Songklanakarin J. Sci. Technol.
31 (5), 567-576, Sep. - Oct. 2009

Songklanakarin Journal
of Science and Technology

http://www.sjst.psu.ac.th

Original Article

Treatment efficiency in wastewater treatment plant of Hat Yai Municipality by quantitative removal of microbial indicators

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Received 16 September 2008; Accepted 11 September 2009

Abstract

The efficiency of treatment in a wastewater treatment plant of Hat Yai Municipality through stabilization ponds and constructed wetlands was monitored by using the bacterial indicators, total coliforms (TC), fecal coliforms (FC), *Escherichia coli* and fecal streptococci (FS), and photosynthetic microbes. The sequence of water flow in the wastewater treatment plant is as follows: primary or anaerobic pond (P), facultative pond (F), maturation pond (M), constructed wetlands (W1, W2 and W3), and an effluent storage pond (S for the treated wastewater). The wastewater treatment plant has an approximate area of 3,264,000 m^2 (2,040 rai) and its dry weather flow was running at only 40,000 m^3/ day. There were 10 sampling times used for all the 7 ponds during July-October, 2006.

Statistical analysis using a Two-Factorial Design model, indicated that pond types significantly affected temperature, dissolved oxygen (DO), and pH ($p<0.05$), whereas the time of sampling during the day had a significant effect ($p<0.05$) only on the temperature and light intensity available to the ponds. There were also significant different removal efficiencies of the different bacterial indicator groups tested ($p<0.05$). The overall performance of the wastewater treatment plant effectively removed TC, FC, *E. coli*, and FS as follows, 99.8%, 99.8%, 75.8% and 98.8%, respectively. The amounts of bacterial indicators, except for *E. coli*, showed a negative correlation with levels of light intensity and DO, whereas there was no correlation between the pH and the different indicator bacteria. There was a positive middle level correlation between pH and chlorophyll *a*.

There were five different divisions of photosynthetic organisms detected throughout the plant as follows, Cyanophyta, Chlorophyta, Bacillariophyta, Euglenophyta, and Pyrrhophyta. The least diversity was found in the anaerobic pond (P) as there were only 15 genera. *Euglena,* an indicator of dirty water, was detected only in this pond. The greatest diversity was found in the effluent storage pond (S) with 24 genera and *Peridiniopsis* normally found in fresh water, was only detected in this pond. Increased diversity can be equaled with increased purification.

Keywords: microbial indicator, photosynthetic microbe diversity, stabilization ponds, physicochemical property, removal efficiencies

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1. Introduction

Waste stabilization pond technology is a quite useful natural method for wastewater treatment as it is cost-effective with a high efficiency for removing pathogenic microorganisms. Waste stabilization ponds (WSP) are intensively used for treating domestic sewage in tropical and subtropical countries due to sufficient sunlight and temperatures that are normally key factors for the efficient removal of potential pathogens (Nascimento, 1987). In addition, constructed wetlands (CSW) are man-made systems designed, built, and operated to remediate polluted waters especially for tertiary wastewater treatment and this system has been used in different parts of the world for the successful treatment of agricultural, municipal, and industrial wastewaters (Verhoeven and Meuleman, 1999).

Hat Yai municipality is a major city in southern Thailand with an estimated population of 191,200 residents and a wastewater treatment plant (WTP) consisting of both WSP and CSW. Effluent from the wastewater treatment plant is discharged into Songkhla Lake. Hence, the quality of the treated effluent, particularly its microbiological quality is of considerable concern because the Songkhla Lake is also a major fish farming area and its water is used for crop irrigation and recreation. It has long been known that the discharge of sewage effluents into the environment with the possibility of enhanced pathogen content can have serious consequences by causing threats to the aquatic life and humans.

As pathogens are only intermittently present in the WSP effluent, it is not useful to directly measure them for routine monitoring of water quality. Bacteria are however the most frequently used indicators for the routine monitoring of fecal contamination and therefore the possibility of the presence of pathogens. Total coliforms (TC) and fecal coliforms (FC) are used as indicators of the possible presence of viral or bacterial pathogens in the WSP effluent and receiving waters (Bitton, 2005). Coliform removal efficiencies in wastewater treatment plants are utilized as indicators of the ability of the process to effectively remove pathogenic viruses and bacteria. However, *Escherichia coli* (one member of the FC) or fecal enterococci (formerly named fecal streptococci: FS) are recommended for use as fecal indicators in fresh waters. The presence of specific photosynthetic organisms are also used to indicate the quality of the water, for example, *Oscillatoria* and *Euglena* are indicators for dirty water, while *Pediastrum* is an indicator for clean water (Baruah and Das, 2001). Thus, a study of the photosynthetic microbial communities (algae and cyanobacteria) in the WSP and CSW might establish that photosynthetic microbes can be used as an effective tool to assess the quality of the effluent.

