



BIOACTIVE COMPOUNDS OF SPONGE FOR AQUACULTURE AND POTENTIAL METHODS FOR SPONGE CULTIVATION

Ruzkiah Asaf^{1*}, Andi Nur Samsi², Admi Athirah¹ and Mudian Paena¹

¹The Research Institute and Development of Brackishwater Aquaculture - Maros

²College of Teacher Training and Education Pembangunan Indonesia - Makasar

*corresponding Author E-mail : qiaasaf@gmail.com

Abstract. Aquaculture is an important sector for Indonesian economic, however the spreads of diseases during farming remains a problem in developing Indonesian aquaculture. Sponge is known as the source of potential bioactive to combat pathogenic diseases in aquaculture. However, the intensifying exploitation of wild sponge tends to depress population of sponges in nature. This article reviews bioactive originating from sponges and potential methods in developing sponges cultivations under in-situ and ex-situ methodologies.

Keywords: sponge, bioactive compounds, aquaculture, sponge cultivation

I INTRODUCTION

Aquaculture is one in the field of fisheries that contribute to the country's foreign exchange; therefore, the Indonesian government is trying to increase the production of aquaculture fishery either in the form of pond revitalization which started from 2010 until the application of super intensive pond technology [1], however, the disease caused by pathogen is still a constraint affecting the success of cultivation [2]. For example, disease attacks on shrimp culture led to the failure of massive shrimp farming in Java [3]. Several shrimp diseases caused by bacteria in Asia have been identified, including Acute Hepatopancreatic Necrosis Disease (AHPND) caused by *Vibrio* bacteria [4,5]. Efforts to prevent disease attacks on aquaculture have been conducted such as: introduction of shrimp aquaculture procedures, the improvement of fish genetics to obtain disease-resistant commodities, tightening of shrimp introduction regulations especially from endemic areas of shrimp disease as well as supporting the prevention of fish disease [6], efforts to eradicate fish diseases during aquaculture activities need to be done, because the disease is still a problem that is often encountered. The natural bactericidal application for treating diseases in humans and animals is a way that is widely promoted today. This is because the use of bactericide from chemicals can cause bacteria to be resistant, but it also has many negative impacts both on living things and the environment [7]. One source of natural bactericide that can be developed is the sponge, which is the most diverse types of

invertebrates of one type of aquatic organism, not only because of the number of species but by its morphological character [8]. Around 8000 different species have been described and estimated to be present, twice the number [9,10]. This diversity is attributed to the fact that invertebrate sessile results in a large array of secondary metabolites, so sponges are targets in the search for high value-added molecules [11]. This article aims to provide information on the bioactive sponge that can be used as a bactericide in the field of aquaculture and sponge cultivation technology.

II NATURAL ACTIVE SPONGE MATERIAL

The sponge is a marine invertebrate macro which is the source of new bioactive compounds with various biomedical potentials. There are 5300 products of natural materials that have been isolated from marine sponges around the world [12], the number is increasing every year [13,14]. Eribulin mesylate is the first drug derived from natural sponge products that entered the market in 2011 as anticancer [15]. Most of the halichondrin B, molecules, avarol, crambescidins, have the high biological activity to produce valuable products for medical drugs as the anti-inflammatory, antitumor, anti-microbial, immunosuppressive or neuro suppressive,

antiviral or antibiotic [12, 16, 17, 18, 19, 20]. The sponge can be an appropriate target, as the basic ingredients of the drug, because this type of marine biota has a high diversity of bioactive compounds [21, 22, 23]. Studies on sponge symbiotic microorganisms, such as bacteria and fungi, have found that this symbionary biota produces bioactive compounds [24]. Since the bioactive compounds present in the sponge are strongly influenced by the symbiotic microbes, the variations of the sponge bioactive compound are very high [23]. There are ten bacterial phyla that are symbiotic with sponges namely *Proteobacteria*, *Nitrospira*, *Cyanobacteria*, *Bacteroidetes*, *Actinobacteria*, *Chloroflexi*, *Planctomycetes*, *Acidobacteria*, *Poribacteria* and *Verrucomicrobia* [24]. Symbiotic bacteria with sponge produce secondary active metabolite compounds as potential antibacterial pathogens *Staphylococcus aureus*, *Bacillus subtilis* and *Vibrio eltor* [26]. Furthermore, the natural material produced by the symbiont bacteria is a type of chlorinated, neuroactive, antimicrobial compound that can be used as a raw material for antibiotic drugs [27]. From these symbiotic results and producing several compounds proves that marine organisms are an important source of research and development of new drugs.

