STUDY ON POLYVINYL ALCOHOL GEL BEADS AS BIOCARRIER APPLIED IN ANAEROBIC SLUDGE IMMOBILIZATION IN THE UASB REACTOR

To Tien Tai, Tran Phuong Thao, Dang Minh Hang, Nguyen Lan Huong *

School of Biotechnology and Food Technology, Hanoi University of Science and Technology, No.1 Dai Co Viet, Hai Ba Trung, Hanoi

*Email: huong.nguyenlan@hust.edu.vn

Received: 15 August 2016; Accepted for publication: 6 October 2016

ABSTRACT

In this study, the biocarrier was made of 8% (w/v) polyvinyl alcohol (PVA) solution dropped into saturated solution of boric acid (6%, w/v), then immersed for 2 hours to form PVA-boric bead. In order to maintain the crosslinked beads, it is subsequently immersed in 1M sodium sulfate solution for 1 hour. PVA gel beads have the diameter of 3.5 - 5.0 mm, a specific gravity of 1.03 - 1.08 g/cm³, high mechanical strength and good elasticity. The surface and internal structure particle were observed under a scanning electron microscope (SEM). The application of PVA beads in microbial immobilization was initially examined in three UASB reactors treating rubber wastewater with sludge and PVA beads ratios of 1/1, 2/1, 3/1 (v/v), respectively. Promising result of bio-immobilization was achieved, at ratio of 1/1, the SMA of PVA beads was 0.133 g COD/gVSS/day, corresponding to 17% of the suspended sludge’s value in the same reactor and the attached biomass was 0.936 g VSS/g dried PVA bead after 90 days.

Keywords: polyvinyl alcohol, PVA-gel beads, UASB, natural rubber processing wastewater, anaerobic granular sludge, immobilization.

1. INTRODUCTION

Rubber industry is one of the key economic sectors in Vietnam’s economy. Apart from the profits, there are environmental problems associated with this industry. The high concentration of organic pollutants discharged from preliminary processes of latex has to be removed prior to release into receiving sources. Currently, UASB reactor as an anaerobic biological process is commonly implemented to remove effectively high organic loads. However, the sludge used in this reactor takes a long time to be activated due to its slow development, and there is difficulty in maintaining the dispersed sludge without sludge agglomeration during the start-up period. Therefore, the granular sludge formation is a key factor for successful operation of a UASB. Until now, granule formation has been investigated and documented by many literatures [1, 2], however, the mechanisms that trigger granulation are yet poorly understood. Based on the theories summarized by Hulshoff Pol et al. [2], the granulation process could be divided into two
steps: inert particles formation and biofilm formation of microorganisms on the inert particles. In order to get the inert particles, a lot of biocarrier particles was made from natural biopolymers (alginate, gelatin, chitosan) or synthetic polymers (polyvinyl alcohol, polyether). Recently, polyvinyl alcohol (PVA) gel which is low cost polymeric gel with a porous microstructure that is well suited for retention of bacteria and having a specific gravity similar to that of biomass granules, has demonstrate effectiveness in wastewater treatment [3, 4, 5]. Using this carrier as a growth nucleus to enhance granulation, the startup of UASB reactors for treatment of corn steep liquor [5] or ethylene glycol [4] was successfully performance. The PVA gel beads in most of recent researches were supplied by Kuraray Company (Tokyo, Japan). However, the Kuraray bead is currently not popular in practical usages due to its high price and difficulty in purchase from outside of Japan. Therefore, in this study, the biocarrier was made by cheap and available material and equipment in laboratory-scale. There was no significant difference found in characteristics between these two kinds of bead. Up to now, the most effective method to produce elastic PVA gel bead of high strength and durability by crosslinking the PVA solution using boric acid solution was reported by Bach and Dinh [6]. However, the technique of bead formation has not been clarified, as well as the immobilization process has not been investigated thoroughly.

Therefore, this study aimed to investigate the PVA bead making process with different concentrations of PVA solution to overcome the agglomeration and the additional solutions to maintain the crosslinked structure afterwards, and then initially evaluate its immobilization performance in laboratory-scale UASB reactor treating natural rubber processing wastewater according to the specific methanogenic activity of PVA beads.

2. MATERIALS AND METHODS

2.1. Materials

Natural rubber processing wastewater (NRPW)

The NRPW was produced in laboratory-scale based on the rubber company’s procedure in Thanh Hoa by coagulating the 60% concentrated latex (Merufa joint-stock company). The components of raw NRPW was pH of 4.8, total chemical oxygen demand (COD) of 14000 mgCOD/L, biological oxygen demand of 10000 mgBOD/L, suspended solid (SS) of 1000 mgSS/L and total nitrogen of 3000 mgN/L. High level of volatile fatty acids (VFAs) of 8000 mgCOD/L occurred in this wastewater. The raw NRPW diluted to the COD concentration of 1800 mgCOD/L and adjusted to pH of 6.8-7.5 was used as influent.

