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Research Article

Estimation of Biochemical Oxygen Demand Based on Dissolved Organic Carbon, UV Absorption, and Fluorescence Measurements

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Determination of 5-d biochemical oxygen demand (BOD_5) is the most commonly practiced test to assess the water quality of surface waters and the waste loading. However, BOD_5 is not a good parameter for the control of water or wastewater treatment processes because of its long test period. It is very difficult to produce consistent and reliable BOD_5 results without using careful laboratory quality control practices. This study was performed to develop software sensors to predict the BOD_5 of river water and wastewater. The software sensors were based on the multiple regression analysis using the dissolved organic carbon (DOC) concentration, UV light absorbance at 254 nm, and synchronous fluorescence spectra. River water samples and wastewater treatment plant (WWTP) effluents were collected at 1-hour interval to evaluate the feasibility of the software sensors. In short, the software sensors developed in this study could well predict the BOD_5 of river water ($r = 0.78$) and for the WWTP effluent ($r = 0.90$).

1. Introduction

The determination of 5-d biochemical oxygen demand (BOD_5) is the standardized experimental procedure to determine the relative oxygen requirements for aqueous microbes to consume organic materials in wastewaters, wastewater treatment plant (WWTP) effluent, or natural waters [1]. BOD_5 has been used as an indicator for the amount of organic pollutants in most aquatic systems, especially a good indicator for biodegradable organic compounds [2]. Due to the 5-d test period, however, BOD_5 is not considered as a suitable parameter for a process control of water treatment processes and for a real-time water quality monitoring system, in which a rapid feedback is essential [3]. The BOD_5-based biodegradation test that relies upon the presence of a viable microbial community has a difficulty in consistently acquiring accurate measurements [4]. BOD_5 generally has an uncertainty of 15%∼20%.

In order to overcome the shortcoming of the conventional BOD_5 test, biosensors, UV-visible spectrophotometry, fluorescence measurements, and software sensor (virtual sensors) have been suggested as an alternative method to determine the BOD_5 of a water sample.

Most BOD_5 biosensors rely on the measurement of the respiratory activity of cells by a suitable transducer. In addition, the ones using an oxygen electrode, a carbon dioxide analyzer, an optical transducer or a microbial fuel cell have recently been reported [5]. Biosensors allow the researchers to conveniently and rapidly (15 minute) obtain the BOD_5 result, compared with the official BOD_5 method [6].

Single or mixed cultures are used in biosensors. Since a single strain is not able to oxidize the entire range of organic contaminants in water samples, and the DO consumption is, thus, not always directly proportional to the concentration of biodegradable organics, mixed cultures like activated sludge have been preferred [7]. Even in the case of mixed cultures, however, the activity of the microbes is easily affected by the changes of environmental condition, such as concentrations of nutrients, temperature, and pH resulting in inaccurate BOD_5 values [8].

Dissolved organic compounds with aromatic structures strongly absorb UV radiation [9]. Based on the principle, the UV-visible spectrophotometry of a water sample is hypothesized to have a linear relation with water total organic carbon (TOC), nitrate, suspended solids (SSs), chemical oxygen demand (COD), BOD_5 or dissolved organic carbon (DOC) [10]. Alternatively, the UV light absorbance at 254 nm has been utilized to directly estimate the aggregate organic content of a water sample [11]. If this approach is to be applied for the BOD_5 determination, target water samples should not contain other light-absorbing chemicals or materials like nitrate or SS [3].

Fluorescence measurements have been applied to determine the presence of humic substances and organic matters in natural waters. Among a few fluorescence analysis methods, synchronous fluorescence spectroscopy is the best way to scan the entire section of excitation wavelengths by fixing the excitation and emission wavelengths uniformly. This method allows obtaining a better resolution and producing various information regarding the DOMs in water [12]. Recently, the method has been successfully applied to identify microbial communities in water and to establish the correlation between water BOD_5 and the microbial activity [4, 13]. Since the BOD_5 test is a microbial assessment of organic substance load, the "microbial" tryptophan-like fluorescence was found correlated with the activity of a microbial community and the absolute BOD_5 values of water samples [4]. The optical parameters of tryptophan-like fluorescence use diverse specific excitation/emission wavelengths: for example, 248 nm/340 nm, 280 nm/350 nm, 220–230 nm/340–370 nm, 220 nm/350 nm, 280 nm/350 nm, and so forth. However, the BOD_5 determination based on the fluorescence peaks obtained from water samples is still infancy. In order to estimate the BOD_5, however, more information should be obtained in addition to the tryptophan-like fluorescence, since real environmental water contains other oxidizable minerals and carbohydrates as well as biodegradable organic matter [14]. Even the water collected near the discharge of an industrial wastewater treatment plant (WWTP) may contain toxic substances such as heavy metals that can inhibit the oxidation of organic compounds by bacteria [15]. Moreover, the water fluorescence is often affected by water pH, temperature, and SS. In fact, the approach has been applied only to wastewater samples, the BOD_5 of which varies wide [2].

