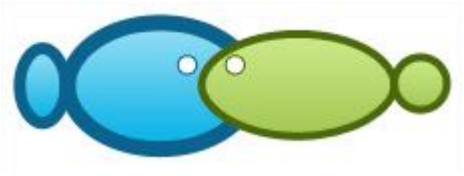


# The selected facultative mixotrophic sulphur-oxidizing bacteria from intensive shrimp ponds

[1]Ardiansyah, [1]Amrullah, [1]Dahlia, [1]Andi Asdar Jaya, [2]Indrayani, [1]Sri Wahidah, [1]Rimal Hamal, [3]Firmansyah Bin Abdul Jabbar

[1] Aquaculture Department, Pangkep State Polytechnic of Agriculture, Makassar-ParePare Km. 83, Sulawesi Selatan 90655, Indonesia; [2] Faculty of Fisheries and Marine Science, Haluoleo University, Postal Code 93132, South East Sulawesi, Indonesia; [3] Faculty of Animal Science & Fishery, Sulawesi Barat University, West Sulawesi, Indonesia. Corresponding author: Ardiansyah, ardi_kimsan@yahoo.com

**Abstract**. The research was conducted to characterize and screen sulphur-oxidizing bacteria (SOB) from three intensive shrimp farms in the province of South Sulawesi, Indonesia. Bacteria were isolated using sulphur-oxidation medium (SOM) with the direct plating method. A volume of 0.1 mL of the diluted sample was poured into SOM agar medium and incubated at 30°C for 24 hours. A total number of 48 facultative mixotrophic SOB isolates were obtained and considered as positive SOB due to their abilities using thiosulphate for growth. Three SOB isolates such as SOB15, SOB26, and SOB31 were selected based on their abilities to grow faster, produce the highest sulphate ion and reducing the pH in the growth medium. Results showed that the optimum pH of all SOB isolates occurred at pH 8.0. Meanwhile, the optimum temperature for SOB15, SOB26, and SOB31 isolates occurred at 35°C. The three isolates were classified as facultative mixotrophic with the capability of growth in thiosulphate medium supplemented with yeast extract. This research reveals the ability of the selected SOB in the oxidation of thiosulphate, temperature and pH adaptabilities, with the metabolic flexibilities of isolates SOB15, SOB26, and SOB31 can be the prospective $H_2S$ removal agent in intensive shrimp farms.
**Key Words**: sulphur-oxidation bacteria, bioremediation, ponds, vannamei shrimp.

**Introduction**. Many studies reported the decline in shrimp culture production is mainly due to disease attacks, triggered by deteriorating water quality (Primavera 2006; Ling et al 2010; Chatterjee & Haldar 2012). The accumulations of inorganic nitrogen, carbon organic compounds, and sulphides from the uneaten feed, shrimp faeces, and fertilization in the long term have a direct impact on the compounds of $NH_3$, $NO_2$, and $H_2S$, which are toxic substances to shrimp. Increased $H_2S$ concentrations may lead to unbalanced movement and death of the aquatic organisms (Boyd 2017). Thus, maintaining water quality in optimum conditions for shrimp growth is one of the main keys to successful shrimp farming. Lim et al (2014) claimed the use of biological techniques is more efficient when compared to mechanical and chemical methods. The use of biological techniques by utilizing micro-organism (bioremediation) for the recovery of waters from pollutants and toxic substances is mostly performed due to its easy and inexpensive application (Zhang et al 2008).

Various commercial products of bioremediation are available in the form of autotrophic and heterotrophic bacteria. The products have been used to mitigate the accumulation of organic matter in the pond bottom. However, from several cases of shrimp disease, it was reported that many of these bioremediation agents were not effective in mitigating organic matters pollution and maintaining good water quality in the pond (Abd El-Rahman et al 2009; Ferguson et al 2010; Nimrat et al 2012; Caia et al 2019).

The microbiological decomposition of the organic matter is an important component for water quality control and nutrient recycling in the shrimp pond. Better control of culture conditions and the sustainability of shrimp ponds is possible with an

improvement of the microbiological processes. In the pond, the primary decomposers, such as bacteria, release extracellular hydrolytic enzymes into the environment and these catalyse organic matter decomposition (Cunha et al 2010). The utilization of decomposers of organic matter, which is capable of acting as autotrophs and heterotrophs or commonly known as mixotrophic bacteria (Crane & Grover 2010) provides new hope for overcoming the problem of organic pollution and deteriorating pond water quality and other shrimp culture environments.

Data on shrimp toxicity exposed to $H_2S$ at sub-lethal concentrations are loss of appetite for several weeks (Boyd 2017). This certainly can cause shrimp death and result in large economic losses. Therefore, $H_2S$ needs to be oxidized so that it does not accumulate in the pond. $H_2S$ oxidation can be carried out by sulphur-oxidizing bacteria (Hou et al 2018).

Based on these problems, it is necessary to develop and utilize sulphur-oxidizing bacteria (SOB) that can survive in conditions of wide environmental fluctuations, survive the limited availability of energy and certain nutrients in the waters, and have a specific or multifunctional role capable of creating balanced conditions in water and sediment. Microbial mats grow and survive in shallow water environments where available sunlight forms multilevel communities that find their optimal conditions for living along with the different levels of chemical availability (Seckbach & Oren 2010). Niches for aerobic organisms are on the mat where enough oxygen is available for photosynthesis. In the lower layer, purple and green-sulphur bacteria make anoxygenic photosynthesis oxidize reduced sulphur compounds. Other SOB are located where $H_2S$ and $O_2$ are available at the surface area of the bottom of the pond. The use of facultative mixotrophic bacteria is expected to release $H_2S$ in the form of sulphate ions and sulphur oxide compounds. Therefore, characterization and screening of SOB from intensive shrimp farms were conducted in this present study.

