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Full Length Research Paper

Some properties on the application of *Candida utilis* TISTR No. 5001 into sequencing batch reactor (SBR) System

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The use of *C. utilis* TISTR No. 5001 as a substitute for bacterial sludge in the SBR system was examined in this study. To stimulate and maintain *Candida utilis* TISTR No. 5001 as the dominant strain in the SBR system, the initial pH of wastewater had to be controlled in the acidic range (5-6). The laboratory scale SBR system (10-L reactor volume) with *C. utilis* TISTR No. 5001 (Y-SBR system) operating at 1 cycle/day, ambient temperature (25-29^{o}C), impeller speed of 60 rpm, dissolved oxygen of 2.0-2.5 mg/l and replacement volume of 2.50 L/d, showed the highest COD, BOD_5 and total nitrogen (TN) removal efficiencies of 97.2±1.6, 97.6±1.6 and 53.1±0.6%, respectively, with synthetic waste water, SWW (pH5), under an organic loading of 0.45 kg BOD_5/m^3-d. The effluent NO_3^- and effluent NO_2^- of the system were only 0.42±0.06 mg/l and 0.05±0.007 mg/l, respectively. The sludge age (SRT) and sludge volume index (SVI) of the system were 19±2.3 days and 72±8.2 ml/g, respectively. The SBR system with a mixed culture of *C. utilis* TISTR No. 5001 and bacterial sludge (Y-SBS-SBR system) showed high organic removal efficiencies of higher than 95% with both acidic-SWW and alkaline-SWW. Furthermore, it showed the highest total nitrogen (TN) removal efficiency with SWW (pH 5). The TN removal efficiency with SWW (pH 8) under the lowest organic loading of 0.13 kg BOD5/m3-d was only 22.1±10.2%, while it increased to 35.4±1.0% for SWW (pH 5).

Key words: Sequencing batch reactor (SBR) system, seafood wastewater, *Candida utilis*, sludge volume index (SVI), organic loading.

INTRODUCTION

The seafood industry is one of the most important exporting industries of Thailand. The volume of export products has increased every year due to the increasing world market demand. Most of the Thai seafood products are exported to Japan, USA, Canada and Australia (Sirianuntapiboon and Nimnu, 1999). The seafood industry uses large volumes of water for many purposes, such as washing, thawing, cutting and butchering, and other processes (Sirianuntapiboon and Nimnu, 1999; Klinpikul and Wongsajja, 1983; Nair, 1990). Thus, a large volume of wastewater is produced (Klinpikul and Wongsajja, 1983; Nair, 1990; Roeckel and Aspe, 1996). Sirianuntapiboon and Nimnu (1999) reported that seafood factories in Thailand produced about 20-30 m^3 of wastewater per ton of product.

The most common and suitable wastewater treatment systems used by seafood factories are facultative ponds, aerated lagoons, and activated sludge systems (AS). Among them, AS is popular due to its high removal efficiency and low area requirement (Benefield and Randall,

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Abbreviations: BOD_5, Biochemical oxygen demand at 5 days; COD, chemical oxygen demand; HRT, hydraulic retention time; LSD, least significant difference; MLSS, mixed liquor suspended solids; SBR, sequencing batch reactor; SBS, bacterial-sludge from central domestic wastewater treatment plant of Bangkok city Thailand (Sriphaya plant); SBS-SBR, sequencing batch reactor with bacterial-sludge; SD, standard deviation; SRT, solids retention time, sludge age; SVI, sludge volume index; SWW, synthetic wastewater; TKN, total kjeldahl nitrogen; TN, total nitrogen; Y, yeast strain, *C. utilis*; Y-SBR, sequencing batch reactor with *C. utilis*; and Y+SBS-SBR, sequencing batch reactor with *C. utilis* and bacterial-sludge.

Table 1. Chemical properties and compositions of seafood wastewater and synthetic wastewater.

Seafood wastewater			Synthetic wastewater			
			Chemical properties		Chemical composition	
Chemical properties	Concentrations		Properties	Concentration	Compositions	Concentration
	Range	Average±SD				
COD, mg/l	1,450-2,900	2,200 ± 723	COD, mg/l	2,200±120	Glucose, mg/l	1875
BOD_5, mg/l	1,200-2,100	1,500 ± 412	BOD_5, mg/l	1,500±40	Urea, mg/l	120
TKN, mg/l	30-95	85 ± 37	TKN, mg/l	100±15	$FeCl_2$, mg/l	3.5
					$NaHCO_3$, mg/l	675
					KH_2PO_4, mg/l	55
					$MgSO_4.7H_2O$, mg/l	42.5

Remark: The pH of SWW was adjusted to be 5, 6, 7 and 8 by 6N H_2SO_4 and 6N NaOH solutions

1980; Metcalf and Eddy, 1991; Fortes and Wase, 1987). However, the use of AS for treating seafood wastewater containing a high concentration of nitrogen compounds has many problems, such as high operation cost, fluctuation of effluent qualities, and rising and bulking of bio-sludge (Benefield and Randall, 1980; Metcalf and Eddy, 1991; Fortes and Wase, 1987). Also, the effluent still contains high total nitrogen.

The sequencing batch reactor (SBR) system is a fill-and-draw activated-sludge treatment system that can be applied to treat seafood wastewater (Irvine and Busch, 1979), increase efficiencies, and reduce both investment and operating costs (Metcalf and Eddy, 1991). Unfortunately, the use of SBR system to treat wastewater containning high concentrations of nitrogen compounds, such as that from seafood factories, still has many problems, such as high excess bio-sludge production and high total nitrogen in the effluent.

To reduce the amount of excess bio-sludge production, the system must be operated with a high bio-sludge concentration (Metcalf and Eddy, 1991; Kagi and Uygur, 2002; Antonio, 1986). Then the amount of oxygen supplied and hydraulic retention time (HRT) of the system must be increased (Metcalf and Eddy, 1991; Kagi and Uygur, 2002; Antonio, 1986). The nitrogen compounds of wastewater in a conventional aerobic treatment system, such as SBR, are normally removed by both assimilation and nitrification/denitrification (Metcalf and Eddy, 1991; Abraham, 1983; Bernard and Klapwijk, 1996; Bernet et al., 2000). The nitrification activity increases with the increase of the bio-sludge concentration of the system (mixed liquor suspended solid: MLSS) (Furumai et al., 1999; Rao and Robert, 1986). However, the use of SBR system for treating seafood wastewater still has many problems regarding bio-sludge quality and removal efficiency.

