Journal of Biotechnology **14**(4): 777-784, 2016

TREATMENT OF ACIDIC WASTEWATER FROM THIEN KE TIN PROCESSING FACTORY BY SULFATE REDUCING BIOREACTOR: A PILOT SCALE STUDY

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Received: 09.11.2016
Accepted: 20.12.2016

SUMMARY

A pilot-scale system of a total volume of 6 m^3 using sulfate reducing (SR) bioreactor technology was established for the treatment of acidic wastewater from Thien Ke tin processing factory in Tuyen Quang province, Vietnam. In the system, the acidic wastewater with high metal content went first to a collecting tank filled with limestone gravels to increase pH to a value favorable for SRB growth, and at the second step to a SR bioreactor where sulfate reduction occurred to produce sulfide for metal precipitation. To activate the SR bioreactor, a laboratory SRB mixed culture dominated by *Desufovibiro*, *Desulfobulbus* and *Desulfomicrobium* species was added at a cell density of ~10^6 cell/ml so that a full activation was achieved just after a week of incubation. Molasses was added to the SR bioreactor at 0.5 ml/L as substrate for the SRB growth during the operation. The performance of the system was studied under batch and continuous modes. The batch mode showed good results after three day-operation. The pH increased from 2.8 – 3.2 to 7 – 7.2, and a total of 750 mg/L sulfate was reduced to sulfide presumably by the SRB. The produced sulfide efficiently removed metals from the wastewater, such as iron from 143.1 mg/L to 0.3 mg/L, copper from 16.32 mg/L to 0.04 mg/L and manganese from 10.9 mg/L to 0.05 mg/L. The continuous mode with a hydraulic load of 100 l/h and an according retention time of three days showed constitutive contaminant removal. The effluent pH of the system was around 7 within six-day period. The sulfate reduction was active, keeping sulfate concentration in the final effluent as low as ~ 150 mg/L. Accordingly, the three most metal contaminants (iron, copper and manganese) were found at concentrations below the regulated limits. The results showed the possibility of applying SR bioreactor technology for the treatment of AMD is feasible and the use of previously enriched mixed culture of SRB could be a good approach to shorten the activation period of the SR bioreactor.

Keywords: *Acid mine drainage (AMD), heavy metals, sulfate-reducing bacteria (SRB), sulfate-reducing bioreactor*

INTRODUCTION

Mining activities bring sulfidic ores to contact with oxygen, resulting in oxidation of pyrite-containing rocks to sulfuric acid and dissolved ferrous iron. Along with this, other metals in the ores can be dissolved, leading to the production of acidic water with high metal content called Acid Mine Drainage (AMD). Thus, AMD is characterized by low pH (2 – 3), dissolving variety of heavy metals at high concentration (up to hundred ppm) and is very toxic to aquatic life or soil ecosystems in mining areas (Johnson, Hallberg, 2005).

Sulfate reducing (SR) bioreactor applied to the AMD treatment is a passive treatment technology that employs sulfate-reducing bacteria (SRB) to produce sulfide for metal sulfide precipitation while generating alkalinity. The chemical basis of SRB remediation involves microbially mediated sulfate reduction coupled with organic carbon (expressed as CH_2O) oxidation, and consequent metal sulfide (MeS) precipitation (Gusek, Knight, 2002):

$$2\ CH_2O + SO_4^{2-} + H^+ \rightarrow H_2S + 2HCO_3^-$$
$$H_2S + Me^{2+} \rightarrow MeS + 2H^+$$

Such a technology is highly efficient for removing heavy metals from AMD. Iron, lead, copper, nickel, cadmium and zinc can readily precipitate as metal sulfides, whereas some other metals and metalloids such as molybdenum, arsenic and antimony form more complex sulfide minerals

(Figueroa, 2005). In addition, arsenic and uranium can also be reduced by SRB to a more insoluble form, for example uranium (VI) to uranium (IV) (Spear *et al*., 2000), arsen (VI) to arsen (V) (Macy *et al*., 2000). Increasing the pH due to the SRB metabolic products HCO_3^- and HS^- create favourable condition for metal precipitation as hydroxides and carbonate salts. For an efficient operation, a sulfate-reducing bioreactor requires a redox potential less than −200 mV, at which sulfate reduction as well as ferric iron reduction can take place (Cabrera *et al*., 2006).

