DISCUSSION

Biodegradation is an important process contributing to the maintenance of biogeochemical cycles and it greatly depends on the microorganisms of a system. Biodegradation/transformations of complex organic molecules like aromatic compounds is not widespread in anaerobic environments, resulting in their accumulation, which is of environmental concern (Berry et al., 1987a). Thus, recent research on biodegradation/transformation of aromatic compounds is focussed on anaerobic metabolism (Gibson and Harwood, 1994; Sasikala and Ramana, 1998; Harwood et al., 1999). Flooded soils, sediments, landfills, lagoons, anaerobic fresh/ocean and ground waters are some of the anaerobic environments where only a limited number of microorganisms are known to degrade aromatic compounds, compared to their widespread utilization under aerobic conditions.

Like in the present study, studies on biodegradation/transformation of aromatic compounds are mostly done under laboratory conditions using pure cultures, mainly to understand the biochemical pathways involved, evaluate the capability of individual organisms and assess their probable role in the natural environments. Alternatively, mixed cultures (Gottschalk and Knackmuss, 1993), sludges (Balba and Evans, 1980) or in situ biodegradations (Suflita and Miller, 1983) are also known which are mostly used for the degradation of more complex compounds like polycyclic aromatic compounds (PAHs) (Cerniglia, 1984) or for bioremediation applications (Rasul Chaudry, 1994).

Photosynthetic microorganisms viz., oxygenic phototrophic bacteria (OPB), anoxygenic phototrophic bacteria (APB) and microalgae have ecological significance not only as primary producers of an ecosystem, but also as biodegraders (Gibson and Harwood, 1994; Sasikala and Ramana, 1998; Harwood et al., 1999; Semple et al., 1999), thus helping in the maintenance of the biogeochemical cycles. Though a few microalgae, including OPB
are known to biodegrade aromatic compounds, their metabolism is restricted to aerobic environments (Semple et al., 1999). Anoxicogenic phototrophic bacteria, particularly the PNSB are a major group of phototrophic microorganisms capable of degrading a wide range of aromatic compounds under anaerobic conditions (Harwood et al., 1999) which depends upon the availability of light and thus commonly referred to as photometabolism (Harwood and Gibson, 1988). However, the role of light in the photometabolism of aromatic compounds is still not understood (Sasikala and Ramana, 1998). While the initial studies on the photometabolism of aromatic compounds were mostly with low molecular weight and naturally occurring aromatic compounds (Dutton and Evans, 1969), recent years have seen a surge in research on biodegradation and biotransformation of recalcitrant and xenobiotic molecules (Sasikala and Ramana, 1998). The studies on aromatic compound utilization by PNSB reported so far were with methoxylated (Harwood and Gibson, 1988; Wright and Madigan, 1991 and Ahmed and Mohamed, 1994), hydroxylated (Khanna et al., 1992), amino (Sasikala et al., 1994b), nitro (Blasco and Castillo, 1992), halogenated (Geissler et al., 1988; Kamal and Wyndham, 1990; van der Woude et al., 1994) and alkoxylated (Madigan and Gest, 1988; Harwood and Gibson, 1988; Khanna et al., 1992) derivatives of homocyclic aromatic compounds and so, it was of interest to study the capability of PNSB to degrade the heterocyclic aromatic compounds.

**CAN PNSB DEGRADE HETEROCYCLIC AROMATIC COMPOUNDS?**

Purines (Busse et al., 1984), pyrimidines (Kaspari 1979) and pyrazines (Sasikala et al., 1994a) are some of the N-containing heterocyclic aromatic compounds so far reported to be degraded by PNSB. In order to understand more about the utilization of N-containing heterocyclic aromatic compounds, pyridine, pyrazine, quinoline, imidazole, indole, purine (guanine), pyrimidine (uracil), benzimidazole (carbendazim) and isoindole (captan) and/or their derivatives were screened for their ability to support growth of a few PNSB viz., Rba.
sphaeroides OU5, Rps. palustris OU11 and Rps. palustris JA1 (Table 12) [abbreviations of names of genera used is according to Trüper and Imhoff, 1999]. The increase in biomass of Rps. palustris JA1 along with disappearance of N-containing heterocyclic aromatic compounds from the growth medium indicates the biodegradation and subsequent assimilation as cell mass. Increased cell mass with other species also may be due to the degradation and assimilation of the compound. In contrast, though disappearance of the compound was observed with reference to indole, imidazole, captan, pyrazine-2-carboxylic acid, 2,4,6 tri methyl pyridine (Figs 13, 14), their incorporation into the cell mass must not have occurred, since there was no increase in biomass when compared to that of control (Table 12). Furthermore, there was no noticeable change in initial and final absorption spectra of the culture supernatant (Figs 13, 14), clearly indicating that there was no photobiotransformation either. Under such conditions, the disappearance of the compound can plausibly be explained by assuming adsorption by the organism.

