

# CHAPTER 4

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### SUMMARY AND CONCLUSION

The photosynthetic bacteria represent a tool of great potential in various fields of biotechnology. The bacterial cells abundant in nutrients provide a possible feed supplement to animal feed and aquaculture. The rapid growth of bacteria consuming organic compounds contributes to the purification of polluted organic wastewaters. The ability of photosynthetic bacteria to produce molecular hydrogen has attracted great attention and stimulated research into the possibility of using these organisms as solar energy converters. The effective combination of hydrogen production with biomass production or organic wastewater purification has also been studied (Vincenzini *et al.*, 1982b; Jouanneau *et al.*, 1985)

In the present work, we attempted to characterize an indigenous strain for biomass production and wastewater purification.

Purple photosynthetic bacteria are remarkably versatile with regards to their capacities for obtaining growth energy through alternative mechanisms. With the vast development in sago industry in Sarawak, the amount of wastes produced is becoming alarmingly high. The use of phototrophic bacteria which can grow at an acidic pH may be a suitable choice for the treatment and utilization of this starchy effluent. *Rhodospseudomonas palustris* strain B1 was observed to grow very well in sago starch processing wastewater (Getha, 1995).

The morphologies of *R. palustris* strain B1 and ATCC 17001 were essentially identical. However, electron microscopic examination showed that *R. palustris* strain B1 were slightly smaller in size and had ridges on the cell surface.

The two isolates were also very similar in their physiological characteristics except for maximum growth temperature, utilization of organic compounds and growth factor requirements. It is able to photometabolize starch and one-carbon organic compounds like methanol and formate effectively. The cells also contain carotenoid components of the spirilloxanthin series. The lower pigment concentrations observed under aerobic conditions was due to the effect of oxygen on the synthesis of carotenoids and bacteriochlorophyll; oxygen inhibits the pigment synthesis and acts as a bleaching agent (Cohen-Bazire *et al.*, 1957).

Environmental parameters have also been found critical in the optimal development of photosynthetic activity of purple non-sulphur bacteria (Sawada and Rogers, 1977). Optimum growth of *R. palustris* strain B1 can be achieved in synthetic medium under anaerobic-light conditions. Maximum biomass of about 8.1 g/L was obtained on glutamate-starch medium after three days of growth. The conditions for cultivation were at pH 7, temperature 25 °C, light intensity of 5 klux with a 5% inoculum of 48 h old. These results indicate that *R. palustris* strain B1 differs from the type strain.

### Description of *Rhodospseudomonas palustris* strain B1

Cells are gram negative facultatively anaerobic, nonspore-forming rods with rounded ends; cells are 1.5 to 2.0  $\mu\text{m}$  long and 0.3 to 0.6  $\mu\text{m}$  wide. Cell multiplication is by budding and there was a tendency to form rosette-like clusters. Both photoheterotrophic growth under anaerobic conditions in the light and chemoorganotrophic growth under aerobic conditions in the dark are possible. Anaerobic cultures are red while aerobic cultures are pale grey-pink. Only biotin is required as the growth factor and yeast extract stimulates growth. The optimum pH is 5.5 and the optimum temperature is 25  $^{\circ}\text{C}$ . Highest growth was obtained at 4 klux light intensity.

The following organic substrates are photoassimilated: malate, succinate, tartrate, formate, pyruvic acid, starch and amylopectin. No growth occurs on simple sugars, acetate, lactate and glycerol. No growth occurs on media containing more than 2.0% NaCl. The cells contain the following photopigments: bacteriochlorophyll *a* (379, 591, 805 and 863 nm) and carotenoids (spirilloxanthin, lycopene and rhodopin).

