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Start-up test on anaerobic sequencing batch biofilm reactor treating mustard tuber wastewater of the Three Gorges Reservoir in China

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ABSTRACT

Mustard tuber wastewater is characterized by high salinity and high organics concentration that is potentially detrimental to the biological treatment systems and accordingly affects the treatment efficacy. The start-up test of anaerobic sequencing batch biofilm reactor aimed at sorting out the bacteria that were adaptive to the wastewater with high salinity by gradient increase in the influent salinity to enhance the treatment efficiency and deciding the optimal acclimation condition. The results showed that the chemical oxygen demand removal rate, gas production rate, and dehydrogenase content of the reactor increased by 8.89, 500, and 200%, respectively, when the system reached the stable state, indicating that both the quantity and the quality of the inoculated anaerobic sludge have been improved through acclimation. Besides, four parallel reactors inoculated with anaerobic sludge at four different acclimation periods (1 month, 2 months, 6 months, and 10 months, respectively) were setup to determine the influence of the length of acclimation period on treatment efficiency. The results revealed that the acclimation period with six months was optimum. Besides, the methane production, which was estimated to around 57.5 million m^3, was a considerable part of resources that cannot be ignored.

Keywords: ASBBR reactor; Acclimation; Anaerobic sludge; Saline wastewater; The three Gorges Reservoir

1. Introduction

Fuling district of Chongqing is the largest mustard tuber manufacture area in which there are more than 200 manufacturers with annual production of over 250 kt and produces approximately 1.5 million m^3 wastewater with salinity of 2–15%, organics concentration of 10–20 g/L, and suspended solid of 7 g/L. Due to the limited self-purification capacity of natural water bodies, direct discharge of the wastewater into the Three Gorges Reservoir will cause detrimental effects on the water ecosystem. Therefore, a cost-effective and energy-efficient technique for pre-treating this kind of industrial wastewater is urgent.

Biological methods have been widely used as an efficient way to treat wastewater. This method also treats wastewater with high salinity and high organics

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concentration. Therefore, anaerobic biological processes were considered as the prior option to treat mustard tuber wastewater [1–3]. Through the comparison of different kinds of anaerobic processes, anaerobic sequencing batch biofilm reactor (ASBBR) was chosen due to its substantial stability at a wide adaptive range of temperature, relatively high capacity to resist the impacts of water quality fluctuation, and comparatively low operation cost [4,5]. Since anaerobic sludge plays an important role in degrading organic matters, the activity of the sludge in the reactor would affect the treatment efficiency significantly. However, the high salinity has been demonstrated to cause severe damages to the biological treatment systems in the ASBBR reactor, such as the inhibition of enzyme activity of most micro-organisms, the decrease of respiration rate, and the increase of effluent suspended solids. Besides, under high salinity conditions, the dehydration and plasmolysis of microbial cells would collapse and kill the cells. Consequently, the efficiency of biological treatment systems treating saline wastewater would decrease sharply [6–10].

But some studies [11] have shown that halobacteria, a kind of micro-organism, which exist in the microflora. This kind of micro-organisms could adjust osmotic pressure through osmoregulatory mechanism and improve the biological activity and degrading ability under a high salinity condition. Le Borgne et al. [12] studied the biodegradation of organic pollutants by halophilic bacteria and archaea and pointed out that 5% of the industrial effluent was saline water and both hypersaline and halophilic micro-organisms were good candidates for the bioremediation of hypersaline environment and treatment of saline effluent. Besides, it was also observed that both the moderately halophilic bacteria and the extremely halophilic archaea had a broader catabolic versatility and capability than previous thought; and a diversity of contaminants were susceptible to be degraded by halotolerant and halophile bacteria. Woolard and Irvine [13] screened out halophiles from the soil of the salt lake and added into SBBR reactor treating phenol wastewater with salinity of 15% after acclimation. It showed very promising results with a stable and highly qualified effluent. Kargi and Uygur [14] studied the biological treatment of saline wastewater in an aerated percolator unit utilizing halophilic bacteria. The results indicated that inclusion of salt-tolerant bacteria in activated sludge culture resulted in high chemical oxygen demand (COD) removal rate especially at high salt concentration such as 5%. Other studies [15,16] such as the application of salt-tolerant bacterial strains to the treatment of tannery wastewater with salinity of 1–10% and the effect of the application of halotolerant micro-organisms on the efficiency of a pilot-scale constructed wetland for saline wastewater treatment were also carried out. Besides, the adaptation of halobacteria to grow and get enriched in the water bodies with high salinity implies the possibility of its practical application in wastewater treatment in large scale.

