Gaseous alkanes,like methane,propane and butane, are widespread in nature and many microorganisms have evolved mechanisms to utilize these substrates as sole source of carbon and energy. The growth on these hydrocarbons causes certain adaptive(physical as well as biochemical) changes in the microorganisms. Although,metabolism of methane is well studied , knowledge about the metabolism and uptake of other gaseous alkanes particularly C2-C4 is very limited. The key enzyme in methane metabolism,methane monooxygenase (MMO) had been purified,characterized and crystal structure elucidated. Propane or butane monooxygenase, on the other hand,proved to be a difficult system for purification and characterization. Virtually, nothing is known about genetic and molecular basis of regulation of the pathways.

It was postulated earlier,(Nair,1991) that a high molecular weight protein was involved in metabolism of propane and butane. It was shown to be consisting of at least four distinct proteins/polypeptides of approximate molecular weight 58kDa,42kDa,37 kDa and 32 kDa respectively. Out of these four polypeptides,two (58 kDa and 42 kDa) were reasonably well studied. Antibodies against these proteins were found to be useful as probes for detection of propane and butane utilizing microorganisms. The present study is primarily focussed on the 32 kDa protein. Since there was no clue about the function(s) of the 58 kDa protein, this aspect has also been included in this work . The study also includes physical changes associated with growth on gaseous alkanes in bacteria.

The main organism chosen for the present study is a soil isolate from Gujrat oil fields. It is a gram positive bacteria, belonging to genus ***Rhodococcus*** and named as ***Rhodococcus*** sp. IMT-35. Two other gaseous alkane utilizers (***Pseudomonas*** sp. IMT-40 and ***Pseudomonas*** sp. IMT-37) have also been used for comparison and to make specific observations.

The physical changes observed upon growth on propane and butane are increased cell size and the presence of surface projections in the form of 'blebs' on the cell surface. The 'blebs' formation appears to be growth related . These structures were not seen in the cells grown on glucose, rich medium and on some of the intermediates of propane/butane catabolism . Circular membranous structures were also seen in the culture filterate of the alkane grown cells. These membranous structures were also seen in the cytoplasm of the propane grown cells. Whether these three strucures are homologous to each other can not be said with certainity at present.

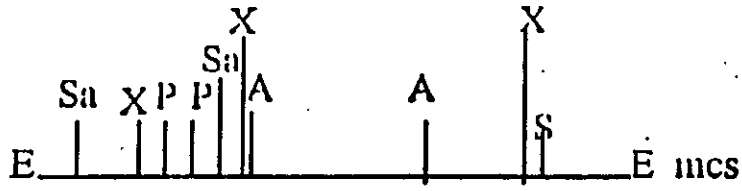
The proteins corrosponding to 58 kDa and 32 kDa were purified from the soluble extract of propane grown cells. Antibodies against these proteins were raised in

rabbits. By western immunoblotting procedure, it was established that both these antibodies were specific for the proteins against which they were raised. The anti 58 kDa antibody was found to be reacting with two (IMT-35 and IMT-40) of the three hydrocarbon utilizing bacteria tested. The anti -32 kDa antibody, on the other hand, showed reaction with all these three organisms (IMT-35, IMT-40 and IMT-37) only when propane or butane was their growth substrate. This indicated the inducible nature of these two proteins. This observation suggested their involvement in metabolism of gaseous alkanes.

The 58 kDa protein was localized by immunogold labelling and electron microscopy on the surface of propane or butane grown cells. Cells grown on glucose or rich medium showed no gold labelling. Using the same technique, this protein was also found on the 'blebs' isolated from the culture supernatant of propane and butane grown cells. This suggested that these two structures may share antigenically similar protein (s).

