Influence of Temperature and Salinity on Anaerobic Sequencing Batch Biofilm Reactor (ASBBR) Treating Mustard Tuber Wastewater

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(Received: 03 March 2013; accepted: 14 April 2013)

Characterized by high level of salinity, organic concentration and biodegradability, mustard tuber wastewater was chosen to be treated by anaerobic sequencing batch biofilm reactor (ASBBR) in this study. Previous researches have proved that the treatment performance of ASBBR is temperature and salinity depended. Orthogonal experiments were carried out to assay the more specific relationship of temperature, salinity and effluent COD. High salinity showed inhibition towards the treatment effect of microorganisms, while suitable temperature played a catalytic role. Under appropriate condition, the COD removal rate could reach 95.1%. However, under adverse condition, the COD removal efficiency could be as low as 42.2%. The linear regression equation of temperature (T), salinity (\hat{n}) and effluent COD was: COD=1515+ 0.072 \hat{n} -69.1T. The expression could be a guide for engineering design and operation.

Key words: Mustard tuber wastewater, ASBBR, temperature, salinity, activated sludge.

Mustard, one of Chinese famous special products, is a semi-dry-state non-fermented pickle. The main mustard origin is the Chongqing city, China. Large amount of wastewater generated from the large-scale mustard production process is discharged directly, which puts great pressure on the environment. With the impoundment of Three Gorges Dam and the increase of environmental awareness, more and more attention is being paid to the treatment of high salinity wastewater. Currently the main treatment methods include: 1. MBR treatment (Artiga et al., 2008); 2.Combination of biological and chemical process (Ahn et al., 1999); 3.SBR treatment (Lefebvrea et al., 2005); 4. Electrochemical method (Lin et al., 1998); 5. Ion exchange treatment (Metcalf et al., 2003);

* To whom all correspondence should be addressed. TeleFax: +86-023-65120980; E-mail: chaihx@cqu.edu.cn 6.Electroflotation process (Bande *et al.*, 2008), etc. In recent years, researches on salt wastewater treatment have made great progress, but most are still limited to laboratory scale. That how to widely use and popularize the findings into practical engineering application needs deeply study and explore.

Biological treatment of wastewater with high salinity has become a difficult and hot spot in the world research field, owing to the great impact of salt on¹ microorganisms. Researchers around the world have conducted many experiments in this regard, and the main conclusions are shown as following: 1.High salinity leads to high cell osmotic pressure, and causes cell dehydration and protoplast isolation. Besides, it induces lack of filamentous microorganisms and protozoa (Reid *et al.*, 2006; Jeison *et al.*, 2008; Abou-Elela *et al.*, 2010); 2.Activated sludge readily floats upward and run off. In addition, the flocculation activity wanes (Bassin *et al.*, 2011); 3.Nitrification and denitrification activities abate, furthermore, the removal efficiency of organic matter and phosphorus decreases (Fontenot *et al.*,2007; Tsuneda *et al.*, 2005; Uygur, 2006; Kartal *et al.*, 2006); 4. It is helpful to exploit halophilic bacteria for the treatment of high salinity industrial wastewater (Abou-Elela *et al.*, 2010). Microbial treatment has advantages such as low cost, thorough clean, environmental protection and easy operation. However, difference in water quality, operating condition or microbe domestication method may lead to distinct results.

Some studies show that high salinity has inhibition on common microbiology. Ingram (Ingram, 1939) found that the respiration rate of microbiology decreased when the NaCl concentration exceeded 10g/L. Rene (Rene et al., 2008) confirmed that increasing the salt concentration lowered the nitrogen removal significantly, which was attributed to salt induced stress (Rene et al., 2008). However, some other studies showed that high salinity improved the flocculation efficiency and removal rate, what's more, made the treatment system more stable. Woolard (Woolard et al., 1995) indicated that the remove rate of phenol was about 99% when the NaCl concentration reached 15%. Hamoda (Hamoda et al., 1995) held that microbial activity and organic matter removal rate increased in high salinity condition.

