Appl Biochem Biotechnol (2009) 152:405–417
DOI 10.1007/s12010-008-8237-9

Reactive Blue 4 Decolorization under Mesophilic and Thermophilic Anaerobic Treatments

A. Boonyakamol · T. Imai · P. Chairattanamanokorn · T. Higuchi · M. Sekine · M. Ukita

Received: 17 January 2008 / Accepted: 31 March 2008 /
Published online: 10 June 2008

Abstract Anaerobic decolorization of anthraquinone dye represented by Reactive Blue 4 (RB4) was studied to evaluate the factors involved in dye-reducing behaviors such as dye concentration, co-substrate, treatment temperature, salt content, and dye-reducing microbial consortia. The experiment was conducted using digested sludge treated under mesophilic (35 °C) and thermophilic (55 °C) conditions. The results indicated that the thermophilic treatment gave higher potential for this dye decolorization compared with the mesophilic one. A reduced form of RB4 did not show an auto-oxidizing reaction but treated RB4 dye was shown in light yellow color, the intensity of which was related to the initial concentration of the dye used in the treatments. Starch, which showed similar decolorizing efficiency under thermophilic conditions, could be used as a co-substrate instead of glucose for the purpose of operating cost reduction. Due to the high content of salt contained in dye wastewater, the effect of salt (NaCl) was investigated. Results showed that decolorization could be accelerated with a concentration of NaCl lower than 200 mM, but the decolorization was inhibited by high concentrations of salt. The presence of RB4 inhibited methane productivity, while total organic carbon (TOC) reduction was similar to control, without dye addition. Increasing the temperature accelerated the decolorizing potential and TOC reduction. The evaluation of dye-reducing microbial consortia was done with acidogen and methanogen inhibitors which acidogenesis microorganism was dominant in RB4 decolorization.

Keywords Dye decolorization · Mesophilic · Thermophilic · Dye-reducing microbial consortia

A. Boonyakamol · T. Imai (✉) · T. Higuchi · M. Sekine · M. Ukita
Graduate School of Science and Engineering, Yamaguchi University,
Tokiwadai, 2-26-1, Yamaguchi 755-8611, Japan
e-mail: imai@yamaguchi-u.ac.jp

P. Chairattanamanokorn
Department of Environmental Science, Faculty of Science, Kasetsart University,
Bangkok 10900, Thailand

Introduction

Anthraquinone dyes are used for the coloration of cotton and cellulose fibers as well as of hydrophobic, synthetic materials [1]. During dye processing, as much as 2–50% of dyestuffs applied may be lost to wastewater ultimately released into the environment [2]. Due to the high water solubility of dye, recalcitrance under the typical aerobic conditions found in conventional biological treatment systems, and very poor absorbance to biological solids, this results in residual color in discharged effluents [3–5]. The impact of dye wastewater discharged into the environment is a matter of concern because: (1) they cause water bodies to become colored, absorbing and reflecting sunlight which, in turn, interferes with photosynthesis and the aquatic ecosystem, and (2) a wide range of textile dye toxicity has been reported and may cause chronic and acute toxicity [6].

Dye decolorization can be readily achieved under anaerobic conditions using either chemical or biological treatment. Biological dye decolorization involves unspecific enzymes ubiquitously found in a wide diversity of microorganisms. However, little is known about the microbiological aspects of anaerobic consortia from wastewater treatment plants in the reductive decolorization of azo and anthraquinone dyes. A large portion of textile wastewaters, mainly from the dyebath and rinsing steps, is discharged at high temperatures (40–70 °C). However, thermophilic anaerobic textile dye decolorization by biological treatment has only been briefly examined [7, 8].

In this study, anthraquinone dye represented by reactive blue 4 (RB4) was investigated for comparative decolorizing efficiency by mesophilic (35 °C) and thermophilic (55 °C) anaerobic biological treatments with the addition of co-substrate without other supplements. Glucose and starch were used as a co-substrate to study the effects on operating cost. The effects of dye concentration and treatment temperature on decolorizing microorganisms were also observed. Due to a high concentration of salt also being found in dye wastewater, the effect of NaCl was studied by using starch under thermophilic conditions. The dye-reducing microbial consortia were also studied using acidogen and methanogen inhibitors, vancomycin, and 2-bromoethane sulfonic acid (BES) to evaluate the contribution of the microbial group which dominated in RB4 decolorizing processes.

Materials and Methods

Chemicals

RB4 (IC 61205), of the anthraquinone dye classes, was used as a target dye (Fig. 1). Vancomycin and BES were used as acidogen and methanogen inhibitors, respectively. Glucose and starch were used as co-substrate or electron donor substrate. All chemicals used were purchased from Wako and Sigma–Aldrich, Japan.

