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Bioconversion of Fish Hatchery Waste as Feed in the Production of Live Feed

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Abstract

Purple Non-Sulfur Bacteria (PNSB), also known as phototrophic bacteria are widely distributed in both freshwater and marine environment and capable to grow in wide range of substrates. In this study, Bacterium *Rhodobacter sphaeroides* strain UMS2, a freshwater isolate was used in this study in utilization of fish hatchery waste. This study was conducted to determine the nutritional values of bioprocess product that was grown in fish hatchery waste. Finally, the waste bio-converted product was used as feed supplement to monitor the growth performance of live feed *Tubifex* spp. Inoculum of *Rhodobacter sphaeroides* strain UMS2 was developed in 112 synthetic media and 48-h culture of 30% (v/v) inoculum was used in fish hatchery waste during the bioprocess. The nutritional values of bio-converted product, except total ash (%), were not significantly improved with 30% (v/v) inoculum of *Rhodobacter sphaeroides*, strain UMS2. Feeding trial in bloodworm (*Tubifex* spp.) with bioconversion product conducted for 15 days to monitor growth (w/v) of live feed. Initial growth 1.42 ± 0.001 g/L of *Tubifex* spp. was stocked in 15 × 75 × 15 cm plastic tray connected with recirculated system. *Tubifex* spp. was observed to be comparatively higher (1.55 ± 0.12 g/L) while fed in the product that contained bacterium than the growth (1.44 ± 0.15 g/L) of *Tubifex* spp. fed in the bioconversion product of without bacterium. The inoculums size (30%) of bacterium not enough to support the growth of *Rhodobacter sphaeroides*, strain UMS2 in the bioconversion process to improve the nutritional values. However, while used as feed supplement it improved the growth performance of the *Tubifex* spp. So, bacterium *Rhodobacter sphaeroides*, strain UMS2 has potentiality to be used as feed supplement in the production of live feed.

Keywords

Hatchery Waste, Bioprocess, Microbe and Live Hatchery Waste, Bioprocess, Microbe and Live Feed Growth

1. Introduction

The waste generated from fish hatchery mainly contained 7% - 32% of the total nitrogen and 30% - 84% of the total phosphorus [1]. Nutrient rich waste in long run if discharged that ultimately causes eutrophication problem. Most of the hatchery designed to save water in a recirculation system. The conventional method for the treatment of wastewater in a recirculation process which includes solids removal, ammonia oxidation, aeration and disinfection [1]. Two types of waste generated from UMS finfish hatchery, soluble and solid waste and they are both organic and inorganic. The wastewater from UMS finfish hatchery is not different from that of a production farm in terms of quality and quantity of waste. The wastewater from UMS finfish hatchery not only generated from larval rearing and fry production units, but also from other culture units [2]. The concentration of dissolved inorganic nutrients, such as ammonia of 4.38 mg/L, nitrate (3.37 mg/L) and phosphate of 8.55 mg/L in UMS hatchery were observed higher [2] compared to that reported by other researchers [3]. The nutrients rich non-conventional resource can properly be utilized into value added substances. It is therefore necessary to develop new research application of converted this waste to a value-added product rather than to use uneconomic conventional method for treatment.

