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Full Length Research Paper

Study on the triphenyl tetrazolium chloride–dehydrogenase activity (TTC-DHA) method in determination of bioactivity for treating tomato paste wastewater

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A quick analysis of the sludge activity method based on triphenyltetrazolium chloride-dehydrogenase activity (TTC-DHA) was developed to change the rule and status of the biological activity of the activated sludge in tomato paste wastewater treatment. The results indicate that dehydrogenase activity (DHA) can effectively facilitate the biochemical reaction of tomato paste wastewater treatment upon analysis of the influences of various DHA and kinetic factors. The biological activity of the activated sludge by TTC-DHA was changed to become applicable to aeration and wastewater treatment operation and management.

Key words: Tomato paste wastewater, TTC-DHA, bioactivity, active sludge.

INTRODUCTION

In recent years, new technologies and methods were developed in environmental water monitoring (Bihan, 2000). Physicochemical analysis was accurate and reliable. However, one of the major problems was that the analysis could only be used for a single component of pollutant concentrations in wastewater (Bihan and Lessard, 1998). Therefore, finding a broad and indispensable biological indicator to analyze the sludge activity for tomato paste wastewater treatment was necessary.

Biological oxidation of organic compounds is a dehydrogenation process (Tabatabai, 1982) mediated by many different intracellular and specific dehydro-genases. The dehydrogenase activity (DHA) of the activated sludge supposedly reflects sludge activity (Nielsen, 1975). DHA estimation is conducted using redox-sensitive tetrazolium dye, which is reduced to insoluble formazan inside the cells as a result of respiratory activity. Tetrazolium chloride-dehydrogenase activity (TTC-DHA) method is recommended as a very sensitive and simple methodology to determine sludge activity (Lazarova and Manem, 1995). Triphenylte-trazolium chloride (TTC) is a kind of colorless soluble dye, which serves as terminal acceptor in biochemical reactions. Red triphenyl formazan (TF) salt forms in microbial cells when TTC irons react with H atoms and can be extracted from the cells using an organic solvent (Yang and Jiang, 2002; Yu et al., 1990). In previous studies, DHA assays were used to evaluate the activated sludge by counting bacterial colonies (Bihan, 1998, 2000; Zhou and Li, 1995; Zinbei and Henrietle, 1994) and measure water toxicity. Therefore, the TTC-DHA method has been widely used

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Abbreviations: TTC-DHA, Triphenyltetrazolium chloride-dehydrogenase activity; **DHA,** dehydrogenase activity; **COD,** chemical oxygen demand; **MLSS,** mixed liquor suspended solids; **DO,** dissolved oxygen.

in the study of biological activity of the activated sludge.

In this study, red TF is an index that reflects sludge activity. Its influence was analyzed in the wastewater treatment plant of Shihezi Tianye Tomato Products Co., Ltd. The DHA from the biochemical analyses will play an important role in tomato paste wastewater treatment and in the daily management of the tomato paste wastewater treatment plant.

MATERIALS AND METHODS

Reactor and operation

The wastewater treatment system of Shihezi Tianye Tomato Products Co., Ltd consists of an equalization tank, seven aeration tanks and a secondary sedimentation tank as shown in Figure 1. Every tank measured 19 × 10 × 5 m (L × W × H), and the volume was 950 m^3. Aeration tanks of nos. 3, 4 and 5 added fiber packing. Seven aeration tanks were divided into three regions by the filler: A region including aeration tanks 1 and 2; B region including aeration tanks 3, 4 and 5; and C region including aeration tanks 6 and 7. The aeration was 405 m^3/min, which was provided by two roots blower (24 kW/h). The influent flow was 216.12 m^3/h on average (ranging from 175.8 to 209.6 m^3/h). The hydraulic retention time (HRT) in every reactor tank and total HRT were 4.40 and 30.77 h, respectively.