Decolorizing Microorganism

Digested sludge used as the decolorizing microorganisms was obtained from an anaerobic wastewater treatment plan, Western Purification Center, Ube City, Yamaguchi Prefecture, Japan. The inoculums of 1,300 mg SSL^{-1}, approximately, were cultivated in serum bottles sealed with rubber and capped with an aluminum cap overnight without supplements to reduce the nutrients remaining in the sludge solution, initial pH was adjusted to about 7–8 and purged with Ar gas. The preincubated conditions were prepared separately at

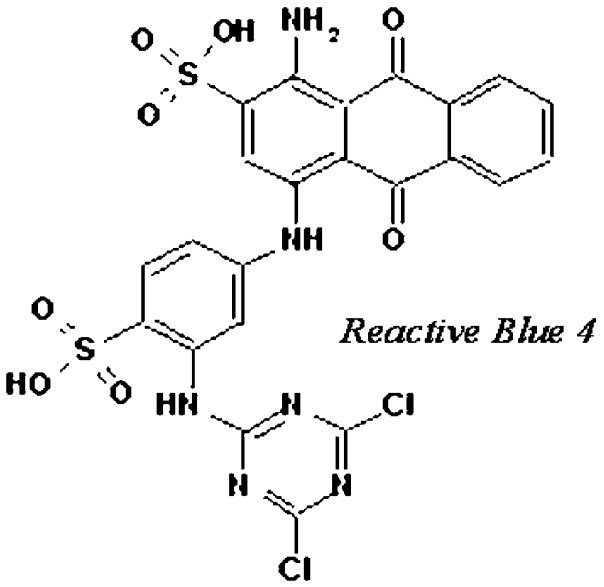

Fig. 1 Chemical structure of RB4

35 and 55°C in temperature-controlled incubators without shaking before being used in all experiments.

Dye Decolorizing Efficiency of Digested Sludge

To reveal the decolorizing efficiency of digested sludge on RB4 decolorization under anaerobic mesophilic and thermophilic conditions, the variation of RB4 at concentrations of 100, 200, 300, 400, 500, and 600 mg L^{-1} supplemented with 1 g L^{-1} glucose as a co-substrate without other supplementary nutrients was tested in batch treatment. The initial pH was adjusted to approximately 7.5 before adding to a serum bottle containing preincubated digested sludge. Treatment temperatures were 35 and 55 °C. Samples were taken every 6 h during the treatment period, and the color reduction was measured by spectrophotometer at 598 nm. The decolorizing extent was represented by percentage of decolorization.

Effect of Glucose on Dye Decolorizing Efficiency

To evaluate the need of a co-substrate as an electron donor, the effect of glucose on dye decolorizing efficiency was studied. The concentration of glucose was varied at 0, 0.5, 1.0, 2.0, and 3.0 g L^{-1} in batch treatment without other supplementary nutrients. The dye concentration added was 200 mg L^{-1}, and the treatment conditions were conducted as described above.

Decolorizing Efficiency of Dye Decolorization using Starch as a Co-substrate

For the study on using of starch as a co-substrate in dye decolorization and also for the purpose of operating cost reduction, 1 g L^{-1} starch was added to the treatment containing 200 mg L^{-1} of RB4 in a batch experiment compared with using 1 g L^{-1} glucose without

other supplementary nutrients, and pH was stabilized in the range of 7–8 by the addition of 4 g L^{-1} $NaHCO_3$. All the mixture solution was added to a preincubated serum bottle. After that, starch was varied at 0, 0.2, 0.4, 0.6, 0.8, and 1.0 g L^{-1} to study the minimum concentration of starch required.

Limitation of Salt on Dye Decolorization using Starch as a Co-substrate

Due to another problem originating mainly in the dye wastewater, which is the salt loads needed for dyebath additives onto fabrics, the effect of salt on RB4 decolorization was studied. The concentration of salt represented by NaCl was varied from 0 to 1 M in treatment containing 200 mg L^{-1} of RB4 and 1 g L^{-1} of starch. The treatment was carried out under conditions of 55 °C without shaking.

Effect of Treatment Temperatures on Dye Decolorization

To explain the effect of treatment temperatures of 35 and 55 °C on decolorizing microorganisms during the decolorizing process, 100 mg L^{-1} of RB4 was used in a batch experiment. Glucose was added at the concentration of 0.1 g L^{-1} without other supplementary nutrients and pH was stabilized in the range of 7–8 by the addition of 4 gL^{-1} $NaHCO_3$. All the mixture solution was added to a serum bottle of preincubated sludge. The control was the treatment without the addition of dye in the mixture solution. A sample was taken to measure the reduction of dye color, volatile fatty acids (VFAs), gas production, and total organic carbon (TOC). The reduced form of dyes after being treated by digested sludge was studied by monitoring the change of spectrum wavelength between 300 and 650 nm. The appearance of a new peak will be presented as a reduced form of treated dye compared with the reduction of the original peak of untreated dye.