To address the above problems, such as excess bio-sludge production, high SVI values, rising and bulking of bio-sludge, and low nitrogen removal efficiencies, *Candida utilis* TISTR No. 5001 was applied into an SBR system instead of bacterial bio-sludge. The experiments were carried out in SBR systems with *C. utilis* TISTR 5001, SBR systems with bacterial-sludge from the central domestic wastewater treatment plant of Bangkok city, Thailand (SBS), and SBR systems with mixed cultures of *C. utilis* TISTR 5001 and SBS, under fixed MLSS concentrations of 1,500 mg/l, 3,000 mg/l, and 3,000 mg/l, respectively, and with synthetic wastewater (SWW) with initial pH values of 5, 6, 7 and 8. These systems were observed in terms of the efficiencies and bio-sludge quality of the systems under various organic loading rates.

MATERIALS AND METHODS

Synthetic wastewater (SWW)

SWW was prepared according to the BOD_5 and TKN concentrations of the seafood wastewater that was collected from a seafood factory in Samuthsakorn province, Thailand, as shown in Table 1. The chemical composition of SWW is shown in Table 1. The pH values of the SWW were adjusted to 5, 6, 7, and 8 with 6N H_2SO_4 and 6N NaOH solutions for the various experiments.

Microbial cultures

The two types of microorganisms used as the inoculum of the SBR system were the bacterial-sludge (SBS) and yeast culture (*C. utilis* TISTR No. 5001). The SBS was collected from the central domestic wastewater treatment plant of Bangkok city, Thailand (Sriphaya domestic wastewater treatment plant). *C. utilis* TISTR No. 5001 was obtained from the culture collection of Bangkok MIRCEN, Thailand Institute of Scientific Research and Technology, Ministry of Science and Technology, Bangkok, Thailand.

Acclimatization of the microbial cultures

SBS was acclimatized in SWW for 1 week. The acclimatized SBS suspension (the concentration of 10,000 mg/l) was used as the inoculum of the SBR system. C. utilis TISTR No. 5001 was cultivated in 100 ml of malt yeast broth medium (Difco Laboratories, 1967) in the reciprocal shaker (shaking speed of 125 rpm) at 30°C for 3

Table 2. Volume and concentration of bacterial-sludge (SBS) and *C. utilis* TISTR No.5001 suspension used as the inoculum of SBR system with *C. utilis* TISTR No. 5001 (Y-SBR), bacterial-sludge (SBS-SBR) and mixed culture of *C. utilis* TISTR No. 5001 and bacterial bio-sludge (Y+SBS-SBR).

Parameters	Types of SBR system		
	Y-SBR	SBS-SBR	Y+SBS-SBR
Working volume of reactor, L	7.5	7.5	7.5
Concentration of BS suspension, cells/ml	-	$4.0x10^{14}$	$4.0x10^{14}$
Volume of SBS suspension for SBR system, L	-	2.25	1.13
Concentration of yeast suspension, cells/ml	$1.0x10^{10}$	-	$1.0x10^{10}$
Volume of yeast suspension for SBR system, ml	300	-	150
Total MLSS, mg/l	70	3,000	1,570
MLSS of yeast, mg/l	70	3,000	1,500
MLSS of SBS, mg/l	-		70
Total yeast cell concentration in the reactor, cells/ml	$4.0x10^{8}$	-	$2.0x10^{8}$
Total bacterial cell concentration in the reactor, cells/ml	-	$1.2x10^{14}$	$6.03x10^{13}$

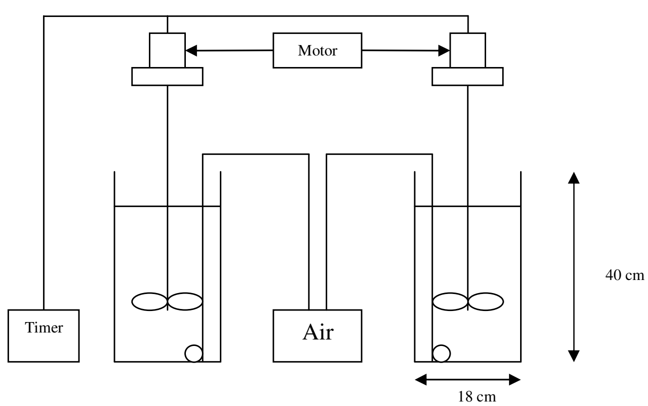

Figure 1. Sheme of the SBR system.

days. The culture broth (cell concentration of approximately 1x109 cells/ml) was used as the inoculum of the SBR system.

Sequencing batch reactor (SBR) System

Six 10-L reactors, made from acrylic plastic (5 mm thick), as shown in Figure 1, were used in the experiments. Each reactor had a diameter of 18 cm, a height of 40 cm, and a working volume of 7.5 liters. Low speed gear motor, model P 630A-387, 100V, 50/60 Hz, 1.7/1.3 A (Japan Servo Co. Ltd., Tokyo, Japan) was used for driving the paddle-shaped impeller. The speed of impeller was adjusted to 60 rpm for complete mixing. One air pump system, model EK-8000, 6.0 W (President Co. Ltd., Bangkok, Thailand), was used for supplying air to the 2 sets of reactors. The dissolved oxygen in the reactors was controlled at 2-2.5 mg/l, indicating that the oxygen supply was sufficient. The excess sludge was drawn during draw and idle period to control the suspended MLSS of the system.

Operation of SBR systems

Three types of SBR systems, SBR system with SBS (SBS-SBR), SBR system with *C. utilis* No. 5001 (Y-SBR), and SBR system with mixed culture of SBS and *C. utilis* TISTR No. 5001 (Y+SBS-SBR), were used in this study. The acclimatized microbial cultures were inoculated in the SBR reactors, as shown in Table 2. SWW was added (final volume of 7,500 ml) within 1 h. During the feeding of the wastewater, the system was fully aerated and the aeration continued for 19 h.. The aeration system was then shut down for 3 h. After full settling of the bio-sludge, the supernatant was removed (the removed volume of the supernatant was based on the operating program as mentioned in Table 3) within 0.5 h and the system was kept under anoxic conditions for 0.5 h. Then the raw wastewater was filled into the reactor up to the final volume of 7,500 ml and the above operation was repeated.

The operation parameters of each type of SBR are described in Table 3. The operation temperature was controlled at ambient temperatures (27-29°C). The excess sludge was drawn during draw and idle period to control the suspended MLSS of the system.