III ACTIVE SPONGE MATERIAL AS ANTI MICROBA IN CULTIVATION

Over the last 60 years, the sponge has been extensively researched by chemists, biologists, and pharmaceuticals, as it has a secondary metabolite content and a very useful bioactive content. Various types of pathogenic bacteria against humans include *Staphylococcus aureus* and *Escherichia coli*, while pathogenic bacterial organisms are *Vibrio harveyi* and *Aeromonas hydrophilla* (Figure 1). To overcome various diseases have been found avarol of sponge *Dysidea avara* [28]. *Staphylococcus aureus* is one of the bacteria that is resistant to several types of antibiotics, especially β -lactam groups such as vancomycin [29]. *E.coli* pathogenic bacteria is one type of bacteria that normally live in the digestive tract of both healthy humans and animals and is a strain that causes some types of diarrhea in humans. Disease disturbance in fish culture is also a problem in cultivation which includes infection and non-infection. Infectious diseases are caused by pathogenic attacks, such as viruses, bacteria, fungi, and parasites [30]. Types of pathogenic bacteria that cause disease in many aquaculture organisms are from the genus *Aeromonas* sp and *Vibrio* sp. *Aeromonas hydrophilla* is a bacterium that causes Motile Aeromonas Septicemia (MAS) disease or Haemorrhagic Septicemia that attacks freshwater fish [31], *Aeromonas hydrophilla* is a bacterium

found mostly in freshwater as well as in seawater. These bacteria include gram-negative, motile and rod-shaped bacteria, while *Vibrio* sp. causing vibriosis that often affects shrimp and fish [32]. the red-spot disease is a disease of ulcers or red spots on freshwater fish. Fish attacked by these bacteria have low survival [33]. Antibiotics have been widely found to be good as antifungal, antibacterial, antiviral, antitumor even as anticancer. However with repeated use may increase resistance to pathogenic bacteria, for which the use of bioactive materials is necessary to overcome this.

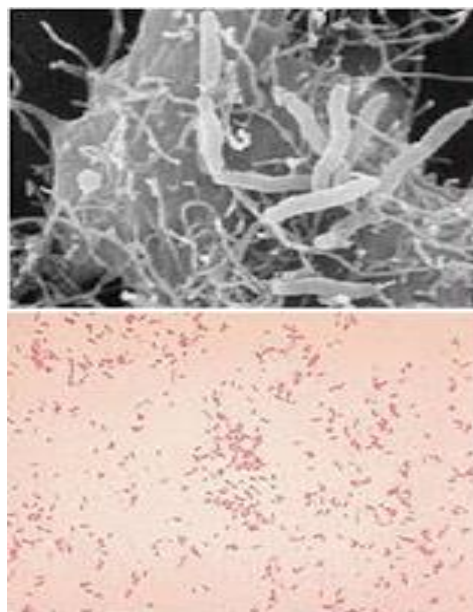


Figure 1 Bacterial Cells Form *Aeromonas hydrophilla* [14]

Vibrio harveyi is a pathogenic bacteria in fish and sea shrimp that most often cause serious problems in cultivation. The presence of *Vibrio harveyi* in $> 10^4$ cells/mL can cause mass mortality in a relatively short time (Figure 2). *Vibrio harveyi* is one of the most common species causing illness and death in crustacean cultivation.



Figure 2. *Vibrio harveyi* Bacterial Cell Form [22]

This bacterium is the cause of the disease of fireflies or fluorescent disease. The infected crustaceans will look bright in the dark (night). This bacterium is the main cause of high mortality rate in crustacean larvae. *Vibrio harveyi* is a bacterial species of luminescent vibriosis in tiger shrimp larvae (*Penaeus monodon* Fabr). Windu shrimp in the zoea phase are most vulnerable to the attack of *V. harveyi* bacteria. So far, synthetic antibacterials are widely used in aquacultures such as Chloramphenicol and Oxytetracycline to inhibit or kill pathogenic bacteria in tiger shrimp. Several studies have been conducted in the utilization of sponge marine biota as an active ingredient to overcome the problems caused by diseases in aquaculture. The sponge is expected to become a standard bactericide in the field of fishery and can be applied for the use of food and in the field of cultivation. Sponge *Geodia* sp has antibacterial activity against *E. coli* and *Vibrio parahaemolyticus* [34]. Sponge *Dendrilla nigra* can control pathogen bacteria *Vibrio harveyi* and *Vibrio alginolyticus* in shrimp and can replace conventional antibiotics [7]. Rough extract of sponge *Hyrtios erectus* can deal with disease problems caused by bacteria in tiger shrimp especially from the genus *Vibrio* [35]. The bioactivity test of the isolate compound from the content of n-hexane extract from *P. alfiani* sponge proved active against shrimp larvae *Artemia salina* Leach with LC50 value of 5,6872 µg / mL (ppm), besides this n-hexane extract and also active against bacteria *E. Coli*, *S. aureus* bacteria and against *Candida albicans* fungi [36]. The content of peptide compounds on the sponge that has many benefits is an important advantage to be utilized as a natural medicine [37].