Thus, the study on the utilization of N-containing heterocyclic aromatic compounds by PNSB indicate their capability to degrade these compounds. However, the observed growth inhibition with some of these compounds (Table 12) led to a detailed study on their toxicity.

- Are N-containing heterocyclic aromatic compounds toxic? What could be the mode of action?

Effect of N-containing heterocyclic aromatic compounds on PNSB is shown in Table 11. A threshold of 10 mM was kept as an upper limit in assessing the toxicity of N-containing heterocyclic aromatic compounds on PNSB, because toxicity on microorganisms by N-substituted aromatic compounds fall within this range (Razo-Flores et al., 1997). The organisms were not inhibited by pyridine, pyrazine, imidazole, uracil (except Rps. palustris) and guanine (except Rps. palustris) (Table 11) suggesting that these compounds are not toxic to PNSB at low concentrations used. The highest toxicity was observed with indole
(Table 11), which is known for its antimicrobial activity against gram negative bacteria (Kubo 1993). When cultures exposed to the heterocyclic aromatic compounds were transferred to regular growth media, the organism grew at the same rate as that of control (without heterocyclic compound), indicating the effect of these compounds to be only growth inhibitory, reversible and not lethal.

Toxicity of N-containing heterocyclic aromatic compounds was observed only with those compounds having a homocyclic ring attached to the heterocyclic ring (Fig. 12). Toxicity among these compounds increased in the following order: benzimidazole<quinoline<isoindole<indole for Rba. sphaeroides (Fig. 12) and a near similar pattern was observed with the other two species also (Fig.12), suggesting common toxicity pattern among PNSB. Thus, pyridine and imidazole were not toxic, while quinoline and benzimidazole were found to be toxic at low concentrations. The reason for such toxicity with respect to the compounds with homocyclic ring may be due to several reasons. While the major reason appears to be the hydrophobicity of the homocyclic ring (Razo-Flores et al., 1997) others could be their effect on the bacterial membranes (Sikkema et al., 1995; Razo-Flores et al., 1997) and photosynthetic pigments (Wright and Madigan, 1991). Since indole has shown the highest toxicity, an in depth study was conducted to establish its mode of action.

- ARE INDOLES ANTIMICROBIAL? HOW DOES IT AFFECT PNSB?

Literature survey (introduction page 19) reveals that antimicrobial activity of indole is only on gram negative bacteria as also observed in the present study (Table 13). A detailed study was taken up to evaluate the possible mode of action on PNSB, using Rba. sphaeroides OU5 as test organism.

Growth studies in the presence and absence of indole were done to know the effect of these compounds on Rba. sphaeroides OU5. The observed prolonged lag period of 6 h in
the presence of indole compared to control and a doubling time of 20 h compared to 9.2 h indicate that indole not only reduced the growth rate but also resulted in early onset of stationary phase ultimately reducing growth yields (Fig. 15). The effect of indole (Fig 17) is more pronounced in the logarithmic phase (Fig. 16), where cells are metabolically active and more susceptible to environmental influence. Such an effect of indole was reversible as evidenced by restoration of growth at similar rates when indole was removed from the assay medium (Fig. 16). Thus, contamination of soils with heterocyclic aromatic compounds appears to have a growth inhibitory effect, which is temporary till compound exists and natural microflora is likely to restore once these compounds are bioremediated.

