CHAPTER 2
LITERATURE SURVEY
2.1 Sediment P chemistry

P is a limiting macronutrient, whose concentration often determines the productivity of aquatic systems (Boström et al., 1982). Sediment plays a fundamental role in phosphorus metabolism determining its concentration, distribution, and final fate (Fytianos and Kotzakioti, 2005). Sediments may act as sink as well as source of P for the overlying water (Baudo et al., 1990). This dissolved P is used by the biotic community, starting from bacteria to organisms of higher trophic level. Internal loading by sediment release plays an important role in the phosphorus status of lakes (Granéli, 1999; Scharf, 1999), and thereby supports the trophic status of water bodies. This dual role of the sediment in P turnover has been known for a long time. Einsele (1936, 1938) and Mortimer (1941, 1942) described the P chemistry in relation to iron chemistry. They demonstrated that oxygenated sediment retained P by fixing it to iron (III), while reduced sediment released P by reduction of iron and subsequent dissolution of Fe-P. However, this mechanism was quite not obvious in calcareous soil.

Four major different mechanisms determine the extent and rate of deposition of P in wetland sediment, viz.

I. Sedimentation of detrital P minerals derived from rapidly settling material of the water shed.

II. Adsorption or co-precipitation of P with various inorganic compounds like compounds of iron and manganese.

III. Sedimentation of P with allochthonous organic matter or autochthonous organic matter.

IV. Direct uptake and assimilation of P from water column by biotic community in the sediment.
Phosphorus retention in a wetland is dependent on the sedimentation characteristics of the particulate P carriers. Differences between wetlands in P retention status are reflected in the composition of sedimentary P. The fractional composition of sedimentary P is usually estimated using sequential chemical extractions. For study of biogeochemical cycle of phosphorus, these chemical fractionation methods have been predominantly used. These methods involve a successive addition of different extractant to sediment samples, each of which is expected to extract a particular fraction of phosphorus, and P fractions identified by this procedure are operationally defined based on the extractant (Abdel-Satar and Sayed, 2010). The fractions thus obtained correspond neither to specific retention, nor to bindings with sedimentary particles. Thus the loosely bound P was represented as NH₄Cl-P, the P associated with Al is represented as NH₄F-P. P bound to metal oxides, mainly those of Fe is represented by NaOH-P. The concentration of the NaOH-P fraction can be used for the estimation of both short-term and long-term available P in sediments and it is a measure of available algal P (Zhou et al., 2001). The P fraction that is assumed to consist mainly of apatite P is represented by HCl-P. The sediment can be divided into different categories, according to the significance of different P fractions.

The high pool of loosely bound P or the NH₄Cl-P indicates saturation of the P binding site in the sediment, while in the iron rich region the excess P may be deposited with the Fe. Calcareous sediment constitutes a significant portion of HCl extractable P. The NaOH-P and HCl-P seem to be the most important inorganic P pools (Golterman, 2004). However, when the inorganic P concentration is very low, most of the P in the sediment is either organically bound or refractory in nature (Boström at al., 1988).
2.1.2 Release of P from wetland sediment (P mobilization)

P release from the wetland sediment to the overlying water occurs either as mobilization of the P species from the re-suspended sediment particles, or by mobilization of the to the dissolved sediment particles (as in case of reduced Fe(II)-P particles) and thereafter through the upward transfers of the dissolved species (Fig. 2.1). This mobilization process is generally governed by certain environmental factors, however, the type of physical, chemical, and biological reactions that occur in the sediment have pronounced effect on P mobilization. P as in the form of orthophosphate may be bound to sediment particles by various physical and chemical interactions, starting from simple adsorption (physical interaction) over the particles, to different types of chemical bonds like covalent or ionic bonds which even vary over their strength. The mobilization process may also be physical, chemical or biological. The physical and chemical mobilization processes include, desorption, dissolution of P containing particles to water column, and functional group exchange reactions like exchange of orthophosphate with hydroxyl or chloride or any other group. The biological mobilization includes mineralization processes by the hydrolysis of phosphoester bonds by various enzymatic reactions, release of stored P from living cells as a result of changed environmental conditions (such as formation of anaerobiosis) leading to changed cellular metabolism, and autolysis of cells after their death.
The effects of pH vary over the constituent of sediment. In non-calcareous sediment, with the increased primary productivity, the water column pH increases. This increased pH at the sediment water interface decreases the P holding capacity of Fe. Thus at this higher pH, anion exchange reactions take place where OH\textsuperscript{-} ions are exchanged with PO\textsubscript{4}\textsuperscript{3-} with the concomitant liberation of P to the water column (Boström et al., 1982). Moreover, at higher pH the binding of water soluble P with sediment Fe(III) is also inhibited (Boström et al., 1988).
However, in calcareous or Ca rich sediment the phenomena are quite different. Here iron plays less important role in the P exchange process. At higher pH and temperature solubility of CaCO$_3$ decreases, causing them to precipitate. The P mobilization can be affected by CaCO$_3$ in several ways. Either the P being adsorbed to the surface may be co-precipitated with CaCO$_3$ or sedimentation of plankton (which are considerable P user in wetlands environments) and bacteria along with CaCO$_3$ crystals reduce the use of P-thus making P available to water column (Stauffer, 1985).

