SUMMARY

1. *Rhizobium japonicum* grown on 0.1g% of glucose attained stationary phase by 16 hours of growth, while at higher concentrations (0.25g%, 0.5g% and 1.0g%), the stationary phase was delayed by six hours.

2. Omission of nitrogen source from the medium resulted into two fold increase in the Poly β-hydroxybutyrate (PHB) content of *R. japonicum*.

3. The rate of PHB accumulation in the cells was found to increase with the increase in glucose concentration till 0.5 g% glucose in the medium. The accumulation was found to occur in the early stationary phase of the growth.

4. Supplementation of acetate or pyruvate in the medium along with glucose or β-hydroxybutyrate as the source of carbon caused marked increase in the PHB accumulation.

5. Addition of lower fatty acids also stimulated PHB accumulation while, higher fatty acids were without effect.

6. The activity of β-hydroxybutyrate dehydrogenase, an enzyme involved in the degradation of the polymer,
increased with the age of the culture. Maximum activity of this enzyme at 28 hours of the growth was found to be responsible for the decrease in the PHB content after 24 hours of growth in *R. japonicum*.

7. With the increase in the activity of \( \beta \)-hydroxybutyrate dehydrogenase, number of isoenzymes of \( \beta \)-hydroxybutyrate dehydrogenase also increase. A single band of \( \beta \)-hydroxybutyrate dehydrogenase activity was observed at 8th hour of growth. In contrast, 16 hours and 24 hours old culture extract showed four activity bands.

8. About 11 fold purification of \( \beta \)-hydroxybutyrate dehydrogenase was achieved with 44% recovery of the total units.

9. The kinetic studies of the purified enzyme showed specificity for NAD, pH optima of 8.0 and the \( Km \) values for \( \beta \)-hydroxybutyrate and NAD of 1.89 mM and 0.23 mM, respectively.

10. The purified enzyme showed inhibition by some of the organic acids tested (eg. succinate, fumarate, lactate, malate and malonate). Maximum inhibition (66.6%) was noted with 1 mM malonate.

11. Higher concentrations of acetoacetate (0.1 mM) and \( \alpha \)-Ketoglutarate (5.0 mM) inhibited \( \beta \)-hydroxybutyrate dehydrogenase activity in a non-competitive manner.
while lower concentrations of acetoacetate (0.05 mM) and α-ketoglutarate (1.0 mM) showed competitive type of inhibition.

12. NADH inhibited β-hydroxybutyrate dehydrogenase activity in a non-competitive manner, while ATP was found to be activating the enzyme β-hydroxybutyrate dehydrogenase.

13. Maximum activity of acetoacetyl CoA reductase, an enzyme involved in the biosynthesis of PHB, was observed when 0.5g% glucose or 0.37 g% β-hydroxybutyrate was supplied in the medium as the carbon source.

14. Supplementation of nitrogen sources, eg. ammonium sulphate or casaminoacids inhibited acetoacetyl CoA reductase activity.

15. The levels of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase increase till 24 hours of growth in R. japonicum. The levels of other NADPH generating enzymes e.g. glyceraldehyde-3-phosphate dehydrogenase, malic enzyme and isocitrate dehydrogenase also increased till 30 hours of the growth.

16. The activity of glucose-6-phosphate dehydrogenase was
found to be 50% less in β-hydroxybutyrate grown culture as compared to that in glucose grown culture.

17. β-hydroxybutyrate grown cells showed higher activity of transhydrogenase and lower activity of NADH oxidase, as compared to that in glucose grown cells.

18. The enzyme acetoacetyl CoA reductase was purified to about 25 fold, using ammonium sulphate precipitation and Ca₃(PO₄)₂ gel adsorption techniques.

19. This enzyme showed the specificity for NADPH and the pH optima of 5.5.

20. The apparent Km values of 10.1 mM and 9.5 mM were observed for the substrate acetoacetyl CoA and NADPH, respectively.

21. Higher concentration of acetoacetyl CoA was found to be inhibitory for the enzyme activity.

22. Acetate (30 mM) in vitro, activated the enzyme acetoacetyl CoA reductase by 16%, while 28% inhibition in β-hydroxybutyrate dehydrogenase activity was observed with 20 mM acetate.

23. Pyruvate at 10 mM concentration showed 14% activation in acetoacetyl CoA reductase and 31% inhibition in β-hydroxybutyrate dehydrogenase activity. Higher
concentration of pyruvate (20 mM) completely inhibited the activity of \( \beta \)-hydroxybutyrate dehydrogenase.

24. The intracellular concentration of acetate was found to be in the range of 5 to 11 mM, while that of pyruvate was in the range of 5 to 13 mM.

25. \textit{R. japonicum} was found to synthesize haem when succinate was provided as the source of carbon in the medium.

26. A pyridine haemochrome of rhizobial haemoglobin extracted from \textit{R. japonicum} showed absorption maxima at 410 nm.

27. Maximum haem synthesis was found to occur when 0.75g\% succinate and 0.05g\% glycine were used in the growth medium of \textit{R. japonicum}.

28. The enzyme \( \Delta \)-aminolevulinate synthetase, involved in haem synthesis, was found to be oxygen sensitive.

29. This enzyme showed pH optima of 7.5 (tri-HCl buffer) with \( K_m \) values of 1.54 mM and 5.9 mM for succinate and glycine, respectively.

30. Marked correlation between the haem content and \( \Delta \)-aminolevulinate synthetase activity was observed, reaching their maxima at 28 hours of the growth.
31. Ammonia was assimilated mainly via glutamate dehydrogenase in *R. japonicum*, when ammonium sulphate or casaminoacids was used as the nitrogen source in the medium.

32. With potassium nitrate as the nitrogen source and glucose, sucrose or xylose as the carbon source, ammonia assimilation was found to occur mainly via glutamine synthetase/glutamate synthase pathway.

33. Both routes of ammonia assimilation, glutamate dehydrogenase and glutamine synthetase/glutamate synthase, were found to be operating in *R. japonicum* in presence of succinate and potassium nitrate as the source of carbon and nitrogen, respectively.

34. The patterns of ammonia assimilatory enzymes with different carbon sources remained almost same in shake and static cultures but, the levels of enzymes were low in static culture as compared to those in shake culture.