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ORIGINAL ARTICLE

Use of *Rhodopseudomonas palustris* P1 stimulated growth by fermented pineapple extract to treat latex rubber sheet wastewater to obtain single cell protein

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Abstract Latex rubber sheet wastewater (non sterile wastewater: RAW) was treated efficiently using a stimulated *Rhodopseudomonas palustris* P1 inoculum with added fermented pineapple extract (FPE) under microaerobic light conditions. Optimization of wastewater treatment conditions using a central composite design (CCD) found that a 3 % stimulated P1 inoculum with 0.9 % added FPE and a 4-day retention time (RT) were the most suitable conditions. Calculations from CCD experiments predicted that a chemical oxygen demand (COD) of 3,005 mg/L could be 98 % removed, together with 79 % of suspended solids (SS) and 72 % of total sulfide (TtS). No H_2S was detected, production costs were low and single cell protein (SCP) was a by-product. The results of the verification test had an error of only 4–8 % and confirmed removal of COD (initial COD 2,742 mg/L), SS and TtS at 94 %, 75 % and 66 %, respectively. These values were less than the best set obtained from the CCD experiment (2 % stimulated P1 inoculum, 0.75 % FPE and 4 days RT); upon repeating, this set could reduce 96 % of the COD, 78 % SS and 71 % TtS. The treated wastewater met the standard guidelines for irrigation use and no H_2S was detected. The biomass obtaining after wastewater treatment from the best set consisted mostly of *R. palustris* P1; the biomass of this set had 65 % protein, 3 % fat, 8 % carbohydrate, 14 % ash and 10 % moisture. The results demonstrated that an inoculum of stimulated P1 grew well in RAW supplemented with FPE and could be considered to be an appropriate technology for effectively treating wastewater, with SCP as a by-product.

Keywords Fermented pineapple extract · Hydrogen sulfide · Latex rubber sheet wastewater · Purple nonsulfur bacteria · Single cell protein · Response surface methodology

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Introduction

Nowadays there are many cooperative rubber sheet factories (CRSFs) throughout all parts of Thailand (Kantachote et al. 2010). Wastewater from CRSFs contains organic and inorganic matter including the ammonia, formic acid, sodium metabisulfite and sodium sulfite used during the manufacturing process (Kantachote et al. 2005; Chaiprapat and Sdoodee 2007). Open lagoons or natural oxidation ponds are used for treatment of wastewater, with little attention due to the lack of skilled personnel and budgets to look after the system. The systems are, thus, unable to consistently produce effluent that can comply with industrial effluent or irrigation standards. In addition, serious problems arise from such lagoons due to incomplete oxidation of wastes, causing a rotten-eggs smell

of hydrogen sulfide (H_2S) and emission of greenhouse gases such as methane (CH_4) and carbon dioxide (CO_2) (Nakajima et al. 1997). H_2S gas is toxic to human health at high levels (Yalamanchili and Smith 2008) and causes nuisance odor at low concentrations. Therefore, an appropriate technology for treatment of CRSF wastewater should ideally have low maintenance and operating costs while still being effective. In this regard, the use of indigenous microbes to consume organic matter and sulfides would meet these objectives and could be maintained by CRSFs themselves.

An interesting group of microbes that meet the above requirements is the anoxygenic phototrophic bacteria, the purple nonsulfur bacteria (PNSB), which have been studied extensively for the treatment of various wastewaters as they are versatile organisms able to grow photoorganotrophically under anaerobic and microaerobic light conditions, and chemoorganotrophically under aerobic dark conditions (Kim et al. 2004; Okubo et al. 2006; Lu et al. 2011; Luo et al. 2012). PNSB not only show high efficiency in wastewater treatment while releasing fewer greenhouse gases and less odor, but also produce biomass that can be utilized as single cell protein (SCP) for animal feed or biofertilizer (Kantachote et al. 2005; He et al. 2010; Kantha et al. 2012). The role of PNSB such as *Rhodopseudomonas*, *Rhodobacter* and *Rhodospirillum* to remove the odorous H_2S has been reported (Kim et al. 2004; Belila et al. 2009; Kantachote et al. 2010). However, blooms of PNSB in lagoons of CRSFs are rare, suggesting that stimulating native/indigenous PNSB or applying inoculant PNSB is required. Our previous studies showed that indigenous PNSB (PNSBsi) stimulated by fermented pineapple extract (FPE) were able to treat latex rubber sheet wastewater (RAW) (Kornochalert et al. 2013); however, the efficiency was less than that obtained using inoculant *Rhodopseudomonas palustris* P1 prepared by sterile RAW (Kantachote et al. 2010). Hence, it would be worth investigating the possibility of stimulating the growth of inoculant P1 by FPE in RAW for treating wastewater at lower cost and obtaining SCP as a by-product.

Effective microorganisms (EM) have been used widely in various countries including Thailand since first developed by Dr. Teuro Higa in the 1970s at the University of Ryukyus, Okinawa, Japan (Okuda and Higa 1999). However, few scientific reports have been published on the use of EM although the product has been used heavily in some areas of agriculture and the environment, particularly in wastewater treatment systems (Shrivastava et al. 2012). In Thailand, EM products have been replaced by fermented plant extracts (FPlEs), especially in agricultural applications as farmers can produce FPlEs by themselves (Kantachote et al. 2009) from various organic plant or animal residues. The main products in FPlEs are organic acids (lactic acid and acetic acid), which are the preferred substrates of PNSB (Kantachote et al. 2010). However, high amounts of FPlEs will also increase the organic matter in wastewater; therefore an optimal concentration should be determined for successful wastewater treatment. In addition, FPlEs could be used to stimulate the growth of indigenous PNSB for treating RAW; however, efficiency is limited by the need for a high inoculum sizes for successful treatment (Kornochalert et al. 2013). Hence, in this work, we examined the use of the selected *R. palustris* P1 with growth stimulated by FPE as an inoculum for treating wastewater.

To determine an effective method for wastewater treatment, the concentrations of inoculant P1 and FPE, and retention time (RT) should be optimized. Response surface methodology (RSM)—a collection of mathematical and statistical techniques—is useful for analyzing the effects of several independent variables (Bas and Boyaci 2007). The eventual objective of RSM was to determine the optimal operating conditions for the treatment of CRSFs wastewater, and to determine a region that satisfies the operating specifications, and where the stimulating effect was most effective. The growth of the selected strain, *R. palustris* P1 (P1), was correlated to FPE concentration and RT, with the goal of finally producing P1 cells as SCP alongside achieving wastewater treatment.

Materials and methods

Latex rubber sheet wastewater used

Latex rubber sheet wastewater was collected from a lagoon pond of a CRSF at Pichit in Hat Yai district, Songkhla province, Thailand. The collected wastewater was filtered through cheesecloth into a 25 L plastic tank until nearly full to prevent aerobic conditions and stored in a cold room at 6±2 °C while not in use. Based on our preliminary work, the wastewater was supplemented with 0.05 % (w/v) NH_4Cl as an extra nitrogen source to support microbial growth; specifically, PNSB and this wastewater was used as the medium for all the experiments in this work. The wastewater medium was named RAW because it was not sterilized; therefore indigenous microbes were still present.