Recently, a few researchers have utilized both UV-visible spectrophotometry and fluorescence measurements together to estimate the BOD_5 in waters. Applying the sensors to the environmental monitoring is advantageous since they are rapid and versatile. In addition, they require low operating costs, no chemicals, and no sample pretreatment for measurements. However, their application to water samples can be very limited if the SS concentration of the samples is high [16].

A software sensor (in other words, virtual sensors) generates virtual signals for the water quality parameter of interest through the calculation of a model fed with real signals from reliable, available sensors for other parameters [17]. It rapidly predicts the effect of changes in other water quality parameters on the target parameter. Since the software sensor does not obtain its result from physical measurements, however, the uncertainty associated with its result can be large [3]. Hence, it has been suggested that a software sensor should generate its data based on signals from as many real relevant sensors as possible.

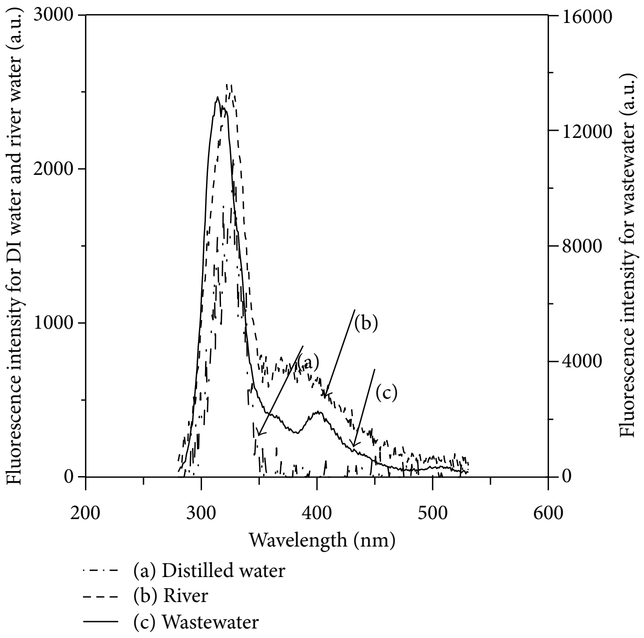


Figure 1: Synchronous fluorescence spectra of (a) DI water (b) river water, and (c) wastewater.

In this study, the synchronous fluorescence spectra, the UV light absorbance at 254 nm, and DOC of water samples were analyzed to predict their BOD_5 values. Since the fluorescence spectra vary depending on the characteristics of DOMs that are site specific in this study, therefore, all the fluorescence spectra of a sample were utilized.

The specific purposes of this study are as follows: (1) the analysis of synchronous fluorescence spectra of river waters and wastewaters, (2) correlation analyses between water BOD_5 and organic parameters (i.e., DOC) and between water BOD_5 and optical parameters (i.e., UV light absorbance at 254 nm, synchronous fluorescence spectra (at 270~300 nm, 310~370 nm, 370~400 nm, and 400~530 nm)), and (3) development of multiple regression models for the BOD_5 prediction using DOC, UV absorbance at 254 nm, and synchronous fluorescence spectra.

2. Experimental

2.1. Sampling Locations and Sample Pretreatment. A total of 23 river samples were collected from the Gyeong-An River which flows through the City of Yong In, Korea, at 1-h intervals. In addition, a total of wastewater samples were collected from the Hwa-Do WWTP in the City of Namyang-ju, Korea, at 1-h intervals. The river samples contained low concentrations of BOD_5, while the wastewater samples contained a wider range of BOD_5. Once the samples were collected, they were stored under refrigerated condition (4°C) and transported to the laboratory, in which they were analyzed immediately. Using prewashed GF/F filters (Whatman, USA; nominal pore size: 0.7 μm), SS was removed from

