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Developing Correlation Equations for Converting Among Coliforms, *E. coli* and HPC for Rainwater Disinfection by Sunlight

(Persamaan Korelasi untuk Menukar Koliform, *E. coli* dan HPC untuk Nyahinfeksi Air Hujan Menggunakan Cahaya Matahari)

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ABSTRACT

The application of solar disinfection for treating stored rainwater was investigated by the authors using indicator organisms. The multiple tube fermentation technique and pour plate method were used for the detection of microbial quality indicators like total and fecal coliforms, E. coli and heterotrophic plate count. These techniques have disadvantages mainly that these are laborious and time consuming. The correlation of total coliform with that of exposure time is proposed under different factors of weather, pH and turbidity. Statistical tools like root mean square error and coefficient of determination were used to validate these proposed equations. The correlation equations of fecal coliform, E. coli and heterotrophic plate count with total coliform are suggested by using four regression analysis including Reciprocal Quadratic, Polynomial Regression (2 degree), Gaussian Model and Linear Regression in order to reduce the tedious experimental work in similar types of experiments and treatment systems.

Keywords: Correlation; microbial parameters; rainwater; sunlight

ABSTRAK

Penggunaan nyahinfeksi solar untuk merawat air hujan tertakung telah dikaji oleh penyelidik kertas ini menggunakan organisma penunjuk. Teknik fermentasi berbilang tiub dan kaedah plat tuang telah digunakan untuk mengesan penunjuk kualiti mikrobial seperti jumlah dan koliform tinja, E. coli dan bilangan plat heterotrofik. Teknik-teknik ini mempunyai kekangan seperti mengambil masa yang lama dan kerja yang rumit. Korelasi koliform jumlah dengan masa dedahan dicadangkan di bawah faktor yang berlainan seperti cuaca, pH dan kekeruhan. Parameter statistik seperti ralat punca kuasa dua dan koefisien penentuan telah digunakan untuk menentusahkan persamaan yang dicadangkan. Persamaan korelasi koliform tinja, E. coli dan bilangan plat heterotrofik dengan koliform jumlah dicadangkan menggunakan empat analisis regresi termasuklah kuadratik resiprokal, regresi polinomial (darjah 2), model Gauss dan regresi linear bagi mengurangkan kerja uji kaji yang menjemukan pada masa hadapan bagi sistem rawatan dan uji kaji.

Kata kunci: Air hujan; cahaya matahari; korelasi; parameter mikrobial

INTRODUCTION

There are various point-of-use water treatment methods to reduce the exposure to microbial pathogens (Clasen et al. 2008) and solar disinfection (SODIS) is considered an effective point-of-use treatment method for household water supplies (Gelover et al. 2006; Ubomba-Jaswa et al. 2009). It is very simple and cost-effective method of treatment where the contaminated water is simply exposed to the direct sunlight for several hours of daytime (McGuigan et al. 2006; Sichel et al. 2007). Roof-top rainwater harvesting (RWH) can be regarded as an alternative source of potable/non-potable water supplies especially in areas where there are problems of centralized water supply systems or in areas of the poor developing world where people do not have access to safe water supplies (Al-Salaymeh et al. 2011; Amin et al. 2013; Amin & Han 2009a; Ghisi & Ferreira 2007; Han 2007; Han & Mun 2008; Handia et al. 2003; Hatibu et al. 2006; Heyworth et al. 2006; Ibrahim 2009; Kim et al.

2005; Kumar 2004; Lee et al. 2010; Meera & Ahammed 2006; Nazer et al. 2010; Roebuck et al. 2010; Simmons et al. 2001; Słys 2009; Spinks et al. 2006; Sturm et al. 2009). The roof-top harvested stored rainwater was treated by SODIS and the efficiency of this method was previously evaluated by authors (Amin & Han 2009b). The simple SODIS was later modified targeting the enhanced optical and thermal effects of the sunlight radiations in a solar collector disinfection (SODO-DIS) system (Amin & Han 2009c, 2011). In all these experiments, weather condition i.e. the sunlight irradiance in Watt/m² was the natural variable in addition to the experimental variables of pH and turbidity. To examine the bacteriological quality of water, indicator organisms like total coliform (TC), fecal coliform (FC) and E. coli were used in addition to heterotrophic plate count (HPC). The rainwater was exposed to direct sunlight in both S1 and S2 for about 8-9 h by starting the exposure at about 8-9 am and thus ending at about 5-6 pm.