SR bioreactors operate not only at mesophilic conditions but also at lower temperatures, even as low as 4 °C, where mesophilic and cold-adapted SRB belonging to *Desulfovibrio* spp., *Desulfobulbus* spp. are often found to be the key players (Zhang, Wang, 2016; Bomberg *et al*., 2015). Substrates for SRB are short chain fatty acids such as lactate, acetate, propionate and alcohols (Logan *et al*., 2005). In SR bioreactors, substrate and growth matrixes for SRB usually are more complex materials like wood chips, from which cellulosic compounds undergo hydrolysis and subsequent fermentation to provide simple organic carbons for the SRB (Logan *et al*., 2005). In some cases, liquid organic carbons such as methanol or ethanol have been used in field application, however this would increase the operation cost of the system (Tsukamoto *et al*., 2004).

Since AMD is very acidic, acid tolerance is an advantageous characteristic for SRB involving in the treatment process. It has been shown that biological sulfate reduction decreased significantly when pH dropped from 6 to 4 (Jong, Parry, 2006). Therefore, the introduction of acid-tolerant SRB strains to AMD treating SR bioreactors may improve the performance (Johnson, Hallberg, 2005). On the other hand, mix cultures of SRB have been shown for higher tolerance to heavy metals that are often found in AMD at high concentrations (Utgikar *et al*., 2011).

In this study, SR bioreactor technology is demonstrated at a pilot scale for the treatment of AMD from Thien Ke tin processing factory in Tuyen Quang, Vietnam. A mixed culture of SRB previously enriched in the laboratory was applied to the pilot system to activate the sulfate reducing bioreactor. The sulfate reduction rate and operational conditions are discussed in details to establish an optimum operation regime that would serve as basis for the full scale application in near future.

MATERIALS AND METHODS

Chemical composition of wastewater from Thien Ke tin mine

Chemical composition of AMD from Thien Ke tin processing factory was determined repeatedly during the study period from July to November 2015, the average values are presented as following:

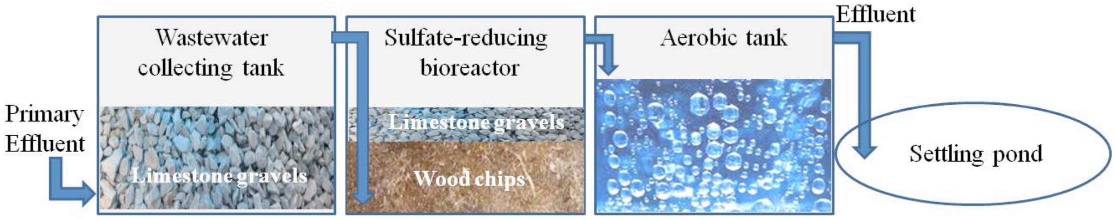
The chemical composition of, AMD from Thien Ke tin factory is characterized by low pH and organic carbon, high concentration of sulfate, iron, manganese and copper. The high concentration of sulfate (982.7 mg/L) is a good prerequisite for the activity of SRB in the system.

Table 1. Chemical composition of AMD from Thien Ke tin mine.