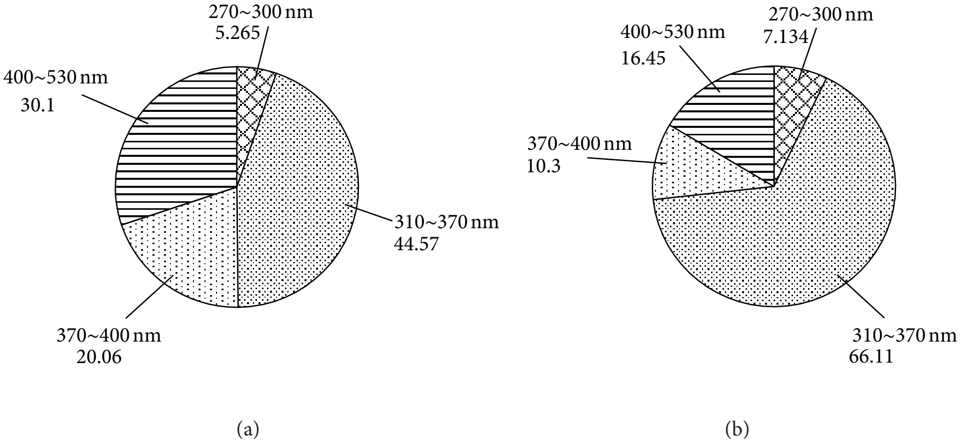


Figure 2: Pie chart of synchronous fluorescence spectra for (a) river water and (b) WWTP effluent.

the samples. SS in water samples often interferes accurate measurements of water quality by scattering light, when the UV spectrophotometry or the synchronous fluorescence measurement is applied to the water samples.

2.2. Analytical Methods. The dissolved organic matters in the filtered samples were measured with an UV spectrophotometer (Shimadzu UV-1800) at 254 nm. The BOD_5 of each sample was determined by calculating the decreased amount of the DO over 5 d (APHA, 2010). The DOC of the samples was calculated by subtracting dissolved inorganic carbon (DIC) from dissolved carbon (DC). The DIC and DC were measured using a TOC analyzer (Shimadzu TOC-V-CPH, Japan). DC concentration of a water sample was determined by combusting a water sample at 680°C in the presence of a platinized alumina catalyst and by measuring the resulting CO_2 production. On the other hand, the DIC of a water sample was determined through the phosphoric acid digestion of the water followed by the determination of the CO_2 production.

The fluorescence spectra of a water sample were measured using a fluorescence spectrometer (Scinco FS-2, Korea). For each sample, synchronous fluorescence spectra for excitation wavelengths ranging from 200 to 600 nm were recorded using a constant offset (i.e., $\Delta\lambda = 30$ nm). The excitation and emission slits were adjusted to 5 nm and 5 nm, respectively. Blank spectrum made by deionized water was subtracted from those of each sample to remove the Raman scattering. The UV-visible spectrum of samples was measured using a UV spectrophotometer (Shimadzu UV-1800). The operating conditions of the spectrophotometer are as follows: a resolution of 2 nm, a response of 0.5 s and a scan speed of 60 nm min^{-1}.

2.3. Development of Multiple Regression Models. The correlation coefficients between the BOD_5 of water samples and the UV light absorbance at 254 nm and between the BOD_5 and fluorescence spectra were analyzed using the correlation function of Microsoft Excel (Microsoft, USA). The multiple regression with the parameters (i.e., DOC, UV absorbance, and fluorescence spectra) for the development of a model to predict the water BOD_5 were carried out using the Data Analysis function of Microsoft Excel.