Temperature induced P exchange involves the aerobic/ anaerobic shift of the sediment. Increased temperature decrease the solubility of air in the overlying water, creating partial anaerobic condition. This is further facilitated by temperature induced increased microbial activity, which demands for enhanced oxygen uptake. Temperature also causes evaporation of water from wetlands, decreasing the depth of water column. Surface sediments of these non-stratified wetlands are sometimes exposed to more heterogeneous conditions due to exposure to circulating overlying water- which rapidly forms and destroys micro-stratification. The high temperature and rapidly formed micro-stratification produces small anaerobic zones in the sediments that favor various P mobilization processes (Boström et al., 1988).

Bioturbation also increases the exchange of pore water and wetland water. The pore water which often contains the loosely bound P increases the mobilization of P in the wetland (Boström et al., 1988).

### 2.1.3 Role of microbial processes in P mobilization

Microbial activities in wetlands’ sediment affect the P mobilization process over sediment water interface in a number of direct or indirect ways. The mineralization process involves the transfer of P from detritus matters to biological community, and then to the mobile P pool. This process is dependent on the type of organic matters
present in the sediment, as well as, the type of respiration prevails. Changes in the chemical environment of the sediment, that controls the P exchange, are by and large determined by the microbial activities in the sediment.

Massive accumulation of inorganic polyphosphate, in form of volutin granules in cytoplasm, has been reported in many microorganisms. Microorganisms are able to take up excess of P under aerobic condition, and release them under anaerobic condition. This rapid uptake of P is coupled with the storage of polyphosphate granules. Under anaerobic condition, microorganisms use this P storage for utilization of the orthophosphates for ATP synthesis. This produced ATP is used for the formation of poly β-hydroxy butyrate (PHB) granules. These PHB granules are utilized as an energy source during aerobic condition (Wentzel et al., 1986). The breakdown of ATP thus increases the orthophosphate concentration in the cytoplasm. This accumulated orthophosphate increases the osmotic pressure inside the cell favoring diffusion of P out of the cell. Thus change in metabolism and respiration process of microbes coupled with the aerobic-anaerobic shift of sediment control P mobilization in the sediment.

Besides the release of intracellular P, microbes are often treated as catalyst in P regeneration from allochthonous organic matter. Net P yield from organic matter to mobilized P pool depends on the type of P present in the organic matter, and the growth yield of bacteria (Fenchel and Blackburn, 1979). When the growth yield is low, the availability, as well as, the mobility of mineral nutrients relative to carbon substrate is higher. Settling organic matter may also act as a sink rather source of P (Gächter and Meyer, 1993) through accumulation of SRP during the process of sedimentation. Thus C:P ratio of settling organic matte decreases with depth. Bacteria with its high internal P loading than algae or higher plant sources, exhibits C:P ratio 20:1. Thus, with high growth rate of bacteria during aerobic condition, net P mobilization only occurs when
C:P ratio of the settling organic matters is less than 40:1 (Gächter and Meyer, 1993). The impact of mineralization process on P mobilization depends on several factors-

I. The degree of decay of settling organic matter.

II. The type of mineralization process prevailing in the sediment.

III. The initial P content, as well, as, C:P ratio of settling organic matters.

IV. The initial P availability for growth of bacteria.

V. The continuity of the settling event.

VI. Factors like pH, temperature, redox potential etc. which usually affect the P mineralization processes.

Specific metabolic reactions are involved in this P release from organic matters. Extracellular products of biomass, in particular, exoenzymes have their role in P mobilization. Other products, like chelating agents, e.g. organic acids are often released for the solubilization and mineralization of inorganic P sources by bacteria, especially by phosphate solubilizing bacteria or PSB.