The Evaluation of the Contribution of Dye-reducing Microbial Consortia

In addition, the contribution of decolorizing microorganisms was monitored by the experiment in the presence of vancomycin (1 g L^{-1}) and BES (10.5 g L^{-1}). Vancomycin is an inhibitor used for inhibiting the activity of acidogenesis microorganisms and BES is for methanogenesis microorganism inhibition. RB4 at a concentration of 100 mg L^{-1} was tested with 0.1 g L^{-1} of glucose or sodium acetate to assess its decolorizing efficiency as a substrate in the organic matter conversion pathway. The reduction of dye was measured at the end of the experiment.

Analyses

Dye reduction was determined photometrically by spectrophotometer (Hitachi U-2001). The appropriate dilution was made in distilled water. The absorbance was read at the maximum absorbance wavelength of 598 nm. The decolorizing efficiency was defined as decolorizing extent as a percentage of differentiation of initial and final absorbance decolorizing efficiencies of the treatment.

TOC concentration was measured by total organic carbon analyzer (Shimadzu TOC-5000). Gas chromatography (Shimadzu GC-8APT combined with Shincarbon T 60/80 column) was used for gas composition measurement and Shimadzu GC-8APF with Packed Column Unisol F-200 30/60 was used for VFA concentration measurement.

Results and Discussion

Dye Decolorizing Efficiency of Digested Sludge

The decolorizing potential of digested sludge on RB4 was studied with variations of dye concentration and showed the different extents of decolorizing efficiency of the tested dyes (Fig. 2). RB4 decolorization with increasing dye concentration at 35 °C caused a decrease in decolorizing efficiency, whereas when the treatment was conducted at 55 °C, the decolorization reached 90% in all concentrations used. The limitation of digested sludge on RB4 decolorization at 35 °C may be due to the dye component being absorbed on sludge cells which showed the blue color of the cell during the experimental period. The absorbed dye may block the substrate transportation pathway involved in the decolorizing mechanism; however, the absorption did not present when decolorizing efficiency reached about 70%. Consequently, at 55 °C, increasing the treatment temperature caused pore sizes to expand in the cell membrane that supports the substrate transportation, and increasing the potential of digested sludge on dye decolorization, which revealed that with biological dye decolorization using anaerobic digested sludge at a high temperature, the decolorizing extent could be accelerated and increased.

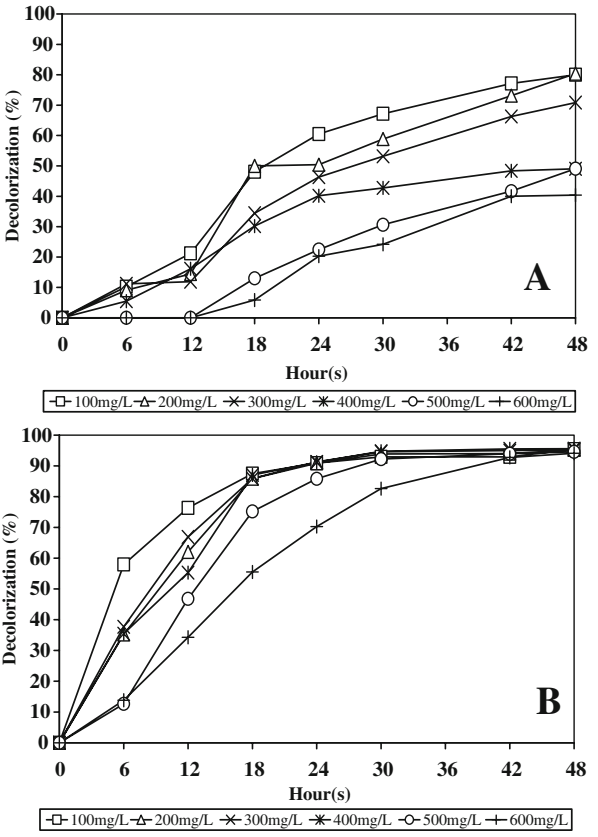

Fig. 2 Decolorizing efficiencies of RB4 under 35 °C (**A**) and 55 °C (**B**) conditions with variations of dye concentration

Effect of Glucose on Dye Decolorizing Efficiency

Due to glucose being the substrate that was easily metabolized and taken up into cells and being known as the best electron donor for the dye decolorizing process, the effect of glucose on RB4 dye decolorizing efficiency with variations of glucose concentrations at 0, 0.5, 1, 2, and 3 g L^{-1} was investigated. Figure 3 reveals that the presence of glucose in the decolorizing system was needed. In the treatment at 35 °C, increasing the glucose concentration showed an increase in decolorizing efficiency contrasted with treatment at 55 °C; the decolorizing efficiency of color reduction was not significantly different in the presence of various glucose concentrations. However, in the absence of glucose, only 20% decolorization occurred which was caused by cell absorption. According to the results of pH changes during decolorization, pH was decreased from 7.5 to 4.0 caused by the accumulation of VFAs in liquid phase. And due to the dye-reducing microorganisms were mixed culture of anaerobic digested sludge, pH was buffered in neutral to reduce the inhibition to methane-producing bacteria caused by low pH.