In this case, there was another issue to sort out the microbes that could be adaptive to saline environment and not susceptible to the fluctuation of water quality. Recently, there were many researches focused on the acclimation of active sludge. Studies on the possibilities, characteristics, and processes of sludge acclimation were carried out. The results of the comparison of the sludge acclimation processes under different salinity level indicated that the halobacteria with high degradation efficiency could be sorted out by using salinity gradient as the selection pressure with a longer acclimation period [17]. Also, it has revealed that the biodiversity and acclimation conditions were the determinant factors in sludge acclimation [18]. Researches on the acclimation stages, the microbial structure of the sludge, and the effect of the biodegradability on the performance were also carried out. It was found that the acclimation period could be divided into four stages with various predominant micro-organisms and high treatment efficiency could be reached by extended hydraulic and sludge retention time [19,20]. Meanwhile, a mathematical model formulated degrader formation by the conversion of indigenous microbial cells was established to serve the need for a clear expression of microbial acclimation to a xenobiotic [21].

Goncalves et al. [22] conducted a research aimed at coming up with a suitable strategy to accelerate the start-up of the anaerobic treatment of olive mill wastewater and enhancing the biogas production. The results showed that the application of acclimated microbes was a promising strategy to accelerate the start-up of digest process and to improve the overall anaerobic treatment. Ren and Gong [23] also conducted a research on the acclimation strategy of a biohydrogen-producing microbe in a continuous-flow reactor with carbohydrate fermentation, which pointed out that the feed to micro-organisms ratio was the key parameter for the enrichment of hydrogen-producing microbes. Zhou et al. [24] studied the rapid acclimation of methanogenic granular sludge into denitrifying sulfide removal (DSR) granules and the results demonstrated that methanogenic granules could be readily acclimated into DSR granules in a short period, removing all $1.30\,kg\,m^3\,d^{-1}$ sulfide and converting more than 90% of $0.56\,kg\,Nm^3\,d^{-1}$ nitrate into dinitrogen gas. Also, there were some researches on the sludge acclimation in the field of microbial fuel cell.

Kim et al. [25] evaluated the procedures of acclimating microbial fuel cells for electricity production. Other researches [26,27] related to the effect of acclimation of micro-organisms to heavy metals on the performance of activated sludge processes, the effect of high-strength ammonia nitrogen acclimation on sludge activity in sequencing batch reactor, and the effect of temperature change on the treatment efficiency during the acclimation period were also carried out.

Based on the aforementioned research findings, the objective of this study was to explore the influence of acclimation and the length of acclimation period of anaerobic sludge to saline water on the efficiency of treating mustard tuber wastewater by ASBBR reactor, and subsequently find out the proper acclimation conditions and sort out the bacteria that were adaptive to the wastewater with high salinity by gradient increase of the influent salinity to enhance the treatment efficiency. The results could provide a practically technical guidance for treating saline wastewater using biological methods.