## Material and Method

***Sample collection***. Water and sediment were collected on April 2018, from three different intensive shrimp farming located in Bone Regency, South Sulawesi, Indonesia. The samples were collected in 100 mL sterile plastic tubes from the surface and bottom water layers and transported to the research laboratory. The samples were stored at 4°C until further analysis in laboratories.

***Isolation of facultative mixotrophic sulphide-oxidizing bacteria***. SOB were isolated according to the method of Visser et al (1997) and Behera et al (2014) on medium sulphur-oxidation medium (SOM), which consisted of 10 g Bacto-Peptone, 1.5 g $K_2HPO_4$, 0.75 g iron ammonium citrate and 1.0 g $Na_2S_2O_3.5H_2O$. The pH of the media was set to 7.0 using 1 M HCl. Isolation of SOB was conducted using a direct plating method. A volume of 0.1 mL of diluted sample (serial dilution) was poured into SOM and incubated at 30°C for 24 hours. Based on visible physical characteristics; the shape and size of colonies, colour, elevation, and edges, each different colony was re-passaged to obtain a single colony. Furthermore, the pure isolates were then cultured in a test tube containing agar (to be slanted). The identification of SOB was carried out using the API 20E assay, which was validated by conventional methods (biochemical tests) (Chen et al 2004).

***Screening of facultative mixotrophic sulphur-oxidizing bacteria***. Five mL of each pure culture was inoculated into a flask containing 50 mL sulphate-screening medium (SSM) consisting of 1.5 g $KNO_3$, 1.5 g $KH_2PO_4$, 1.5 g $NH_4Cl$, 1.4 g $MgSO_4.7H_2O$, 1.5 g $NaHCO_3$, 4.0 g $Na_2S_2O_3.5H_2O$ and 1.0 mL mineral solution pH 6 with 1 N KOH. Mineral solution containing (g $L^{-1}$): 50 g $Na_2$-EDTA, 7.34 g $CaCl_2.2H_2O$, 2.2 g $ZnSO_4.7H_2O$, 5.0 g $FeSO_4.7H_2O$, 0.2 g $CaSO_4.5H_2O$, 2.5 g $MnCl_2.4H_2O$, 0.5 g $(NH_4)$ $6Mo_7O_{24}.4H_2O$ and 11.0 g NaOH (DSMZ 2002). Then 10 g of agar was added to freeze the medium and incubated in a rotary shaker (100 rpm) at room temperature for 7 days. Turbidity in the medium indicated bacterial growth. The culture was purified into a solid SSM medium and incubated at 30ºC for 7 days. After dilution, 100 µL of bacterial culture was inoculated on

solid SSM medium and incubated at 25°C for 48 h. Every different colony growth was carried out by purification of the culture by inoculating into a new screening medium. The procedure was carried out in five replicates and the sulphate ion concentration resulting from bacterial growth was determined (Ullah et al 2013).

***Metabolic characterization of the potential SOB isolates on SSM medium***. Purified cultures were grown using SSM medium containing $Na_2S$ as a substitute for $Na_2S_2O_3$. Three mL of bacterial culture (culture) was centrifuged at 10,000 rpm for 10 minutes, the supernatant was removed, bacterial pellets washed with pH 6 phosphate buffer and then centrifuged again. Bacterial culture (1% (v/v)) was inoculated into a medium containing $Na_2S$ as above and then incubated at 30°C for 7 days. A 500 µL culture medium was put into 50 mL of a new medium in a 100 mL capacity conical flask. Controls (without inoculum) were incubated at the same temperature. All experiments were performed in five replicates. Metabolic characterization of SOB was determined on SSM medium supplemented with 0.05% (w/v) of yeast extract (Chen et al 2004), and incubated aerobically at 30°C for 7 days. The optical density of cell growth was measured every 24 h with a UV-vis spectrophotometer (Jasco, Tokyo, Japan) at 660 nm. The specific growth rate of the isolate was determined and sulphate ion production was measured by using $BaCl_2$ (Cha et al 1999).

***Screening of SOB for growth at different pH and temperature***. The effect of pH on the growth of SOB was performed in six different pH range from 4 to 9 (4.0, 5.0, 6.0, 7.0, 8.0, and 9.0). The culture was incubated at room temperature (28±2°C) with 160 rpm agitation speed for 7 days. To determine the effect of temperatures on the growth of bacteria, the culture was incubated at four different temperatures of 25, 30, 35, and 40°C. The culture medium was set at pH 8.0 and incubated with 160 rpm agitation speed for 7 days. The sulphate concentration resulting from bacterial growth was determined at the beginning and end of the growth period (Behera et al 2014).

## Results and Discussion

***Isolation and screening of facultative mixotrophic sulphur-oxidizing bacteria***. A total number of 48 isolates were obtained and the colonies were selected based on the size, colour, and shape. At this point, the colonies obtained from the isolation were considered as potential SOB due to their abilities using sulphate for growth. The 48 isolates were subjected to a screening process to choose the potential facultative mixotrophic SOB, and six isolates were found to have the capability to grow faster based on the development of turbidity observed (Figure 1).

Of forty-eight visually different bacterial isolates, 6 bacterial strains were selected based on pH reduction from the initial 8.0 to less than 5.0. The pH reduction of the medium was due to sulphuric acid production and pH reduction, in which SOB15, SOB26, and SOB31 isolates were observed a final pH range of 5.5-6.0 from the initial pH 7.5. SOB15 formed a yellowish-brown, a wrinkled colony, SOB26 formed a greyish-white, smooth colony, while SOB31 formed a regular white-greyish, smooth colony. Light microscopy examination showed that cells of SOB26 were gram-negative bacilli, SOB31 were gram negative cocci, while SOB15 were gram positive cocci. The results showed that the potential SOB isolates are cocci-shaped gram negative bacteria as the main group of bacterial populations found in this study, followed by gram-positive bacilli, while gram-positive cocci are the lowest density bacteria. Their morphological and biochemical characteristics are summarized in Table 1.