The organic concentration in mustard wastewater is high (COD=3000-4000mg/L), and it is the same with biodegradability (BOD/CODH"0.5). It can greatly reduce the effluent COD and the burden of follow-up treatment plants to take ASBBR as a treatment unit. Also ASBBR is an energy efficient treatment process. The starting time can be greatly shortened by inoculating efficient salt-tolerant bacteria filtered out from high-salt mustard marinated wastewater into the reactor.

It has been revealed that ASBBR is temperature and salinity depended (Mohan *et al.*, 2007; Bergamo *et al.*, 2009), whereas there are few specific relationships in this direction. In this case, orthogonal experiments were conducted to test the comprehensive influence of salinity and temperature on the reactor performance. The regression equation of temperature, salinity and effluent COD is then established. And it is helpful for engineering design and operation.

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MATERIALSAND METHODS

Experiment setting

The ASBBR test device is shown in Fig.1. The effective volume of the reactor was 24L and the size was 30.0cm×16.0cm×50.0cm (L×B×H). The reactor was made of plastic, in which filled semisoft fiber filler. CH₄ was collected by serum bottle liquid replacement system. The volume of the serum bottle was 2L, and the liquid used to replace biogas was NaOH solution (1%).



1-ASBBR reactor 2-bracket 3-intake 4-sampling ports 5-biogas collection device 6-biological filter media

Fig. 1. Experiment equipment of ASBBR

The commercial available semi-soft fiber (Fig.2) was made of fiber and reinforcing material. It was processed by simulating the form of nature aquatic plants and then shaped into circular. The diameter was 150mm and the specific surface area was 25m2/m3. The porosity was 99% and the specific gravity after biofilm formation was 40g/m3. The installation distance was 100mm.



Fig. 2. Semi-soft filler

The microbe inoculated in ASBBR was efficient salt-tolerant bacteria screened from mustard marinated wastewater. Activated sludge acclimation was achieved in a way of gradually increasing the salinity and organic load. Since experimental water salinity was proportional to COD, the decrease of the dilution factor would increase the organic load. In the acclimation process, salinity and COD were increased when the COD removal rate reached 80% steadily. In order to reduce the impact of salinity on the anaerobic activated sludge, water salinity and COD were respectively increased by 2000mg/L and 600mg/L every time when acclimation met the intended demand. The water quality in the domestication process is shown in Table.1

Table 1. Water quality during domestication process

COD (mg/L)	salinity (Cl ⁻)(mg/L)	pН
600	2000	6.8-7.2
1200	4000	6.8-7.2
1800	6000	6.8-7.2
2400	8000	6.8-7.2
3000	10000	6.8-7.2
	COD (mg/L) 600 1200 1800 2400 3000	COD (mg/L) salinity (Cl)(mg/L) 600 2000 1200 4000 1800 6000 2400 8000 3000 10000

Four identical ASBBRs were used for parallel trials. The ASBBR worked in accord with sequencing batch reactor. The reactors ran with 2d sequential cycles. In each cycle, feeding, reacting and discharging last for 0.5h, 47h and 0.5h, respectively. The excess activated sludge was extracted by pump from the bottom regularly every 45 days. In each reactor, biofilm density was 50%, drainage rate was 1/2. COD volume load is 0.5 Kg/ m³·d and influent COD is 4000mg/L.

Experiment water quality

In the mustard tuber production process, waste liquid generated in the second and third stage ([Cl⁻] =70-80g/L and [Cl⁻] =140-160g/L, respectively) was used to produce mustard tuber sauce owing to the high salinity and nutrient concentration. Wastewater in the first stage ([Cl-]=10-30g/L), difficult to reuse due to the impurity and relatively low salinity, was usually collected and treated uniformly before being discharged. Experimental wastewater used in this work was the synthetic wastewater from Fuling Mustard Tuber Group Co., Ltd, Chongqing, China. The wastewater composition is listed in Tab.2. Prior to the experiments, influent pH was adjusted to 6.8 to 7.2 in order to reduce the impact of pH on anaerobic process (Bergamo et al., 2009).