2. Materials and methods

2.1. Wastewater characteristics

Mustard tuber production flowchart is shown in Fig. 1. The wastewater sample obtained from mustard tuber manufacture in Fuling China was a mixture of the wastewater produced during the different production processes. There were many steps in total to produce mustard tuber. The salinity of the effluent of the first pickling step, the salinity of mustard tuber washing water, and the salinity of the desalination water were as low as 3, 2, and 5–6%, respectively, which were not significantly different, herewith they can be collected and treated together lately. However, the effluent of the second and the third pickling steps were of high salinity as 7–8 and 14%, respectively. Therefore, it was economically favorable to further process the effluent into soy sauce. The characteristics of the comprehensive wastewater sample are shown in Table 1.

Table 1
The characteristics of the comprehensive wastewater sample

COD mg/L	Total nitrogen mg/L	Ammonia nitrogen mg/L	pH value	Salinity mg/L
4,000	500	150	6.2 ± 0.3	10,000

Note: The salinity mentioned in this paper was measured by the concentration of chloride ions.

2.2. The sludge inoculation

The inoculated sludge was selected from the effluent of mustard tuber production process.

2.3. Experimental device

The ASBBR was adopted in the experiment and its structure is shown in Fig. 2. The size of the reactor was L × B × H = 30.0 × 16.0 × 50.0 cm, with the effective volume of 24 L. The reactor was made of plastic filled with semi-soft fibrous filter. The CH_4 gas generated was collected by a serum bottle liquid displacement system. The volume of the serum bottle was 2 L and the liquid used for replacing biogas was 1% NaOH solution. Specifically speaking, 1% NaOH solution was used to absorb the gas of CO_2 produced in the reactor in which pure methane gas was obtained.

The anaerobic sludge with high activity would produce massive extracellular polymeric substances, a contributor to the adhesion of activated sludge particles to the membrane inside the reactor, whereas the

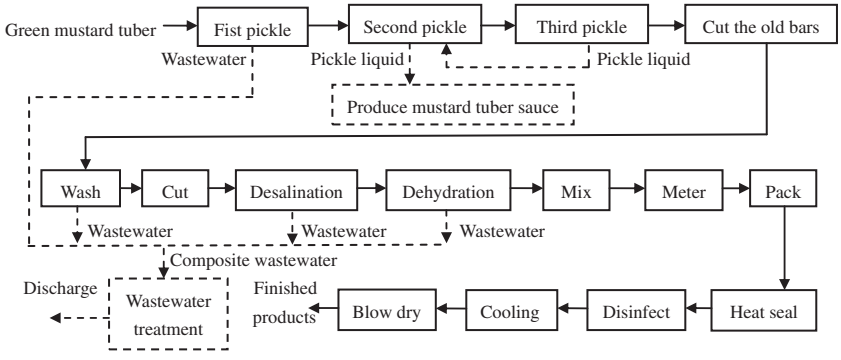

Fig. 1. Wastewater sources of mustard tuber production process [28].

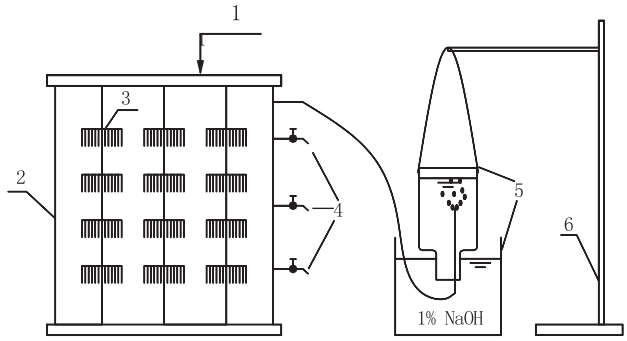

Fig. 2. Experiment equipment of ASBBR. (1) Influent, (2) ASBBR reactor, (3) Biological packing, (4) Sampling and effluent, (5) Biogas collection devices, and (6) Bracket.