Disinfection procedures in WSP and CSW are natural processes and are therefore inconsistent, as they depend on the physicochemical properties of the water column. As exposure to sunlight is the main factor for disinfection in those systems (Mayo, 1989) pond depth is a critical limiting factor. In addition, the algae population and degree of mixing are factors affecting penetration of solar radiation into the pond water (Moeller and Calkins, 1980). Inactivation of microbes, particularly fecal coliforms by a photo-oxidation mechanism are influenced by environmental variables i.e. pH and dissolved oxygen (DO) that can be governed by the photosynthetic microbial population in the ponds (Curtis *et al.*, 1992). Hence, the aims of this study were to assess the efficiency of the Hat Yai WTP to remove bacterial indicators and to investigate the diversity of the photosynthetic microbial population.

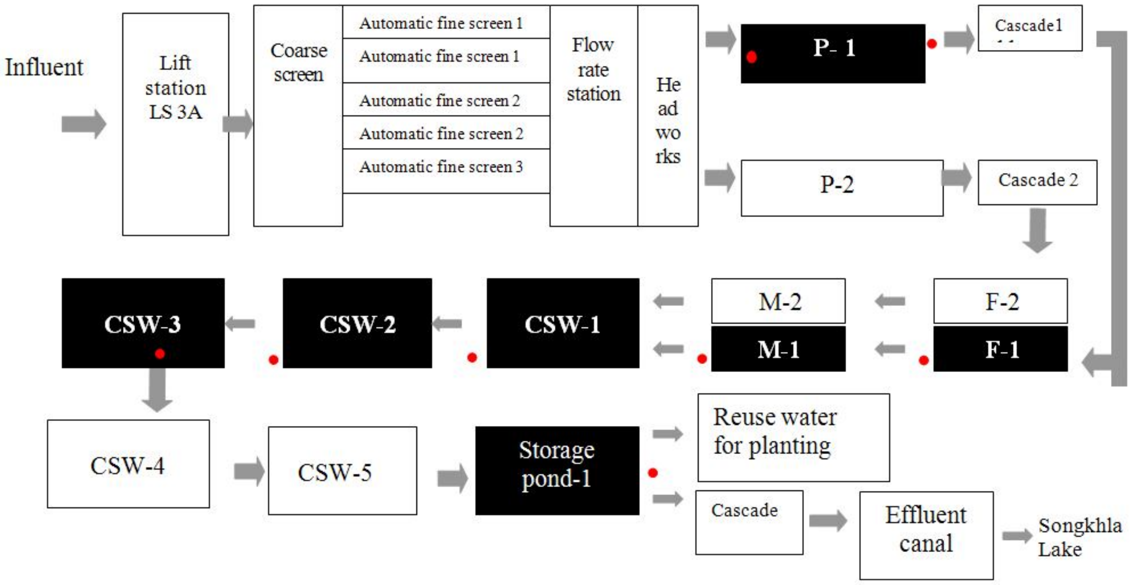
2. Materials and Methods

2.1 Wastewater treatment plant

The wastewater treatment plant of Hat Yai Municipality comprises two series of anaerobic or primary (P), facultative (F), and maturation (M) ponds in parallel and as a final process constructed wetlands (W1-W5) (Figure 1). The wastewater treatment plant has a full dry weather flow capacity of 138,000 m^3/day; however, only 40,000 m^3/day was running in one line of the system during this study (Figure 1; P-1: P, F-1: F, M-1: M, CSW-1: W1, CSW-2: W2, CSW-3: W3 and S). Details of the dimensions of each pond in the system are provided in Table 1. The design volumetric BOD loading rate of P pond was 191.67 g BOD/d-m^2 (100 mg/l x 138,000 m^3/72,000 m^2); thereby the design loading capacity was 13,800 kg BOD/d (191.67 g BOD/d-m^2 x 72,000 m^2). However, during the study period, an average influent BOD_5 (five day BOD) of 45 mg/l into P pond, BOD loading to the system was only 1800 kg BOD/d (45 mg/l x 40,000 m^3/day). The system in the CSW was designed to remove nutrients, especially phosphorus and nitrogen by the use of plants. These wetlands support a diverse wildlife, particularly water birds, and they are used for environmental education and observation. A small amount of effluent from the storage pond is used for watering plants in areas surrounding the wastewater treatment plant (personal communication from wastewater treatment staff member).

2.2 Wastewater sampling and field study

Wastewater samples were collected 10 times during July-October 2006 from each operating pond at a water depth of 30 cm from the water surface at the outlet from each pond; however for the P pond, samples were collected at the inlet and at the outlet of the pond. This study was limited to four months in a period at the beginning of the rainy season in southern Thailand. Hence, it was possible that heavy rain might affect the results obtained. The wastewater samples for laboratory investigations of bacterial indicators, algae diversity, and chlorophyll *a* were sampled between 9.30 am and 12.00 noon, whereas the physicochemical properties were measured in the field for every pond at different time intervals as follows: 9.30-11.00, 12.00-13.55, 14.00-15.55, and 16.00-17.55. Because of the budget and time limitations



Note ● Sampling points are at either the inlet or the outlet of each pond, black color rectangular ponds indicate they were in use during the experimental period, while the white rectangles indicate those that had been closed by controlling the valve at the inlet flow division box.