Bolliger *et al.* (1985) have demonstrated the importance of using locally isolated strains in the purification of untreated waste substrates to produce value added products. Photosynthetic bacteria have been used in wastewater treatment by several workers. An advantage of the bacteria is that they are capable of growing in

high COD undiluted wastewaters. Another advantage of treating wastewaters using phototrophic bacteria is that this system produces a useful bacterial biomass instead of sludge (Balloni *et al.*, 1980; Sasaki *et al.*, 1981). Kobayashi and Kurata (1978) suggested that wastewaters from food industries could be used as sources for biomass production of phototrophic bacteria which could concomitantly reduce the COD of the wastewaters. Single cell protein production by treatment of sago effluent by *R. palustris* strain B1 showed that the biomass contained about 40% of crude protein with a balanced amino acid profile and rich in carotenoid pigments (Getha, 1995). Thus, the bacterial treatment system may represent an important complement to present day agronomy. A cell mass yield of about 0.59 g dry wt/g COD of *R. palustris* strain B1 was obtained during the purification of sago effluent (Getha, 1995). The COD removal of 86% of undiluted sago effluent indicates the advantages of using free cells of strain B1 for the primary treatment of the sago effluent (Ch.3,pp93). Furthermore, effective COD removal and biomass production are achieved by *R. palustris* strain B1 at a shorter period compared to other anaerobic-light systems (Getha, 1995).

In this study, the use of immobilized cells to treat sago effluent was also studied. The entrapment of microbial cells in agar was favoured over other matrices for the treatment of sago effluent. Entrapment in alginate and carrageenan resulted in fragile beads that tended to deteriorate in its physical nature after a few days. Agar was found to be suitable because it offers the possibility of continuous

operation and higher stability at higher organic loading rates. It is possible that the secondary sludge settling and recycling facilities required by the conventional biological wastewater treatment process can be eliminated.

In this study, both the free cells and immobilized cells exhibited similar COD removal patterns for the treatment of sago effluent. The free cells recorded a COD removal of 86% after 3 days of treatment whilst the immobilized cells recorded a COD removal of 82% after 4 days of treatment (Ch 3, pp 93). However, when compared to liquid cultures, treatment of sago effluent by immobilized cells were preferred because of their superior stability over prolonged use and the elimination of cell separation at the end of the treatment process. The immobilized cells retained 89% of their activity after the third consecutive treatment and 58% after the fifth consecutive treatment.

However, the use of immobilized cells to treat wastewaters is normally coupled with the production of hydrogen gas. As compared to liquid cultures, immobilized cells exhibited improved product characteristics and stability in many systems (Fukui and Tanaka, 1982). Production of molecular hydrogen from liquid cultures of free cells was also much lower than the ones of immobilized material (von Felten *et al.*, 1985). Entrapment in polymeric matrices anchors the living cells in a network, but allows free diffusion of the substrate and product. Immobilized growing cells are attracting worldwide attention because of the superior stability that results from the self-regenerating nature of catalytic systems (Fukui and Tanaka,

1982). When living microbial cells were immobilized and cultivated with a supply of adequate nutrients, the immobilized cells were found to form a dense layer near the gel surface. This result indicates that cells grow where nutritive balance is optimal for growth. An advantage of the immobilized cell systems is that the reactor volume is smaller because active cellular concentration is higher than in fermentations employing free cells. Furthermore, the inhibition by substrate or product concentration can be reduced in the continuous systems.

Biological hydrogen evolution is still in an exploratory stage in bioenergy production research, unlike methane (biogas) and alcohol production. Bennett and Weetall (1976) concluded that hydrogen cannot be produced economically on a substrate having a price of even 10 cents  $\text{lb}^{-1}$ . From this point of view, a process employing wastewater as electron donors offers clear advantages because the substrate has a negative cost corresponding to the depuration expense. According to von Felten *et al.* (1985), the projected costs of hydrogen production would be about four times higher than the equivalent fuel from anaerobic digestion (methane formation). Amounts, availability, seasonality and geographic distribution of suitable waste streams would thus limit practical applications of hydrogen production. Also, the successful immobilization with all its advantages over suspension cultures would not change this evaluation.

## **FUTURE RESEARCH WORK**

With regards to future prospects of developing the photoutilization of sago effluent by purple non-sulphur bacteria and converting them to commercial by-products, the following areas need to be considered:

### **Nonsterile Culture System**

In this study, the sago effluent was autoclaved prior to tests. However, it is in our best interest to study further the ability to grow phototrophic bacteria directly in raw sago effluent in an outdoor nonsterile culture system. According to Sasaki *et al.* (1991) sterilization of the waste prior to inoculation with the bacteria is no longer considered desirable. On the other hand, to maintain the predominance of phototrophic bacteria throughout the culture, heavy inoculation is required to achieve the cultivation within a short period.