Seed sludge

Seed sludge was collected from an UASB reactor with effective working volume of 20 L which treated natural rubber processing wastewater at Hanoi University of Science and Technology. They had a total suspended solid content of 49.6 gSS/L and dispersed sludge (size under 0.5 mm) accounted for over 90% of dry mass.

Chemicals

Polyvinyl alcohol – PVA powder (C\textsubscript{2}H\textsubscript{4}O)n grade 22-99 (China), which has hydrolysis of 98 % - 100 %, viscosity of 40 - 48 mPa.S, pH 5.0 - 7.0; Calcium Chloride (CaCl\textsubscript{2}); Boric acid (H\textsubscript{3}BO\textsubscript{3}); Sodium Sulfate (Na\textsubscript{2}SO\textsubscript{4}); Sodium Nitrate (NaNO\textsubscript{3}); Monosodium Phosphate (NaH\textsubscript{2}PO\textsubscript{4}) were purchased in Hanoi, Vietnam.
2.2. Experimental setup

**PVA-gel bead**

The bead-formation was carried out by crosslinking process between PVA solution (at concentration of 6 %, 8 %, 10 % and 12 %, w/v) and saturated boric acid (6 %, w/v). After being dissolved at 80 – 95 °C, the PVA solution was cooled to 50 °C and dropped into saturated boric acid solution afterward. Subsequently, the bead was immersed for 2 hour to form PVA beads, then removed and transferred to either 1M Na₂SO₄ solution, 0.5 M NaH₂PO₄ solution or 50 % NaNO₃ solution for 1 hour to maintain the cross-link bond. Finally, all beads were washed in tap water to remove any chemical residue and stored at room temperature for further use.

**System for immobilizing anaerobic sludge**

The three laboratory-scale UASB reactors were constructed by cylinder polyvinyl chloride (PVC) tube with an inner diameter of 65 mm, height of 90 mm and a working volume of 2.5 L, total volume of 3L. A PVC U-tube was fixed to the effluent port to avoid sludge wash-out, as well as provide a liquid lock, and hence anaerobic conditions. The schematic diagram of the system is shown in Figure 1. The system was fed by a peristaltic pump into the bottom of the reactors. The total solid, containing suspended sludge and PVA beads with ratios of 1/1, 2/1 and 3/1 (v/v) in reactor UASB 1, 2 and 3, respectively, had a volumetric packing ratio of 40 % in all reactors. The flow rate was 15 mL/min and the hydraulic retention time (HRT) was kept at 8 - 12 hours in each reactor. Before immobilization, the bead was characterized for the size, the structure by scanning electron microscopy (SEM), the mechanical strength and the specific gravity. After 90-day period, the PVA-beads were collected to measure volatile suspended solid (VSS), specific methanogenic activity (SMA) and SEM observations.

![Figure 1. Schematic diagram of the system for immobilizing anaerobic sludge.](image)

2.3. Analytical methods

The pH value of influent and effluent were measured using a portable pH meter (AS-212, Horiba), TN and COD were determined using a HACH water quality analyzer (DR-2800, HACH). The suspended solid (SS) and volatile suspended solid (VSS) were measured using standard methods APHA [7]. Biogas composition was analyzed using a gas chromatograph equipped with a thermal conductivity detector (GC-8A, Shimadzu).

The amount of dispersed sludge in seed sludge was achieved by wet sieving with 0.5 mm sieves. The TSS of the fraction passing through the sieve mesh over total mass was expressed as dispersed sludge.
The mechanical strength of PVA bead was analyzed by the modified methods of Bach and Dinh [6], in which 20 representative beads were put in 15 ml falcon having 10 ml distilled water, then centrifuged with speed of 3000 to 10000 rpm by Tomy MX-350 centrifuge (Tomy Seiko Co., Ltd.) in 5 min. The mechanical strength of beads was determined by the number of unchanged beads.

The specific gravity of PVA bead was the ratio of its weight in air to its volume immersed in gasoline. An amount of PVA beads added into falcon nearly level of 2 ml is weighed first in air, then adjust gasoline in the falcon up to 2 ml and weight the gasoline in falcon without beads.

