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Modelling inactivation rates of indicator microorganisms based on laboratory determinations of T_{90} for different temperature and salinity levels

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ABSTRACT

In coastal areas, treated or pre-treated domestic wastewaters are commonly discharged into marine environment through outfall systems as a final disposal application. The main parameters used to design a marine outfall system are; treatment level, length of outfall manifold and discharge depth. One major aim is to preserve seawater quality especially in sensitive areas where aquaculture and recreational activities take place. It is vital to comply with the bathing water quality standards in such areas to protect public health against water borne diseases originated from pathogens. In this manner, it is very important to define the case specific bacterial inactivation rates and/or the time needed to inactive 90% of bacteria (T_{90}) in design of marine outfall systems. In this study, T_{90} values have been determined for four different temperatures to represent different seasonal conditions in the Mediterranean Sea. The experiments were conducted in dark at 16 °C, 20 °C, 24 °C and 28 °C with wastewater inoculated seawater samples with different salinity levels between 20 ppt and 40 ppt. The selected temperatures represent the mean seasonal seawater temperatures in the bay of Antalya, located on the Turkish Mediterranean coast. The dark conditions represent a common case of submerged wastewater field below Secchi depth that is commonly observed in many deep sea outfall systems located along the coastal area. The seawater samples were taken from an offshore location in Antalya Bay and the inoculated wastewater was from a domestic wastewater treatment plant. T_{90} values have been determined for faecal coliform (FC) bacteria which are commonly used as bacteriological indicators in seawater. Multiple linear regression analysis was applied to develop a model to predict T_{90} values for different salinity and temperature levels.

Keywords: Bacterial inactivation; Marine outfall; Salinity; T_{90}; Temperature

1. Introduction

Disposal of domestic wastewaters to the marine environment has been a sanitary procedure for decades. The principle idea of a marine disposal system is to dilute the wastewaters by a vast quantity of seawater in marine environment. The marine disposal systems are still the best feasible option for the disposal of wastewaters in coastal areas. The discharged effluents from a marine outfall system are subject to initial/near field dilution, and far field dilutions due to dispersion and bacterial inactivation. Bacterial inactivation rate and the related T_{90} parameter (the time required to inactivate 90% of the coliform bacteria) are important parameters for predictive calculations of indicator bacteria.

Total coliform (TC), faecal coliform (FC), faecal streptococci (FS) and *E. coli* (EC) are the commonly used indicator bacteria groups for water quality purposes worldwide and are currently the preferred compliance organisms in the regulatory standards for recreational

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water quality [1–4]. In most of the bacterial inactivation studies, faecal bacteria are considered to be a good indicator organism of human originated contamination of water bodies as they are present in the faeces of humans and warm-blooded animals. Therefore, the presence of faecal bacteria in water may also indicate the presence of intestinal pathogens [5–8].

The bacterial inactivation process is strongly influenced by a number of factors such as solar irradiation, salinity, temperature, turbidity, predation by natural micro biota, infection by bacteriophages and nutrient deficiencies besides sedimentation with particulates [9–12]. However, numerous studies revealed that the inactivation process is strongly dominated by the solar irradiation which is a mixture of irradiance wavelengths of UV-B (280–320 nm), UV-A (320–400 nm), visible light (400–780 nm) and infra-red (780 to several thousand nm) [13,14]. Gameson and Gould stated that nearly half of the bactericide effect of the solar irradiance is originated from 280 to 370 nm portion of the whole spectrum [14]. Additionally, Sinton et al. reported that inactivation of the indicator bacteria at wavelengths beyond 550 nm was not really different than in the dark [15]. Bell et al. showed that the T_{90} value changes dramatically depending on the received solar irradiation both seasonally and diurnally, and reaches the maximum values at night for both summer and winter seasons [16]. According to their in-situ studies at 0.7 m depth, T_{90} values were obtained to vary between 2–65 h in summer and 5–80 h in winter seasons [16].

Depending on the light attenuation capacity of the water environment, intensity of solar irradiation reduces with increasing depths which decreases rate of the bacterial inactivation process. As a consequence of decreasing inactivation rates, the value of T_{90} increases considerably. Therefore, investigating the changes of T_{90} values and dilution due to bacterial inactivation along the depth of the water column bears a special interest for the design of sea outfalls with submerged effluents. In density stratified water bodies, discharged wastewaters remain submerged below the surface and the depth of submergence depends on the strength of the density stratification. Intensity of solar irradiation becomes negligible below a certain depth in water and if the wastewater field remains below this depth, particularly in dark, the effect of solar radiation on bacterial inactivation process becomes negligible. In this manner, salinity and temperature have more influence on the bacterial inactivation process.