Growth inhibitory effect of indole was observed not only when it served as sole nitrogen source (Fig 23) but also when ammonium chloride was also provided (Table 11) with malate as sole carbon source. Similarly, indole was growth inhibitory even when malate was replaced with a number of other carbon compounds in the presence of ammonium chloride as nitrogen source (Table 14). However, replacement of ammonium chloride with other inorganic or organic nitrogenous substrates in the presence of malate as carbon source resulted in varied growth effects (Table 16, 14). Inhibitory effect of indole was observed only with respect to the inorganic nitrogenous sources used (Table 16) with almost no effect in the presence of organic nitrogenous substrates (amino acids), exception being glycine (Table 14). These studies indicate that the growth inhibitory effect of indole on Rba. sphaeroides OU5 is due to its effect on assimilation of inorganic nitrogen and by extrapolation on glutamine dehydrogenase (GDH) or glutamine synthetase (GS)/glutamine oxoglutarate amino transferase (GOGAT) (Sasikala et al., 1993) systems which mediate inorganic nitrogen assimilation, rather than on transaminase activity which is responsible for organic nitrogen (amino acid) assimilation (Hug & Weckmann, 1957). Studies on the effect of indole on the macromolecular composition of Rba. sphaeroides OU5 (Table 15) shows
that indole has no effect on the contents of either carbohydrate or protein. However, it showed an inhibitory effect on photosynthetic pigment content as observed with other aromatic structures (Sasikala and Ramana, 1998) which increased with increasing concentrations of indole (Fig 18). Surprisingly, this decrease in BCHl-a synthesis was accompanied by enhancement in yellow carotenoid content (Fig. 18). This indicates that carotenoids are serving functions other than photosynthetic, which may be protective as observed not only in purple bacteria (Cohen-Bazire and Stanier, 1958) but also in other bacteria (Mathews and Sistrom, 1959) and in green plants (Goodwin, 1959, 1960).

The above results indicate the effect of indole to be multifactorial as also evidenced from earlier reports of indole’s effect on porphyrin/pigment synthesis (Umirikhina & Krasnovski, 1969; present study), oxidative and photophosphorylation (Buechel and Draber, 1968), anthraquinone biosynthesis (El-Shagi et al., 1984), cytochrome P-450 (Evarts and Mostafa, 1981) and on some enzymes (Evarts and Mostafa, 1981) together on carbon uptake (Kamath and Vidyanathan, 1990). Though indole is a known antimicrobial compound, its biodegradation was also demonstrated in a few anaerobic bacteria (Bak and Widdel, 1986; Madsen and Bollag, 1989 and Gu and Berry, 1991, 1992). Hence, a detailed study was conducted by altering cultural conditions to see if PNSB were also capable of indole biodegradation.

- **DO PNSB HAVE THE CAPABILITY TO DEGRADE INDOLE?**

Two cultures of PNSB (*Rba. sphaeroides* OU5; *Rps. palustris* OU11) were used to evaluate their capability to degrade indole (sole carbon/nitrogen source) under both aerobic/dark and anaerobic/light conditions (Figs. 20, 22, 23). Lack of growth on indole under various experimental conditions used (Figs. 20, 22, 23) indicate the inability of the organisms to use indole as a growth substrate. However, degradation of indole (Figs. 20, 23)
formation (Fig. 24). The compound with absorption maxima at 291 nm is tentatively identified as 2-aminophenylacetic acid (2APA) is proposed as a hypothetical intermediate in the photometabolism of indole. The peaks corresponding to the hydroxylated indoles like indoxyl, dihydroxy indole, isatin and oxindole could not be detected in the present study under both aerobic and anaerobic conditions (Fig. 21.24) when indole was used as sole 'C' or 'N' sources by Rba. sphaeroides OU5.

The initial steps leading to the formation of anthranilate from indole varies with microorganisms. The proposed pathways leading to the formation of anthranilate include: indole → 3-hydroxyindole (indoxyl) → 2,3-dihydroxyindole → isatin → anthranilate (Sakamoto et al., 1953) or indole → 2,3-dihydroxyindole → N-carboxyanthranilate → anthranilate (Fujioka and Wada, 1968) or indole → indoxyl → N-formylantranilic → anthranilate (Kamath and Vaidyanathan, 1990). While these represent the aerobic pathways, the anaerobic bacteria degrade indole via oxindole, which is later converted to anthranilate (Berry et al., 1987b). On the other hand, rats metabolize indole by two pathways (King et al., 1966), one via isatin → N-formylantranilic → anthranilate occurring under aerobic conditions, while under anaerobic conditions with oxindole → 2-aminophenyl acetate as an intermediate before anthranilate formation. A similar pathway is tentatively proposed for the photobiodegradation of indole by Rba. sphaeroides OU5 via 2-aminophenyl acetate (Fig 44). Further biodegradation of anthranilate leading to the formation of either benzoate (Berry et al., 1987b), salicylate (Sakamoto et al., 1953) or catechol (Kamath and Vaidyanathan, 1990) could not be demonstrated under the present experimental conditions even after 15 days of prolonged assay.