The microbial analysis of rainwater was carried out by following the Standard Methods (APHA 1999). TC, FC and E. coli were measured using the multiple tube fermentation technique (MPN Method) while HPC was determined by the pour plate method. These techniques and methods have disadvantages; these are laborious, time consuming and error prone. For analysis, 15 tubes were used for three dilution of one sample which was not easy to handle for minimum of three samples at each time the experiment was performed. The MPN method requires 24 ± 2 or even 48 ± 4 h of incubation for the presumptive phase of TC, FC and *E.coli* and requires additional 24±2 or up to 48±4 h of incubation for the confirmation phase of both TC and FC. Additional 24±2 h are needed for the confirmation phase of the E. coli. So, about 3-4 days are required for the final detection and counting of the positive tubes indicating detection. These methods were also error prone as the water sample to be tested should be diluted accurately, to get countable number of colonies on the agar plates and detect the microbial growth in test tubes. The defined substrate method detects the enzymes, β -galactosidase and β -glucuronidase produced by fecal coliforms and E. coli, respectively (Rice & Edberg 1990) and changes the color of a chromogenic substrate which is added to the water to be analyzed (Edberg et al. 1990).

The objectives of this paper were to present the mathematical correlated equations in order to minimize the tedious experimental work whereby 30 samples were analyzed for 4 microbial parameters in each S1 and S2 for only one variable with three different experimental conditions. So, there was about 30*3*4*2*3=2160 samples which were analyzed in a time span of about two years by the methods described above. So, it will be of interest and useful to develop the mathematical correlating equations for the dependency of microbial parameters on each other to reduce the tedious work when such treatment methods will be used for the stored rainwater.

METHODS

STUDY LOCATION AND SAMPLING

In the experimental setup, the stored rainwater in polyethylene terephthalate bottles in both S1 and S2 were exposed to direct sunlight on the rooftop of an engineering department building at Seoul National University campus in Seoul, Republic of Korea, as shown in Figure 1. The rainwater was collected from the underground storage tanks of the RWH facility and the detailed description of this system including both SODIS (S1) and SOCO-DIS system (S2) is already published (Amin & Han 2009a, 2009b).

All rainwater samples were collected directly into the sterile 1L plastic bottles and transported to the laboratory for the analyses which usually commenced within 24 h of the sampling time. The initial concentrations of investigated bacteriological water-quality parameters including TC, FC, *E. coli* and HPC in parent rainwater samples are shown in Table 1. Figure 2 is the simple established relationship among coliforms and *E. coli*. Bacteriological parameters were measured at appropriate time intervals usually after every 1.5 h during 9 h of exposure.

EXPERIMENTAL VARIABLES AND CONSTANTS

The sunlight disinfection efficiency was evaluated for both S1 and S2 under different weather conditions which was categorized as weak, moderate and strong weather. The radiation intensities range between <100 and >1000 W/m² with three average values of 300, 550 and 880 W/m² (Table 1). A SP-110 Pyranometer (Apogee Instruments Inc., Logan, USA) connected to a datalogger (DT80 Series 2) recording 1 min averages in Watt/m² (W/m²) was used to monitor the on-site sunlight radiations.