Some of the studies carried out so far showed that the indicator bacteria may survive in dark for considerably long times than in light. Therefore, T_{90} values in dark may reach several times of the same value under solar irradiation and could be defined in the order of days or even weeks [17]. The research studies for Marmara Sea of Turkey have shown that T_{90} value in the dark was nearly twenty times higher than the value on the surface during the summer season, being 35 h [18].

In the experimental study of Noble et al., effects of temperature, nutrients, suspended solids and initial bacteria concentrations were investigated in two different ambient temperatures at dark [19]. The findings of the study showed that there are no significant effects of nutrients and suspended solids on the decay process. The T_{90} values for the *E. coli*, member of FC group, have been found as 109.7 h at 14 °C and 79.4 h at 20 °C. Additionally, Canteras et al. have studied the relationship between T_{90}, salinity, temperature and light intensity [12]. The obtained values of T_{90} have fitted to the exponential model of die-off for the studied parameters. At a salinity level of 8.5%, obtained T_{90} values in dark were between 8.38–36.05 h for temperatures ranging from 10 °C to 42 °C.

In fresh waters where the effect of salinity on the decay process becomes negligible, the values of T_{90} may reach several days or weeks [15]. In literature, there are a few studies related to bacterial inactivation process in dark and also the combined impact of temperature and salinity were not discussed in a systematic way. The principle aim of the current study is to investigate the bacterial inactivation rates and/or T_{90} values under different temperature and salinity levels in dark and to generate a model to predict T_{90} for variable ambient conditions.

2. Materials and methods

2.1. Experimental approach

The effects of salinity and temperature on T_{90} values were studied in controlled laboratory conditions to obtain case specific T_{90} values under different temperature and salinity levels for the Mediterranean region in general. Four different temperatures were chosen to represent the average *in-situ* seawater temperatures in winter, spring, summer and fall seasons for the Mediterranean Sea conditions. In order to determine the four experimental temperatures, *in-situ* seawater temperatures have been measured seasonally at five stations (P0, P1, P2, P3, P4) in Antalya Bay (see Fig. 1) in the vicinity of Antalya Sea Outfall between July 2008 and August 2009. Antalya Bay and Antalya City are located in the south of Turkey on the Mediterranean Sea coast. Experimental temperatures were selected as 16 °C, 20 °C, 24 °C and 28 °C based on the results of *in-situ* temperature measurements. The levels of temperature and salinity parameters investigated in the scope of the experimental study are given in Table 1. The seawater samples used in the laboratory experiments were taken