Purple non-sulfur bacteria the largest group in phototrophs are the potential species to convert wastes into values added product. Bioconversion of waste has been proven to be effective in the increase of nutrients in culture media. Purple non sulfur bacteria (PNSB) capable in bio-conversion of agro-based industrial waste into nutritionally value added product [4]. This group of bacteria grew well in wastewater could have two folds benefit: production of single cell protein and the cost of wastewater treatment could be reduced. PNSB bacterium *Rhodovulum sulfidophilum* for production of bacterial biomass with concomitant treatment of sardine processing effluent [5], *Rhodocyclus gelatinosus* as best substrate for biomass production dilution of tuna condensate 1:10 (v/v) with shrimp blanching [6], *Afifella marina* strain ME (KC205142) production of exopolymeric substances [7], *Rhodobacter sphaeroides* strain UMSFW1 production of biomass with the reduction of chemical oxygen demand in Palm Oil Mill effluent [8], *Rhodopseudomonas* spp. for the production of biomass and carotenoids in synthetic sugar wastewater [9], *Rhodobacter blasticus* and *Rhodobacter capsulatus* in the reduction of pollutant load in swine wastewater [10], production of *Rubrivivax gelatinosus* in poultry slaughterhouse wastewater [11] and mixed photosynthetic bacteria in treatment of agrobased industrial wastewater with the production of single cell protein [12] are well documented. In addition, waste grown PNSB biomass are rich in especially crude protein can be used as feeding additive and diet of cultured animal to reduce the cost and provide better nutritional composition of cultured animals. The self-flocculated bacterium *Rhodovulum* sp. is not only rich in high quality protein, but also contains significantly large amounts of carotenoid pigments, biological co-factor and vitamins

that might enhance the growth and survival in fish [13]. Bacterial cells *Rhodobacter sphaeroides*, grown in pineapple wastewater and *Rhodocyclus gelatinosus* cells grown in cassava waste used in diets improves the growth red tilapia and fancy carp [14], fish growth hormone gene into *Rhodobacter* sp. strain NKPB0021 in diets accelerate the growth of fish [15], *Rhodospirillum rubrun* with fish meal improves the growth and survival of juvenile fresh water prawn [16], *Rhodovulum sulfidophilum* combined with commercial tilapia feed improves the skin pigmentation and growth [17], *R. sulfidophilum* with *Skeletonema costatum* supported better growth and survival of *P. monodon* larvae reared from the nauplliar to post-larval stage [18] and improvement in growth and survival in Asian sea bass larvae in *R. sulfidophilum* sp. [19] have reflected the uses of PNSB in aquaculture industry. However, Information is limited in the use purple non-sulfur bacteria to utilize fish hatchery waste in the bioprocess to improve the nutritional values of biomass and possibility to be uses as feed supplement in live feed production for aquaculture industry. Aquatic worm, *Tubifex* spp. is one of the potential live feed in aquarium industry. Until now this live feed comes from the wild harvest which is unreliable, inadequate, have many parasite and diseases and hazardous to collect for unhealthy conditions [20]. It is essential to develop a technique for mass production of Tubificid to get reliable supply of the growing demand of this live feed in controlled environment. No information is available on the use of live feed, *Tubifex* spp. grown on derived product from bioprocess fish hatchery waste. This study was conducted to determine the nutritional values of products derived from the bioprocess of hatchery waste with *R. sphaeroides* Strain UMS2 and suitability to use as feed supplement in production of live feed.

2. Materials and Methods

2.1. Collection of Fish Hatchery Waste (FHW)

Fish Hatchery Waste (FHW) was collected from the Fish Hatchery of University Malaysia Sabah, Sabah. Only discarded or thrown FHW was collected from the area such as in tank culture of freshwater species, Tilapia and Catfish. The collected FHW was immediately transported to Borneo Marine Research Institute (BMRI) Biotechnology Laboratory for further process. The FHW then placed into the oven for complete dry at 70˚C for 24 h and grinded into fine powder (smaller than 300 mm). Dried Hatchery Waste Powder (HWP) was used in bioconversion process.

2.2. Preparations of Inoculums

Purple non-sulfur Bacterium, *Rhodobacter sphaeroides* Strain UMS2 was taken from the Borneo Marine Research Institute (BMRI) culture collection, which was isolated from mud of College Excellent in Campus of University Malaysia Sabah (UMS). The bacterium was incubated in 112 synthetic media for inoculums preparation. Synthetic 112 media is specific media used for the better

