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ORIGINAL PAPER

Hydrogen production and anaerobic decolorization of wastewater containing Reactive Blue 4 by a bacterial consortium of *Salmonella subterranea* and *Paenibacillus polymyxa*

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Abstract Anaerobic biodegradability of wastewater (3,000 mg CODcr/l) containing 300 mg/l Reactive Blue 4, with different co-substrates, glucose, butyrate and propionate by a bacterial consortium of *Salmonella subterranea* and *Paenibacillus polymyxa*, concomitantly with hydrogen production was investigated at 35°C. The accumulative hydrogen production at 3,067 mg CODcr/l was obtained after 7 days of incubation with glucose, sludge, the bacterial consortium. The volatile fatty acids, residual glucose and the total organic carbon were correlated to hydrogen obtained. Interestingly, the bacterial consortium possess decolorization ability showing approximately 24% dye removal after 24 h incubation using glucose as a co-substrate, which was about two and eight times those of butyrate (10%), propionate (12%) and control (3%), respectively. RB4 decolorization occurred through acidogenesis, as high volatile fatty acids but low methane was detected. The bacterial consortium will be the bacterial strains of interest for further decolorization and hydrogen production of industrial waste water.

Keywords Hydrogen · Decolorization · RB4 · *Salmonella subterranea* · *Paenibacillus polymyxa* · Wastewater

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Introduction

Hydrogen is a clean and environmentally friendly fuel, when it burns, it only produces water as the by product. Hydrogen can be produced in many ways including; chemically (e.g., gasification of coal), electrochemically (e.g., electrolysis of water) or by the use of microorganisms (Takabatake et al. 2004; Wang et al. 2007; Bothe et al. 2008). There are two main systems of microbial hydrogen production, photochemical system using photosynthetic microorganisms such as algae and photosynthetic bacteria (Melis and Happe 2001) and fermentative system using facultative anaerobes and obligate anaerobes (Nandi and Sengupta 1998). The fermentation substrates most studied in the laboratory are glucose (Mu et al. 2006) and sucrose (Tao et al. 2007). In addition,

it is well known that carbohydrates are the main source of hydrogen during fermentative processes and therefore, renewable energy sources, wastes/wastewater or agricultural residues rich in carbohydrates can be considered as potential sources of hydrogen (Kapdan and Kargi 2006). Hydrogen could be converted into electricity via fuel cells or directly used in internal combustion engines. It can also be used for the syntheses of ammonia, alcohols and aldehydes, as well as for the hydrogenation of petroleum, coal, shale oil, and edible oil (Hart 1997). Many believe that hydrogen will replace fossil fuels as the next generation of energy supply. A hydrogen-based economy will impose no risk of global warming, and will improve significantly the urban air quality (Fang et al. 2004).

Dyes are annually produced and applied in textiles, cosmetics, pharmaceutical, photographic, plastics, paper, and food industry. They are classified as acidic, basic, azo, diazo, disperse, metal complex and anthraquinone-based dyes (Fu and Viraraghavan 2001; Aksu and Tezer 2005) according to their structural varieties. Due to the increased demand for textile products, the textile industry and its wastewater have been increasing proportionally, making it one of the main sources of severe environmental problems (Vandevivere et al. 1998). Reactive Blue 4 (RB4), an anthraquinone-based chlorotriazine dye, is very important in dyeing of cellulosic fabrics. Reactive dyes have environmental implications since up to 50% of the initial dye mass used in the dyeing process remains in the spent dyebath in its hydrolyzed form which no longer has an affinity for the fabric, and therefore cannot be reused in the dyeing process (Laszlo 1995). They are not readily removed by typical wastewater treatment processes due to their stability and resistance towards light or oxidizing agents (Lee et al. 2005). In addition, the high pH and high salt concentrations under typical reactive dyeing conditions further complicate the management of used reactive dyebaths (Rys and Zollinger 1989). Therefore, establishing dye removal technologies is an urgent problem. The most commonly used method for the treatment of textile wastewater is the combination of physicochemical treatment and biological oxidation (Manu and Chaudhari 2002; Singh et al. 2008; Omar 2008). However, these processes are quite ineffective in wastewater color removal since dyestuff, such as anthraquinone-based dye, are biorecalcitrant owing to their aromatic structure. RB4 was selected as a targeted dye for this research due to its relatively slow biodecolorization kinetics (Lee et al. 2005).

