Microbiol. Biotechnol. Lett. (2019), 47(1), 116–124
http://dx.doi.org/10.4014/mbl.1801.01005
pISSN 1598-642X eISSN 2234-7305

MBL
Microbiology and Biotechnology Letters

Reduction of Hydrogen Sulphide in Chicken Manure by Immobilized Sulphur Oxidising Bacteria Isolated from Hot Spring

M. Y. Hidayat[1], H. M. Saud[2], and A. A. Samsudin[1]*

[1]*Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*
[2]*Department of Agriculture Technology, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*

Received: January 10, 2018 / Revised: October 2, 2018 / Accepted: October 17, 2018

The rapid development of the poultry industry has led to the production of large amounts of manure, which produce substances like hydrogen sulfide (H_2S) that contribute to odor pollution. H_2S is a highly undesirable gas component and its removal from the environment is therefore necessary. Sulfur-oxidizing bacteria (SOB) are widely known to remove contaminating H_2S due to their ability to oxidize reduced sulfur compounds. In this study, three potential SOB (designated AH18, AH25, and AH28) that were previously isolated from a hot spring in Malaysia were identified by 16S rRNA gene analysis. Laboratory-scale biological deodorization experiments were conducted to test the performance of the three isolates—in the form of pure or mixed cultures, with the cells immobilized onto alginate as a carrier—in reducing the H_2S from chicken manure. On the basis of 16S rRNA phylogenetic analysis, isolate AH18 was identified as *Pseudomonas* sp., whereas isolates AH25 and AH28 were identified as *Achromobacter* sp. The most active deodorizing isolate was AH18, with an H_2S reduction rate of 74.7% ($p < 0.05$). Meanwhile, the reduction rates for isolates AH25 and AH28 were 54.2% and 60.8% ($p > 0.05$), respectively. However, the H_2S removal performance was enhanced in the mixed culture, with a reduction rate of 81.9% ($p < 0.05$). In conclusion, the three potential SOB isolates were capable of reducing the H_2S from chicken manure in the form of a pure culture immobilized on alginate, and the reduction performance was enhanced in the mixed culture.

Keywords: **Sulphur oxidising bacteria, *Pseudomonas* sp., hydrogen sulphide reduction, odour removal, poultry manure**

Introduction

One of Malaysia's National Agro-food Policy, 2011–2020 [1] goals is to ensure an adequate domestic supply of egg and poultry. The poultry sector has become an integral part of the livestock industry in Malaysia, however, the rapid development of the poultry industry could become a great threat to the environment. Tonnes of poultry manure produces daily have the potential hazards to the environment and could have a detrimental effect on health and safety of living creatures. Hydrogen sulphide (H_2S) is one of the main malodorous compounds emits from a poultry farm which generates an obnoxious odour smell like a rotten egg even at lower concentration [2]. Apart from that, H_2S also may cause serious adverse effects on human and animals as well as the environment due to its toxic characteristic [3]. Therefore, the removal of H_2S is necessary.

Several methods have been employed to overcome the problem such as through physicochemical methods by using odorant oxidising agents like ozone or permanganate [4]. However, these methods required high investment, high capital cost and often led to secondary pollution. Thus, the demand for a more economical and

***Corresponding author**
Tel: +0389474878, Fax: +0389432954
E-mail: anjas@upm.edu.my

environmentally friendly method has led to an investigation into microbiological alternatives. In the microbiological method, H_2S is oxidised into a stable compound such as sulphate as the major oxidation product [5] by sulphur oxidising bacteria (SOB). Most of the studied SOB group is belong to *Thiobacillus*. This autotrophic microorganism is efficient in the utilised oxidation of sulphide, sulphur, and thiosulphate for energy and growth [6]. Moreover, a number of heterotrophic microorganisms have the capability to generate energy from the oxidation of reduced sulphur compounds such as *Limnobater thiooxidans* [7] and *Bosea thiooxidans* [8]. Besides that, *Pseudomonas* sp. [9, 10] have also been recognised able to oxidise H_2S into a stable compound.

Recently, the microbiological alternative employing microorganisms to solving the environmental pollution problem has rapidly grown due to its effectiveness. However, the key point to ensure the effectiveness of the process is to maintain the high biomass of bacterial population [11]. Free microbial cells may expose to various tremendous stress including survival, proliferation, nutrition utilisation and competition with indigenous microorganisms from the surrounding [12]. Thus, in order to improve the survival of the microorganisms, the cells must be immobilised.

Immobilisation of bacterial cells can be defined as a restriction of movement in a space or making it incapable of movement while preserving their viability and retained their catalytic functions [13]. There is various method and carriers have been discussed for cell immobilisation including adsorption, covalent bonding, and entrapment [14, 15]. Among these methods, the entrapping immobilisation has been widely studied. The method is based on capturing the microbial cells within a matrix in which limit the cells to move only within a support matrix and thus, prevents the cells from release into surrounding medium while allowing the penetration of nutrients and metabolites. This method is very simple, rapid and cost-effective, however, there are some drawbacks including the cells leakage from the support and diffusional problems between substrates and products depending on the type of porous carrier used [16]. Generally, there are various types of the carrier have been used in cells immobilisation such as alginate, agar, cellulose, carrageenan, gelatin, polyacrylamide, polyester, polystyrene, and polyurethane [17]. However, a good carrier must meet certain criteria to ensure the effectiveness of application such as non-toxic, high porosity, long shelf life, and cheaper [18]. In this current study, alginate was chosen as a carrier for cell immobilisation due to its chemical stability, rapid method, simple and low-cost immobilisation method [19]. Therefore, this study was aimed to evaluate the H_2S reduction performance in chicken manure by using the potential SOB isolated from hot spring immobilised on alginate.