growth of purple non-sulfur bacteria. The composition of 112 media was yeast extract (10 g), di-potassium hydrogen sulphate (1 g) and magnesium sulphate (0.5 g). All the ingredients of 112 synthetic media were mixed well in one-liter distilled water and 29 ml was dispensed into several 30 ml universal bottles. The bottles were autoclaved at 121˚C for 15 minutes for inoculum preparation. From the stock one ml of the stock culture was dispensed in 29 ml of previously autoclaved universal bottles contained 112 media and incubated anaerobic under 2500 lux of light intensity at 30˚C ± 2˚C for 72-h. Subsequently inoculum was developed in 1 L Schott bottle. The inoculum developed in 30 mL was used to make 100 mL inoculum and 48-h culture of 100 mL inoculum was used to prepared 1 L inoculum.

2.3. Bioprocess of Hatchery Waste Powder (HWP)

20 g of previously dried hatchery waste powder (HWP) was mixed in 800 ml of 112 synthetic media in 1-liter Schott bottle. *Rhodobacter sphaeroides* inoculums of 30% from 48-h culture was inoculated into each bottle. The bottles were incubated aerobically under 2500 lux of light intensity at 30˚C ± 2˚C for 6 days. The products derived from the bioprocess of HWP was collected after centrifuged at 4000 rpm for 30 minutes. The obtained biomass was dried to a constant moisture level in oven at 60˚C ± 1˚C. The powder biomass was packed in airtight seal plastic bag and kept at room temperature until used.

2.4. Feeding Trial in Bloodworm (*Tubifex* sp.) Using Bioprocess HWP Derived Product

Two diets were used in feeding trial. Diet 1 HWP derived product with bacterium (*Rhodobacter sphaeroides*) and Diet 2 without bacterium of *Rhodobacter sphaeroides*. The diets without bacteria content (*Rhodobacter sphaeroides*) that derived from the bio-converted products used as the control. The both diets were grinded and used to feed *Tubifex* spp. in powder form. Feeding trial was conducted in Integrated Multi-trophic Aquaculture (IMTA) research area at University Malaysia Sabah, Malaysia.

2.5. Other Experimental Protocols in Feeding Trial

Culture unit: *Tubifex* spp. was in plastic tray, size of 15 × 75 × 15 cm, and with water depth of 10 cm with closed recirculating system. Fine sand was used as substrate to settle *Tubifex* spp.

Collection: *Tubifex* worm was purchase from local markets. They were placed in a beaker and randomly taken for stocking. Initial growth of 1.42 ± 0.001 g/L of *Tubifex* spp and was stocked at the rate of 2.5 mg/cm^2 in culture tray.

Feeding: The worms was fed with 10 g (dry wright) of diets two times in a day (8:00 am and 4:00 pm). During feeding the water flow was stopped for 20 min.

Culture condition: The water flow rate of 1.23 ± 0.33 L/min was maintained to keep up dissolved oxygen between 5 - 6 mg/L. The temperature 25˚C - 27˚C was maintained in production of *Tubifex* worm.

Duration: Experiment was conducted for 15 days, in the month of July 2019 with three replications. Final total weight (g/L) was taken with balance at the end of experiment.

2.6. Analytical Parameters

The proximate compositions of initial and bioprocessed product were carried out with the standard methods [21]. Estimation of crude protein (%) was done using KjeltecTM 2300 Auto-analyzer Unit, crude fiber (%) after hydrolysis with strong acid and alkali using FibertecTM 1020 and crude lipid (%) extracted in petroleum ether using SoxtecTM System 2043 Extraction Unit of Foss Tecator, Sweden, and crude ash (%) was determined using muffle furnace.

2.7. Statistical Analysis

T-test was used to determine significance difference (5% level) between the growth performances of bloodworm (*Tubifex* sp.) fed with two diets using SPSS version 24.

