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Effects of Na_2SO_4 Concentration on Pollutants Removal and Microbial Metabolic Characteristics in the Hydrolysis-Aerobic Treatment of Dyeing Wastewater

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ABSTRACT

Hydrolysis-aerobic biological treatment process shows low efficiency in removal inorganic salts from the dyeing wastewater, and the accumulation of inorganic salts in dyeing wastewater reuse process may inhibited the pollutants removal. To understand this problem, a dyeing hydrolysis-aerobic biological treatment process was established, and the influence of interval increasing Na_2SO_4 concentration on treated effluent quality was investigated. Biolog technology was used to study the microbial metabolic characteristics under different Na_2SO_4 concentrations. The results indicated that the concentration of COD_{Cr}, NH_4^+-N and TP in the effluent increased as the increase of Na_2SO_4 concentration. When the Na_2SO_4 concentration in the influent increased to 2700 mg/L, the concentration of COD_{Cr} and TP in sedimentation tank effluent was 2567.94 mg/L and 1.94 mg/L, respectively, which exceeded the requirement of effluent quality for printing and dyeing process. After 144 h, MLSS decreased to 1148 mg/L, while SV_{30} increased to 78% with increasing salt content and sludge run off with the effluent. The system acclimated seed could tolerate a maximum shock of 2400 mg/L Na_2SO_4. The high Na_2SO_4 concentration would increase the ability of microbial community to utilize carbon source, especially the use of acids and saccharides.

Keywords: Dyeing wastewater; Na_2SO_4; biolog; microbial metabolic characteristics

1. INTRODUCTION

The wastewater quantity generated from textile and dyeing industry occupies about 35% of the wastewater discharge in the whole industry of China (Zhou, 2012). However, the recycle rate of the wastewater in the entire textile and dyeing industry is less than 10% at present, which is the minimum one among all industries nationwide in terms of recycle rate (Wei et al., 2009). The contradiction between water resource shortage and high water consumption in textile and dyeing process has hampered the development of dyeing industry.

Currently the aerobic biological treatment process utilized by the domestic dyeing wastewater treatment is in the majority (He, 2007). The process of common aerobic biological treatment is unable to eliminate

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plenty of salts, which acts as the dye-fixing agent. Therefore, the salinity will be accumulated during recycle, and the biological treatment system will be impacted (Peng et al., 2006; Verma et al., 2012; Zhao et al., 2010). Na_2SO_4 and NaCl was the main of the key dye-fixing agents in dyeing industry. Panswad and Anan (1999) found that the removal rate of chemical oxygen demand (COD_{Cr}) in the non-naturalized biological treatment system had reduced to 60% from 97% in A^2/O treatment process, when the NaCl content in the synthetic wastewater was raised from 0 g/L to 30 g/L. Uygur and Kargi (2004) studied nutrient removal in a small-scale SBR system discovered that the removal rate of COD_{Cr} and ammonia nitrogen (NH_4^+-N) declined to 32%, and the removal rate of total phosphorous (TP) declined to 22%, when the NaCl concentration was up to 60 g/L. Li et al. (2007) found that the activity of the nitrosobacteria and nitrobacterium in the common activated sludge system had been inhibited completely, when the NaCl concentration in wastewater was higher than 30 g/L.

Past researches mainly focused on the NaCl inhibition on pollutants removal. Although Na_2SO_4 is widely used in dyeing industry as a kind of dye-fixing agent, the researches about Na_2SO_4 inhibition on the removal of organic matter and nutrients in dyeing wastewater are still few. In addition, so far the analyzing technology of investigating the change of microbial community and metabolic characteristics as the increase concentration of salinity in the dyeing wastewater influent is still lack.

Aiming the above-mentioned problems, this paper laid emphasis on investigating the influence of increasing Na_2SO_4 on the purifying efficient of dyeing wastewater hydrolysis-aerobic biological treatment process. Biolog technology was used to analysis the microbial metabolic characteristics under different concentrations of sodium sulfate. The purpose of our study is to provide the reference basis knowledge of safe operation for the system model with high-ratio cyclic utilization of dyeing wastewater.