The sponge has the potential of large bioactive compounds, but the stability of sponge production is a problem that can inhibit the development of commercial processes [38]. Sponge exploitation in the utilization of basic ingredients of the drug is a threat of sponge extinction as a producer of bioactive compounds [16, 39, 40]. Therefore, alternative sponge development is required, in order that the active ingredients derived from the sponge can be used sustainably [38].

IV SPONGE CULTIVATION FOR SUSTAINABLE USE

Sponge cultivation has been conducted since the 1800s in the Mediterranean region, then developed extensively in the Americas, especially Cuba and the Caribbean Islands in the 1970s [41]. In the 19th and early 20th centuries, sponge cultivation technology has been developed and disseminated; but in that period, sponge cultivation only aims to satisfy the demand for bathing equipment [39].

Development of sponge cultivation technology is done because the quality of sponge from nature is so varied that it can not be utilized optimally [41]. Sponge cultivation method is very important to be done so that the conservation of sponge can be maintained so that it can solve the problems in fishery cultivation and support the fishery sector in a sustainable manner.

Some research results in the method of sponge cultivation are:

1. In Situ Cultivation

Sponge cultivation conducted at sea is called in situ cultivation, by conditioning the environment where sponges grow in nature, using longlines [42]. The sponge is cultivated in the form of small pieces that are fragmented from the wild parent sponge (obtained in nature). The explant is then implanted into an artificial substrate, for example, a rope, nylon or plastic plate, or positioned onto a net. This method has been done in the Western Mediterranean [43]. In the Eastern Mediterranean a similar method was performed on the sponge *D. avara* [44], using a stainless steel frame (height: 50 cm) placed on the seafloor and explants of *D. avara* grown with frames on horizontal nylon, with adhesion [43] and by placing the explant on a plastic plate [44]. In addition, explant also has grown on stainless steel containers [43]. The results showed that survival in the Eastern Mediterranean reached (90-100%), while in the Western Mediterranean only (11-70%). This success depends on the method used. Both studies explain that the rate of growth depends on the methodology applied [43, 44] and varies from 40% to 800% increase per year. In addition, has been applied a technique called "spike method" for species sponge *Rhopaloeides odorabile* and *Coscinoderma* sp [42]. The technique concludes that it is not recommended to cultivate this species (a much lower growth rate than other techniques such as cultivation in webs and ropes). Thus it is affirmed that the optimization of aquaculture sponge is a specific species process [42]. The same study also shows an external factor of vulnerability in sponge cultivation [44]. A comprehensive study of sponge cultivation has also been conducted [45,46]. The cultivation of sponges with a cloning system with three experiments, explants obtained from wild parent sponges (called F0), the next two cultivations were made by preparing the explants from the harvested sponge from previous culture experiments

(called F1 and F2) [46]. But from several studies of marine aquaculture is still vulnerable to biological pests. Several studies have demonstrated the feasibility of marine aquaculture for sponge production on a commercial scale, from which it can be concluded that marine aquaculture requires a method suitable for its sustainability. Another conclusion is that successful cultivation procedures are for most specific species [42] and certain sites, the latter shown in different results obtained on *D. avara*, in two different places of the Mediterranean [43,44]. To start sponge cultivation in new species, it is recommended to first conduct a 1-year trial to evaluate productivity in different locations using different methods. The season factor becomes one of the most important considerations in conducting sponge cultivation since growth explant varies greatly throughout the year. Season-related studies have been conducted on four types of sponge by cultivating at the beginning of the experiment at least one complete annual cycle. Recent studies describe year-round growth in *Haliclona oculata* [47], *Corticium wax* [48], *Discodermia dissoluta* [49], *D. erythraenus* [50], and *Mycale hentscheli* [46]. The influence of the seasons is shown in the subtropical climate and from each zone. The growth of subtropical temperate species (*H. oculata*, *C. wax* and *M. hentscheli*) was positively correlated with temperature, while the growth of two tropical species (*D. dissoluta* and *D. erythraenus*) did not show significant seasonal effects.