3. Results

Ash and crude protein are the major component obtained in Hatchery Waste Powder (HWP) was used in the bioprocess (Table 1). At the end of 6th day, the derived product that was harvest after bioprocess with *Rhodobacter sphaeroides* shows very little improvement except ash (Table 2). On the other hand, reduction of crude fiber from 1.02% to 0.03% as observed same in the derived product bioprocessed with bacterium and without bacterium. However, proximate compositions of the bioprocess HWP were increased, except crude fiber while PNSB bacterium *Rhodobacter sphaeroides* was used as inoculum (Table 2).

At the end of 15 days experiment the mean final weight of *Tubifex* spp. was observed significantly higher while fed with Diet 1 (derived product with *Rhodobacter sphaeroides*) than fed with Diet 2 (derived product without *Rhodobacter sphaeroides*). There observed significantly differences ($F = 15.63$; $p = 0.00$) in the growth performance in live feed fed with Diet 1 and Diet 2 (Table 3).

Table 1. The proximate compositions of Hatchery Waste Powder (HWP).

Type of Waste:	Crude protein (%)	Crude ash (%)	Crude lipid (%)	Crude fiber (%)	Moisture (%)
Hatchery Waste Powder (*HWP*)	19.78	34.58	5.85	0.07	5.73

Table 2. Proximate composition of derived products after bioconversion of Hatchery Waste Powder (HWP) with *Rhodobacter sphaeroides*.

Rhodobacter sphaeroides	Crude protein (%)		Crude ash (%)		Crude lipid (%)		Crude fiber (%)		Moisture	
	Day 0	Day 6	Day 0	Day 6	Day 0	Day 6	Day 0	Day 6	Day 0	Day 6
With bacterium	19.78	20.90	34.58	89.52	5.85	5.90	1.02	0.32	5.73	4.65
Without bacterium	19.78	19.88	34.58	59.90	5.85	5.88	1.02	0.32	5.73	3.12

Table 3. Growth performance of live feed, *Tubifex* spp while fed with two types of diets.

Diets	Initial weight of Tubifex (g/l)	Final weight of Tubifex (g/l)
Diet 1 derived product with *Rhodobacter sphaeroides*	1.42 ± 0.001^{a} g/L	1.55 ± 0.12^{a} g/L
Diet 2 derived product without *Rhodobacter sphaeroides*	1.42 ± 0.001^{a} g/L	1.44 ± 0.15^{b} g/L

Values are express mean ± SD, different subscript shows significant differences.

4. Discussion

The variation in the organic and inorganic composition of finfish wastes are influenced by certain factors, such as, types of rearing tank, culture techniques, types of species and sizes of species, feed types and feed management, dynamics of nutrient in circulation and utilization, species handling techniques and the other physical chemical environment of the culture areas [22]. The concentration of nutrients in aquaculture system increased with excess use of feed that remains un-eaten as well from the accumulation of fish excreta until bacterial activity. Total solids generated in any aquaculture system from the uneaten fraction of feed as well as the excreted product from the fish gradually dissolved in water and increase the inorganic component so nutrients. The solid generated in UMS hatchery in the range of 75 - 82 mg/L comparatively higher [2] than reported by other researchers. The concentration of DIN, such as ammonia of 4.38 mg/L, nitrate (3.37 mg/L) and phosphate of 8.55 mg/L in UMS hatchery were observed higher [2]. Normally the concentration of nutrients in were in the range of 0.12 - 14.7 mg/L of NH_4-N, 0.02 - 1.5 NO_2-N mg/L, 0.01 - 5.3 mg/L of NO_3-N, and 3.1 - 17.7 PO_4-P mg/L [3]. In addition, opportunistic microbes in the system start to breakdown solids and converted into bacterial biomass while reducing the DIN in water. Nutrients compositions are important in further use of these wastes to convert value added products in bioconversion process. Purple non-sulfur bacteria (PNSB) are well known with its bio-transformation characteristic due availability in wide range of natural inhabitant [23] PNSB also known as well with their characteristic organic transformation [24]. The purple non-sulfur bacterium, *Rhodobacter sphaeroides* was expected to be increased the nutritional value of hatchery waste; in term of dry biomass such as crude protein, crude lipid and crude ash; under standard liquid state fermentation with light intensity of 2500 lux at 30˚C ± 2˚C temperature [25]. Thus, the UMS fish hatchery effluent possesses more than sufficient nutrients to support the growth PNSB that can be used as substrate in culturing *Rhodobacter sphaeroides* Strain UMS2 for the production of bacterium biomass in controlled environment [2]. Under the presence of *Rhodobacter sphaeroides*, the higher amount crude protein of hatchery waste biomass was expected, but it shows little improvement in present study. It indicated bacteria was unable to growth in this bioprocess system. The bacteria that inoculated might convert some hatchery waste protein or other nitrogenous compounds that available in environment into cell protein for