In addition to sunlight radiations, the other two experimental variables include the initial pH and turbidity values of stored rainwater. Three different initial pH values of 5, 7 and 10 were chosen representing the acidic, neutral



FIGURE 1. A typical RWH system with current sunlight experimental sketch

	Experimental parameters					Microbial parameters			
Varying parameter	Sunlight intensity Avg. (range), W/m ²	рН	Turbidity (NTU)	Exposure time (h)	Total samples	TC CFU/100 mL	FC CFU/100 mL	E. coli CFU/100 mL	HPC CFU/mL
Weather	300 (200-450) 580(450-700) 880(650-1000)	7	5	9	30	880-1080	415-450	210-240	1700-2000
рН	580(450-700)	5 7 10	5	9	30	880	415	210	2000
Turbidity	300 (200-450)	7	5 20 100	9	30	1080	420	240	1700

TABLE 1. Experimental conditions and initial microbial concentrations in both S1 and S2



FIGURE 2. Relationship among the investigated three microbial parameters

and basic rainwater samples. The pH was adjusted by using the diluted HCl and NaOH and the comparison for the disinfection efficiency was performed only at moderate weather. Rainwater collected from underground storage tank had turbidity of less than 5 NTU and for the comparison purposes higher values of 20 and 100 NTU were adjusted by adding kaolin particles. A Turbidimeter (Hach 2100, USA) was used to measure the turbidity while pH was measured using a pH meter (Hach Sension 1, USA).

PROPOSED EQUATIONS AND STATISTICAL ANALYSIS

At first, the equation is proposed for the TC (Y in (1)) depending upon the weather factor (X1 in (1)) and exposure time (X2 in (1)) with three associated constants a, b, c, and d being the intercept value (Table 2). The proposed equation can be written in the following form;

$$Y = aX_1 + bX_2 + cX_2^2 + d.$$
 (1)

In addition, four regression analysis including Reciprocal Quadratic, Polynomial Regression (2 degree), Gaussian Model and Linear Regression were used for the correlation equations of fecal coliform, *E. coli* and heterotrophic plate count with that of total coliform. These four equations can be written in the following form:

Linear regression,
$$Y = a + bX$$
. (2)

Reciprocal quadratic, $Y = 1/(a + bX + cX^2)$. (3)

Gaussian model, $Y = ae^{-(X-b)^2/2c^2}$. (4)

Polynomial regression (2 degree),
$$Y = a + bX + cX^2$$
.
(5)

The suitability of these proposed equations were confirmed by the statistical tools like root mean-square error (RMSE), correlation coefficient and coefficient of determination. The RMSE is a kind of generalized standard deviation and is a measure of knowing how well the curve fits the data.

For each data point, CurveExpert Professional (software used to draw the graphs and calculate the statistical and experimental parameters) calculates the value of 'y' from the formula. It subtracts this from the data's y-value and squares the difference. All these squares are added up and the sum is divided by the number of data and finally it takes the square root. RMSE can be written mathematically as follows:

$$RMSE = \sqrt{\frac{\sum \left(f(x_i) - y_i\right)^2}{n}}.$$
(6)

The corresponding weather, pH and turbidity factors were calculated by assigning 0 to the lowest value and 1

to the maximum value. For example, in case of weather, 0 was assigned for 200W/m² and 1 for 1000W/m² and accordingly 0.125, 0.475 and 0.850 were assigned to the corresponding average values of sunlight radiations at 300, 580 and 880W/m², respectively, by simple mathematical method.

RESULTS AND DISCUSSION

For reducing the tedious experimental work in both systems with different experimental conditions, correlation equations are proposed for relating among different microbial parameters.

CORRELATING TC WITH TIME UNDER DIFFERENT EXPERIMENTAL CONDITIONS

To relate the FC, *E. coli* and HPC with TC, TC was first related with time under different weather, pH and turbidity conditions, keeping in mind the time-dependant nature of these parameters (O'Hogain et al. 2010). Three different weather factors were assigned to weak, moderate and

strong weather conditions. These values were adjusted based on the minimum and maximum sunlight intensities. Figure 3 shows the TC relationship with time and weather factor based on the proposed equation (1).