2. Ex Situ Cultivation

Sponge cultivation with an ex-situ system in an aquarium is an experiment that remains a challenge, although many have succeeded in the cultivation but are still rarely done. The influential factor in this cultivation is the flow rate. Some research development in this cultivation has been done, by better understanding the requirements of sponge cultivation (Table 1). The continuous addition of iron ferric (Fe^{3+} as a supplier of citrate iron) to water in aquariums in oscule formation and pumps for sponge activity in the Mediterranean, *C. reniformis* and *Acanthella acuta* [51]. The present invention is consistent with the results of other studies which show that the ferric iron regulates genes involved in channel formation in primmorphs from sponge *Suberites domuncula* [52]. In addition, it was found that variations in concentration in Fe^{3+} significantly affected the successful cultivation of *Hymeniacidon perlevis* [53]. In addition, there are also studies that examine the function of water pump flow at the level of life of *D. avara* [45]. This species shows the formation of fragile exhalant structures, by the force of the currents caused by higher flow rates of 7.5 cm s^{-1} . In nature, *D. avara* is found to grow only in sheltered habitats where the mean (oscillating) with the flow does not exceed 5.9 cm s^{-1} [54]. From the results of the study, it was concluded that the flow

rate in cultivated containers designed for *D. avara* should not exceed $8\text{-}10 \text{ cm s}^{-1}$ [54]. Furthermore, evidence for flow function has also been investigated, the sponge *M. cecilia* explants have been successfully cultivated in an aquarium of waterlift with the continuous flow of water (unknown flow velocity) [55]. However, the growth of sponges in the aquarium system was three times lower than the growth of marine aquaculture: 65% and 207% in 60 days for each aquarium culture and marine aquaculture [55]. The interaction of microorganisms to sponges in primary and secondary metabolites in situ cultivation in an aquarium is important to know. Analog with in situ has also been studied [50]. The results also show changes in microbial composition when *R. odorabile* explants are transferred from the ocean to the aquarium system [56]. Although some species do not exhibit such changes in experiments using aquariums [57], the aquarium effect appears to be a common phenomenon that occurs among marine animals, some reef aquariums also show dramatic changes in bacterial composition when compared with species existing in nature [58]. Sponge cultures and other invertebrates outside the natural environment can disrupt symbiotic relationships and may inhibit the formation of natural products, as these may be associated with secondary metabolite associations with symbionts. Although sponge cultivation in situ for commercial purposes cannot be realized in the near future, only provides a brief perspective on future design studies. In an ex-situ cultivation system, the influence of the seasons should be noted, therefore, the ex-situ system may be operated in semi-continuous methods (regular seeding with a small harvest of cultivation) rather than taking in large quantities. It has advantages in terms of volumetric productivity and easier maintenance routine [44].

V METABOLITE PRODUCTION IN AQUACULTURE

The important thing to do for the commercial production of aquaculture sponge metabolites is the cultivation of spores to maintain their ability to produce the desired metabolites (Figure 3). Researchers have succeeded in finding a comparable level of avarol and norsesterterpene peroxide acid in sponges in nature and cultivation yields (*D. avara* and *D. erythraenus*) [44,50]. A researcher has

conducted a qualitative test of mycalazal type metabolite in a crude extract from nature, the result of ex-situ cultivation of sponge [55].

Table 1 Growth of Sponge Metabolite of Cultivation [59]

Sponge Species	Cultivation Metabolite	Source
<i>Acanthella cavernosa</i>	The effect is the same as that obtained directly in nature, there is no stress level	Mendola (2003)
<i>Aplysina aerophoba</i>	The resulting alkaloid is slightly greater than that obtained in nature, the light factor slightly inhibits growth	Kloppel et al. (2008)
<i>Axinella corrugata</i>	The level of Stevensine in an aquarium culture is 110-157% of the levels found in nature	Duckworth et al. (2003)
<i>Callyspongia biru</i>	The same level of amphitoxin in marine aquaculture and species obtained in nature; high variability in between Individual	De Voogd (2007)
<i>Diacarnus erythraenus</i>	The same rate of norsesiterpene peroxide acid in aquaculture and the type obtained in nature	Bergman et al. (2011)
<i>Mycale cecilia</i>	Mycalazals are found in species that are in nature, aquaculture and aquarium culture (qualitative analysis)	Carballo et al. (2010)
<i>Negombata magnifica</i>	The concentration of latrunculin B on the cultivated sponge rose three times higher than previously reported in the literature	Hadas et al. (2005)