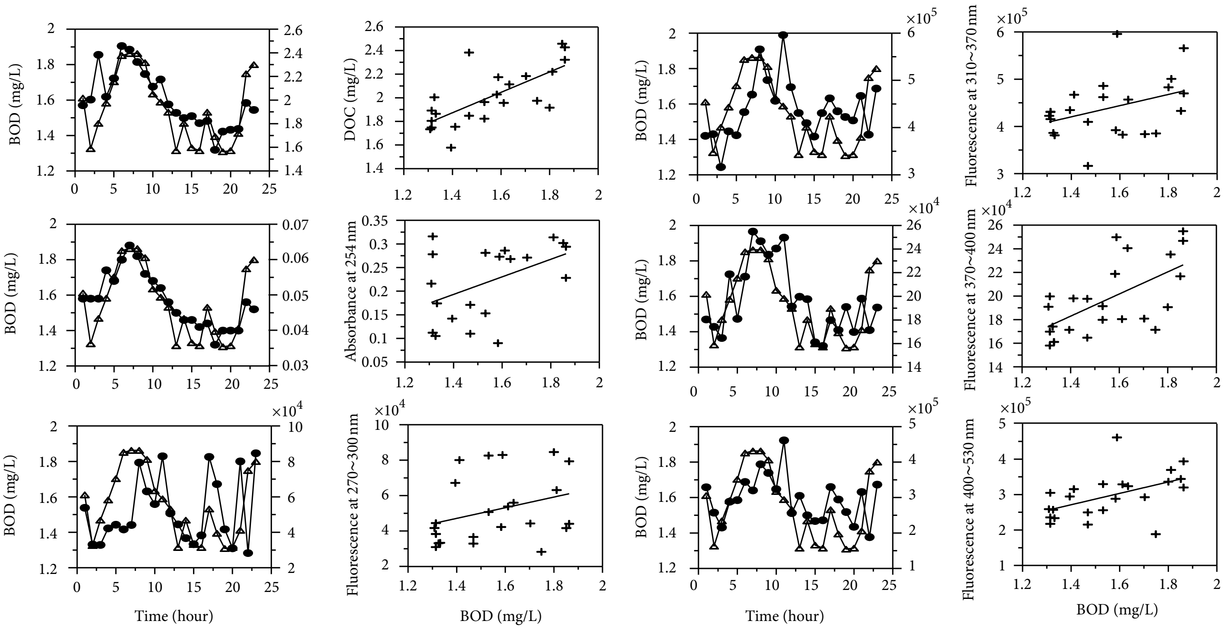
3. Results and Discussion

3.1. Measurement of Synchronous Fluorescence Spectra. In this study, all the spectra obtained at the wavelengths of 270 nm~300 nm, 310 nm~370 nm, 370 nm~400 nm, and more than 460 nm for monoaromatic compounds and tryptophan, diaromatic compounds, fulvic acid, humic acids, and other compounds, respectively, were selected as fluorescence parameters after the synchronous fluorescence spectra of 200~600 nm had been examined. Ferrari and Mingazzini [12] also used these spectra to analyze the compounds in natural DOMs in their study.

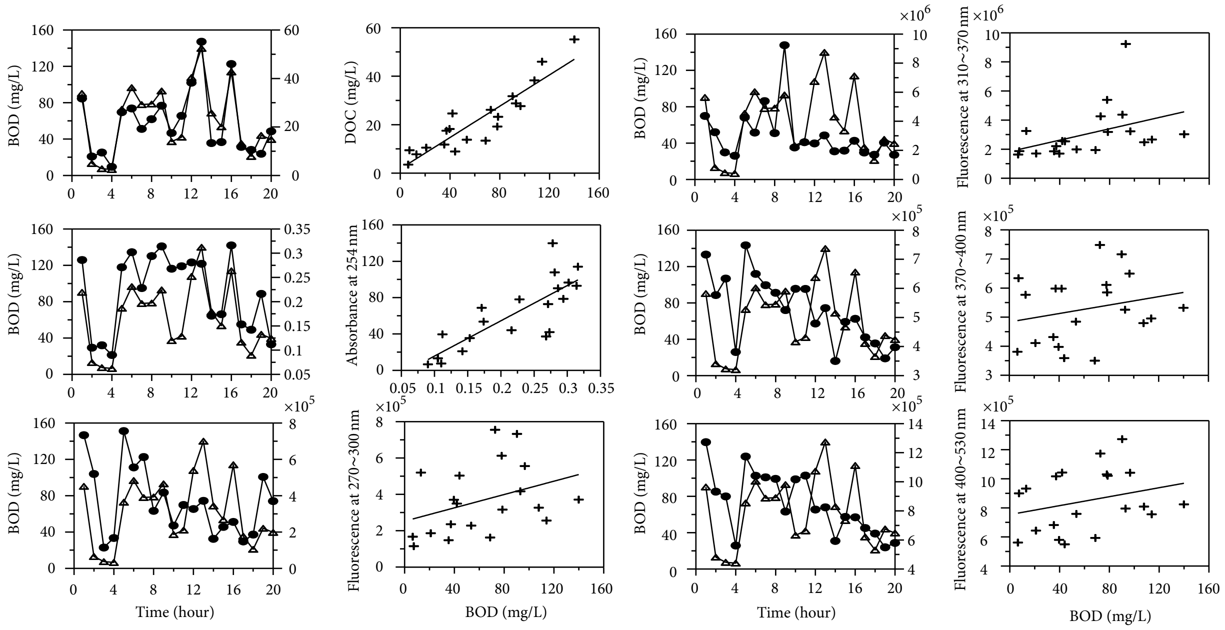
Figure 1 shows the average of the values measured by synchronous fluorescence spectra for a blank, 23 stream waters, and 20 wastewaters. Examining the average spectrum of sample excitation wavelength values by samples, the river waters showed peaks at the wavelengths of 310 nm and 380 nm while the wastewaters showed peaks at 320 nm and 400 nm. A peak at the wavelength of 310~320 nm appeared common for all the water samples including the blank. However, the peak occurring at the wavelength between 350~530 nm appeared common only for river waters and wastewaters.

