INTRODUCTION

1.0 General

The increasing world population has led to a continuous demand for food. More emphasis has been given to agriculture and food sectors. Further, encouraging achievements have been made in aquaculture biotechnology to increase its production. Genetically-improved farmed fishes for high yielding, development of pigmentation in fish with the manipulation of artificial feed and production of single cell protein (SCP) as aquaculture feed (yeast, bacteria and microalgae) have been successfully carried out by many workers (Lyons 1990; Coutteau et al. 1990; Kiessling & Askbrandt 1993; Rodriguez et al. 1994; Manju & Dhevendran 1997). On the other hand, fish nutritionists are looking for suitable and cheap non-conventional protein sources to be incorporated in the feed to reduce dependence on animal protein, such as fishmeal, which accounted for 80% of the costs in formulated diets. Replacements of fishmeal by single cell protein by 55-80% have been reported to be encouraging (Atack et al. 1987).

Attempts have been made to use single cell protein (SCP) from yeast, bacteria and algae as sole protein sources in mirror carp diet (Atack et al. 1987). Among the unconventional protein sources, SCP of methane origin appeared to be a promising substitute for fish meal (Kaushik & Luquet 1980). Alkane yeast as SCP was an acceptable partial substitution at 40% for fishmeal in rainbow trout's diet (Mahnken et al. 1980). Plasmolysed lactic yeast has been used as a partial replacement of fishmeal in a diet of shrimp juveniles (Millamena & Triflo 1994). Most of the SCP sources are
derived from yeast in a microbial fermentation of raw hydrocarbon substitutes or organic waste products (Davies & Wareham 1988). *Rhizopus arrhizus*, a fungus was used as a protein source in initial diet for snakehead larvae (Hashim et al. 1994). These reports have spearheaded the exploration of other unexploited microbes for SCP in aquaculture sectors.

Further, there have been reports that shrimp larval diet could be supplemented with microbial live feed including alga. Shrimp larval survival could be increased if the planktonic food was supplemented with live feed, including nutritive bacterial strain. Aujero et al. (1985) had reported that *Penaeus monodon* larvae fed with a diet consisting of algae and bacteria had higher survival rates than larvae fed with a diet of algae or yeast alone.

Phototrophic bacteria are reported to be one of the potential bacteria that can be considered as an aquaculture feed supplement. Phototrophic bacteria also known as photosynthetic bacteria are widely distributed and can be isolated from diverse habitat. Their unique characteristics, which include the ability to grow in different cultural conditions, both in synthetic and naturally occurring substrates. Phototrophic bacteria are characterized as photoautotrophic, chemoautotrophic or chemoheterotrophic and a few are chemolithotrophic (Imhoff 1995). The biotechnological applications of phototrophic bacteria include bioremediation of wastewater (Kobayashi & Kobayashi 1995), deodorization of swine sewage (Lee & Kobayashi 1992) and uptake of heavy metal form polluted water (Vatsala 1987). Phototrophic bacteria were reported as producers of antiviral substances (Hirotani et al. 1991), herbicides (Sasaki et al. 2002), ubiquinone Q10 as medicine (Yoshida 1998), enzymes (Buranakarl et al. 1988) and vitamins (Sasaki et al. 1991). Other applications
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include the production of biodegradable plastics (Suzuki et al. 1995), fertilizer (Sasikala et al. 1994) and fuel (Tsygankov et al. 1998). They have been used for production of single cell protein, too (Sasaki et al. 1991).

The widely cultured species for SCP are *Rhodobacter sphaeroides*, *Rhodobacter capsulatus*, *Rubrivivax gelatinosus* and *Rhodocyclus gelatinosus*. Though *Rhodopseudomonas palustris*, *Rhodospirillum rubrum* and *Rhodocyclus tenue* have also been cultured, their protein profiles were low both in quality and quantity (Sasikala & Ramana 1995). Although phototrophic bacteria have been isolated from various sources and cultured for different purposes, their potential is still largely untapped and not wholly exploited for commercial purposes (Sasikala & Ramana 1995).

