CONCLUSIONS
5.0 Conclusions

Aromatic compounds are the most abundant group of compounds that exist in the environment. Bacteria utilise these aromatic compounds for growth under oxic and anoxic conditions. Aromatic amino acids are one such group of naturally occurring aromatic compounds found abundantly in nature. Bacteria utilise aromatic amino acids for its growth as sole source of carbon or nitrogen. Among bacteria, anoxygenic phototrophic bacteria are metabolically diverse group of bacteria that occupy diverse habitats and thrive on wide array of organic compounds. *Rubrivivax benzoatilyticus* JA2, one such anoxygenic phototrophic bacteria thrive under oxic and anoxic conditions by utilising different organic compounds. *Rubrivivax benzoatilyticus* JA2 utilise aromatic amino acids as sole source of nitrogen but not as carbon source. *Rubrivivax benzoatilyticus* JA2 has remarkable ability to transform aromatic amino acids into phenolic and indolic compounds. In the present work *R. benzoatilyticus* JA2 was used as model system to study the L-phenylalanine catabolism under oxic and anoxic conditions by using stable isotope based metabolomics approach.

*Rubrivivax benzoatilyticus* JA2 could not utilise L-phenylalanine as sole source of carbon neither under oxic nor in the anoxic conditions. However, *R. benzoatilyticus* JA2 utilise L-phenylalanine as sole source of nitrogen both under oxic and anoxic conditions and produce phenols (aryl metabolites) indicating the metabolic potential of *R. benzoatilyticus* JA2 to utilise L-phenylalanine under different growth modes. Comparative HPLC and LC-MS based metabolic profiling revealed the production of different aryl metabolites under oxic and anoxic conditions and this suggests catabolic diversity of L-phenylalanine by *R. benzoatilyticus* JA2 under oxic and anoxic conditions. Stable isotope studies and enzyme activities revealed the existence of multiple catabolic pathways such as Ehrlich’s, N-acetyl L-phenylalanine pathway, 4-hydroxyphenylpyruvic acid pathway and some unknown pathways under anoxic conditions. However, under oxic conditions apart from Ehrlich’s pathway, L-phenylalanine catabolised into pyomelanin and anthocyanin-like pigments. Existence of multiple catabolic pathways of L-phenylalanine under oxic and anoxic conditions indicated the catabolic multitasking of *R. benzoatilyticus* JA2. L-Phenylalanine is mainly fluxed towards Ehrlich’s pathway under anoxic conditions whereas under oxic conditions towards pigments production indicating the differential flux of L-phenylalanine. Multiple pathways with differential flux of L-phenylalanine under oxic and anoxic conditions suggest plausible oxygen dependent regulation of L-phenylalanine in *R. benzoatilyticus* JA2.
L-Phenylalanine catabolised via 4-hydroxyphenylpyruvic acid followed by homogentisic acid only under oxic conditions by *R. benzoatilyticus* JA2 and this is the first report among anoxygenic phototrophic bacteria. However, 4-hydroxyphenylpyruvic acid under anoxic conditions remained as a dead-end molecule, when shifted to oxic conditions 4-hydroxyphenylpyruvic acid is further catabolised into homogentisic acid and finally to pyomelanin. This suggests that presence of oxygen evoked the pyomelanin biosynthesis by activating oxygen dependent enzymes and metabolic shift of L-phenylalanine under oxic conditions. In addition to pyomelanin, *R. benzoatilyticus* JA2 also produced different colored compounds under oxic conditions in presence of L-phenylalanine. Among those pigments, blue pigment tentatively identified as anthocyanin-like pigment derived from L-phenylalanine. However, genome sequence revealed the absence of homologous genes of anthocyanin biosynthetic pathway suggesting the possible alternative pathway of anthocyanin-like pigment biosynthesis which needs further investigation.

Metabolomics study based on the multivariate statistical analysis such as hierarchical cluster analysis (HCA), principal component analysis (PCA), and partial least squares discriminant analysis (PLS-DA) analysis revealed the metabolic variations of *R. benzoatilyticus* JA2 under oxic and anoxic conditions. The relative fold change analysis and functional classification explained the differential regulation of key metabolites and their metabolic pathways influenced under anoxic and oxic conditions. Metabolic variations under oxic and anoxic conditions suggests metabolic shift in *R. benzoatilyticus* JA2 to survive under changing conditions.

Finally metabolic shift and catabolic multitasking of L-phenylalanine in *R. benzoatilyticus* JA2 suggest a possible adaptive strategy to survive and utilize L-phenylalanine under oxic/anoxic transition zones. Diverse metabolic adaptations of *R. benzoatilyticus* JA2 to utilise L-phenylalanine under anoxic and oxic conditions was clearly demonstrated and similar kind of adaptations may exist in other anoxygenic phototrophic bacteria for metabolizing organic compounds which may help in carbon recycling in the oxic/ anoxic environments.