Table 2
The characteristics of the influent during the acclimation period

Working parameters stages of the acclimation period	COD mg/L	Salinity mg/L	pH
The first stage	600	2,000	6.8–7.2
The second stage	1,200	4,000	6.8–7.2
The third stage	1,800	6,000	6.8–7.2
The fourth stage	2,400	8,000	6.8–7.2
The fifth stage	3,000	10,000	6.8–7.2

Note: According to the preliminary experiment result, pH value has significantly affected the ASBBR reactor treatment efficiency on the high-salinity mustard tuber wastewater, and the optimum pH range has proved to be 7.0 ± 0.2, so the pH value of the raw water has been adjusted to 7.0 ± 0.2 before the experiments.

aged sludge would fall off from the surface of membrane. The deciduous sludge, namely the excess sludge, was discharged from the bottom of the reactor by regular pump events.

2.4. The setup of acclimation test

The aim of this study was to explore the influence of acclimation of anaerobic bacteria on the biodegradability. He et al. [29] have studied the acclimation of activated sludge from the secondary settling tank in a wastewater treatment plant by gradually increasing the influent salinity and keeping the influent salinity stable, respectively. The results showed that former method was more conducive to the cultivation of salt-tolerant bacteria and the effluent quality was better than the latter. In a paper which focused on the salt-tolerant sludge acclimation and its mechanisms, it also pointed out that, with salt as the selection pressure, salt-tolerant bacteria could be screened out with good degradation ability by gradually increasing the influent salinity [17]. So, in this study, the influent salinity and COD concentration were gradually increased to sort out the halobacteria. As salinity of the wastewater sample was correlated with COD concentration, both salinity and COD concentration were increased when the dilution multiple of the wastewater sample was gradually decreased. When the COD removal rate of the reactor under specific influent COD concentration and salinity reached 80% and kept stable for two weeks, the influent COD concentration and salinity could increase and move to the next acclimation stage. The whole acclimation period would not finish until the fifth acclimation stage was completed and treated for the hypersaline wastewater effectively. In order to reduce the impact of salinity change on anaerobic sludge, the increasing extent of the influent salinity and COD concentration was controlled at 2,000 and 600 mg/L, respectively, during the acclimation period as the influent of the reactor was obtained by the dilution of the wastewater sample. The characteristics of the influent during the acclimation are presented in Table 2. Parameters measured included effluent COD, gas production rate, and dehydrogenase content, which reflected the activity of the anaerobic sludge inside.

2.5. The influence of the length of acclimation period

Based on the former studies, it was shown that the activity of the anaerobic sludge had been improved through acclimation. So whether the length of acclimation period had an influence on the sludge activity under the same acclimation conditions was worth studying. Rodrigo et al. [20] pointed out that with high hydraulic and sludge retention time, it was possible to obtain a very efficient process with 90% COD removal rate and practically total conversion of COD into electricity. So, in this study, four parallel reactors inoculated with anaerobic sludge at four different acclimation periods (1 month, 2 months, 6 months, and 10 months) under the same operation conditions were setup to determine the optimal acclimation conditions. All the reactors ran under the following conditions: biofilm density at 50% which indicated that the volume of the packing occupied the 50% capacity of the whole reactor, drainage ratio at one-third which meant the ratio between the discharged water and the volume of the reactor, HRT of 2 d, and the temperature of 15˚C. The characteristics of the influent are shown in Table 1. Parameters measured included

effluent COD, gas production rate, and dehydrogenase content.

2.6. Experimental parameters and analytic methods

Parameters such as the effluent COD concentration, gas production rate, and dehydrogenase content were measured and analyzed by standard methods [30]. While parameters like temperature and pH value were tested by thermometer and HACH sension 2 precision acidometer respectively. Biofilm density is the ratio between the volume of the packing and the capacity of the whole reactor, and the biofilm density was obtained by measuring the volume of the packing inside the reactor since the capacity of the reactor was known.