 Table 2. Schedule of inspect results of wastewater

COD	TN	NH ₄ -N	pH	Salinity
(mg/L)	(mg/L)	(mg/L)	(mg/L)	
4000	500	150	6.2±0.3	10000

Note: The salinity was metered in the concentration of Cl[.].

EXPERIMENTAL

In order to assay the comprehensive influence of temperature and salinity on ASBBR treating mustard tuber wastewater, orthogonal experiments were carried out according to literature (Geramita *et al.*, 1979).

Salinity and temperature were selected as the orthogonal experiment factors. The maximum value of salinity was 20000 mg/L and the minimum was 10000 mg/L. The maximum and the minimum value of temperature were 30°C and 10°C, respectively. The factors were showed (Table 3): where Z_1 expressed salinity ρ (mg/L) and Z_2 expressed temperature T (°C)

 Table 3. Levels of factors

Studied parameters	Z_1	Z ₂
Zero Level Z _{0j}	15000	20
Constant Intervals ³ %	5000	10
Maximum Value +1	20000	30
Minimum Value -1	10000	10

The two-factor and two-level linear regression orthogonal table L4 (2^3) was chosen. The experiment arrangement was shown in Tab.4. The interaction between the two factors was taken into account, and repeated experiments were carried out. The experiments were repeated in the same condition for three times, and the results were used for orthogonal regression calculation. The four reactors ran in a parallel way.

Table 4. Experiment arrangement

Reactor number	Salinity ρ (mg/L)	Temperature T (°C)	Influent COD (mg/L)
1	20000	30	4000
2	20000	10	4000
3	10000	30	4000
4	10000	10	4000

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In the regression orthogonal table, +1 and -1 indicated the factor levels. Factor levels must been encoded. Both factors were expressed by Z_{ij} , the level zero and the change interval could be expressed as:

$$z_{qj} = \frac{z_{2j} + z_{1j}}{2} \ \Delta_j = \frac{z_{2j} - z_{1j}}{2}$$

Factor values were used for the following

linear transformation: $x_j = \frac{z_{ij} - z_{oj}}{\Delta_j}$

So, in the test:

$X_1 = (Z_1 - 15000)/5000 = (\rho - 15000)/5000$	(1)
$X_{2} = (Z_{2} - 20)/10 = (T - 20)/10$	(2)

Thus factors levels coding table was obtained and showed in Table 5.

Table 5. Coding of factors levels

X _j	Z_{i1}	Z_{i2}
1	20000	30
0	15000	20
-1	10000	10

RESULTS AND DISCUSSION

Results and analysis

The results were shown in Fig. 3. When salinity was a constant value, with the rise of temperature, COD removal rate was higher. When temperature was a definite value and salinity was lower, COD removal efficiency was higher. When temperature was 30°C and salinity was 10000mg/L,





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COD removal rate was highest (95.1%). When temperature was 10°C and salinity was 20000 mg/ L, COD removal efficiency was lowest (42.2%).

According to the calculation result, the influence of salinity and temperature on effluent COD was highly significant, but the interaction could be neglected. Thus the linear regression equation was COD= $1208.75+358.75X_1-691.25X_2$ (3).

DISCUSSION

Temperature and salinity affect biological treatment system significantly. The reasons may be as following: (i) within the general environmental temperature range, when the temperature rises, the activity of microorganism enzymes enhances. The metabolic activity of microorganisms enhances, which causes greater consumption of organic matter. (ii) When salinity increases, microbial activities are inhibited, and even excess Cl⁻ shows toxicity towards the microorganisms; the increase of wastewater density leads to the decrease of activated sludge settleability. After being treated by ASBBR, most organic matters have been removed, but the concentration of the remaining organic matters in the wastewater is still high (Fig.3). In addition, combinations of ASBBR and aerobic reactors or physic-chemical units would be necessary to meet stricter emission standards or to remove nutrients such as nitrogen and phosphorus (Sarti et al., 2007). Mustard tuber wastewater treated by ASBBR cannot be discharged into water body directly. It needs to be discharged into sewer followed by wastewater treatment plants. If there are no municipal pipe networks near the high salinity wastewater production enterprises, the ASBBR effluent needs further treating before being discharged.