Figure 1. Flow chart of the wastewater treatment plant of Hat Yai Municipality, consisting of waste stabilization ponds (WSP: P, F, and M) and constructed wetlands (CSW).

Table 1. Dimensions and hydraulic retention time (HRT) of each pond in the wastewater treatment plant of Hat Yai Municipality.

Pond	Surface area (m^2)	Depth (m)	HRT (day)*
P (Primary Pond)	72,000	3.4	6.12
F (Facultative Pond)	220,800	1.7	9.38
M (Maturation Pond)	124,800	1.3	4.06
W1 (Constructed wetland 1)	340,800	0.7	5.96
W2 (Constructed wetland 2)	118,400	1.4	4.14
W3 (Constructed wetland 3)	68,800	1.4	2.41

* A dry weather flow of 40,000 m^3/day was used to calculate HRT. Dimensions and the dry weather flow were provided by an officer of the Wastewater Treatment Plant of Hat Yai Municipality.

for this study, investigations of bacterial indicators, algae diversity and chlorophyll *a* were carried out between 9.30-12.00 as a compromise sampling time because the photosynthetic activities should be occurred closer to 6.00-9.30 am. In addition, bacterial populations in the ponds should be measured closer to night time, and again in the early morning, when they probably reach equilibrium rather than in the afternoon when other variables e.g. light, temperature, DO, etc have an influence. In order to support the above reasons it is well recognized that natural disinfection in WSP is governed by sunlight exposure and photosynthesis by algae as mentioned in the introduction. Hence as sunlight exposure increases in the afternoon the efficiency of destroying bacteria is much higher than in the early morning. Stable bacterial populations will therefore be reached very early in the morning. The algal populations will also be at a more stable level at this time rather than later when they might multiply rapidly. The following parameters, temperature, pH, DO, and light intensity were monitored. A thermometer was used to measure temperature, whereas a pH meter was used to determine pH. DO and light intensity were measured using a YSI Ecosen DO and Denki light meter DK-211, respectively.

2.3 Determinations of microbial indicators

Water samples were collected in sterile screw capped

100 ml glass bottles and carried to the laboratory in an insulated icebox. Microbiological examinations of the samples were conducted as promptly as possible after collection or were stored at 4°C in a refrigerator until use. A multiple tube fermentation technique or most probable number (MPN technique) was used to determine bacterial indicators as follows; total coliforms (TC), fecal coliforms (FC), *Escherichia coli* and fecal streptococci (FS) according to standard methods as described in APHA (1998). The removal efficiency of bacterial indicators was calculated using the following formula:

$$\text{Removal efficiency} = \frac{\text{Number in influent - Numbers in effluent}}{\text{Numbers in influent}} \times 100$$

To examine chlorophyll *a*, wastewater was collected in a 1 liter plastic bottle and placed in a black plastic bag to exclude light and prevent photosynthesis. Chlorophyll *a* was examined by a standard method following APHA (1998), in brief by using a 90% acetone extraction of a filtered sample and measuring its absorption at 750, 664, 647, and 630 nm by a spectrophotometer. To preserve wastewater samples for investigation of photosynthetic microbes, 90% formalin was used. A light microscope was used to identify photosynthetic microbes based on their external appearance, color, and cell shape including cell arrangements using the key book of Bold and Wynne (1985).

2.4 Statistical analysis

Means and standard deviations of each parameter are presented. Two factorial designs were used to analyze the effects of pond types on the physicochemical properties of the wastewater and also the types of ponds and bacterial indicators on the removal efficiency of organisms. There were in total 28 treatment combinations of pond types, with the first factors of (P, F, M, W1, W2, W3, and S), while the second factor was the time period (9.30-11.30, 12.00-13.55, 14.00-15.55, and 16.00-17.55). In addition, a Spearman Rank correlation was used to analyze the correlation coefficients between the numbers of bacterial indicators and the values of the physicochemical properties due to the data distribution not being parametric. Statistical analysis was carried out by the SPSS version 10 for Windows.

3. Results and Discussion

3.1 Effects of pond types and time periods on the physicochemical properties of wastewater

Exposure of wastewater to sunlight has a major effect on the temperature and rate of photosynthesis by photosynthetic microbes. This consequently affects the DO and pH. Therefore, light intensity, temperature, DO, and pH were monitored in each pond. The influences of pond types and sampling times on the physicochemical properties of the waters in the treatment plant are presented in Table 2. Pond type had a significant effect on the following investigated parameters; temperature, DO and pH, while the sampling times had a significant effect only on the temperature and surface light intensity. In general, light intensity and photosynthesis in water ponds depends on the surface light intensity during the day, therefore light measurement at the surface

Table 2. Effects of pond types and sampling times on the physicochemical properties of the wastewater column in the WTP of Hat Yai Municipality.

