removal rate of ammonia nitrogen was found to be 32.45 and 36.84%. Moreover, the removal rate of phosphate was recorded to be 91.67 and 50.00 %; for turbidity 93.97and 87.89% and for TDS 13.86 and 17.43. During the experimental period, no markedly change in pH value was observed as compared to control sample (untreated sewage water). These results are in agreement with those found by [5] who stated that, the immobilized bacterial cells were of high efficiency in removing a large fraction of biodegradable organic compounds present in sewage water. Moreover, the biological treatment is much cheaper than chemical and physical treatments. In addition, immobilized microorganisms can be used several times without significant loss in their efficiency [6], [7].

TABLE II
REMOVAL PERCENTAGES (%) OF BIO-TREATMENTS FROM WASTEWATER TREATED WITH FREE BACTERIAL CELLS AND IMMOBILIZED CELLS AT DIFFERENT INCUBATION PERIODS

Parameters	BOD		COD		P		NH_4		Turbidity		TDS		pH	
Treatments Time (hrs)	Free cells	Immobilized cells	Free cells	Immobilized cells	Free cells	Immobilized cells	Free cells	Immobilized cells	Free cells	Immobilized cells	Free cells	Immobilized cells	Free cells	Immobilized cells
4	68.13	63.85	55.06	62..08	52.78	19.44	16.98	5.26	83.20	83.26	2.86	11.14	7.27	7.40
8	69.60	65.68	60.39	63.76	72.22	27.78	24.16	6.02	86.64	83.69	4.57	11.71	7.35	7.70
12	69.23	68.10	72.75	64.61	86.11	38.33	24.16	22.56	93.10	84.98	10.57	14.43	7.14	7.71
24	76.92	69.45	79.78	65.45	91.67	41.67	32.45	30.08	93.97	87.12	13.86	16.57	7.73	7.91
28	74.73	76.70	72.75	79.70	91.67	50.00	31.69	36.84	91.81	87.89	11.43	17.43	7.78	7.90
32	71.32	63.11	60.39	67.98	77.77	30.56	20.38	22.56	90.08	78.11	7.29	13.29	7.48	7.80
36	62.09	62.53	43.82	63.20	77.77	25.00	18.49	13.16	88.36	70.38	6.71	12.14	7.59	7.80
48	60.81	59.71	41.01	62.36	77.77	19.44	15.09	10.53	86.64	61.80	4.57	11.14	7.76	7.80

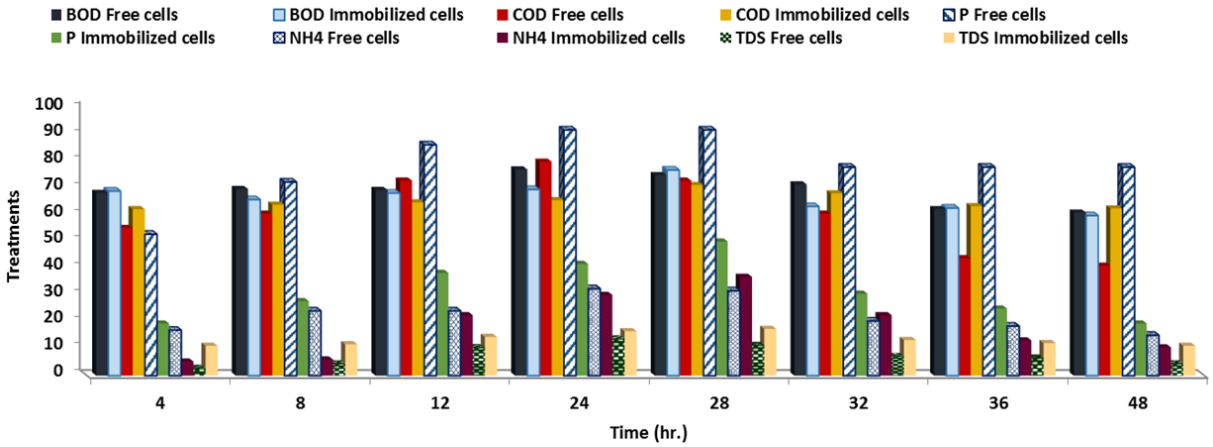

Fig. 1 Removal percentages of bio-treatment and bio-sorbent of heavy metals from wastewater treated with free bacterial cells and immobilized cells at different incubation periods

