Chapter 1.
Introduction
General introduction

The tidal forests of coastal wetlands, existing in the intertidal zones of sheltered shores, estuaries, tidal creeks, backwaters, lagoons, marshes and mud-flats of the tropical and sub-tropical regions are commonly referred to as 'Mangroves'. Though, mangroves generally prefer shallow sheltered intertidal swampy regions, they are capable of establishing and growing in the shallow sheltered sandy and rocky coasts. They form an important ecological asset and economic resource of the coastal environment. The mangroves are the most productive and ecologically sensitive ecosystems, which can efficiently fertilize the sea and potentially protect the coastal zone. Mangrove regions being rich in detritus (organic matter), serve as a natural nursery and feeding grounds for a variety of fishes and shellfishes, and hence are also used for aquaculture practices [McIntosh, 1982; Achuthankutty and Nair, 1983]. The mangroves exist under very hostile and inhospitable conditions like higher salinity, tidal extremes, wind velocity, high temperature and muddy anaerobic soil. The plants have peculiar adaptations such as support roots, viviparous germination, salt-excreting leaves, breathing roots, knee roots, etc., by which the plants are well-adapted to water-logged, anaerobic saline soils of coastal environment. The mangrove flora can also adapt to climatic changes (precipitation and temperature), sea level rises and to the incidence of solar ultraviolet-B radiation [Rahaman, 1990; Swaminathan, 1991; Moorthy, 1995; Moorthy and Kathiresan, 1996]. They play a significant role in sedimentation, helping in land building process, and also
protect the same by reducing erosion with the help of their specialized root network.

The mangrove area in Asia equals more than 5.8 million hectares and accounts for some 38 percent of global mangrove area, representing the highest percentage of mangroves worldwide. Indonesia is the country with the largest extent of mangroves in the region (and in the world), accounting for about half the regional extent of mangrove area. Other Asian countries with a significant extent of mangroves are (in order of mangrove area) Malaysia, Myanmar, Bangladesh and India, which, together with Indonesia, account for more than 80 percent of total Asian mangrove area. Asia has the largest mangrove area of any region, and the mangroves are exceptional for their high biodiversity (especially in South and Southeast Asia). The edaphic and coastal features of South and Southeast Asian countries, together with the high rainfall and significant riverine inputs, are particularly favorable to the development of well-structured mangrove forests. Some of the largest mangrove forests in the world are found in Asia, the best known being the Sundarbans, a transboundary forest covering approximately 1 million hectares in Bangladesh and India.

**Distribution and ecology of mangroves in Goa (south-west coast of India):**

Mangroves are restricted to the lower latitude 32°S-38°N in the tropical regions of which the maximum diversity and area cover lies between 25°S-25°N. Indian mangroves are distributed in about 6,740 km² [Krishnamurthy et al, 1975] which constitute 7% of the total Indian coastline [Untawale, 1987]. Along the
central west coast, approximately 21,000 hectares of mangrove area have been estimated, while along Goa it was estimated to be ~ 2,000 hectares [Jagtap 1985; Jagtap et al, 1993,1994]. The Goa coastline is approximately 110 km long and within the latitude 15° 00’N - 15° 52’N and longitude 73° 30’E - 74° 44’E. The inter-tidal zones of two major (Mandovi and Zuari) and seven minor estuaries in Goa are mostly flanked on both sides by rocky cliffs formed with silty-sand and silty-clay along with copious amounts of organic matter. Mandovi and Zuari are the two major estuaries that flow over an area of 2500 km² that is about 68% of the total geographical area and are important for the economy of the territory. They flow through the mining areas and are heavily used for transporting ferromanganese ores to the Marmugao harbor (Goa). About two-third of the total ferromanganese ores of Goa come from the mines located in the basins and watersheds of these two estuaries. In fact, 90% of ferromanganese ores are transported through these estuaries in barges [Nair et al, 2003].