The results showed a slight increase in metabolite production in *Aplysina aerophoba* on ex-situ cultivation, but sponges did not grow on methods with conditioned aquariums [60]. In addition, it has found a fourfold increase in discodermolide concentration in specimens from *D. dissoluta* from cultivation for 6 months [61]. Table 1 shows the production data of sponge metabolites of in situ and ex-situ cultivation, high variability values, reported values ranging from the fourfold increase to the absence of bioactivity. Factors that determine the metabolites in some sponges are the seasons, depth and location and the existence of the inducing effort with stimuli. But the concentration of metabolites varies in time and space, and there are some species that are independent of the above factors, depending on the technique we use. Recent advances in marine aquaculture to produce natural bioactive sponge producers, using a multitrophic cultivation system (Fig. 3) [42, 44, 46]. Sponges are targeted to grow more rapidly around the ocean [44] and shells [46] when compared to where they came from, where visibility (qualitative measure for organic food availability) is 5-10 times higher [44].

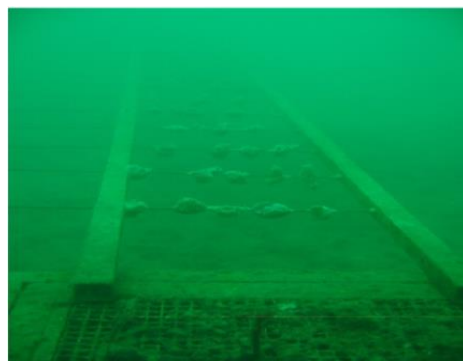


Figure 3 Multitrophic Sponge Cultivation [22]

CONCLUSION

The sponge has a very useful bioactive compound as antimicrobial. Problems in aquaculture are often caused by the existence of several types of microbes that cause failure in cultivation, the prohibition of the use of antibiotics as disease prevention, causing the importance of natural materials research as a basic material anti-microbial so that success in aquaculture can be achieved. Production of bioactive components from the market is commercially limited by the problem of raw

material supply, excessive exploitation can inhibit sponge preservation, in situ and ex-situ cultivation techniques are a feasible method, but several factors affecting growth should also be considered, sponge preservation can be maintained so as to support the success of aquaculture in a sustainable manner.