In terms of ASBBR treating high salinity wastewater, when two parameters among temperature, salinity and effluent COD are given, expression (4) can be used to predict the left one. Two applications are shown as following:

When ASBBR is designed as a pretreatment plant for high salinity wastewater, expression (4) is useful to calculate the influent salinity in different temperature conditions according to the effluent COD based on different follow-up treatment processes.

If the effluent is to be treated after being discharged into sewer, the COD must be less than 500 mg/L. According to expression (4), the design salinity of the reactor running in summer (30° C), spring or autumn (20° C) is 14.6kg/m³ and 5.09kg/m³, respectively. While the design salinity is less than 0 in winter (10° C), which means ASBBR effluent cannot meet the standard and must be further treated by aerobic process.

ii. If the effluent is discharged directly into natural water body, it needs to be further treated by aerobic process. In order to protect the receiving waters, nitrogen and phosphorus and other organic compounds should be removed. The total phosphorus content is too high, and it can be removed by chemical process (Tran et al., 2012). While total nitrogen can be remove through aerobic biological process (Casellas et al., 2006). From Tab.2, the total nitrogen content is 500 mg/L. In order to meet the C/N ratio of biological nitrogen removal, the effluent COD should be about 1500 mg/L. From expression(4), the design salinity in summer (30°C), spring and autumn (20°C) and winter (10°C) are 28.58kg/m³, 18.99kg/m³ and 9.39kg/ m³, respectively.

When ASBBR is used to pretreat high salinity wastewater, expression (4) is helpful to direct the engineering operation. When the reactor is running in a certain temperature condition:

- i. The effluent COD can be predicted according to the influent salinity so the engineering operation can be adjusted timely.
- According to the follow-up processes, the effluent COD is determined. From equation (4), the influent salinity of the ASBBR is identified. Thus the wastewater can be desalinated appropriately.

CONCLUSIONS

When using ASBBR to treat mustard tuber wastewater, both temperature and salinity have a significant impact on the treatment effect, and their interaction is not significant. In appropriate circumstances, the COD removal efficiency can reach more than 95%. While under adverse case, the COD removal rate could be as low as 42.2%. Linear regression equation of temperature T, salinity \tilde{n} and effluent COD is: COD=1515+ 0.072 \tilde{n} -69.1T. This equation can be used to predict the effluence COD in different temperature and salinity conditions, so it can be used to direct engineering operation, also it can be used to guide engineering design according to the treatment effect that meets the desired requirements.

ACKNOWLEDGEMENTS

This study was supported by the National Natural Science Foundation of China (Grant No. 51008318).

REFERENCES

- 1. Abou-Elela SI, Kamel MM, Fawzy ME, Biological treatment of saline wastewater using a salt-tolerant microorganism. *Desalination*. 2010; **250**: 1-5.
- Ahn DH, Chang WS, Yoon TI., Dyestuff wastewater treatment using chemical oxidation, physical adsorption and fixed bed biofilm process. *Process Biochem.* 1999; 34: 429-439.
- APHA., Standard methods for the examination of water and wastewater, 21st ed. American Public Health Association, Washington DC, 2005.
- 4. Artiga P, García-Toriello G, Méndez R, Garrido JM (2008), Use of a hybrid membrane bioreactor for the treatment of saline wastewater from a fish canning factory. Desalination. 221:518-525
- Bande RM, Prasad B, Mishra IM, Wasewar KL., Oil field effluent water treatment for safe disposal by electroflotation. *Chem. Eng. J.* 2008; 137: 503-509.
- Bassin JP, Dezotti M, Jr. GLS., Nitrification of industrial and domestic saline wastewaters in moving bed biofilm reactor and sequencing batch reactor. J. Hazard. Mater. 2011; 185: 242-248.
- Bergamo CM, Monaco RD, Ratusznei SM, Rodrigues JAD, Zaiat M, Foresti E., Effects of temperature at different organic loading levels on the performance of a fluidized-bed anaerobic sequencing batch bioreactor. *Chem. Eng. Process.* 2009; 48:789-796.
- 8. Casellas M, Dagot C, Baudu M, Set up and assessment of a control strategy in a SBR in order to enhance nitrogen and phosphorus removal. *Process Biochem.* 2006; **41**:1994-2001.
- 9. Eldon R. Rene, Sung Joo Kim, Hung Suck Park., Effect of COD/N ratio and salinity on the performance of sequencing batch reactors. *Bioresour. Technol.* 2008; **99**: 839-846.