The mangroves grow luxuriantly in alluvial soil substrate, which are fine textured, loose mud or silt, rich in humus and sulphides [Rao, 1987]. Their distribution is limited by temperature [Duke, 1992] and they prefer moist atmosphere and freshwater inflow, which brings in abundant nutrients and silt from terrestrial sources. Repeatedly flooded but well-drained soils support good growth of mangroves, but impeded drainage is detrimental [Gopal and Krishnamurthy, 1993]. The Indian mangrove flora is comprised of more than 60 species belonging to 41 genera and 29 different families and of these; about 50% are reported from the west coast [Deshmukh, 1991]. About 25 species reported
from east coast are not found along the west coast. Similarly, about 8 species that characterize the west coast are absent on the east coast. *Rhizophora*, *Sonneratia*, *Avicennia*, *Excoecaria*. etc are some of the dominant mangrove genus found along the Mandovi and Zuari estuaries, while *Bruguiera*, *Acanthus*, *Derris*, *Clerodendrum* etc are less abundant.

**Nitrogen cycling in mangrove ecosystem:**

An overall perspective of the nitrogen cycle is summarized and illustrated in Figure 1. In mangrove ecosystems, the nitrogen flux is dynamic and partitioned between terrestrial, aquatic and benthic compartments. Studies on the seasonal variation in nitrogen fluxes in mangrove sediments and waters along the west coast of India have been done by Dham (2000) and Heredia (2000) respectively. Though mangroves are considered to be productive coastal marine ecosystems [Qasim and Wafar, 1990], nutrient measurements, especially that of nitrogen, an important factor sustaining this production has been sparse Dham *et al* [2002]. Additionally, the concepts of new and regenerated production [Dugdale and Goering, 1967], has triggered an entire gamut of elemental flux studies, principally that deals with the key element, nitrogen. Separate estimates for new and total production are crucial for quantifying carbon and nitrogen fluxes into the sea [Platt *et al*, 1991]. Most of the nutrient flux studies in mangroves have been confined to a limited period of the annual cycle [Boto and Wellington, 1988; Trott and Alongi, 1999; Harrison *et al*, 1983; Rivera-Monroy *et al*, 1995; Krishnamurthy *et al*, 1975]. Recently, Dham *et al* [2002] have reported the seasonal changes in...
uptake of nitrogenous nutrients and regeneration in the plankton fraction of a mangrove ecosystem on the west coast of India.

**Benthic nitrification: Process and Controls**

Nitrification occupies a central position within the global nitrogen cycle. It is a microbial process by which ammonium is sequentially oxidized to nitrite and nitrate. It is an important process in the nitrogen cycle, particularly because it links nitrogen mineralization to potential nitrogen loss from the benthic system through denitrification [Seitzinger, 1990; Sloth et al., 1992]. It is the dominant process converting reduced inorganic nitrogen to its oxidized form and mitigating ammonium levels from being toxic [Hall, 1986; Sloth et al., 1992], thus maintaining homeostasis. The oxidation of ammonium is a two-step process catalyzed by ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO). AMO catalyzes the oxidation of ammonium to hydroxylamine and HAO catalyzes the oxidation of hydroxylamine to nitrite. HAO is located in the periplasm and is a homotrimer with each subunit containing eight C-type hemes [Daniel et al., 2002].

Despite the potential importance of nitrification, only a few studies have explored the factors regulating this process in mangroves [e.g., Dham, 2000 and Heredia, 2000], and no single set of factors has emerged consistently as the regulator of nitrification rates.
Diversity and abundance of nitrifiers:

Nitrifying bacteria are the only organisms which are capable of converting the most reduced form of nitrogen (ammonium), to the most oxidised form (nitrate) and also can carry out a range of other processes within the nitrogen cycle. Though nitrification is an autotrophic process, heterotrophic nitrification is also reported to occur in various groups of bacteria and fungi, though at a slower rate than that found among autotrophic organisms [Verstraete and Alexander 1973; Watson et al, 1981]. *Nitrosomonas, Nitrosococcus, Nitrosospira* etc. are the most frequently observed genus associated with the process of ammonium oxidation and *Nitrobacter, Nitrospina, Nitrococcus, Nitrospira* etc. are involved in nitrite oxidation [Watson et al, 1981]. In the recent times, several studies have reported the use of molecular probes by Fluorescence In Situ Hybridization (FISH) for detection and enumeration of the nitrifying community. 16S rRNA probes for FISH have been successfully used for identification and quantification of nitrifier populations in nitrifying fluidized bed reactors [Wagner et al, 1998] and autotrophic nitrifying biofilms [Kindaichi et al, 2004]. Most Probable Number (MPN) method is also employed [Whitby et al, 2001] to quantify nitrifiers in fresh water lake sediments.