Seed sludge

The flora consisted of seven aerobic strains (Wen et al., 2009). These strains were screened from acclimated sludge which originated from the tomato paste wastewater drainage pipe in Shihezi, China. The seven strains were divided into two types: degradation dominant bacteria including *Bacillus subtilis* (JH642), *Pseudomonas putida* (KT2440), *Bacillus megaterium* (DSM319) and *Citrobacter koseri* (BAA-895), and settlement dominant bacteria including *Bacillus cereus* (F65185), *Bacillus sp.* (B-14911) and *Pantoea agglomerans* (WAB1913). Strains were cultured separately in a shake flask and subsequently mixed in a 140 L container. The polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) analysis showed that the major microbes of the sludge were the added strains in the treatment system.

Sample preparation

Activated sludge samplings were done upwardly from aeration tanks sampling ports every day. Bioactivity measurements were immediately done after sampling. Moreover, all the materials used were sterilized.

Analytical methods

The treatment continued for 50 days (from August 11, 2011 to September 29, 2011). Chemical oxygen demand (COD) and mixed liquor suspended solids (MLSS) were measured by standard methods (Wei, 2002). pH was measured using a pH electrode connected to a pH meter (Sension156, Hach Company, America). The dissolved oxygen (DO) was measured using a DO electrode connected to a DO meter (Sension156, Hach Company, America). The temperature was measured using a temperature electrode connected to a temperature meter (Sension156, Hach Company, America). All measurements were replicated three times.

DHA analytical measurement steps

The TTC-DHA analytical measurements were performed base on the method proposed by Yu et al., 1990). Briefly, the tubes with the detached active sludge samples were added with 1.5 mL of Tris-HCl buffering liquid (pH 8.4), 0.5 mL Na_2SO_3 solution, 1.5 mL distilled water and 1.5 mL of TTC solution. The tubes were lightly cap, and then placed in water bath at 37°C. After subjecting the tubes to centrifugation at 10000 rpm for 1 min at room temperature, 1 mL concentrated sulfuric acid was added to the tubes to stop the reaction, and 5 mL of chloroform was used to extract the TF crystal. The mixture was further centrifuged at 10000 rpm for 1 min and the supernatant was separated to measure the TF content colorimetrical at 485 nm. The amount of TF was determined from the standard TF curve drawn with $Na_2S_2O_4$ as reduction agent. All samples were analyzed at least in duplicate. DHA was expressed in terms of 10^{-6}g TF/(ml·h). The DHA standard curve is shown in Figure 7.

RESULTS

DHA in the three regions during sludge culture

DHA in the three regions during sludge culture is shown in Figure 2. These samples were collected after six days of the aeration tanks operation times (from August 5 to 10, 2011). As indicated in Figure 2, DHA in the three regions were relatively low during the early stage (one to two days). As the culture continued, DHA began to increase. The main reason for this trend was the relatively low MLSS (average MLSS was 540 mg/L) of the aeration tank, which caused DHA to become relatively low. However, DHA in the B region was higher than the DHA in the other regions after two days. The high concentration of TTC irons can penetrate deeply into the cells within filler during bioactivity detection, and reacted with hydrogen atoms which were removed from organic matters catalyzed by the dehydrogenase enzyme, generating the red TF crystal. Whether the cells of the organism were fixed on the support media or kept free in the liquid, the red TF product can be extracted by organic solvent from the cells and measured quantitatively. Therefore, the TTC-DHA activity was determined for tomato paste wastewater treatment without necessarily unfixing the fibred from the support media for the B region (Tian et al, 2006).

DHA in the three regions during the continuous treatment

Figure 3 shows the trend in the three regions during the continuous treatment. DHA in the A region was slightly higher than the other two regions during the early stage of the treatment. Two reasons were attributed to the higher DHA in the A region. One was the lower COD

