CHAPTER 1
INTRODUCTION
Phosphorus (P) is one of the essential nutrients for every life form, from bacteria and plankton to organisms of higher trophic levels, and has been extensively investigated in relation to plant and aquatic productivity. Thus the P cycle in aquatic environments has been a topic for scientific study for several decades. Einsele (1936, 1938) and Mortimer (1941, 1942) were the first limnologists to recognize the importance of iron phosphorus interactions and the coupling between mobilization and redox conditions. Their observations form the basic of many studies which concern the precise nature of iron phosphorus bindings in sediments and suspended matter and their effect on the phosphorus buffering system, i.e. the ability of sediment to constrain phosphate concentrations within narrow limits (Froelich, 1988). The sorptive properties of soil minerals were first investigated by agronomists and soil scientists to assess ‘phosphate fixation’ which makes part of the phosphorus applied as fertilizers unavailable to plants (Kittrick and Jackson, 1956). In sediment-water systems phosphate fixation has been studied to determine the long term loss of phosphorus from the ecosystem to the sediment. Soil scientists also initiated the development of reliable sequential extraction schemes to distinguish between phosphorus compounds bound to different complexes and minerals (Chang and Jackson, 1957). Sequential extraction has proven to be a useful tool in quantifying metal-P associations as non-mobilizable phosphorus. The fundamental mechanism for the kinetics of phosphate sorption on solids was described by Carritt and Goodgal (1954) in estuarine sediments. The mechanism they proposed consists of a rapid surface adsorption reaction followed by a much slower diffusion controlled process towards the interior of the solids; the slow step is particularly important to predict sedimentary phosphorus binding capacity and future behavior of sedimentary P complexes after changing external loadings.

1.1 Background of the study
All the discussed aspects are largely of an inorganic geochemical nature. Yet, microbiology forms a substantial part of the field of research. The most obvious reason is that mineralization of organic phosphorus is the first and driving step in benthic P cycling and sediment-water exchange processes. Consequently, mineralization and biogeochemistry of P compounds are important subjects.

1.2 P and its occurrence

Phosphorus (P), a multivalent nonmetal element of the nitrogen group with atomic number 15, is the eleventh most abundant element and comprises approximately 0.1% by mass (Fuller, 1972; Klein and Hurlbut, 1999) in the Earth’s crust. It occurs in the form of inorganic phosphate minerals and organic phosphate derivatives in rocks, soil and sediment. It is almost always present in its maximally oxidised state, and apatite \( \text{Ca}_5(\text{PO}_4)^{3-} (\text{F, Cl, OH}) \) is the most common naturally occurring P containing mineral in the Earth’s crust (over 95% of P); however, approximately 300 additional minerals that contain phosphate (\( \text{PO}_4^{3-} \)) have been described (Jahnke, 1992). Elemental phosphorus exists in two major forms- white phosphorus and red phosphorus, but due to high reactivity phosphorus is never found as a free element on Earth.

In biological sense, P is essential for most (http://www.nasa.gov/topics/universe/features/astrobiology_toxic_chemical.html) life. As phosphate, it is present in all living things as a component of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) - the major hereditary molecules of life, adenosine triphosphate (ATP) - a fundamental energy source in living things, and also as phospholipids that form all cell membranes (http://www.enviroliteracy.org/article.php/480.htm). Demonstrating the link between phosphorus and life, elemental phosphorus was historically first isolated from human
urine, and bone ash was an important early phosphate source. There is over 1 lb (454 grams) of phosphorus in the human body. It is found in complex organic compounds in the blood, muscles, and nerves, and in calcium phosphate, the principal material in bones and teeth. Phosphorus compounds are essential in the diet - organic phosphates, ferric phosphate, and tricalcium phosphate are added to foods. Dicalcium phosphate is added to animal feeds.

1.3 Various forms of P in ecosystems

P is found in various forms in nature. They are briefly described in the following section-

1.3.1 Forms of P in soil

There are many types of P found in soils, the biologically available form is orthophosphate (Lindsay, 1979). The inorganic P constituents in mineral soils can generally be classified into two groups based on the most significant contribution to P solubility, namely, calcium phosphates and iron and aluminum phosphates. Among the calcium phosphate $\text{Ca}_{10}F_2(\text{PO}_4)_6$ (fluorapatite), $\text{Ca}_{10}(\text{OH})_2(\text{PO}_4)_6$ (hydroxyapatite), $\text{Ca}_{10}O(\text{PO}_4)_6$ (oxyapatite) and $\text{Ca}_{10}\text{CO}_3(\text{PO}_4)_6$ (carbonate apatite) are the most abundant. $\text{FePO}_4.2\text{H}_2\text{O}$ (strengite) and $\text{AlPO}_4.2\text{H}_2\text{O}$ (variscite) are the other main P-bearing minerals.