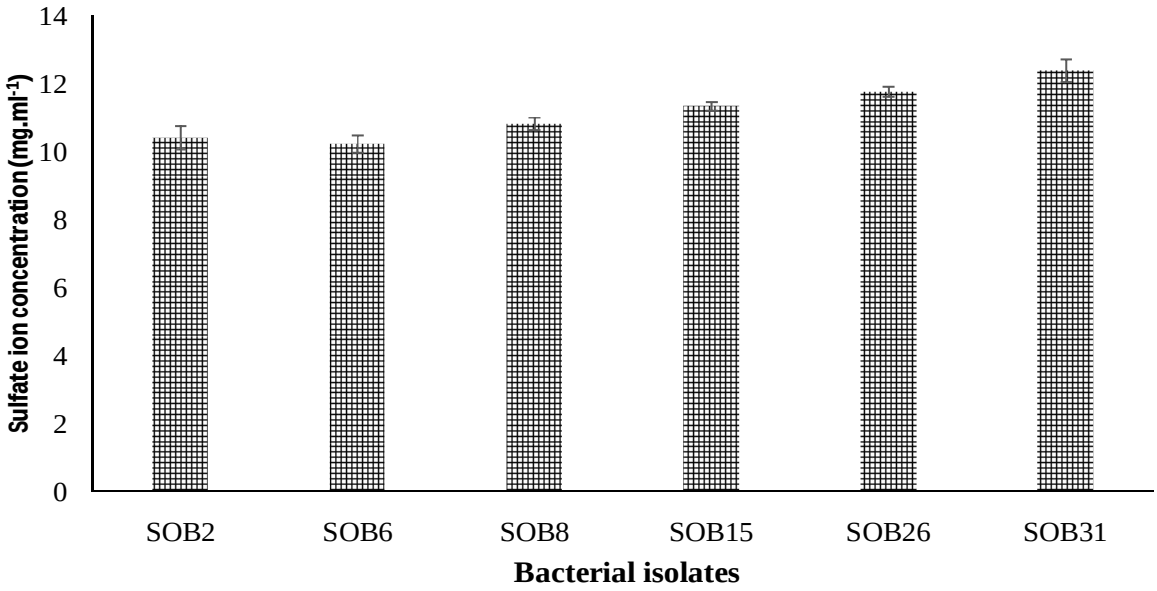


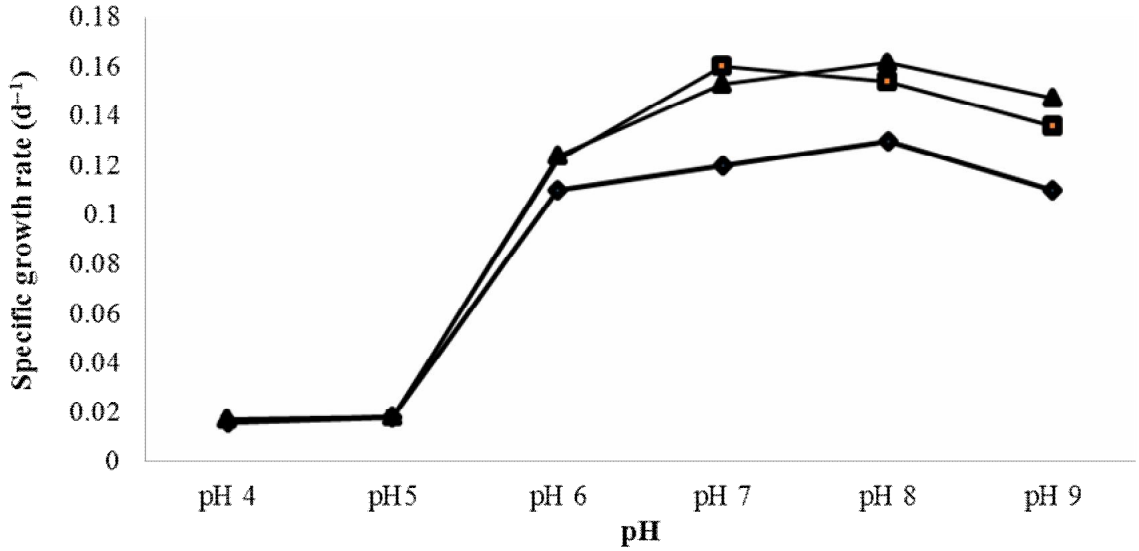

Figure 1. Screening results of sulphur-oxidizing bacteria for sulphate ion production. Each value represents the mean of five replicates ±SE.