microbial biomass production. Low performance in the improvement of protein reflects the growth of other opportunistic bacteria that remain in the HWP. The inoculum sizes used in present study was 30% (v/v), might not enough to support to utilize the substrate in bioconversion process, fail to convert nutrients into biomass protein. Not only protein other nutritional components like fiber and lipid also did not improved at desired levels, with 30% (v/v) inoculum. Nutritional values of vegetable waste during bioprocess with PNSB bacterium was observed better with 30% level of inoculum *Afifella marina* strain ME. The highest crude protein of 18.95% was recorded with 30% inoculum size. The crude lipid increased the highest value at 1.70%, 1.65% and 1.49% for 10%, 20%, and 30% inoculum respectively. The maximum crude ash yielded 32.55% with inoculum size of 30% level of inoculum *Afifella marina* in bioprocess of vegetable waste [25]. Optimum size of inoculum is important because generally, the bacterial population is strongly heterogeneous and it takes time for all sub-populations to adapt to the new conditions. Soon *et al.* (2013) [26] The inoculum size of 20% (v/v) *Afifella marina* used to observe the effect of light intensities and photoperiod of production of extracellular nucleic acids. Growth characteristic *of Afifella marina* strain ME (KC205142), as well as production of exopolymeric substances like enzymes and nucleic acid has been documented [7]. The increment of crude protein observed under the presence of bacteria might be due to the increasing of biomass of with higher level of inoculum [27]. The inoculated bacteria might convert some waste protein or other nitrogenous compounds available in the environment into cell protein for microbial mass production [28]. Other than inoculum sizes the optimum period in bioprocess for crude protein production is day 6 in current study was shorter than reported by other researcher [28], which suggested best harvesting on day 8 and day 7 for maximum crude protein production. The optimum proteolytic activity was recorded at 48 h of incubation, which also explains the high level of crude protein at Day 4 [7]. In addition, the extracellular proteases within the bacterium extracellular polymeric substances matrix play an important role in providing nutrients and alter extracellular polymeric substances composition [7]. However optimum time for incubation in present study was not taken in account which need further investigation.

Growth characteristics of live feed like *Tubifex* spp. depend on the media or substrate and other environmental parameters of the culture condition. The mean final weight of *Tubifex* spp. in present feeding trial was observed 1.55 g/L significantly higher while fed with Diet 1 (derived product with *Rhodobacter sphaeroides*) than growth of 1.44 g/L while fed with Diet 2 (derived product without *Rhodobacter sphaeroides*) at the end of 15 days. *T. Tubifex* grew slowly and attained a body weight of about 1.5 mg during the initial period of 28 days; this was followed by the logarithmic growth phase for a subsequent period of 14 days; after the 42nd day the maximum body weight stabilized at around 7.5 mg [29]. In this aspect the growth observed in present is seems satisfactory. The op-