The fluorescence intensity ratio of river waters to wastewaters is 1 to 5.6. With synchronous fluorescence spectra ($\Delta\lambda =$ 25 nm), a number of compounds present in natural DOM can be identified [12]. Fluorescence spectra of 310 nm ~ 320 nm include naphthol and indoxyl quinoline compounds and 350 nm ~500 nm includes 1-amino-2-naphthol-4-sulfonic acid, fulvic acid, flavin adenine dinucleotide, riboflavin, and humic acid.

To estimate compounds in the DOM of samples, the whole spectrum area obtained for each sample type was divided into four subareas for the excitation wavelengths of 270–300 nm, 310–370 nm, 370–400 nm, and more than 460 nm (Figure 2). The components of the DOMs in river



(a) River waters



(b) Wastewaters

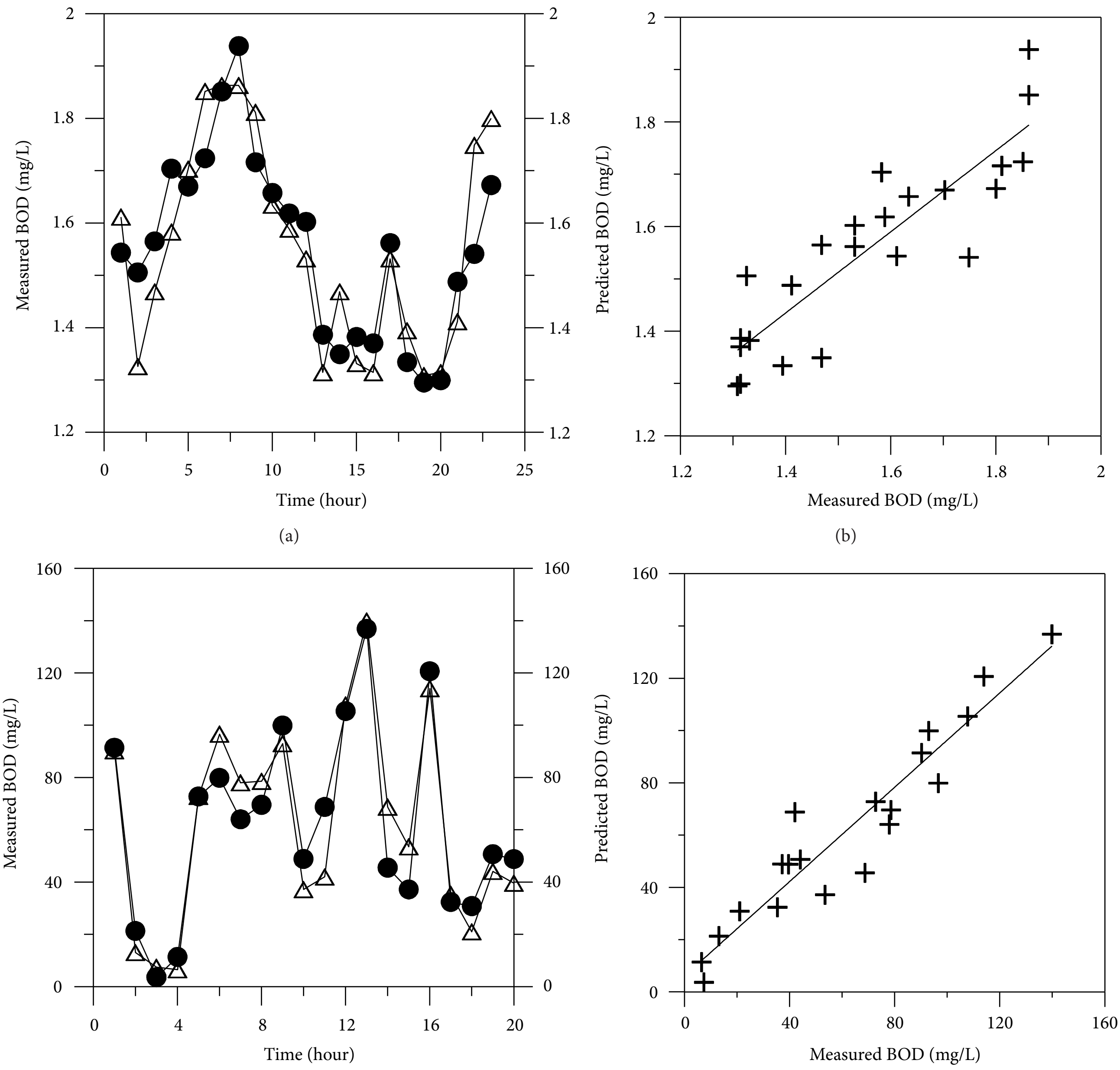
Figure 3: Time profile of BOD_5 and correlation between measured BOD_5 and other parameters for (a) river waters and (b) wastewaters.

waters were estimated in the following order: diaromatic compounds (44.6%), humic acids (30.1%), fulvic acids (20.6%), and monoaromatic compounds and tryptophan (5.3%). Those in wastewaters were estimated in the following order: diaromatic compounds (66.1%), humic acids and other compounds (16.5%), fulvic acids (10.3%), and monoaromatic compounds and tryptophan (7.1%).