Table 1

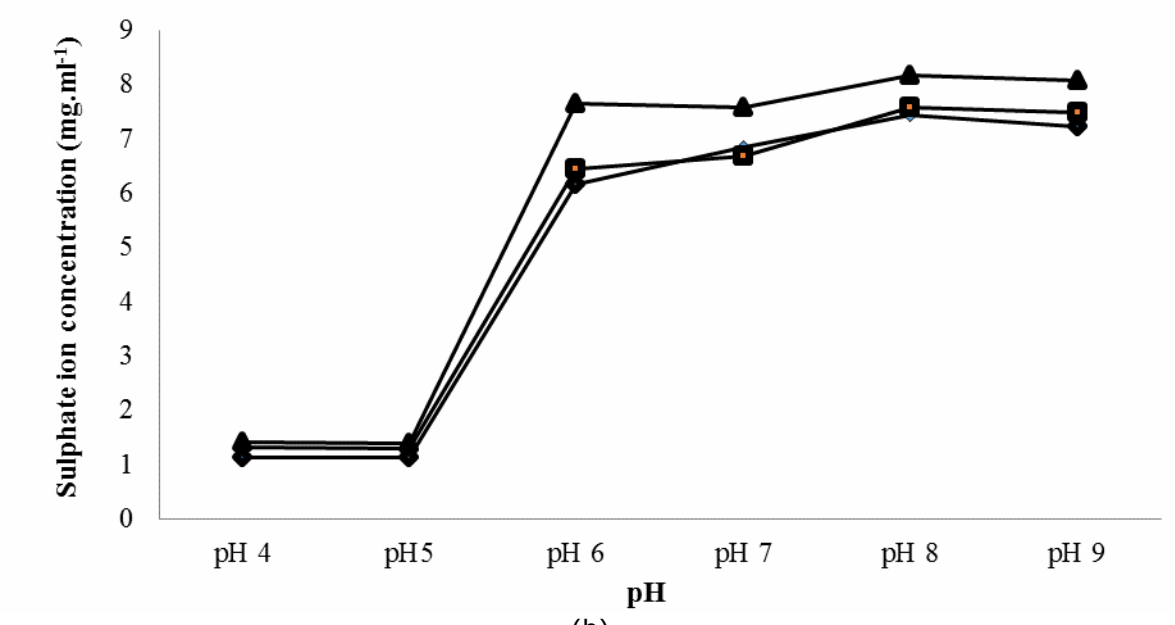
The morphology and physiological characteristic of facultative mixotrophic SOB from intensive shrimp farms in Bone Regency, Indonesia

| *Traits* | *SOB isolates* | | | | | |
|---|---|---|---|---|---|---|
| | *SOB2* | *SOB6* | *SOB8* | *SOB15* | *SOB26* | *SOB31* |
| Morphology | Cocci | Bacilli | Cocci | Cocci | Bacilli | Cocci |
| Gram stain | - | - | - | + | - | - |
| Pigmentation | White-greyish | Translucent | White | Yellowish-brown | Greyish-white | White-greyish |
| Spore | - | - | + | - | + | - |
| Motility | - | - | - | + | + | - |
| Oxidase | - | - | - | + | + | + |
| Catalase | - | - | - | - | - | - |
| Citrate utilisation | - | - | + | + | - | - |
| Nitrate reduction | + | - | + | + | + | + |
| $H_2S$ production | - | - | - | + | - | - |
| Indole production | + | + | + | - | + | + |
| Methyl red | + | - | + | + | + | - |
| Voges-Proskauer | + | + | + | - | + | - |
| Urease | + | - | + | + | + | + |
| Tryptophan | - | + | - | + | + | + |
| Gelatinase | + | + | + | + | + | + |
| Ornithine | + | + | - | + | + | + |
| Starch | + | + | - | + | + | - |
| Glucose | + | - | - | + | + | + |
| Mannitol | - | - | + | + | + | + |
| Fructose | + | - | + | + | + | + |
| Lactose | - | - | - | - | - | - |
| Maltose | - | - | + | + | + | + |
| Arabinose | - | - | - | - | - | - |
| Sucrose | - | - | + | + | + | + |
| No. of clones (%) | 6.25 | 15.63 | 25.00 | 6.25 | 21.88 | 25.00 |

***Specific growth rate*, *sulphur-oxidation activity*, *and pH***. The effects of pH on SOB growth rate and sulphur-oxidation activity are shown in Figure 2. The three potential SOB isolates were preferred to grow in the range of pH 6.0 to 9.0 accompanied by the production of sulphate ion in the growth medium. Based on the results, it was observed that the optimal pH of growth and sulphur oxidation activity was 8.0. However, in the pH medium of 4.0 and 5.0, the three isolates showed less growth activity and sulphate ion production ($p > 0.05$).




(a)




(b)

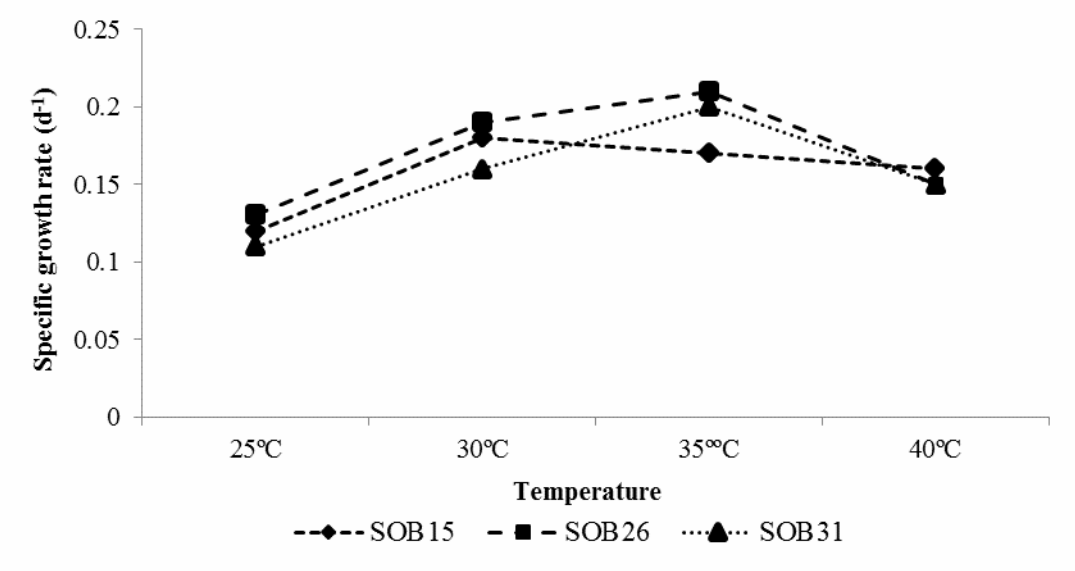
Figure 2. Bacterial growth rate (a) and sulphur oxidation activity (b) by SOB15 (-•-), SOB26 (-■-), and SOB31 (-▲-) isolates at six different pH levels. Each value represents the mean of five replicates ±SE.