Fermented pineapple extract preparation

Fermented pineapple extract (FPE) was used as a medium for preparing the P1 inoculum and also for treating RAW due to the large amount of waste from the coring process of pineapple canning. The FPE was produced in our laboratory (Kantachote et al. 2009). The fermentation process was stopped after 2 months and the FPE was kept in a cold room until use. After 2 months the FPE had no sugar but contained 1.90 % total acidity (0.58 % lactic acid, 0.15 % acetic acid, etc.) with 3.51 mS/cm electrical conductivity (EC) and a pH of 3.61. In addition to nutrients, FPE contained populations of heterotrophs that were counted by a heterotrophic plate count (HPC), lactic acid bacteria (LAB) and yeasts, at roughly 10^6 CFU/mL for each group.

Inoculant preparation

Due to its ability to utilize H_2S, *Rhodopseudomonas palustris* P1 was used to treat latex rubber sheet wastewater (Kantachote et al. 2010). One loopful of isolate P1 from a stab culture was inoculated into a screw cap test tube (20×150 mm: 30 mL) containing 28 mL GM (glutamate-malate) broth (Kantachote et al. 2005), leaving a small space at the top of the medium to provide microaerobic conditions. The culture was incubated with a light intensity of 3,500 lux, generated by a 60 W incandescent lamp for 48 h. The light intensity was measured using a Denki light meter Model DK-211. The culture broth was centrifuged at 6,000 rpm (Sorvall RC 5C Plus, Du-pont, Wilmington, DE) for 15 min and the cell pellet was washed twice with 0.85 % NaCl, then adjusted to obtain a 0.5 OD_{660} nm in sterile distilled water. Distilled water was used instead of NaCl to provide the same conditions between the control and treatment sets with no extra NaCl. With regard to our preliminary work, the optimal conditions for stimulation the growth of the isolate P1 was as follows: RAW in which the initial chemical oxygen demand (COD) had been adjusted to 2,000 mg/L then supplemented with 2 % FPE (v/v); the final pH was adjusted to 7 using 5N NaOH because the addition of FPE decreased the pH value, and a 2 % cell suspension of P1 (v/v) was transferred into the adjusted RAW, and incubated under the same conditions as above for 48 h to yield stimulated P1 inoculum (P1) for treating wastewater.

Analytical methods

The standard methods used in this study are described in APHA (1998). The dichromate reflux method was used to determine the chemical oxygen demand (COD) and the settleable COD was determined by placing all effluent samples including RAW in a cold room for 2 h to allow sedimentation prior to determination of COD. The amount of sulfate was examined by a turbidimetric method. Sulfide in wastewater was measured in three forms—total sulfide (TtS), dissolved sulfide (DsS) and un-ionized hydrogen sulfide (UHS: H_2S)—using an iodometric method. However, H_2S in the air space of the treatment bottles (bioreactors) was measured using a portable multi gas detector (MX 2100, Oldham, France). As settleable COD was measured, the supernatant (clear liquid near the water surface) was sampled and analyzed. Hence, the microbial cells had settled and were not present in the suspended solids (SS) measurement. SS and total dissolved solids (TDS) were determined after filtration using a standard glass fiber filter and then the residue retained on the filter was dried to a constant weight at 103–105 °C to obtain SS while the filtrate was dried to constant weight at 180 °C for determination of TDS. Values of pH and EC were measured using a pH meter (Seven multi, Metler Toledo, Columbus, OH). An oxidation-reduction potential (ORP or redox) probe was used to measure the redox values with the data recorded after obtaining a constant value. The phosphate content of the wastewater was measured photometrically using a test kit (Spectroquant® 1.14842.0001, Merck, Darmstadt, Germany) according to the manufacturer's instructions. Elements such as Mn, Cu and Cd were measured by inductively coupled plasma-optical emission spectroscopy (ICP-OES; 4300 DV, Perkin-Elmer, Uberlingen, Germany). Samples of RAW before and after treatment were analyzed directly and the protocol used for the ICP-OES followed the instruction manual for the instrument.

Total acidity was presented as lactic acid and determined by a titration method, whereas the actual amounts of lactic acid and volatile fatty acids such as acetic acid were determined using gas chromatography according to the method of Yang and Choong (2001). Viable cell counts of PNSB were enumerated by spreading on GM agar and incubating under anaerobic light conditions for 5 days (Kantachote et al. 2005) and were assumed to be the inoculant P1 based on morphology (colony appearance: size and shape) and cell shape under a light microscope after Gram staining. Yeasts, HPC and LAB were counted on potato dextrose agar (PDA), plate count agar (PCA) and de Man Rogosa and Sharp (MRS) agar, respectively, for 3 days. All plates were incubated at 30 °C in an incubator to match the wastewater temperature in the bioreactors. HPC, LAB and yeasts were also counted because they were part of the initial flora of the FPE while HPC were also indigenous microbes found in the wastewater. Proximate analysis was performed according to methods described in AOAC (2000). The moisture content was measured by drying the

samples overnight at 100 °C to constant weight. Crude protein content was determined by the Kjeldahl method, and crude lipid content was determined by the acid hydrolysis method. The ash content was determined by burning samples overnight at 550 °C. The carbohydrate content was calculated from the difference (carbohydrate=100−% protein−% fat −% ash−% moisture) while total energy content was calculated from the sum of energy obtained from the energy sources.

Experimental design and data analysis

Central composite design (CCD) was chosen as the experimental design in this study because this method is suited to fitting a quadratic surface and helps optimize the effective parameters with a minimum number of experiments. It also enabled an analysis of the interaction between the parameters (Montgomery 2001). The independent variables studied were the amounts of stimulated inoculant P1 (X_1), FPE (X_2) and RT (X_3), and the levels of each independent variable investigated (Table 1). These three independent variables together with their respective ranges were chosen based on our preliminary studies (data not shown). The experimental sequence was randomized in order to minimize the effect of light intensity and temperature by the distance from the light source and bioreactors. The CCD consists of 2^n factorial runs with $2n$ axial runs and nc center runs. For each categorical variable, a 2^3 full factorial CCD for the three independent variables, consisting of eight factorial points, six axial points and six replicates at the center point were employed. The total number of experiments with three variables was 20 ($=2^n+2n+6$), where n is the number of independent variables. The center point with six runs was used to determine the experimental error and the reproducibility of the data. To evaluate the efficiency of the wastewater treatment; COD, SS and TtS were the important key parameters for monitoring and were considered as responses, in particular for the latex rubber sheet wastewater. The responses (dependent variables) were reductions of COD (Y_1) SS (Y_2) and TtS (Y_3) with the statistics program. For the three factors, the following equation was used.

Table 1 Experimental range and coded levels of independent variables for treating latex rubber sheet wastewater (RAW). *FPE* Fermented pineapple extract, *RT* retention time, *COD* chemical oxygen demand

Variable	Code	Units	Coded variable levels				
			−α	−1	0	+1	+α
Inoculum P1	X_1	% (v/v)	1	1.5	2	2.5	3
FPE[a]	X_2	% (v/v)	0.5	0.625	0.75	0.875	1
RT	X_3	days	2	3	4	5	6

[a] Low concentrations are designed to prevent any significant increase of the COD in the wastewater

$$Y_N = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_1X_2 + b_5X_2X_3 + b_6X_1X_3 + b_7X_1^2 + b_8X_2^2 + b_9X_3^2 \quad (1)$$

Where Y_N is the predicted response; b_0 intercept; b_1, b_2, b_3 linear coefficient; b_4, b_5, b_6 interaction coefficients, and b_7, b_8, b_9 square coefficient.