3.2. Correlation between BOD_5 and Fluorescence Parameters.

In order to rapidly estimate BOD_5 of a water, the UV spectra [14], the optical scattering (i.e., fluorescence) [18], the UV light absorption at 280 nm [16], COD [15], and so forth, were utilized. These parameters are divided into organic material parameters (e.g., COD, etc.) and optical parameters (e.g., UV light absorbance, fluorescence spectra, etc.). In fact, none of the parameters has been able to successfully predict the BOD_5 of water samples perfectly.

Thomas et al. [14] suggested that organic matter be classified into BOD_5, COD, TOC, and substances absorbing UV light. The BOD_5 is related to oxidizable minerals, carbohydrates, and biodegradable organic matters. The COD is related to oxidizable minerals, carbohydrates,



(a)

(b)

(c)

(d)

Figure 4: Correlation between manually measured BOD_5 and model predictions for (a) river waters and (b) wastewaters.

biodegradable organic matters, and humic substances. The TOC is related to carbohydrates, biodegradable organic matters, humic substances, aromatic hydrocarbons, and aliphatic hydrocarbons. Lastly, the UV light absorption is related to biodegradable organic matters, humic substances, aromatic hydrocarbons, and UV light-absorbing minerals. Since each of COD, TOC, and the UV absorption only identifies some of the organic matter related to BOD_5, the predicted BOD_5 values based on the parameter should be erroneous.

Therefore, this study used both organic parameter and optical parameters to improve the accuracy of the BOD_5 estimation: DOC as the organic parameter, UV absorbance at 254 nm, and fluorescence spectra at 270~300 nm, at 310~370 nm, at 370~400 nm, and at 400~530 nm as the optical parameters (Table 1). The river waters contained 1.3~1.9 mg L^{-1} of BOD_5 and 1.6~2.5 mg L^{-1} of DOC. In addition, 0.036~0.64 of the UV absorbance at 254 nm and 28252~84575 of fluorescence spectrum intensity at 270~300 nm, 381099~595808 at 310~370 nm, 158019~254822 at 370~400 nm, and 188096~461216 at 400~530 nm were observed for the water.

The wastewater samples contained 6.5~140 mg L^{-1} of BOD_5 and 3.5~55.2 mg L^{-1} of DOC. In addition, 0.090~0.310 of the UV absorbance at 254 nm and 114609~755116 of fluorescence intensity at 270~300 nm, 1629452~9233331 at

Table 1: BOD_5 and different parameters measured for river waters and wastewaters.

Sample	BOD_5 mg L^{-1}	DOC mg L^{-1}	Absorbance at 254 nm	Fluorescence intensity (AU)			
				270~300 nm	310~370 nm	370~400 nm	400~530 nm
River waters							
Mean	1.6	2.0	0.049	53600	450202	201142	300654
S.D.	0.2	0.2	0.008	19294	57116	30772	64592
Min	1.3	1.6	0.036	28252	381099	158019	188096
Max	1.9	2.5	0.064	84575	595808	254822	461216
Waste-waters							
Mean	62.0	21.8	0.219	366183	3050004	528135	849118
S.D.	37.9	13.4	0.079	192547	1771048	116813	213009
Min	6.5	3.5	0.090	114609	1629452	350192	549098
Max	139.9	55.2	0.310	755116	9233331	748122	1272352

Table 2: Correlation coefficients between parameters for (a) river waters and (b) wastewaters.

(a) River waters

Parameter	BOD_5 (mg L^{-1})	DOC (mg L^{-1})	Absorbance at 254 nm	Fluorescence at 270~300 nm	Fluorescence at 310~370 nm	Fluorescence at 370~400 nm	Fluorescence at 400~530 nm
BOD_5 (mg L^{-1})	1						
DOC (mg L^{-1})	0.72	1					
Absorbance at 254 nm	0.80	0.91	1				
Fluorescence at 270~300 nm	0.32	−0.05	0.02	1			
Fluorescence at 310~370 nm	0.38	0.15	0.26	0.75	1		
Fluorescence at 370~400 nm	0.62	0.58	0.73	0.41	0.70	1	
Fluorescence at 400~530 nm	0.50	0.37	0.46	0.78	0.80	0.75	1