Most bacteria prefer a specific pH range in which a change in pH can affect their growths and activities. The microbial growth and sulphur-oxidation activity of all facultative mixotrophic SOB isolates were observed occurred under the neutral and slightly alkaline conditions with the optimum pH was 8.0. The result was in line with the finding of Hidayat et al (2017), which reported that an optimum pH level of SOB isolated was observed at pH 8.0. This may be due to the fact that the optimum pH level in the water for shrimp farming ranged from 6.5 to 9.5 (Njoku et al 2015). However, the optimum pH

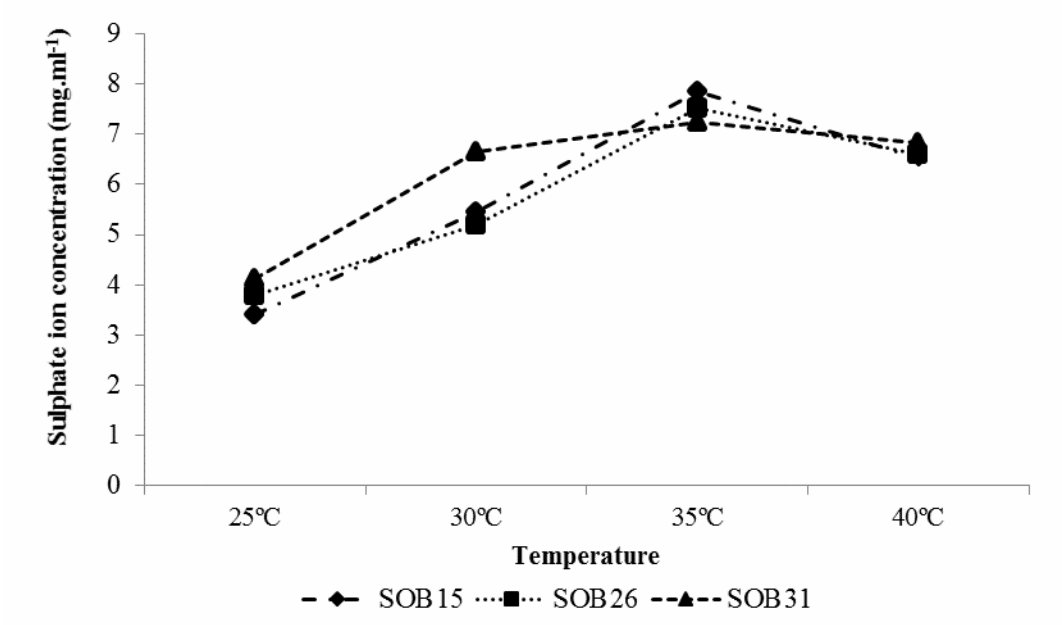
for SOB was varied depending on the bacterial species and habitat. SOB isolated from Soda Lakes strain AL-2 and AL-3 grew over a pH range 8.0-10.4 with an optimum at 9.5-9.8 (Sorokin 2000), while other species, *Acidithiobacillus thiooxidans* produced high sulphate concentration in pure liquid batch at an initial pH value of 4.5 (Huber et al 2016). Bacteria that grow optimally in a near-neutral pH range require a strong mechanism for their cytoplasmic pH homeostasis to survive and grow in exposure to acidic or alkaline conditions that are outside the pH range that are tolerated for their cytoplasmic pH (Krulwich et al 2011). Expectedly, the growth of all facultative mixotrophic SOB isolates was inhibited under acidic conditions. This phenomenon may be due to the deterioration of bacterial growth and metabolism under the inappropriate pH conditions and thus indicated the neutrophilic character of all the potential isolates. Several sulphur bacteria such as *Thiobacillus* sp are characterized to have a neutrophilic character such as *T. novellus* and *T. thioparus* with an optimal pH of 7.0 and 7.5, respectively (Pokorna & Zabranska 2015). Therefore, from the practical point of view, the ability of potential SOB to grow on varies pH conditions would help the isolates to deal with fluctuation of pH level in shrimp ponds under neutral to an alkaline condition.

***Specific growth rate, sulphur-oxidation activity, and temperature***. The effects of temperature on the growth rate and sulphur-oxidation activity of SOB15, SOB26, and SOB31 isolates are illustrated in Figure 3. The results suggested that the three SOB isolates were able to grow over a broad range of temperatures investigated. Interestingly, the bacterial growth of SOB15 and SOB26 isolates were observed to decrease with the increase in temperature and the optimal temperature was at 30°C ($p < 0.05$) and 35°C ($p > 0.05$) respectively, while sulphate ion production of the SOB15 and SOB26 isolates increase up to 35$^{o}$C. Meanwhile, the isolate SOB31 shows a different bacteria growth and sulphur-oxidation behaviour towards the temperature investigated. The bacterial growth and sulphate ion production increased with the increase of temperature up to 35°C ($p < 0.05$) and was observed starts to decrease beyond that temperature.