timum parameter to grow are concentration of dissolved oxygen in the range of 6 - 7 mg/L, pH 7.0 - 7.2, water temperature 27.5 - 28.0, with flow rate of 200 to 250 ml/min [30], which was also maintained in present study. The worms selectively ingest silt and clay particles at depth and digest the attached microflora, primarily bacteria. They fed on the organic material, algae and bacteria lived in the sediment. Tubificid fed on the organic debris and bacteria lived in the sediment, but also fed on decayed vegetable waste [31]. Species of tubificid worms able to feed on waste organic materials such as sewage sludge and cattle excrement. The use of organic fertilizers in culture media including the wastes/faeces of quail, goat and chicken mixed with the rejected bread and tofu so far been conducted as the use of organic fertilizer could impact the growth performance and nutrients content of *T. Tubifex* [32]. Growth performance in present study indicated that diet composed of purple non-sulfur bacterium, *Rhodobacter sphaeroides* performed better than the diet composed without *Rhodobacter sphaeroides* in feed. Tubifex worms fed with decayed vegetables and yeast had growth higher than fed with minced fish and jellies, but no significant differences were observed between fed with decayed vegetables and yeast [31]. Fermented organic matter as substrate for the production and improve the nutritional values of Tubificid worms. The fermented process includes preparation of fermented molasses, water, and probiotic activator which are bacteria *Sacharomy cescerevisae* and *Lactobacillus* sp. [32]. The fermentation of the organic matter has been proven to be effective in the increase of the nutrient of culture media. During fermentation the organic matter would be easily digested and used for having experienced alteration by the bacteria [33]. No information available regarding the probiotic effects of PNSB live feed sector. However, PNSB while used as feed additive it accelerated the growth and survival of aquaculture species. The nutritional value of phototrophic bacteria clearly indicated that it could be used as a potential protein supplement. Survival rate of carp increased 96.5% when PSB was fed at 0.1% supplemented with commercial feed [13]. Phototrophic bacterial cells that were used in the purification of wastewater utilized as food by plankton, fish, and could also be used as a feed for the cultivation of *Artemia salina* (brine shrimp). Phototrophic bacteria have some anti-virus compounds that suppress viral diseases of shrimp as well as shellfish [34]. In Japan, gill disease of prawn was prevented completely by adding the anoxygenic phototrophic bacteria in the tank [13]. Addition of three species of PNSB such as, *Rhodobacter sphaeroides*, *Rhodobacter capsulatus* and *Rhodopseudomonas palustris* observed to be increase weight in prawn during grow-out culture and improve water quality when fed with phototrophic bacteria, as ammonia nitrogen was observed to be significantly lower [35]. Seed production of *Penaeus chinensis* fed a with mixture of four strains of *Rhodopseudomonas* sp. shows grazing ability of shrimp. The most striking fact was faster metamorphosis (one day earlier) than the pond supplied with normal diet [36]. Further the water quality was observed to be improved with the addition of waste grown PNSB bacterium *Rhodovulum sulfi-*

dophilum in of *Penaeus monodon* larval rearing period [18]. However, the use of various strains of PNSB in production and improvement of nutritional values of live feed need more investigation. Decomposition of waste has been proven to be effective in the increase of nutrients in *Tubifex* culture. *Tubifex* spp. grown on such bioprocessed product might have a better organoleptic characteristics and nutritional components, which need further investigation. More comprehensive and detail studies should be conducted to evaluate the bioprocess of hatchery waste as complete diet for the live feed production.

5. Conclusion

Purple non-sulfur bacterium, *Rhodobacter sphaeroides* strain UMS2 play a prominent role in improving the nutritional value of fish hatchery waste during in the bioprocess. The harvesting of bioprocess product from fish hatchery waste could be improved only with the optimum inoculum size and incubation period, 30% (v/v) and six days incubation not suitable to increase the nutritional values. Based on the current study, the ability of *Rhodobacter sphaeroides* strain UMS2 in the utilization of fish hatchery waste is promising. The advantages of using of bioprocess product in live feed supplement to increase growth performance in *Tubifex* spp. have potentiality for production in controlled environment. The only limitation of this study was duration of the feeding trial. It would be better to have continuous production of *Tubifex* spp. to get enough biomass for live feed for aquaculture species. The product might have potential as supplement or feed additive in the aquaculture live feed industry.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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