The reduction of anthraquinone dye took place by the mechanism of reversible quinone reduction to hydroquinone in two steps: benzoquinone ⟷ semiquinone ⟷ hydroquinone [9]. In the reduction process, transformation of RB4 in terms of the anthraquinone form to hydroquinone was related to H^+ generated from glucose degradation in the organic matter conversion process and reductive transformation of the anthraquinone nucleus [10].

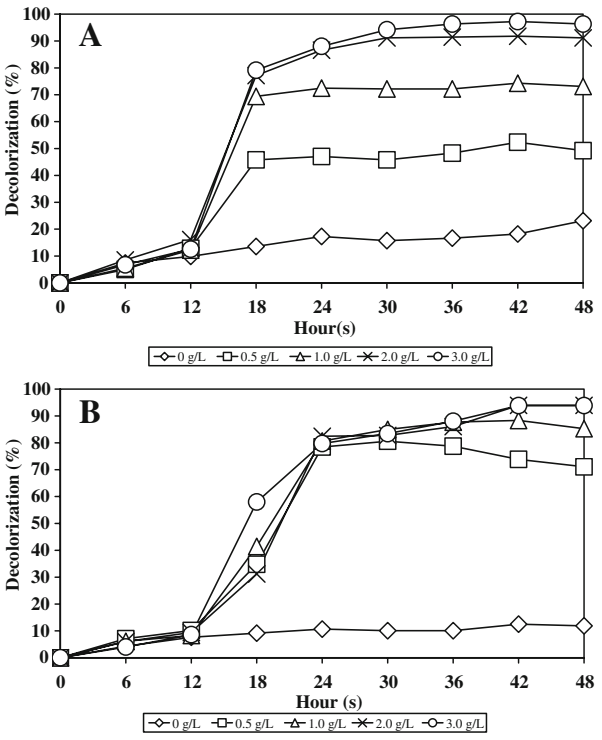

Fig. 3 Decolorizing efficiencies of RB4 at 35 °C (**A**) and 55 °C (**B**) with variations of glucose concentration

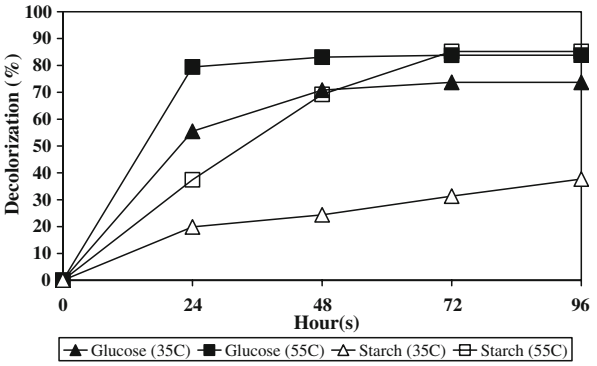

Fig. 4 Comparative decolorizing efficiencies of RB4

Decolorizing Efficiency of Dye Decolorization using Starch as a Co-substrate

Figure 4 shows the decolorizing efficiencies using glucose and starch as co-substrates. Low decolorizing efficiency, 30% decolorization within 96 h, occurred at 35 °C when starch was added. This indicated that the degradation of starch was slow, caused by the low activity of the glycoside hydrolase enzyme, while glucose could be used directly. However, treatment at 55 °C showed results similar to those using glucose as a co-substrate, which showed at 70–80% that even the decolorizing potential was slower in the first period of decolorization. The reason for high decolorizing efficiency of treatment using starch at 55 °C may be that the treatment temperature of 55 °C was more suitable for the glycoside hydrolase enzyme than 35 °C. By using glucose as a co-substrate, the VFAs produced were higher because the glucose was directly utilized and converted to VFAs, whereas when using starch in the treatment, the hydrolyzes of starch were released and slowly converted to VFAs in the hydrolysis stage (Table 1). On the other hand, because of the high concentration of VFAs, the use of glucose caused VFA accumulation and affected the methane-producing bacteria. The slow rate of VFA production was confirmed by slowly increasing the TOC in the treated solution, in which nonhydrolyzed starch was separated by centrifugation. The increasing of TOC was caused by hydrolyzed starch as long and short chain sugar content in treated solution (Fig. 5).