Chemical and biological analysis

The chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), total nitrogen (TN), total kjeldahl nitrogen (TKN), ammonia nitrogen (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) of influent and effluent, mixed liquor suspended solids (MLSS), and sludge volume index (SVI) of the system were determined by using standard methods for the examination of water and wastewater (APHA, AWWA, WPCF, 1998). SRT (solid retention time: sludge age) was computed as the ratio of total MLSS of the system to the amount of excess sludge waste per day. F/M was calculated as the ratio of BOD_5 loading to the total bio-sludge of the system. The number of bacterial and yeast cells were determined by total plate count method (Ronald, 1946). The nutrient agar plate (Ronald, 1946) and malt yeast agar plate (Difco Laboratories, 1967) were used to cultivate the bacteria and *C. utilis*, respectively.

Statistical analysis method

Each experiment was repeated at least 3 times. All the data were subjected to two-way analysis of variance (ANOVA) using SAS Windows Version 6.12 (SAS Institute, 1996). Statistical significance was tested using least significant difference (LSD) at the $p<0.05$ level. The results are shown as the mean ± the standard deviation.

Table 3. Operation parameters of sequencing batch reactor (SBR) system with SWW.

Parameters	Hydraulic retention time: HRT (days)											
	Y-SBR under various HRT (days)				SBS-SBR under various HRT (days)				Y+SBS-SBR under various HRT (days)			
	3	5	7	10	3	5	7	10	3	5	7	10
Working volume of reactor, L	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Replacement volume, L/d	2.50	1.50	1.08	0.75	2.50	1.50	1.08	0.75	2.50	1.50	1.08	0.75
SBR operating cycle, cycles/d	1	1	1	1	1	1	1	1	1	1	1	1
Flow rate, L/d	2.50	1.50	1.08	0.75	2.50	1.50	1.08	0.75	2.50	1.50	1.08	0.75
Hydraulic loading, m^3/m^3-d	0.33	0.20	0.14	0.10	0.33	0.20	0.14	0.10	0.33	0.20	0.14	0.10
MLSS, mg/l	1,500	1,500	1,500	1,500	3,000	3,000	3,000	3,000	3,000	3,000	3,000	3,000
BOD_5 loading, g BOD_5/d	3.38	2.03	1.43	0.98	3.38	2.03	1.43	0.98	3.38	2.03	1.43	0.98
Volumetric BOD_5 loading (kg BOD_5/m^3-d)	0.45	0.29	0.19	0.13	0.45	0.29	0.19	0.13	0.45	0.29	0.19	0.13
F/M, d^{-1}	0.33	0.20	0.14	0.10	0.17	0.10	0.07	0.05	0.17	0.10	0.07	0.05
TKN loading, g TKN/d	0.25	0.15	0.11	0.08	0.25	0.15	0.11	0.08	0.25	0.15	0.11	0.08
Volumetric TKN loading, kg TKN/m^3-d	0.030	0.020	0.015	0.011	0.030	0.020	0.015	0.011	0.030	0.020	0.015	0.011
Operating Temperature, ^oC*	26-29	26-29	26-29	26-29	26-29	26-29	26-29	26-29	26-29	26-29	26-29	26-29

*: Operation temperature of the system was ambient temperature, 26-29^{o}C.

Table 4. Qualities of bio-sludge of Y-SBR, SBS-SBR and Y+SBS-SBR systems with SWW (pH of 5, 6, 7 and 8) under BOD_5 loading of 0.13 kg BOD_5/m^3-d (HRT of 10 days).

Properties	Types of SBR system											
	Y-SBR system				SBS-SBR system				Y+SBS-SBR system			
Initial pH of SSIWW	5	6	7	8	5	6	7	8	5	6	7	8
Controlling MLSS, mg/l	1,500±280	1,500±305	1,500±295	1,500±270	3,000±332	3,000±313	3,000±294	3,000±273	3,000±312	3,000±301	3,000±286	3,000±270
Excess sludge, mg/d	150±27	150±31	150±19	150±33	320±45	341±40	362±34	393±49	380±51	338±33	390±42	345±40
SVI, ml/g	70±9.3	73±11.2	85±13.5	89±15.3	100±20.1	85±18.3	75±10.7	76±12.1	92±19.6	90±17.6	85±18.1	85±15.3
SRT, days	60±8.3	60±9.5	60±10.1	60±8.4	28±4.8	26±5.9	25±3.1	23±2.8	24±3.9	27±3.0	23±3.6	26±3.8
Bacterial concentration, X 10^{14}cells/ml	3.1±0.42*	3.3±0.50*	12±0.37*	11±0.40*	2.3±0.35	2.4±0.30	2.4±0.38	2.2±0.34	1.2±0.21	1.7±0.22	1.7±0.18	2.0±0.27
Yeast concentration, X10^9 cells/ml	9.4±1.32	9.1±1.10	8.3±0.91	6.7±0.77	-	-	-	-	0.21±0.04	0.16±0.04	0.15±0.03	0.007±0.0

Remark: *The unit of microbial cells was multiplied by 10^4cells/ml.

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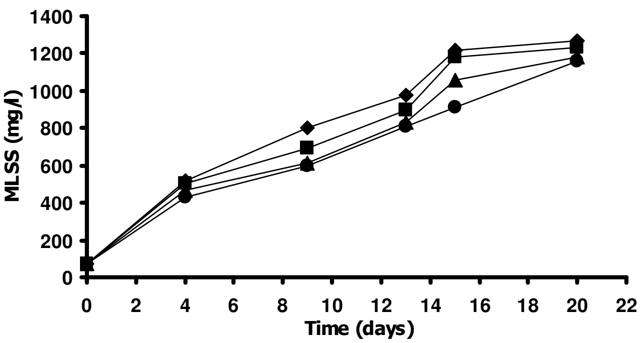

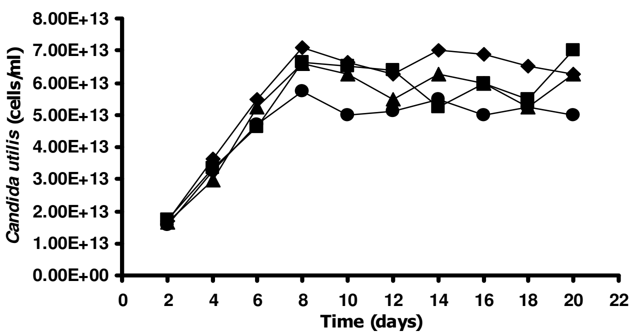

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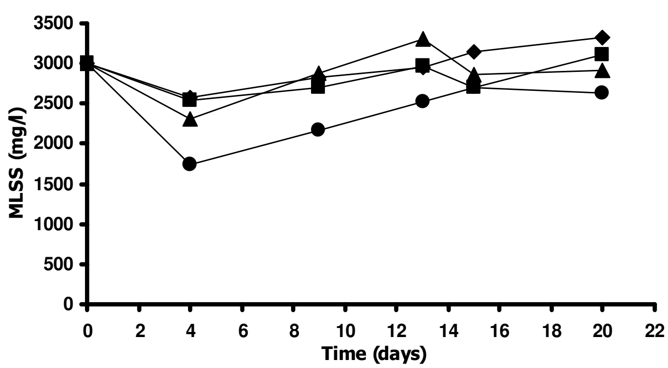