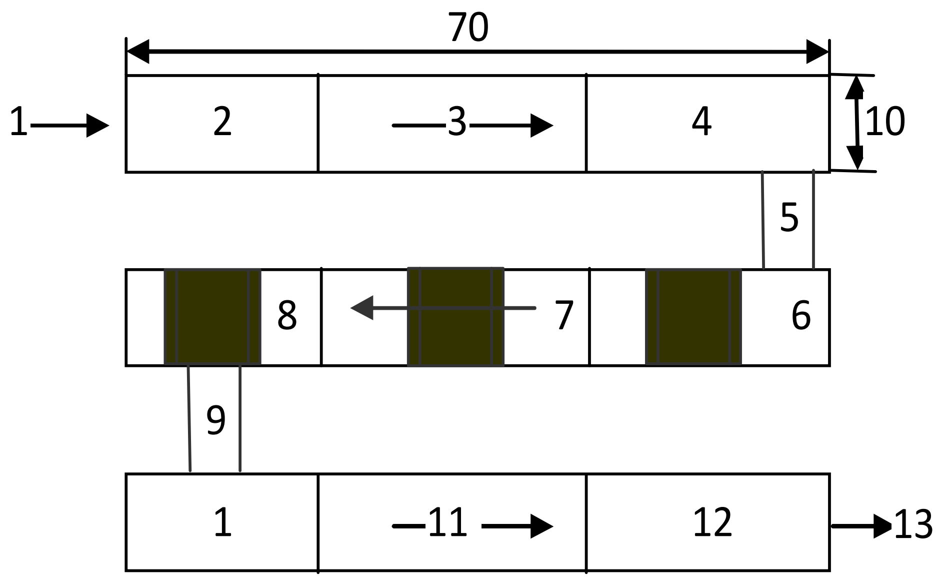

Figure 1. Schematic diagram of the system. 1, Influent water; 2, equalization tank; 3 and 4, aeration tank (A region); 5, aqueduct I; 6, 7 and 8, fibre tank (B region); 9, aqueduct II; 10, 11 and 12, aeration tank (C region); 12, secondary settling tank; 13, effluent water.

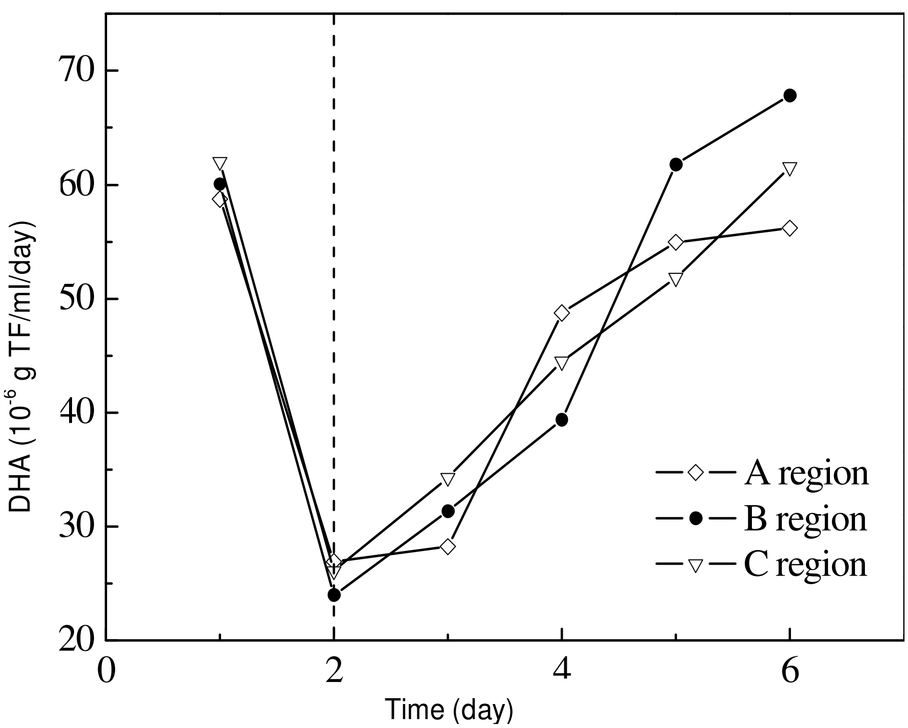

Figure 2. The dehydrogenase activity (DHA) in the three regions during the sludge culture.

loading of the influent, which was lower than 1000 mg/L, thus resulting in the increase of the sludge activity, while the other was the increase in the sludge return ratio resulting in the increase of sludge concentration. DHA in the B region gradually increased halfway through the treatment. The sludge concentration in the B region was also increased compared with the other two regions. In the last stage of the treatment, DHA in the region became