No.	Chemical composition	Concentration (mg/L)	**QCVN 40: 2011/BTNMT** (National Technical Regulation on Industrial Wastewater), **B level** (mg/L)
1	pH	2.8 – 3.2	5.5 - 9
2	COD	28.6	50
3	BOD_5	17.6	100
4	SS	67	100
5	Pb	0.1	0.5
6	Cu	16.32	2
7	Ni	0.05	0.5
8	Mn	10.9	1
9	Fe	143.1	5
10	Sulfate	982.7	–

Set-up of the pilot-scale system

The pilot-scale AMD treatment system comprised from a set of three tanks with the same size of 1.5 m × 1.25 m × 1.5 m (L × W × D), corresponding to a volume of 2.8125 m^3 each (Fig. 1). The first tank collected wastewater from the factory by mean of a pumper. The tank contained limestone gravels (2 – 3 cm in size) to a half of the volume. The second tank was a SR-bioreactor, containing up to half volume a matrix layer, two third of that was made of wood chips (at the bottom) and one third of limestone gravels (on the top). Wood chips in this tank served as (i) growth matrix for the SRBs in the bioreactor and (ii) a type of slowly released energy and carbon source for the SRBs. The third tank was an aerobic treatment unit, equipped with a gas blowing device at the bottom to promote manganese and aluminum removal since these metals do not precipitate as sulfide salt efficiently. At the final stage, the wastewater went to a settling pond where it could be stabilized before discharging to common receiving system outside.



A

B

Figure 1. Pilot- scale AMD treatment system. A – Operational scheme; B – The onsite system

SRB source

In the SR bioreactor, sulfate-reducing bacteria (SRB) were introduced from a laboratory mixed SRB culture at the initial cell density of ~10^6 cells/ml (estimated according to the dilution factor of the microbial seed in the wastewater volume in each tank, accepting that the number of SRB present in the AMD was negligible). The mixed culture of SRB was previously enriched from sludge of an AMD storage pond using the selective anoxic lactate-sulfate medium (Widdel, Bak, 1992). The key SRB species of the mixed culture were determined as *Desufovibrio* sp., *Desulfobulbus* sp. and *Desulfomicrobium* sp. (Nguyen, Dinh, 2016).

The culture was grown in 2 L-Schott bottles containing 1.8 L medium, flushed with a gas mixture N_2/CO_2 90/10 (vol/vol) to remove oxygen. After 2 – 3 days of incubation, the liquid culture with OD_{600} of 0.7 – 0.8 was applied to the SR bioreactor of the pilot system at a dilution factor of 10^{-3}. To support growth of SRB in the SR bioreactor during the operation, molasses was added to the pilot system at the concentration of 0.5 ml/L.

Analytical methods

Environmental parameters of wastewater from Thien Ke tin processing factory during the treatment process were analyzed using standard analytical methods provided by MONRE (QCNV40:2011/BTNMT). The parameters had been analyzed included COD (TCVN 6491:1999), Pb, Cu, Ni (TCVN 6193:1996), Mn (TCVN 6002:1995), Fe (TCVN 6177:1996), sulfate, (TCVN 6494-1:2011). The analytical work was carried out at the laboratory of the Department of Informatics, Technology and Environment, Vinacomin.

RESULTS AND DISCUSSION

Start up the pilot-scale system

Before operating the treatment system, the collecting tank and SR bioreactor were filled to half volume (flooded the matrix beds) with wastewater from Thien Ke tin processing factory. Afterwards, the enriched mixed culture of SRB was added to the SR bioreactor at the dilution factor of 10^{-3} in order to provide the bacterial seed at a cell density of $\sim 10^6$ cells/ml. The system was then incubated for a week to activate the SRB community.

It was shown that after contacting with the limestone gravel bed in the collecting tank, pH of the wastewater instantly increased from 2.8 to 4.7 due to the chemical effect of carbonaceous compounds. In the SR bioreactor, however sulfate reduction could be clearly observed after two days, the sulfate concentration dropped gradually from 982.7 mg/L to 213 mg/L after one week incubation, and at the same time, pH increased from 4.7 to 6.8 (Fig. 2).