Materials and Methods

Chicken manure sample preparation

Fresh solid non-litter chicken manure samples were collected from Poultry Unit, Ladang 2, Universiti Putra Malaysia, Malaysia (3° 2' 0" North, 101° 43' 0" East). The chicken manure was randomly picked and placed into the close plastic sample bag and then transported to the laboratory. Afterwards, the manure was homogenised and mixed well before the commencement of the experiment.

Cultivation of microorganisms

The thiosulphate mineral medium (TSM) composition is as followed (g/*l*): 1.5 g K_2HPO_4, 1.5 g KH_2PO_4, 0.4 g NH_4Cl, 0.8 g $MgCl_2 \cdot 6H_2O$, 0.1 g $CaCl_2 \cdot 2H_2O$, and 10 g $Na_2S_2O_3 \cdot 5H_2O$. The pH of the medium was adjusted with 1 M NaOH or HCl to 8.0. The potential SOB isolates of AH18, AH25 and AH28 which was isolated and screened in our previous study [20] were cultivated in TSM medium and incubated at 30℃ with 160 rpm of agitation speed until the medium turned turbid. The growth was followed by measuring the turbidity at 660 nm using a spectrophotometer and when the initial OD reaches 0.5–0.8, the cultures were ready to use as inoculums by transferring into another broth medium.

Molecular identification of the potential SOB isolates

The three potential SOB isolates were identified based on the 16S rRNA gene analysis. The bacteria were grown in 15 ml TSM broth at 30℃ for 24 h. The genomic DNA of each culture was extracted using PureLink® Genomic DNA Kits Invitrogen™ (Thermo Fisher Scientific, USA). The DNA of SOB isolates was obtained for amplification and sequencing of the 16S rRNA gene. The PCR mixture contained 17.9 µl dH_2O, 2.5 µl 10x PCR

buffer, 1.0 µl $MgCl_2$, 0.5 µl dNTPs, 1.0 µl of each universal primers, 0.1 µl PlatinumTaq polymerase and 1.0 µl DNA template, resulting in 25 µl of reaction. The 16S rRNA gene from the genomic DNA was amplified directly with primer pair 27F/1492R (27F: 5' AGA GTT TGA TCM TGG CTC AG 3'; 1492R: 5' TAC GGY TAC CTT GTT ACG ACT T 3') [21]. The amplification was carried out as follows: one cycle at 95℃ for 3 min, followed by 35 cycles at 95℃ for 30 sec, 55℃ for 25 sec, 72℃ for 2 min and a final extension step at 72℃ for 10 min. The reaction product was analyzed using agarose gel electrophoresis and the PCR product was sent to a commercial sequencing company (First BASE Laboratories Pte Ltd., Malaysia) for purification and sequencing. The forward and reverse sequences obtained were combined by using BioEdit Sequence Alignment Editor 7.1.9 to get the full-length sequence. Afterwards, the full sequences in FASTA form were compared by using Basic Local Alignment Search Tool (BLASTn) in the National Centre for Biotechnology Information (NCBI) website (http://www.ncbi.nih.gov) to search for a similar sequence in the GenBank. Sequences and their closet relatives were retrieved from the database and were aligned with ClustalW and a phylogenetic tree was constructed with Neighbour-Joining method [22] by using Mega 7 software. Evolutionary distances of nucleotide sequences were computed using the Jukes-Cantor model [23] (bootstrap values: 1000 re-sampling).

Cell harvesting for immobilisation

The potential SOB isolates were grown separately in 500 ml TSM liquid media (pH 8.0). However, for this study 0.1% (w/v) of yeast extract was amended in the medium to increase the amount of cell biomass. The bacterial culture was incubated aerobically at 30℃ with 160 rpm agitation until the initial OD reaches 0.8 (stationary phase). The bacterial cultures of SOB were then harvested by centrifugation at 7500 g for 10 min at 4℃ and then the harvested cells were washed twice with 25 mM phosphate buffer at pH 7.4.

Number of bacteria

The colony forming units (CFU) of each of the SOB isolates was performed before the cells harvesting procedure in order to estimate the density of cells in alginate beads for the immobilisation process. About 1 ml of broth was discarded from the culture flask (stationary phase) and was serially diluted (10^{-1} to 10^{-9}). Afterwards, 0.1 ml of each serial dilution media was transferred and was aseptically inoculated (spread plate method) onto sterilised TSM agar which was prepared with the addition of 0.1% (w/v) yeast extract. The agar was then incubated aerobically at 30℃ until the colony viable observed and the number of viable colonies was counted.