Therefore, this study aims to investigate hydrogen production and decolorization potential of waste water containing RB4 by facultative anaerobic bacterial consortium of, *Salmonella subterranea* and *Paenibacillus polymyxa*, which possess both hydrogen production and dye decolorization properties using different co-substrates, glucose, butyrate and propionate.

Materials and methods

Chemicals

Reactive Blue 4 (RB4, IC 61205), the anthraquinone dye class, was used as a targeted dye. Glucose, butyrate and propionate were used as co-substrates or electron donor substrates. All chemicals were purchased from Sigma–Aldrich and Wako, Japan.

Bacterial strains used were *Salmonella subterranea* and *Paenibacillus polymyxa* capable of hydrogen production and dye decolorization.

Preparation of culture medium containing wastewater and RB4

Salmonella subterranea and *Paenibacillus polymyxa*, capable of hydrogen production were obtained from Dr. Sungwan Kanso. The two bacterial strains were isolated from Mae Khong River, Ubolratchathani Province, Thailand. Screening, purification, morphological and biochemical characterization and phylogenetic analysis of 16SrRNA sequences were conducted and are currently under preparation for publication by Dr. Kanso's group. Interestingly, we later found that the bacterial strains also showed dye decolorization property using 1% molasses in agar plates and then also preliminary experiment with molasses wastewater decolorization (data not shown).

Anaerobic sludge obtained from municipal wastewater treatment plant, Western Purification Center, Ube City, Yamaguchi Prefecture, Japan was centrifuged to separate the wastewater and sludge cell. Sludge was concentrated to 3,000 mgVSS/l and separated wastewater was supplemented with co-substrate

and nutrient to make up the chemical oxygen demand (COD) concentration to 3,000 mg CODcr/l. The co-substrates used were glucose (9.4 g/l), propionate (6.6 g/l) and butyrate (5.5 g/l) and supplemented with yeast extract (0.1 g/l), and K_2HPO_4 (4 g/l). In order to understand the effect of RB4 on microbial activity, neutral pH (pH 7) was obtained by the addition of buffering chemical, $NaHCO_3$ (4 g/l). For bacterial consortium used, 1×10^{10} cell of each of *S. subterranea* and *P. polymyxa* were mixed with anaerobic sludge before use.

Before the experiment was conducted, the 500 ml serum bottle contained 50 ml bacterial consortium was sealed with butyl rubber stopper, capped with aluminium crimp cap and purged with Argon gas to deplete the oxygen accumulated in bottle, then incubated at 35°C overnight to reduce the nutrients remaining in the sludge solution before the addition of 200 ml wastewater (3,000 mg CODcr/l) and 300 mg/l RB4. The detailed experimental condition is shown in Table 1. The operational parameters used were optimized previously in our laboratory.

Analysis of samples

During the incubation period, a 5-ml sample was taken every 24 h from each bottle, and centrifuged at 12,000*g* for 10 min. The supernatant was collected for measuring of pH, accumulated fatty acids (VFAs), total organic carbon (TOC) and decolorizing efficiency. The gas evolved was measured volumetrically by water displacement in a burette and the volume was calculated using the mass balance equation (Zheng and Yu 2005). As from equation $H_2 + 1/2\ O_2 \rightarrow H_2O$, so 1 mole H_2 is equal to 16 g COD/l, the unit of H_2 was used as mg CODcr/l so that it will be the same unit used for VFAs and co-substrate, i.e., glucose. Gas samples were taken from the headspace of each bottle by a gas-tight syringe. The biogas composition was analyzed by a gas chromatograph (Shimadzu GC-8APT) equipped with a thermal conductivity detector (TCD) and 1.5 m stainless column packed with activated charcoal 60/80, Shinwa Co. Ltd, Japan.