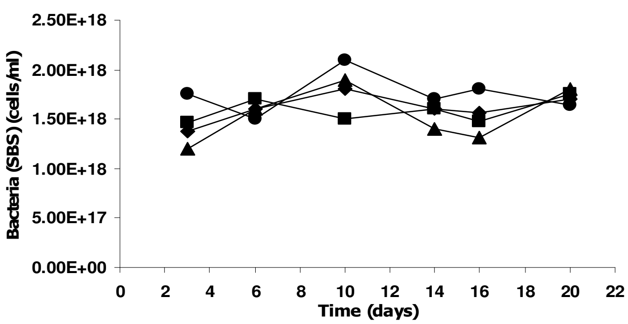

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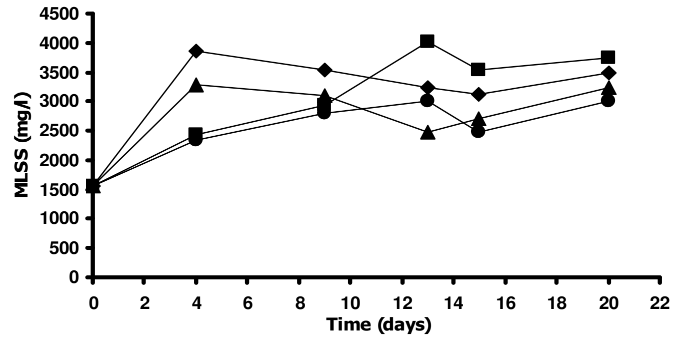
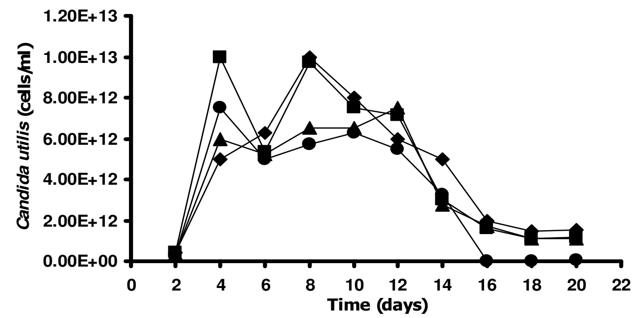




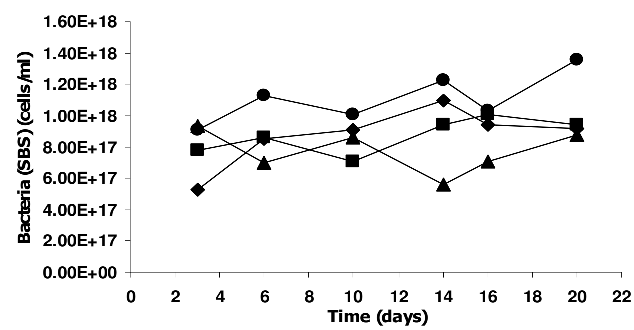


Figure 2. MLSS and numbers of *C. utilis* and bacterial cells of Y-SBR (A), SBS-SBR (B) and Y+SBS-SBR (C) systems under HRT of 10 days tested with SWW with various initial pH of 5, 6, 7, and 8. Symbols: (◆); pH5, (■); pH6, (▲); pH7, (●); pH8.

RESULTS

Effects of initial pH of SWW on the performance of SBR systems

The experiments were carried out in three types of SBR systems (SBS-SBR, Y-SBR and Y+SBS-SBR) with SWW that had initial pH values of 5, 6, 7, and 8 under HRT of

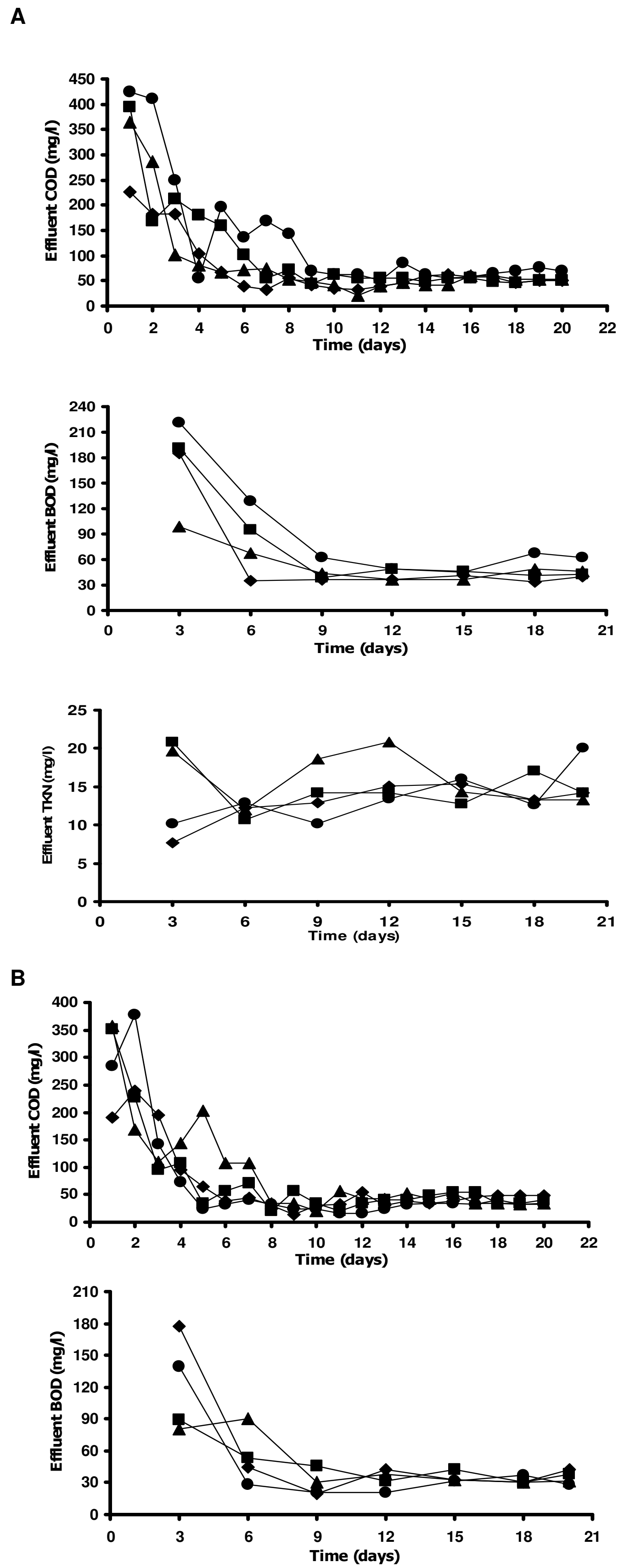
10 days (organic loading of 0.13 kg BOD_5/m^3-d). The bio-sludge quality and efficiency of the system were determined.