Cellular immobilisation in alginate beads

The cell immobilisation process was following the protocol describes by Yañez-Ocampo *et al.* [24]. The harvested cell pellet of each of the potential SOB isolates (10^8 cells per ml) were immersed in 100 ml of sterilised 4% Na-alginate solution and then mixed thoroughly with a vortex mixer to form a homogenous paste of cells. Afterwards, the Na-alginate containing cells were dropped into a 0.1 M $CaCl_2$ solution by using 20 ml needleless syringe and 2–3 mm diameter of immobilised beads were formed immediately (Fig. 1). The beads were incubated in the $CaCl_2$ for 1 h at room temperature (28 ± 2℃). Furthermore, a mixture of SOB isolates was mixed together by mixing equal proportions of the harvested cell (10^8 cells per ml of each SOB isolates) to form a group of bacteria. Additionally, the same procedure was followed for the control (beads without bacteria).

Biological deodorisation experiments

The laboratory scale experiments were conducted in a 500 ml Erlenmeyer flask as presented in Fig. 2. Each flask was closed with a rubber stopper and a small hole at the top was equipped with a tube to facilitate gas measurement. Two replicates of each treatment were prepared and approximately 100 g of chicken manure

Fig. 1. Photograph of alginate beads used as a carrier for cell immobilisation of the potential SOB isolates.

http://dx.doi.org/10.4014/mbl.1801.01005

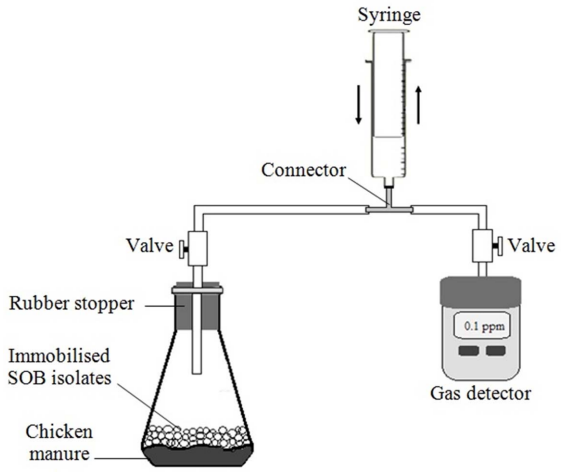

Fig. 2. Schematic diagram of experimental equipment for the evaluation of potential SOB isolates in biological deodorisation of H_2S in chicken manure.

was placed in each flask. About 50 g of alginate beads containing the microorganisms were applied on the surface of the manure by pouring and then the flask was tightly closed. The valve of the adjacent tube was open and headspace air was sampled by pulling the syringe up and the valve was closed and allowed the gas flow to the gas detector by pushing the syringe down. The sampling was done at 0 h and was again recorded every 24 h for 7 days consecutively. The concentration of H_2S gas was determined by Tango X1 (Industrial Scientific, USA) portable gas detector which can detect H_2S gas within the range of 0–200 ppm with 0.1 ppm increments. The H_2S concentration for each treatment was recorded and plotted against the incubation time and the reduction rate of H_2S was calculated. In order to evaluate the ability of the potential SOB isolates and the SOB mixed cultures carried on alginate, the following treatments were prepared (Table 1):

Table 1. Treatments for H_2S biological deodorisation by the immobilised potential SOB isolates and SOB mixed culture.

Treatment	Alginate bead
Control	CM + Empty alginate
A1-AH18	CM + Alginate + AH18
A2-AH25	CM + Alginate + AH25
A3-AH28	CM + Alginate + AH28
A4-Mixed	CM + Alginate + SOB mixed cultures

CM = chicken manure.

Calculation of H_2S reduction

The decrease of the H_2S concentration was calculated as the reduction R (%) according to the following formula:

$$R = 100\% - (C_7 \times 100\% / C_0)$$

Where C_7 is the concentration of H_2S in the sample collected from headspace after 7 days of deodorisation with treatment, C_0 is the concentration of H_2S in the sample collected from headspace at the beginning of deodorisation process. All mathematical calculations were made using Microsoft Excel 2007 and the graph was generated using GraphPad Prism 7.04 software.

Statistical analysis

All data were analysed using one-way ANOVA of Statistical Analysis System Package (SAS) Version 9.4 software. Duncan's Multiple Range Test was performed to determine differences between the mean of treatments. P value was set at 0.05.

Results

Identification of potential SOB isolates using 16S rRNA gene analysis

In this study, three potential SOB isolates have been selected from our previous study due to their efficiency in the oxidation of reduced sulphur compound and their ability to survive in various environmental factors including metabolic flexibility, pH and temperature [20]. The genomic DNA of potential SOB isolates was successfully extracted and was then subjected to PCR amplification using the universal primers 27F and 1492R. The gel electrophoresis of 16S rRNA gene obtained in this study give an intact band of 1500 bp DNA fragment, indicating that the 16S rRNA was successfully amplified. BLASTn search of these 16S rRNA gene sequences indicated that the three potential SOB isolates belonged to phylogenetically different groups as shown in generated phylogenetic tree (Fig. 3), whereas, SOB isolate AH18 is closely related to a member of the genus *Pseudomonas* (Fig. 3A) with the highest 95% similarity to *Pseudomonas stutzeri* strain Gr50. Meanwhile, for SOB isolates AH25 and AH28 were both closely related to the genus *Achromobacter* (Fig. 3B) with the highest 92% and 93% similarity to *Achromobacter marplatensis* strain RY27, respectively.