3. Results and discussion

3.1. The influence of acclimation on COD removal rate

It can be obtained from Fig. 3 that the COD removal rate of ASBBR reactor gradually increased during the acclimation period. But, the growth rate tended to decrease with the increase of influent salinity. As influent salinity increased from 2,000 to 6,000 mg/L, the COD removal rate increased from 83.33 to 92.22%, which increased by 8.89%. Then the COD removal rate kept stable though the influent salinity was even higher than 6,000 mg/L, which indicated the success in accomplishing the acclimation period and sorting out the halobacteria which was highly effective in treating hypersaline wastewater. The results were similar to the first picture of Fig. 4 in the paper written by Abou-Elela et al. [31]. And it also explained the reasons for the variation of COD removal rate as different micro-organisms had different optimum salinity ranges to adapt. With the increase in salinity, the activity of activated sludge gradually restrained, while halophilic micro-organisms stimulated and was of high activity. So, the COD removal rate was increased with the decline in increase rate.

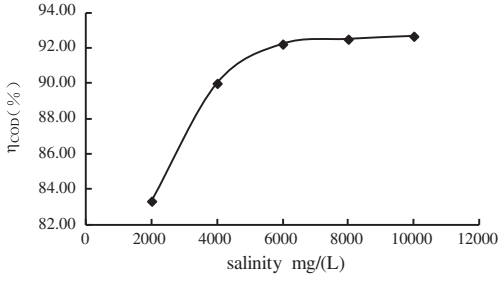

Fig. 3. Influence of acclimation period on COD removal rate.

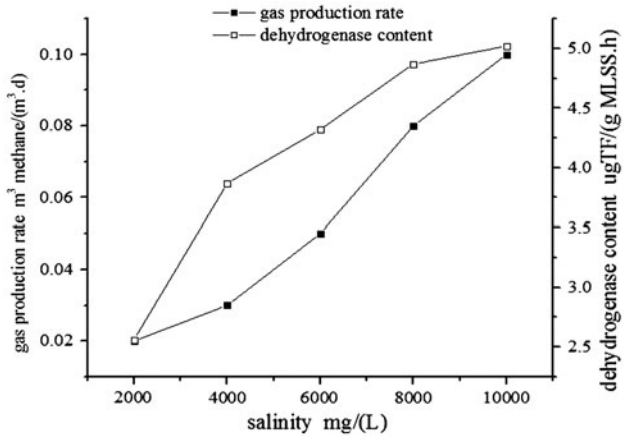

Fig. 4. Influence of acclimation period on gas production rate and dehydrogenase.

3.2. The influence of acclimation on gas production rate and dehydrogenase content

The influence of acclimation on gas production rate and dehydrogenase content were presented in Fig. 4. The upside curve represented the changes of dehydrogenase content with the increase of influent salinity. It can be seen from the profile that the dehydrogenase content increased by almost 200%, from 2.56 to 5.02 μg TF/(g MLSS) while the increase rate of dehydrogenase content decreased as the influent salinity increased from 2,000 to 10,000 mg/L. The downside profile reflected the changes of gas production rate with the increase of influent salinity. As the influent salinity increased from 2,000 to 10,000 mg/L, gas production rate of the inoculated anaerobic sludge increased from 0.02 to 0.1 m^3 methane/(m^3 d), which increased nearly 500%. It also can be found that the increase rate tended to increase along with the increase of the influent salinity.

Researches have demonstrated that the content of dehydrogenase could be used as an indicator of the amount of living cells and the activity of the cells. And the effect of altering substrate load on the gas production was also well followed by the enzyme activity data [32–34]. Therefore, it can be obtained from Fig. 4 that both the number and the activity of living anaerobic bacteria have increased. This was presumably because the proliferation of the adaptive bacteria outcompeted the others by obtaining more nutrients under high salinity [18]. The phenomena that COD removal rate was increased with the increase of influent salinity also can be explained as the increase of sludge quantity and the improvement of sludge activity. And this kind of sludge has made a full use of the organic matters within the influent which led to

the increase of COD removal rate. Besides, it can be found that more biogas was produced as more organic matter was converted which was in accordance with the results shown in the paper entitled biogas production from anaerobic co-digestion of food waste with dairy manure in a two-phase digestion system [35].