The growth of C. utilis TISTR No. 5001 and bacteria-sludge in SBR systems

The results are shown in Figure 2 and Table 4. The growth of both *C. utilis* TISTR No.5001 and bacteria in the SBR systems were affected by the initial pH of SWW. *C. utilis* TISTR No.5001 of the Y-SBR system showed optimal growth with acidic-SWW (pH 5-6) and it reached steady state within 6-8 days of culture. The number of *C. utilis* TISTR No.5001 and MLSS of Y-SBR system with SWW (pH 5) was increased up to $9.4 \pm 1.32 \times 10^9$ cells/ml and 1,500±280 mg/l, respectively. However, the SWW (pH 7-8) had a negative effect on the growth of *C. utilis* TISTR No.5001 and a positive effect on the growth of bacteria in the Y-SBR system. The number of *C. utilis* TISTR No.5001 was decreased down to $6.7 \pm 0.77 \times 10^9$ cells/ml and the number of bacteria was increased up to $1.1 \pm 0.40 \times 10^5$ cells/ml when the system was operated with SWW (pH 8).

For the SBS-SBR system, bacterial sludge showed optimal growth with SWW having initial pH values higher than 6 and the system became steady state within 3-4 days cultivation. The number of bacteria and MLSS of SBS-SBR system with SWW (pH 7) was increased up to $2.4 \pm 0.38 \times 10^{14}$ and 3,000±294 mg/l, respectively. Y-SBS-SBR system showed high removal efficiency with both acidic-SWW and alkaline-SWW. However, the *C. utilis* were dominant in acidic-SWW, while the bacteria were dominant in alkaline-SWW. The number of *C. utilis* and bacteria were $2.1 \pm 0.4 \times 10^8$ cells/ml and $1.2 \pm 0.21 \times 10^{14}$ cells/ml, respectively with SWW (pH 5), while the number of *C. utilis* and bacteria were 7×10^{11} cells/ml and $2.0 \pm 0.27 \times 10^{14}$ cells/ml, respectively, with SWW (pH 8). However, the MLSS of Y+SBS-SBR system with both alkaline-SWW and acidic-SWW were increased up to approximately 3,000 mg/l within 5-7 days, but the population of *C. utilis* was decreased after 10 days operation. For the excess sludge and SRT determination, MLSS of Y-SBR system could be increased up to only 1,500 mg/l, while both BS-SBR and Y+SBS-SBR systems could be increased to values greater than 3,000 mg/l. The amount of excess sludge of the Y-SBR system was about 2.0-2.5 times lower than those of both the SBS-SBR and Y+SBS-SBR systems. Thus, the sludge age of the Y-SBR system was double those of the SBS-SBR and Y+SBS-SBR systems.

For the determination of bio-sludge quality of the system, SVI of the Y-SBR system was increased with an increase in the initial pH of SWW. However, the SVI of the Y-SBR system with SWW (pH 8) under HRT of 10 days was 89±15.3 ml/g.



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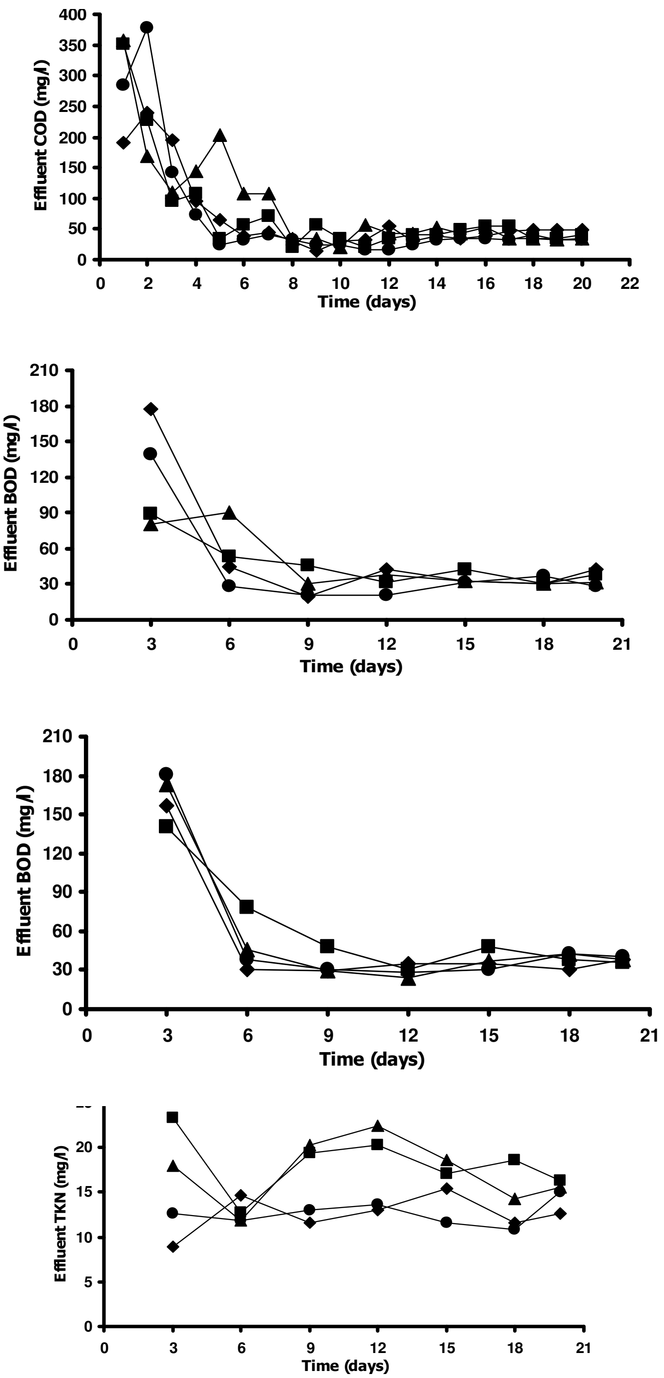

Figure 3. Chemical properties (COD, BOD_5, and TKN) profiles of effluents from Y-SBR (A), SBS-SBR (B) and Y+SBS-SBR (C) systems tested with SWW with various initial pH of 5, 6, 7 and 8. Symbols: (◆); pH5, (■); pH6, (▲); pH7, (●); pH8.