Factor	Temperature (°C)	Light intensity (Lux)	DO (mg/l)	pH
Pond type				
P	31.50±1.83^{a}	6756±3406^{ns}	1.99±1.89^{de}	7.37±1.18^{b}
F	30.86±1.24^{bc}	6531±3328^{ns}	5.02±3.44^{a}	8.08±1.03^{a}
M	31.08±1.78^{ab}	6149±2923^{ns}	3.39±2.15^{bc}	7.96±1.63^{a}
W1	30.74±0.83^{bc}	6826±3255^{ns}	3.98±3.58^{ab}	7.95±1.42^{a}
W2	29.80±0.60^{d}	5730±3165^{ns}	1.90±1.42^{de}	7.03±0.44^{bc}
W3	29.76±0.43^{d}	5532±3058^{ns}	1.41±0.75^{e}	6.92±0.43^{c}
S	30.29±1.12^{cd}	5779±3311^{ns}	2.85±1.76^{cd}	7.02±0.48^{bc}
Sampling time				
9.30-11.30	30.2±0.86^{b}	7782±3679^{a}	2.63±2.17^{ns}	7.30±0.48^{ns}
12.0-13.55	30.7±1.34^{a}	8785±4024^{a}	3.04±2.71^{ns}	7.54±0.60^{ns}
14.0-15.55	31.0±1.32^{a}	5346±3290^{b}	3.05±2.70^{ns}	7.60±0.67^{ns}
16.0-17.00	30.5±0.96^{ab}	2829±1813^{c}	3.0±2.66^{ns}	7.47±0.94^{ns}

Means ± standard deviations from ten determinations. ns = no significant difference. The different superscripts in the same column indicate a significant difference at $p<0.05$.

by a Denki light meter could reflect the effective light intensity in the water ponds. In addition, it has long been known that the upper layers of pond water absorb light (Hagreaves, 2003) and turbidity decreases the penetration of light into the water column, where collected water samples should be different due to the turbidity. Hence, it was likely that light intensity will rapidly decrease from the surface in these ponds where the turbidity is so high.

Among the ponds, the temperature was in a range of 29.8°C to 31.5°C and the highest temperature was found in the anaerobic pond (P) due to its greater depth and highest organic loading (Table 1 and personal communication from wastewater treatment staff member). The pH values among ponds varied from 6.92 to 8.08. Ponds F, M, and W1 had the highest values of 8.08, 7.96, and 7.95 respectively. DO values between 1.41-5.02 mg/l were found with the highest in the F pond. During the day time investigations, the highest temperature of 31°C was found between 14.00-15.55 hr. The highest light intensity (8785 Lux) was detected between 12.00-13.55 hr while the lowest value (2829 Lux) was found between 16.00-17.00 hr. The DO values ranged from 2.63 to 3.05 mg/l and the pH values ranged from 7.30 to 7.60. It should be noted that all the results in this study were obtained in a period of only four months, therefore for a more complete picture further studies over a year long period should be carried out.

3.2 Removal efficiency of indicator bacteria

The influences of pond types on the numbers of the various bacterial indicators and their final removal efficiency in the wastewater samples are shown in Table 3. Both, the pond types and the group of bacterial indicators tested had significant effects on the rates of reduction. In all cases the biggest reductions occurred between pond P and F; Total coliforms showed the biggest reduction from 294x10^3 to 1x10^3 MPN/100 ml. Besides, *E. coli* was reduced from 7x10^3 to only 18. Although there were minor fluctuations of numbers in the sequence of ponds following F, i.e. ->M-> W1-> W2-> W3-> S; none of the differences were statistically significant (Table 3). There was an indication that some post loading of fecal organism did occur perhaps by exposure to birds.

Comparing the numbers of bacterial indicators in the water of any pond, like P pond, with those of the next ponds in the flow sequence provided an indication of the removal efficiency of each pond. The biggest drop in numbers for all indicator groups occurred from pond P to F, and the F pond was the most effective for removing bacteria (Table 3). Additionally, this pond was also the pond with the highest hydraulic residence time (Table 1) and this is one explanation for the high efficiency of removal of bacteria (discussed later). The overall removal efficiency of indicator bacteria was classified into 5 levels; the greatest in the F pond followed by the P pond, the third in M and W1 ponds, the fourth in W2 and W3 ponds and the fifth in S pond (Figure 2). At the end of the treatment process values for reduction of TC, FC, *E. coli*, and FS were 99.8%, 99.8%, 75.8%, and 98.8%, respectively (Table 3 and Figure 2).