REFERENCES

1. Kementerian Kelautan dan Perikanan. KKP, Revitalisasi 80 ribu Ha tambak udang, <http://www.kkp.go.id/index.php/arsip/c/2417/KKP-Revitalisasi-80-Ribu-Ha> Tambak-Udang. Diakses 19 Oktober 2015.
2. Ahn, T.A., Kroeze, C., Bush, S.R., dan Mol, A.P.J., 2010, Water pollution by intensive brackish shrimp farming in south-east Vietnam: causes and option for control. *Agriculture water management*, **97**: 872-882.
3. Hanafi, A. dan Ahmad, T., 1999, Shrimp culture in Indonesia: key sustainability and research issue. In: Smith, P.T. (ed.). *Toward sustainable shrimp culture in Thailand and the region, Thailand. Australian Centre for International Research*.
4. Han, J. E., Mohney, L. L., Tang, K. F., Pantoja, C. R., dan Lightner, D. V., 2015, Plasmid mediated tetracycline resistance of *Vibrio parahaemolyticus* associated with acute hepatopancreatic necrosis disease (AHPND) in shrimps, *Aquaculture Reports*, **2**:17-21.
5. Lafferty, K.D., Harvell, C. D., Conrad, J.M., Friedman, C.S., Kent, M.L., Kuris, A.M., Powell, E.N., Rondeau, D. dan Saksida, S.M., 2015, Infectious Diseases Affect Marine Fisheries and Aquaculture Economics, *Annual Review of Marine Science*, **7**: 471 -496.
6. Cock, J., Salazar, M. dan Rye, M., 2015, Strategies for managing diseases in non-native shrimp populations, *Reviews in Aquaculture*, **1**–16.
7. Selvin, J., dan Lipton, A.P., 2003, Leaching and residual kinetics of chloramphenicol incorporated medicated feed treated to juvenile black tiger shrimp *Penaeus monodon Fabricius*, *Fish. Technol.*, **40** (1):13– 17.
8. Hooper JNA., dan Van Soest, R.W.M., 2002, *Systema Porifera. A guide to the classification of sponges*, New York: Kluwer Academic/Plenum Publishers.
9. Hooper JNA., dan Lévi C., 1994, Biogeography of Indo-west Pacific sponges: microcionidae, raspailiidae, axinellidae. In: Soest RWMv, K TMGv, B JC, editors. *Sponges in time and space*, Balkema, Rotterdam.
10. Thakur, N.L., dan Müller, W.E.G., 2004, Biotechnological potential of marine sponges, *Curr Sci.*, **86**:1506–12.
11. Leal, M.C., Puga, J., Serôdio, J., Gomes, N.C.M., dan Calado, R., 2012, Trends in the discovery of new marine natural products from invertebrates over the last two decades--where and what are we bioprospecting? *PLoS ONE*
12. Sipkema, D., Franssen, M.R., Osinga, R., Tramper, J., dan Wijffels, R., 2005, Marine sponges as pharmacy, *Mar. Biotechnol.*, **7**:142–62.
13. Blunt, J.W., Copp, B.R., Keyzers, R.A., Munro, M.H.G., dan Prinsep, M.R., 2012, Marine natural products, *Nat Prod Rep.*, **29**(2):144–222.
14. Blunt, J.W., Copp, B.R., Keyzers, R.A., Munro, M.H.G., dan Prinsep, M.R., 2013, *Nat Prod Rep.*, **30**(2):237–323
15. Huyck, T.K., Gradishar, W., Manuguid, F., Kirkpatrick, P., 2011, Eribulin mesylate, *Nat Rev Drug Discov.*, **10**(3):173–4.
16. Bergman, O., Mayzel, B., Anderson, M.A., Shpigel, M., Hill, R.T., Ilan, M., 2011, Examination of marine based cultivation of three demosponges for acquiring bioactive marine natural products. *Mar. Drugs.*, **9**(11): 2201–19.
17. Bondu, S., Genta, J. G., Leiròs, M., Vale, C., Guigonis, J.M., dan Botana, L.M., 2012, Additional bioactive guanidine alkaloids from the Mediterranean sponge *Crambe crambe.*, *RSC Adv.*, **2**:2828–35.
18. Mayer, A. M. S., Rodriguez, A. D., Tagliatalata Scafati, O., dan Fusetani, N., 2013, Marine pharmacology in 2009-2011: marine compounds with antibacterial, antidiabetic, antifungal, antiinflammatory, antiprotozoal, antituberculosis, and antiviral activities; affecting the immune and nervous systems, and other miscellaneous mechanisms of action, *Mar. Drugs.*, **11**:2510-2573.
19. Newman, D.J., dan Cragg, G.M., 2004, Marine Natural products and related compounds in clinical and advanced preclinical trials, *J Nat Prod.*, **67**(8):1216–38.
20. Pabel, C. T., Joachim, V., Wilde, C., Franke, P., Hofemeister, J., Adler, B., Bringmann, G., Hacker, J., dan Hentschel, V., 2003, Antimicrobial activities and matrix assisted laser desorption/ ionization mass spectrometry of *Bacillus* isolated from the marine sponges *Aplysina aerophoba*, *Marine Biotechnology*, p. 424-434.