The initial COD of RAW used in this study was 3,005 mg/L while the SS and TtS were 42 and 11.11 mg/L. All experimental runs as shown in Table 2 were carried out in 120 mL serum glass bottles (bioreactors) and three replicates were used in each run. All bottles were incubated in microaerobic light conditions as described previously for varying the RT. The parameters COD, SS and TtS were determined. In addition to RSM, ANOVA (Tukey HSD post-hoc test) was also used to analyze data in this work.

Verification test

The CCD results were used to calculate the optimal conditions for levels of FPE, inoculum size of the stimulated P1 inoculum (P1) and RT. Optimal conditions based on CCD calculations were then confirmed. Moreover, the experimental conditions at the center point that produced the highest efficiency for treatment of RAW based on Table 2 were also confirmed in this work and named "best run". A control set without addition of P1 and FPE was included in the experimental design in order to help explain the roles of the treatment sets by using a combination of P1 and FPE according to the calculation and actual results of the best run. After 4 days, the loss of COD, SS, TDS, sulfate ion (SO_4^{2-}), phosphate ion (PO_4^{3-}) TtS, DsS, UHS (H_2S in wastewater) and H_2S in the head space were monitored to assess the efficiency of the treatment. The amounts of HPC, LAB and PNSB (mostly P1) were also enumerated to confirm the efficiency of wastewater treatment with an initial COD of 2,742 mg/L.

Biomass was separated from the effluents of the best set and the control set in this study and weighed. The measured values were used to calculate the biomass yield and also to examine the cell composition. The biomass yield or the cell yield (Yx/s) was calculated based on the consumed COD whereas the cell composition as an approximate analysis

Table 2 Removal of COD, suspended solids (SS) and total sulfide (TtS) from RAW (initial COD 3,005 mg/L) by treatment with a combination of stimulated P1 inoculum and FPE under microaerobic light conditions. Different lowercase letters in each column indicate significant differences with ANOVA (Tukey HSD post-hoc test, $P<0.05$)

Run no.	%P1	%FPE	RT (day)	COD (mg/L)		SS (mg/L)		TtS (mg/L)	
	(X_1)	(X_2)	(X_3)	Actual	Predicted	Actual	Predicted	Actual	Predicted
1	1	0.5	6	307 b	190	18.33 cd	16.53	5.11 h	4.75
2	1	0.5	2	1,895 m	1,837	53.33 h	50.80	7.56 n	7.24
3	2	0.75	7	405 d	445	13.33 ab	13.75	5.11 h	5.10
4	3	0.5	2	1,483 k	1,481	31.67 e	33.19	5.78 j	5.66
5	0	0.75	4	634 g	744	20.00 d	25.12	6.44 m	6.86
6	1	1	2	1,679 l	1,669	40.00 f	37.47	6.00 k	5.88
7	2	1.17	4	810 i	723	18.33 cd	19.61	3.56 c	3.25
8*	2	0.75	4	170 a (13)	169	13.33 ab (0.96)	13.29	3.45 b (0.26)	3.44
9	3	1	2	673 h	781	16.67 bcd	17.36	3.16 a	3.44
10	2	0.75	1	2,215 n	2,188	45.00 g	46.16	6.22 l	6.34
11	3	0.5	6	464 e	465	15.00 abc	16.42	4.44 f	4.48
12	1	1	6	621 f	613	18.33 cd	15.70	5.56 i	5.59
13	4	0.75	4	327 c	229	11.67 a	8.12	4.89 g	4.58
14	2	0.33	4	856 j	956	33.33 e	33.62	4.00 d	4.41
15	3	1	6	307 b	356	11.67 a	13.09	4.22 e	4.46

*The experiment was repeated 6 times and the responses represented average values with their standard deviation in parenthesis

was conducted to assess their biomass as SCP. In order to compare the efficiency of RAW treatment by P1 or PNSBsi, the biomass obtained from effluent treated by PNSBsi from our previous work (Kornochalert et al. 2013) was used to determine biomass yield and also cell composition. Moreover, all completed treatments as mentioned above were also used to evaluate their potential as effluents for use as irrigation water based on Thai standard guidelines (Pollution Control Department 1994; Royal Irrigation Department 1989) and the amounts of heavy metals were also determined. ANOVA (Tukey HSD post-hoc test) was also used to analyze data in this verified test. The mean of three determinations and its standard deviation are reported.

Results

Efficiency of wastewater treatment using FPE to stimulate the growth of the P1 inoculum

In this study, CCD was used to determine the optimal conditions for treating RAW. Actual and predicted values of COD, SS and TtS in wastewater after treatment using varying levels of stimulated P1 inoculum, FPE and RT under microaerobic light conditions are shown in Table 2. The actual data obtained from the experiments were analyzed by multiple linear regression to provide predicted values. Among the experimental runs, the actual values averaged from six runs at the center point to be run no. 8 had the lowest COD of 170 mg/L, while the SS of 13.33 and TtS of 3.45 (in mg/L) were a little higher than in run nos. 9 (TtS, 3.16), 13 and 15 (SS, 11.67), respectively. However, there was no significant difference in SS value in run nos. 8, 13 and 15. The conditions of run no. 8 were : 2% stimulated P1 inoculum, 0.75 % FPE and 4 days RT, while the conditions of run no. 9 were (3 % P1, 1 % FPE and 2 days), run no. 13 (4 % P1, 0.75 % FPE and 4 days) and run no. 15 (3 % P1, 1 % FPE and 6 days) were different. The predicted values of COD, SS and TtS in run no. 8 were 169, 13.29 and 3.44 mg/L (corresponding to a reduction percentage of 94, 68 and 69, respectively). Run no. 8 produced the same removal percentages for COD, SS and TtS in both actual and predicted values. Among runs 8, 9, 13 and 15, run no. 8 was considered to be the best run as the minimal dose of inoculant P1 was used with best removals of COD and SS.

According to actual data, as shown in Table 2, COD values were in the range of 170–2,215 mg/L, while values of SS and TtS were between 11.67 and 53.33 mg/L and 3.16–7.56 mg/L. Design Expert software was used to analyze the relationship of the variables to the responses using the regression model with the significance level $\alpha=0.05$. The P-value is a tool for evaluating the significance and thus quadratic models were appropriate by considering the P-value ($P<0.5$), lack of fit ($P\geq0.05$) and the test statistics (Std. Dev PRESS lower and higher R^2 and adjusted R^2) combination. The F-value was high and P-value was low, which indicated that the model was good. For wastewater treatment conditions, the P-value of

COD reduction; X_1, X_2, X_3, X_1^2, X_2^2 X_3^2, X_1X_2, X_1X_3 and X_2X_3 were less than 0.05 (Table 3). In addition, the *P*-values of dependent variables for SS and TtS that were less than 0.05 can be found in the following equations.