In general, native inorganic and organic sources of soil P are highly stable and of little short-term consequence for commercial crop production. Organic P compounds in mineral soils can be found in the form of inositol phosphates, of which phytic acid is the most significant component, phospholipids, nucleic acids, nucleotides and unidentified sugar phosphates. However, part of the organic P pool may undergo mineralization or occur as dissolved materials in the soil solution.
Wetland sediments act generally as sinks for phosphorus. Consequently a mass balance for a given wetland shows a net sedimentation of phosphorus; only during a recovery do sediments act as a source of phosphorus on an annual basis. The source of sediment phosphorus is partly settled particulate phosphorus of allochthonous origin or of autochthonous origin and partly dissolved phosphate sorbed to the surface sediment and accumulated in the interstitial water. In rare cases dissolved phosphate derived from seepage is accumulated in the sediments.

The allochthonous contribution of P to the sediments consists mainly of organic matter produced along the shoreline or in the drainage area and transported to the wetlands with its tributaries. In drainage areas with high rate of soil erosion, a considerable part of allochthonous phosphorus input is in the form of inorganic particulate phosphate sorbed to mineral particles or in the form of phosphorus containing minerals. The autochthonous settling material consists of dead or live planktonic organisms, excretion products, organic detritus, precipitated humic acid substances and calcium carbonate acting as vehicle for phosphate.

In the sediments, diagenetic processes transform the sedimented phosphorus according to biological activity, redox conditions and pH into the final sediment composition. Hence the quantitative and qualitative composition of aquatic sediments is determined by the source of the sediments and the bio-, physico-, chemical environment in the sediments.

1.4 P cycling

The P cycle is a biogeochemical cycle that describes the movement of phosphorus through the lithosphere, hydrosphere, and biosphere. Unlike many other biogeochemical cycles, the atmosphere does not play a significant role in the movement of phosphorus,
because phosphorus and phosphorus-based compounds are usually solids at the typical ranges of temperature and pressure found on Earth. The production of phosphine gas occurs only in specialized, local conditions.

1.4.1 The soil P cycle

P is present in 3 basic forms (or stores) in soil: biomass (living tissue), organic matter (dead and decaying tissue), inorganic matter (minerals, and simple ions). Biological and chemical processes constantly change soil phosphorus from one form to another, in what is known as a "biogeochemical cycle". Plants and other soil organisms take up inorganic phosphate and incorporate it into their tissue. When these organisms die, their remains accumulate as soil organic matter. As this organic matter decays, organic compounds are broken down into simpler inorganic molecules and ions. The release of inorganics from decaying organic matter is known as "mineralization."

Plant uptake of soil phosphorus occurs only from the inorganic store. Plant roots will absorb phosphate ions ($H_2PO_4^-$, $HPO_4^{2-}$) dissolved in soil water. $H_2PO_4^-$ is the dominant phosphate ion in our soils. $HPO_4^{2-}$ becomes dominant at soil pH's greater than 6.5 (neutral and basic soil reactions).

Losses of soil phosphorus occur mainly by runoff/erosion and harvesting of crops. Phosphorus tends to concentrate in the organic matter store, which accumulates on the soil surface. Erosion, therefore, tends to take a disproportionate amount of soil phosphorus. More important than the loss of soil phosphorus fertility is the deposition of this eroded phosphorus into surface waters where it poses an environmental hazard of eutrophication. Inputs of phosphorus can be provided through fertilizer, livestock manure and other sources of phosphorus.
An important transformation of soil phosphorus results from the reaction between soluble inorganic phosphates and other soil inorganic components. These reactions produce insoluble mineral compounds and make the native and applied soil phosphorus less available for plant uptake. These reactions are known collectively as "phosphorus fixation". The opposite chemical reaction, "weathering" also occurs, converting fixed phosphorus to more soluble, available, forms, but weathering is usually a much slower process than fixation. In acid soils (pH < 6.5) phosphorus is fixed mainly by aluminum and iron. In basic (alkaline) soils (pH > 7.2) phosphorus is fixed mainly by calcium. The more acidic the soil, the greater is the reactivity of Al and Fe and the greater degree of phosphorus fixation. It is estimated that as much as 90% of added fertilizer phosphorus is fixed in acid soils (http://nsac.ca/pas/staff/dlynch/ext_bull/exten_p2.htm).