21. Bhakuni, D.S., dan Rawat, D.S., 2005, *Bioactive Marine Natural Products*, Berlin/Heidelberg: Springer-Verlag.
22. Faulkner, D.J., 2001, Marine natural products. *Natural Product Reports*, **18**:1–49.
23. Pawlink, J.R., 1993, Marine Invertebrate Chemical Defenses, *Chemical Reviews*, **93**:1911–1922.
24. Uria, A., dan Piel, J., 2009, Cultivation-independent approaches to investigate the chemistry of marine symbiotic bacteria, *Phytochemistry Reviews*, **8**: 401–414.
25. Thomas, T.R.A., Kavlekar, D. P., dan LokaBharathi, P.A., 2010, Marine Drugs from Sponge-Microbe Association—A Review, *Mar. Drugs*, **8**:1417- 1468.
26. Muniarsih dan Rasyid, 2010, Potensi Bakteri Yang Berasosiasi Dengan Spons Asal Barrang Lompo (Makassar) Sebagai Sumber Bahan Antibakteri, *Jurnal Oseanologi dan Limnologi di Indonesia*, **36** (3): 281-292
27. Lee, Y.K., Jung, H.L., dan Hong, K.L. , 2001, Microbial Symbiosis in Marine Sponges, *The Journal of Microbiology*.
28. Tommonaro, G., Iodice, C., Hady, F.K.A., Guerriero, G dan Pejin, B., 2015, The Mediterranean Sponge *Dysidea avara* as a 40 Year Inspiration of Marine Natural Product Chemists, *J Biodivers Endanger Species*.
29. Hastari, R., 2012, Uji Aktivitas Antibakteri Ekstrak Pelepah Dan Batang Tanaman Pisang Ambon (*Musa paradisiaca* var. *sapientum*) terhadap *Staphylococcus aureus*, *Tesis*, Program Pendidikan Sarjana Kedokteran Fakultas Kedokteran Universitas Diponegoro, Semarang.
30. Rume, M.I., Rantetondo, A dan Latama, G., 2011, Isolasi bakteri dari Usus Udang Windu dan Aplikasinya Dalam Upaya pengendalian *Vibrio harveyi* yang menginfeksi Larva Udang Windu (*Penaeus monodon* Fabricius), *Tesis*, Pasca sarjana Universitas Hasanuddin. Makassar.
31. Ismail, N., El, D., Atta, I. N. S., Ahmed, A.E.A.M., 2001, Oral Vaccination of Nile Tilapia (*Oreochromis niloticus*) Against Motile *Aeromonas septicaemia*, *Nature and Science*, **21**-26.
32. Rinawati. N. D., 2010, Daya Antibakteri Tumbuhan Majapahit (*Crescentia cujete* L.) Terhadap Bakteri *Vibrio alginolyticus*, *Skripsi*, Jurusan Biologi, Fakultas Matematika Ilmu Pengetahuan Alam Institut Teknologi Sepuluh November. Surabaya.
33. Badjoeri, M., Identifikasi Bakteri Patogen Pada Sistem Karamba Jaring Apung (KJA) Di Danau Maninjau, *Oldi*, **34** (2) : 1-3.
34. Faikoh, E.N., Yuliana, D.E., Suhendriani, S., dan Aini, H.Q., 2013, Studies of Antibacterials effect of Fresh Soft Coral (*Geodia* sp.) Extract Against *Escherichia coli* and *Vibrio parahaemolyticus* and The Content of Active Compounds, *Jurnal Teknologi Pertanian*, **14**(3):201-208.
35. Youssef, D.T.A., 2005, Department of Pharmacognosy, Faculty of Pharmacy, Suez Canal University, Ismailia 41522, *Egypt, J. Nat. Prod.*, **68** (9):1416–1419.
36. Aqa'id, M.S., 2015. Isolasi, identifikasi, dan uji bioaktivitas metabolit sekunder ekstrak N- Heksana spons *Petrosia alfiani* dari Kepulauan Barrang Lompo, *Skripsi*. Jurusan Kimia, Fakultas Matematika Ilmu Pengetahuan Alam Universitas Hasanuddin, Makassar.
37. Cheung, R.C.F., Ng, T.B., dan Wong, J.H., 2015, Marine Peptides: *Bioactivities and Applications*. **13**: 4006-4043.
38. Murray, P.M., Moane, S., Collins, C., Beletskaya, T., Thomas, O.P., dan Duarte, A.W.F., 2013, Sustainable production of biologically active molecules of marine based origin, *New Biotechnol.*, **6**:839–50.
39. Osinga, R., Tramper, J., dan Wijffels, R.H., 1999, Cultivation of marine sponges, *Mar. Biotechnol.*, **1**:509 32.
40. Pomponi, S.A., 2001, The oceans and human health: the discovery and development of marine-derived drugs, *Oceanography*, **14**(1):78–87.
41. Yi, Q., Wei, Z., Hua, L., Xingju, Y. dan Meifang, J., 2005, Cultivation of marine sponges, *Chin. J. Oceanol. Limnol.*, **23**(2):194 – 198.
42. Duckworth, A., 2009, Farming sponges to supply bioactive metabolites and bath sponges: A review, *Marine Biotechnology*, **11**: 669–679.
43. De Caralt, S., Sanchez-Fontenla, J., Uriz, M. J., dan Wijffels, R. H., 2010, In situ aquaculture methods for *Dysidea avara* (*Demospongiae*, *Porifera*) in the Northwestern Mediterranean, *Marine Drugs*, **8**:1731–1742.
44. Osinga, R., Sidri, M., Cerig, E., Gokalp, S. Z., dan Gokalp, M., 2010, Sponge aquaculture trials in the East-Mediterranean sea: New approaches to earlier ideas, *The Open Marine Biology Journal*, **4**:74–81.
45. Page, M. J., Northcote, P. T., Webb, V. L., Mackey, S., dan Handley, S. J., 2005, Aquaculture trials for the production of biologically active