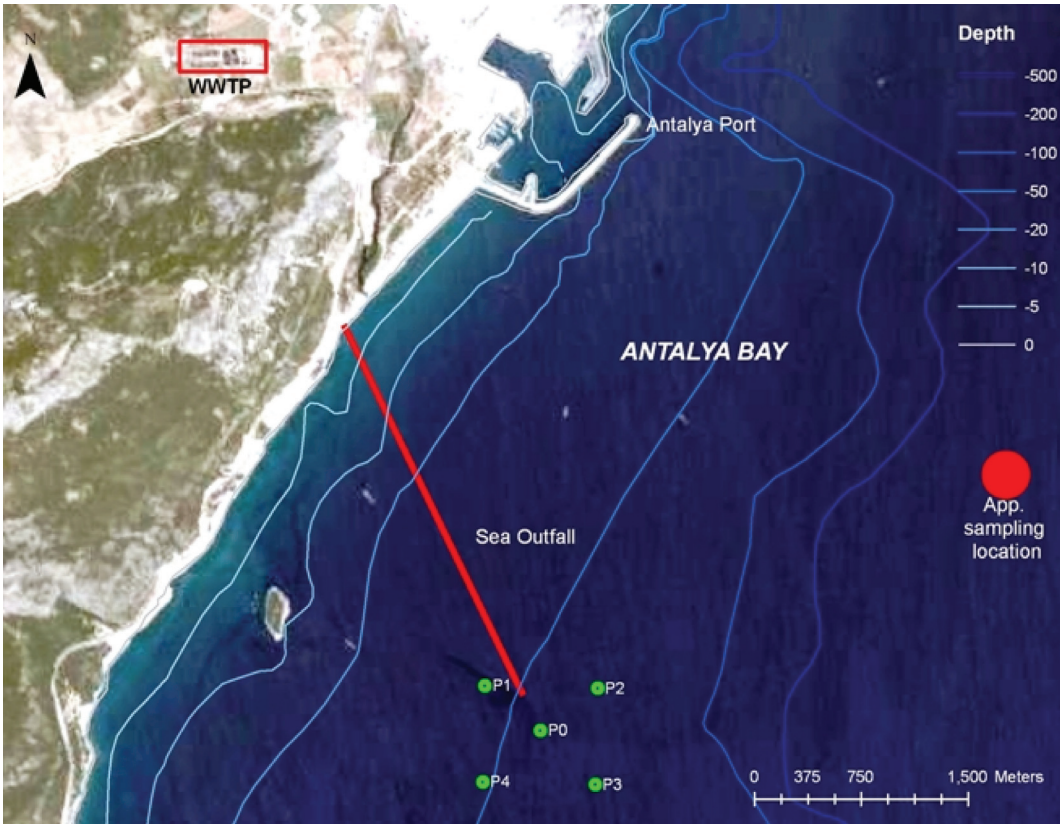

Fig. 1. Locations of *in-situ* measurement stations, Antalya Hurma Wastewater Treatment Plant, Antalya Port, Antalya sea outfall and seawater sampling location.

Table 1
Levels of the studied temperature and salinity parameters

Salinity (ppt)	20	25	30	35	40
Temperature (°C)	16–20–24–28	16–20–24–28	16–20–24–28	16–20–24–28	16–20–24–28

approximately 4 km away from Antalya Port to avoid any microbiological contaminations from shoreline. Fig. 1 depicts Antalya Bay, Antalya Port, Antalya Hurma Wastewater Treatment Plant (WWTP) and the locations of stations where *in-situ* seawater temperatures were measured and seawater samples were collected.

The effluent of Antalya Hurma WWTP was used to inoculate the seawater samples in the experiments. As part of an ongoing research project, the bacteriological characteristics of the influent and effluent of Antalya Hurma WWTP have been weekly monitored for one year starting from August 2008. The effluent samples were analyzed for total coliform (TC) and fecal coliform (FC) using membrane filtration technique and all the microbiological analyses were conducted in two replicates. The analysis results have shown that, the averages of TC and FC numbers in the treatment plant effluent were 10^6 TC/100 ml and 10^4 FC/100 ml, respectively. The temporal variations of TC and FC numbers in the treatment plant effluent starting from the first of week of August 2008 are presented in Fig. 2 for a period of 18 weeks which corresponds to the end of 2008. The inoculation volume of the wastewater and the dilution ratio of 1/100 have been derived from FC analyses results.

2.2. Sample preparation and analysis

Seawater sample has been collected from the prescribed offshore area and it has been divided into smaller volumes in the laboratory. Each sample volume was adjusted to different salinity level using distilled water. The adjusted salinity levels were varied between 20–40 ppt. Afterwards the salinity adjusted samples were transferred into 2 l glass borosilicate bottles and

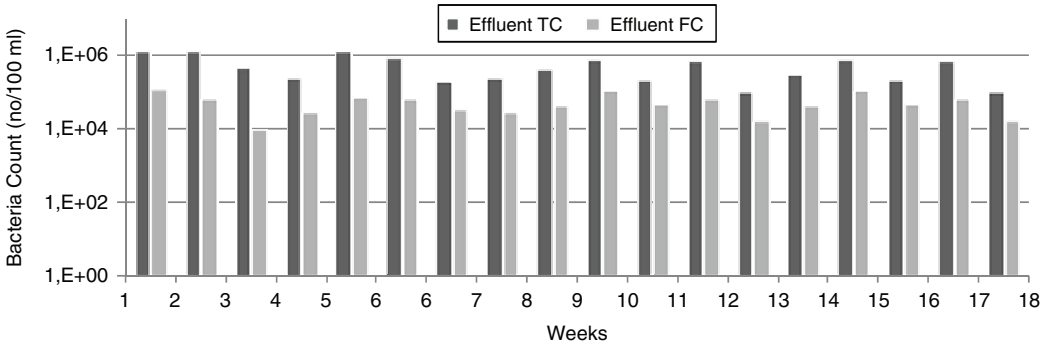

Fig. 2. Temporal variations of TC and FC in the effluent of Antalya Hurma WWTP between August–December 2008.

inoculated with the effluent of Antalya Hurma WWTP with a dilution rate of 1/100. A series of experiments were carried out in a constant temperature water bath which was placed in a specially designed laboratory cabinet to avoid any light penetration into the samples. The initial bacteriological test samples have been taken from each experimental bottle and all the test samples were analyzed for FC concentrations at the beginning of each experiment and at a time interval of 4 h for a duration of 48 h. The aim was to observe bacterial inactivation rate constants for each set of temperature and salinity in dark.