Efficiencies of SBR systems

The results are shown in Figure 3 and Table 5. Y-SBR system showed high removal efficiency with SWW (pH 5), but Y+SBS-SBR and SBS-SBR systems showed high removal efficiency with both acidic-SWW and alkaline-SWW. The COD, BOD_5 and TN removal efficiencies of Y-SBR system with SWW (pH 5) were highest, with values of 97.6±1.4%, 96.5±2.0%, and 36.5±1.0%, respectively. Y+SBS-SBR and SBS-SBR systems showed the highest BOD_5 removal efficiencies of 97.0±1.7% and 97.4±1.6%, respectively, with SWW (pH 8), but they showed TN removal efficiencies of only 22.1±1.0% and 25.5±1.1%, respectively. The effluent NO_3^- of Y-SBR system with all types of SWW was lower than those of the SBS-SBR and Y+SBS-SBR systems, as shown in Table 5. Additionally, the effluent pH of Y-SBR system with SWW (pH 5) was neutral, but the effluents pH of all systems were increased to alkaline levels (8-9) when the initial pH values of SWW were greater than 6.

In the above experiments, both Y-SBR and Y+SBS-SBR systems showed high removal efficiencies with acidic-SSWW (pH 5). For further study, the Y-SBR, Y+SBS-SBR and SBS-SBR systems were tested with acidic-SWW (pH 5) under various HRT operations of 3, 5, 7, and 10 days (organic loading of 0.45, 0.27, 0.19 and 0.13 kg BOD_5/m^3-d).

Effects of organic loading on the efficiency of SBR systems

The results are shown in Table 6 and Table 7. The Y-SBR, Y+SBS-SBR and SBS-SBR systems did not show much difference in COD and BOD_5 removal efficiencies. The COD and BOD_5 removal efficiencies were higher than 95%, even when the systems were operated under a high organic loading of 0.45 kg BOD_5/m^3-d. The TN removal efficiency increased with the increase of organic loading. However, Y-SBR system showed marginally higher TN removal efficiency than both SBS-SBR and Y+SBS-SBR systems, as shown in Table 6. Additionally, the effluent NO_3^- of Y-SBR system under every organic loading was lower than those of the Y+SBS-SBR and SBS-SBR systems.

The excess sludge of Y-SBR system was about 2-3 times lower than those of the SBS-SBR and Y+SBS-SBR systems, as shown in Table 7. The excess sludge of Y-SBR system under the lowest organic loading of 0.13 kg BOD_5/m^3-d was only 150±24 g/d, while it was 393±63 g/l in SBS-SBR system under the same organic loading. The bio-sludge age (SRT) of Y-SBR and SBS-SBR systems under the lowest organic loading of 0.13 kg BOD_5/m^3-d were 60±5.4 and 23±2.1 days, respectively, as shown in Table 7. Also, the number of bacteria in all types of SBR systems increased with the increase of organic loading as, shown in Table 7. For the bio-sludge determination, the SVI of Y-SBR system with SWW (pH 5) was increased with an increase in organic loading or a decrease in HRT. However, the SVI of Y-SBR system

Table 5. Effluent qualities and removal efficiencies of Y-SBR, SBS-SBR and Y+SBS-SBR systems with SWW (pH of 5, 6, 7 and 8) under BOD_5 loading of 0.13 kg BOD_5/m^3-d (HRT of 10 days).

Type of SBR	Chemical properties													
	Dissolved oxygen in reactor	pH		COD		BOD_5		Nitrogen content of SWW						SS mg/l
		Influent	Effluent					Total nitrogen: TN		TKN		NO_3^--N	NO_2^--N	
				Effluent mg/l	% Removal	Effluent mg/l	% Removal	Effluent mg/l	% Removal	Effluent mg/l	% Removal	Effluent mg/l	Effluent mg/l	
Y-SBR	2.0-2.5	5.0±0.2	7.1±0.4	53±8.3	97.6±1.4	38±4.5	96.5±2.0	65.8±8.6	36.5±1.0	51.2±6.3	48.2±0.7	14.50±1.97	0.05±0.007	254±30
	2.0-2.5	6.0±0.3	8.3±0.5	51±7.6	97.6±1.5	43±4.3	95.8±1.9	67.4±7.8	35.0±1.1	54.7±4.8	45.2±0.5	12.55±2.11	0.06±0.008	192±32
	2.0-2.5	7.0±0.2	9.0±0.3	56±7.9	97.3±1.8	44±4.9	95.7±1.9	93.1±7.9	10.2±1.0	50.6±5.9	49.3±0.5	42.34±2.15	0.10±0.008	134±38
	2.0-2.5	8.0±0.3	9.0±0.4	68±8.1	96.8±1.4	58±5.1	95.3±1.3	96.6±8.2	6.9±1.2	60.3±6.3	39.6±0.5	36.13±2.23	0.09±0.009	115±41
SBS-SBR	2.0-2.5	5.0±0.2	7.6±0.4	46±8.0	97.9±1.2	35±5.2	96.8±1.7	85.5±8.6	17.6±1.0	49.1±6.1	50.8±0.5	36.22±1.89	0.09±0.010	162±35
	2.0-2.5	6.0±0.3	8.0±0.5	44±7.6	97.9±1.1	37±4.6	96.4±1.9	88.1±8.4	15.1±1.0	64.4±5.7	35.5±0.6	23.55±1.88	0.09±0.011	128±36
	2.0-2.5	7.0±0.2	8.7±0.3	38±8.2	98.2±1.9	32±4.7	97.0±1.4	96.7±8.2	6.8±1.2	59.9±4.9	40.0±0.5	36.65±1.97	0.08±0.012	94±34
	2.0-2.5	8.0±0.3	9.1±0.2	34±7.8	98.4±1.8	32±5.4	97.4±1.6	77.2±8.1	25.5±1.1	46.1±5.0	53.8±0.6	30.98±2.05	0.08±0.009	78±33
Y+SBS-SBR	2.0-2.5	5.0±0.2	7.5±0.4	37±7.7	98.3±1.6	34±5.1	97.0±1.4	67.0±7.1	35.4±1.0	47.3±5.1	52.7±0.6	19.67±2.04	0.06±0.009	165±31
	2.0-2.5	6.0±0.3	7.9±0.3	45±7.3	97.8±1.3	41±4.8	96.1±1.5	85.5±7.5	17.5±1.4	69.6±6.0	30.3±0.5	15.83±2.00	0.06±0.011	130±37
	2.0-2.5	7.0±0.2	8.7±0.3	49±7.1	97.6±1.5	39±4.2	96.2±1.2	85.1±8.6	18.0±1.3	60.7±5.3	39.3±0.5	24.27±2.21	0.07±0.010	112±28
	2.0-2.5	8.0±0.3	9.0±0.2	43±7.0	98.0±1.6	38±5.3	97.0±1.7	80.8±8.1	22.1±1.0	61.2±5.2	40.1±0.6	19.49±1.50	0.08±0.009	90±27