Table 1 Volatile fatty acids' concentration of dyes.

	Decolorization (%)	Volatile fatty acids' concentration ($mgCODL^{-1}$)			
		HAc	HPr	*i*-HBu	*n*-HBu
Mesophilic treatment (35 °C)					
Glucose	73.7	341	231	38.7	12.4
Starch	37.7	91.1	12.0	0	0
Thermophilic treatment (55 °C)					
Glucose	83.8	232	23.4	5.97	11.4
Starch	85.2	115	13.3	5.77	0

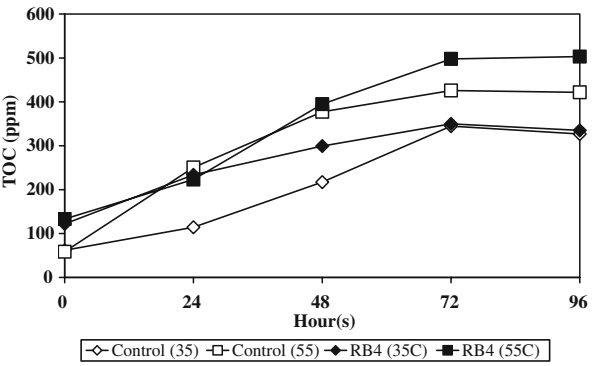

Fig. 5 Total organic carbon of treated solution using glucose and starch as co-substrates control and dye wastewater

These indicated that starch could be used as a co-substrate in the dye decolorization process instead of glucose and that it is possible to reduce the inhibitory effects of VFA accumulation in the treatment. In addition, due to the increase in the treatment's temperature, the advantages were shown in the increase of decolorizing efficiency on dye decolorization, especially the recalcitrant dye which is difficult to decolorize under normal temperatures, as shown in the result.

In addition, the minimum concentration of starch needed in thermophilic decolorization is shown in Fig. 6. The result indicated that the content of starch concentration should be more than 0.6 g L^{-1} in thermophilic decolorization of RB4 dye, in which high decolorizing efficiency is related with higher starch concentration addition. Varying temperature was resulting into a shift of microbial composition. It would be interesting to examine the effect of temperatures between 35 and 55 °C to find a compensation of effects and results. Obviously, higher temperature promoted the activity of starch-degrading bacteria. The operating cost could also be reduced with the combination of using starch-containing wastewater from the starch production process with dye-containing wastewater. Furthermore, using a mixture of starch and glucose as co-substrates would give no difference with using only glucose as co-substrate. However, if a mixture of glucose and starch was added as co-substrates, resulting cost performance would be decreased compared with using only starch addition.

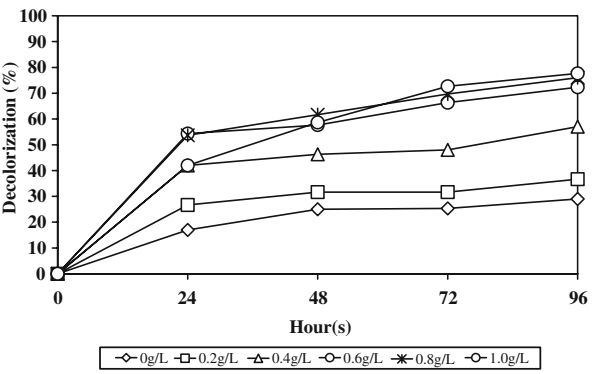

Fig. 6 Decolorizing efficiencies of RB4 decolorization with variations of soluble starch concentration under thermophilic treatment

Limitation of Salt Concentration on Dye Decolorization using Starch as a Co-substrate

The salt content of wastewater from batch dyeing is usually very high because the dyeing processes demand a significantly high concentration of salt, approximately 100 g L^{-1} [11]. The effect of NaCl was studied by using starch under thermophilic condition. It was possible to use starch as a co-substrate instead of using glucose under thermophilic treatment for the purpose of operating cost reduction in textile dye decolorization process, which gave similar decolorizing efficiency to that of glucose. The advantage of using starch was represented by the slow rate of VFA production, which presented low effects on the methane-producing bacteria by the accumulation of VFAs. The result shown in Fig. 6 indicates that with low concentrations of NaCl applied to the treatment, 20–100 mM, decolorization was accelerated and higher decolorizing efficiency achieved than in the control (0 mM). However, at concentrations over 200 mM, the decolorizing efficiency was decreased and the decolorization was inhibited at concentrations over 500 mM. The effect of salts on the decolorizing process was the cause of the change in osmotic pressure in the wastewater, in which the decolorizing microorganism could be harmed, as a concentration of salt much over 1% is harmful to bacteria, and microorganisms are inhibited by a high concentration of salt of 10–15% (Fig. 7) [12].