2. MATERIALS AND METHODS

2.1 Device and operation condition of hydrolysis-aerobic biological treatment system

2.1.1 Experimental device

The simulation biological treatment system in this study was established on the basis of the practical dyeing wastewater treatment plant located in Tongxiang City, Zhejiang Province of China. It consists of inlet pump, storage water tank, peristaltic pump, hydrolysis acidification tank, aerobic tank, aeration pump, sedimentation tank, pipeline and accessories. Fig. 1 shows the experiment device and technological process.

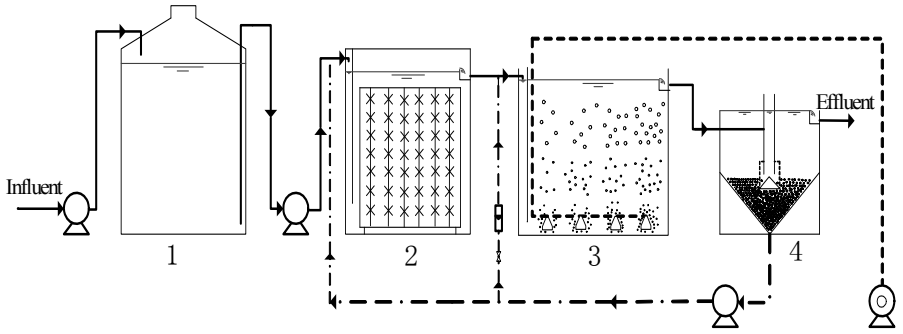

Figure 1 Schematic flow diagram of hydrolysis-aerobic process (1) Storage water tank (2) Hydrolysis acidification tank (3) Aerobic tank (4) Sedimentation tank

Table 1 Effluent quality of primary sedimentation tank

pH	COD (mg/L)	NH_4^+-N (mg/L)	TP (mg/L)	BOD_5/COD_{Cr}
6.8~7.6	467.9~657.3	3.99~7.03	0.14~2.73	0.2~0.3

2.1.2 Operating conditions for the experimental device

The influent of this hydrolysis-aerobic simulation biological treatment system is the discharged effluent from the primary sedimentation tank in a printing and dyeing wastewater treatment process, located in Tongxiang City of Zhejiang Province.

Table 1 shows the quality characteristics of effluent. It was due to dyeing wastewater contains a large number of macromolecular organic degradation-resistant matters; result in BOD/COD ratio is low. The operational parameters of the experimental device were: the rate of influent water was 10 L/h, the effective volume of hydrolysis acidification tank was 120 L, the effective volume of aerobic tank was 190 L, the dissolved oxygen concentration in aerobic tank was controlled to 2.5 mg/L, the sludge quantity that flew back to aerobic tank was 7 L/h, and the sludge quantity that flew back to hydrolysis acidification pool was 2 L/h.

To elevate the effect of salinity on the operation of biological treatment system, Na_2SO_4 was added to in the influent of hydrolysis acidification pool, the concentration gradients of Na_2SO_4 were 1800 mg/L, 2100 mg/L, 2400 mg/L and 2700 mg/L, which meant the concentration of Na_2SO_4 was increased by 300 mg/L during every 48 h in the influent. Before adding Na_2SO_4 in the influent, we firstly ensure the biological treatment system under stable state, namely, the removal rates of COD_{Cr}, NH_4^+-N and TP in the system should be stabilized at approximate 80%, 90% and 61% for at least 7 days, respectively. Mixed liquid suspended solids (MLSS) and SV_{30} kept at 1400 mg/L and 55% in the aerobic tank, respectively.