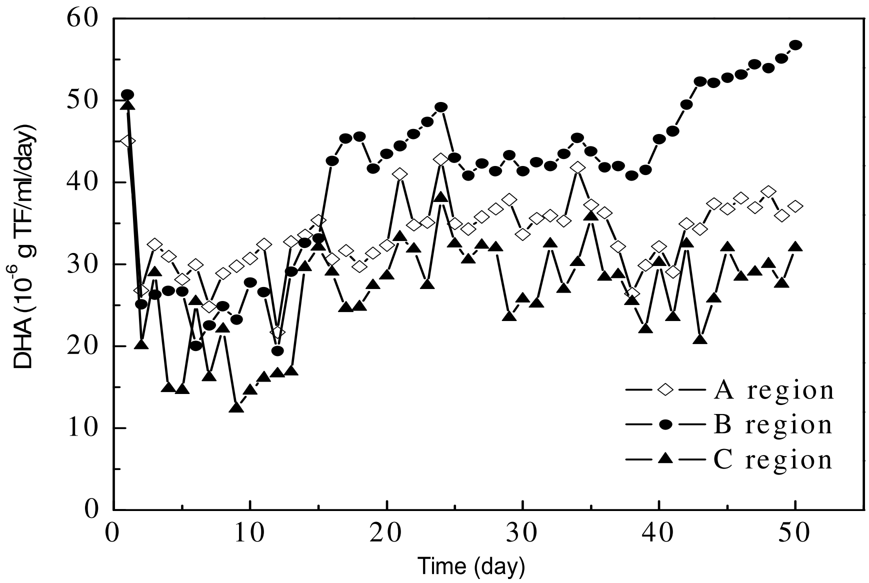

Figure 3. The dehydrogenase activity (DHA) in the three regions during the continuous treatment

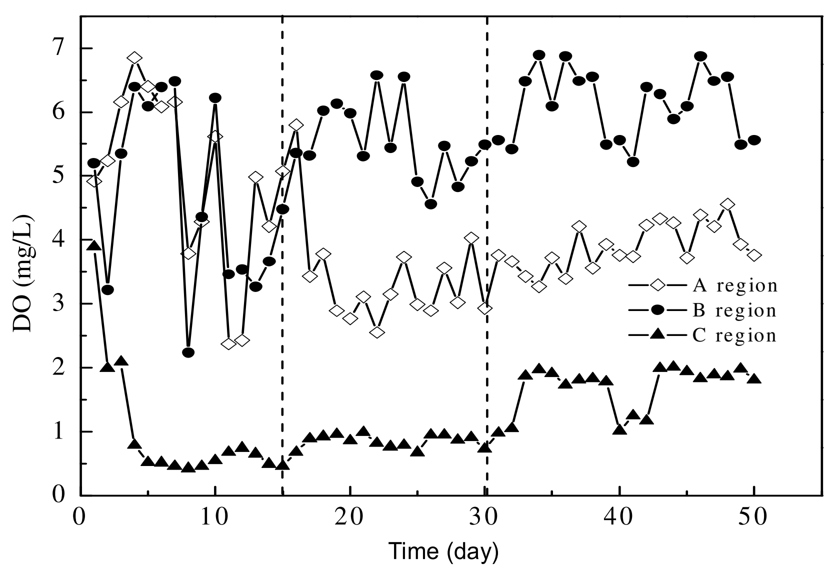

Figure 4. Dissolved oxygen (DO) concentration in the three regions during the continuous treatment.

significantly higher than the DHA in the other two regions because of the fiber added into the treatment system, which led to the increase of MLSS in the B region and also because of the higher COD removal and removal rate (Gao et al., 2009).

In general, the DHA value obtained in the present study is much higher than the DHA value obtained in the previous study, especially in the last treatment, where the value ranged from 12 (10^{-6} g/(mL·day)) to 56 (10^{-6} g/(mL·day)) (Gao et al., 2009). This difference is possibly due to the high biological activity of the activated sludge in the treatment system of this study.