TABLE III
BIO-REMOVAL PERCENTAGES (%) OF DIFFERENT HEAVY METALS IN WASTEWATER

Parameters	Cu		Mn		Fe		Pb		Zn	
Treatments Time (hrs)	Free cells	Immobilized cells	Free cells	Immobilized cells	Free cells	Immobilized cells	Free cells	Immobilized cells	Free cells	Immobilized cells
4	0.00	0.00	33.33	76.66	10.00	80.00	0.00	0.00	0.00	0.00
8	0.00	0.00	60.00	86.66	30.00	90.00	0.00	0.00	50.00	0.00
12	0.00	0.00	83.33	96.66	70.00	90.00	90.00	50.00	55.00	50.00
24	90.00	30.00	97.00	99.00	98.00	94.00	90.00	60.00	80.00	55.00
28	90.00	50.00	86.67	99.33	96.00	98.00	80.00	90.00	65.00	75.00
32	80.00	20.00	86.67	97.67	80.00	95.00	60.00	70.00	50.00	60.00
36	70.00	10.00	83.33	80.00	20.00	91.00	60.00	70.00	50.00	50.00
48	50.00	10.00	83.33	70.00	10.00	91.00	60.00	50.00	50.00	50.00

Furthermore, the efficiency of COD removal increased to 93% in swage water with increasing NaCl concentration up to 3% and application of free cells of *Staphylococcu* combined with activated sludge [16]. The TDS in saline sewage wastewater was reduced to 39.0 % and 36.0% after 4 and 8 days, respectively as a result of inoculation with free cells of bacteria and algae [13].

Application of mixed bacteria in forms of free and immobilized cells to sewage wastewater was effective in bio-removal of different heavy metals as compared to control

sample (untreated wastewater). Data presented in Table III and Fig. 2 showed the percentage of bio-removal was 90.00 and 50.00% for Cu; 98.00 and 98.00% for Fe; 97.00 and 99.33 % for Mn; 90.00 and 90.00 % for Pb, as well as 80.00 and 75.00% for Zn after 24 and 28 hrs, respectively. These results are in agreement with those obtained by [17]-[20]. The biological treatment of sewage wastewater with free cells of bacteria and algae successfully removed different heavy metals after 48 and 96 hrs as reported by [13].

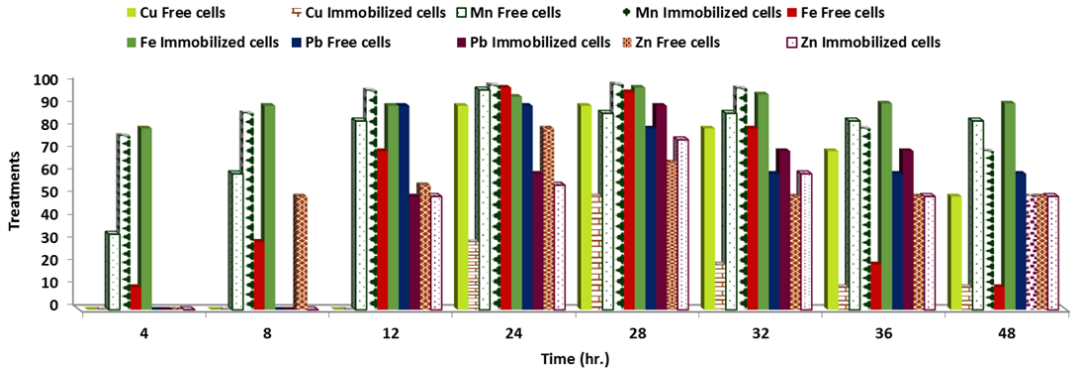

Fig. 2 Percentages of bio-removal of different heavy metals from wastewater treated with free bacterial cells and immobilized cells after different incubation periods

Comparison between the Efficiency of Different Bio-Treatments of Wastewater

Data presented in Tables IV and V indicated that application of mixture of different bacteria as bio-treatment to wastewater significantly reduced COD, BOD, ammonia nitrogen, phosphate, turbidity, TDS and significantly removed different heavy metals, e.g. copper, iron, manganese, lead and zinc as compared to the control (untreated wastewater sample collected from the same location).