During the process of simulating biological treatment system, the concentrations of COD_{Cr}, NH_4^+-N and TP in the effluent from sedimentation tank should satisfy the requirement of effluent quality for printing and dyeing process. Under the circumstance, any water quality index exceeded the safe operation index, the treatment efficiency of this system could not reach actual demand. At this moment, the concentration of Na_2SO_4 in the influent water of biological treatment system was the tolerant limitation for the microorganisms in such system. The safe operation indexes in the system were formulated according to the Discharge Standard of Water Pollutants for Textile and Dyeing Industry (GB4827-2012): $COD_{Cr} \leq 150$ mg/L, NH_4^+-N ≤ 10 mg/L, TP ≤ 1.0 mg/L.

2.2 Measurement of water quality indexes and analysis of microbial metabolic characteristics in activated sludge

During the experiment, the water samples were collected from the influent of hydrolysis acidification pool, the effluent of hydrolysis acidification pool aerobic tank and sedimentation tank to measure COD_{Cr}, NH_4^+-N, TP and pH, the sludge from aerobic tank were collected to measure MLSS and SV_{30}. The chemical parameters (COD_{Cr}, NH_4^+-N and TP) and some physical parameters (MLSS and SV_{30}) were measured according to the standard methods (State Environmental Protection Administration, 2002).

The microbial metabolic characteristics in activated sludge were analyzed by Biolog-ECO plate. S1, S2 and S3 represent the different activated sludge which the Na_2SO_4

content in the influent was 1800 mg/L, 2400 mg/L and 2700 mg/L, respectively. The inoculated solution was prepared using a modified method according the previous reports of Chen et al. (2009) and Smalla et al. (1998). The detailed method was as follows: the inoculated sludge was precipitated at room temperature for 30 min, 1 mL mixed solution of fresh activated sludge was taken and put into 1.5 mL centrifuge tube. Each sample made three parallel samples. The sludge was collected by repeating centrifugation twice 10000 rpm for 10 min and washing with PBS. The liquid supernatant was discarded and the sludge was suspended in PBS to adjust the absorbance to 0.1~0.15 OD_{590nm}. Then 150 μL bacterium suspensions were added to the Biolog-ECO plate and incubated at 28˚C for 10 d. After inoculation, the inoculated plates were scanned at 590 nm (color and turbidity) and 750 nm (turbidity) with a Biolog microplate reader at 24 h intervals for 10 d.

The overall metabolic condition of the microorganisms to carbon source under the state of the same salts with different concentrations (Rogers and Tate, 2001) had been expressed by utilizing the Average Well Color Development (AWCD).

The computational formula is:

$$AWCD = \frac{\sum(C_i - R)}{n}$$

Where C_i is the absorbance value of the reaction hole and R is the absorbance value of the contrast hole.

Shannon-Wiener Index (H´)

$$H' = -\sum \left(P_i \text{gln } P_i\right)$$

Where P_i is the proportion of total microbial metabolic capability (blanked absorbance values of contrast well in this study) on a particular carbon source (Garland, 1997). Simpson dominance index (D) is the concentrated measurement in terms of diversity.

$$D = 1 - \sum P_i^2$$

Pielou evenness index (J) is the ratio between the diversity of community actually measured and the maximum diversity (Benci and Menconi, 2005).

$$J = \frac{H'}{\ln S}$$

Where S is the hole number while C-R value is larger than 0.25.

2.3 Data Analysis

SPSS 19.0 software was applied to perform the correlation analysis between microbial community and the use of carbon source, and the difference in different sludge samples. The results were considered statistically significant at a P value less than 0.05. During the Biolog measurement, the data at cultivating for 144 h (Haack et al., 1995) had been adopted to calculate the biodiversity index in the microbial community of activated sludge, and the principal component analysis (PCA) had been used to analysis the difference of microbial community's carbon source utilization pattern under different salt concentration.