Though light appears to be a major factor influencing the biodegradation of indole by Rba. sphaeroides OU5, its role in photobiodegradation of aromatic compounds remains to be understood (Sasikala and Ramana, 1998) as also enhancement in the biodegradation of
indole with respect to some of the inorganic nitrogen sources (Table 16) which needs
detailed in depth study. The differences in the products observed in the presence of different
organic substrates (Table 17) indicate the capability of *Rba. sphaeroides* OU5 to
photobioltransframe indole in addition to its biodegradation.

![Chemical Structure](image)

**Fig 44.** Tentative new pathway for the photobiodegradation of indole
by *Rba. sphaeroides* OU5 via 2-aminophenyl acetate as
hypothetical intermediate.

The above discussions lead to the following conclusions on the photometabolism of
indole.

1. Indole does not support growth of PNSB.
2. Indole nucleus can be broken by certain PNSB in a light dependent process,
which is incomplete and partial.
3. Major product identified in indole photobiodegradation by *Rba. sphaeroides*
   OU5 was anthranilate with 2-aminophenyl acetate as a hypothetical intermediate.
4. Further degradation of anthranilate to benzoate or any of such intermediates of anaerobic metabolism (Sakamoto et al., 1953; Kamath and Vaidyanathan, 1990) could not be demonstrated.

5. Presence of inorganic nitrogen sources enhance the indole photobiodegradation, through the basis of such effect is not understood.

6. Identification of the end products of photobiotransformation of indole by *Rba. sphaeroides* OU5 in the presence of organic substrates has to be carried out.

- **WHAT ARE THE DIFFERENT INDOLE DERIVATIVES FORMED DURING PHOTOBIOTRANSFORMATION OF INDOLE BY *Rba. sphaeroides* OU5?**

  L-Tryptophan as confirmed through TLC was a major end product in the presence of different precursors (Table 18). The quantity of L-tryptophan photoproduced was high in the presence of L-serine compared to in the presence of DL-alanine or glycine or pyruvate/lactate + NH₄Cl (Table 18). This is the first report of L-tryptophan biosynthesis from indole with DL-alanine, glycine and lactate + NH₄Cl and L-serine is most common and widely used precursor for L-tryptophan production by microorganisms (Nyeste et al., 1983). Altering the medium conditions by omitting the yeast extract (source of vitamins including pyridoxal phosphate essential for L-tryptophan synthesis [Snell, 1975; Miles et al., 1987]) resulted in the formation of end products other than L-tryptophan (Table 19).

- **WHAT FACTORS INFLUENCE L-TRYPTOPHAN FORMATION BY *Rba. sphaeroides* OU5 IN THE PRESENCE OF L-SERINE?**

  L-Tryptophan is one of the essential amino acids and its production by microbial processes is of high commercial significance. Though most of the microbial processes reported were by genetically engineered organisms through over-expression of metabolic capabilities, these organisms proved to be very unstable and thus using precursors and regulating the biochemical pathways is an alternate choice (Nyeste et al., 1983). However, this process depends and is influenced by several factors (Bang et al., 1983a) and an attempt
was made to look into some of the possible factors influencing the L-tryptophan formation by *Rhodobacter sphaeroides* OU5.

For L-tryptophan formation, indole appears to be an obligate requirement (Fig. 29) compared to the other precursor, L-serine (in the presence of yeast extract) (Fig. 28). However, lack of L-tryptophan formation in the absence of L-serine when yeast extract was omitted from the medium (Table 22) reveals the obligate requirement for precursors which can be provided by yeast extract. *Rhodobacter sphaeroides* OU5 did not require long adaptation time for L-tryptophan biosynthesis to be induced (Fig. 26). pH of the medium had no major influence either on indole consumption or L-tryptophan formation at neutral to alkaline pH and the unchanged final pH of the assay medium (Table 21) indicate that buffers may not be required for L-tryptophan photoproduction by *Rhodobacter sphaeroides* OU5 unlike for other organisms as observed earlier (Bang *et al.*, 1983a).