The SEM observations of the PVA beads structure: a PVA bead was cut into two pieces and washed twice, for 5 min each time, with 0.1 M cacolydate buffer (pH 7.4). The PVA bead pieces were hardened for 90 min in a 2.5 % glutaraldehyde solution prepared with 0.1 M cacolydate buffer at 4 °C. Next, the samples were washed in the buffer solution three times, for 5 min each time, and then fixed for 90 min in a 1.0 % OsO₄ solution prepared with 0.1 M cacolydate buffer at room temperature. After washing the samples three times for 5 min each in the buffer solution, they were dewatered for 5 min each in serially graded solutions of ethanol at concentrations of 50°, 70°, 80°, 90° and 100°. Then the samples were sputter coated with platinum/palladium (Pt/Pd) and observed by a scanning electron microscope (Hitachi S-4800, Japan).

The SMA of PVA beads was determined in anaerobic serum vials at 35 °C using a modified method according to that proposed by Tsuyoshi Imai [8], which is 25 ml of samples (suspended sludge and PVA bead) cultivated in 25 ml of synthesis wastewater containing 20 ml of sodium acetate, 4.5 ml of nutrients and 0.5 ml of Na₂S. The composition of nutrients was same as that reported by Dolfing and Bloemen [9]. Suspended sludge in each of three UASB (approximately 20 to 50 mg VSS) as positive samples, 10 g unused PVA bead as negative sample and 10 g PVA beads in each of three UASB was added into each vial under the anaerobic condition. Sodium acetate used as substrate solution was added into the vials to obtain a desired concentration (approximately 2000 mg COD/L). These vials were then sealed with butyl rubber caps and aluminum caps, then the headspace of 120 ml-serum vials was made anaerobic by flashing with oxygen-free nitrogen gas and the pH was adjusted to 7.0. All vials were incubated in shaking water bath at 35 °C. Gas samples were withdrawn from the vials periodically to determine methane concentration and production. SMA was calculated based on methane production rate and VSS content in each vial and expressed as g COD/gVSS/d. A factor of 350 ml CH₄/gCOD under standard temperature (273 K) and pressure (1 atm.) condition was used for this calculation.

The relative activity of SMA between PVA beads and suspended sludge in each UASB is calculated by the percentage of the SMA of bead over the SMA of the corresponding sludge.

3. RESULTS AND DISCUSSION

3.1. Biocarrier formation

3.1.1. The bead formation process

Figure 2 shows the morphology of PVA bead at various concentrations. The PVA solution with concentration of 8 % and 10 % resulted in homogeneous sphere beads, meanwhile, 6 % and 12 % PVA solutions resulted in agglomerated beads or long beads with tail. In addition, this study reported that the temperature of PVA solution allowed easy dropping was under 60 °C due
to the increase of viscosity, hence bigger beads. Moreover, the saturated boric acid solution, into which PVA solution was dropping, was stirring vigorously and continuously by a magnetic stirrer to accelerate the crosslinking all over the surface of these beads, preventing adhesion (data not shown).

![Figure 2. Shape of beads at various PVA concentrations of 6 %, 8 %, 10 % and 12 %.

After being immersed for 2 hours in saturated boric acid, the beads still could re-dissolve in water (Fig. 3d). Thus, the beads subsequently were introduced to NaH$_2$PO$_4$, Na$_2$SO$_4$ and NaNO$_3$ solution.

![Figure 3. PVA bead immersed in (a) 1 M Na$_2$SO$_4$, (b) 0.5 M NaH$_2$PO$_4$, (c) 50 % NaNO$_3$ solutions and (d) water.

As can be seen in Fig. 3, the beads immersed in NaNO$_3$ solution deformed, re-agglomerated into a mass, while those immersed in Na$_2$SO$_4$ solution and NaH$_2$PO$_4$ solution could maintain the shape and keep the bead separated. Due to its softer surface, the bead soaked in Na$_2$SO$_4$ solution was chosen to use in further experiments. The study of Bach and Dinh [6] reported that PVA bead immersed in 0.5 M Na$_2$SO$_4$ solution was stronger than both those immersed in 0.5 M NaH$_2$PO$_4$ and 50 % NaNO$_3$ in relative mechanical strength test by centrifuge and in swelling test [6].

3.1.2 The characteristics of PVA-gel beads

The morphology of PVA bead and the surface and the internal microstructure from a cutting cross-section is shown in Fig. 4.