3. RESULTS AND DISCUSSION

3.1 Na_2SO_4 inhibition on biological pollutants removal

In the simulating biological operation system, the changing situation of sedimentation tank effluent COD_{Cr} after the influent water Na_2SO_4 concentration increased in the system was shown in Fig. 2. Within the 96 h system operation, although the influent Na_2SO_4 concentration varied from 1800 mg/L to 2400 mg/L, and the sedimentation tank effluent COD_{Cr} was stabilized at around 100 mg/L all the time. At 144 h, when the influent Na_2SO_4

concentration raised to 2700 mg/L, the sedimentation tank effluent increased to 257.94 mg/L suddenly, this value could not meet the discharge criterion of printing and dyeing process for water quality ($COD_{Cr} \leq 150$ mg/L). During the process, the Na_2SO_4 in the influent raised from 1800 mg/L to 2400 mg/L, the sedimentation tank effluent NH_4^+-N firstly increased and then declined, whereas when the concentration of Na_2SO_4 in the influent reached 2700 mg/L at 144 h, the sedimentation tank effluent NH_4^+-N reached to the maximum value, which was 2.36 mg/L. It is different from the NH_4^+-N removal, the sedimentation tank effluent TP raised along with the increase of Na_2SO_4 in the influent, when it reached 2700 mg/L at 144 h, the TP of treated wastewater reached 1.94 mg/L, this value had surpassed the safe operation index in system ($TP \leq 1.0$ mg/L).

Previous many researches indicated that the rise of salinity in wastewater would influence the removal ability of biological system towards pollutants (Cui et al., 2003; Glass and Silverstein, 1999; Zhou, 2011). For example, Huang et al. (2002) found that when the Na_2SO_4 in silk mill reached 4.5%, the removal rate of COD_{Cr} by common activated sludge biological treatment system was lower than 40%. However, due to the specificity in industrial wastewater treatment, the rise of salinity in different wastewater showed various extents effect on the pollutants removal of biological treatment system. In this experiment, the low Na_2SO_4 concentration content in the influent showed fewer declines in the removal ability of COD_{Cr}, NH_4^+-N and TP by biological treatment system. While the system operated for 144 h, the Na_2SO_4 in the influent was 2700 mg/L, the sedimentation tank effluent COD_{Cr} and TP would rise to 257.94 mg/L and 1.94 mg/L (Fig. 3). It is obvious that the COD_{Cr}, NH_4^+-N and TP removal efficiencies decreased when the salt concentration went up 2700 mg/L. It is apparent that the system started with high salt content and acclimated seed could tolerate a maximum shock of 2400 mg/L Na_2SO_4.

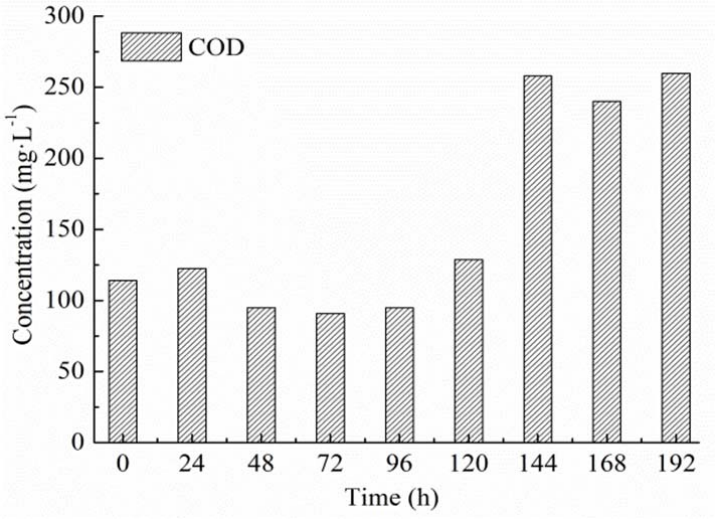

Figure 2 The COD_{Cr} concentrations of sedimentation tank effluent in the biological treatment unit