### 2.2 Phosphate solubilizing bacteria (PSB)

Several workers have examined the ability of different bacterial species to solubilize insoluble inorganic phosphate compounds such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate (Goldstein, 1986) and are commonly designated as P solubilizing bacteria (PSB). P solubilizing microorganisms are mostly bacteria and fungi that cause solubilization of mineral-bound insoluble P compounds by producing various organic acids (Goldstein, 1994; Nautiyal, 1999; Lin et al., 2006). Most or majority of the PSB are effective in solubilization of Ca-bound P (Ca-P), rather than the Fe-, Al- and Mg-bound forms (Kucey et al., 1989; Fankem et al., 2006). These bacteria are termed as Phosphate solubilizing Bacteria or PSB. Among several bacterial
genera with this capacity *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Microccocus*, *Aereobacter*, *Flavobacterium* and *Erwinia* are important.

There are reports of considerable populations of phosphate-solubilizing bacteria in soil and in plant rhizospheres (Sperberg, 1958; Katznelson et al., 1962; Raghu and MacRae, 1966; Alexander, 1977), belonging to both aerobic and anaerobic genera, with a prevalence of aerobic strains in submerged soils (Raghu and MacRae, 1966).

Sediment also contains various P solubilizing microbes. Occurrence of these microbes, including bacteria (De Souza et al., 2000; Seshadri et al., 2002), actinomycetes (Sahu et al., 2007) and yeast have been documented in marine and estuarine ecosystems indicating their role in sediment P release; similar studies in freshwater environments, are, however, lacking. Because of salinity and other differences between freshwater and marine environments, the marine PSM isolates may not be suitable for inhabiting in ponds, lakes or other freshwater ecosystems.

### 2.2.2 Mechanisms of microbial phosphate solubilization

It is generally thought that the major mechanism of mineral phosphate solubilization is the action of organic acids synthesized by soil or sediment microorganisms (Banik and Dey, 1982; Halder et al., 1990). Production of organic acids results in acidification of the cell surroundings (Fig. 2.2). Consequently, inorganic phosphate (Pi) may be released from mineral phosphate by proton substitution for Ca$^{2+}$ or other cations (Goldstein, 1994). The production of organic acids by phosphate solubilizing bacteria has been well documented. Among them, gluconic acid seems to be the most efficient mechanism of mineral phosphate solubilization. This acid was reported as the principal organic acid produced by phosphate solubilizing bacteria such as *Pseudomonas* sp. (Illmer and Schinner, 1992), *Erwinia herbicola* (Liu, 1992), *Pseudomonas cepacia* (Goldstein, 1993).
and *Burkholderia cepacia*. Another organic acid identified in PSB with phosphate-solubilizing ability is 2-ketogluconic acid and has been detected in various organisms like *Rhizobium leguminosarum* (Halder et al., 1990), *Rhizobium meliloti* (Halder and Chakrabarty, 1993), *Bacillus firmus* (Banik and Dey, 1982), and other unidentified soil bacteria (Duff and Webley, 1959). Strains of *Bacillus liquefaciens* and *Bacillus amyloliquefaciens* were found to produce mixtures of lactic, isovaleric, isobutyric, and acetic acids. Other organic acids, such as glycolic, oxalic, malonic, and succinic acid, have also been identified among various phosphate solubilizers (Illmer and Schinner, 1992; Banik and Dey, 1982).

There is experimental evidence that supports the role of organic acids in mineral phosphate solubilization. Halder et al. (1990) showed that the organic acids isolated from a culture of *Rhizobium leguminosarum* solubilized an amount of P nearly equivalent to the amount that was solubilized by the whole culture. Besides this, treatment of the culture filtrates from several *Rhizobium* strains with pepsin or removal of proteins by acetone precipitation did not affect phosphate release capacity, showing that this was not an enzymatic process. However, neutralization with NaOH destroyed the solubilization activity (Halder and Chakrabarty, 1993). Based on these findings and by cloning of
mineral phosphate solubilization genes Goldstein (1994, 1995) proposed that the direct periplasmic oxidation of glucose to gluconic acid, and less often 2-ketogluconic acid, forms the metabolic basis of the mineral phosphate solubilization phenotype in some Gram negative bacteria.