Though L-tryptophan formation from indole using L-serine is observed in aerobic bacteria (Table 9), this study appears to be first report of such a biotransformation by anaerobic bacteria and under both anaerobic (Table 18) and aerobic conditions (Table 20) by the same organism. Significant variation was observed with respect to indole consumption and L-tryptophan formation under both the conditions (Table 18, 20) and the presence of oxygen reduced the yields and conversion efficiencies (Table 20). The purple non-sulfur bacterium used in this study, *Rhodobacter sphaeroides* OU5, has the capability to grow under both aerobic (data not shown) and anaerobic (Fig. 15) conditions and thus the possibility of oxygen effect on the bacterial metabolism in general is ruled out. The other alternative could be the oxygen sensitivity of the enzyme responsible for L-tryptophan formation as observed in some organisms (Nyeste *et al.*, 1983), although no definitive conclusion can be drawn from the experimental data available.
Tolerance to indole concentration of up to 4 mM (Table 23) with an optimum of 2 to 3 mM (Fig 29, Table 23) observed in this organism is comparable to other L-tryptophan producing organisms (Bang et al., 1983a; Nyeste et al., 1983). However, for complete utilization of indole a ratio of 1:25 mM indole to L-serine was required (Fig. 28). Maintenance of the same ratio at higher concentration of indole (2 and 3 mM) resulted not only in decreased indole consumption, but also decreased conversion efficiencies (Table 23). Either for growth (data not shown) or for L-tryptophan formation there was no obligate requirement for externally added vitamins (Fig 30) and enhancement in the presence of vitamin B₆ (pyridoxal phosphate) (Fig 30) or yeast extract (Table 22, 26) may be explained as due to its obligate requirement for the enzymes involved in biotransformation (Snell, 1975; Miles et al., 1987). Thus, yeast extract appears to provide the required vitamins in sufficient quantities (Difco Manual, 1998) in addition to being a good source of precursors.

The other parameters, which influenced L-tryptophan formation by Rba. sphaeroides OU5 were culture age (Fig. 27) and culture density (Table 22). L-Tryptophan formation was dependent on the culture age with highest production occurring at logarithmic phase (Fig 27) suggesting that mid logarithmic cultures are more efficient at photobiotransformation. Though culture densities did not affect L-tryptophan yields significantly (except at very low densities), the conversion efficiencies fell markedly at higher densities (Table 22) probably due to self shading, which will impede light availability to individual cells.

Among all the parameters influencing L-tryptophan formation by Rba. sphaeroides OU5, organic and inorganic combined nitrogen sources greatly affected L-tryptophan formation (Table 24). Among the substrates tested (Table 24) inorganic combined nitrogen sources have either decreased or completely inhibited indole uptake and L-tryptophan formation. Explanation for such an effect needs further detailed experimentation, which had not been a part of this thesis nor was discussed earlier by other workers. In contrast to
inorganic nitrogen substrates, presence of organic nitrogenous substrates had no effect or enhanced L-tryptophan yields from indole + L-serine (Table 24). This enhancement may be explained as due to acceleration in the enzyme activity by compounds like anthranilate (Freundlich and Lichstein, 1962) or due to the direct participation of compounds as additional precursors since L-tryptophan could also be produced directly from DL-alanine (Table 18). However, such an enhancement could not be observed with glycine, though it could also serve as a precursor and this is the only organic nitrogen source in whose presence growth inhibitory effect of indole was evident (Table 14).

- DOES IMMOBILIZATION ENHANCE THE L-TRYPTOPHAN PHOTOPRODUCTION?

