Nanobubble technology in synbiotics production for animal husbandry and fisheries by the minari small business group

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Abstract. This activity aims to carry out the process of dissolving oxygen improved in a symbiotic mass culture media using nanobubble injectors conducted by the MINARI small business group under the supervision of Universitas Hasanuddin. Synbiotic is used by fish farmers, breeders, and farmers to enhance growth, immunity in fish and shrimp, and host resistance to pathogenic infections. Observations were made in January - August 2019. The results of the activity showed that Bacillus subtilis cultured was aerated using a nanobubble could increase double of dissolved oxygen concentration. Applying the nanobubble at culture media also increasing the growth of B. subtilis bacteria and speeding up harvest time of synbiotic. The nanobubble aeration technique could increase synbiotics production by 5 times. Increase synbiotic production causes the number of sales also increase and expanding the marketing area. Before using Nano Bubble Technology, this group only supplied 8 regencies-cities totalling 289 L synbiotics (January-April). After using nanobubble technology, production increased to 709 L and supplied 13 districts-cities (May-August). The three regions with the highest demand for synbiotics are Soppeng Regency (262 L), Palopo City (142 L), and Sidrap Regency (110 L).

1. Introduction

Nowadays, synbiotics have become an important commodity in the livestock, fisheries and agriculture industries. Synbiotics are nutritional supplements that combine probiotics and prebiotics in the form of synergism [1]. Probiotic is a term used in living microorganisms that can give good effects or health to other organisms. Whereas Prebiotics are food that cannot be digested by the host but has a beneficial effect on the host by stimulating the growth and activity of normal microflora in the host digestive tract [2].

Based on several studies showing that synbiotics given through feed can effectively increase growth [3], increase immunity in fish and shrimp, and increase host resistance to pathogenic infections. [4], from the results of his study reported that the administration of synbiotics through feeds resulted in improved growth performance, immune response, and resistance of vannamei shrimp to infection of the IMNV virus (infectious myonecrosis virus). Synbiotics as feed additives are also good for chicken
growth performance [5]. Synbiotics are also widely used in aquaculture to improve water quality in aquaculture.

Synbiotics are easy to produce if they know important bacterial groups and how to handle them. There are hundreds of bacteria and microorganisms that have been identified as good for health such as the Bacillus, Thiobacillus, yeast (Saccharomyces cerevisiae) and micro-algae (Tetraselmis sp) groups. The combination of certain microorganisms and with certain prebiotics can produce high-quality synbiotics for various organisms.

With the increasingly widespread use of synbiotics, production technology and products are also growing. Synbiotics industry is now starting to grow among the people because this industry does not need a large place so that it can be done at home. The MINARI group is a micro-business group engaged in the production and sale of synbiotics for agriculture, animal husbandry and fisheries.

However, the problems experienced by partners, especially in terms of production. The biggest problems are mainly in the supply of oxygen for culture media and aerobic bacterial inoculums and stirring is still done using aquarium aerators and is stirred manually. Oxygen is needed for the development of an individual/cell and its population. Oxygen limitations cause a long culture period with a maximum capacity of 20 L for each culture period or 100 L per month.

Therefore, this activity aims to carry out the process of dissolved oxygen enrichment in probiotic mass culture media using nanobubble technology. The presence of a nanobubble is expected to enrich the concentration of dissolved oxygen in the water thereby increasing the quality of probiotics.

2. Material and methods

2.1 Starter bacteria
This study was conducted from January to August 2019 at Makassar. Bacteria were used for a starter to produce symbiotic was Bacillus subtilis that has been isolated at Microbiology Laboratory, Faculty of Marine Science and Fisheries, Universitas Hasanuddin.

2.2 The process for producing symbiotic
Bacillus subtilis bacteria as a starter were cultured at 100 L bucket using enriched water with nanobubble and nutrient (molasses). The process to produce synbiotic was 4 hours/100 L. After 4 hours of culture, the media was enriched by the nanobubble, the cultured container was closed tightly to create an anaerobic condition for 7 – 10 days. After this process, synbiotic bacteria were harvested.