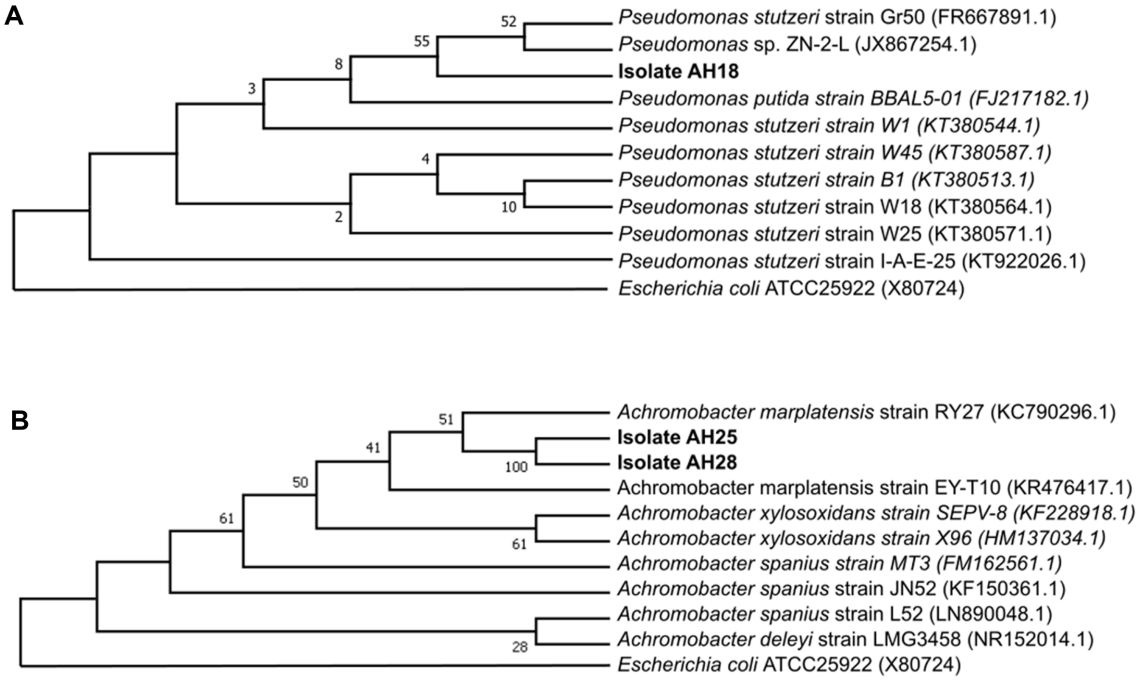

Fig. 3. Phylogenetic tree derived from 16S rRNA sequence data of potential SOB isolates and other closely related species. The evolutionary history was inferred using Neighbour-Joining method. (A) Isolate AH18. The analysis involved 10 nucleotide sequences. (B) Isolate AH25 and AH28. The analysis involved 10 nucleotide sequences. Bootstrap values (expressed as percentages of 1000 replications) are shown next to the branches. All positions containing gaps and missing data were eliminated. The tree was rooted with *E. coli* ATCC25922.

Reduction of H_2S by the potential SOB isolates and the mixed cultures carried on alginate

Based on the results obtained (Fig. 4A), the H_2S emission from the chicken manure in the control treatment was observed initially increased at day 0 and then fluctuated around that level. Meanwhile, the other four treatments in this study showed a reduction of H_2S concentration throughout the 7 days of the biological deodorisation process. Fig. 4B shows the percentage of H_2S reduction rate for all the treatments. Based on the results, the control treatment showed a 16.7% reduction of H_2S concentration after 7 days. Moreover, the use of

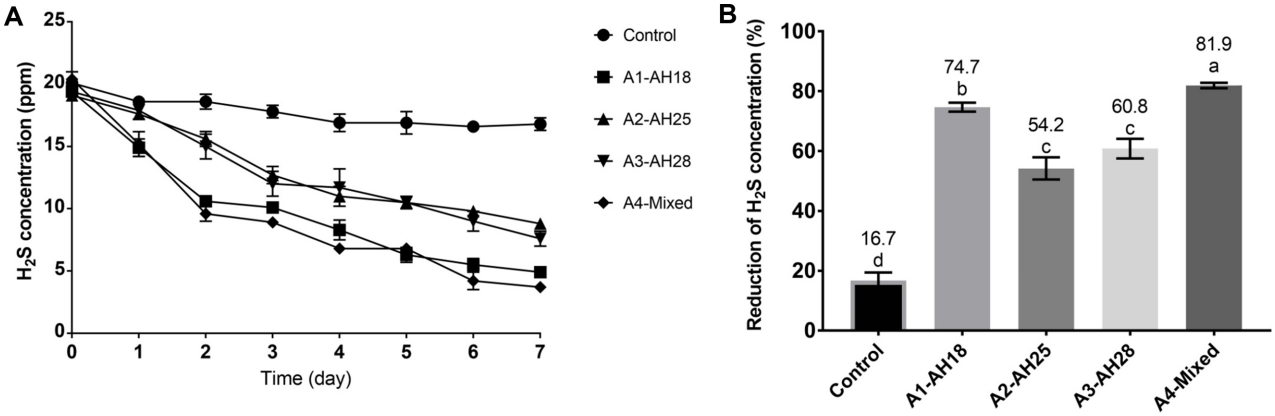

Fig. 4. (A) H2S concentration from chicken manure during biological deodorisation process, (B) H2S concentration reduction from chicken manure after 7 days of biological deodorisation. Each point represents the mean of duplicate ± SE. [a,b,c,d]different letters indicate the values are significantly different between the treatment groups.