DISCUSSION

The relationship between dissolved oxygen concentration and DHA

Figure 4 shows the DO concentration in three regions during continuous treatment. The DO concentration in the A region essentially increased, whereas DO

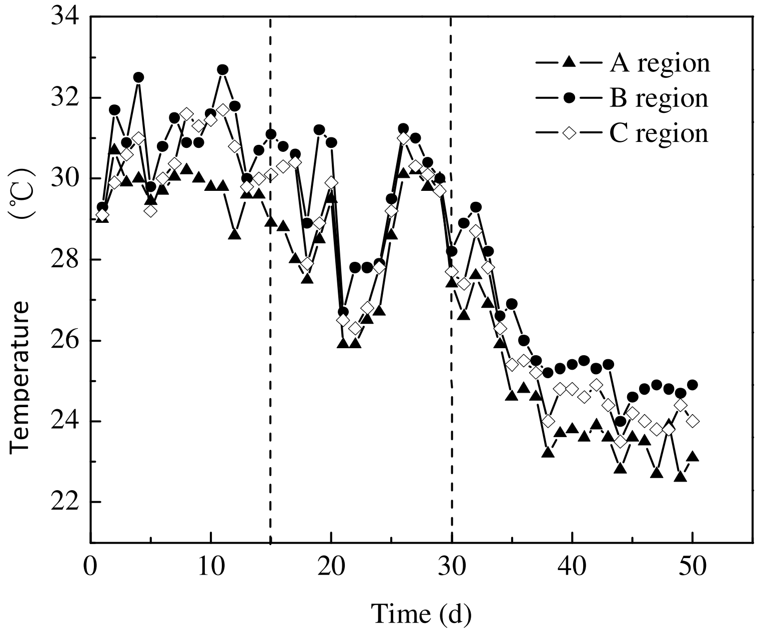

Figure 5. Temperatures of the three regions during the continuous treatment.

concentration in the C region decreased. During the treatment in 2010, all aeration tanks had the same aeration rate. DO concentration in the A region was lower than 2.0 mg/L, which was lower than the lower limit of the DO concentration in the activated sludge system, thus indicating insufficient aeration. On the other hand, the DO concentration in the C region was higher than 3.4 mg/L, which denoted a slight waste of energy costs. Consequently, DO concentration in the two regions was adjusted during the treatment in 2011. However, the higher COD loading of the influent decreased the sludge activity during the early treatment, and the higher COD loading of the influent was mainly caused by a large amount of peeled tomato flesh. Most of the flesh precipitated in the A region, which caused a higher burden. As a result, the average DHA in the treatment system became lower during the early treatment, with a positive correlation coefficient of 0.5. The positive correlation coefficient of DHA and DO concentration was 0.85 when the treatment system stabilized with the adjustment of the DO concentration. DHA was evidently by DO concentration and a positive correlation. Compared with other studies, DHA in the current study was higher because of a larger positive correlation resulting in higher biological activity of the flora.

The relationship between temperature and DHA

Figure 5 shows the temperature of the three regions during continuous treatment. The temperature of the A region was lower than the temperatures of the B and C regions during continuous treatment. However, DHA in the A region was slightly higher than the DHA in the other two regions because of the increase in sludge return ratio. The main reason for this finding was that the temperature of the clean tomato wastewater was low, while the endogenous respiration of microorganisms was limited in the A region compared with the endogenous respiration of microorganisms in the B and C regions. The temperature of the B region was higher than the temperatures of the other two regions halfway through the treatment. The sludge concentration in the region was higher than the sludge concentration in the other regions, which increased along with the microbial endogenous respiration and temperature.

Compared with DHA, temperature did not increase with the microbial endogenous respiration while the exothermicity of the aeration equipment continually reduced. The higher COD loading of the influent was caused by the non-achievement of the desired concentration of microorganisms Therefore, DHA and tomato paste wastewater treatment were not affected by temperature. However, in the later stage of the treatment, temperature decreased, resulting in the increase in DHA. This result was apparently influenced by the decrease of local temperature and measurement time, especially during the last stage of the treatment.