The average numbers of bacterial indicators (MPN/100 ml) in the effluent of S pond prior to discharge into the Songkhla Lake, were as follows, TC: 704±509, FC: 116±117, *E. coli* 74±110, and FS: 232±142. WHO guidelines recommend that an effluent is suitable for unrestricted irrigation when the FC do not exceed 1000 FC per 100 ml (WHO 1989); therefore, this effluent can be safely used for both

Table 3 Effects of types of pond and bacterial indicators on the removal efficiency of different indicator bacteria measured as MPN/100ml in the effluents collected from the WTP of Hat Yai Municipality.

Pond type	Total coliforms (TC)	Fecal coliforms (FC)	*E. coli*	Fecal streptococci (FS)
Influent	294,000±60,222	51,200±14,459	7,098±21,405	19,600±843
P	19,700±5,122^{aA}	2,800±1,033^{bA}	375±654^{cNS}	3,920±169^{cA}
F	1,091±664^{aB}	166±284^{bB}	18±14^{bNS}	108±78^{bB}
M	740±646^{aB}	30±38^{bB}	13±16^{bNS}	550±616^{aB}
W1	1,146±620^{aB}	160±280^{cB}	29±28^{cNS}	531±445^{bB}
W2	1,282±535^{aB}	175±272^{bcB}	42±54^{cNS}	461±519^{bcB}
W3	752±535^{aB}	133±174^{bB}	99±166^{bNS}	432±456^{abB}
S	704±509^{aB}	116±117^{bB}	74±110^{bNS}	232±142^{bB}
% Removal*	99.8±0.19	99.8±0.22	75.8±24.07	98.8±0.77

Means ± standard deviations from ten determinations over a 4 month period. NS = no significant difference. The different lowercase letters in the same row indicate significant differences ($p<0.05$) for effect of bacterial indicators. The different capital letters in the same column indicate significant differences ($p<0.05$) for the effect of pond types. *Overall percentage removal bacterial indicators from P pond to S pond.

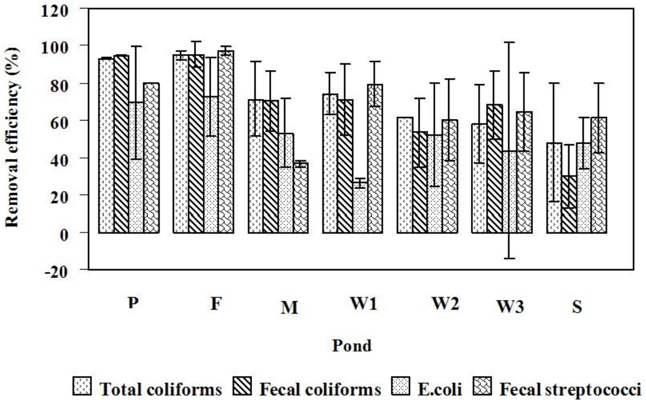


Figure 2. Removal efficiency of bacterial indicators in each pond of the WTP of Hat Yai Municipality (means and standard deviations from 10 sampling times over a period of 4 months).

unrestricted and restricted irrigation. However, this study was involved mainly with biological data and for a complete analysis, chemical properties of the effluent, including soil analysis after using the effluent for irrigation, should be investigated. Thus, it is possible to use the effluent from the S pond for only onsite watering of plants in areas surrounding the Hat Yai WTP. However, the TMDL (total maximum daily load) of discharge water into receiving water such as Songkhla Lake should also be considered. In addition, the best time to discharge the effluent is when the tide was high so that seawater would dilute any pollutants or potential bacterial pathogens.

Amongst the ponds in the WSP, the F pond showed the best average percentage removal of all types of bacterial indicators followed by the P and M ponds. The principal factors involved in bacterial reduction are retention time (Lloyd *et al.*, 2002), the exposure to sunlight (Curtis *et al.*, 1992), and temperature. Turbidity has a large effect on the penetration of light into the water column and therefore a lower turbidity increases the light effectiveness (Qin *et al.*, 1991; Curtis *et al.*, 1992). Photosynthesis depends on photosynthetic microbial mass, a suitable temperature, incident light penetration, nutrient supply, and induced vertical mixing by wind as these are the primary supports for the removal mechanisms at WSP.

It is possible that, P pond could have no DO and no oxygen generating photosynthetic microbes, although in this study occasionally a thin film of mainly *Oscillatoria* could be seen at the surface with a low DO level of < 2 mg/l (Table 4 and 5). However, the P pond did remove bacterial indicators, except for FS with high efficiencies close to that of the F pond (Figure 2). We suspect that this is probably due to sedimentation. Anaerobic ponds are commonly 2-5 m deep and in this study the pond was 3.4 m deep with a hydraulic retention time (HRT) of 6.12 d (Table 1). Potential pathogens attached to sedimenting particles were therefore being constantly removed from the sampling regions.