1.4.2 P cycling in aquatic environments

According to the classical view of P cycling in lakes, phosphate (PO$_4^{3-}$) flushed into a lake by its tributaries or released from the sediments is taken up from the water by living and non-living suspended particles and lost to the lake bottom in proportion to the settling velocities of the particles. In the sediment, organic P is liberated as soluble phosphate to interstitial water during decomposition by bacteria, or it becomes buried as refractory organic P. The liberated P may be adsorbed to inorganic surfaces, complexed by refractory organic materials, or precipitated as apatite or vivianite. Part of it may remain in sediment or be recycled into the overlying water. In many cases, the flux of P from the sediment to the water is largely controlled by the prevailing redox conditions at the sediment/ water interface. An oxidized sediment surface often prevents or strongly diminishes release of P from the sediment to the water. It forms an efficient trap for dissolved iron (Fe), manganese (Mn) and P moving the interstitial water from reduced
sediments to the oxic surface. When the hypolimnion and, hence, the sediment surface become anoxic, this barrier disappears and release rates of Fe, Mn and \( \text{PO}_4 \) often increase markedly, indicating reduction of ferric hydroxide complexes and subsequent release of ferric ion and adsorbed \( \text{PO}_4 \) (Mortimer, 1941, 1942; Einsele, 1936). In lakes with too low a Fe:P ratio or with too high a pH, release and binding of inorganic P cannot be controlled exclusively by iron. Boström et al. (1982) discussed additional chemical, biological and physical mechanisms (e.g., sorption of P to clay minerals and humic substances, bacterial activity, bioturbation by benthic organisms, mobilization of P by rooted macrophytes, wind induced turbulence in the sediment overlying water, re-suspension gas ebullition and temperature) controlling the cycling of P at the sediment water interface.

### 1.4.3 P availability in sediment

P is one of the major essential growth-limiting macronutrients for biological growth and development, and unlike the case for nitrogen, there is no large atmospheric source that can be made biologically available (Ezawa et al., 2002). Phosphorus (P) is proven to be one of the key limiting factors of bacterial activity and for primary production in aquatic systems. With the constant input of sewage and fertilizer in past years, large amounts of P have been detected accumulating in lake sediments and would be released into overlying water under suitable environmental conditions, a process known as internal P loading (Ribeiro et al., 2008). Only about 10% of phosphate fertilizers applied in aquaculture becomes available in water column which may trigger acute undesired eutrophication; the rest majority gets bound to sediment and converted into insoluble compounds and become unavailable to primary producers (Jana, 2007).
The transition and transformation of P at the water-sediment are of physical, chemical, and biological nature. A substantial amount of research has been carried out in the chemical and physical fields, such as the impact of changes in pH, dissolved oxygen (Edlund and Carman, 2001), and disturbances (Wang, 2009). In the biological field, microbial activity can significantly influence the release of P (Jacobs et al., 2002; Khoshmanesh et al., 2002). Bacteria can dissolve insoluble inorganic P with the action of low molecular weight organic acids which are produced in the periplasmic space of some Gram-negative bacteria through a direct glucose oxidation pathway (Anthony, 2004). Mineralization of organic forms of P is mainly dependent on bacterial activity since the mineralization of these compounds is carried out by means of several phosphatases secreted by microorganisms (Gautier et al., 2005). These groups of bacteria that can directly influence P release have been termed as P releasing bacteria.

1.5 Role of bacteria in P mobilization

In the scenario described above, bacteria are treated only as ‘catalysts’ that accelerate solubilization of P, by oxidation of organic detrius and reduction of various oxidants. By consuming O$_2$, NO$_3^-$ and SO$_4^{2-}$, they provide the necessary conditions for abiotic or biotic reduction of ferric iron (Fe$^{3+}$), subsequent release of P and precipitation of iron sulfide (FeS). The concentration of dissolved P in the interstitial water is assumed to be controlled by mineralization, by abiotic physical/chemical equilibria or precipitation/dissolution or sorption/desorption, and by diffusion.

By definition, catalysts are neither produced nor consumed during a chemical reaction, and their composition and pool size are constant. Thus, it is assumed that the amount of P bound to bacteria does not vary (i.e., uptake is always compensated by release, independent of varying environmental conditions). As a consequence, uptake and release
of P by bacteria and, hence, their potential role in P mobilization across the sediment/water interface, often is disregarded or considered to be negligible in comparison to mineralization rate and chemical equilibrium. Bacteria are not, however, only in corporal sites of enzyme production. More likely, as other organisms, they depend on P as a nutrient; hence, they are able to take it up from the organic substrate or from the water. Net release seems to be controlled by their demand for P.