The temperature requirements of facultative mixotrophic SOB are different and mostly they were found as mesophilic or thermophilic bacteria. In the present study, all SOB isolates grew and survived in the range of 25-40°C. However, the activity of all SOB isolates was observed to decrease at the temperature higher than 35$^{o}$C. All bacterial growth were observed slower at a temperature of 40°C. This is understandable because all SOB isolates inhabit shrimp farms and have undergone physiological adaptation to shrimp pond conditions, in which the optimal range of water temperature for white Pacific shrimp may be greater than 30°C (Kakoolaki et al 2015). This result was similar to the finding of Watsuntorn et al (2017), which reported an optimal temperature of SOB isolates at 35°C. Furthermore, the ability of facultative mixotrophic SOB isolates to survive and grow up to 40°C was due to the natural habitat of bacteria itself which is from the tropical region. Thus, all SOB isolates can be classified as mesophilic bacteria which would be an advantage for these isolates for the use in bioremediation of aquaculture environments.



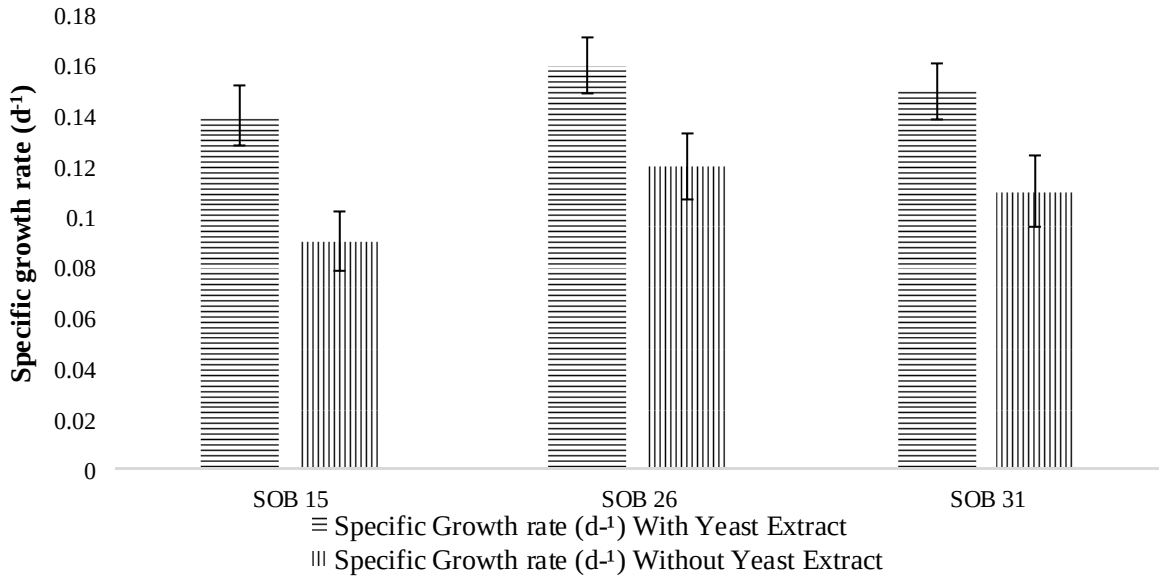

(a)



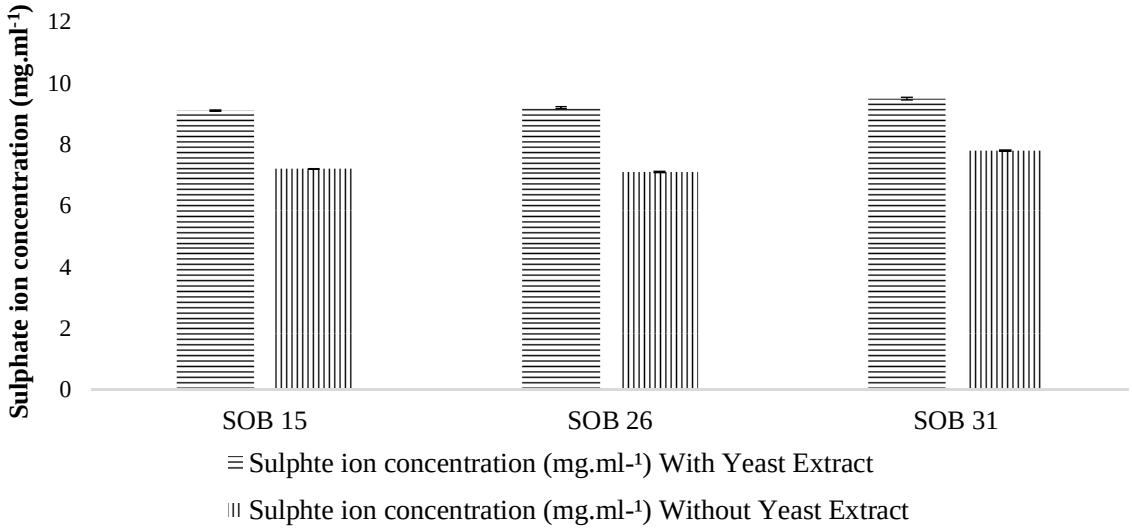

(b)

Figure 3. Bacterial growth rate (a) and sulphur-oxidation activity (b) by SOB15 (-•-), SOB26 (-■-), and SOB31 (-▲-) isolates at four different temperatures. Each values represents the mean of five replicates ±SE.

***Metabolic characteristics of facultative mixotrophic SOB***. All the isolates were observed to grow rapidly when the SSM medium was amended with yeast extract for mixotrophic growth. As presented in Figure 4, specific growth rate and the production of sulphate ion were significantly higher ($p < 0.05$) in medium supplemented with yeast extract compared to the medium without yeast extract for all the isolates.