(b) Wastewaters

Parameter	BOD_5 (mg L^{-1})	DOC (mg L^{-1})	Absorbance at 254 nm	Fluorescence at 270~300 nm	Fluorescence at 310~370 nm	Fluorescence at 370~400 nm	Fluorescence at 400~530 nm
BOD_5 (mg L^{-1})	1						
DOC (mg L^{-1})	0.91	1					
Absorbance at 254 nm	0.81	0.76	1				
Fluorescence at 270~300 nm	0.36	0.27	0.42	1			
Fluorescence at 310~370 nm	0.42	0.30	0.51	0.54	1		
Fluorescence at 370~400 nm	0.24	0.31	0.47	0.61	0.39	1	
Fluorescence at 400~530 nm	0.27	0.32	0.54	0.63	0.37	0.97	1

310~370 nm, 350192~748122 at 370~400 nm, and 549098~1272352 at 400~530 nm were observed (Table 1).

In Figure 3, BOD_5 and other parameters were drawn for (a) river waters and (b) wastewaters to analyze the correlation between the measured BOD_5 and each parameter. Moreover, the time profile of BOD_5 was provided to illustrate its hourly variation.

The correlation coefficients between BOD_5 and the UV absorption or other fluorescence intensity at different wavelengths were obtained for river waters and wastewaters (Table 2). For the river waters, the parameter that has a high correlation with the measured BOD_5 was in order of the UV absorbance at 254 nm ($r = 0.80$), DOC ($r = 0.72$), fluorescence intensity at 370~400 nm ($r = 0.62$), fluorescence

TABLE 3: Summary of model development for predicting BOD_5 of (a) river waters and (b) wastewaters.

(a) River waters

Number in Model	Variables in model	R-square	Adjust R-square	C_P	MSE
1	UV_{254}	0.6322	0.6147	11.10	0.9839
2	DOC	0.5117	0.4885	7.86	1.3062
3	F1, F2, F3, and F4	0.4163	0.2866	0.71	0.3904
4	DOC, UV_{254}	0.6326	0.5959	9.11	0.4914
5	DOC, F1, F2, F3, and F4	0.6636	0.5646	−3.95	0.1500
6	UV_{254}, F1, F2, F3, and F4	0.7770	0.7114	7.00	0.1193
7	DOC, UV_{254}, F1, F2, F3, and F4	0.7770	0.6934	7.00	0.0994

(b) Wastewaters

Number in model	Variables in model	R-square	Adjust R-square	C_P	MSE
1	UV_{254}	0.6538	0.6345	3.29	9441.1632
2	DOC	0.8310	0.8217	7.61	4607.0759
3	F1, F2, F3, and F4	0.2271	0.0210	35.52	5268.8259
4	DOC, UV_{254}	0.8609	0.8445	7.45	1896.5335
5	DOC, F1, F2, F3, and F4	0.8901	0.8509	−3.24	599.2478
6	UV_{254}, F1, F2, F3, and F4	0.7140	0.6118	7.59	1559.9272
7	DOC, UV_{254}, F1, F2, F3, and F4	0.9024	0.8574	7.00	443.5252

TABLE 4: Multiple linear regression models for predicting BODs of river waters and wastewaters.

Sample	Multiple regression model
River waters	$BOD_5\ (\mathrm{mg\,L^{-1}}) = 49.93536 \cdot UV_{254}{}^{a} + 1.23 \cdot 10^{-5} \cdot FI_{270\sim300\,\mathrm{nm}} + 3.32 \cdot 10^{-7} \cdot FI_{310\sim370\,\mathrm{nm}} - 2 \cdot 10^{-06} \cdot FI_{370\sim400\,\mathrm{nm}} - 2.4 \cdot 10^{-06} \cdot FI_{400\sim530\,\mathrm{nm}} + 0.612293$
Wastewaters	$BOD_5\ (\mathrm{mg\,L^{-1}}) = 2.066723 \cdot DOC + 113.2703 \cdot UV_{254}{}^{a} + 2.93 \cdot 10^{-5} \cdot FI_{270\sim300\,\mathrm{nm}} + 1.96 \cdot 10^{-6} \cdot FI_{310\sim370\,\mathrm{nm}} - 0.00011 \cdot FI_{370\sim400\,\mathrm{nm}} + 2.14 \cdot 10^{-5} \cdot FI_{400\sim530\,\mathrm{nm}} + 16.6394$

intensity at 400~530 nm ($r = 0.50$), fluorescence intensity at 310~370 nm ($r = 0.38$), fluorescence intensity at 270~300 nm ($r = 0.32$). In case of wastewaters, the measured BOD_5 had a higher correlation with DOC ($r = 0.91$) and the UV absorbance at 254 nm ($r = 0.81$).