$$\mathrm{COD}: Y_1 = 169.50 - 153.11X_1 - 69.26X_2 - 518.03X_3 - 133.12X_1X_2 + 157.62X_1X_3 + 147.82X_2X_3 + 112.13X_1^2 + 236.87X_2^2 + 405.49X_3^2 \quad (2)$$

$$\mathrm{SS}: Y_2 = 13.29 - 5.05X_1 - 4.17X_2 - 9.64X_3 + 4.37X_1X_3 + 3.12X_2X_3 + 4.71X_2^2 + 5.89X_3^2 \quad (3)$$

$$\mathrm{TtS}: Y_3 = 3.44 - 0.68X_1 - 0.34X_2 - 0.37X_3 + 0.33X_1X_3 + 0.55X_2X_3 + 0.81X_1^2 + 0.81X_3^2 \quad (4)$$

The experimental results were analyzed by regression analysis, which consisted of the effect of linear, quadratic and interactions that provided regression equations for COD, SS and TtS as a function of the stimulated inoculant P1 (X_1), FPE

Table 3 ANOVA analysis for the full quadratic equations; COD, SS and TtS

Source	Degree of freedom	Sum of squares	Mean square	*F*-value	*P*-value (Prob>*F*)
Model (COD)	7.54E+06	9	8.38E+05	6.88E+01	<0.0001
X_1	3.20E+05	1	3.20E+05	2.63E+01	0.0004
X_2	6.55E+04	1	6.55E+04	5.38E+00	0.0429
X_3	3.66E+06	1	3.66E+06	3.01E+02	<0.0001
X_1^2	1.81E+05	1	1.81E+05	1.49E+01	0.0032
X_2^2	8.09E+05	1	8.09E+05	6.64E+01	<0.0001
X_3^2	2.37E+06	1	2.37E+06	1.94E+02	<0.0001
X_1X_2	1.42E+05	1	1.42E+05	1.16E+01	0.0067
X_1X_3	1.99E+05	1	1.99E+05	1.63E+01	0.0024
X_2X_3	1.75E+05	1	1.75E+05	1.43E+01	0.0036
Model (SS)	2.84E+03	9	3.15E+02	2.47E+01	<0.0001
X_1	3.49E+02	1	3.49E+02	2.74E+01	0.0004
X_2	2.37E+02	1	2.37E+02	1.86E+01	0.0015
X_3	1.27E+03	1	1.27E+03	9.95E+01	<0.0001
X_1^2	2.00E+01	1	2.00E+01	1.57E+00	0.2391
X_2^2	3.20E+02	1	3.20E+02	2.51E+01	0.0005
X_3^2	5.00E+02	1	5.00E+02	3.92E+01	<0.0001
X_1X_2	3.12E+00	1	3.12E+00	2.45E−01	0.6312
X_1X_3	1.53E+02	1	1.53E+02	1.20E+01	0.0061
X_2X_3	7.81E+01	1	7.81E+01	6.13E+00	0.0328
Model (TtS)	3.04E+01	9	3.38E+00	2.10E+01	<0.0001
X_1	6.25E+00	1	6.25E+00	3.89E+01	<0.0001
X_2	1.62E+00	1	1.62E+00	1.01E+01	0.0099
X_3	1.85E+00	1	1.85E+00	1.15E+01	0.0069
X_1^2	9.35E+00	1	9.35E+00	5.82E+01	<0.0001
X_2^2	2.73E−01	1	2.73E−01	1.70E+00	0.2212
X_3^2	9.35E+00	1	9.35E+00	5.82E+01	<0.0001
X_1X_2	3.76E−01	1	3.76E−01	2.34E+00	0.1572
X_1X_3	8.60E−01	1	8.60E−01	5.35E+00	0.0432
X_2X_3	2.42E+00	1	2.42E+00	1.51E+01	0.0030

(X_2), and RT (X_3) with each response. The equations were used to predict the removal of COD, SS and TtS values. The fit of the models was further checked by the coefficient of determination, R^2. According to the ANOVA results, the COD model showed a high R^2 value of 98.4 %, which implied that only 2 % variation could not be explained by this model. A higher R^2 value indicated a higher representing capability of the full quadratic equation for COD under the given experimental domain. The adjusted R^2 value of 97.0 % indicated that the model was meaningful and it was in agreement between the actual and predicted values of wastewater treatment. The models of SS and TtS had R^2 values of 95.7 % (adjusted R^2 91.8 %), and 95 % (adjusted R^2 90.5 %), respectively. These results indicate that the accuracy of the polynomial models was good as those equations could be used to predict the value of COD, SS and TtS. However, the polynomial model for COD was selected to use in a verified test as this model gave the highest R^2 and adjusted R^2 values. The results also showed that the stimulated P1 inoculum (X_1), FPE (X_2) and RT (X_3) were the main factors that affected the COD, SS and TtS values. In contrast, the interaction of the terms of X_1X_3 and X_2X_3 was minor.

Design-Expert software was used to build the 3D surface plots shown in Fig. 1 and to analyze the interaction effects of the three variables—inoculum P1, FPE and RT—on wastewater treatment efficiency. This figure shows that COD and SS decreased significantly as influenced by RT (Fig. 1b, c, e, f), but decreased only slightly for TtS (Fig. 1h, i). It was also observed that P1 and FPE individually had less impact on COD, SS, and TtS removal as the response surface did not show much change with their variations (Fig. 1a, d, g). However, there was a strong interaction between P1 and FPE (Fig. 1a). FPE may have stimulated the activity of our inoculum. The TtS contour plot versus inoculum P1 and RT (Fig. 1h) shows the zone of minimum response located in the middle of the figure, suggesting that minimum effluent values of TtS could be found in our parameter ranges. The optimal conditions that minimized COD reduction were calculated by setting the partial derivatives of the function to zero, with respect to the corresponding variables. The optimum condition for COD removal was found at 3 % stimulated P1 inoculum, 0.90 % FPE and 4 days RT. Based on our model, this optimal condition gives the removal of COD, SS and TtS at 98 %, 79 % and 72 %, respectively.

Verification of the model and optimum conditions

Based on the results in Table 2 using a numerical optimization method as previously described (Fig. 1), the optimum operating conditions calculated (3 % stimulated inoculant P1, 0.9 % FPE and 4 days of RT) were confirmed in RAW (initial COD 2,742 mg/L) under microaerobic light conditions (verified test) and the removal percentages for COD, SS and TtS under the designed experiment were 94 %, 75 % and 66 %, respectively (Table 4). It should be noted that the experimental values obtained were in good agreement with the values predicted from the models, with relatively small errors between predicted and actual values, of only 4 %, 5 % and 8 %, for COD, SS and TtS removals, respectively. Therefore, it can be concluded that the generated model has sufficient accuracy to predict the efficiency of rubber sheet wastewater treatment as the error is less than 10 %.

Results of the verification experiments under microaerobic light conditions with optimal prediction of design (verified set) and the best conditions from experimental run no. 8 (Table 2) are shown in Table 5 and Fig. 2. Under the verified set, the removal percentages of COD, SS and TtS were 94 %, 75 % and 66 % as previously described, whereas in the best condition run, the removal percentages of COD, SS and TtS were 96 %, 78 % and 71 %, respectively. Again, removal of 68 % of the sulfate and 32 % of the phosphate were found in the best condition run, but in the verified set removals of sulfate and phosphate were only 66 % and 30 %, respectively. A control set without addition of the stimulated P1 inoculum and FPE produced the lowest efficiency of RAW treatment as the removal percentages for COD, SS, TtS, sulfate and phosphate ions were 45, 24, 31, 24 and 20, respectively. No significant differences were found for the numbers of HPC and stimulated P1 inoculum in the verified set (7.20 and 8.46 log CFU/mL) when compared with the best run (7.18 and 8.41 log CFU/mL). In contrast, for the control set, no PNSB were detected whereas the amount of HPC was higher at 7.68 log CFU/mL. No LAB were found in any treatment sets or the control set. Based on the above results, the best condition run was called the best set and this was studied further for its yield of biomass and cell composition and to consider its use as an SCP.