Markedly significant differences were detected in reduction of BOD, COD, phosphate, ammonia nitrogen, TDS and turbidity which were 102.111, 160.815, 1.174, 21.425, 642.815 and 4.648 after 24 hrs respectively in case of free cells treatment, while in case of using immobilized cells were 108.626, 145.70, 2.618, 21.911, 610.185 and 5.722 after 28 hrs respectively. Moreover, slight differences were recorded in removing heavy metals *e.g.* Cu, Fe, Mn, Pb and Zn from wastewater in case of using free bacterial cells which were 0.025, 0.069, 0.112, 0.026 and 0.026 after 24 hrs respectively, while in case of using immobilized cells were 0.031, 0.041, 0.069, 0.039 and 0.035 after 28 hrs, respectively. These results are in agreement with those found by [13], [17]-[20].

IV. Conclusion

On the basis of the obtained results, it can be concluded that the use of mixture of different bacteria in form of free cells or alginate immobilized cells was found to be highly effective as bio-treatment of wastewater and bio-sorbent of heavy metals from wastewater. The free cells were found to be more effective than the immobilized cells. Therefore, the use of these microorganisms for wastewater treatment as alternative for chemical and physical treatments is highly recommended, since the cost of biological treatment is much lower than chemical and physical treatments.

TABLE IV
Significances Difference between Free Cells and Immobilized Cells as Bio-Treatments of Wastewater

Parameters	BOD		COD		P		NH_4		TDS		Turbidity	
Treatments Time (hrs)	Free cells	Immobilized cells	Free cells	Immobilized cells	Free cells	Immobilized cells	Free cells	Immobilized cells	Free cells	Immobilized cells	Free cells	Immobilized cells
Zero	270.00^{a}	270.00^{a}	351.67^{a}	351.67^{a}	3.47^{a}	3.47^{a}	26.10^{a}	26.10^{a}	656.67^{a}	656.67^{a}	21.00^{a}	21.0^{a}
4	82.00^{fg}	85.60^{bcdef}	155.33^{c}	121.33^{ed}	1.77^{cde}	2.07^{bcd}	21.40^{cd}	24.73^{ab}	675.33^{ab}	$619.00^{e,f,g}$	3.17^{cde}	3.07^{cde}
8	82.33^{fg}	89.07^{cdefg}	159.33^{cd}	124.67^{ed}	1.74^{cde}	2.09^{bcd}	22.87^{bcd}	24.83^{ab}	663.00^{bc}	609.33^{fghi}	3.27^{bcde}	3.87^{bcde}
12	84.33^{fg}	88.70^{cdefg}	192.00^{g}	124.67^{ed}	0.70^{f}	2.37^{bc}	20.26^{de}	21.20^{cd}	621.33^{ef}	584.67^{ij}	3.40^{bcde}	4.57^{bcd}
24	60.33^{h}	87.00^{defg}	71.00^{h}	128.33^{cd}	0.33^{f}	2.27^{bc}	18.00^{ef}	18.13^{ef}	591.33^{ghij}	586.00^{hij}	3.83^{bcde}	5.36^{bc}
28	63.67^{h}	61.20^{h}	94.33^{fg}	118.67^{e}	0.50^{f}	2.37^{bc}	18.07^{ef}	16.27^{f}	610.00^{efghi}	573.00^{j}	2.93^{cde}	6.30^{b}
32	67.00^{g}	95.63^{bcde}	138.33^{cd}	119.63^{fg}	0.83^{ef}	2.70^{abc}	22.03^{bcd}	20.07^{ed}	629.67^{def}	615.33^{efgh}	1.67^{de}	2.77^{cde}
36	101.33^{bc}	99.10^{bcd}	198.67^{b}	120.67^{ef}	0.87^{ef}	2.83^{ab}	21.53^{cd}	22.23^{bcd}	639.33^{cde}	614.33^{fghi}	1.37^{e}	2.33^{cde}
48	104.00^{b}	98.83^{bcd}	206.67^{b}	129.67^{ed}	0.90^{ef}	3.00^{ab}	22.57^{bcd}	23.63^{abc}	658.67^{bcd}	614.33^{fghi}	1.20^{e}	2.33^{ed}
Means of treatments	102.111^{B}	108.626^{A}	160.815^{A}	145.70^{B}	1.174^{B}	2.618^{A}	21.425^{A}	21.911^{A}	642.815^{A}	610.185^{B}	4.648^{B}	5.722^{A}
Means of time		7.897		11.775		0.621		1.835		18.271		1.940
HSD at 5%		2.29		3.414		0.180		0.532		5.298		0.563
HSD interaction between time & treatments		12.704		18.941		0.999		2.952		29.392		3.121