Table 1 The experimental condition of controls and treatments for hydrogen production and RB4 decolorization at 35°C

	Bacteria consortium	Sludge	Co-substrate	300 mg/l RB4
Control 1	X	O	X	O
Control 2	O	O	X	O
Sample 1	O	O	Glucose	O
Sample 2	O	O	Propionate	O
Sample 3	O	O	Butyrate	O

Decolorization of the samples was determined using a spectrophotometer (Hitachi U-2001), pH of all samples were adjusted to 7.6 with NaOH or H_2SO_4, before measuring the absorbance at the maximum wavelength of 598 nm. Dye removal defined as a percentage of differences between the initial and final absorbance (% decolorization). The reduced form of treated dye was detected by monitoring the changes of spectral wavelengths between 200 and 800 nm. The appearance of a new peak indicated a reduced form of treated dye. Residual glucose was measured spectrophotometrically (Hitachi U-2001) using dinitrosalicylic acid (DNS) method. Chemical oxygen demand (COD) was measured as the corresponding oxygen consumption during oxidation with dichromate, defined as CODcr (Pitwell 1983).

The biogas composition was analyzed by a gas chromatography (Shimadzu GC-8APT) equipped with a thermal conductivity detector (TCD) and 1.5 m stainless column packed with activated charcoal 60/80, Shinwa Co. Ltd, Japan. The temperature of injector, column and detector were kept at 50, 60, and 50°C, respectively. Argon was used as carrier gas at a flow rate of 20 ml/min. Volatile fatty acids (VFAs) concentrations were detected using Shimadzu GC-8APF with Packed Column Unisol F-200 30/60. The temperature of injector, column and detector were kept at 250, 140, and 140°C, respectively. Nitrogen was used as carrier gas at a flow rate of 30 ml/min. Total organic carbon (TOC) were also determined using total organic carbon analyzer (Shimadzu TOC-5000).

Results and discussion

Hydrogen production

Hydrogen production by biological methods, determined from various renewable resources, are less energy intensive than chemical or electrochemical

Table 2 Decolorization (%), volatile fatty acid concentrations, gas production and TOC removal after 7 days of incubation at 35°C of RB4 and wastewater in the presence and absence of glucose, propionate and butyrate, and the bacterial consortium

Treatments	Decolorization (%)	VFAs (mg COD l^{-1})				Gas production (mg COD l^{-1})		TOC removal (%)
		Hac	HPr	i-Hbu	*n*-Hbu	CH_4	H_2	
Control 1	32.3	97.7	18.7	3.6	0	1,015.5	33.7	16.9
Control 2	64.8	270.8	215.8	9.1	41.5	1,065.5	436.3	3.04
Sample 1	74.3	1,522.8	1,190.3	39.9	330.4	395.7	3,067.5	41.8
Sample 2	82.5	199.8	143.0	0	2,702.6	987.7	711.9	20.4
Sample 3	79.7	392.7	4,263.1	0	202.9	468.6	1,374.0	15.4

ones since they are carried out at ambient temperature and pressure (Elam et al. 2003). Therefore, hydrogen production by the bacterial consortium, mixed culture of anaerobic sludge, *S. subterranea* and *P. polymyxa*, was determined. The accumulative H_2 production after 7 days was as high as 3,067.5 mg CODcr/l, in the presence of the bacterial consortium and glucose (sample 1), as compared to propionate (1,374.0 mg CODcr/l) (sample 2) and butyrate (711.9 mg CODcr/l) (sample 3), which are 3 times and 5 times less H_2 was detected (Table 2). The results were correlated with the VFAs produced (Table 2). In general, after glucose hydrolysis, VFAs were then produced by acidogenic activity of the bacterial consortium and hydrogen production occurred. Table 2 indicated that in the presence of glucose, H_2 and acetic acid (Hac) was increased. On the other hand, methane production using glucose and propionate as substrate was low, implying that methanogenic activities may be inhibited by color adsorption to the bacterial consortium. In general, methanogenic activity could be inhibited by low pH and high VFA, however, in this study pH was not less than 6 (Fig. 1) and VFAs are not so high to cause inhibition of methanogenic activities. Dye adsorption to the cells leading to methanogenic activity inhibition is a possible explanation. The total organic carbon (TOC) was shown in Table 2, supporting carbon degradation by the anaerobic bacterial consortium.