Effect of Treatment Temperatures on Dye Decolorization

As in the previous experiments, the results confirmed that decolorization of RB4 could be accelerated by treatment under thermophilic conditions (55 °C) and gave a better result of decolorizing potential when compared with mesophilic treatment. However, the effect of dye and temperature on decolorization can still not be understood. In this experiment, even the decolorizing efficiency was examined, and other factors including VFA concentration, gas production, TOC reduction and also the reduction of dye were investigated. The obtained results of decolorization of 100 mg L^{-1} of RB4 used in the experiment were compared with the control, which had no dye addition.

Results indicated that the inhibitory effect of the temperature increasing and the presence of dye were associated with inhibition of the organic matter conversion pathway caused by the accumulation of volatile fatty acids in the treatment system. In the case of increasing temperature, Fig. 8 shows the different organic matter conversion pathways under

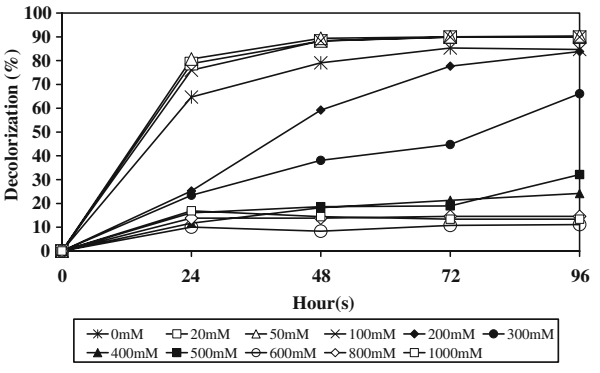

Fig. 7 Decolorizing efficiencies of RB4 decolorization applied with variations of NaCl concentration

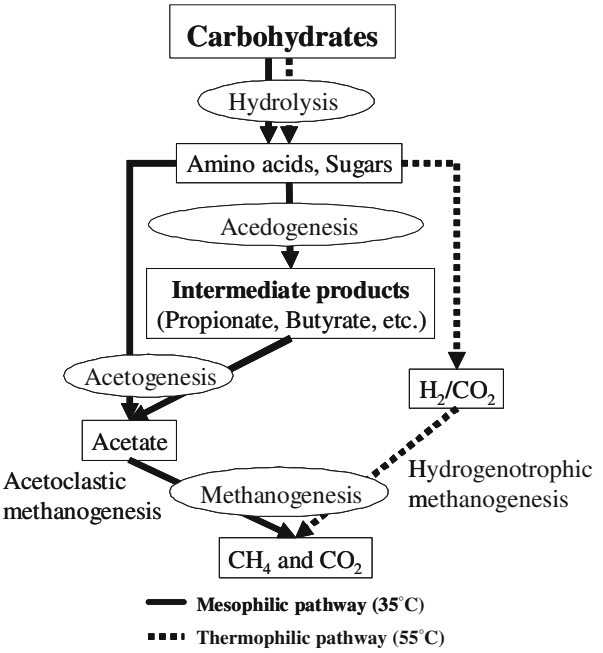

Fig. 8 Suggestion of the organic matter conversion pathways at 35 °C and 55 °C

mesophilic and thermophilic conditions. Under mesophilic conditions, the conversion was the degradation of glucose to intermediate products (acetate, butyrate, propionate, etc.) by acedogens and acetogens to be used as a substrate for methane production, whereas under thermophilic conditions, the major pathway was the hydrogen production by hydrogenotrophic microorganisms combined with the minor pathway of VFA production, which was indicated by the high volume of H_2 production and the low VFA concentration produced (Table 2).

Table 2 Batch decolorization of RB4 by anaerobic treatment under mesophilic and thermophilic conditions at the 72nd and 168th hour of the experiment.

Decolorization (%)		72nd hour							168th hour
		VFAs ($mgCODL^{-1}$)				TOC removal (%)	Gas prod. (mlL^{-1})		CH_4 production (mlL^{-1})[a]
		HAc	HPr	*i*-HBu	*n*-HBu		CH_4	H_2	
Mesophilic conditions (35 °C)									
Control	–	255	220	19.4	46.4	72.3	4.8	1.22	13.4
RB4	73.7	337	116	10.2	152	73.2	0.67	2.40	15.4
Thermophilic conditions (55°C)									
Control	–	240	17.8	0	7.56	70.6	3.72	12.48	2.64
RB4	83.8	192	28.6	9.54	33.1	63.4	0.48	1.31	3.57

[a] CH_4 production was total production during the 72nd to 168th hours of the experiment.