Different species of phototrophic bacteria have been cultured in a wide range of agro-industrial wastes. *Rhodocyclus gelatinosus* was successfully cultured in wheat bran (Shipman et al. 1975), in cassava starch (Noparatnaraporn et al. 1986) and in sea food processing wastewater (Prasertsan et al. 1993). The bacterium, *Rhodobacter sphaeroides* has been cultured in soybean waste (Sasaki et al. 1991) as well as in pineapple waste (Noparatnaraporn & Nagai 1986). Culture of *Rhodobacter sphaeroides* and *Rhodobacter capsulatus* in clarified biogas plant slurry was reported by Vrati (1994). The phototrophic bacterium strain *Rhodopseudomonas palustris* strain B1 was cultured in sago wastewater and the biomass was non-toxic to *Artemia* nauplii (Getha et al. 1998).

There are, however, few studies on the utilization of phototrophic bacteria in the bio-utilization fish processing wastes (Gaigher et al. 1982, Prasertsan et al. 1993). These
wastes contained mainly blood, tissues and extrudes rich in carbon and nitrogen sources (Martin 1994).

Fish and fish products consumption in Malaysia is expected to increase with an increasing population. To meet these demands industries have to expand to increase their production capacity. The fish canning industry plays a significant role in processing fisheries products to fulfill consumer requirement. The major problem in canning industry is the generation of liquid wastes (Prasertsan et al. 1997) and their disposal. The liquid wastes from canning industries are different from the wastes generated by other fish processing industries and contains cooked fish skin, tissue, soft bone, fish extracts and nitrogenous organic compounds. The high organic and inorganic loading in the wastes resulted in high chemical oxygen demand (COD). These industries have to treat the wastewater before disposal into the environment to meet the regulations under the Environment Quality Act, 1974. Kobayahi & Kobayashi (2001) reviewed the successful utilization of phototrophic bacteria for both pollution loading reduction and SCP production

The advantages:

(i) raw waste materials are free of costs or cheap
(ii) bacteria can reduce organic loading of waste
(iii) contamination by other microorganisms can be control
(iv) bacterial biomass contains high protein, good amino acids profile and rich in vitamins
(v) bacteria have soft cell walls, which can be easily digested by feeders
(vi) bacteria are not reported to be pathogenic and toxic to animals
(vii) energy saving as they assimilate CO₂ and fix nitrogen
The culture of *Rhodocyclus gelatinosus* in tuna condensate, diluted tuna condensate, shrimp blanching and frozen seafood processing waste has been encouraging (Prasertsan et al. 1993). The main objective of that study was to treat and utilize the wastewater and to optimize the growth parameters. Further, information on production of bacterial biomass in fish canning industrial wastewater and nutritional assessment of the waste grown bacterial biomass are lacking.

There have been no intensive studies on the production of SCP as aquaculture feed by *Rhodovulum sulfidophilum* biomass in sardine processing wastewater (SPW), an effluent from the fish canning industry with simultaneous reduction of chemical oxygen demand (COD) of wastewater. Similarly, no information is made available on the use of the waste grown phototrophic bacterial biomass in rearing the shrimp larvae.

The present study was therefore to evaluate the possibility for the production of purple non-sulfur bacteria *Rhodovulum sulfidophilum* in sardine processing wastewater (SPW) and to assess the biomass produced as a potential feed for rearing shrimp larvae.

1:1 OBJECTIVES

The specific objectives of the present study were:

(i) to identify and characterize the bacterial strain and SPW as substrate in culturing bacteria

(ii) to optimize growth conditions for biomass production in sardine processing wastewater (SPW)
(iii) to evaluate the nutritional profile of the waste grown bacterial biomass under optimized conditions

(iv) to assess its potential as an aquaculture feed for rearing *Artemia salina* (brine shrimp) and *Penaeus monodon* (tiger prawn)

(v) to develop a conceptual design to utilize sardine processing wastewater for production of aquaculture feed with simultaneous reduction in pollution load