The higher efficiency of glucose as a co-substrate for H_2 production after 7 days (Table 2) in the presence of the bacterial consortium as compared to butyrate and propionate was supported by many studies on hydrogen production using *Clostridia* (Taguchi et al. 1993) and *Enterobacteria* (Kumar and Das 2000). In this study, during the experimental period of 7 days, pH was relatively constant for each

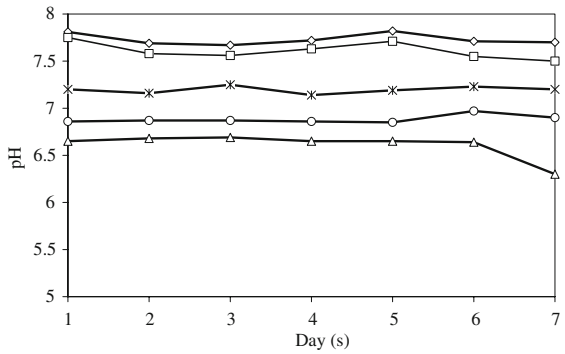

Fig. 1 pH changes during anaerobic decolorization at 35°C of wastewater with sludge and 300 mg/l reactive blue4 in the absence (◇) and presence (□) of the bacterial consortium. Different substrates were added; glucose (Δ), butyrate (*) and propionate (◯)

sample (Fig. 1), therefore, hydrogen production was not affected by pH variation. Since fermentative hydrogen production is affected by pH, temperature as well as the nature of the microorganisms. pH is crucial due to its effects on hydrogenase activity, metabolism pathways (Lay 2000; Jun et al. 2008; Liu et al. 2008) and microbial communities (Fang and Liu 2002). In general, the dominant metabolism in a mixed acidogenic culture depends strongly on pH of the microbial culture and hydrogen production is suppressed by both low and high pH (Chen et al. 2002). Thus, it is important to control the pH in order to maintain satisfactory hydrogen production.

In general, degradation of sugars is accompanied by the production of hydrogen and different metabolic products, mainly VFAs (acetic acid, Hac; propionic acid, HPr; *n*-butyric acid, *n*-Hbu; isobutyric acid; i-Hbu), lactic acid and ethanol, during the fermentation process. Different organic matter conversion pathways under mesophlilic condition, of which glucose degradation by acidogens and

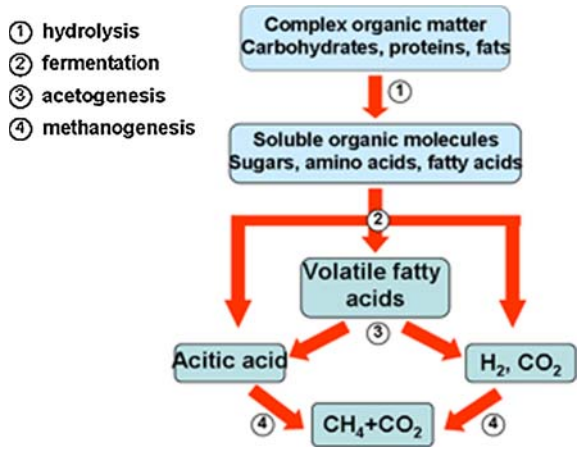

Fig. 2 Suggestion of the organic matter conversion pathways at 35°C (mesophilic condition)