In laboratory studies as well as practice, SRB seed was often attained from natural sources such as cow manure or anaerobic sludge (Doshi, 2006). However in such cases, the SR bioreactor activation process would take much longer, from several weeks to months (Doshi, 2006; Kieu Quynh Hoa *et al.*, 2013). Using the enriched mixed culture of SRB as microbial seed in this study has significantly shortened the adaptation and activation time for the SR bioreactor.

It has been reported that the thickness of matrix layer filled in SR reactors is considered of prime important for the set-up of anaerobic condition for the SRB, and hence can strongly influence the treatment efficiency of the system (Doshi, 2006). Generally, a filling level of half bioreactor volume with some variations has been suggested (Doshi, 2006). In this pilot system, the recommended filling level of ½ volume was applied, leading to a good anaerobic condition which was proven by the active sulfate reduction.

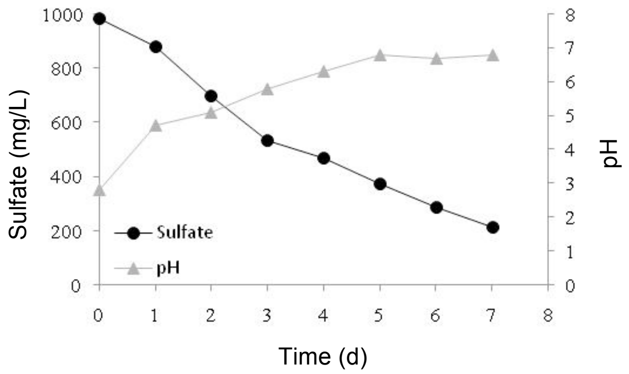


Figure 2. Activation of biological sulfate reduction in the SR bioreactor

Performance at the batch mode

After the SRB community in SR bioreactor had been activated, the system was operated under batch mode, i.e. the wastewater was filled in the system at a hydraulic load of 100 L/h (the filling process took approximately four days), then the system was kept untouched for two days. Effluent samples (water out coming from the aerobic tank) were collected for analyzing chemical parameters, for each an average value was adopted from two parallel measurements (Fig. 3).

The analyzed results indicated that the system has efficiently removed contaminants from the wastewater. Thus, pH increased right after the contact with limestone grave bed in the collecting tank, and latter due to the alkali products of biological sulfate reduction HS^- and HCO_3^-. Since the wastewater had been filled in the system at a rate of 100 L/h, it got contact with the limestone gravel bed for about 24 h (containing volume of the tank was ~2,4 m^3). As observed at graph A, pH reached ~5.4 after the first day and ~7 after the second day, then slowly increased during the last four days of monitoring period, reaching ~7.3 at the end.

Active sulfate reduction as an indicator for the growth of SRB in the SR reactor was observed from the first day, continued for the next three days, then slowed down (Fig. 3B). It was supposed to be due to the limitation of organic carbon serving as growth substrate for SRB in the SR bioreactor. The sulfate reduction rate was in accordance with the rate of COD removal (Fig. 3C). It should be noted that the original acidic wastewater from Thien Ke factory had very low COD content (Table. 1), most of which was not biodegradable (low BOD value). The addition of molasses at the concentration of 0.5 ml/L increased the COD content to 305 mg/L, better favoring SRB in reducing sulfate to sulfide and precipitating metal ions. In previously published data, the COD/sulfate ratio could vary significantly, depending on types of SR bioreactors, SRB communities and nature of the organic carbons as well (Kousi *et al*., 2015). Generally, the COD/sulfate ratio should be kept as low as possible, in the range favoring SRB activity (Kousi *et al*., 2011). Although being lower than the optimal theoretical COD/sulfate ratio which has been calculated for 0.67 (Hao *et al*., 1999; Neculita, Zagury, 2008), the ratio at 0.31 in this study still allowed an active sulfate reduction since another part of COD had came from wood chips matrix in the SR bioreactor as slowly released organic carbons.