* The same letter is not significantly different.

TABLE V
SIGNIFICANCES DIFFERENCE BETWEEN FREE CELLS AND IMMOBILIZED CELLS AS BIO- REMOVAL TO HEAVY METALS FROM WASTEWATER

Parameters	Cu		Fe		Mn		Pb		Zn	
Treatments Time (hrs)	Free cells	Immobilized cells	Free cells	Immobilized cells	Free cells	Immobilized cells	Free cells	Immobilized cells	Free cells	Immobilized cells
Zero	0.073^{a}	0.073^{a}	0.267^{a}	0.267^{a}	0.367^{a}	0.367^{a}	0.073^{a}	0.073^{a}	0.073^{a}	0.073^{a}
4	0.073^{a}	0.073^{a}	0.113^{ab}	0.033^{b}	0.267^{ab}	0.050^{c}	0.073^{a}	0.040^{a}	0.060^{a}	0.035^{a}
8	0.010^{a}	0.073^{a}	0.063^{b}	0.013^{b}	0.133^{bc}	0.056^{c}	0.040^{a}	0.043^{a}	0.013^{a}	0.020^{a}
12	0.010^{a}	0.017^{a}	0.017^{b}	0.030^{b}	0.046^{c}	0.023^{c}	0.006^{a}	0.005^{a}	0.006^{a}	0.007^{a}
24	0.001^{a}	0.007^{b}	0.002^{b}	0.005^{b}	0.008^{c}	0.003^{c}	0.002^{a}	0.004^{a}	0.003^{a}	0.007^{a}
28	0.002^{a}	0.006^{a}	0.004^{b}	0.002^{b}	0.066^{c}	0.001^{c}	0.003^{a}	0.001^{a}	0.007^{a}	0.005^{a}
32	0.004^{a}	0.008^{a}	0.020^{b}	0.005^{b}	0.060^{c}	0.006^{c}	0.004^{a}	0.041^{a}	0.013^{a}	0.023^{a}
36	0.016^{a}	0.009^{a}	0.060^{b}	0.007^{b}	0.055^{c}	0.040^{c}	0.005^{a}	0.054^{a}	0.023^{a}	0.076^{a}
48	0.032^{a}	0.013^{a}	0.083^{b}	0.009^{b}	0.057^{c}	0.083^{c}	0.028^{a}	0.085^{a}	0.045^{a}	0.083^{a}
Means of treatments	0.025^{A}	0.031^{A}	0.069^{A}	0.041^{A}	0.112^{A}	0.069^{B}	0.026^{A}	0.039^{A}	0.026^{A}	0.035^{A}
Means of time		0.1113		0.0996		0.0955		0.1035		0.0545
HSD at 5%		0.0323		0.0289		0.0277		0.0300		0.0158
HSD Interaction between time & treatments		0.1791		0.1602		0.1537		0.1664		0.0876

* The same letter is not significantly different.

ACKNOWLEDGMENT

The authors would like to express their thanks for the financial support of Sustainable forestry in desert lands of Egypt using treated sewage water project (Implemented by Ain Shams Univ. for funded by Science & Technology Development Fund - STDF).

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