acetogens to intermediate products (acetate, butyrate, propionate, etc.), which further be used as substrate for methane production (Fig. 2). Since, the hydrogen yield varies proportionally to the final metabolic products, acetic and butyric acid production favors hydrogen production (Nandi and Sengupta 1998; Hawkes et al. 2002) according to Eqs. (1) and (2) with the fermentation to acetic acid giving the highest theoretical yield of 4 mol H_2/mol hexose. The final products of fermentation (acetate, hydrogen and CO_2) are the precursors of methane formation (methanogenesis) (McCarty and Smith 1986). In addition, lowering the pH to 4.5 or below may shift the VFA-producing pathway to an alcohol-producing pathway. As the study by Khanal et al. 2004, showing that if there is a shift of pH to be more acidic, this will affect VFA and metabolic alteration, which was not occurred in our experiment.

Therefore, our results obtained in Table 2 showed correlation between hydrogen production and the VFAs produced, showing increased acetic acid production accompanied by increased hydrogen production as of sample 1 (glucose, the bacterial consortium).

Acetic acid production

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (1)$$

Butyric acid production

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \quad (2)$$

Decolorization of RB4

Apart from H_2 production, the bacterial consortium also possesses decolization ability. The spectra of RB4 after 24 h treatment with the bacterial consortium scanned at 400–800 nm, with maximum absorption at 598 nm revealed the evident reduction of absorbance in the presence of glucose as compared to controls (Fig. 3a). It is readily apparent from Fig. 3b that in the presence of glucose, the bacterial consortium, at day 1, 24% dye removal was achieved, which is approximately 2 times and 8 times higher than those of butyrate (10%), propionate (12%) and control (3%), respectively, implying that the presence of glucose in the decolorizing system was needed for the fast decolorization efficiency. This result was supported by the TOC removal and decreased residual glucose

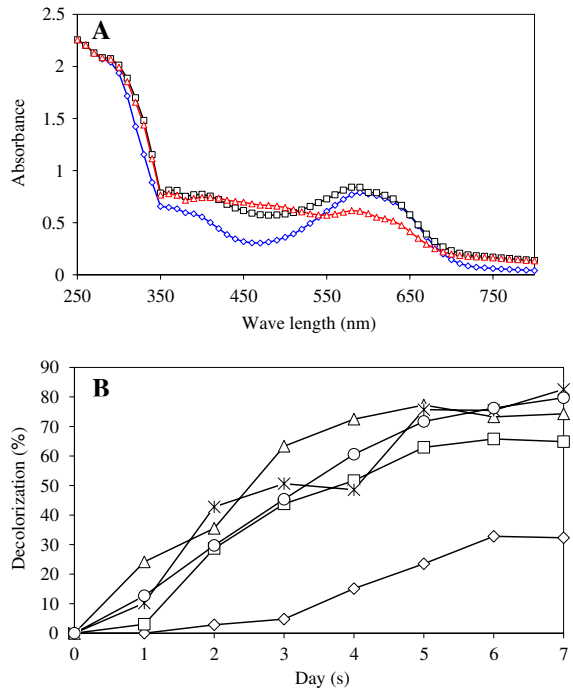

Fig. 3 **a** Wastewater spectra after 24 h of incubation with RB4, sludge and the bacterial consortium at 35°C in the presence of glucose, sample 1 (Δ) as compared to control 1, no bacterial consortium (◇) and control 2, with the bacterial consortium (□). **b** Percentage of anaerobic decolorization of waste water with sludge and 300 mg/l RB4 in the absence (◇) and presence (□) of the bacterial consortium. Different substrates were added; glucose (Δ), butyrate (*) and propionate (◯), measured at 598 nm

concentration (Table 2, Fig. 4). The dye removal was increased continuously until day 7, however, the increase in dye decolorization of control was not so evident as in the presence of glucose, regardless of the bacterial consortium addition. Approximately 32.3–82.5% total color removal was obtained after 7 days of decolorization in different types of substrates used as demonstrated in Table 2.