(a)




(b)

Figure 4. The specific growth rate (a) and sulphate ion production (b) of SOB15, SOB26, and SOB31 isolates on SSM medium supplemented with 0.05% (w/v) of yeast extract and without yeast extract. Each value represents the mean of five replicates ±SE.

All SOB isolates were characterized as a facultative mixotrophic bacteria because the highest sulphate ion production was observed in the medium containing both thiosulphate and yeast extract compared to the medium containing only thiosulphate. Yeast extract has become favourable organic compound by the three SOB isolates due to several amino acids and peptides contents in yeast extract and the water-soluble characteristic of vitamins and carbohydrates molecule, which are enough to support the bacterial growth. However, by supplementing SSM liquid medium with an organic compound such as yeast extract, the bacterial growth was significantly increased.

The results indicate that SOB isolates preferred mixotrophic growth because the bacteria grow better with the presence of both reduced inorganic sulphur compound and an organic compound. Similar findings have also reported that mixotrophic growth with yeast extract was found to be a great condition that promotes bacterial growth and sulphur oxidation resulting in higher production of sulphate ion (Vardanyan & Vardanyan

2014). Thus, the metabolic flexibility of SOB isolates may ensure higher survival and growth of SOB in many different environments (Graff & Stubner 2003).

It is important to determine the adaptability and feasibility of the SOB in the various environmental conditions as described above to ensure their effectiveness in biological deodorization performance. Therefore, it was well-established from this present study that the potential SOB isolates have possessed a good application potential for the biological removal of $H_2S$ in intensive shrimp farms due to their ability to perform the oxidization of sulphur compound at various parameters tested.

**Conclusions**. In summary, three potential SOB isolates were successfully isolated from intensive shrimp farms have remarkable potentials for application in the biological removal of $H_2S$ in intensive shrimp ponds. The results revealed that the potential isolates of SOB15, SOB26, and SOB31 have optimum pH at 8.0 and optimum temperatures of isolate SOB16, SOB26, SOB31 were at 35°C which makes them appropriate candidates for bioremediation of shrimp farming ponds. Moreover, all potential isolates were characterized as facultative mixotrophic and this metabolic versatility may be an advantage to survive in various environmental conditions. All the data obtained in this study can be useful information in developing a feasible bioremediation strategy for aquaculture environments.

**Acknowledgements**. This study was funded by the Ministry of Research, Technology and Higher Education of the Republic of Indonesia under the National innovation system of the research incentive program. We also appreciate the help of colleagues at Aquaculture Department during sample collections.

## References

Abd El-Rahman A. M., Khattab Y., Shalaby A., 2009 *Micrococcus lutes* and *Pseudomonas* species as probiotics for promoting the growth performance and health of Nile tilapia, *Oreochromis niloticus*. Fish and Shellfish Immunology 27(2):175-180.

Behera B. C., Patra M., Dutta S. K., Thatoi H. N., 2014 Isolation and characterisation of sulphur oxidising bacteria from mangrove soil of Mahanadi River Delta and their sulphur oxidising ability. Journal of Applied and Environmental Microbiology 2(1):1-5.

Boyd C. E., 2017 Fish diseases: prevention and control strategies. Chapter 6: General relationship between water quality and aquaculture performance in ponds. Academic Press, pp. 147-166.

Caia Y., Yuana W., Wanga S., Guoa W., Lia A., Wua Y., Chena X., Rena Z., Zhoua Y., 2019 *In vitro* screening of putative probiotics and their dual beneficial effects: to white shrimp (*Litopenaeus vannamei*) postlarvae and to the rearing water. Aquaculture 498:61-71.

Cha J. M., Cha W. S., Lee J. H., 1999 Removal of organ-sulphur odour compounds by *Thiobacillus novellus* SRM, sulphur-oxidizing microorganisms. Process Biochemistry 34(6-7):659-665.

Chatterjee S., Haldar S., 2012 *Vibrio* related diseases in aquaculture and development of rapid and accurate identification methods. Journal of Marine Science: Research and Development S1:002.

Chen X. G., Geng A. L., Yan R., Gould W. D., Ng Y. L., Liang D. T., 2004 Isolation and characterization of sulphur-oxidizing *Thiomonas* sp. and its potential application in biological deodorization. Letters in Applied Microbiology 39(6):495-503.

Crane K. W., Grover J. P., 2010 Coexistence of mixotrophs, autotrophs and heterotrophs in planktonic microbial communities. Journal of Theoretical Biology 262(3):517–527.

Cunha A., Almeida A., Coelho F., Gomes N., Oliveira V., Santos A., 2010 Bacterial extracellular enzymatic activity in globally changing aquatic ecosystems. Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, pp. 124-135.