Table 6. Effluent qualities and removal efficiencies of Y-SBR, SBS-SBR and Y+SBS-SBR systems with SWW (pH 5) under various organic loadings.

Type of SBR	BOD_5 loading kg/m^3-d	Dissolved oxygen in reactor mg/l	Chemical properties												
			pH		COD		BOD_5		Nitrogen content in the SSIWW						SS mg/l
									Total Nitrogen		TKN		NO_3^--N	NO_2^--N	
			Influent	Effluent	Effluent mg/l	% Removal	Effluent mg/l	% Removal	Effluent mg/l	% Removal	Effluent mg/l	% Removal	Effluent mg/l	Effluent mg/l	
Y-SBR	0.45	2.0-2.5	5.0±0.2	7.3±0.2	55±7.5	97.2±1.6	31±5.6	97.6±1.6	47.2±5.3	53.1±0.6	46.8±5.2	53.2±0.4	0.42±0.06	0.05±0.007	164±21.3
	0.27	2.0-2.5	5.0±0.2	7.2±0.3	60±7.1	97.0±1.7	64±7.3	94.8±1.5	50.4±6.7	49.9±0.6	49.9±5.4	50.1±0.4	0.41±0.05	0.06±0.008	186±22.7
	0.19	2.0-2.5	5.0±0.2	7.2±0.2	61±6.8	97.0±1.7	54±4.8	95.7±1.5	53.9±5.3	46.5±0.6	53.3±5.9	46.7±0.5	0.51±0.06	0.08±0.008	220±25.1
	0.13	2.0-2.5	5.0±0.2	7.1±0.3	53±6.1	97.6±1.5	38±5.2	96.5±1.5	65.5±5.7	36.7±0.6	51.4±6.3	48.6±0.5	14.05±0.05	0.05±0.009	154±24.0
SBS-SBR	0.45	2.0-2.5	5.0±0.2	7.2±0.3	56±5.9	97.2±1.6	35±4.9	97.2±1.6	49.9±5.1	50.4±0.5	49.2±6.4	50.8±0.5	0.66±0.07	0.09±0.010	135±21.6
	0.27	2.0-2.5	5.0±0.2	7.0±0.3	56±6.3	97.2±1.6	63±5.3	95.0±1.6	57.9±5.9	42.5±0.6	57.4±5.7	42.6±0.5	0.46±0.04	0.08±0.007	147±22.4
	0.19	2.0-2.5	5.0±0.2	7.0±0.4	53±6.2	97.3±1.7	50±5.7	96.0±1.6	64.6±5.7	35.8±0.6	60.0±5.5	40.0±0.5	4.60±0.05	0.07±0.007	162±21.9
	0.13	2.0-2.5	5.0±0.2	7.6±0.3	46±5.8	97.9±1.8	35±4.6	96.8±1.6	99.6±6.1	3.7±0.6	64.4±6.2	35.6±0.4	35.09±0.04	0.09±0.010	165±20.7
Y+SBS-SBR	0.45	2.0-2.5	5.0±0.2	7.1±0.2	55±5.4	97.2±1.4	36±4.7	97.1±1.6	48.0±5.7	52.3±0.6	47.3±6.1	52.7±0.5	0.61±0.05	0.08±0.008	124±21.8
	0.27	2.0-2.5	5.0±0.2	7.1±0.2	56±5.9	97.2±1.6	56±3.9	95.5±1.7	61.8±5.8	38.5±0.5	61.1±5.7	38.9±0.5	0.68±0.04	0.08±0.009	134±22.1
	0.19	2.0-2.5	5.0±0.2	7.1±0.3	55±6.2	97.2±1.6	52±3.8	95.8±1.7	76.0±5.4	24.4±0.6	71.9±5.4	28.1±0.5	4.04±0.04	0.10±0.010	151±22.0
	0.13	2.0-2.5	5.0±0.2	7.6±0.3	37±6.1	98.3±1.4	34±2.7	97.0±1.7	94.7±5.9	8.4±0.6	75.6±6.1	24.4±0.4	19.06±0.06	0.06±008	162±21.8

Remarks: BOD_5 loading of 0.45 kg/m^3-d = HRT of 3 days, BOD_5 loading of 0.27 kg/m^3-d = HRT of 5 days, BOD_5 loading of 0.19 kg/m^3-d = HRT of 7 days, BOD_5 loading of 0.13 kg/m^3-d = HRT of 10 days.

Table 7. Qualities of bio-sludge of Y-SBR, SBS-SBR and Y+SBS-SBR systems operated with SWW (pH5) under various organic loadings.