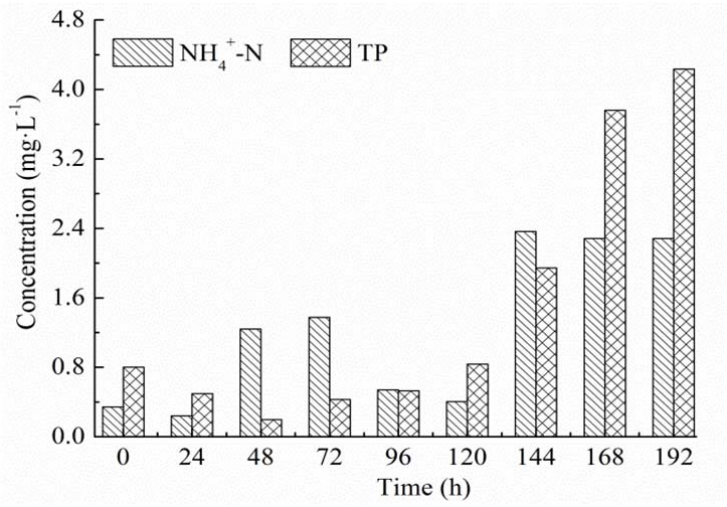

Figure 3 The NH_4^+-N and TP concentrations of sedimentation tank effluent in biological treatment systems

3.2 The influence of influent sodium sulfate concentrations on characteristics of activated sludge

During the process of system operation, the characteristics change of activated sludge in the aerobic tank is shown in Fig. 4. When the system ran until 96 h, the concentration of Na_2SO_4 in the influent raised to 2400 mg/L, and the MLSS in aerobic tank started to decline. At 144 h, the sedimentation tank effluent COD_{Cr} and TP concentrations exceeded the safe operation index, at this moment the MLSS in aerobic tank was 1148 mg/L and the concentration of Na_2SO_4 in system influent was 2700 mg/L. SV_{30} raised slowly along with the increase of the concentration of Na_2SO_4 in the influent during 96 h operation, while SV_{30} increased quickly after 120 h. The SV_{30} reached 78% at 144 h and the influent Na_2SO_4 concentration was 2700 mg/L. Thus it can be seen that the unceasingly elevated salt concentration would worsen the settling property of sludge in the aerobic tank, run off the floating activated sludge and cause the sludge concentration drop drastically. High Na_2SO_4 concentrations (> 2700 mg/L) could lead to the sludge bulking.

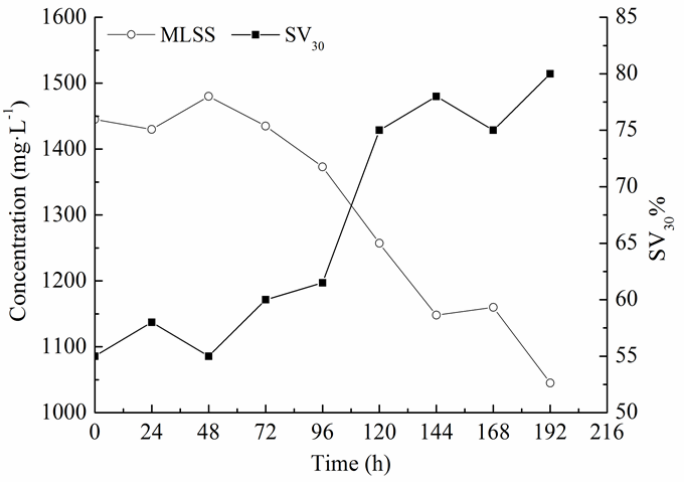

Figure 4 The characteristics of the activated sludge in aerobic tank

3.3 Change of microbial metabolic characteristics

Fig. 5 revealed the AWCD change of activated sludge microorganisms when the concentration of Na_2SO_4 in the influent was 1800 mg/L, 2400 mg/L and 2700 mg/L. AWCD value could represent the microbial average activity (Elfstrand et al., 2007). Overall, the order of AWCD in activated sludge was as follows: S2> S3>S1, suggesting that the sludge in the influent 2400 mg/L Na_2SO_4 had higher microbial metabolic activities than that of the influent 1800 mg/L and 2700 mg/L Na_2SO_4. Consequently, it can be concluded that the sodium sulfate could improve the microbial metabolic activity to some extent. Moreover, when the concentration of Na_2SO_4 in the influent raised from 2400 mg/L to 2700 mg/L, the metabolic activity in activated sludge microorganisms began to decline.