### **Optimization of Temperature and pH**

The preliminary investigation has shown promising results which could be applied on a larger scale. Malaysia is in a good position to take advantage of this treatment because good conditions for bacterial growth are available all year round. The temperature and humidity conditions are fairly constant and sunlight can replace tungsten lighting during outdoor bacterial cultivation.



In this present study, photoutilization has been carried out in room temperature under anaerobic-light conditions. Further studies should include temperature changes and its effect on the growth rate, the yield and the efficiency in reducing COD value in the wastes.

Another process variable of interest is the pH for optimum COD reduction and hydrogen production. Hydrogen photoproduction did not occur at pH values below 6.5 or above 8.0 (Sasikala *et al.*, 1993). Therefore, future studies should include the effect of buffers on the COD reduction and hydrogen production as well as maintenance of optimum pH if possible.

#### **Optimizing the mechanical strength of the immobilized cell**

The concentration of gel is an important factor to be considered when a balance has to be established between optimum product formation, substrate diffusion, light penetration and the mechanical strength of the matrice.

The mechanical strength of the matrice, however, depends on the pH, ionic strength and the dynamics of the flow rate of the medium through the reactor (Chibata *et al.*, 1974; Marcipar *et al.*, 1978).

Further experiments would include effect of gel concentration, pH and addition of inorganic metal ions or use of inorganic supports such as glass, ceramic, mineral silicates or organic supports such as polyacrylamide to enhance the mechanical stability of the carrier.

### **Optimizing the Production of Hydrogen**

Important factors regulating hydrogen photoproduction include pH, temperature, light intensity and wavelength, the organic substrate used as electron donor, age of culture, cell density and nutritional history of the cells. Hydrogen production can be studied as a two-step process involving (1) a growth phase and (2) a production phase (Hillmer and Gest, 1977b). It can also be monitored as a single step, namely simultaneous photoproduction of hydrogen during growth (Kim *et al.*, 1982a,b).

It is noted that using a single-step process for the treatment and photoutilization of sago effluent offers numerous additional sources of revenue within the sago industry. This includes single-cell protein production with simultaneous photoproduction of hydrogen and reduction in the COD of the waste. Nonsterile outdoor culture systems with high inoculum density and without temperature, pH or light control may have to be considered.

## CONCLUSIONS

This study showed that:

- (1) *Rhodopseudomonas palustris* strain B1 was able to utilize starch particularly those with high amylopectin content such as potato, sago and tapioca starch at an optimum concentration of 1.0 g/L.
- (2) *Rhodopseudomonas palustris* strain B1 required only biotin of about 100 µg/mL for growth.
- (3) Addition of yeast extract significantly increased the growth yield of *R. palustris* strain B1.
- (4) The optimum temperature for growth was 25 °C.
- (5) The general pH range which supported good growth was 5.5 to 8.5.
- (6) Tungsten lamps with a light intensity of 4 klux was chosen as the suitable light source for biomass production.
- (7) *Rhodopseudomonas palustris* strain B1 could not tolerate salinities above 2.0%.

- (8) Optimum inoculum size was 10% whereas the inoculum age does not significantly affect the growth rate.
- (9) Maximum biomass of 8.14 g/L *R. palustris* strain B1 was obtained with starch as the electron donor under optimized conditions.
- (10) The biomass was successfully immobilized using 4% (w/v) agar (in distilled water).
- (11) A COD removal of 82% from the unsettled sago effluent was achieved after 96 h of treatment using agar-immobilized *R. palustris* strain B1.
- (12) A 100% effluent concentration was necessary for the efficient utilization of the immobilized cells during the COD removal process.
- (13) Continuous mixing or agitation during treatment does not increase the COD removal rates.
- (14) Inoculum size of the immobilized beads had no effect on the COD removal capability of the immobilized cells.
- (15) The immobilized cells of *R. palustris* strain B1 retained 89% of their activity after the third consecutive batch and 58% after the fifth consecutive batch.