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- Fontenot Q, Bonvillain C, Kilgen M, Boopathy R, Effects of temperature, salinity, and carbon: nitrogen ratio on sequencing batch reactor treating shrimp aquaculture wastewater. *Bioresour*. *Technol.* 2007; 98:1700-1703.
- Geramita AV, Seberry J, Orthogonal designs. M. Dekker, New York, 1979; 439-455.
- Hamoda MF, Al-atlar IMS, Effects of high sodium chloride concentration on activated activated sludge treatment. *Water Sci. Technol.* 1995; **31**: 61-72.
- 13. Ingram MS, The influence of sodium chloride and temperature on the endogenous respiration of bacillus cereus. *J. Bacteriol.* 1939; **38**: 613-618.
- Jeison D, Kremer B, Lier JBV, Application of membrane enhanced biomass retention to the anaerobic treatment of acidified wastewaters under extreme saline conditions. *Sep. Purif. Technol.* 2008; 64:198-205.
- 15. Kartal B, Koleva M, Arsov R, Star WVD, Jetten MSM, Strous M, Adaptation of a freshwater anammox population to high salinity wastewater. *J. Biotechnol.* 2006; **126**: 546-553.
- Lefebvrea O, Vasudevan N, Torrijosa M, Thanasekaran K, Moletta R, Halophilic biological treatment of tannery soak liquor in a sequencing batch reactor. *Water Res.* 2005; 39:1471-1480.
- Lin SH, Shyu CT, Sun MC, Saline wastewater treated by electrochemical method. *Water Res.* 1998; **32**:1059-1066.
- 18. Metcalf, Eddy, Treatment and reuse, fourth ed.

McGraw-Hill, Inc., New York, 2003.

- Mohan SV, Babu VL, Bhaskar YV, Sarma PN, Influence of recirculation on the performance of anaerobic sequencing batch biofilm reactor (AnSBBR) treating hypersaline composite chemical wastewater. *Bioresour. Technol.* 2007; 98: 1373-1379
- 20. Reid E, Liu XR, Judd SJ, Effect of high salinity on activated sludge characteristics and membrane permeability in an immersed membrane bioreactor. *J. Membr. Sci.* 2006; **283**:164-171
- 21. Sarti A, Garcia ML, Zaiat M, Foresti E., Domestic sewage treatment in a pilot-scale anaerobic sequencing batch biofilm reactor (ASBBR). *Resour. Conserv. Recy.* 2007; **51**: 237-247
- 22. Tran N, Drogui P, Blais JF, Mercier G, Phosphorus removal from spiked municipal wastewater using either electrochemical coagulation or chemical coagulation as tertiary treatment. *Sep. Purif. Technol.* 2012; **95**:16-25
- Tsuneda S, Mikami M, Kimochi Y, Hirata A, Effect of salinity on nitrous oxide emission in the biological nitrogen removal process for industrial wastewater. J. Hazard. Mater. 2005; 119: 93-98
- 24. Uygur A, Specific nutrient removal rates in saline wastewater treatment using sequencing batch reactor. *Process Biochem*.2006; **41**:61-66
- 25. Woolard CR, Iruine RL, Treatment of hypersaline wastewater in the sequencing batch reactor. *Water Res.* 1995; **29**: 1195-1168.