Alternative possibilities other than organic acids for mineral phosphate solubilization have been proposed based on the lack of a linear correlation between pH and the amount of solubilized P (Thomas, 1985; Asea et al., 1988). In addition, no significant amounts of organic acid production could be detected from a phosphate solubilizer fungus, *Penicillium* sp. (Illmer and Schinner, 1992). Studies have shown that the release of H\(^+\) to the outer surface in exchange for cation uptake or with the help of H\(^+\) translocation ATPase could constitute alternative ways for solubilization of mineral phosphates. Many other mechanisms of P solubilization was also considered, such as the production of chelating substances by microorganisms (Sperberg, 1958; Duff and Webley, 1959), the production of inorganic acids, such as sulphidric acid (Sperberg, 1958), nitric acid, carbonic acid etc. However, the effectiveness of these processes has been questioned by several researchers and their contribution to P release in soil appears to be negligible.

### 2.2.3 Genes involved in mineral phosphate solubilization

The genetic basis of mineral phosphate solubilization (i.e. the Mps1 phenotype) (Goldstein and Liu, 1987) is not well understood. Since the production of organic acids is considered to be the principal mechanism for mineral phosphate solubilization, it could be assumed that any gene involved in organic acid synthesis might have an effect on this character. Goldstein and Liu (1987) cloned a gene from *Erwinia herbicola*, involved in mineral phosphate solubilization, by screening the antibiotic-resistant recombinants from a genomic library in a medium containing hydroxyapatite as the source of P. The expression of this gene allowed production of gluconic acid and mineral phosphate
solubilization activity in *E. coli* HB101. Sequence analysis of this gene (Liu et al., 1992) suggested its probable involvement in the synthesis of the enzyme pyrroloquinoline quinone (PQQ) synthase, which directs the synthesis of PQQ, a co-factor necessary for the formation of the holoenzyme glucose dehydrogenase (GDH)-PQQ. This enzyme catalyzes the formation of gluconic acid from glucose by direct oxidation pathway.

Following a similar strategy, another mineral phosphate solubilization gene from *Pseudomonas cepacia* was isolated. This gene (*gabY*), whose expression also allowed the induction of the mineral phosphate solubilization phenotype via gluconic acid production in *Escherichia coli* JM109, showed no apparent homology with the previous cloned PQQ synthetase gene (Liu et al., 1992), but it did with a permease system membrane protein. The *gabY* gene could play an alternative role in the expression and/or regulation of the direct oxidation pathway in *Pseudomonas cepacia*, thus acting as a functional mineral phosphate solubilization gene *in vivo*.

Various other genes may be involved in the P solubilization. A genomic DNA fragment from *Enterobacter agglomerans* showed P solubilizing activity in *E. coli* JM109, although neither was it related to PQQ gene nor the pH of the medium was altered (Kim et al., 1998a). This results was indicative of non-exclusiveness of the acid production during P solubilization. It is an important but not the only mechanism of P solubilization by bacteria (Illmer and Shinnera, 1992; Rodríguez et al., 2006).

Very little is known regarding the genetic regulation governing the mineral phosphate solubilization trait. In fact, the information about the genetic or biochemical mechanisms involved in the synthesis of the GDH-PQQ holoenzyme is scant, and variations between constitutive and inducible phenotypes are observed among several bacterial species (Goldstein, 1994). Glucose, gluconate, manitol, and glycerol are among the possible inducers of the holoenzyme activity (van Schie et al., 1987).
2.2.4 P solubilizing efficacy of bacteria

Visual detection and even semiquantitative estimation of the phosphate solubilization ability of microorganisms have been possible using plate screening methods, which show clearing zones around the microbial colonies in media containing insoluble mineral phosphates (mostly tricalcium phosphate or hydroxyapatite) as the single P source. In some cases, there have been contradictory results between plate halo detection and P solubilization in liquid cultures (Ostwal and Bhide, 1972). However, the method can be regarded as generally reliable for isolation and preliminary characterization of phosphate-solubilizing microorganisms (Illmer and Schinner, 1992; Goldstein and Liu, 1987). Gupta et al. developed an improved procedure using a medium containing bromophenol blue. In this medium, yellow colored halos are formed around the colonies in response to the pH drop produced by the release of organic acids, which are responsible for phosphate solubilization. With this method, the authors reported more reproducible and correlated results than with the simple halo method.

In vitro studies of the dynamics of phosphate solubilization by bacterial strains have been carried out based on the measurement of P release into culture broth, from cultures developed using an insoluble compound as the only P source. The rate of P solubilization is often estimated by subtracting the final P concentration (minus that of an inoculated control) from the initial theoretical P supplied by the P substrate. This estimation has the disadvantage of not taking into account the P utilized by the cells during growth.