The slow RB4 decolorization of the control at 35°C, showing blue cells, may be due to the dye adsorption to the cells during experimental period. Dye adsorption to the cells leading to methanogenic activity inhibition is a possible explanation. The result showed that the TOC removal was decreased from 90% (start-up condition) to 30%, approximately, during the decolorization (Table 2), which corresponded with other researches. The inhibition of decolorizing microorganisms also occurred by textile dye and its intermediates, the VFAs accumulation was mainly in the form of acetate and propionate with traces of iso-butyric, *n*-butyric and iso-valeric acid when RB4 or RB19 was amended in culture (Lee et al. 2004). In addition, the methanogenic culture amended with 250–300 mg/l of Brilliant Red Resolin (BLS) showed 78.9% inhibition of specific methane yield and 59.6% production via aceticlastic methanogenesis (Melpei et al. 1998).

The adsorbed dye may also block the substrate transportation pathway involved in decolorizing mechanism. The increased dye removal in the presence of glucose may be due to glucose utilization, as glucose is the best electron donor for the dye decolorizing. This result was correlated with our previous study that

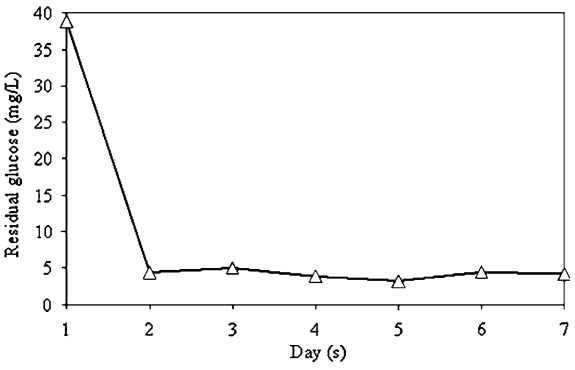

Fig. 4 Residual glucose (mg/l) of anaerobic decolorization at 35°C of sample 1 containing waste water, sludge and 300 mg/l RB 4 in the presence of glucose (300 mg CODcr/l) and the bacterial consortium

increasing the glucose concentrations showed an increase in decolorizing efficiency, at 35°C (data not shown). In general, anthraquinone dye reduction occurs by the mechanism of reversible quinone reduction to hydroquinone in two steps: benzoquinone ↔ semiquinone ↔ hydroquinone (Zollinger 1991). Thus, in the reduction process, transformation of RB4 in terms of the anthraquinone to hydroquinone was related to H^+ generated from glucose degradation and reductive transformation of the anthraquinone nucleus (Revenga et al. 1994). For RB4 decolorization mechanism, the results indicated evidently that acidogenesis was involved, as high decolorization efficiency, high VFAs but low methane were obtained when using glucose as compared to control and other co-substrates. This can be explained by Fig. 2, under mesophilic condition, degradation of glucose by acidogens and acetogens produced acetate, butyrate and propionate etc., which then will be used as substrates for methane production. These indicated that the reduction of RB4, anthraqionone form to hydroquinone, was related with H^+, generated from organic matter conversion process, and reductive transformation of anthraquinone nucleus (Revenga et al. 1994). Reducing byproduct of RB4 did not show any auto-oxidizing reaction, as the treated wastewater containing RB4 showed light yellow due to the unsubstituted antraquinone. The slower dye removal found when using other co-substrate, may be due to dye adsorption or accumulation to the cells.

Anthraquinone and phthalocyanine dyes are shown to be rather recalcitrant (Lee et al. 2005; Dos Santos et al. 2005). Therefore, the bacterial consortium is beneficial for enhanced the RB4 removal efficiency.

In conclusion, the isolated microbial cultures of the bacterial consortium shown in this study, deserve attention as a new biomass media, which can be utilized with combined anaerobic sludge treatment in the decolorization of wastewater effluents containing dyes as well as hydrogen production.