So, conclusions can be made from Figs. 3 and 4 that COD removal rate, gas production, and the content of dehydrogenases in the reactor were increased along with the gradient increase of influent salinity. Based on the variety of bacteria, bacteria needed could be screened out by controlling the acclimation condition. And this kind of bacteria has made a full use of the nutrient in the influent of ASBBR reactor and obtained a fast growth, which led to the increase of COD removal rate and methane production in the reactor after acclimation. The results obtained could provide guidance for the acclimation of other kind of bacteria in wastewater treatment.

3.3. The influence of the length of acclimation period on effluent COD

The influence of the length of acclimation period on effluent COD is presented in Fig. 5. The anaerobic sludge inoculated in the four parallel reactors with four different lengths of acclimation period, i.e. 1 month, 2 months, 6 months, and 10 months, respectively. All the reactors inoculated with acclimated sludge presented an effective removal of influent COD and longer the reaction time was, the more significant the removal rate was. In the paper written by Sivaprakasam et al. [16] similar results have been presented. It also can be obtained from Fig. 5 that the COD removal efficiency with anaerobic sludge acclimated for six months was better than that with the sludge acclimated for shorter time. On top of that, the removal efficiency did not increase significantly even with the sludge acclimated for more than six months. Accordingly, from the economic point of view, six months of acclimation was optimal to get the fullest use of the bacterial functions under high salinity.

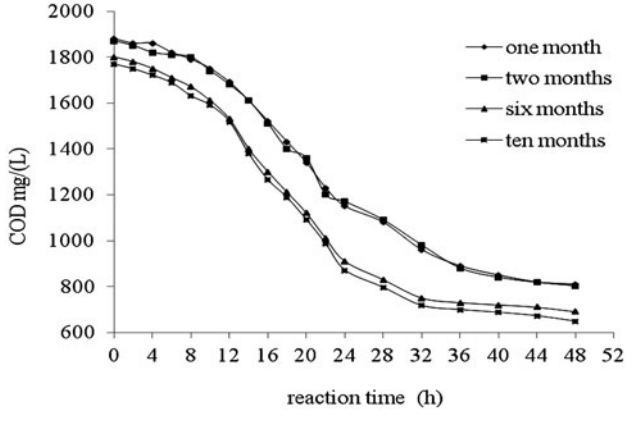

Fig. 5. The influence of the length of acclimation period on effluent COD.

3.4. The influence of the length of acclimation period on gas production rate

The influence of the length of acclimation period on gas production rate can be indicated from Fig. 6. The four parallel reactors inoculated with anaerobic sludge at four different acclimation periods presented a different gas production rate when the operation of the reactors reached the stable state. The gas production rate of the reactor inoculated with sludge acclimated for one month was 0.089 m^3 methane/(m^3 d) and increased as the acclimation period prolonged. And the gas production rate of the reactor increased to 0.105 m^3 methane/(m^3 d) and varied insignificantly when the reactor inoculated with sludge acclimated for six months.

The results were coherent with the point presented in the paper written by Abou-Elela et al. [31]. In that paper, it was pointed out that the time required to reach the steady-state conditions after acclimation period under the different salinity conditions was different and it was increased along with the increase of salt content. Also, it was pointed out that the acclimation period was 25–35 d when salt concentration was 10 g/L as aerobic biological degradation was chosen. As anaerobic reactor was chosen in the start-up test in this study, the time required to get the steady-state conditions after acclimation period may be different. And it was also pointed out in the paper written by Yu et al. [19] that there were four stages: the early stage of sludge acclimation, the stage of sludge formation, the stage of sludge proliferation and the stage of