Babenko et al. (1984) isolated and grouped phosphate-solubilizing bacteria into four different types according to kinetics and rate of P accumulation, and the groups ranged from a linear increase of P concentration along with the growth of the culture to oscillating behavior with variations in the soluble P levels, giving rise to several peaks and troughs of P concentration. This type of kinetic behavior was also observed by others.
These changes in P concentration could be a consequence of P precipitation of organic metabolites (Babenko et al., 1984) and/or the formation of organo-P compounds with secreted organic acids which are subsequently used as an energy or nutrient source, and the event being repeated several times in the culture (Illmer and Schinner, 1992) showed the peaks and troughs of P concentration. An alternative explanation could be the difference in the rate of P release and uptake. When the rate of uptake is higher than that of solubilization, a decrease of P concentration in the medium could be observed. When the uptake rate decreases (for instance as a consequence of decreasing growth or entry into stationary phase), the P level in the medium increases again. More probably a combination of two or more phenomena could be involved in this behavior. Thus, the P concentration in the culture broth as an indication of phosphate solubilization capacity should be viewed with caution, and a kinetic study of this parameter would offer a more reliable picture of cellular behavior toward P.

The physiology of phosphate solubilization has not been studied thoroughly. Some studies indicate that certain mineral elements play a role in this process. A critical K concentration is necessary for optimum solubilization rates (Beever and Burns, 1980; Illmer and Schinner, 1992), while Mg and Na seem to be important in some fungi (Beever and Burns, 1980) but not in bacteria like Pseudomonas strains (Illmer and Schinner, 1992).

Instability of the phosphate-solubilizing character of some strains after several cycles of inoculation was reported by several researchers like Kucey (1983), Halder et al. (1990) or Illmer and Schinner (1992). However, for most of the isolates the trait seems to remain stable (Arora and Gaur, 1979).
2.2.5 Phosphate solubilizing bacteria as biofertilizer in crop soil

Biofertilizers are organisms that enrich the nutrient quality of soil. The main sources of biofertilizers are bacteria, fungi, and cyanobacteria (blue-green algae). Plants have a number of relationships with fungi, bacteria, and algae. After the introduction of chemical fertilizers during the last century, an increased yield in agriculture was obtained. But slowly chemical fertilizers started displaying their ill-effects such as leaching out, polluting water basins, destroying flora and fauna including friendly organisms, making the crop more susceptible to the attack of diseases, reducing the soil fertility and thus causing irreparable damage to the ecosystem (Rodríguez and Fraga, 1999).

The principle behind the biofertilization strategy is that microbes have various abilities which could be exploited for better farming practices. Some of them help in combat diseases while some have the ability to degrade soil complex compounds into simpler forms which are utilized by plants for their growth. They are extremely beneficial in enriching the soil by producing organic nutrients for the soil. To convert insoluble phosphates to a form accessible to the plants, like orthophosphate is an important trait for a plant growth promoting bacteria (PGPB) for increasing plant yields. Microbes having the ability to dissolve appreciable amount of phosphates is not rare. Some of them are already used as commercial biofertilizers for agricultural improvements. The use of microbial products has certain advantages over conventional chemicals: they are considered safer than many of the chemicals now in use, they do not accumulate in the food chain, the target organisms seldom develop resistance as is the case when chemical agents are used, and biofertilizing agents are not considered harmful to ecological processes or the environment (Rodríguez and Fraga, 1999).
2.2.6 PSB from aquatic resources of India

Meager works have been done on PSB in aquatic environment with wide variations in their findings. Occurrence of P solubilizing actinomycetes in Vellar estuary sediment was very low (0.09 - 0.59 CFU g$^{-1}$) (Sahu et al., 2007). Whereas 10-15% of heterotrophs in Indian marine sediment solubilize Ca-P (De Souza et al., 2000), Seshadri et al. (2002) observed that southwest coastal and estuarine waters contained as high as 1000 - 1300 CFU PSB ml$^{-1}$. De Souza et al. (2000) also observed that more occurrence of PSB in sea islands and coasts than in sandy beaches and offshore region, and hypothesized that offshore organisms were poor solubilizers probably due to their low carbon uptake and less acid production. In marine environments *Pseudomonas* and *Bacillus* are the predominant PSB (De Souza et al., 2000; Seshadri et al., 2002). Other recorded species of PSBs belong to the genera *Vibrio* and *Klebsiella* (De Souza et al. 2000), *Flavobacterium* and *Acinetobacter* (De Souza et al., 2000), *Alkaligenes*, *Flavobacterium*, *Corynebacterium* and *Micrococcus* (Seshadri et al., 2002.).