The relationship between MLSS and DHA

Figure 6 shows the MLSS of aqueducts I and II, and aeration tanks 4 and 7. During the early treatment, MLSS experienced a transient decrease because of the dilution effect. With the large population of microorganisms,

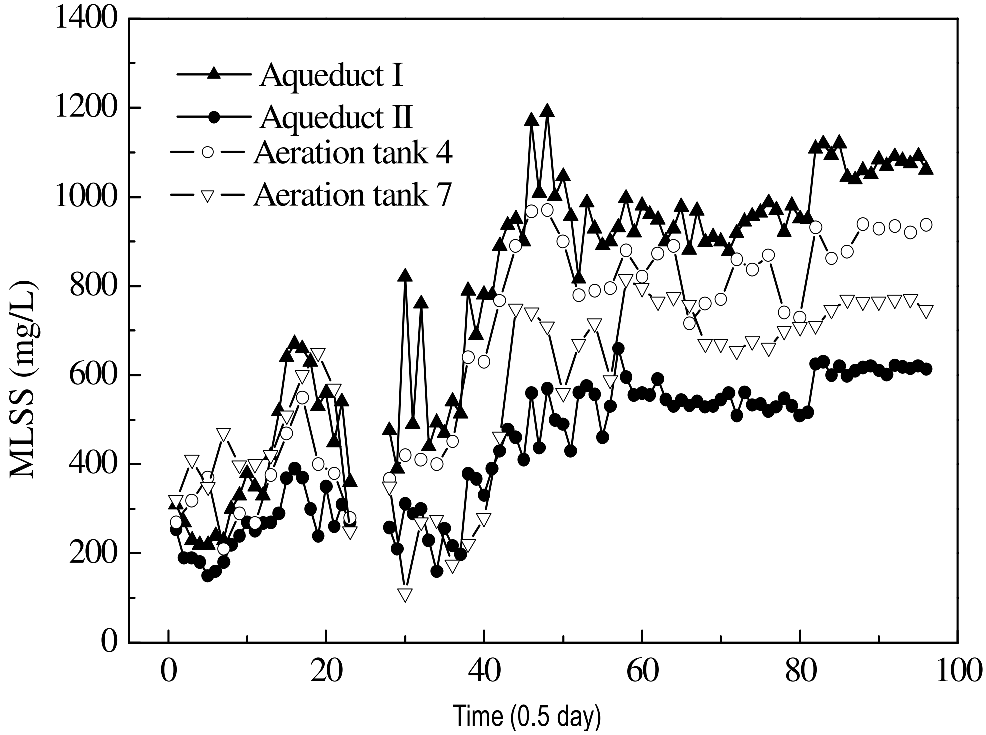

Figure 6. MLSS of aqueducts I and II, and aeration tanks 4 and 7. MLSS, Mixed liquor suspended solids.

MLSS in the system quickly increased. At first, MLSS in the A and B regions were stable at 500 to 700 mg/L. Along with the quick growth of the influent COD, MLSS in the A and B regions increased again and eventually stabilized at 800 to 1200 mg/L. Considering the sludge adhering on the filler (0.1189 kg/1.5 m, approximately equal to 461 mg/L), sludge concentration in the B region could reach to 1250 to 1650 mg/L. Sludge concentration in the 2011 treatment was significantly higher than the sludge concentration in the 2010 treatment which was only 400 to 450 mg/L (the treatment effect radically improved). However, sludge concentration only reached the lower limit of that in the conventional activated sludge process (Tchobanoglous and Burton, 1991; Galapate et al., 1999). The flora added into the system dominated and the proportion of degrading dominant strains in the sludge became higher than the strains in the conventional activated sludge. This potentially contributed to the better effect reached under lower MLSS.

On the other hand, MLSS in the C region was lower (stable at 600 to 800 mg/L) than the MLSS in the other regions. Since the filler could hold up the sludge, the amount of sludge entering into the C region was lower (MLSS of aqueduct II in Figure 6). Furthermore, the sludge growth was limited by the lower pollution loads. The load of secondary sedimentation tank could be decreased because of the lower MLSS in the C region. At the same time, the sludge recycle flow rate could also be decreased if the sludge was intercepted more effectively in the system.