The amounts of some heavy metals and cations found in the wastewater sets of the control and treatment sets (best set, P1 and PNSBsi) are shown in Table 6. According to the results, only the effluent treated by P1 passed the standard guidelines set by the Pollution Control Department and Royal Irrigation Department, Thailand. However, the PNSBsi-treated wastewater passed in almost all parameters, with the exception of UHS. In contrast, the control set did not pass standard guidelines for levels of COD, SS and UHS. The biomass at day 4 was 865 mg/L for the best set of the stimulated P1 inoculum, and the corresponding wastewater COD removal was 2,626 mg/L. Hence, the calculated cell yield was 0.33 (Table 6). A lower cell yield was obtained (0.30) in the PNSBsi set, but a higher cell yield (0.42) was found in the control set. The biomass obtained after 4 days treatment of RAW had the maximum protein content in the biomass from the best P1 treatment followed by the biomass from a control set; the lowest biomass was from PNSBsi (Table 6). The biomass from the best P1 condition set had

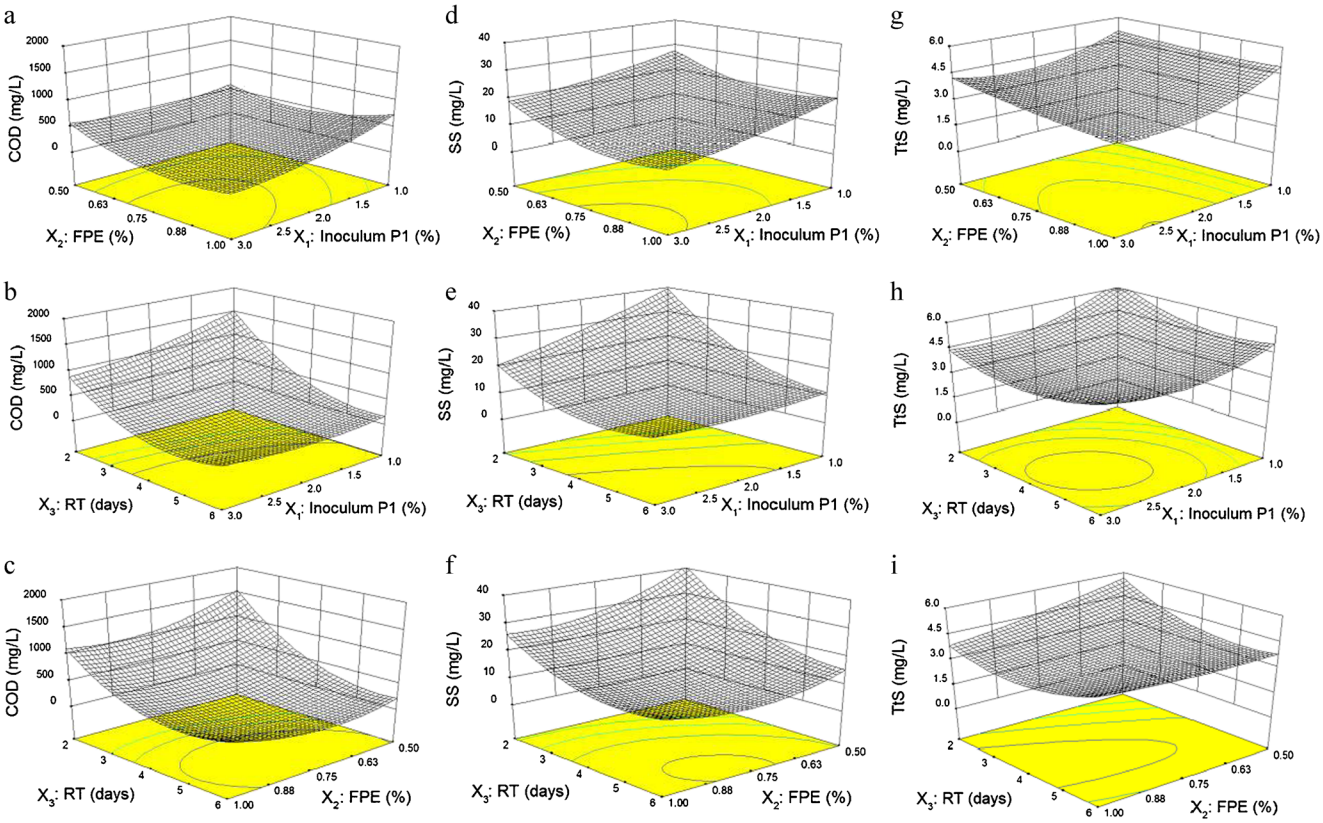

Fig. 1 Three-dimensional (3D) response surfaces illustrating the values of chemical oxygen demand (COD) (**a**, **b**, **c**); suspended solids (SS) (**d**, **e**, **f**) and total sulfide (TtS) (**g**, **h**, **i**) as functions of inoculum P1, fermented pineapple extract (FPE) and retention time (RT). Each graph displays the interaction effect of two variables while the third variable was fixed at its central level shown in Table 2

65 % protein, 8 % carbohydrate, 3 % crude fat, 14 % ash, 10 % moisture and 319 kcal (see details of cell composition of other biomass in Table 6).

Discussion

According to the CCD experiment, run no. 8 (Table 2) provided the best conditions for treating RAW based on the COD and SS removals by using a 2 % stimulated P1 inoculum, 0.75 % FPE and 4 days RT. The result of using the stimulated P1 inoculum and the 2 % FPE with the non sterile wastewater (RAW) resulted in the inoculant P1 becoming the major bacterium involved in the treatment process. It is not surprising that the P1 inoculum became the dominant organism both in the preparation of the inoculum and in the RAW treatment due to the provision of the most suitable conditions for its growth as a photoheterotroph under microaerobic light conditions. To explain why FPE stimulated the growth of PNSB but had little effect on HPC in RAW, our previous studies had clearly shown that the FPE contained organic acids that stimulated the growth of PNSB including the isolate P1 under low dissolved oxygen (DO) and the availability of good light (Kantachote et al. 2010; Kornochalert et al. 2013). This is because the role of FPE stimulating the growth of PNSB under light condition is to lower the ORP value and to provide reducing conditions. In our previous study (Kornochalert et al. 2011), the ORP value reportedly was the most effective parameter for stimulating growth of PNSB, and was −340 mV in the treatment with 2.5 % FPE and 235 mV in the control set (RAW). This proved that the lactic acid and acetic acid in the FPE were being used preferentially as electron donors for photosynthesis in the partially anaerobic light conditions. These results are in agreement with those of Okubo et al. (2006), who reported that the lower chain length fatty acids such as acetate and propionate in a swine wastewater ditch stimulated the growth of PNSB, particularly *Rhodopseudomonas* and *Rhodobacter* spp., to form visible microbial mats. One explanation of why microaerobic