3.4 Comparative analysis of diversify index of microbial community

The diversify index of microbial community could characterize biological community types and the number of individual value, which used to reflect the change of the microbial community characteristics under the influence of external factors. Table 2 indicated the diversity index change of microorganisms in activated sludge when the concentration of Na_2SO_4 in the influent was 1800 mg/L, 2400 mg/L and 2700 mg/L. Along with the increase of the concentration of Na_2SO_4 in the influent, Shannon-Wiener index (H`) firstly rose and then reduced. However, H` index in S2 had improved obviously compared to S1, which meant the microorganism species had been added. The variation tendency of Peilou evenness index (J) was similar with H`. The rise of J index represented that microbial community function became diversity and abundant gradually. Each microorganism among species distributed more evenly. The results indicated that the microbial community get abundant and distribute more evenly by adding 2400 mg/L sodium sulfate. Considering the dominance index (D), Simpson index was used for evaluating the advantage of common species. The higher of the value represents the more dominance species. The change tendency of D index was similar with H` and J, which accounted for the percentage of dominance species in the microbial communities, had been improved by adding sodium sulfate. Hence, the high sodium sulfate concentration especially for 2400 mg/L had intensified the activity and diversity of microbial community and enhanced their competitive capacity.

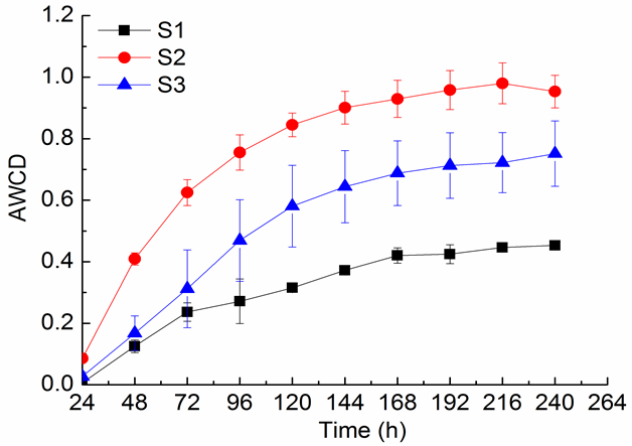

Figure 5 AWCD changes of Activated sludge microorganisms in different concentration of Na_2SO_4, (S1) Na_2SO_4 concentration=1800 mg/L, (S2) Na_2SO_4 concentration=2400 mg/L, (S3) Na_2SO_4 concentration=2700 mg/L

Table 2 Diversity indices of activated sludge microbial communities in different concentrations of Na_2SO_4

Sample	H`	D	J
S1	2.04±0.44	0.83±0.07	0.69±0.15
S2	3.19±0.02	0.96±0.01	0.74±0.01
S3	3.00±0.13	0.94±0.01	0.62±0.03

S1: Na_2SO_4 concentration=1800 mg/L; S2: Na_2SO_4 concentration=2400 mg/L; S3: Na_2SO_4 concentration=2700 mg/L