Although M pond was designed to ensure that the final effluent had the highest bacteriological quality and having as its primary function the removal of all pathogens, the Hat Yai WTP has an additional supplementary system of three constructed wetlands (Figure 1) to facilitate removal of nutrients. It is well recognized that the numbers of pathogens and fecal coliforms are governed by the activity of photosynthetic microbes in synergy with photooxidation (Curtis *et al.*, 1992; Davies-Colley *et al.*, 1994). Amongst the ponds, the average chlorophyll *a* concentration ranged from 64 µg/l to 321 µg/l and the highest value of chlorophyll *a* was always in F pond (data not shown). Therefore the highest removal efficiency of the F pond was generated by photosynthetic microbial photosynthesis (chlorophyll *a*: 321±152 µg/L) and their abundant growth in the facultative (F) pond colored the water dark green. This high rate of photosynthesis in the F pond (Table 2) also produced the highest pH (8.08±1.03), another factor that helped to reduce bacterial indicators.

In this study, ponds that produced effluents with average pH values of approximately 8.0 produced the largest

Table 4. Correlations among the monitored parameters in all investigated ponds during 9.30-11.30 for ten sampling times.

Parameter	Temperature	Light	DO	pH	Chlorophyll *a*	Coliforms	Fecal coliforms	*E. coli*	Fecal streptococci
	r	r	r	r	r	r	r	r	r
Temperature	-	-	0.465**	0.362**	-	-	-	-	-
Light	-	-	-	-	-	-0.230*	-	-	-0.246*
DO	0.465**	-	-	0.493**	-	-0.247*	-0.344**	-	-0.283*
pH	0.362**	-	0.493**	-	0.525**	-	-	-	-
Chlorophyll *a*	-	-	-	0.525**	-	-	-	-	-
Coliforms	-	-0.230*	-0.247*	-	-	-	0.614**	-	0.642**
Fecal coliforms	-	-	-0.344**	-	-	0.614**	-	0.582*	0.571**
E. coli	-	-	-	-	-	-	0.582*	-	-
Fecal streptococci	-	-0.246*	-0.283*	-	-	0.642**	0.571**	-	-

r = Correlation; r > 0.70 (high correlation), r = 0.40-0.69 (medium correlation), r = 0.20-0.39 (low correlation). p = Probability- value, * Significant difference (p<0.05),** Significant difference (p<0.01). - = No correlation.

Table 5. Diversity of photosynthetic organisms collected over 10 sampling times in the various ponds of the WTP of the Hat Yai Municipality (+ = present, - = not detected).

Photosynthetic microbe	P	F	M	W1	W2	W3	S
Cyanophyta							
Anabaena	-	-	-	+	+	+	+
Aphanocapsa	-	-	+	-	-	-	-
Aphanothece	-	-	+	-	-	-	-
Chrorococcus	-	-	+	-	-	-	-
Merismopedia	+	+	+	+	+	+	+
Oscillatoria	+	-	-	+	+	+	-
Planktolyngbya	+	+	+	+	-	+	+
Pseudanabeana	+	-	+	+	-	-	+
Synechococcus	-	-	-	-	+	-	-
Nostoc	-	-	-	+	+	+	+
Microcystis	-	-	-	-	+	-	-
Chlorophyta							
Actinastrum	-	+	-	-	-	-	+
Closterium	-	+	-	+	+	+	+
Coelastrum	+	+	-	+	-	-	-
Cosmarium	+	+	+	+	+	+	+
Crucigenia	-	+	+	+	-	-	+
Crucigenilla	-	-	+	-	-	+	+
Golenkinia	-	+	+	+	+	+	+
Kinchneriella	+	+	+	+	+	+	+
Monoraphidium	+	+	-	-	-	+	+
Staurodesmus	-	-	-	+	-	-	-
Pediastrum	-	+	+	+	-	-	+
Scenedesmus	+	+	+	+	+	+	+
Ankistrodesmus	+	-	+	-	-	-	-
Strarastrum	-	-	-	+	-	+	-
Spirogyra	-	+	+	-	-	-	-
Tetraedon	-	+	+	+	-	-	+
Volvox	-	+	+	-	-	-	-
Pandorina	-	+	-	-	-	-	-
Chlorella	+	+	-	-	-	-	-
Chlamydomonas	-	+	+	-	-	-	-
Dictyosphaerium	+	+	-	+	+	+	+
Euglenophyta							
Euglena	+	-	-	-	-	-	-
Phacus	+	+	+	-	-	+	+
Trachelomonas	-	-	-	+	+	-	+
Bacillariophyta							
Cyclotella	+	+	+	+	+	+	+
Diatom	-	+	+	+	-	+	+
Diatomella	-	-	-	+	+	-	+
Pyrrhophyta							
Peridiniopsis	-	-	-	-	-	-	+
Peridinium	-	-	-	-	+	-	+
Total Genera	**15**	**22**	**21**	**22**	**16**	**17**	**24**

efficiencies for removal of bacterial indicators (Table 2 and Figure 2). Although effluents from both the F and M ponds had a pH about 8.0, F pond was more effective in removing bacterial indicators and the F pond had a higher HRT (9.38 days) than the M pond (4.06 days), so the longer HRT is probably an additional benefit. This is in an agreement with Travieso *et al.* (2006) who reported that an increase of HRT favored the development of photosynthetic organisms, therefore providing better conditions to remove the bacterial indicators. Based on the results of this study the M pond could be modified to improve its pathogen removal efficiency. Bracho *et al.* (2006) have suggested the use of channels in the M pond to increase FC removal and reduce investment and maintenance costs as no additional land will be required.