As shown in Figure 3, the concentrations of TC decreased with exposure time and with increased weather factor due to the effects of sunlight i.e. both UV and thermal disinfecting effects. TC was related with the time and the weather factor by the (1) and the values of four constants are given in Table 2. If the time is denoted by 't' and weather as 'Wf' then (1) can be re-written in the following form;

$$TC = a^*(Wf) + bt + ct^2 + d.$$
 (1a)

The values of Wf depend upon the weather conditions and the average sunlight radiations which would remain constant over specific weather time period of few months. The validation of the above proposed equations can be seen by the corresponding values of the coefficient of determination, \mathbb{R}^2 , which was about 94.3% and 94.5% for both S1 and S2, respectively, with RMSE values of about 66 and 90 for both S1 and S2, respectively (Table



FIGURE 3. The correlation of TC with time and weather factor under different weather conditions for (a) SODIS and (b) SOCO-DIS system

TABLE 2. Statistical parameters for TC correlation with time under different experimental conditions of weather, pH and turbidity based on the propose equation

Different parameters	Weather		pl	Н	Turbidity	
Treatment system	S 1	S2	S1	S2	S1	S2
a	-1.85 *10 ²	-3.33 *10 ²	-5.75 *101	9.81 *10 ¹	9.25 *10 ¹	9.43 *10 ¹
b	-1.35 *10 ²	$-1.70 * 10^{2}$	-1.10 *10 ²	-1.55 *10 ²	-1.19 *10 ²	$-1.42 * 10^{2}$
С	6.13	6.08	3.75	4.88	5.65	4.01
d	1.03 *103	1.23 *103	9.92 *10 ²	9.21 *10 ²	1.12 *103	1.14 *103
Standard Error	6.63 *10 ¹	9.05 *10 ¹	5.86 *10 ¹	7.59 *10 ¹	5.99 *10 ¹	8.06 *10 ¹
Coefficient of Determination, R^2	0.94	0.95	0.94	0.95	0.93	0.94
Correlation Coefficient, R	0.97	0.97	0.97	0.98	0.96	0.97

2). For the same values of weather factor and time for both S1 and S2, higher values of TC were calculated for S2 as compared with S1 and this difference increased with increasing time showing the effects of concentrated sunlight and increased exposure.

The similar equations can be reduced for different pH and temperature conditions by denoting the pH factor as 'pHf' and turbidity factor as 'Tf' as shown in 1b and 1c, respectively.

$$TC = a^*(pHf) + bt + ct^2 + d.$$
 (1b)

$$TC = a^*(Tf) + bt + ct^2 + d.$$
 (1c)

The corresponding coefficient of determination for S1 and S2 under different weather conditions is about 94% and 95%, respectively, with corresponding RMSE values of about 59 and 76, respectively, as shown in Table 2. Almost similar results are obtained for the relations of TC with time and turbidity factor with slightly lower and higher coefficient of determination and RMSE, respectively,

as compared to different initial pH conditions. The dependency of TC on time in addition to corresponding variable factor demonstrate that it is possible to reduce the tedious experimental work and to predict the removal of TC and hence the disinfection efficiency of sunlight for treating rainwater or other types of water.

CORRELATION BETWEEN FC AND TC

Having developed the TC correlation with time and weather conditions, correlation of FC with that of TC is computed under different weather conditions for both S1 and S2 by using the Reciprocal Quadratic and Linear Regression (Table 3). The suitability of the regressions is represented by the Figure 4 and the correlation equations can be written based on (2) and (3) and the corresponding constant values from Table 3.

For S1, $FC = 1/(0.105 + b^* TC + c(TC)^2)$. (7)

For S2,
$$FC = 0.433^* TC - 0.82$$
. (8)

TABLE 3. Statistical parameters for the correlation of FC, E. coli and HPC with TC under different weather conditions

Correlating parameter	FC		Е. с	oli	HPC	
Treatment system	S1	S2	S1	S2	S 1	S2
a	1.49 *10-2	-8.20	2.46 *10 ²	-6.02	2.02 *10-3	6.44 *10 ¹
b	-2.59 *10-5	4.33 *10-1	1.18 *10 ³	1.99 *10-1	-3.21 *10-6	2.80
С	1.33 *10-8	-	5.08 *10 ²	3.59 *10-5	1.74 *10-9	-1.06 *10-3
Standard error	3.44 *101	3.06 *101	1.03 *101	9.08	2.01 *102	$1.70 * 10^2$
Coefficient of determination, R ²	0.93	0.97	0.98	1	0.81	0.94
Correlation coefficient, R	0.97	0.98	0.99	1	0.9	0.97
Regression kind	Reciprocal Quadratic	Linear	Gaussian Model	Polynomial Regression (degree=2)	Reciprocal Quadratic	Polynomial Regression (degree=2)