The technique of immobilization has become a method of choice to improve yields, rates and stabilize formation of desired products (Ramakrishna and Prakasham, 1999). This is true even in the case of PNSB where this technique was used for the production of H₂ (Sasikala et al., 1993). Various immobilization methods were used for photoproduction of H₂ (Sasikala et al., 1993) and entrapment was the method of choice (Hallenbeck, 1983) attempted at laboratory or pilot plant levels (Sasikala et al., 1993). Thus in this study also entrapment of *Rba. sphaeroides* OUS5 in agar and alginate was used for the production of L-tryptophan from precursors (Table 25). In contrast to earlier studies on H₂ production, where an enhancement was demonstrated (Sasikala et al., 1993), immobilization had no effect on L-tryptophan production (Table 25). In addition, immobilized matrix itself (without microorganisms) has expressed a tendency to adsorb indole as well as L-tryptophan (Table 25). Thus, about 35 % of L-tryptophan formed was recovered from the assay medium, though, neither indole consumption nor conversion to L-tryptophan themselves were affected. This clearly indicate a physical adsorption of the substrate as well as product by the immobilized matrix which will interfere in product recovery and thus immobilization is not a method of a choice of L-tryptophan production by *Rba. sphaeroides* OUS5.
CAN L-TRYPTOPHAN BE PRODUCED FROM ALTERNATE AND CHEAPER SUBSTRATES?

Even though L-tryptophan can be produced from indole + L-serine/pyruvate + NH₄Cl by *Rba. sphaeroides* OU5, the precursors used were costly and thus a study had been taken up to evaluate alternate and cheaper precursors. Glycine, a less expensive amino acid and biochemically closely related to L-serine was used for photoproduction of L-tryptophan from indole by *Rba. sphaeroides* OU5 (Fig. 33). The yields of L-tryptophan thus obtained in the presence of glycine and glycine + glucose [0.61 mM and 0.54 mM, respectively] (Fig. 33, 39) were comparable to the yields obtained in the presence of 5 mM of L-serine (Fig. 26). Further, a detailed study was conducted on glycine photometabolism by *Rba. sphaeroides* OU5 in the presence of indole for L-tryptophan formation.

Glycine is known to be metabolized by *Rba. sphaeroides* through a process of transamination which results in the intermediary formation of DL-alanine and glyoxalate (Tsuki and Kikuchi, 1962). Similar results were obtained with this organism also with the formation of DL-alanine as an intermediate in glycine metabolism even in the presence of indole (Fig. 34). However, a further formation of L-serine was also observed with glycine consumption and simultaneous formation of DL-alanine (Fig. 34). Thus, probably indole induces L-serine synthesis required for the L-tryptophan formation (Fig. 34).

Transamination of amino group of glycine is possible only in the presence of keto acids (Hug and Wekkmann, 1957) which are probably derived by *Rba. sphaeroides* OU5 from intracellular carbohydrates (Fig. 35) or externally supplied carbohydrate (Fig. 39). Contribution of intracellular reserves for L-tryptophan formation from indole + glycine by *Rba. sphaeroides* OU5, can be evidenced even from experiments with varying biomass (Fig. 36). Increasing cell biomass [and hence keto acids] (Fig. 36) and glycine concentration (Fig. 37 and 40) has increased L-tryptophan formation and maximum yields of about 1.2 mM of L-tryptophan with about 80% consumption of indole could be obtained.
Mobilization of intracellular carbohydrates (Fig. 35) as the source for ketoacids could also be confirmed from the stoichiometric molar ratios of the products formed from the consumed intracellular carbohydrates. This is also further confirmed by the formation of pyruvic acid in assay medium in the presence of indole + glycine + glucose (Fig. 39).

The above experimental evidences confirm the findings of Tsuki and Kikuchi (1962) that suggest strongly that glycine metabolism in *Rba. sphaeroides* occur only in a metabolic pool of dicarboxylic acids. APB, particularly PNSB grow photoheterotrophically using organic acids and a few carbohydrates as photosynthetic electron donors/carbon sources (Imhoff, 1995). While photoanaerobic assimilation of organic acids proceeds via citric acid cycle, carbohydrate metabolism varies with carbohydrates provided, ultimately leading to the formation of a ketoacid, pyruvate (Sasikala et al., 1993). This was further confirmed by the enhanced L-tryptophan formation in the presence of all organic substrates tested [except with α-KGA, ribose and glucose] (Table 27), which increased with the concentration of substrate (Fig. 38) and biomass (Fig 36). Differences in the indole biotransformation to L-tryptophan in the presence of organic substrates may be explained as due to the difference in the assimilation capabilities of these substrates (Table 28) and also IAA formation observed in the presence of α-KGA, ribose and glucose. Thus in the presence of some of the externally supplied keto acids (or their sources), the biotransformation does not stop at L-tryptophan formation, but proceeds further to IAA. Further, a close look in to the carbon number of the keto acids provided (Table 27), reveals that only those compounds with more than four carbon number resulted in IAA formation and not with other substrates in a light dependent process.