SOB pure culture and mixed culture carried on alginate was able to reduce H_2S concentration in the range of 54.2–81.9%. According to the results obtained (Fig. 4B), A4-Mixed treatment showed a higher reduction rate ($p < 0.05$) when compared to the other treatment groups (control, A1-AH18, A2-AH25, and A3-AH28) which effectively reduced 81.9% of H_2S and then followed by A1-AH18 treatment with 74.7%. Meanwhile, there is no significant difference ($p > 0.05$) between treatment A2-AH25 and A3-AH28 which have recorded a 54.2% and 60.8% of reduction rate, respectively. In term of SOB pure culture performances, isolate AH18 ($p < 0.05$) was observed to be the most effective in reducing the H_2S compared to isolate AH25 and AH28 ($p > 0.05$). However, the reduction performance was observed enhanced when the SOB pure culture was mixed together as a consortium ($p < 0.05$).

Discussion

Microbiological approaches employing sulphur oxidising bacteria (SOB) for the biological deodorisation of H_2S have gained much attention in this recent years as the promising alternatives to conventional physicochemical methods. Three potential SOB isolates which were coded as AH18, AH25 and AH28 have been isolated from hot spring water in Malaysia [20] which have remarkable potentials for the application of biological deodorisation of H_2S. The three SOB isolates were immobilised in alginate bead to investigate the reduction performances of H_2S in chicken manure. In this present study, the released of H_2S from the chicken manure was observed immediately occurred right after the flask was closed which can be confirmed by the increases of the initial H_2S concentration recorded at day 0 (Fig. 4A). Generally, chicken manure consists of a mixture of urine and faeces which contains undigested nutrient including nitrogen, phosphorus, potassium and protein [25]. The undigested protein and sulphur containing amino acids such as methionine and cysteine were then decomposed and metabolised by sulphur reducing bacteria (SRB) anaerobically and aerobically condition in chicken manure which resulted in the released of H_2S gas [26].

The application of potential SOB isolates carried on alginate was observed active in reducing the H_2S in the flasks within 24 h after the application of treatments without a lag time. This may attribute to the fact that, the SOB isolates has been harvested at the stationary phase for cell immobilisation. During this phase, cells switch to a survival mode of metabolism and undergone a series of physiological changes including enabling the cells to rapidly adapt to stress and new condition [27]. Furthermore, it can be explained that when the H_2S gas penetrating the bead, it was immediately oxidised by the starving SOB cells. This phenomenon can be seen in Fig. 4A, where the H_2S gas is drastically decreased from day 0 to 1 compared to the control treatment. This observation was similar in Honma and Akino [28] works which reported the removal of H_2S by the *Pseudomonas* sp. is rapidly occurred without a lag time. However, in this study, a reduction of H_2S concentration was also observed occurs in the control treatment without the presence of SOB isolates and this could be explained by the spontaneous oxidation of H_2S gas and the inhibition of SRB which in turn prevents the production of H_2S. Furthermore, a similar observation was reported from several studies which had found the reductions of H_2S were affected with time in livestock manure [29, 30].

It was ascertained from this present study that potential SOB isolate AH18 has significantly effective in reducing the H_2S compared to the other pure SOB isolates. From the phylogenetic analysis study, isolate AH18 was identified closely related to the member of the genus *Pseudomonas*. The *Pseudomonas* sp. is widely distributed in the environment which occupying various ecological niches. Additionally, it has been studied for its ability to metabolise H_2S effectively [9, 28, 31]. Chung *et al.* [10] reported that *P. putida* CH11 which was isolated from livestock farming wastewater was effectively removed H_2S gas up to 96% in the biofilter treatment of H_2S containing gas. Beside H_2S, the genus *Pseudomonas* is widely known in their ability to degrade other environmental contaminants including polyaromatic hydrocarbons (PAHs) [32], removal of arsenite [33] and phenol [34] from the environment. Thus, this is evident that *Pseudomonas* sp. is a well-studied microorganism in the biodegradation and bioremediation of environment pollutant. Additionally, the potential SOB isolate AH18 in this study was reported to have highest 95% similarity with *Pseudomonas stutzeri* strain Gr50. It has been reported that *P. stutzeri* species are involved in environmentally important metabolic activities and is

one of the known species which able to perform autotrophic oxidation of sulphur compounds coupled to the reduction of nitrogen compounds [35]. Mahmood *et al.* [35] isolated *P. stutzeri* QZ1 from anoxic sulphide-oxidising bioreactor which reported to actively involved in the oxidation of sulphide in wastewater. Moreover, Singh *et al.* [34] reported in their study that, *P. stutzeri* have the ability to degrade toxic materials including phenol and cyanide.