Kinetic analysis of the sludge activity

A linear relationship existed between DHA of the activated sludge and the substrate concentration for the biodegradation process of organic matter (Tchobanoglous and Burton, 1991). These phenomena can be described by Equation 1, which is expressed by the Michaelis-Menten equation where DHA (U^T) and Max DHA (U_M) of the activated sludge have a similar relationship with maximum specific growth rate (μ_m) and specific growth rate (μ):

$$U^T = \frac{U_M}{K^T + S} \qquad (1)$$

Where, U^T is DHA of the activated sludge (TF10^{-6} g/(mL·day)), U_M is the max DHA of the activated sludge (TF(10^{-6} g/(mL·day)), K^T is the Michaelis constant (mg/L) and S is the organic matter concentration (mg/L).

Figure 8 shows the relationship between $1/U^T$ and 1/S for the three regions. U_M in the A region was higher than U_M in the other two regions (Table 1). The main reason for this trend is the higher COD loading in the A region

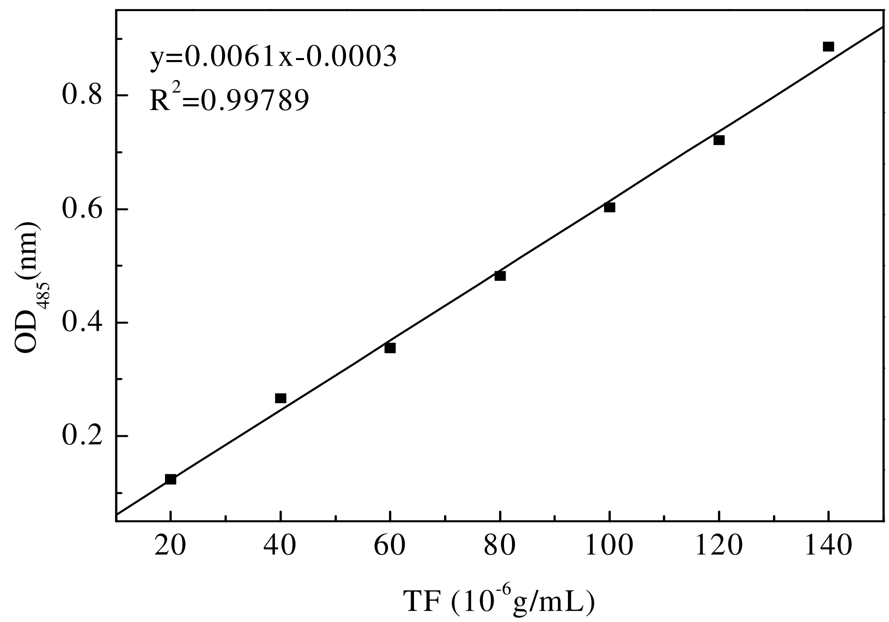

Figure 7. The dehydrogenase activity (DHA) standard curve.