- **CAN IAA ALSO BE PRODUCED FROM CHEAPER SUBSTRATES?**

The conversion of indole has not stopped at L-tryptophan formation but it proceeds further to IAA in the presence of some of the externally provided keto acids. The yield of
IAA from L-tryptophan (3 mM) + α-KGA (0.1 % w/v) (Fig 42) was 530 mg IAA l⁻¹, and from L-tryptophan (3 mM) + glucose (0.1 % w/v) 210 mg IAA l⁻¹ was produced (Table 29). These are higher yields than previous reports in various bacteria: Pseudomonas fluorescens [from L-tryptophan + glucose, 102 mg IAA l⁻¹ (Oberhansli et al., 1991)], Enterobacter cloacae [from peptone, 82 mg IAA l⁻¹ (Koga et al., 1994)], Arthrobacter globiformis [from L-tryptophan, 2.4 mg IAA l⁻¹ (Forni et al., 1992)] and Streptomyces sp. [from L-tryptophan + glucose, 35 mg IAA l⁻¹ (Manulis et al., 1994)].

So far reports on the production of IAA by microorganisms were only from L-tryptophan as a precursor, which is itself an expensive compound used in pharmaceutical formulations and feedstock modifications (Bang et al., 1983a). Since, Rba. sphaeroides OU5 could photoproduce L-tryptophan itself from cheaper precursors, an attempt was made to produce IAA also directly from indole and inexpensive substrates such as glycine and glucose. During this process yields of about 61 mg IAA l⁻¹ were observed in less than 30 h (Fig 43). Only about 50% of indole added (1.5 mM) was transformed to L-tryptophan and further to IAA resulting in 100% molar yields. The above results suggest that the photobiotransformed intermediates of indole (and not L-tryptophan itself) may have an inhibitory effect on tryptophan synthesis, since, when L-tryptophan (3 mM) itself was used, it was all converted to IAA (Table 29). Yields of IAA photoproduction from indole, glycine and glucose by Rba. sphaeroides can be improved by knowing the nature of product inhibition and optimizing the process. Anoxygenic phototrophic bacteria being phototrophic, use light as the source of energy and thus offer several advantages over chemotrophs (Sasikala and Ramana, 1995 a, b), particularly in the production of indole derivatives by not utilizing the precursors either for energy or as a source of carbon or nitrogen, thus resulting in high yields and substrate conversion efficiencies.
IAA can be produced by microorganisms by two pathways, indole-3-pyruvate pathway (Koga et al., 1994) and indole-3-acetamide pathway (Manulis et al., 1994). Occurrence of indole-3-pyruvate pathway mainly depends upon ketoacid, α-KGA which leads to the formation of indole-3-pyruvate (Koga et al., 1994). Higher yields of IAA obtained with α-KGA compared to other precursors (Table 29) can plausibly be explained by assuming the presence of indole-3-pyruvate pathway for the formation of IAA in this microorganism.

- **IS INDOLE PRODUCTION FROM L-TRYPTOPHAN COMMON AMONG PNSB?**

  PNSB are known to synthesize L-tryptophan and there are no reports of wild strains showing obligate requirement for this amino acid. Production of indole from L-tryptophan is normally used to differentiate the members of Enterobacteriaceae particularly between *Escherichia coli* and *Enterobacter aerogenes*. Similar experiment was also conducted using 18 strains of PNSB belonging to the genera *Rhodobacter* and *Rhodopseudomonas*. While all *Rhodobacters* gave positive reaction to indole production (Table 30), inability of the genus *Rhodopseudomonas* to do so indicates the taxonomic importance of this test for differentiation of the two genera (earlier placed in same genus *Rhodopseudomonas*; Pfennig and Trüper, 1978) in addition to the structural similarities of macromolecular cell constituents which led to their rearrangement (Imhoff et al., 1984).