3.3 Influences of physicochemical properties on removal efficiencies of bacterial indicators

Table 4 shows the statistical analysis for correlations between physicochemical properties and indicator bacteria. TC had a positive middle level correlation with FC and FS ($r = 0.614$, $p<0.01$; $r = 0.642$, $p<0.01$). In contrast, TC had a negative low level correlation with light intensity and DO ($r = -0.230$, $p<0.05$; $r = -0.247$, $p<0.05$). FC were closely correlated with *E. coli* and FS ($r = 0.582$ $p<0.05$; $r = 0.571$, $p<0.01$), and the FC had a low inverse correlation with DO ($r = -0.344$, $p<0.01$). FS had a negative correlation with light intensity and also DO ($r = -0.246$, $p<0.05$; $r = -0.283$, $p<0.05$). It is not surprising that the amounts of TC are related to the amounts of FC and FS because both FC and FS are from the same original fecal source; in addition, some of the FC are be included in the TC group (APHA, 1998). As *E. coli* is one of the members of FC, therefore again the numbers of the FC are closely correlated to the numbers of *E. coli* (APHA, 1998; Bitton, 2005).

With regard to the correlations amongst the physicochemical properties of the water column, temperature had a positive correlations in a middle level with DO and a low level with pH ($r = 0.465$, $p<0.01$; $r = 0.362$, $p<0.01$). Whilst chlorophyll *a* had a positive middle level correlation with pH ($r = 0.525$, $p<0.01$) and the pH also had a positive correlation with DO ($r = 0.493$, $p<0.01$). These correlations could be explained as follows. Sunlight is the energy source for photosynthesis and has a direct effect on water temperature in the ponds. The warm water under sunlight provides a suitable condition for photosynthesis by photosynthetic microbes in the WSP and also in the CSW.

Among the different chlorophylls, chlorophyll *a* is vital for photosynthesis, as it is the pigment used by photosynthetic microbes to obtain energy from light. It is well recognized that the photosynthetic activity of photosynthetic microbes results in a diurnal variation in the concentrations of DO and pH (Curtis *et al.*, 1992). As oxygen is generated from photosynthesis by algae and cyanobacteria in WSP, it result is in an increase of DO. Additionally, the photosynthesis also increases the pH of the ponds due to the removal of carbonate and bicarbonate ions for the growth of photosynthetic microbes, leaving an excess of hydroxyl ions. The F pond that showed the highest level of chlorophyll *a* also had the highest values of pH and DO (Table 2). Consequently, as previously mentioned this pond gave the best removal efficiency of bacterial indicators (Figure 2).

Sunlight exposure is considered to be the most important cause of natural disinfection in the WSP and thus we investigated the effect of DO, pH, and temperature on the WSP effluent on inactivation of indicator bacteria. Survival of bacterial indicators, except for *E. coli,* decreased as the values of DO increased (Table 4). However, pH did not have a significant influence over the range detected (6.92-8.08). Results in this study have also been supported by Davies-Colley *et al.* (1999) who reported that sunlight inactivation of FS is strongly dependent on DO and on light-absorbing WSP constituents, but independent of pH in the range of 7-10. That means that sunlight probably has its major effect as the result of photo-oxidative damage to the cell membrane (Davie-Colley *et al.*, 1997).

The finding that the physicochemical properties investigated in this study had little effect on *E. coli* can be explained as the pH values in the ponds were not high enough to kill these organisms. A pH above 8.5 is required to strongly inactivate the organisms (Davies-Colley *et al.*, 1999). Thus at the lower pH, sunlight inactivation is independent of the WSP constituents and damage is mainly caused by the ultraviolet B in the sunlight that induces internal photo-oxidation damage (Davies-Colley *et al.*, 1999). Based on results in this study, sunlight exposure is the main disinfection factor at WSP and even more effective disinfection could be achieved by increasing sunlight exposure through increasing the residence time in the M pond as previously described or facilitating mixing of the water column. At present the residence time in the M pond is too short at only 4 days yet the main aim in this pond is to remove pathogens.

3.4 Photosynthetic microbe diversity in the WTP

The distribution of photosynthetic microbes in different ponds is presented in Table 5. Various genera belonging to 5 divisions namely, Cyanobacteria (blue green bacteria), Chlorophyta (green algae), Euglenophyta (motile green algae), Bacillariophyta (diatoms), and Pyrrhophyta (dinoflagellates) have been identified at WTP (Salwa *et al.*, 1996; Shanthala *et al.*, 2008; this study). Dinoflagellates were found in pond W2 and S. Dinoflagellates are common in fresh water habitats and sea water (Berman-Frank and Zohary, 1994). Within the recorded 5 divisions there were 40 genera, 11 genera of Cyanobacteria, 21 genera of green algae, 3 genera of motile green algae, 3 genera of diatoms, and 2 genera of dinoflagellates (see details in Table 5). The diversity of the genera in the ponds was in order of S (24) > W1 (22) ~ F (22) > M (21) > W3 (17) > W2 (16) > P (15).