The P releasing bacteria include both the inorganic phosphate solubilizers (collectively known as phosphate solubilizing bacteria), and organic P mineralizer. The inorganic phosphate solubilizing bacteria are microorganisms that solubilize mineral-bound insoluble phosphate forms through production of organic acids like gluconic acid, ketogluconic acid, citric acid, lactic acid etc. (Nautiyal, 1999; Lin et al., 2006); enzymes like glucose dehydrogenase, citrate synthase, and lactate dehydrogenase involved in oxidation of glucose and other intermediates of energy metabolism pathways are important in production of these organic acids (Goldstein, 1994; Chen et al., 2006). P mineralizers release P through decomposition of organic matter and enzymes like phosphatase, phytase, nucleotidase etc. have been associated with process (Rodríguez and Fraga, 1999). Microbes having these enzymes have been widely studied in agricultural soil. In lakes, microbial decomposition is considered as one of the most important process of sediment P release; however, microbes involved in the process in aquatic environments have been meagerly characterized.

The P releasing bacteria has shown their wide application in crop production as cost effective biofertilizer. This group of bacteria is also known as ‘plant growth promoting rhizobacteria’ (PGPR) and they include genera like Pseudomonas, Azospirillum, Burkholderia, Bacillus, Enterobacter, Rhizobium, Erwinia, Serratia, Alcaligenes, Arthrobacter, Acinetobacter and Flavobacterium (Rodríguez and Fraga, 1999), and are commercially used
in agriculture practice to enrich the nutrient status of terrestrial soil. Use of these bacteria as biofertilizer in aquaculture mobilizes the sediment bound P (Chang and Yang, 2009), thus helping in nutrient enrichment or restoration of aquatic environments in an ecologically benign manner. However, this trend is very limited and requires further studies so as to properly manage the aquaculture practices in biological way.

### 1.6 Nutrient imbalance in aquatic environments: Indian scenario

Global aquaculture practices can be subdivided on marine and freshwater basis where the freshwater aquaculture occupies 59.9% of total global aquaculture (http://www.fao.org/docrep/014/ba0132e/ba0132e.pdf). Globally, freshwater occupies 0.003% of total water fills (http://en.wikipedia.org/wiki/Fresh_water), while India alone has 8.05 million ha of freshwaters (10% area of the world's fresh water supply) (http://en.wikipedia.org/wiki/Fishing_in_India), and 4.03 million tones of freshwater productivity (http://www.fao.org/fishery/countrysector/naso_india/en). The freshwaters include ponds, reservoirs, wetlands etc. Wetlands, especially floodplain wetlands, are integral part of riverine ecosystems and are hot spots of biological activities, playing essential functions like water reserve and recharge, flood control, purification, supporting biological diversity etc. These wetlands are also breeding ground of fish fauna and provide substantial fish food for a nation. India has 0.88 million ha wetland area, of which 19550 ha are cut off meanders (http://moef.nic.in/downloads/public-information/NWIA_National_brochure.pdf), and 0.202 million ha of floodplain wetland areas, concentrated in the states of Assam, West Bengal and Bihar (Sinha, 2003), which are rich in endemic fish species and considered highly productive with fish production potential of about 1000-1500 kg ha⁻¹ and surpass reservoir productivity (73.63 kg ha⁻¹) (http://www.fao.org/docrep/003/v5930e/V5930E01.htm) but quite less than the pond
productivity. However, actual production from these wetlands is often <200 kg ha\(^{-1}\), excepting in some well managed wetlands, where production has exceeded 800 kg ha\(^{-1}\) (Vinci, 2003). The low productivity is mainly due to improper management and distribution in terms of content related imbalances of P and N (Das, 2003).

1.7 Objective of the study

In the present world, when there is regardless concern about the anthropogenic phosphorus input in water bodies in the form of fertilizers either applied directly to enhance the aquatic productivity, or by agricultural runoff, at this moment our work is to generate some microbial species that can be selectively used for augmenting of internal P loading in wetlands for better productivity management.

The objective of this study is to isolate and identify microbes from freshwater environments those can release phosphorus from inorganic and organically-bound forms, determine their activity in sediment microcosm towards sustainable P release for aquatic productivity enhancement, and thus predict some bacteria as good candidate for bio-fertilization application.