The three most significant metal contaminants of the wastewater, i.e. iron, copper and manganese, were also monitored. Results showed that concentrations of these three metals decreased significantly within the first three days of treatment, reaching values below 1 mg/L after the fourth day of operation (Fig. 3D, E, G).

The obtained results from the batch operation study showed that an efficient treatment of the acidic wastewater from Thien Ke tin processing factory by the SR bioreactor system designed above could be possible if the system operated with COD content of ~300 mg/L (by adding molasses at 0.5 ml/L) and a hydraulic retention time of 3 – 4 days.

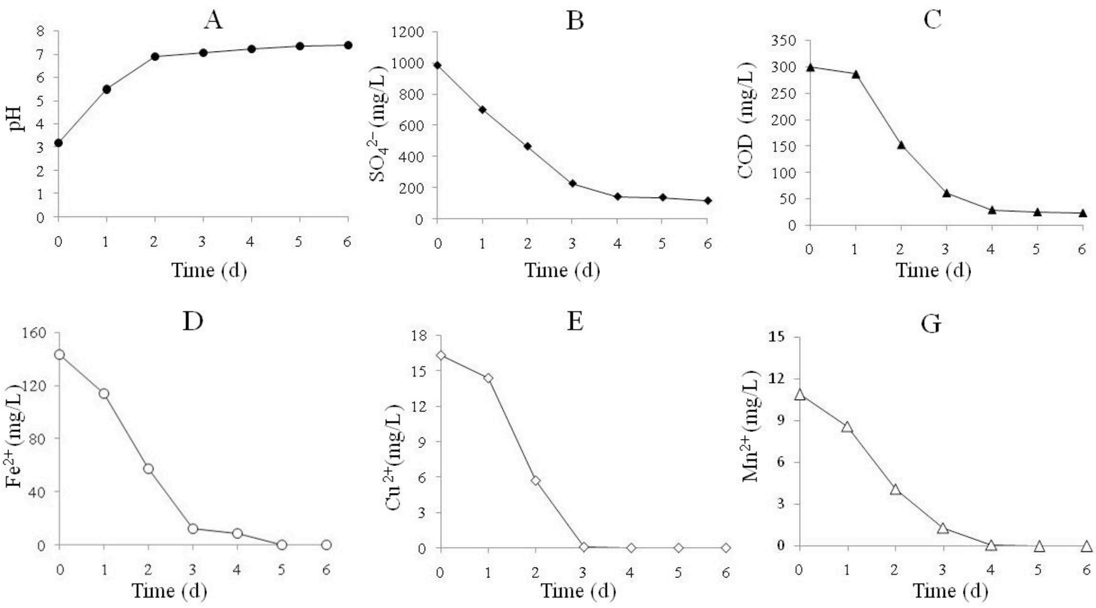


Figure 3. Removal of the most significant contaminants in AMD from Thien Ke tin processing factory by SR bioreactor operated in a batch mode with time. A: pH; B: SO_4^{2-}; C: COD; D: Fe^{2+}; E: Cu^{2+}; G: Mn^{2+}.