Meanwhile, the potential SOB isolate of AH25 and AH28, both were identified closely related to the genus *Achromobacter*. Previous studied by Graff and Stubner [36] had reported *Achromobacter* sp. was able to oxidise elemental sulphur and thiosulphate and produced sulphate as an end product of the oxidation process. However, to date, no study has reported the capability of *Achromobacter* sp. in the oxidation of H_2S and in this present study, it was the first time *Achromobacter* sp. involved in the biological deodorisation of H_2S in chicken manure. However, several studies had discovered *Achromobacter* sp. was found to have the capability of degrading other environmental contaminants including the degradation of aromatic hydrocarbons [37] and the biodegradation of catechol [38].

During the biological deodorisation experiment, a synergistic effect was observed when the three isolates were mixed as bacterial consortia (A4-Mixed) in which, the reduction rate of H_2S was significantly higher ($p < 0.05$) compared to the pure culture treatments (A1-AH18, A2-AH25, A3-AH28). A possible explanation employing mixed culture more efficient than a pure culture is it could be due to the multiple metabolic capacities among the SOB in removing the toxic compounds. Similar results have been obtained in Gutarowska *et al.* [39] and Matusiak *et al.* [40] studied on the removal of odorant compounds from poultry manure by using a mixed culture of bacteria immobilised on perlite-bentonite which able to reduce 17.5% and 78% of H_2S, respectively. Moreover, it is may also due to the cell density in the mixed culture which was prepared by adding an equal amount of each harvested pure culture cells (10^8 cells per ml) and then mixing the three SOB cells together for immobilisation process which attributed to higher cell density trapped in alginate bead compared to the pure culture. However, the reduction rates of H_2S by SOB mixed cultures apparently did not increase 3 fold higher as the upsurge of the cells. It can be postulated that besides the synergetic effects between the SOB, these bacteria also competing for the same energy source (H_2S). This could have resulted in the growth restriction of other SOB cells which in turn led to the slight reduction of H_2S oxidation activity. In this regards, it is interesting to note that, mixed culture has remarkable potentials to be used in the biological deodorisation process due to the different bacteria have different mechanisms and metabolic activity. Liu *et al.* [41] have demonstrated a removal treatment of phenol employing the pure culture of *Acinetobacter* sp. XA05 and *Sphingomonas* sp. FG03 and also the mixed cultures of both strains. The results showed the efficiency of phenol degradation by mixed cultures is better than pure culture and they suggested that the different enzyme and metabolic activity among the bacteria have influenced the removal efficiency. Therefore, from this study, it can be elucidated that the reduction rate of H_2S is influenced by the cell density of SOB trapped in the carrier and suggested that the performance of pure culture can be enhanced by increasing the number of cells. Furthermore, mixed culture treatment was preferred due to its diversity in the oxidation mechanisms and physicochemical properties [20].

The biological deodorisation of H_2S in chicken manure was assessed by using alginate as the carrier. Alginate is one of the most frequently used carriers for cell immobilisation owing to its several advantages including cost-effective, simple one-step method, and eco-friendly which is non-toxic to human and environment [19]. The use of alginate bead could entrap high bacterial cells inside the bead in which contributed to higher cell density which played the main role in the degradation of H_2S since the high-density cell could trap high H_2S as their electron donors and as the consequence resulted in the higher H_2S removal. Furthermore, the effective use of alginate as the carrier is possibly due to its material which could act as a shelter to protect the bacterial cells against loss of cell viability and toxicity from the environment [42]. Liu *et al.* [42] reported that *Leucobacter* sp. JW-1 immobilised on polyvinyl alcohol-sodium alginate (PVA-SA) had better adaptability and tolerance to environmental stress factor than free cells application to biodegrade prometryn in wastewater. Several studies have been conducted in biological deodorisation of H_2S by using SOB carried on alginate. In Chung *et al.* [43]

http://dx.doi.org/10.4014/mbl.1801.01005

studies, *Thiobacillus thioparus* CH11 was immobilised in Ca-alginate could remove H_2S effectively up to 98% reduction rate. Similar to Park *et al.* [44] works where *Thiobacillus* sp. IW was encapsulated inside the Ca-alginate could remove H_2S completely up to 600 ppm in the inlet stream. Additionally, alginate has been used extensively for other bioremediation and biodegradation of numerous toxic pollutant including catechol [38] and organophosphate pesticides [24]. In conclusion, the present study showed the feasibility of using active SOB immobilised in alginate beads for the removal of H_2S in chicken manure. The most active isolate in the reduction of H_2S turned out to be isolate AH18 compared to isolate AH25 and AH28. However, the deodorisation ability was enhanced when the three isolates were mixed together as a consortium. Additionally, the use of alginate as a carrier for the cell immobilisation was found to enhancing the performance of the potential SOB isolates.

Acknowledgments

The authors extend their appreciation to Universiti Putra Malaysia (UPM) for financing this work through Putra Graduate Initiative (IPS).

Conflict of Interest

The authors have no financial conflicts of interest to declare.