The samples were analyzed for FC using membrane filtration technique. After membrane filtration, the filter papers were placed in petri dishes having mFC (Sartorius brand disposable Nutrient Pad Sets) type incubation media and incubated at 44.5 °C for 24 ± 2 h. Following the incubation period, colonies developed on the filter paper were counted to calculate specific inactivation rate constants. All FC analyses were conducted in two replicates and the standard deviations were calculated. Additionally water quality parameters which may interfere with the inactivation process such as pH, conductivity, dissolved oxygen concentration (DO), dissolved oxygen percent saturation (DO sat) and turbidity have been measured prior to the experiments. The measured values of water quality parameters are given in Table 2 with their mean and standard deviations for each salinity group.

3. Results and discussions

In this study, salinity and temperature controlled laboratory experiments were carried out in dark to define the effects of salinity and temperature on bacterial inactivation process. The decay of the bacteria could be expressed using first order kinetics, also known as Chick's Law of disinfection, which describes the relationship between the bacteria concentration and the time [20]:

$$\frac{dc}{dt} = -k_d C \tag{1}$$

Table 2
Mean values and standard deviations (SD) of the measured water quality parameters for the experimental sets

Parameter		Group I (20 ppt)	Group II (25 ppt)	Group III (30 ppt)	Group IV (35 ppt)	Group V (40 ppt)
pH	mean	8.16	8.24	8.25	8.23	8.25
	SD	0.09	0.12	0.13	0.15	0.12
Conductivity (mS/cm)	mean	31.93	38.90	46.13	52.20	58.55
	SD	0.84	0.80	0.86	0.77	0.15
DO (mg/l)	mean	8.42	8.43	8.45	8.44	8.15
	SD	0.42	0.38	0.47	0.42	0.53
DO sat (%)	mean	99.10	99.10	99.40	99.40	95.40
	SD	5.56	5.48	4.52	6.26	2.39
Turbidity (NTU)	mean	0.89	0.99	1.41	1.64	1.13
	SD	0.17	0.36	1.03	1.47	0.43

In Eq. (1), k_d term denotes the first-order inactivation rate constant in the dark. Eq. (2) is derived by integration of Eq. (1):

$$\frac{C}{C_0} = \exp(-k_d t) \quad (2)$$

The term C in Eq. (2) denotes the bacteria concentration in time t where the C_0 term is the initial bacteria concentration. In order to linearize Eq. (2), the natural logarithm of the equation has been taken and the experimental data versus time has been fitted to the derived equation to obtain k_d values. Additionally, T_{90} term which denotes the time needed for 90% removal of the coliform has been calculated using Eq. (3) which has also been derived from Eq. (1):

$$T_{90} = \frac{2.303}{k_d} \quad (3)$$

The experimental studies were conducted at four different temperatures between 16–28 °C to represent temporal variation of T_{90} values with respect to four different seasons. The standard deviations of FC analyses have been computed and averaged for each experimental temperature to represent a season. The resultant standard deviations and their ranges are presented in Table 3. The bacterial inactivation rate constants k_d and the corresponding T_{90} values are given in Table 4 for all the experimental sets representing winter, spring, summer and fall seasons.

As a result of the experimental studies, it is clearly observed that T_{90} values decrease with increasing salinity levels, which is a consequence of the increased rates of the bacterial inactivation process. To observe the relation between salinity and T_{90} values, T_{90} values obtained for each experimental set was plotted against the salinity values. A strong correlation has been found for all studied seasonal cases as given in Fig. 3. The bacterial inactivation rates increase when the same salinity levels are compared for different temperatures. As an example,

Table 3
Standard deviations of bacteriological analyses and their ranges

Sample	Average standard deviation (CFU/100 ml)	Ranges of standard deviations (CFU/100 ml)
Winter case (16 °C)	4.10	3.50–4.50
Spring case (20 °C)	10.74	8.10–17.25
Fall case (24 °C)	7.87	3.04–20.09
Summer case (28 °C)	3.36	1.80–4.85