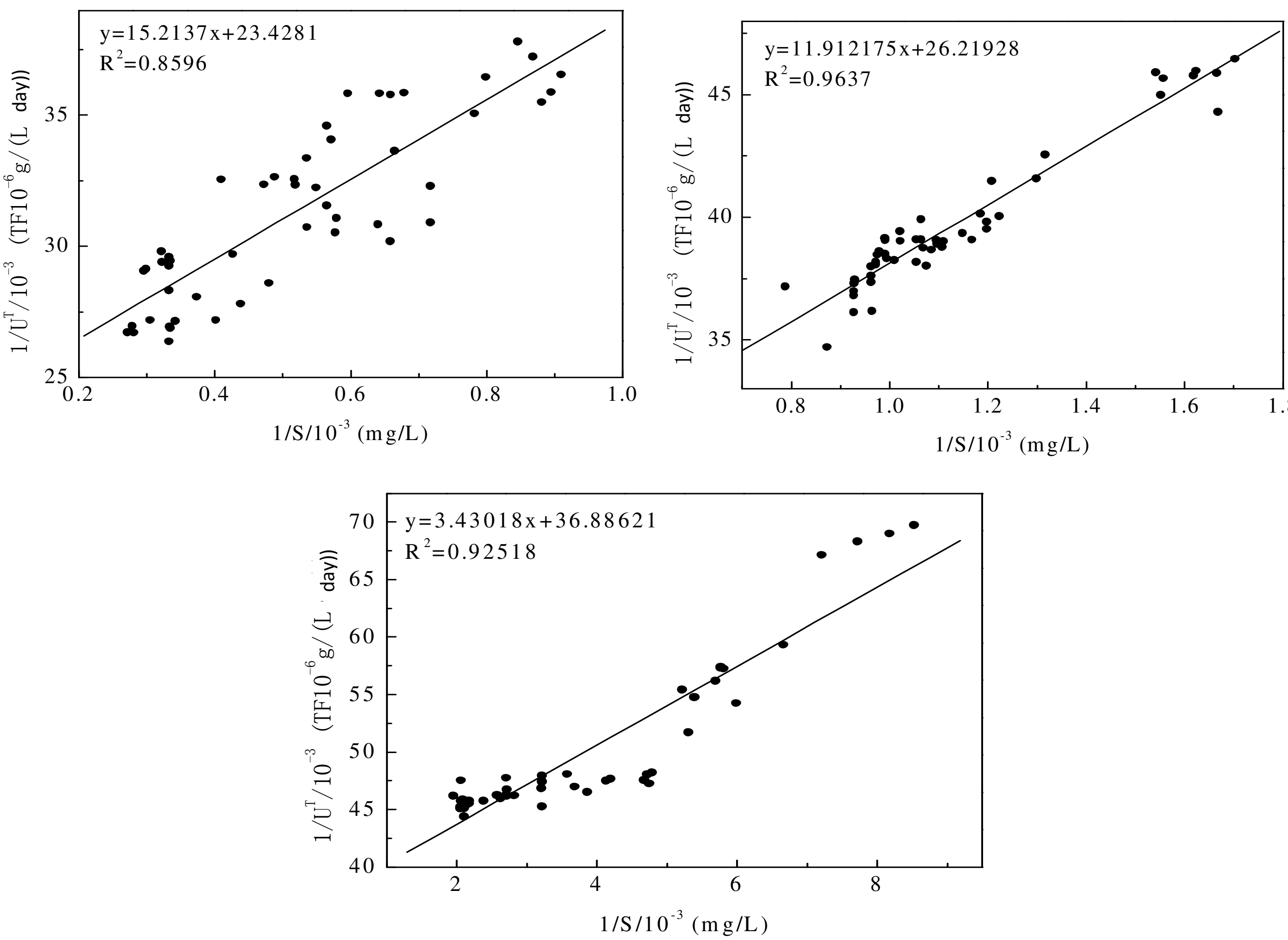

Figure 8. The relationship between (a) $1/U^T$ and 1/S for A region; (b) $1/U^T$ and 1/S for B region; and (c) $1/U^T$ and 1/S for C region.

Table 1. The U_M and K^T of values for the three regions.

Parameter	A region	B region	C region
U_M	0.0427	0.0381	0.0271
K^T	0.6496	0.4543	0.0930

U_M, Max DHA of the activated sludge (TF (10^{-6} g/(mL·day)), while K^T, Michaelis constant (mg/L). TF, Triphenyl formazan.

compared with the other regions. On the other hand, the microbial endogenous respiration increased, which led to the increase in the microorganism growth rate. The value of U^T indicates the removal rate of COD over a range of influent COD concentrations and thus indicates the stability of the process. U^T obtained in the present study is comparable with the results in a previous literature (Tan et al., 2005), indicating that the addition of the aerobic degrading strains was appropriate to the treatment of wastewater with different concentrations in the treatment system. The system is also suitable to high and shock loadings.

Conclusion

By the change of the sludge activity in the treatment system, the state of the treatment system is indicated and provides a good basis to improve the water quality of the treatment system. This experiment shows that the TTC-DHA method is suitable to be used for the treatment of tomato paste wastewater, where DO and sludge concentrations are not affected by temperature. The kinetic analysis of the sludge activity shows that the TTC-DHA method was reliable for the treatment of tomato paste wastewater.

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