2.2.7 Application of PSB as biofertilization in aquatic environments

Although application of PSB as biofertilizer is common in rhizoplane for improving plant productivity, paucity of information is there about their use in aquatic productivity enhancement. The limiting source of P fertilizer in environment always surge for a good alternative, which can be well served by PSB. As such, PSB isolated from and suitable for freshwater environments are not available for aquaculture production enhancement. Only recently, a few PSM from freshwater pond ecosystems has been reported from China (Hu et al., 2010). Thus study should be carried on this regard to evaluate their role and activity as biofertilizer in aquaculture/ freshwater ecosystems.
Phytic acid (Fig 2.3) [known as myo-inositol hexakisphosphate (IP6), or phytate when in salt form] is the principal storage form of phosphorus in many plant tissues, especially bran and seeds. About 60-80 % of the organically bound phosphorus in seed is present in the form of phytic acid (Asada et al. 1969). It was discovered as early as in 1872 by Pfeffer. Phytic acid plays other important roles in the plants such as it may act as an energy store, it may also compete for ATP, it may complex with multivalent cations and even may act to regulate inorganic phosphate level.

Fig. 2.3 Phytic acid structure

Phytate is not digestible to humans or nonruminant animals. It is neither a source of inositol nor a source of phosphate if eaten directly. Rather phytic acid is considered to be an antinutritive part of human and animal diets because-

- it forms complexes by chelating with some multivalent metal ions and thus interferes with the assimilation of important trace metals such as zinc and iron, and to a lesser extent, also macro minerals such as calcium and magnesium, making certain important minor minerals unabsorbed.
- it binds to proteins and makes them more resistant to proteolytic digestion, and
- phytate phosphorus is poorly available to monogastric animals.
Catabolites of phytic acid are called lower inositol polyphosphates. Examples are inositol penta- (IP5), tetra- (IP4), and triphosphate (IP3).

The soil chemistry of inositol phosphates has been reasonably well investigated over the past quarter century (Turner et al., 2002). The paucity of information on the transformation of myo-inositol hexakisphosphate to plant nutrient P presents the biggest challenge to a thorough understanding of the P-cycling process in natural ecosystems, including P-burdened manure amended soils. In high P soils, rates of phosphate release in excess of crop uptake will lead to an increase in P transport to nearby surface waters and/or groundwater. Over long term, increased rates of phytase-catalyzed P release will influence the amount of P transported from high P soils to open waterways accelerating eutrophication. Quantification of myo-IP₆ in soil extracts was recently achieved using solution ³¹P nuclear magnetic resonance (NMR) spectroscopy (Turner et al., 2003). However, sediment chemistry of phytic acid is not so well understood. Complete understanding of the P-cycling process in natural environments will not be achieved until an idea of sedimentary phytate is developed.

### 2.3.1 Phytate mineralizing enzyme: Phytase

Utilization of phytate phosphorus and increase in nutritive value of feed and food can be well achieved by dietary phytase supplementation. Phytase (myo-inositol hexakisphosphate phosphohydrolase) belongs to the group of phosphoric monoester hydrolases; it catalyzes the hydrolysis of myo-inositol hexakisphosphate (phytic acid, myo-inositol-P6) to inorganic monophosphate and lower phosphoric esters of myo-inositol, or in some cases to free myo-inositol.

A first note on phytase can be found in the literature in 1907. This enzyme is suggested as a mean for lowering of phytic acid content in rice-bran (by its hydrolysis producing
myo-inositol and inorganic phosphate). This very first application accounts for the industrial importance of phytase.

The Enzyme Nomenclature Committee of the International Union of Biochemistry distinguishes two types of phytase: 3-phytase and 6-phytase. This classification is based on the first phosphate group attacked by the enzyme. 3-Phytase is typical for microorganisms and 6-phytase for plants.