- **WHAT ENZYMES ARE INVOLVED IN L-TRYPTOPHAN PHOTOMETABOLISM OF *Rba. sphaeroides OU5?**

  Tryptophan synthase and L-tryptophanase are the two enzymes reported to be involved in L-tryptophan metabolism by microorganisms. Tryptophan synthase catalyses the reaction of indole to L-tryptophan in the presence of L-serine (Miles et al., 1984). L-Tryptophanase is a multifunctional enzyme which mostly catalyses the L-tryptophan biodegradation to indole + pyruvate + ammonium chloride and this reaction is reversible if
the concentration of the precursors is high (Snell, 1975). Thus from the precursors used and products formed during the photometabolism of indole (Table 18), it appears that this organism may be having both these enzymes. The confirmation for the existence of tryptophan synthase comes from the production of \( L \)-tryptophan from indole + \( L \)-serine (Table 18). Though formation of \( L \)-tryptophan from indole + pyruvate + \( \text{NH}_4 \text{Cl} \) indicates the presence of tryptophanase, the formation of \( L \)-serine and \( \text{DL} \)-alanine as intermediates (Fig. 31) shows that it may be \( L \)-tryptophan synthase itself which is involved in \( L \)-tryptophan formation with these precursors also. Further evidence for the same comes from the formation of \( L \)-serine as an intermediate during \( L \)-tryptophan biodegradation (Fig. 41) which is not an intermediate during the action of \( L \)-tryptophanase. Additional evidence is presented by lack of catabolite repression for \( L \)-tryptophan degradation in the presence of glucose, which inhibits the activity of \( L \)-tryptophanase (Isaacs et al., 1994). The results from indole + glycine and indole + lactate + \( \text{NH}_4 \text{Cl} \) (Fig. 34, 32), which also lead to the formation of \( L \)-serine, strongly support the above observation that only tryptophan synthase-like enzyme may be involved in \( L \)-tryptophan synthesis in \textit{Rba. sphaeroides} OU5 (Fig. 45), though the purification of the enzyme provides the conclusive evidence.

- **ECOLOGICAL SIGNIFICANCE OF THE STUDY.**

PNSB are widely distributed in environments rich in organic matter (Imhoff, 1992, 1995). \textit{Their survival depends on light as source of energy and organic matter as electron donor/carbon source} (Imhoff, 1992, 1995). Though they greatly depend on tricarboxylic acid cycle intermediates, their ability to utilize other organic compounds including aromatic molecules in light dependent anaerobic process is of a great ecological significance (Sasikala and Ramana, 1998).
Recent understanding of the capability of complex and xenobiotic aromatic compounds metabolism by PNSB (Sasikala and Ramana, 1998) coupled with utilization of heterocyclic aromatic compounds observed in this study indicate their probable role as de-pollutants in nature. In addition, biotransformation of toxic indole to less toxic and more widely utilized anthranilate (Anderson and Dagley, 1981; Braun and Gibson, 1984) indicate their additional role as detoxifiers also. This study also signifies the importance of PNSB in biotransformation of indole to its value added derivatives like IAA and L-tryptophan, which may help in plant productivity (Sasikala and Ramana, 1995a, b) in addition to helping other microorganisms which are auxotrophic to L-tryptophan (Anderson and Dagley, 1981; Bak and Widdel, 1986).

- **BIOTECHNOLOGICAL SIGNIFICANCE OF THE STUDY.**

The capability of PNSB to degrade a wide range of heterocyclic aromatic compounds necessitates their exploitation in bioremediation of contaminated environments.
The biotransformation of indole to its value added products, L-tryptophan and IAA adds to the biotechnological applications of anoxygenic phototrophic bacteria (Sasikala and Ramana, 1995 a, b). Such photoproduction using less expensive glucose and glycine has the potential to replace the conventional methods in commercial production of L-tryptophan (Nyeste et al., 1983) and IAA (Okamoto, 1988) by chemotrophic bacteria.

**CONCLUSION**

In conclusion,

1. Hitherto unknown metabolic potential of PNSB in metabolizing indole and other heterocyclic aromatic compounds was discovered and
2. New biochemical pathways of indole photometabolism are proposed along with a description of ecological and biotechnological significance of such a metabolism.

Much of the work presented here is published in standard refereed journals of international repute (copies enclosed).