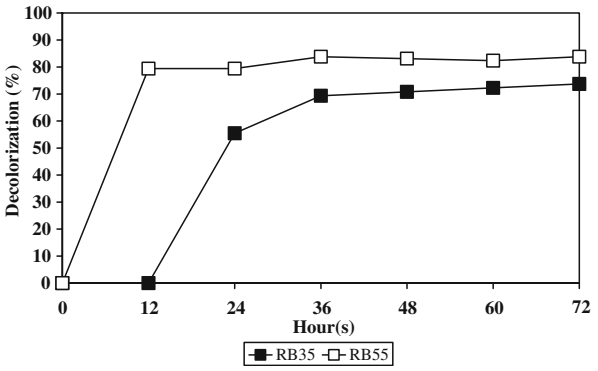

Fig. 9 Decolorizing efficiencies of RB4 decolorization at 35 °C (*filled square*) and 55 °C (*empty square*)

For the dye itself, the presence of RB4 inhibited methane productivity. TOC reduction of the treatment of RB4 was similar to the control. Due to the increase in the treatment's temperature (thermophilic condition), the advantages were shown in the increase in decolorizing efficiency on RB4 decolorization and TOC reduction. Inhibition of decolorizing microorganisms by textile dye and its intermediate products has been reported. The accumulation of VFAs was mainly in the form of acetate and propionate with traces of iso-butyric, n-butyric and iso-valeric when RB4 or RB19 was amended in the culture [13]. Methanogenic culture amended with 250–300 mg L^{-1} of Brilliant red Resolin showed 78.9% and 59.6% inhibition of specific methane yield and production via aceticlastic methanogenesis [14].

Figure 9 shows the higher efficiency obtained under thermophilic conditions (80% decolorization) compared with mesophilic conditions (70%), which confirms the acceleration by increasing the treatment's temperature. Figure 10 shows the spectrogram of RB4 and treated RB4: the reducing by-products of RB4 did not show any auto-oxidizing reaction and treated wastewater containing RB4 turned light yellow in presence of unsubstituted anthraquinone. The reduction of anthraquinone dye takes place by the mechanism of reversible quinone reduction to hydroquinone in two steps: benzoquinone ⟷ semiquinone ⟷ hydroquinone [9]. In general, under anaerobic conditions, a lower

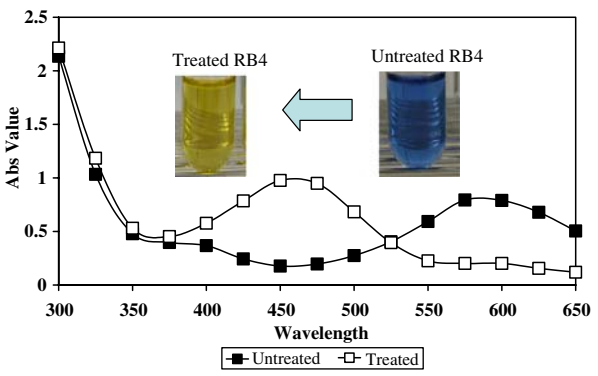

Fig. 10 Wavelength scanning of untreated (*filled square*) and treated (*empty square*) RB4 and apparent color

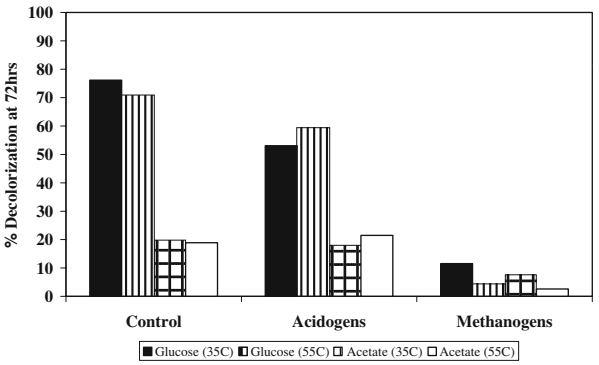

Fig. 11 Decolorizing efficiencies of RB4 decolorizations at 72 h of experiment employed with vancomycin (acidogeninhibitor) and BES (methanogen inhibitor)

rate and extent of decolorization of anthraquinone dyes have been observed as compared with azo dye, which corresponds to the presented results [15, 16].