![Figure 4. The morphology of PVA beads before immobilization (a), SEM observation of surface of a PVA bead (2000 magnification) (b) and inner part of PVA bead (10000 magnification (c).
The beads in Fig. 4a appeared white in color and had a roughly spherical form with an approximate diameter of 3.5 - 5.0 mm. The biocarrier beads also appeared insoluble and mechanically stable in water, showed no misshaped beads after centrifuging with a high speed of 10000 rpm (data not shown). The beads had many craters on an uneven surface, which can be viewed at a magnification of 2000 times (Fig. 4b), suggesting that beneficial for biofilm formation of microorganisms. SEM images in Fig. 4c indicate that the inner structure was porous, with holes' diameter ranging from 0.5 - 2 µm, appropriate for trapping and retaining bacteria. The PVA bead in this study has a similar sphere shape and size with Kuraray bead having a diameter of 3.0 - 4.0 mm, but the specific gravity is quite higher (1.03 - 1.08 g/cm³) than the Kuraray bead with specific gravity of 1.025 ± 0.01 g/cm³ [10].

### 3.2 Initial immobilization performance

**The activity of PVA-gel**

After 90 days of immobilization, the color of almost beads changed from white to yellow and some turned black (data not shown), probably indicating biomass immobilization. The activity of immobilized microorganisms on PVA beads was confirmed by comparing the SMA of immobilized bead and the suspended sludge in the same reactors and determining the amount of biomass attached to PVA-beads in Table 1.

Table 1. The SMA of beads, the SMA of suspended sludge and the biomass attached onto PVA-beads in each reactor after 90 days.

| Reactors | Sludge/PVA beads (v/v) | SMA of PVA beads (gCOD/gVSS/day) | SMA of sludge (gCOD/gVSS/day) | Relative activity (%) | gVSS/g dried PVA beads |
|----------|------------------------|----------------------------------|-------------------------------|-----------------------|------------------------|
| UASB 1   | 1/1                    | 0.133                            | 0.779                         | 17.07                 | 0.936                  |
| UASB 2   | 2/1                    | 0.106                            | 0.861                         | 12.29                 | 0.698                  |
| UASB 3   | 3/1                    | 0.128                            | 0.933                         | 13.76                 | 1.407                  |

The SMA of beads in UASB reactor 1, 2 and 3 were 0.133, 0.106, and 0.128 g COD/gVSS/day, respectively and the amount of biomass attached on PVA beads were 0.936, 0.698 and 1.407 g VSS/g dried PVA beads. The SMA of suspended sludge in each reactor was from 0.779 to 0.933 g COD/gVSS/day, therefore the relative activity of of SMA between PVA beads and sludge in each UASB reached from 12 to 17 %. After 90 days, the SMA of PVA beads in UASB 1 had the highest activity, but the biomass attached to PVA-gel did not show significantly higher than that of others. It is suggested that the immobilization period should be prolonged, so that the SMA of immobilized bead would reach the SMA of sludge. Wenjie et al., [5] reported that PVA beads cultivated in UASB reactor treating synthetic corn steep liquor on day 115, which had the average attached biomass of 1.02 g VSS/g PVA beads [5].

**The SEM observation of immobilized PVA-gel**

The SEM observation proved the biomass retained onto the PVA gel bead. After 90 days, there are colonizations of microorganisms on the surface (Fig. 5a and 5b) and interior (Fig. 5c) of the PVA beads. The NRPW includes high concentration of volatile fatty acids, thus the methane-producing bacteria is abundant in anaerobic reactors [11]. Moreover, the uneven
surface and porous structure of PVA bead as can be seen in Fig. 5 seems to be appropriate to trap cocci, which maybe *Methanosarcina* (Fig. 5b) as mentioned in the study of Supawat Chaikasem [10]. Furthermore, fluorescence in situ hybridization (FISH) should be used to identify the immobilized microorganisms in the PVA beads. However, the observation of microorganisms in the interior of the gel bead suggest that colonization progresses well toward the core of the carrier.

![Figure 5. SEM observation of immobilized PVA bead after 90 days of immobilization: (a) microorganism on the surface of PVA bead (5000 magnification), (b) microorganism on the surface (12000 magnifications), (c) microorganism in the inner part of PVA bead (5000 magnification).](image)

4. CONCLUSIONS

The present study showed that 8% PVA solution as the main component and 1M Na₂SO₄ solution for complete crosslinking was applied to produce effectively PVA-gel bead as biocarrier. The initial application of these beads in UASB treating NR PW reported that the immobilization of anaerobic sludge using PVA beads is going well to enhance granule formation. Further research is needed to optimize the conditions for rapid cultivated PVA beads in UASB reactor, as well as to evaluate the effect of biocarrier for performance of reactor treating natural rubber processing wastewater.

Acknowledgements. This research is supported by grants of Ministry of Education and Training of Vietnam (B2016-BKA-16) project.