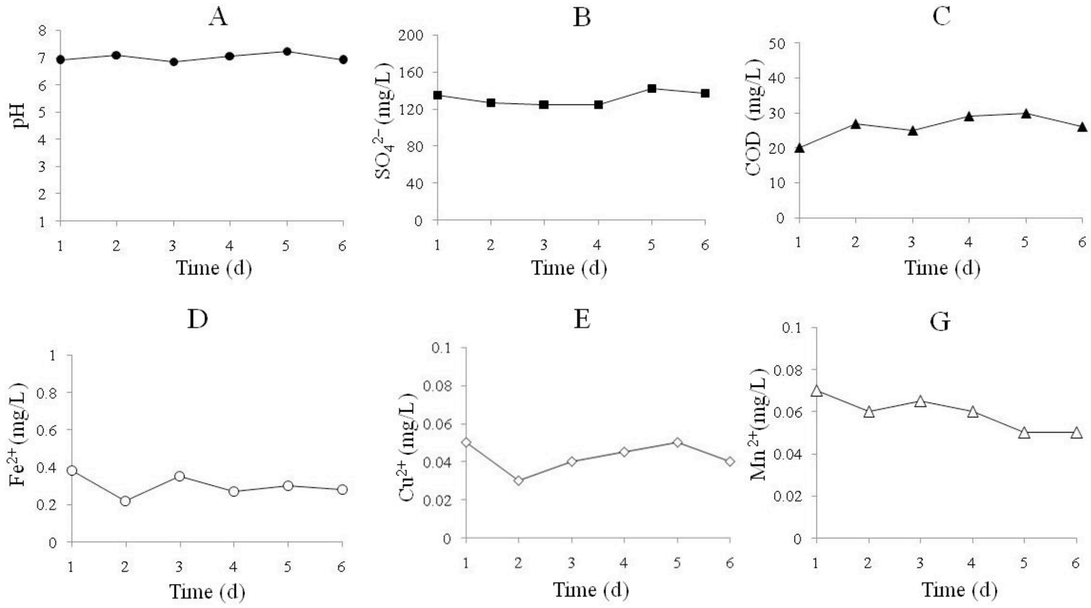


Figure 4. Removal of the most significant contaminants in AMD from Thien Ke tin processing factory by SR bioreactor operated in a continuous mode with time. A: pH; B: SO_4^{2-}; C: COD; D: Fe^{2+}; E: Cu^{2+}; G: Mn^{2+}. Each value was an average adopted from two parallel measurements.

Performance at the continuous mode

After operating under the batch mode, the system was switched to the continuous mode. Here the acidic wastewater was filled into the system at the rate of 100 L/h, leading to the hydraulic retention time of 3 days in the whole pilot system (the settling pond was not included). Effluent samples were collected daily over a week for analyzing chemical parameters, including pH and concentrations of sulphate, COD, iron, copper and manganese (Fig. 4).

The results showed that pH of the effluent was relatively stable around 7 over the monitored period (Fig. 4A). Sulfate was measured at concentrations ~ 120 mg/L (1.25 mM) and was nor reduced further (Fig. 4B), obviously due to the limitation of organic carbons. COD content in the effluent varied between 20 – 30 mg/L (Fig. 4C), much lower than the set limit at 50 mg/L. Concentrations of iron, copper and manganese in the effluent were below 1 mg/L during the whole monitoring period, satisfying the limits set by MONRE at QCVN:40/2011 for industrial wastewater.

Thus, such a pilot scaled treatment system as showed in the present study would provide basic design and performance conditions for development of AMD treatment plants in the near future in Vietnam. Obviously, for advantages of the technology accounted the simple design, easy and safe operation, high treatment efficiency and being applicable at different scales, just depending on the size of AMD sources.

CONCLUSIONS

The present study demonstrated the efficiency of a pilot scale SR bioreactor operated under batch and continuous regimes for the treatment of acidic wastewater from Thien Ke tin processing factory. The wastewater was characterized by low pH (2.8 – 3.2), high content of iron (143.1 mg/L), copper (16.32 mg/L) and manganese (10.9 mg/L). A high sulfate content of 982.7 mg/L was suitable for the SRB activity, however the low COD content of 28.6 mg/L was not favorable. The addition of molasses at 0.5 ml/L to the SR bioreactor increased the COD content to 305 mg/L, and in accordance a COD/sulfate ratio of 0.31 was established. Although being lower than the optimal theoretical COD/sulfate ratio which has been calculated for 0.67, this COD/sulfate ratio still allowed an active sulfate reduction since another part of COD had came as slowly released organic carbons from wood chips matrix.