Impact of abiotic parameters on nitrification:

Nitrification was traditionally considered to be restricted to aerobic environments [e.g. Froelich et al, 1979], but recent studies [Mortimer et al, 2004] have shown that nitrification does happen in anoxic environments at the expense
of elements like manganese and/or iron. In general, benthic nitrification rate is regulated by the availability of dissolved oxygen [Caffrey et al, 2003] and ammonium [Henriksen and Kemp, 1988]. It also depends on ammonium regeneration rates, which in turn is positively influenced by temperature [Nixon, 1981]. Ammonium oxidation is also controlled by light intensity; light stimulates ammonium assimilation while it inhibits oxidation [Ward et al, 1984]. Caffrey et al, [2003] have also shown that nitrification rates are negatively influenced by hypersaline conditions. In vitro studies have shown that several compounds like valine, hydroxyproline, threonine, thiourea, thiosinamine, dl-Methionine, chloromycetin, nitrourea and nitromethane [Quastel and Scholefield, 1949] are inhibitory in nature at various concentrations, while a number of organic amendments, including yeast extract, vitamin free casamino acids, acetic acid and some amino acids can stimulate growth and nitrification rates. On the other hand the presence of glucose or glycerol, does not enhance the rate of nitrification, and may diminish the rate and the yield of nitrate formed, by diverting nitrogen from the nitrifiers to the heterotrophs proliferating at the expense of easily assimilable carbon [Delwiche and Finstein, 1965].

From the previous sections it is obvious that nitrification, though a key reaction in the environment is influenced by an array of factors. Some have positive influence while some are negative. Hence, the health and sustainability of the 'Mangrove-Buffer Zones', and there by coastal environments needs to be studied from the perspective of nitrogen cycling and nitrification in particular. To understand this aspect the following aim and objectives were set forth.
Aims and Objectives of the present study:

The aim of the present study is to understand the principle factors influencing nitrification rates in mangrove ecosystems and to delineate the taxonomy and nutritional status of the nitrifier community therein. This study has been conducted with the following objectives:

- to quantify the abundance of nitrifying populations
- to identify the nitrifiers at cellular and molecular level
- to delineate their trophic status
- to quantify nitrification rates and understand the influence of environmental parameters.

Significance:

Mangrove forests that once covered more than 200,000 km$^2$ of sheltered tropical and subtropical coastlines is disappearing worldwide at a rate of 1 to 2% per year. This is very much comparable or greater than the loss in adjacent coral reefs or tropical rainforests. Most of this happens in the developing countries where >90% of the world's mangroves are located. As mangrove areas are becoming smaller or fragmented, their long-term survival is at great risk, and essential ecosystem services may be lost. Therefore, any further decline in mangrove area is likely to be followed by accelerated functional losses. Mangroves act as a CO$_2$ sink as well as an essential source of oceanic carbon. The decline also affect mangrove-dependent fauna, as well as physical benefits like the buffering of seagrass beds and coral reefs against the impacts of river-
borne siltation, protection of coastal communities from sea-level rise, storm surges, and tsunamis.

This study is probably the first of its kind addressing the nitrification issue in mangroves in conjunction with the bacterial flora mediating this process. Very little research has been carried out on benthic nitrification in the marine environment, especially from the mangroves. This study on down core variability in nitrifiers and nitrification rates at a monthly resolution is probably the first of its kind in any mangrove ecosystem. Also, a systematic account on the occurrence and significance of heterotrophic bacteria in nitrification is recorded in a comprehensive way. Though some reports are available on the impact of certain abiotic factors on nitrification rates, this study addresses the influences of key anthropogenic inputs like liquid hydrocarbons, fertilizers and pesticides besides the other well known factors. This work adds a new dimension to ecological management in coastal zones by demonstrating the elements functioning and governing nitrification in these environments. Moreover, the nitrifiers isolated from these environments could be used for bioremediation processes in an efficient and environmentally safe way.
Figure 1. Key redox transformations in the nitrogen cycle