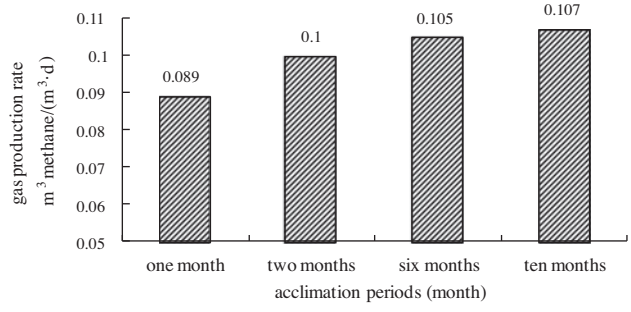

Fig. 6. The influence of the length of acclimation period on gas production rate.

sludge maturation in total to finish the acclimation. The reactor could reach the stable state with the highest treatment efficiency inside when the acclimation period was finished. So, conclusions can be deduced that acclimation period with six months was the best and the annual methane production was figure out as around 57.5 million m^3, which could be utilized for other purposes.

3.5. The influence of the length of acclimation period on dehydrogenase content

The influence of the length of acclimation period on dehydrogenase content can be obtained from Fig. 7. The dehydrogenase contents increased as the acclimation period prolonged. The dehydrogenase content was 4.88 μg TF/(g MLSS) when inoculated with the sludge acclimated for only one month and the dehydrogenase content increased to 5.43 μg TF/(g MLSS) when the acclimation period increased to six months. And after that it increased a little though the acclimation period was much longer, which meant six months of acclimation have won the best bacteria activity. This presumably was due to the inhibitory effect on enzyme activity caused by the high salinity within the influent of the reactor, which led to the decrease of dehydrogenase activity [36]. While, it can be found from the part of 3.2 that the micro-organisms gradually adapted to the saline wastewater and the dehydrogenase content of the reactor also increased through acclimation. But, the increased extent of dehydrogenase content was different due to the different length of acclimation period. This part has discovered that six months was the optimum duration to obtain the best dehydrogenase content.

From Figs. 5–7, it can be concluded that the activity of anaerobic sludge increased by prolonging the acclimation period under the same influent salinity, which led to the increase of COD removal rate and gas production rate. And it can be found that the optimum acclimation period turned out to be six months.

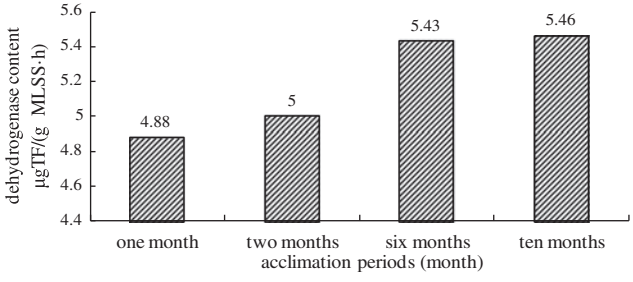

Fig. 7. The influence of the length of acclimation period on dehydrogenase content.

Besides, it also can be pointed out that the anaerobic treatment was more energy efficient than aerobic process as 57.5 million m^3 methane gas has been produced per year during the treatment process.

4. Conclusions

The start-up of ASBBR reactor treating mustard tuber wastewater was systematically investigated in this study. By gradient increase of the influent salinity, it can be found that COD removal rate, gas production rate, and the dehydrogenase content of the reactor increased by 8.89, 500, and 200%, respectively, when the system reached the stable state, which indicated the improvement of both the quantity and the quality of inoculated bacteria after acclimation. Then, experiment about the influence of the length of acclimation period on the activity of anaerobic sludge has been carried out. Results have shown that the sludge activity increased with the extension of acclimation period and six months of acclimation period was optimum by comparing four parallel reactors inoculated with anaerobic sludge at four different acclimation periods under the same operation condition. These results provide some insights on sludge acclimation.

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