### 2.3.2 Source of phytase

Phytase is widespread in nature, occurring in plants, microorganisms, and rarely in some animals. Sourcewise phytase is classified as follows:

#### 2.3.2.1 Plant source of phytase

Phytase occurs very frequently in the plant kingdom. Its activity has been detected in many plant species such as wheat, rye, barley, pea, bean, soybean, maize, rice, white mustard, potato, radish, lettuce, spinach, grass, lilly pollen, etc. Erdman (1977) monitored a rapid increase of phytase activity in plant seeds during germination. Generally, it is assumed that during seed germination phytate, after decomposition by phytase, is utilized in the form of phosphate and inositol (Asada et al., 1969). Some authors described the rise in phytase activity to *de novo* phytase synthesis during germination (Meyer et al., 1971) while others attributed it to a rise of an already existing phytase activity (Eastwood and Laidman, 1971). Nevertheless, it was concluded that seeds contain both constitutive and germination-inducible phytases (Nayni and

#### 2.3.2.2 Animal source of phytase

germinating maize seedlings, and cDNA coding for this phytase was cloned (Maugenest et al., 1997). This would allow the isolation of corresponding genes and the study of their regulation during germination.
Although less common, phytase activity is also reported in some animals. Endogenous phytase enzyme activity was reported in hybrid tilapia (*O. niloticus* × *O. aureus*) (LaVorgna, 1998) and striped bass (*Morone chrysops* × *Morone sexatilis*) (Ellestad et al., 2003).

### 2.3.2.3 Microbial sources of phytase

Microbial phytase activity has been most frequently detected in fungi. Shieh and Ware (1968) tested over 21,300 microorganisms isolated from soil, out of which extracellular phytase activity was observed in only 30 isolates. All phytase producers were filamentous fungi, 28 of them belonged to the genus *Aspergillus*, one species belonging to *Penicillium* and one to *Mucor*. Phytase from the *A. niger* group was the most active. Most of the isolates produced only intracellular phytase. Later on in 1983 screenings by Howson and Davies confirmed *A. niger* strains to be the best producers of extracellular phytase. He also demonstrated that bacterial cultures produced only intracellular enzyme. Industrial production of phytase currently employs the soil fungus. Considerable research has been conducted in this regard on *Aspergillus* (Ullah et al., 1999). However, because of substrate specificity, resistance to proteolysis and catalytic efficiency, and some other properties bacterial phytases may be a real alternative to the fungal enzyme (Konietzny and Greiner, 2004). Major bacterial genera include important phytase producer are *Pseudomonas, Arthrobacter, Staphylococcus* and *Bacillus*.

### 2.3.3 Genes involved in phytase activity

Studies of the genetic basis of phytases began in 1984, and the first commercial phytase, produced by genetically modified microorganisms, appeared on the market in the mid 1990s (Yanming et al., 1999). Several genes may be involved in phytase activity. The growth and phosphorus nutrition of Arabidopsis plants was significantly improved by Richardson et al. (2001), who genetically transformed the tree with the phytase gene.
(phyA) from *Aspergillus niger*. Another novel Phytase gene (phyC) encoding the mature enzyme from *Bacillus subtilis* was isolated by Kerovuo et al. (1998). Thermally stable phytase genes from Bacillus sp. DS11 was isolated and cloned by Kim et al. (1998). A bi-functional acid phosphatase/phytase gene (appA and appA2 genes) was isolated and characterized from *E. coli* (Rodríguez and Fraga, 1999; Golovan et al., 2000). These bi-functional enzymes are attractive for mineralization of organic P in soil. Neutral phytases that have great potential for genetic improvement of PGPB, have also been cloned from *B. subtilis* and *B. licheniformis* (Tye et al., 2002).

However, most genetic engineering studies focused on the search for phytases that are optimal for improving animal nutrition. Another attractive application of these enzymes that is not currently exploited is solubilization of soil organic phosphorus through phytate degradation that may be important for management of productivity in oligotrophic freshwaters- where nutrient imbalance often limits the productivity.

### 2.3.4 Application of phytase

Among them are in food or feed industry.

#### 2.3.4.1 Phytase in human nutrition

Despite having vital positive roles (e.g, as antioxidant, an anticancer agent), dietary phytic acid may have its role in mineral diminution and deficiency. As discussed in the previous portion, phytate binds multivalent cations or essential minerals and thereby prevents their absorption. Phytic acid also has potential for binding positively charged proteins or amino acids, and the resulting complexes are insoluble. These insoluble complexes are difficult for humans to hydrolyze during digestion, and thus, typically are nutritionally less available for absorption. In the cereal grain, phytic acid is deposited in the aleurone and germ, which is also the site for the grain’s main mineral stores. Increased dietary consumption of cereal fibers, legumes and soy protein isolates results
in an increased intake of phytate in the body. Vegetarians eating mostly whole grain products and extruded cereals, elderly people consuming unbalanced food with a lot of cereals, people eating unleavened bread, and babies eating soy based infant formulas take in large amounts of phytate (Simell et al., 1989). Infract bread which is a staple food in the world, are an important source of phytate. Phytase acting as an exceptional breadmaking improver leads to an acceleration of the proofing, improvement in bread shape, a slight increase in specific volume, and also confers softness to the crumb. The supplementation of commercial fungal phytase from *Aspergillus niger* in the dough ingredients containing fiber formulation is often a regular practice. These improvements in bread quality might be associated with an indirect action of phytase on α-amylase activity (Afinah et al., 2010). However, a further hydrolysis of the phytates is reached by adding exogenous phytase, therefore an enhancement in the mineral adsorption can be obtained with the consumption of phytase supplemented food (Haros et al., 2001).