FIGURE 4. FC correlation with TC corresponding to different weather conditions in (a) SODIS and (b) SOCO-DIS system

The corresponding coefficient of determination for S1 and S2 were 96.5 and 98.2%, respectively, with corresponding values of RMSE of about 35 and 30, respectively, as shown in Table 3. Table 3 also shows the parameters for the correlation equations of *E. coli* and HPC with TC for both treatment systems. The regression kind for each type of correlation is also mentioned in Table 3 which was chosen for the best fit of data, keeping in mind the maximum 3 constant values i.e. a regression of second degree or less. The FC concentrations can be correlated with that of time and weather factor by combining (7) and (8) with (1) for both S1 and S2, respectively, under different weather conditions.

CORRELATION BETWEEN E. COLI AND TC

As was the correlation of FC with TC, the *E. coli* correlation with TC and *E. coli* was developed and the corresponding regression equations for S1 and S2 can be written as represented by (9) and (10) and based on the best of the data as shown in Figure 5. The regression analysis in the form of Gaussian model and polynomial regression of second degree are presented as:

For S1, *E*.
$$coli = 246e^{-(TC-1180)^2/516128}$$
. (9)

For S2, *E*.
$$coli = -0.602 + 0.199^* TC + c(TC)^2$$
). (10)

The coefficient of determination was about 98 and 99% for S1 and S2, respectively, as mentioned in Table 3. The RMSE of about 10 was calculated for both systems which show the best fit of b2nd degree polynomial regression for S1 and S2. By combining (9) and (10) with that of (1), *E. coli* can be correlated with weather factor and time.

CORRELATION BETWEEN HPC AND TC

Equations (11) and (12) are the best model fit for the HPC correlation with that of TC as shown in Figure 6. Reciprocal

Quadratic and second degree Polynomial Regression were selected for S1 and S2, respectively.

For S1,
$$FC = 1/(0.105 + b^* TC + c(TC)^2)$$
. (11)

For S2, *E*.
$$coli = -0.602 + 0.199^* TC + c(TC)^2$$
). (12)

From Table 3, it is clear that a relatively high RMSE values are observed although the coefficient of determination was determined as about 94% for S2. From the relative high values of RMSE, the correlation of HPC with TC does not seem valid for both systems but a good correlation of HPC with TC was observed for S1 and S2 under different pH and turbidity conditions as can be seen from Tables 4 and 5, respectively. HPC correlation of time and weather factor for both S1 and S2 can be achieved by combining (1) with that of (11) and (12), respectively.

MICROBIAL CORRELATION UNDER DIFFERENT PH AND TURBIDITY CONDITIONS

The correlation of FC, *E. coli* and HPC with TC under different pH conditions were achieved by using only two regression analysis i.e. Reciprocal Quadratic and Polynomial Regression (2 degree), as shown in Table 4. The coefficient of determination was observed at 97% and even more than 99% in most of the cases except for the HPC correlation with TC for S1 under different pH conditions, as shown in Table 4. A relatively higher RMSE was observed with the corresponding coefficient of determination as about 91%. This shows the poor relationship between HPC and TC, however, reciprocal quadratic proved to be the best in second degree regression analysis.