2.3 Measurement variable and data analysis
In this study, a variable was measured namely, dissolved oxygen concentration, the number of bacteria cells that could be produced for one period of culture. Data were analyzed descriptively using tables and graphs.

3. Results and discussion

3.1. Increasing dissolved oxygen concentration in culture media
Dissolved oxygen concentration in culture media which used conventional aeration was accounting 3 – 4 ppm, meanwhile when culture media was aerated using nanobubble, dissolve oxygen concentration was increasing significantly accounting for 7–11 ppm. Using the nanobubble also increased temperature in culture media, which was 39°C. The increasing temperature was due to the increasing fraction between water and mineral in the culture media. Consequently, this increasing temperature affected several factors, such as affecting dissolved oxygen in culture media, increasing the growth of B. subtilis bacteria and inhibiting the growth of pathogen bacteria.

Pathogen bacteria will die in condition on high temperature and dissolved oxygen in culture media. B. subtilis bacteria were mesophilic that could grow optimally at 25°C–30°C. This bacterium also has eurythermal which could tolerate the high range of temperature changing.
3.2. Growth of Bacillus subtilis
Using the nanobubble in culture media could speed up bacteria growth and increasing the production of bacteria per volume of water. The same amount of *B. subtilis* starter was used for conventional and nanobubble aerator. After 24 hours *B. subtilis* culture using a conventional aerator, it produced $2.2 \times 10^{10}$ CFU/mL. However, the growth of bacteria after applying the nanobubble in cultured media for 4 hours accounting for $2.2 \times 10^{12}$ CFU/mL. This finding also indicated that culture media that aerated by nanobubble could increase *B. subtilis* bacteria growth and speed up harvest time of synbiotics.

3.3. Production and selling of synbiotics

![Production and selling of synbiotics for 8 months of activity.](image_url)

Figure 1 showed that there were lower production and selling of synbiotics for January to April 2019. The low production of synbiotics those months due to the synbiotics culture used a conventional aerator, meanwhile for May to August, the culture used the nanobubble technique. The highest production of synbiotics was found in May accounting for 390 L and the lowest production was 62 L in February when using a conventional aerator. The result showed that the production of synbiotics using nanobubble aeration was almost 5 times higher than those of using conventional aerators. This finding indicated that the production of synbiotics increased significantly when the *B. subtilis* culture used nanobubble aeration. Increasing production of synbiotics ideally followed by increasing of selling. However, our data showed that the increased production is not linear with increasing selling, consequently, a small-scale business of synbiotics could not get a maximum profit. To solve this problem, it was required more promotion regarding this product, especially focusing on the advantage of synbiotics in stimulating growth and improving the immune system of cultured organisms.

Table 1 showed that the selling area of synbiotics was increasing when the production of synbiotics using nanobubble aeration. There were 8 areas of synbiotics selling when the production using a conventional aerator, however, the area of synbiotics selling was increasing accounting for 13 areas. The highest selling of synbiotics production was at Soppeng Regency accounting for 262 L while the lowest area of synbiotics production selling was Gowa, it was only 4 L. The farmer use synbiotics mostly for tilapia culture and chicken poultry.
Table 1. The number of synbiotic sales in several areas in South Sulawesi in the period of January to August 2019.

| Area of selling | Jan - April (L) | May - August (L) |
|-----------------|-----------------|------------------|
| Soppeng         | 110             | 262              |
| Sidrap          | 40              | 110              |
| Makassar        | 15              | 38               |
| Pangkep         | 10              | 70               |
| Palopo          | 55              | 142              |
| Takalar         | 30              | 87               |
| Gowa            | 4               | 4                |
| Mamasa          | 25              | 85               |
| Maros           | 50              |                  |
| Barru           | 20              |                  |
| Pinrang         | 35              |                  |
| Jeneponto       | 60              |                  |
| Malili          | 35              |                  |

4. Conclusions

*Bacillus subtilis* cultured was aerated using a nanobubble could increase double of dissolved oxygen concentration. Applying the nanobubble at culture media also increasing the growth of *B. subtilis* bacteria and speeding up harvest time of synbiotic. The nanobubble aeration technique could increase synbiotics production by 5 times.

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