Table 4 Verification test based on optimal conditions (3 % inoculum P1, 0.9 % FPE and 4 days RT) predicted by central composite design (CCD) for treating RAW (initial COD 2,742 mg/L) under microaerobic light conditions

Removal (%)	Experimental	Predicted	Error (%)
COD	94	98	4
SS	75	79	5
TtS	66	72	8

Table 5 Efficiency of RAW treatments using a combination of FPE and stimulated P1 inoculum under microaerobic light conditions. Different lowercase letters in the same row indicate a significant difference ($P < 0.05$). *TDS* Total dissolved solids, *DsS* dissolved sulfide, *UHS* un-ionized hydrogen sulfide, *HPC* heterotrophic plate count, *LAB* lactic acid bacteria, *PNSB* purple nonsulfur bacteria

Parameter (mg/L)	Property	Percentage reduction		
	[RAW]	Control	0.9 % FPE+3 % P1 (verified set)	0.75 % FPE+2 % P1 (best set)
	T=0	Day 4	Day 4	Day 4
COD	2,742±11	45.3 a	94 b	96 b
SS	42±3	23.8 a	75 b	78 c
TDS	540±20	18.5 a	55 b	56 b
Phosphate	185±1	20 a	30 b	32 b
Sulfate	5.0±0.2	24.0 a	66 b	68 b
TtS	13.11±0.38	30.5 a	66 b	71 c
DsS	12.22±0.38	21.2 a	63 b	66 c
UHS	5.38±0.17	57.4 a	96 b	94 b
H_2S	8±2	100	100	100
Numbers of organisms (log CFU/mL)				
Parameter	T=0	Day 4	Day 4	Day 4
pH	7.03±0.01	7.46 a±0.03	8.15 b±0.01	8.07 b±0.01
HPC	8.15±0.02	7.68 b±0.03	7.20 a±0.01	7.18 a±0.02
LAB	0	0	0	0
PNSB (P1)	0	0	8.46 a±0.01	8.41 a±0.02

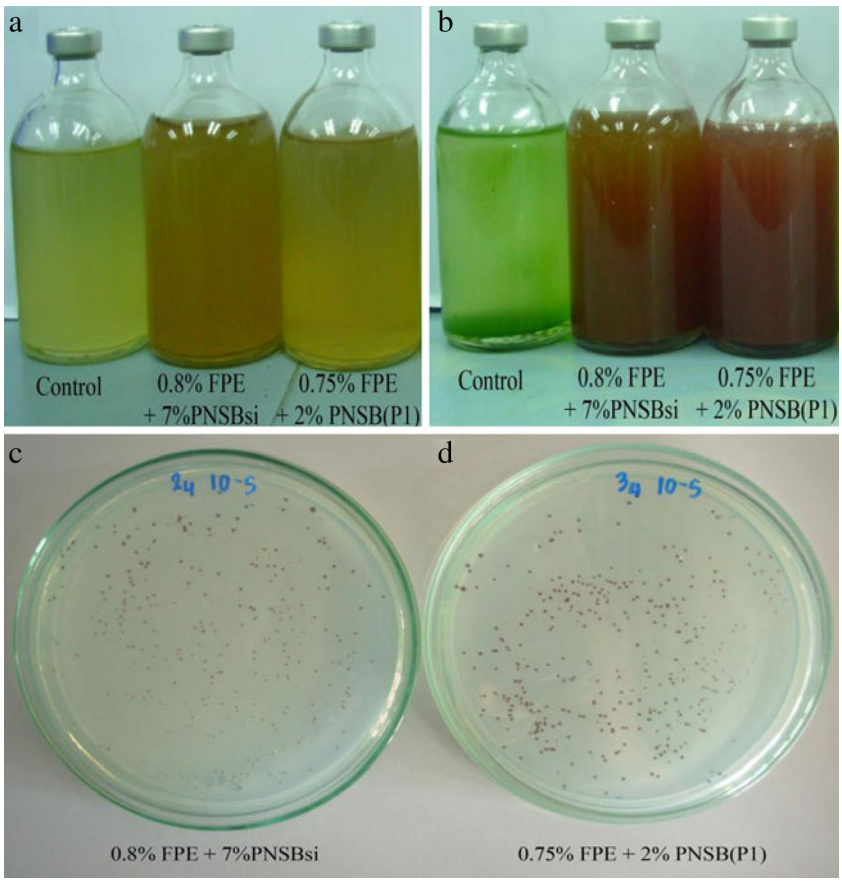

Fig. 2 Photographs showing the treatment process for latex rubber sheet wastewater (RAW) using a combination of stimulated indigenous purple nonsulfur bacteria (PNSBsi) or stimulated inoculant of P1 (P1) and FPE with optimal conditions under microaerobic light conditions (**a**) at the start of the experiment, (**b**) at the end (day 4), (**c**) PNSB from a set of PNSBsi at day 4 and (**d**) PNSB from a set of P1 that was presumed to be isolate P1

Table 6 Proximate analysis of biomass obtained from effluents after 4 days treatment by a combination of stimulated P1 inoculum or stimulated indigenous PNSB with FPE under microaerobic light conditions. Different lowercase letter in the same row indicates a significant difference ($P<0.05$)

Parameter (mg/L)[a]	Control	7 % indigenous PNSB+0.8 %FPE (PNSBsi)	2 % inoculant P1+ 0.75 %FPE (P1)	Guideline level[b], [c]
pH	7.46±0.03[a]	7.35±0.02[a]	8.07±0.01[b]	5.5–9.0, 6.5–8.5
COD	1,500±10[c]	239±11[b]	116±19[a]	120–400, na
SS	42±3[b]	11±3[a]	10±2[a]	≤50, ≤30
UHS	2.29±0.07[c]	1.23±0.09[b]	0.32±0.01[a]	≤1.0, ≤1.0
Cd	0.001±0.000	0.004±0.001	0.001±0.000	≤0.03, ≤0.03
Cr (Hexavalent)	0.001±0.000	0.003±0.001[a]	0.001±0.000	≤0.25, ≤0.30
Pb	<0.0001	< 0.0001	< 0.0001	≤0.2, ≤0.1
Mn	0.199±0.001	0.174±0.001	0.246±0.001	≤5, ≤0.5
Cu	0.007±0.001	0.018±0.001	0.005±0.001	≤2, ≤1
Zn	0.016±0.001	0.058±0.001	0.012±0.001	≤5, ≤5
Effluent quality	Exceed	Nearly pass	Pass	
Biomass (Yx/s)	0.42[c]	0.30[a]	0.33[b]	na[d]
% Protein	64.4[b]	55.5[a]	64.7[b]	na
%Carbohydrate	4.5[a]	11.2[c]	8.0[b]	na
% Fat	0.8[a]	12.6[c]	3.1[b]	na
% Ash	23.3[c]	17.1[b]	14.1[a]	na
% Moisture	7.0[b]	3.6[a]	10.1[c]	na
Energy (kcal)	283[a]	380[c]	319[b]	na

[a] Unless otherwise stated

[b] Criterion of the Pollution Control Department

[c] Criterion of the Royal Irrigation Department, Thailand

[d] Not available

light conditions promote PNSB growth is that these organisms are anoxygenic photosynthetic bacteria; this is due to the PNSB exhibiting higher oxygen tolerance and being able to perform aerobic respiration at full atmospheric oxygen tension (Okubo et al. 2005), unlike purple sulfur bacteria, which fail to grow in the presence of even a low concentration of oxygen.