References

1. Ministry of Agriculture and Agro-based Industry Malaysia. http://www.moa.gov.my/en/objektif. Accessed May 18, 2017.
2. Kim KY, Ko HJ, Kim HT, Kim YS, Roh YM, Lee CM, *et al.* 2007. Sulphuric odorous compounds emitted from pig-feeding operations. *Atmos. Environ.* **41**: 4811-4818.
3. Roth SH, Skrajny B, Reiffenstein RJ. 1995. Alteration of the morphology and neurochemistry of the developing mammalian nervous system by hydrogen sulphide. *Clin. Exp. Pharmacol.* **22**: 379-380.
4. Woestyne MV, Verstraete W. 2007. Biotechnology in the treatment of animal manure, pp. 311-328. *In* Wallace RJ, Chesson A. (eds.), *Biotechnology in Animal Feeds and Animal Feeding.* Willey VCH Verlag GmbH, Weinheim. Germany.
5. Friedrich CG, Rother D, Bardischewsky F, Quentmeier A, Fischer. 2001. Oxidation of reduced inorganic sulfur compounds by bacteria: Emergence of a common mechanism?. *Appl. Environ. Microbiol.* **67**: 2873-2882.
6. Robertson LA, Kuenen JG. 2006. The colourless sulphur bacteria, pp. 985-1011. *In* M. Dworkin, S. Falkow, E. Rosenberg, K.H. Schleifer, E. Stackebrandt (eds.), *The Prokaryotes*, vol. 2, 3rd ed., Springer, New York.
7. Spring S, Kämpfer P, Schleifer KH. 2001. *Limnobacter thiooxidans* gen. nov., sp. nov., a novel thiosulphate-oxidising bacterium isolated from freshwater lake sediment. *Int. J. Syst. Evol. Microbiol.* **51**: 1463-1470.
8. Das SK, Mishra AK, Tindall B, Rainey E, Stacke-Brandt E. 1996. Oxidation of thiosulphate by a new bacterium, *Bosea thiooxidans* (strain BI-42) gen. nov., sp. nov.: Analysis of phylogeny based on chemotaxonomy and 16S ribosomal DNA sequencing. *Int. J. Syst. Bacteriol.* **46**: 981-987.
9. Xu XJ, Chen C, Guo H, Wang A, Ren N, Lee DJ. 2016. Characterisation of a newly isolated strain *Pseudomonas* sp. C27 for sulphide oxidation: Reaction kinetics and stoichiometry. *Sci. Rep.* **6**: 21032.
10. Chung YC, Huang C, Tseng CP. 1996a. Biodegradation of hydrogen sulphide by a laboratory-scale immobilised *Pseudomonas putida* CH11 biofilter. *Biotechnol. Prog.* **12**: 773-778.
11. Huang ZZ, Chen GQ, Zeng GM, Chen AW, Zuo Y, Guo Z, *et al.* 2015. Polyvinyl alcohol-immobilised *Phanerochaete chrysosporium* and its application in the bioremediation of composite-polluted wastewater. *J. Hazard. Mater.* **289**: 174-183.
12. Stelting SA, Burns RG, Sunna A, Bunt CR. 2014. Survival in sterile soil and atrazine degradation of *Pseudomonas* sp. strain ADP immobilised on zeolite. *Bioremediat. J.* **18**: 309-316.
13. Zhang YQ, Tao ML, Shen WD, Zhou YZ, Ding Y, Ma Y, *et al.* 2004. Immobilisation of L-asparaginase of the microparticles of the natural silk sericin protein and its characters. *Biomaterials* **25**: 3751-3759.
14. Dzionek A, Wojcieszyńska D, Guzik U. 2016. Natural carriers in bioremediation: A review. *Electron. J. Biotechnol.* **23**: 28-36.
15. Bayat Z, Hassanshahian M, Cappello S. 2015. Immobilisation of microbes for bioremediation of crude oil polluted environments: A mini review. *Open Microbiol. J.* **9**: 48-54.
16. Trelles JA, Rivero CW. 2013. Whole cell entrapment techniques, pp. 365-374. *In* Guisan J. *Immobilization of Enzymes and Cells. Methods in Molecular Biology (Methods and Protocols),* vol 1051, Humana Press, Totowa, NJ.
17. Ramakrisna SV, Prakasham RS. 2003. Microbial fermentation with immobilised cells. Available from http://www.iisc.ernet.in/currsci/jul10/articles17.htm. Accessed January 10, 2017.
18. Moslemy P, Neufeld RJ, Guiot SR. 2002. Biodegradation of gasoline by gellan gum- encapsulated bacterial cells. *Biotechnol. Bioeng.* **80**: 175-184.
19. Zhou Z, Li G, Li Y. 2010. Immobilisation of *Saccharomyces cerevisiae* alcohol dehydrogenase on hybrid alginate-chitosan beads. *Int. J. Biol. Macromol.* **47**: 21-26.
20. Hidayat MY, Saud HM, Samsudin AA. 2017. Isolation and characterisation of sulphur oxidising bacteria isolated from hot spring in Malaysia for biological deodorisation of hydrogen sulphide in chicken manure. *Media Peternakan.* **40**: 178-187.
21. Lane DJ. 1991. 16S/23S rRNA sequencing, pp. 115-175. *In* Goodfellow M (eds), *Nucleic Acid Techniques in Bacterial Systematics*, Wiley, Chichester, UK.