The Evaluation of the Contribution of Dye-reducing Microbial Consortia

To reveal the contribution of the microorganism group effect on dye decolorization, vancomycin (1 g L^{-1}) and BES (10.2 g L^{-1}) were used as acidogen and methanogen inhibitors, respectively. The results indicated clearly that acidogens were important in the decolorizing mechanism, of which high decolorizing efficiency occurred only by using glucose as a co-substrate for RB4 decolorization (Fig. 11). The decolorizing efficiencies of using acetate as a co-substrate for acidogens and in treatments of methanogens (vancomycin addition) occurred by dye absorption or accumulation, as discussed previously. In addition, acetate was the primary substrate for methanogens and was not utilized by acidogens in the hydrolysis process. Then, when methanogens were inhibited by BES, the decolorization did not occur because the reduction of RB4 in the form of anthraquinone to hydroquinone was related to H^+ generated by the organic matter conversion process and reductive transformation of the anthraquinone nucleus [10].

Conclusions

The results of comparing decolorizing efficiency of RB4 decolorization by mesophilic and thermophilic treatment could be concluded as follows:

1. Thermophilic treatment at 55 °C showed acceleration and effectiveness of RB4 decolorization and did not show a limitation on decolorization even when a high concentration of dye was applied. While under mesophilic treatment at 35 °C, decolorizing efficiency was inhibited by various dye concentrations. The presence of glucose as an electron donor substrate was needed, especially under mesophilic treatment, in which a high concentration of glucose was needed if the wastewater had high dye content.
2. It was possible to use starch as a co-substrate instead of using glucose under thermophilic treatment for the purpose of operating cost reduction in the textile dye decolorization process, which gave similar decolorizing efficiency to that of glucose. The advantage of using starch was represented by the slow rate of VFA production, which presented low effects on the methane-producing bacteria by the accumulation of VFAs.

3. At low concentrations of NaCl contained in the wastewater, the decolorizing process could be accelerated; however, the decolorization was inhibited when the NaCl concentration reached over 200 mM.
4. The effect of temperature at 55 °C did not inhibit dye decolorizing efficiency but affected the inhibition of methanogen activity, confirmed by the accumulation of VFAs and low CH_4 production. The effect of inhibition of dyes was shown on the metabolic pathway of organic conversion; however, after the dye was completely decolorized, microorganism activity could be recovered.
5. Dye-reducing microbial consortia were evaluated with acidogen and methanogen inhibitors, vancomycin and BES, in which acidogenesis microorganisms was dominant in the dye decolorization.

Acknowledgement This study was carried out under the JSPS-NRCT Scientific Cooperation Program and supported by a Japanese government scholarship (MOMBUKAGAKUSHO: MEXT).

References

1. McMullan, G., Meehan, C., Conneely, A., Kirby, N., Robison, T., Nigam, P., et al. (2001). *Applied Microbiology and Biotechnology*, *56*, 81–87.
2. O'Neill, C., Hawkes, F. R., Hawkes, D. L., Lourenco, N. D., Pinheiro, H. M., & Delee, W. (1999). *Journal of Chemical Technology and Biotechnology*, *74*, 1009–1018.
3. Beydilli, I. M., Pavlostathis, S. G., & Tincher, W. C. (2000). *Water Environment Research*, *72*, 692–705.
4. Epolito, W. J., Lee, Y. H., Bottomley, L. A., & Pavlostathis, S. G. (2005). *Dyes and Pigments*, *67*, 35–46.
5. Pearce, C. I., Lloyd, J. R., Guthrie, S. G., & Tincher, W. C. (2003). *Dyes and Pigments*, *58*, 179–196.
6. Sloker, Y. M., & Le Marechal, A. M. (1998). *Dyes and Pigments*, *37*, 335–356.
7. Dos Santos, A. B., Bisschops, I. A. E., Cervantes, F., & Vanlier, J. B. (2004). *Chemosphere*, *55*, 1149–1157.
8. Willets, J. R. M., & Ashbolt, N. J. (2000). *Water Science and Technology*, *42*, 409–416.
9. Zollinger, H. (1991). *Color chemistry* (2nd ed.). New York: VHC.
10. Revenga, J., Rodriguez, F., & Tujero, J. (1994). *Journal of the Electrochemical Society*, *141*, 330–333.
11. Correira, V. M., Stephenson, J., & Judd, S. J. (1994). *Review on Environmental Technology*, *15*, 917–929.
12. Patrick, K. J. (2004). *Environmental microbiology*: *principles and applications*. Enfield: Science.
13. Young, L. H., Spyros, G., & Pavlostathis, S. G. (2004). *Water Research*, *38*, 1838–1852.
14. Melpei, F., Andreoni, V., Daffonchio, D., & Rozzi, A. (1998). *Bioresource Technology*, *63*, 49–56.
15. Delee, W., O'Neil, C., Hawks, F. R., & Pinheiro, H. M. (1998). *Journal of Chemical Technology and Biotechnology*, *73*, 323–335.
16. Panswad, T., & Luangdilok, W. (2000). *Water Research*, *34*, 4177–4184.