- metabolites in the New Zealand sponge *Mycale hentscheli* (Demospongiae: Poecilosclerida), *Aquaculture*, **250**:256–269.
46. Page, M. J., Handley, S. J., Northcote, P. T., Cairney, D., dan Willan, R. C., 2011, Successes and pitfalls of the aquaculture of the sponge *Mycale hentscheli*, *Aquaculture*, **312**:52–61.
47. Koopmans, M., dan Wijffels, R. H., 2008, Seasonal growth rate of the sponge *Haliclona oculata* (Demospongiae: Haplosclerida), *Marine Biotechnology*, **10**:502–510.
48. De Caralt, S., Uriz, M. J., dan Wijffels, R. H., 2008, Grazing, differential size-class dynamics and survival of the Mediterranean sponge *Corticium candelabrum*. *Marine Ecology Progress Series*, **360**:97–106.
49. Zea, S., Castellanos, L., Valderrama, K., Puentes, C. A., Gomez-Leon, J., dan Pomponi, S. A., 2010, *Potential of antitumoral (p)-discodermalide production by the Caribbean sponge Discodermia dissoluta*. In “VIII World Sponge Conference, Girona”.
50. Bergman, O., Haber, M., Mayzel, B., Anderson, M., Shpigel, M., Hill, R., and Ilan, M., 2011, Marine-based cultivation of *Diacarnus* sponges and the bacterial community composition of wild and maricultured sponges and their larvae, *Marine Biotechnology*, p.1–14.
51. Osinga, R. dan Kotterman, M., 2007, *Ferric iron promotes the formation of oscules: Observations on sponges in aquaria*. In “*Porifera Research—Biodiversity, Innovation and Sustainability*” (M. R. Custodio, G. Lo`bo-Hajdu, E. Hajdu and G. Muricy, eds). 2007; p. 497–502. Museo Nacional, Rio de Janeiro.
52. Krasko, A., Schroder, H. C., Batel, R., Grebenjuk, V. A., Steffen, R., Muller, I. M., dan Muller, W. E. G., 2002, *Iron induces proliferation and morphogenesis in primmorphs*.
53. Xue, L., dan Zhang, W., 2009, Growth and survival of early juveniles of the marine sponge *Hymeniacidon perlevis* (Demospongiae) under controlled conditions, *Marine Biotechnology*, **11**:640–649.
54. Mendola, D., 2008, *The importance of water flow for culture of Dysidea avara sponges*, *Bioprocess Engineering Wageningen University, Wageningen*.
55. Carballo, J. L., Yanez, B., Zubia, E., Ortega, M. J., dan Vega, C., 2010, Culture of explants from the sponge *Mycale cecilia* to obtain bioactive mycalazal-type metabolites, *Marine Biotechnology*, **12**:516–525.
56. Webster, N., Cobb, R., Soo, R., Anthony, S., Battershill, C., Whalan, S., dan Evans- Illidge, E., 2011, Bacterial community dynamics in the marine sponge *Rhopaloeides odorabile* under in situ and ex situ cultivation, *Marine Biotechnology*, **13**:296–304.
57. Gerce, B., Schwartz, T., Voigt, M., Ruhle, S., Kirchen, S., Putz, A., Proksch, P., Obst, U., Syldatk, C., dan Hausmann, R., 2009, Morphological, bacterial, and secondary metabolite changes of *Aplysina aerophoba* upon long-term maintenance under artificial conditions, *Microbial Ecology*, **58**:865–878.
58. Kooperman, N., Ben-Dov, E., Kramarsky-Winter, E., Barak, Z., dan Kushmaro, A., 2007, Coral mucus-associated bacterial communities from natural and aquarium environments, *FEMS Microbiology Letters*, **276**:106–113.
59. Schippers, K.J., Sipkema, D., Osinga, R., Smidt, H., Pomponi, S.A., Martens, D.E., Wijffels, R.H., 2012, Cultivation of sponges, sponge cells and symbionts: achievements and future prospects, *Marine Biology*, **62**.
60. Kloppel, A., Pfannkuchen, M., Putz, A., Proksch, P., dan Brummer, F., 2008, Ex situ cultivation of *Aplysina aerophoba* close to in situ conditions: Ecological, biochemical and histological aspects from the marine sponge *Suberites domuncula*, *DNA and Cell Biology*, **21**:67–80.
61. Selvin, J., dan Lipton, A.P., 2004, *Dendrilla nigra*, a marine sponge, as potential source of antibacterial substances for managing shrimp diseases, *Aquaculture*, **236** : 277–283.