We now know that photosynthetic microbes play an important role in improving the water quality of wastewater in WSP and CSW. It is also expected that the photosynthetic microbe diversity will increase from pond to pond along the sequence due to changes in organic loading that led to the disappearance of some species while others are not affected. Additionally, the depth and surface area of the ponds may affect photosynthetic microbial diversity. Thus, the photosynthetic microbial diversity in the M pond might be expected to be greater than that of the F pond. However, in this study the diversity of genera in the F pond was similar to that of the M pond. This may be due to the depth levels being similar with 1.7 m for the F pond and 1.3 m for the M pond (Table 1). In addition, the F pond has a bigger surface area than the M pond, so this may provide more opportunities for a variety of photosynthetic microbes and the highest amount of DO (5.02±3.44 mg/l, Table 2).

The most common genera detected in every pond of the system were namely cyanobacteria: *Merismopedia*; chlorophyta: *Cosmarium*, *Kirchnerriella* and *Scenedesmus*; bacillariophyta: *Cyclotella*. They were found in every pond of the system because those genera are fairly common in several varieties of water habitats (Bukharin *et al.*, 2001). The algae that tend to predominate in the turbid water of the P pond were the motile genera such as *Phacus* and *Euglena*, perhaps because they could optimize their vertical position in the pond to get optimal exposure to sunlight. Besides, euglenoids exhibit a great deal of adaptability to varying levels of BOD and nutrients (Hosetti and Patil, 1987). *Euglena* was detected only in the P pond because this organism can be considered to have both plant and animal features, which allow it to survive in varying environments.

Other research workers have reported that some cyanobacteria i.e. *Synechococcus* and *Synechocystis* in wastewater treatment plants produce toxic compounds that inhibit *E. coli* and other bacteria (Mezrioui *et al.*, 1994; Oufdou *et al.*, 1998). However, in this study only *Synechococcus* was found in only the W2 pond and it may be one of reasons that the Hat Yai WTP had the least ability to remove *E. coli*. On the other hand, cyanobacteria like *Oscillatoria* and *Microcystis aeruginosa* in WTP produce microcystin, a hepatotoxic peptide, toxic to humans (Ueno *et al.*, 1996; Vasconcelos and Pereira, 2001). Fortunately, both toxic cyanobacteria were not detected in the S pond although some *Oscillatoria* was detected in the following ponds, P, W1, W2, and W3 (Table 5). Based on the result in this study, the presence in the effluent of toxic compounds released by cyanobacteria should be checked before discharge into Songkhla Lake.

The least photosynthetic microbe diversity was found in the P pond (15 genera) as this pond had the highest BOD and degree of pollution. *Pediastrum* was found in the S pond. This algae has a cosmopolitan distribution (Tell and Zamaloa, 2004) and is a genus of green algae that is commonly found in many freshwater microhabitats. Additionally, the dinoflagellate, *Peridiniopsis* sp., was also only found in the S pond. In general, the genus *Peridiniopsis* is a minor blooming dinoflagellate that usually appears in lakes towards the end of the *Peridinium* bloom, because *Peridiniopsis* can compete better under conditions of limited CO_2 (Berman-Frank and Zohary, 1994). The genus *Peridinium* was not detected in the S pond and it was only found in the W2 pond. This result indicates that as the S pond has the largest photosynthetic microbe diversity there may be a limitation of CO_2 in this pond. The results indicate that genera diversity increases with the decrease in pollution. In general, genera diversity is used to indicate a more complex and healthier community because a greater variety of genera allows for more genera interactions hence better stability and indicates better aquatic environmental conditions.

4. Conclusions

Results from this study of the removal of bacterial indicators, particularly *E. coli* in the Hat Yai WTP indicate that the overall efficiency of the maturation pond could be improved to further reduce the presence of possible pathogens by decreasing some of its depths and increasing the residence time. Based on WHO guidelines the present effluent from the storage pond could be used for onsite watering of plants. However, the TMDL of the effluent that is discharged into the Songkhla Lake should be studied to confirm its safety for the ecosystems and health of humans. A check on photosynthetic microbe diversity could be used as an indicator of the treatment efficiency in the wastewater treatment system.

Acknowledgements

This study was supported in part, by the Graduate School, Prince of Songkla University, Hat Yai, Thailand. The authors acknowledge officers at WTP of Hat-Yai Municipality who allowed the collection of wastewater and also provided information about the WTP. We are much indebted to Dr. Brian Hodgson for his critical reading of the manuscript.

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