3.5 Principal component analysis of microbial metabolic diversity

Using the method of principal component analysis could transform multivariate vector of different samples into unrelated principal component vectors (PC1 and PC2 is the component of main vectors), dimension reduction after the principal vector space could be used in the location of the point of intuitive reflect the metabolic characteristics of different microbial communities (Xi et al., 2003). The principal component analysis of AWCD value was carried out to determine the microbial community functions towards the use of each carbon source under different influent Na_2SO_4 concentration. The result was shown in Fig. 6. Two principal components had been extracted, and total 27 kinds of carbon sources were distributed. Among the 27 kinds of carbon sources, 22 kinds of carbon sources were distributed on the first principal component (PC1), including 6 kinds of acids, 5 kinds of saccharides, 4 kinds of amino acides, 3 kinds of esters, 3 kinds of amine and 1 kind of alcohol. 5 kinds of carbon sources were distributed on the second principal component (PC2), including 3 kinds of acids, 1 kind of amino acides and 1 saccharides. The rate of contribution in PC1 variance accounted for 48.70% of AWCD variance, whereas the rate of contribution in PC2 variance was 15.81%. As shown in Fig. 6A, the activated sludge samples with three different Na_2SO_4 concentrations had showed obviously distribution difference. Among the three samples, the distribution difference between S1 and S2, S3 was relatively large. Garland and Mills (1991) considered that the utilizing ability of carbon source by each sample had decided its location in space in PCA figure. In the meantime, the difference of each sample on different axes of principal component space was correlative with the utilizing ability of carbon source gathered on the corresponding axes. Under the different sodium sulfate concentration, the utilization ability of activated sludge microbial community towards 31 kinds of carbon sources had showed difference, and this indicated that the microbial activity had been impacted by the high salt. The correlation analysis was performed between the principal component and AWCD, the results showed that the first principal component and AWCD were significantly correlative ($P=0.021<0.05$), and second principle component and AWCD were not significantly correlative ($P= 0.614$). Therefore, the first principal component could more reflect the general activity of the community. T-test was carried out to analysis the difference in the first principal component of different samples responding to the different influent Na_2SO_4 concentration, the results showed a significant difference between S1 and S2 was discovered ($P=0.04<0.05$), while there was no significant difference between S1 and S3 ($P=0.008<0.05$), and S2 and S3 ($P=0.452$). It was indicated that S1 was significant difference from S2 and S3 in terms of carbon source utilization. However, S2 and

S3 were relatively close in terms of carbon source utilization. Hence, high sodium sulfate concentration changed the microbial metabolic characteristics. As shown in Fig. 6B, the main carbon sources with relative high load value in first principal component were in total 22 kinds of carbon sources included β-Methyl-D-Glucoside (C1), α-Cyclodextrin (C3), Glycogen (C4), D-Cellobiose (C5), Glucose-1-Phosphate (C6), L-Arginine (C8), L-Asparagine (C9), L-Phenylalanine (C10), L-Serine (C11), Pyruvic Acid Methyl Ester (C14), Tween40 (C15), Tween80 (C16), D,L-α-Glycerol Phosphate (C19), Phenylethylamine (C21), Putrescine (C22), N-Acetyl-D-Glucosamine (C23), D-Glucosaminic Acid (C24), 2-Hydroxybenzoic Acid (C26), γ-Hydroxybutyric Acid (C27), D-Mannitol (C29), α-Ketobutyric Acid (C30), D-Malic Acid (C31). Therefore, the difference between S1 and S2, S3 samples was mainly in the utilization of acids and saccharides. As above mentioned (Fig. 5), the study indicated that high influent Na_2SO_4 concentration (2400 mg/L) resulted in the enhancing of microbial metabolic ability and the change of microbial community function. The change might mainly reflect the selection of dominance species for the utilizing ability of microbial carbon source, especially for the utilization of acids and saccharides. However, the microbial metabolic ability decreased at higher Na_2SO_4 concentration (2700 mg/L).