22. Saitou N, Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**: 406-425.
23. Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* **33**: 1870-1874.
24. Yanez-Ocampo G, Sanchez-Salinas E, Jimenez-Tobon G, Penninckx M, Ortiz-Hernandez ML. 2009. Removal of two organophosphate pesticides by a bacterial consortium immobilised in alginate or tezontle. *J. Hazard. Mater.* **168**: 1554-1561.
25. Kelleher BP, Leahy JJ, Henihan M, O'Dwyer TF, Sutton D, Leahy MJ. 2002. Advances in poultry litter disposal technology – A review. *Bioresour. Technol.* **83**: 27-36.
26. Aldin S, Tu F, Nakhla G, Ray MB. 2011. Simulating the degradation of odour precursor in primary and waste-activated sludge during anaerobic digestion. *Appl. Biochem. Biotechnol.* **164**: 1292-1304.
27. Zinser E, Kolter R. 2004. Escherichia coli evolution during stationary phase. *Res. Microbiol.* **155**: 328-336.
28. Honma T, Akino T. 2014. Isolation and characterisation of a hydrogen sulphide-removing bacterium, *Pseudomonas* sp. strain DO-1. *Biosci. Biotechnol. Biochem.* **62**: 1684-1687.
29. Arogo J, Zhang RH, Riskowski GL, Day DL. 2000. Hydrogen sulphide production from stored liquid swine manure: a laboratory study. *Trans. ASAE.* **43**: 1241-1245.
30. Clanton CJ, Schmidt DR. 2000. Sulphur compound in gasses emitted from stored manure. *Trans. ASAE* **43**: 1229-1239.
31. Chung YC, Huang C, Tseng CP. 2001. Biotreatment of hydrogen sulphide- and ammonia-containing waste gases by fluidised bed bioreactor. *J. Air Waste Manag. Assoc.* **51**: 163-172.
32. Barman SR, Banerjee P, Mukhopadhayay A, Das P. 2017. Biodegradation of acenaphthene and naphthalene by Pseudomonas mendocina: Process optimization, and toxicity evaluation. *J. Environ. Chem. Eng.* **5**: 4803-4812.
33. Akther M, Tasleem M, Alam MM, Ali S. 2017. *In silico* approach for bioremediation of arsenic by structure prediction and docking studies of arsenite oxidase from *Pseudomonas stutzeri* TS44. *Int. Biodeterior. Biodegradation.* **122**: 82-91.
34. Singh U, Arora NK, Sachan P. 2017. Simultaneous biodegradation of phenol and cyanide present in coke-oven effluent using immobilised *Pseudomonas putida* and *Pseudomonas stutzeri*. *Braz. J. Microbiol.* **49**: 38-44.
35. Mahmood Q, Zheng P, Hu B, Jilani G, Azim MR, Wu D, *et al*. 2009. Isolation and characterisation of *Pseudomonas stutzeri* QZ1 from an anoxic sulphide-oxidising bioreactor. *Anaerobe* **15**: 108-115.
36. Graff A, Stubner S. 2003. Isolation and molecular characterisation of thiosulphate-oxidising bacteria from an italian rice field soil. *Syst. Appl. Microbiol.* **26**: 445-452.
37. Vinas M, Sabate J, Espuny MJ, Solanas AM. 2005. Bacterial community dynamics and polycyclic aromatic hydrocarbon degradation during bioremediation of heavily creosote-contaminated soil. *Appl. Environ. Microbiol.* **71**: 7008-7018.
38. Bramhachari PV, Rama Sekhara Reddy D, Kotresha D. 2016. Biodegradation of catechol by free and immobilised cells of *Achromobacter xylosoxidans* strain 15DKVB isolated from paper and pulp industrial effluents. *Biocatal. Agric. Biotechnol.* **7**: 36-44.
39. Gutarowska B, Matusiak K, Borowski S, Rajkowska A, Brycki B. 2014. Removal of odorous compounds from poultry manure by microorganisms on perlite-bentonite carrier. *J. Environ. Manage.* **141**: 70-76.
40. Matusiak K, Borowski S, Opalinski S, Bakula T, Kolacz R, Gutarowska B. 2015. Impact of a microbial-mineral biopreparation on microbial community and deodorisation of manures. *Acta. Biochim. Pol.* **62**: 791-798.
41. Liu YJ, Zhang AN, Wang XC. 2009. Biodegradation of phenol by using free and immobilized cells of *Acinetobacter* sp. XA05 and *Sphingomonas* sp. FG03. *Biochem. Eng. J.* **44**: 187-192.
42. Liu J, Pan D, Wu X, Chen H, Haiqun C, Li QX, *et al*. 2018. Enhanced degradation of prometryn and other s-triazine herbicides in pure cultures and wastewater by polyvinyl alcohol-sodium alginate immobilised *Leucobacter* sp. JW-1. *Sci. Total Environ.* **615**: 78-86.
43. Chung YC, Huang C, Tseng CP. 1996b. Operation optimisation of *Thiobacillus thioparus* CH11 biofilter for hydrogen sulphide removal. *J. Biotechnol.* **52**: 31-38.
44. Park DH, Cha JM, Ryu HW, Lee GW, Yu EY, Rhee JI, *et al.* 2002. Hydrogen sulphide removal utilising immobilised *Thiobacillus* sp. IW with Ca-alginate bead. *Biochem. Eng. J.* **11**: 167-173.

http://dx.doi.org/10.4014/mbl.1801.01005