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References

Aksu Z, Tezer S (2005) Biosorption of reactive dyes on the green alga *Chlorella vulgari*. Process Biochem 40:1347–1361. doi:10.1016/j.procbio.2004.06.007

Bothe H, Winkelmann S, Boison G (2008) Maximizing hydrogen production by cyanobacteria. Z Naturforsch 63(C):226–232

Chen CC, Lin CY, Lin MC (2002) Acid-base enrichment enhances anaerobic hydrogen production process. Appl Microbiol Biotechnol 58(2):224–228. doi:10.1007/s00253 0100814

Dos Santos AB, Bisschops IAE, Cervantes FJ, Van Lier JB (2005) The transformation and toxicity of anthraquinone dyes during thermophilic (55°C) and mesophilic (30°C) anaerobic treatments. J Biotechnol 15:345–353. doi: 10.1016/j.jbiotec.2004.09.007

Elam CC, Gregoire Padro CE, Sandrock G, Luzzi A, Lindblad P, Hagen EF (2003) Realizing the hydrogen future: the International Energy Agency's efforts to advance hydrogen energy technologies. Int J Hydrogen Energy 28:601–607. doi:10.1016/S0360-3199(02)00147-7

Fang HHP, Liu H (2002) Effect of pH on hydrogen production from glucose by a mixed culture. Bioresour Technol 82(2):87–93. doi:10.1016/S0960-8524(01)00110-9

Fang HHP, Liu H, Zhang T (2004) Bio-hydrogen production from wastewater. Water Sci Technol Water Supply 4(1):77–85

Fu Y, Viraraghavan T (2001) Fungal decolorization of dye-wastewaters: a review. Bioresour Technol 79:251–262. doi:10.1016/S0960-8524(01)00028-1

Hart D (1997) Hydrogen power: the commercial future of "the ultimate fuel". Financial Times Energy Publishing, London

Hawkes FR, Dinsdale R, Hawkes DL, Hussy I (2002) Sustainable fermentative hydrogen production: challenges for process optimisation. Int J Hydrogen Energy 27(11–12):1339–1347. doi:10.1016/S0360-3199(02)00090-3

Jun YS, Yu SH, Ryu KG, Lee TJ (2008) Kinetic study of pH effects on biological hydrogen production by a mixed culture. J Microbiol Biotechnol 18(6):1130–1135

Kapdan IK, Kargi F (2006) Bio-hydrogen production from waste materials. Enzyme Microb Technol 38(5):569–582. doi:10.1016/j.enzmictec.2005.09.015

Khanal SK, Chen WH, Li L, Sung S (2004) Biological hydrogen production: effects of pH and intermediate products. Int J Hydrogen Energy 29(11):1123–1131

Kumar N, Das D (2000) Enhancement of hydrogen production by *Enterobacter cloacae* IIT-BT 08. Process Biochem 35(6):589–593. doi:10.1016/S0032-9592(99)00109-0

Laszlo JA (1995) Electrolyte effects on hydrolyzed reactive dye binding to quaternized cellulose. Textile Chemist Colorist 27(4):25–27

Lay JJ (2000) Modeling and optimization of anaerobic digested sludge converting starch to hydrogen. Biotechnol Bioeng 68(3):269–278. doi:10.1002/(SICI)1097-0290(20000505) 68:3<269::AID-BIT5>3.0.CO;2-T

Lee KK, Kassim AM, Lee HK (2004) The effect of nitrogen supplementation on the efficiency of color and COD removal by Malaysian white-rot fungi in textile dyeing effluents. Water Sci Technol 50:73–78

Lee YH, Matthews RD, Pavlostathis SG (2005) Biological decolorization of reactive anthraquinone and phthalocyanine dyes under various oxidation-reduction conditions. Water Sci Technol 52(1–2):377–383