3.3. Multiple Linear Regression Analysis. Multivariate relationships require a multiple regression analysis involving several explanatory variables for predictors of theoretical interest and control variables [18]. Often multivariate regression is applied to predict a variable (i.e., predictor or dependent variable), which is not easily measurable, with other variables (i.e., independent variables), which are easy to measure. For examples, COD, $NH_4{}^-$, and $NO_3{}^-$ concentrations of water samples were predicted by using pH, temp, conductivity, redox potential DO, and turbidity of the same water [19]. Helling et al. [20] predicted the COD/TOC ratio using CO_2 and O_2. Lee and Ahn [21] utilized protein-like fluorescence intensities at 220/350 nm and 633 nm to predict wastewater COD.

If many independent variables are used to explain a dependent variable, the most appropriate regression model should be selected based on the coefficient of determination such as R_P^2 (coefficient of determination of a multiple regression model), R_{adj}^2 (adjusted coefficient of determination), MSE (residual mean of squares), and C_P of Mallows among many models which could be set up by correlating available independent variables to the dependent variable of interest.

A regression model is selected if it increases R_P^2 slows, if it makes the R_{adj}^2 the maximum and MSE the minimum, and if it makes the C_P of Mallows close to $P + 1$ value [18].

To select an appropriate regression model for the BOD_5 prediction, a total of seven models were developed for river waters and wastewaters and presented in Table 3. From Table 3(a), the Model 6 was found to be the most appropriate in predicting the BOD_5 of river waters since it slowly increased R_P^2 and made R_{adj}^2 the maximum. In fact, the Model 7 appeared equivalently appropriate since the MSE of the model was the minimum and it made C_P of Mallows closest to $P + 1$. However, Model 6 was finally selected for predicting the BOD_5 of river waters since it involves fewer variables. By the same token, a total of seven linear regression models were developed to predict the BOD_5 of wastewaters, and Model 7 was selected as the most appropriate model after reviewing the result of analyzing each linear regression models (Table 3(b)).

In Table 4, the Model 6 in Table 3(a), a linear regression for predicting the BOD_5 of river waters was provided. The input variables for the model are the UV absorbance at 254 nm and fluorescence intensities at 270~300 nm, at 310~

370 nm, at 370~400 nm, and at 400~530 nm. The linear regression model for wastewaters (i.e., Model 7 in Table 3(b)) was also provided in Table 4. Its input variables are DOC, the UV absorbance at 254 nm, and fluorescence intensities at 270~300 nm, at 310~370 nm, at 370~400 nm, and at 400~530 nm.

3.4. Validation of Developed Linear Regression Models for Predicting BOD$_5$. The developed multiple regression models for river waters and wastewaters were, respectively, applied to predict the BOD$_5$ of different sets of river water and wastewater samples. The model predictions were then compared with manual measurements (Figure 4). As shown in the figure, the developed multiple regression models could reasonably well predict the BOD$_5$ of the target water samples. The coefficient for the correlation between manually measured BOD$_5$ and model prediction was calculated 0.78 for river waters, while that for wastewaters was 0.90. The relative lower correlation coefficient for river waters was attributed to the fact that the concentration was within the range from 1.3 mg L^{-1} to 1.9 mg L^{-1}; the BOD$_5$ range for the wastewater samples was 6.5 mg L^{-1} ~139.9 mg L^{-1}.

4. Conclusion

In this study, two multiple regression models were developed to predict the BOD$_5$ of two types of environmental waters: one for river waters and the other for wastewaters. The model for river waters predicts BOD$_5$ using the data of the UV absorbance at 254 nm and fluorescence intensities at 270~300 nm, at 310~370 nm, at 370~400 nm, and at 400~530 nm. The model for wastewaters was utilizing the data of DOC, the UV absorbance at 254 nm, and fluorescence intensities at 270~300 nm, at 310~370 nm, at 370~400 nm, and at 400~530 nm. The developed models reasonably well predicted the BOD$_5$ of the river waters and the wastewater samples; correlation coefficients between the model-predicted and manually measured BODs were 0.78 for the river waters and 0.90 for wastewaters.

In fact, the data used for predicting the BOD$_5$ of two types of water samples can be measured using an on-line optical sensors. Therefore, if the BOD$_5$ is estimated using the approach proposed in this study, its measurement can be done rapidly. In addition, this approach can be applied to develop a software sensor for the BOD$_5$ measurement which can be implemented in an on-line water quality monitoring system for streams or WWTP discharges.

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