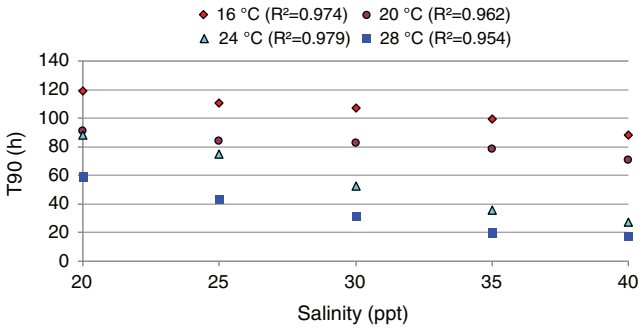

Fig. 3. Seasonal variation of T_{90} values for different seawater temperatures.

Table 4
Bacterial inactivation rate constants (k_d) and T_{90} values of FC for four seasons

Salinity (ppt)	Winter case (16 °C)			Spring case (20 °C)		
	k_d (h^{-1})	T_{90} (h)	R^2	k_d (h^{-1})	T_{90} (h)	R^2
20	0.0193	119	0.967	0.0252	91	0.914
25	0.0208	111	0.918	0.0274	84	0.974
30	0.0215	107	0.95	0.0280	82	0.953
35	0.0232	99	0.814	0.0294	78	0.959
40	0.0261	88	0.433	0.0325	71	0.942
Salinity (ppt)	Fall case (24 °C)			Summer case (28 °C)		
	k_d (h^{-1})	T_{90} (h)	R^2	k_d (h^{-1})	T_{90} (h)	R^2
20	0.0262	88	0.991	0.0387	60	0.963
25	0.0307	75	0.956	0.0531	43	0.847
30	0.0441	52	0.967	0.0723	32	0.885
35	0.0649	36	0.947	0.1146	20	0.863
40	0.0838	27	0.951	0.1325	17	0.99

Table 5
Statistical summary for the multiple linear regression analysis

	Coefficient	Standard error	*t*-Value	*p*-Value	Confidence interval 99%	
					Lower	Upper
Constant	257.669	10.554	24.416	<0.0001	227.083	288.256
Salinity	–1.939	0.227	–8.540	<0.0001	–2.597	–1.281
Temperature	–5.927	0.359	–16.509	<0.0001	–6.968	–4.887

Table 6
Statistical evaluation of the multiple linear regression analysis

Summary	Multiple-*R*	R^2	Adjusted R^2	Standard error of estimate
	0.9763	0.9531	0.9476	7.1808

in the experimental sets with the salinity level of 20 ppt, T_{90} values were determined as 119 and 60 h for 16 °C and 28 °C, respectively. Under the maximum ambient conditions of temperature and salinity (28 °C and 40 ppt), the combined effect of both controlling parameters caused a much more rapid inactivation process which reduced T_{90} value to 17 h with a R^2 of 0.99.

Multiple linear regression analysis was carried out using the data sets to develop a model to predict T_{90} values of FC at different salinity (S) and temperature (T) levels. Summary of the statistical analyses are presented in Tables 5 and 6 while the obtained multiple linear regression model is presented below:

$$T_{90}\,(\mathrm{FC}) = 257.669 - 1.939\,\mathrm{S} - 5.927\,T \quad (4)$$

The developed multiple linear regression model has been tested for the whole experimental data sets to observe the correlation of the predicted T_{90} values versus the observed T_{90} values (Fig. 4). A high correlation has been achieved for the observed and predicted values of T_{90} with a value of R^2 = 0.9531. The residuals of the predicted T_{90} values were in the range of –12.03 to +11.40 h with an absolute mean value of 5.69 h. The mean value of the whole set of observed T_{90} values is calculated as 69.8 h and correspondingly the mean residual value of 5.69 h corresponds to 8.2% of the mean value. According to the statistical results presented in Table 5, the correlation between the observed and predicted T_{90} values (multiple R) was obtained as 0.9763 and the standard error of the estimate was 7.1808 with p-value of less than 0.0001. All the statistical findings support the high correlation of the observed and predicted T_{90} values.