Properties	Types of SBR system											
	Y-SBR system				SBS-SBR system				Y+SBS-SBR system			
HRT, d	3	5	7	10	3	5	7	10	3	5	7	10
BOD_5loading, $kgBOD_5/m^3$-d	0.45	0.29	0.19	0.13	0.45	0.29	0.19	0.13	0.45	0.29	0.19	0.13
Controlling MLSS, mg/l	1,500±153	1,500±162	1,500±167	1,500±170	3,000±278	3,000±268	3,000±224	3,000±277	3,000±231	3,000±245	3,000±258	3,000±264
Excess sludge	480±49	320±36	250±33	150±24	869±88	732±84	556±70	393±63	844±74	682±49	512±51	345±42
SVI, ml/g	72±8.2	75±9.1	80±9.1	82±8.6	82±8.9	70±8.0	70±7.6	100±8.9	71±8.0	70±8.2	100±10.2	92±8.6
SRT, days	19±2.3	28±3.2	36±4.2	60±5.4	10±1.9	12±2.0	16±2.4	23±2.1	11±2.0	13±2.3	18±2.8	26±3.2
Bacterial concentration, 10^{14}cells/ml	15±1.22*	1.4±0.20*	2.0±0.18*	31±2.25*	2.6±0.31	2.7±0.36	2.4±0.34	2.3±0.31	1.8±0.21	1.8±0.24	1.5±0.23	1.2±0.20
Yeast concentration, 10^9cells/ml	12.0±0.17	11.0±0.21	10.0±0.22	9.4±0.78	-	-	-	-	0.27±0.04	0.25±0.03	0.24±0.03	0.21±0.03

Remarks: BOD_5 loading of 0.45 kg/m^3-d = HRT of 3 days, BOD_5 loading of 0.27 kg/m^3-d = HRT of 5 days, BOD_5 loading of 0.19 kg/m^3-d = HRT of 7 days, BOD_5 loading of 0.13 kg/m^3-d = HRT of 10 days

*The unit of microbial cells was multiplied by 10^4cells/ml.

was less than 85 ml/g under organic loadings of 0.13-0.45 kg BOD_5/m^3-d.

DISCUSSION

Our results demonstrate that *C. utilis* can grow in SBR system with both acidic-SWW and alkaline-SWW, but it was dominant and showed the highest removal efficiency with acidic-SWW (pH 5). This is because *C. utilis* had optimum growth in acidic conditions (Hang et al, 1972; Prior, 1984; Elmaleh et al, 1996) and the acidic conditions inhibited the growth of bacterial-sludge (heterotrophic bacteria) in SBR system (Metcalf and Eddy, 1991). Furthermore, the mixed culture of *C. utilis* and bacterial-sludge could be applied to the SBR (Y+SBS-SBR) system to treat both acidic-SWW and alkaline-SWW because the acidic-SWW stimulated the growth of *C. utilis* and repressed the growth of bacteria, while alkaline-SWW stimulated the growth of bacteria and repressed the growth of *C. utilis* (Metcalf and Eddy, 1991; Hang et al., 1972; Prior, 1984; Elmaleh et al, 1996). However, the population of *C. utilis* of Y+SBS-SBR system was decreased after 10 days operation in all treatment conditions due to the high growth rate of bacterial-sludge under pH in the neutral to alkaline range (Metcalf and Eddy, 1991). The effluent pH of Y-SBR system with SWW (pH 5) was neutral due to buffering by $NaHCO_3$.

MLSS of Y-SBR system could be increased up to only 1,500 mg/l when the organic loading was 0.45 kg BOD_5/m^3-d, and the MLSS of Y-SBR system was controlled at 1,500 mg/l. However, the MLSS of both Y+SBS-SBR and SBS-SBR systems could be higher than 3,000 mg/l, so the MLSS of both systems in this study were controlled at 3,000 mg/l. Additionally, the excess sludge of Y-SBR system was 2.0-2.5 times lower than those of the SBS-SBR and Y+SBS-SBR systems.

From the above results, it could be suggested that the Y-SBR system was operated under an F/M 2 times higher than those of both SBS-SBR and Y+SBS-SBR systems, with less excess sludge production due to the metabolism of *C. utilis* (Kurtzman,1984; Prior, 1984; Kagi and Uygur, 2002). The other advantage of Y-SBR system was the good settling bio-sludge with acidic-SWW (SVI of Y-SBR system with acidic-SWW (pH 5-6) under organic loading of 0.13-0.45 kg BOD_5/m^3-d was less than 85 ml/g) because the *C. utilis* cells (3.5-4.5 x 7-13 μm) were 7-9 times bigger than the bacterial cells (SBS) (0.5-1 x 1.5-3.0 μm), which resulted in good settling (Metcalf and Eddy, 1991; Kurtzman, 1984).

Effluent NO_3^- from both SBS-SBR and Y+SBS-SBR systems were 2 times higher than that of Y-SBR system because *C. utilis* TISTR 5001 removed nitrogen compounds from wastewater by assimilation, while SBS, which contained both heterotrophic bacteria and nitrification bacteria, removed nitrogen compounds (organic nitrogen: TKN) by both assimilation and oxidation nitrification (Abraham and Job, 1983;, Metcalf and Eddy, 1991; Sirianuntapiboon and Tondee, 2000).

The effluent pH of all systems was in the neutral to alkaline range, even when the initial pH of SWW was acidic because of the buffering effect of $NaHCO_3$ (Metcalf and Eddy, 1991). The effluents pH of the systems was increased with increases in the initial pH of SWW, especially for the Y+SBS-SBR and SBS-SBR systems. The nitrification ability of SBS increased with increases in the pH of the effluents or wastewater, and this resulted in a subsequent increase in the concentrations of NO_3^- in the effluent (Metcalf and Eddy, 1991; Abraham and Job, 1983, Sirianuntapiboon and Tondee, 2000).

For application, *C. utilis* TISTR No. 5001 can be applied in the SBR system as a pure culture (Y-SBR system) or mixed culture with bacterial-sludge (Y-SBS-SBR system) according to the initial pH of wastewater. Y-SBR system was applied for treating acidic-SWW, while Y+SBS-SBR system was applied for treating both acidic and alkaline-SWW. This is recommended because the application of *C. utilis* TISTR No. 5001 in the SBR system resulted in increases in the removal efficiencies, reductions in excess sludge production, reductions in effluent NO_3^- and NO_2^-, and improvements in sludge quality (low SVI values). However, the initial pH of SWW had to be controlled at values no greater than 8 to control the effluent NO_3^- at low levels.

Conclusion

The results of this study indicated that C. utilis TISTR 5001 could be applied to SBR system as a substitute for bacterial sludge because of its higher organic matter and nitrogen removal rate, low excess sludge production (about half that of conventional SBR (SBS-SBR) system), and good quality bio-sludge (SVI of lower than 85 ml/g). Moreover, the effluent of Y-SBR system contained low NO3- content due to the low nitrification rate in the reactor, which was a result of the low concentration of bacterial sludge. Y-SBR system was operated under an F/M ratio that was 2 times higher than that of conventional SBR (SBS-SBR) system, with the excess sludge being 2.0-2.5 times lower than that of the conventional SBR system under the same organic loading. However, the initial pH of SWW had to be adjusted into the acidic range (not higher than 6) to maintain the C. utilis as the dominant species in the reactor.

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