The batch operation showed good treatment results achieved after three days, i.e. pH increased from 2.8 – 3.2 to 7 – 7.2, sulfate reduction was active and the produced sulfide efficiently removed metals from the wastewater, such as iron from 143.1 mg/L to 0.3 mg/L, copper from 16.32 mg/L to 0.04 mg/L and manganese from 10.9 mg/L to 0.05 mg/L. The continuous mode with a hydraulic load of 100 L/h and a retention time of three days showed constitutive contaminant removal, the effluent monitored within six day period had a pH around 7, and the three most significant metals iron, copper and manganese at concentrations below 1 mg/L.

The results in this study showed a possibility to apply SR bioreactor technology for the treatment of AMD and the use of previously enriched mixed culture of SRB for the activation of bioreactor is feasible.

Acknowledgement: *The study was supported by research project QG.15.33, granted by Vietnam National University Hanoi. The authors would like to thank colleagues at Vinacomin for their supports in the performing research at Thien Ke tin processing factory.*

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XỬ LÝ NƯỚC THẢI ACID TỪ NHÀ MÁY SẢN XUẤT THIẾC THIỆN KẾ BẰNG BỂ SINH HỌC KHỬ SULFATE: NGHIÊN CỨU Ở QUY MÔ PILOT

Nguyễn Thị Hải, Đinh Thúy Hằng

Viện Vi sinh vật và Công nghệ sinh học, Đại học Quốc gia Hà Nội

TÓM TẮT

Mô hình pilot dựa trên công nghệ bể phản ứng khử sulfate sinh học được thiết kế để thử nghiệm xử lý nước thải acid từ nhà máy chế biến thiếc Thiện Kế ở Tuyên Quang, Việt Nam. Trong mô hình, nước thải được đưa vào bể điều hòa chứa đá vôi đập nhỏ chiếm ½ thể tích để nâng pH tới mức phù hợp cho sinh trưởng của vi khuẩn khử sulfate (SRB). Ở bước tiếp theo, nước thải chảy vào bể phản ứng khử sulfate sinh học, tại đó diễn ra quá trình khử sulfate thành sulfide và kết tủa kim loại. Để khởi động bể phản ứng khử sulfate, hỗn hợp SRB gồm các đại diện chính là *Desufovibiro*, *Desulfobulbus* và *Desulfomicrobium* được đưa vào với mật độ ban đầu là ~10^6 TB/ml, nhờ đó hoạt tính khử sulfate đã được thiết lập trong thời gian ngắn chỉ sau một tuần. Rỉ đường được bổ sung vào bể phản ứng ở nồng độ 0.5 ml/L làm cơ chất cho SRB. Vận hành mô hình ở chế độ theo mẻ và chế độ liên tục được nghiên cứu chi tiết. Ở chế độ vận hành theo mẻ, kết quả xử lý cao đạt được sau 3 ngày, theo đó pH tăng từ 2.8 – 3.2 lên 7 – 7.2, khử sulfate diễn ra tích cực và tạo ra lượng sulfide lớn để kết tủa và loại kim loại trong nước thải như sắt từ 143.1 mg/L xuống 0.3 mg/L, đồng từ 16.32 mg/L xuống 0.04 mg/L và mangan từ 10.9 mg/L còn 0.05 mg/L. Vận hành theo chế độ liên tục với tải trọng 100 L/h tương ứng với thời gian lưu của nước thải là ba ngày cũng cho kết quả xử lý ổn định. Nước thải sau khi xử lý có pH xấp xỉ 7 và ba kim loại ô nhiễm chủ yếu là sắt, đồng và mangan được loại tới nồng độ thấp hơn 1 mg/L, dưới mức quy định cho phép. Các kết quả là minh chứng rõ ràng cho tiềm năng ứng dụng công nghệ bể phản ứng sinh học khử sulfate để xử lý AMD và việc sử dụng nguồn SRB đã làm giàu trước đó để khởi động là hiệu quả.

Từ khóa: *Nước thải mỏ acid (AMD), kim loại nặng, vi khuẩn khử sulfate (SRB), bể khử sulfate sinh học*