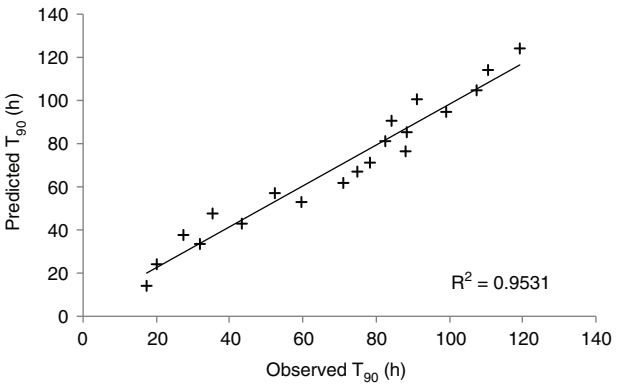

Fig. 4. Scatter plot and correlation of predicted and observed T_{90} values.

In the literature, effects of salinity and temperature on T_{90} values have been studied in a narrow range of the parameter values in dark. However, bacterial inactivation process in dark is a common phenomenon of the submerged wastefield occurrences due to sea outfall discharges in coastal waters. Therefore, it is important to define case specific T_{90} values for a wide range of salinity and temperature values. In this study, five different salinity levels were considered during the experimental studies at four different temperatures to obtain a set of 20 different T_{90} values. The minimum and maximum values of T_{90} have been found as 17 h at 28 °C and 40 ppt and 119 h at 16 °C and 20 ppt, respectively. The results showed that T_{90} parameter is highly variable under the effects of salinity and temperature in dark. Findings of the study are in good agreement with T_{90} values obtained from the literature [6,12,19,21]. Gabutti et al. studied the effect of salinity on the inactivation of feacal coliform bacteria at 22 °C [6]. The values of T_{90} parameter have been found as 72 h at 27 ppt salinity and 48 h at 35 ppt salinity. Noble et al. reported similar results of T_{90} values under different salinity and temperature values [19]. Additionally, Sinton et al. studied dark inactivation rate constants at two different temperature values representing cold and warm sea water temperatures [21]. The findings of the similar literature work have been presented in Table 7. Additionally, the literature data have been used to test the validity of the developed multiple linear regression model and T_{90} values have been computed using the experimental data obtained from the previous studies

Table 7
Findings of the similar literature work and the predicted values of T_{90} using the developed multiple linear regression model

Author	Salinity (ppt)	Temperature (°C)	T_{90} (h)	Model Predictions of T_{90} (h)
Sinton et al. 1994	32.5	10	115	135
		20	82.3	76
Gabutti et al. 2000	27	22	72	74
	35		48	59
Noble et al. 2004	33	14	109.7	110
		20	79.4	75

mentioned above (see Table 7). The presented literature data and the predicted values of T_{90} are close to each other. As could be observed from the presented literature data, the developed linear regression model could predict T_{90} values for different salinity (ranging from 27–35 ppt) and temperature (10–22 °C) levels.

The developed multiple linear regression model could be easily applied in different environmental conditions to predict T_{90} especially in cases of submerged wastewater field from marine outfall discharges. The developed model could be used for design of marine outfall systems located in the coastal areas of the Mediterranean Sea.

4. Conclusions

Bacterial inactivation process in marine environment is strongly dominated by solar radiation, osmotic stress and temperature. Osmotic stress is a consequence of changing salt concentration or the salinity of the receiving environment. Especially in density stratified water bodies, discharged wastewaters remain submerged under the water surface and in some cases below Secchi depth. In such cases bacterial inactivation process undergoes with the effects of salinity and temperature. In this study, the effects of different salinity and temperature levels on the inactivation process have been investigated in dark. It is found that the bacterial inactivation rate at a salinity level of 20 ppt increases with temperature and the resultant T_{90} values almost double for winter when compared with summer cases. When the salinity levels are further increased to 40 ppt, the resultant T_{90} values for different temperatures are observed to vary from 17 h at 28 °C to 88 h at 16 °C. The results achieved in this study show that bacterial inactivation rate constants are proportional to the saline concentration and temperature and a strong correlation exists in between. The obtained data sets were used to develop a multiple linear regression model to predict T_{90} values of faecal coliform for different environmental conditions. The developed model shows a high correlation for the predicted T_{90} values versus the observed ones.

Acknowledgement

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Symbols

T_{90} — time required to inactivate 90% of the coliform bacteria
k_d — bacterial inactivation rate constant
C — bacteria concentration in time t
C_0 — initial bacteria concentration
t — time
S — salinity (ppt)
T — temperature (°C)

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