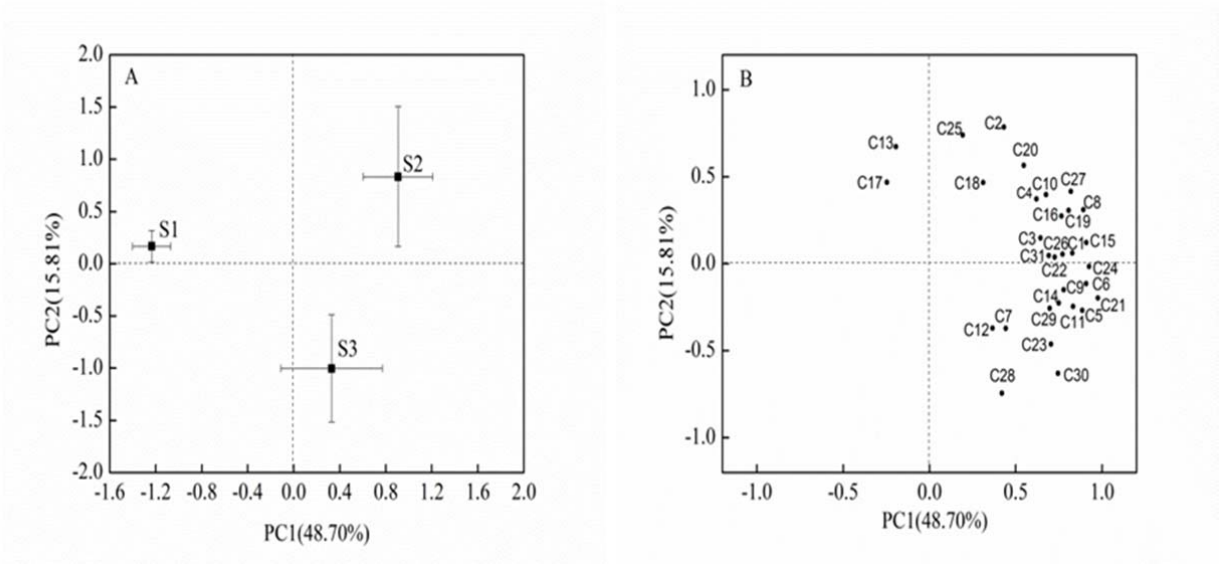

Figure 6 PCA of carbon-source utilization under different Na_2SO_4 concentrations (A) Scores of each group for the first and second PCs (B) Loadings of each carbon-source on the first and second PCs

C1: β-Methyl-*D*-Glucoside; C2: *D*-Xylose; C3: α-Cyclodextrin; C4: Glycogen; C5: *D*-Cellobiose; C6: *α*-*D*-Glucose-1-Phosphate; C7: *α*-*D*-Lactose; C8: *L*-Arginine; C9: *L*-Asparagine; C10: *L*-Phenylalanine; C11: *L*-Serine; C12: *L*-Threonine; C13: Glycyl-*L*-Glutamic Acid; C14: Pyruvic Acid Methyl Ester; C15: Tween40; C16: Tween80; C17: *D*-GalactonicAcid Lactone; C18: *i*-Erythritol; C19: *D*,*L*-*α*-Glycerol Phosphate; C20: Phenylethylamine; C21: Putrescine; C22: *N*-Acetyl-*D*-Glucosamine; C23: *D*-Galacturonic Acid; C24: *D*-Glucosaminic Acid; C25: 2-Hydroxybenzoic Acid; C26: 4-Hydroxybenzoic Acid; C27: *γ*-Hydroxybutyric Acid; C28: Taconic Acid; C29: *D*-Mannitol; C30: *α*-Ketob-utyricAcid; C31: *D*-Malic Acid

CONCLUSIONS

During improving the concentration of Na_2SO_4 in the influent of the hydrolysis-aerobic biological treatment system, the effluent concentrations of COD_{Cr}, NH_4^+-N and TP increased. When the concentration of Na_2SO_4 was 2700 mg/L, the effluent COD_{Cr} and TP were 257.94 mg/L and 1.94 mg/L, respectively, and the effluent quality could not reach the standard.

While improving the influent Na_2SO_4 concentration for 96 h, MLSS gradually declined, the sludge settle ability got worsen and sludge ran off along with the effluent. After 144 h operation, the Na_2SO_4 concentration in the influent was 2700 mg/L, MLSS decreased to 1148 mg/L, while SV_{30} increased to 78%.

Based on the Biolog measurement results, high influent Na_2SO_4 concentration (2400 mg/L) resulted in the enhancing of microbial metabolic ability and the change of community function. The change might mainly reflect the selection of dominance species for the utilizing ability of carbon source, especially for the utilization of acids and saccharides according to the principal component analysis.

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