Immunoscreening of the genomic library of IMT-40 prepared in lambda gt11 was done using anti-32 kDa antibody. Two putative clones giving positive signal were picked up and rescreened till homogenity . They were named as λTA1 and λTA2. Both these clones contained an insert of about 4.1 kb. λTA1 was chosen for further analysis. The first step was to confirm the presence of fusion protein . For this, the λTA1 clone was lysogenized .Two lysogens were obtained. A fusion protein (150 kDa, of which 112 kDa is β-galactosidase part) was detected in crude extract of λTA1 by immunoblotting technique using anti-32 kDa antibody. No corrosponding protein was seen in lysogen extract of λgt11. This confirmed that fusion protein was present and the gene encoding the 32 kDa protein was being expressed. For the sake of convenience in further analysis, the DNA fragment was recloned in pUC 19 vector and recombinant plasmid was named as pTA1. Dot blot analysis involving DNA-DNA hybridization confirmed the presence of DNA fragment in the genomic DNA of IMT-40. The detection of this sequence in two other hydrocarbon utilizers, namely IMT-35 and IMT-37 and its absence in bacteria (a ***Bacillus*** sp. and a ***Pseudomonas*** sp.) that are incapable of catabolizing gaseous alkanes, suggested that this sequence was unique in these organisms and is important for utilization of these alkanes. Conservative nature of the fragment in IMT-35,IMT-40 and IMT-37 was also established by southern hybridization. Southern blot analysis,using the cloned DNA fragment as probe,revealed a positive band at 4.1 Kb region of restriction endonuclease (Eco RI) digested genomic DNA of each of these three organisms. This DNA fragment did not hybridize with 4.9 kb, fragment that encodes the 58 kDa protein or its flanking region. This indicated that this sequence lies somewhere

else in the genome. A detailed restriction map of this DNA fragment was prepared and shown below. The DNA fragment was found to have a single site for enzyme SmaI,two sites each for PstI,ApaI,and Sal I. It had three sites for XhoI.



Alkanes are structurally very similar compounds and follow similar catabolic pathways. It was considered of interest to see whether they involve same enzymes for metabolizing all alkanes or specific enzymes are induced for each alkane. Monooxygenase activities for alkanes were determined in whole cells as well as in cell extract by GC based assay involving quantification of respective epoxide formed . It had been observed that the enzyme system induced by propane showed propane monooxygenase (PMO), butane(BMO) and hexane monooxygenase (HMO) activities. Butane grown cells showed BMO and HMO activity but no PMO activity was observed. The enzyme induced by hexane showed only HMO activity and no PMO or BMO activity was detectable. This suggests that these monooxygenases show some degree of specificity. The reasons for this observed specificity could only be hypothesized at present. It is possible that the core enzyme remains the same and the specificity could be due to a specific carrier protein or due to some conformational change of the enzyme depending on the inducer. When the mixture of alkanes were used for growth, enzyme activity for the higher alkane was detected. When the propane and butane was given separately at different time intervals during growth, both monoxygenase activities were found indicating that two enzymes are separately induced. Genetic experiments involving mutants and their complementation may be helpful for answering this question.

The levels of propanol deydrogenase, propanol-2 dehydrogenase and

propanal dehydrogenase were higher when the cells were grown on propane. Since the anti-58 kDa and anti-32 kDa antibodies were available, an attempt was made to see if these two proteins are components of monooxygenase complex. Antibody against these two proteins were used to see their effect on monooxygenase activities in whole cells as well as in cell extract. The anti-58 kDa antibody significantly (more than 70%) inhibited monooxygenase activity in both whole cells as well as in cell extract. Both propane and butane monooxygenase activities were affected almost to the same extent. No significant inhibition of PMO and BMO activities was observed in whole cells when, anti-32 kDa IgG was used. In cell extract however, about 50% inhibition of both activities was observed with this antibody. These antibodies did not inhibit activities of other enzymes like alcohol and aldehyde dehydrogenase. Control IgG did not affect the activities of PMO,BMO or the dehydrogenases. These results suggested that these two proteins are specifically involved in metabolism of propane and butane.