Table 5 shows the RMSE, coefficient of determination and correlation coefficient along the values of the constants of regression equations used for correlations TC, *E. coli* and HPC with TC under different turbidity conditions for both S1 and S2. Three kinds of regressions including Reciprocal Quadratic and Gaussian Model were used to establish the



conditions in (a) SODIS and (b) SOCO-DIS system



TABLE 4. Statistical parameters for the correlation of FC, E. coli and HPC with TC under different pH conditions

Correlating parameter	FC		Е.	coli	НРС	
Treatment system	S1	S2	S 1	S2	S1	S2
a	-9.78 *10 ¹	2.75 *10-3	2.75 *10-2	-4.42	2.00 *10-3	1.49 *102
b	7.46 *10-1	-4.09 *10-6	-4.73 *10-5	1.65 *10-1	-3.46 *10-6	2.87
С	-1.92 *10-4	1.93 *10-9	2.44 *10-8	8.78 *10-5	1.98 *10-9	-8.77 *10-4
Standard error	1.97 *10 ¹	6.92 *10 ¹	1.00 *101	7.05	$1.52 * 10^{2}$	7.16 *10 ¹
Coefficient of determination, R ²	0.98	0.96	0.97	1	0.91	1
Correlation coefficient, R	0.99	0.98	0.99	1	0.96	1
Regression kind	Polynomial	Reciprocal	Reciprocal	Polynomial	Reciprocal	Polynomial
	Regression	Quadratic	Quadratic	Regression	Quadratic	Regression
	(degree=2)			(degree=2)		(degree=2)

TABLE 5. Statistical parameters for the correlation of FC, E. coli and HPC with TC under different turbidity conditions

Correlating parameter	FO	2	Е.	E. coli		HPC	
Treatment system	S1	S2	S1	S2	S1	S2	
a	1.68 *10-2	4.85 *10 ²	2.49 *10-2	-3.12 *10 ¹	2.75 *10-3	1.84 *10 ³	
b	-2.62 *10-5	1.41 *10 ³	-3.64 *10-5	2.98 *10-1	-4.09 *10-6	1.38 *10 ³	
С	1.19 *10-8	6.05 *10 ²	1.59 *10-8	-4.54 *10-5	1.93 *10-9	7.66 *10 ²	
Standard error	1.86 *10 ¹	1.37 *10 ¹	1.06 *101	2.34 *10 ¹	6.92 *10 ¹	3.21 *101	
Coefficient of determination, R ²	0.97	1	0.97	0.92	0.96	1	
Correlation coefficient, R	0.99	1	0.98	0.96	0.98	1	
Regression kind	Reciprocal Quadratic	Gaussian Model	Reciprocal Quadratic	Polynomial Regression (degree=2)	Reciprocal Quadratic	Gaussian Model	

correlations under said conditions for the investigating parameters. Relatively high values of coefficient of determinations were observed for each case as compared to the pH dependent correlations equations. The highest RMSE, about 70, was observed for HPC correlation with TC (Table 5) for S1 just as was the case under different weather conditions (Table 4) while the lowest value of coefficient of determination, 92%, was observed for *E. coli* correlation with TC for S2 by using second degree polynomial regression.

CONCLUSIONS

Application of SODIS as point-of-use water treatment is becoming wide spread in many parts of the world. RWH and management have also gained acceptability due to climate change, increased population and depleting water resources. Authors have investigated the efficiency of solar based disinfection systems for the removal of microorganisms from the stored rainwater to improve the bacteriological quality of rainwater for potable purposes. Four microbial parameters including TC, FC, E. coli and HPC were used as indicator organisms for the treatment efficiency by multiple tube fermentation technique (MPN Method) for TC, FC and E. coli and Pour Plate method for HPC. Due to the laborious, time consuming and error prone nature of these methods, correlation equations of FC, E. coli and HPC with that of TC were developed and validated by using statistical tools like RMSE, correlation coefficient and coefficient of determination. TC correlation with that of time and weather, pH or turbidity factors were developed for both SODIS and SOCO-DIS systems and the correlating equations are proposed as a first step. The validity of the proposed equation for correlating TC with time was confirmed by the high values of coefficient of determination usually 93 and 94%. Four kinds of regression including Reciprocal Quadratic, Polynomial Regression (2 degree), Gaussian Model and Linear Regression were used for correlating other microbial parameter with TC. The best fit of the data were observed as noted by the low RMSE and high values of corresponding coefficient of determination in each case. This approach can help reducing the experimental work in similar studies of using sunlight for rainwater disinfection.

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