REFERENCES

1. Chen J. and Lun S. Y. - Study on mechanism of anaerobic sludge granulation in UASB reactors, Water Science and Technology 28 (1993) 171–178.
2. Hulshoff Pol L. W., De Castro Lopes S. I., Lettinga G., and Lens P. N. - Anaerobic sludge granulation, Water Research 38 (2004) 1376-1389.
3. Khanh D., Quan L., Zhang W., Hira D. and Furukawa K. - Effect of temperature on low-strength wastewater treatment by UASB reactor using poly (vinyl alcohol)-gel carrier, Bioresource Technology 102 (2011) 11147–11154.
4. Wenjie Z. - Applications of PVA-gel beads as biomass carrier for anaerobic wastewater treatment, PhD. thesis, Kumamoto University, Japan (2008).
5. Wenjie Z., Qinglin X., Rouse J. D., Qiao S. and Furukawa K. - Treatment of highstrength corn steep liquor using cultivated polyvinyl alcohol gel beads in an anaerobic fluidized-bed reactor, Journal of Bioscience and Bioengineering 107 (2009) 49–53.
6. Leu Tho Bach and Pham Van Dinh - Immobilized bacteria by using PVA (Polyvinyl alcohol) crosslinked with Sodium sulfate, International Journal of Science and Engineering 7 (1) (2014) 41-47.

7. APHA - Standard Methods for the Examination of Water and Wastewater, 21st edition, American Water Works Association, Washington, DC. USA (2005).

8. Tsuyoshi I., Masao U., Jun L., Masahiko S., Hiroshi N., Masayuki F. - Advanced start up of UASB reactors by adding of water absorbing polymer, Water Science and Technology 36 (1997) 399-406.

9. Jan D. and Wim G. B. M. B. - Activity measurements as a tool to characterize the microbial composition of methanogenic environments, Journal of Microbiological Methods 4 (1985) 1-12.

10. Supawat Chaikasem - Effect of PVA-gel on performance improvement of a two stage thermophilic anaerobic membrane bioreactor, PhD thesis, Asian Institute of Technology, Thailand (2015).

11. Thanh N. T., Takahiro W., Thao T. P., Hatamoto M., Tanikawa D., Syutsubo K., Fukuda M., Tan N. M., Anh T. K., Yamaguchi T., Huong N. L. - Impact of aluminum chloride on process performance and microbial community structure of granular sludge in UASB reactor for natural rubber processing wastewater treatment, Water Science and Technology 74 (2) (2016) 500-507.

TÓM TÁT

NGHIỆN CỬ TẠO GIẢ THẾ ĐANGER HẠT BÀNG POLYVINYL ALCOHOL NHarehouse ỨNG DỤNG CỔ ĐỊNH BỤN YẾM KHỊ TRONG HỆ THÔNG UASB

Tố Tiên Tài, Trần Phượng Thảo, Đặng Minh Hằng, Nguyễn Lan Hương

Viện CN sinh học và CN thực phẩm, Trường ĐH Bách khoa Hà Nội, Số 1 Đại Cồ Việt, Hà Nội

*Email: huong.nguyenlan@hust.edu.vn

Nghiên cứu này tạo giả thể bằng polyvinyl alcohol (PVA) ở dạng keo nồng độ 8 % (w/v) trong nước, làm biến tính bằng cách nhỏ giọt vào dung dịch axit boric hòa hoá (6 %, w/v) và ngâm trong 2 giờ. Để tăng độ bền, hạt được ngâm trong dung dịch natri sulfat 1M trong 1 giờ. Giả thể PVA dạng hạt có đường kính 3,5 - 5 mm, ti trọng 1,03 - 1,08, hạt có độ bền cơ học cao và tính dần hoá tốt. Câu trúc bề mặt hạt và lỗ xốp bên trong đã được quan sát dưới kính hiển vi điện tử quét (SEM). Bước đầu tiên hành cơ định vi sinh vật trong bùn hoạt tính yếm khí lên giả thể PVA trong hệ thống UASB xử lý nước thải cao su với các tỷ lệ bùn/hạt PVA lần lượt là 1/1, 2/1, 3/1 (v/v). Kết quả thử được ở tỷ lệ bùn/hạt là 1/1 thì hoạt tính sinh metan (SMA) của hạt là 0,133 g COD/gVSS/ngày, đạt 17 % so với hoạt tính SMA của bùn phân tán trong cùng hệ thống và lượng sinh khối bám dinh trên giả thể là 0,936 g VSS/g hạt PVA khó sau 90 ngày.

Từ khóa: polyvinyl alcohol, giả thể dạng hạt PVA, UASB, nước thải cao su, bùn hoạt tính yếm khí, cơ định vi sinh vật.