### 2.3.4.2 Phytase as animal feed additive

Phytases have been mainly used as animal feed additive in diets largely for swine and poultry. The addition of phytase to feed for monogastric animals is commonly used to enhance the digestibility of phytate-associated phosphorus (Pontoppidan et al., 2007). The effectiveness and limitations of phytase supplementation may also depend on substrate specificity (Greiner and Farouk, 2007).

Phytases as feed additives should have the ability to be effective in releasing phytate phosphate in the digestive tract, stable to resist inactivation by heat during feed processing and storage, and cheap to produce (Greiner and Farouk, 2007). Besides increasing the phytate phosphorus availability, phytase also enhances digestibility of amino acids (Mroz et al., 1994), and increases utilization of metals like calcium (Mroz et al., 1994), magnesium and zinc (Pallauf et al., 1994). This phytate supplemented corn-
soybean is used to feed pigs, chickens (Sebastian et al., 1996) and rats (Rimbach et al., 1995). However, calcium present in the diet greatly reduced the efficiency of phytase supplements (Qian et al., 1996). There are two basic ways to use phytase in feed. The first is the replacement of the inorganic phosphorus with phytase in feed. However, as the reaction conditions such as pH, temperature, moisture, incubation time etc. in the animal stomach or intestine are not optimal for phytase activity, the second method of phytase use that is the feed pretreatment with phytase becomes more attractive (Simell et al., 1989).

### 2.3.5 Importance of phytase in aquatic environment

Being an essential nutrient, all the organisms require P for living. However, ruminants, poultry or herbivorous fish those directly depend on plant material for food, are much vulnerable to the anti nutritional effect of phytate. The ruminants digest phytic acid through the action of phytases liberated by the gut fungi and bacteria present in their rumen (Ray et al., 2009). But phytate is not digestible for fishes, since they do not contain phytase, and it is released as fecal matter to the sediment. Thus for monogastric animals, like pig, poultry and fish who are not able to metabolize phytate, inorganic phosphate is added to their diets to meet the phosphorus requirement. As a consequence this contributes to phosphorus pollution problems in areas of intensive livestock production (Kerovuo et al., 1998). Fish excrete phosphorus in soluble and particulate forms. The soluble forms are organic phosphorus and phosphates which affect water quality directly. The particulate forms accumulate in the sludge and the phosphorus is released slowly to the water. Dissolved reactive phosphorus is usually regarded as the most important factor affecting water quality, especially in the context of mesotrophic water bodies.
2.3.6 Phytate mineralizing bacteria from water resources

The search for a phytate mineralizing bacteria (in other words phytase producing) for feed treatment has a long history. But similar search for aquatic phytase producing bacteria was not prominent in early days, however, the increasing cost of synthetic fish feed surge the quest for an alternative fish feed, using plant resources as ingredients- that increases the chance of wetland being polluted by phytate P. Few reports have identified phytase activity in gut bacteria from freshwater teleosts. Roy et al. (2009) identified phytase-producing as *B. licheniformis* strains LF1 and LH1 isolated from *L. rohita*. Khan et al. (2011) isolated an efficient phytase-producing strain CC 1.1 from *C. catla* and identified it phenotypic characterization as *Rhodococcus* sp. MTCC 9508. Khan and Ghosh (2012) isolated two promising strains by evaluation in 14 freshwater teleosts, *B. subtilis* from *L. bata* and *Bacillus atrophaeus* from *Gudusia chapra*. Recently Das et al. (2014) reported *Brevibacillus parabrevis* from four brackish water fishes viz. *Scatophagus argus, Mystus gulio, Terapon jarbua*, and *Etroplus suratensis*.

So far, studies have been carried out for defining applicability of PMB in fish feed and feed digestion. However, the presence and distribution of PMB in nature, especially in aquatic systems needs further evaluations. Sedimented phytate which can be used as an autochthonous source of P, can be degraded by PMB to combat with the P deficient pattern of the Indian wetlands.