Liu D, Zeng RJ, Angelidaki I (2008) Effects of pH and hydraulic retention time on hydrogen production versus methanogenesis during anaerobic fermentation of organic household solid waste under extreme-thermophilic temperature (70°C). Biotechnol Bioeng 100(6):1108–1114. doi:10.1002/bit.21834

Manu B, Chaudhari S (2002) Anaerobic decolorization of simulated textile wastewater containing azo dyes. Bioresour Technol 82:225–231. doi:10.1016/S0960-8524 (01)00190-0

McCarty PL, Smith DP (1986) Anaerobic wastewater treatment. Environ Sci Technol 20:1200–1206. doi:10.1021/ es00154a002

Melis A, Happe T (2001) Hydrogen production. Green algae as a source of energy. Plant Physiol 127(3):740–748. doi: 10.1104/pp.127.3.740

Melpei F, Andreoni V, Daffonchio D, Rozzi A (1998) Anaerobic digestion of print pastes: a preliminary screening of inhibition by dyes and biodegradability of thickeners. Bioresour Technol 63:49–56. doi:10.1016/ S0960-8524(97)00109-0

Mu Y, Zheng WJ, Yu HQ, Zhu RF (2006) Biological hydrogen production by anaerobic sludge at various temperature. Int J Hydrogen Energy 31:780–785. doi:10.1016/j.ijhydene. 2005.06.016

Nandi R, Sengupta S (1998) Microbial production of hydrogen: an overview. Crit Rev Microbiol 24(1):61–84. doi: 10.1080/10408419891294181

Omar HH (2008) Algal decolorization and degradation of monoazo and diazo dyes. Pak J Biol Sci 11(10):1310–1316

Pitwell LR (1983) Standard COD. Chem Br 19:907

Revenga J, Rodriguez F, Tijero J (1994) Study of redox behavior of anthraquinone in aqueous medium. J Electrochem Soc 141(2):330–333. doi:10.1149/1.2054725

Rys P, Zollinger H (1989) Reactive dye-fiber systems. The theory of coloration of Textiles. In: Johnson A (ed) Society of dyers and colorists, West Yorkshire, England, pp 552

Singh S, Chandra R, Patel DK, Reddy MM, Rai V (2008) Investigation of the biotransformation of pentachlorophenol and pulp paper mill effluent decolorisation by the bacterial strains in a mixed culture. Bioresour Technol 99(13):5703–5709. doi:10.1016/j.biortech.2007.10.022

Taguchi F, Chang JD, Mizukami N, Saito-Taki T, Hasegawa K, Morimoto M (1993) Isolation of a hydrogen-producing bacterium, *Clostridium beijerinckii* strain AM21B. Can J Microbiol 39(7):726–730

Takabatake H, Suzuki K, Ko IB, Noike T (2004) Characteristics of anaerobic ammonia removal by a mixed culture of hydrogen producing photosynthetic bacteria. Bioresour Technol 95:151–158. doi:10.1016/j.biortech.2003.12.019

Tao Y, Chen Y, Wu Y, He Y, Zhou Z (2007) High hydrogen yield from a two-step process of dark- and photo-fermentation of sucrose. Int J Hydrogen Energy 32:200–206. doi:10.1016/j.ijhydene.2006.06.034

Vandevivere PC, Bianchi R, Verstraete W (1998) Treatment and reuse of wastewater from the textile wet-processing industry: review of emerging technologies. J Chem

Technol Biotechnol 72:289–320. doi:10.1002/(SICI)1097-4660(199808)72:4<289::AID-JCTB905>3.0.CO;2-#

Wang X, Hoefel D, Saint CP, Monis PT, Jin B (2007) The isolation and microbial community analysis of hydrogen producing baceria from activated sludge. J Appl Microbiol 103:1415–1423. doi:10.1111/j.1365-2672.2007.03370.x

Zheng XJ, Yu HQ (2005) Inhibitory effects of butyrate on biological hydrogen production with mixed anaerobic cultures. J Environ Manage 74:65–70

Zollinger H (1991) Color chemistry, 2nd edn. VHC publishers, New York