Ferguson R. M. W., Merrifield D. L., Harper G. M., Rawling M. D., Mustafa S., Picchietti S., Balcázar J. L., Davies S. J., 2010 The effect of *Pediococcus acidilactici* on the gut microbiota and immune status of on-growing red tilapia (*Oreochromis niloticus*). Journal of Applied Microbiology 109(3):851-862.
Graff A., Stubner S., 2003 Isolation and molecular characterisation of thiosulphate-oxidising bacteria from an Italian rice field soil. Systematic and Applied Microbiology 26(3):445-452.
Hidayat M. Y., Saud H. M., Samsudin A. A., 2017 Isolation and characterisation of sulphur oxidizing bacteria isolated from hot spring in Malaysia for biological deodorisation of hydrogen sulphide in chicken manure. Media Peternakan 40(3):178-187.
Hou N., Xia Y., Wang X., Liu H., Liu H., Xin L., 2018 $H_2S$ biotreatment with sulfide-oxidizing heterotrophic bacteria. Biodegradation 29(6):511-524.
Huber B., Herzog B., Drewes J. E., Koch K., Müller E., 2016 Characterization of sulfur oxidizing bacteria related to biogenic sulfuric acid corrosion in sludge digesters. BMC Microbiology 16:153.
Kakoolaki S., Afsharnasab M., Sharifpour I., 2015 The relation between temperature and salinity with WSSV occurrence in shrimp farms in Iran: an article review. Journal of Survey in Fisheries Sciences 2(1):31-41.
Krulwich T. A., Sachs G., Padan E., 2011 Molecular aspects of bacterial pH sensing and homeostasis. Nature Reviews Microbiology 9(5):330-343.
Lim K. T., Shukor M. Y., Wasoh H., 2014 Physical, chemical, and biological methods for the removal of arsenic compounds. BioMed Research International 2014:503784.
Ling T. Y., Buda D., Nyanti L., Norhadi I., Justin J. J. E., 2010 Water quality and loading of pollutants from shrimp ponds during harvesting. Journal of Environmental Science and Engineering 4(6):13-18.
Nimrat S., Suksawat S., Boonthai T., Vuthiphandchai V., 2012 Potential *Bacillus* probiotics enhance bacterial numbers, water quality and growth during early development of white shrimp (*Litopenaeus vannamei*). Veterinary Microbiology 159(3-4):443-450.
Njoku O. E., Agwa O. K., Ibiene A. A., 2015 An investigation of the microbiological and physicochemical profile of some fish pond water within the Niger Delta region of Nigeria. African Journal of Food Science 9(3):155-162.
Pokorna D., Zabranska J., 2015 Sulphur-oxidising bacteria in environmental technology. Biotechnology Advances 33:1246-1259.
Primavera J. H., 2006 Overcoming the impacts of aquaculture on the coastal zone. Ocean and Coastal Management 49(9-10):531-545.
Seckbach J., Oren A., 2010 Microbial mats: modern and ancient microorganisms in stratified systems. Springer Netherlands, 606 pp.
Sorokin D. Y., Robertson L. A., Huenen J. G., 2000 Isolation and characterization of alkaliphilic, chemolithoautotrophic, sulphur-oxidizing bacteria. Antonie van Leeuwenhoek 77(3):251-262.
Ullah I., Jilani G., Haq M. I., Khan A., 2013 Enhancing bio-available phosphorous in soil through sulphur oxidation by *Thiobacilli*. British Microbiology Research Journal 3(3):378-392.
Vardanyan N. S., Vardanyan A. K., 2014 New sulphur oxidizing bacteria isolated from bioleaching pulp of zinc and copper concentrates. Universal Journal of Microbiology Research 2(2):27-31.
Visser J. M., de Jong G. A. H., Robertson L. A., Kuenen J. G., 1997 A novel membrane-bound flavocytochrome c sulphide dehydrogenase from the colourless sulphur bacterium *Thiobacillus* sp. W5. Archives of Microbiology 167:295-301.
Watsuntorn W., Ruangchainikom C., Rene E. R., Lens P. N. L., Chulalaksananukul W., 2017 Hydrogen sulfide oxidation under anoxic conditions by a nitrate-reducing, sulfide-oxidising bacterium isolated from the Mae Um Long Luang hot spring, Thailand. International Biodeterioration and Biodegradation 124:196-205.
Zhang K., Xu Y. Y., Hua X. F., Han H. L., Wang J. N., Wang J., 2008 An intensified degradation of phenanthrene with macroporous alginate-lignin beads immobilized *Phanerochaete chrysosporium*. Biochemical Engineering Journal 41(3):251-257.

Received: 13 June 2020. Accepted: 28 September 2020. Published online: 28 October 2020.
Authors:
Ardiansyah, Department of Aquaculture, Pangkep State Polytechnic of Agriculture, Pangkep 90655, South Sulawesi, Indonesia, e-mail: ardi_kimsan@yahoo.com
Amrullah, Department of Aquaculture, Pangkep State Polytechnic of Agriculture, Pangkep 90655, South Sulawesi, Indonesia, e-mail: ulla_285@yahoo.com
Dahlia, Department of Aquaculture, Pangkep State Polytechnic of Agriculture, Pangkep 90655, South Sulawesi, Indonesia, e-mail: unga-dahlia@yahoo.co.id
Andi Asdar Jaya, Department of Aquaculture, Pangkep State Polytechnic of Agriculture, Pangkep 90655, South Sulawesi, Indonesia, e-mail: andiasdar_bdp@yahoo.com
Indrayani, Faculty of Fisheries and Marine Science, Haluoleo University, Postal Code 93132, South East Sulawesi, Indonesia, e-mail: indrayani_tajudin@yahoo.com.au
Sri Wahidah, Department of Aquaculture, Pangkep State Polytechnic of Agriculture, Pangkep 90655, South Sulawesi, Indonesia, e-mail: sriwahidah@gmail.com
Rimal Hamal, Department of Aquaculture, Pangkep State Polytechnic of Agriculture, Pangkep 90655, South Sulawesi, Indonesia, e-mail: rimalhamal00@gmail.com
Firmansyah Bin Abd Jabbar, Faculty of Animal Science & Fishery, Sulawesi Barat University, Postal Code 91214, West Sulawesi, Indonesia, e-mail: firmansyahjabbar@unsulbar.ac.id

How to cite this article:
Ardiansyah, Amrullah, Dahlia, Jaya A. A., Indrayani, Wahidah S., Hamal R., Jabbar F. B. A., 2020 The selected facultative mixotrophic sulphur-oxidizing bacteria from intensive shrimp ponds. AACL Bioflux 13(5):2886-2896.