Based on the removal efficiencies of COD and SS, the RT had the biggest influence (Eqs. 2, 3) due to the organisms having enough time to hydrolyze and consume nutrients (COD and SS) in the wastewater, and 4 days RT was confirmed to be the optimal time for PNSB including *R. palustris* P1 to treat RAW (Kantachote et al. 2005, 2010; Kornochalert et al. 2013). However, the RT varied depending on the type of wastewater and PNSB present in the wastewater systems. For example, treating pharmaceutical wastewater by PNSB under microaerobic light conditions took 3–5 days (Madukasi et al. 2010). However, treatment of swine wastewater with an initial COD of 18,700 mg/L by *R. palustris* required 6 days to reduce the COD by 90 % (Kim et al. 2004). With regard to H_2S removal, the stimulated P1 inoculum was the key factor to reduce all forms of sulfide in RAW (Eq. 4, Fig. 1g–h). This is due to sulfide also being used as an electron donor for photosynthesis by PNSB, including isolate P1. The results were supported by our previous work showing that isolate P1 can use sulfide under good microaerophilic light conditions (Kantachote et al. 2010). In addition, *Rhodobacter* and *Rhodopseudomonas* are able to use inorganic electron donors such as sulfide or H_2 as reductants for $NAD(P)^+$ enabled by redoxactive enzymes that are able to accept electrons from these substrates and subsequently donate them to the cyclic electron transport chain (Sinha and Banerjee 1997).

This study has demonstrated clearly that the stimulated P1 inoculum performed highly effectively to treat CRSF wastewater with the complete removal of any odor of H_2S as there was no detection of this gas in the head space of the bioreactors (Table 5). This is because light was applied for treating RAW under microaerobic conditions, which quickly became anaerobic conditions based on redox values (−50 to −110 mV) in the wastewater systems (data not shown). These conditions allow organisms, either microalga in the control set or PNSB in the treatment sets, to grow as photoautotroph/photoorganotroph in RAW and this sulfide may be used as an electron donor by PNSB. In addition, as H_2S was not detected in the head space, this was also related to the pH values as the pH altered the sulfide (<7: H_2S, 7–8: HS^- and> 8: S^-) (Markl 1999). Hence, any H_2S in the head space was changed to HS^- and S^- due to the pH of the wastewater being higher than 7 in both the control and treatment sets (Table 6)

and this might be a reason why no H_2S was detected in the control set with the microalgae.

Based on the results of Table 5, the use of 0.75 % FPE and 2 % stimulated P1 inoculum led to higher efficiency treatment of RAW than that found in the verification set with 0.9 % FPE and a 3 % stimulated P1 inoculant. It might be that the former condition was more suitable than the latter in the case that the initial COD that was 2,742 mg/L and this indicated that inoculums of only 2 % allowed the P1 inoculum to become the dominant organism (8.41 log CFU/mL), as was also the case for the 3 % inoculum size (8.46 log CFU/mL). Moreover, the use of a lower inoculum size meant that a lower amount of FPE was needed for stimulating growth. In this work, the stimulated P1 inoculum was prepared in non-sterile wastewater (RAW) and it was claimed to be the dominant organism as previously described; this was supported by the evidence from checking morphology on a GM agar plate and cell shape (data not shown). Again, when using P1 for treating non-sterile wastewater, the same kind of colonies were found on GM agar (Fig. 2d) and rod-shaped cells were observed, although the colonies were no different from other PNSBsi (Fig. 2c). In this work, a 2 or 3 % inoculum of P1 added to the RAW as previously described was able to compete with other microbes by using the RAW (initial COD 2,742 mg/L) with a high efficacy similar to the inoculum P1 prepared with sterile RAW to treat RAW with an initial COD of 1,457 mg/L (Kantachote et al. 2010) due to the effluent meeting the standard guidelines set by the Pollution Control Department and Royal Irrigation Department, Thailand.

In addition, the stimulated P1 inoculum with lower inoculum size, such as only 2 or 3 %, performed with a higher efficiency to treat RAW than 7 % PNSBsi (Kornochalert et al. 2013) (Table 6). This supports the concept that most of the organisms in the stimulated inoculant P1 were *R. palustris* P1; this isolate is a useful strain previously isolated from latex rubber sheet wastewater (Kantachote et al. 2010). Therefore, it could compete with other microbes in both the preparation of an inoculum and for highly efficient treatment of wastewater. The phosphate removal results (Table 5) indicated that almost all the organisms found in the stimulated P1 inoculum are likely to be isolate P1 as it has a higher efficiency (32 %) to remove phosphate when compared with the control set that produced only a 20 % reduction. This is in accordance with Liang et al. (2010), who reported that PNSB found in activated sludge has the potential to accumulate phosphorus and thereby, to remove phosphorus from the wastewater. According to the above results, a study using molecular methods of the dynamics of bacteria during wastewater treatment should be conducted in the future for a better understanding of the efficacy of the system.

The lowest efficacy of treating RAW was observed in a control set as this set had no additions of either FPE or stimulated P1 inoculum. In the control set, microalgal growth appeared as a green color while the treatment sets had the red color of PNSB (Fig. 2a,b). This can be explained by the lower level of nutrients in the control set under light conditions with microaerobic conditions stimulating microalgal growth. Moreover, this is also the reason why the green color observed in the control set was from microalga not purple sulfur bacteria because the latter organisms cannot survive in O_2. This result was in agreement with previous work that had shown that low nutrients under light condition with a little O_2 supports the growth of microalga (Valderramaa et al. 2002; Kornochalert et al. 2013). LAB were not detected in any sets (Table 5) although LAB are microaertolerant; it is presumed that the initial numbers of LAB in the wastewater that came from FPE were low as only a small volume was used and substrates in the RAW were not suitable for supporting LAB growth when compared with PNSB.

The amounts of heavy metals such as Cd, Cr and Pb were very low in the wastewater treated by PNSBsi or P1, including the control set (Table 6); however, the PNSBsi effluent had a slightly higher amount of UHS than the acceptance level. A lower efficiency of UHS removal in the PNSBsi-treated set might be caused by the lower pH (7.35) compared to the value of 8.07 in the P1 set, as the amount of PNSB was not significantly different (Tables 5, 6). This also indicated that most of the PNSB in the P1 set was isolate P1 because of the higher pH being attributed to the active consumption of sulfide when compared with the PNSBsi. Therefore, only P1-treated wastewater can be considered for use as irrigation water for agriculture, particularly in the dry season. In addition, the PNSB biomass obtained should be considered for use as SCP for animal feed as previously mentioned. Among the effluents, the control set produced maximum biomass yield. The highest biomass yield (Yx/s=0.42) was found in the control set was due to the growth of the microalgae, and this also had the highest HPC (Table 5). In general, the biomass yield of HPC is in the range of 0.45–0.77 (Majone et al. 1999), whereas the biomass yield of PNSB such as *Rhodobacter sphaeroides* Z08 grown in soybean wastewater was 0.28 (He et al. 2010). However, in this study, a higher biomass yield of 0.30 was obtained for the PNSBsi and 0.33 for the P1 set. The amount of crude protein was 65 %; 56 % was found in the biomass of effluents in the best condition set with the use of *R. palustris* P1 and PNSBsi could be considered as SCP. The results of our work are in accordance with those of Honda et al. (2006), which showed that the crude protein of PNSB biomass in mixed culture was between 56 % and 68 %, whereas Kantachote et al. (2005) using *R. blastica* DK6 to treat RAW under microaerobic light conditions produced biomass with 65 % crude protein. However, treatment of soybean wastewater by *Rhodobacter sphaeroides* Z08 under natural conditions produced a biomass of 52 % crude protein (He et al. 2010). This is because the amount of the crude protein depends on the organism used.

Conclusions

Application of PNSB for treating RAW could be possible as this study has successfully developed appropriate technology by using FPE to stimulate the growth of *R. palustris* P1 under light conditions first to use as an inoculum for treating RAW under anaerobic treatment with high efficiency and with no detection of H_2S. The RAW treatment with P1 not only produced effluent that met the standard guidelines for use as irrigation water but also obtained a biomass as a by-product to utilize as SCP.

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References

AOAC (2000) Official methods of analysis of AOAC international, 17th edn. AOAC International, Gaithersburg

APHA (1998) Standard methods for the examination of water and wastewater, 20th edn. American Public Health Association, American Water Works Association (AWWA), and Water Environment Federation (WEF), Washington DC

Bas D, Boyaci HI (2007) Modeling and optimization I: usability of response surface methodology. J Food Eng 78:836–845

Belila A, Gtari M, Ghrabi A, Hassen A (2009) Purple anoxygenic phototrophic bacteria distribution in Tunisian wastewater stabilisation plant exhibiting red water phenomenon. Ann Microbiol 59: 399–408

Chaiprapat S, Sdoodee S (2007) Effects of wastewater recycling from natural rubber smoked sheet production on economic crops in southern Thailand. Resour Conserv Recyl 51:577–590

He J, Zhang G, Lu H (2010) Treatment of soybean wastewater by a wild strain *Rhodobacter sphaeroides* and to produce protein under natural conditions. Front Environ Sci Eng China 4:334–339

Honda R, Fukushi K, Yamamoto K (2006) Optimization of wastewater feeding for single-cell protein production in an anaerobic wastewater treatment process utilizing purple non-sulfur bacteria in mixed culture condition. J Biotechnol 125:565–573

Kantachote D, Torpee S, Umsakul K (2005) The potential use of anoxygenic phototrophic bacteria for treating latex rubber sheet wastewater. Electron J Biotechnol 8:314–323

Kantachote D, Kowpong K, Charernjiratrakul W, Pengnoo A (2009) Microbial succession in a fermenting of wild forest noni (*Morinda coreia* Ham) fruit plus molasses and its role in producing a liquid fertilizer. Electron J Biotechnol 12:1–11

Kantachote D, Kornochalert N, Chaiprapat S (2010) The use of the purple non sulfur bacterium isolate P1 and fermented pineapple extract to treat latex rubber sheet wastewater for possible use as irrigation water. Afr J Microbiol Res 4:2604–2616

Kantha T, Chaiyasut C, Kantachote D, Sukrong S, Muangprom A (2012) Synergistic growth of lactic acid bacteria and photosynthetic bacteria for possible use as a bio-fertilizer. Afr J Microbiol Res 6: 504–511

Kim MK, Choi KM, Yin CR, Lee KY, Im WT, Lim JH, Lee S (2004) Odorous swine wastewater treatment by purple non-sulfur bacteria, *Rhodopseudomonas palustris*, isolated from eutrophicated ponds. Biotechnol Lett 26:819–822

Kornochalert N, Kantachote D, Chaiprapat S (2013) Use of fermented plant extract to enhance the growth of phototrophic bacteria for use in the treatment of latex rubber wastewater. Manuscript submitted

Kornochalert N, Kantachote D, Techkarnjanaruk S (2011) The use of fermented plant extract to stimulate the growth of phototrophic bacteria to use as inoculants for treatment of rubber sheet wastewater. The proceeding of the 21st national graduate research conference. 26 May 2011.

Liang CM, Hung CH, Hsu SC, Yeh IC (2010) Purple nonsulfur bacteria diversity in activated sludge and its potential phosphorus-accumulating ability under different cultivation conditions. Appl Microbiol Biotechnol 86:709–719

Lu H, Zhang G, Wan T, Lu Y (2011) Influences of light and oxygen conditions on photosynthetic bacteria macromolecule degradation: Different metabolic pathways. Bioresour Technol 102:9503–9508

Luo W, Deng X, Zeng W, Zheng D (2012) Treatment of wastewater from shrimp farms using a combination of fish, photosynthetic bacteria, and vegetation. Desalin Water Treat 47:221–227

Madukasi EI, Dai X, He C, Zhou J (2010) Potentials of phototrophic bacteria in treating pharmaceutical wastewater. Int J Environ Sci Technol 7:165–174

Majone M, Dircks K, Beun JJ (1999) Organic wastewater treatment without greenhouse gas addition by photosynthetic bacteria. Water Sci Technol 35:61–73

Markl H (1999) Modeling of biogas reactors. In: Winter J (ed) Biotechnology, vol 11a, 2nd edn. Wiley, New York, pp 527–560

Montgomery DC (2001) Design and analysis of experiments. Wiley, New York

Nakajima F, Kamiko N, Yamamoto K (1997) Organic wastewater treatment without greenhouse gas emission by photosynthetic bacteria. Water Sci Technol 35:285–291

Okubo Y, Futamata H, Hiraishi A (2005) Characterization of phototrophic purple nonsulfur bacteria forming colored microbial mats in a swine wastewater ditch. Appl Environ Microbiol 72:6225–6233

Okubo Y, Futamata H, Hiraishi A (2006) Distribution and capacity for utilization of lower fatty acids of phototrophic purple non sulphur bacteria in wastewater environments. Microbiol Environ 20:135–143

Okuda A, Higa T (1999) Purification of wastewater with effective microorganisms. Proc. 5th International Conf. Kyusi Nature Farming, Senanayake YDA and Sangakkara UR. (ed) APNAN, Thailand, pp 246–253

Pollution Control Department (1994) Laws and Standards on Pollution Control in Thailand. 3rd edn

Royal Irrigation Department (1989) Water characteristics discharged into irrigation system. Summarized from Royal Irrigation Department Order No. 883/2532 (1989), dated 19 December B.E. 2532 (1989).

Shrivastava JN, Raghav N, Singh A (2012) Laboratory scale bioremediation of the Yamuna water with effective microbes (EM) technology and nanotechnology. J Bioremed Biodeg 3:160

Sinha SN, Banerjee RD (1997) Ecological role of thiosulfate and sulfide utilizing purple nonsulfur bacteria of a riverine ecosystem. FEMS Microbiol Ecol 24:211–220

Valderramaa LT, Del Campo CM, Rodriguez CM, de-Bashan LE, Bashan Y (2002) Treatment of recalcitrant wastewater from ethanol and citric acid production using the microalga *Chlorella vulgaris* and the macrophyte *Lemna minuscula*. Water Res 36:4

Yalamanchili C, Smith MD (2008) Acute hydrogen sulfide toxicity due to sewer gas exposure. Am J Emerg Med 26:518.e5–518.e7

Yang MH, Choong YM (2001) A rapid gas chromatographic method for direct determination of short-chain (C2–C12) volatile organic acids in foods. Food Chem 75:101–108