Introduction
Pollution is defined as an undesirable change in the physical, chemical or biological characteristics of air, water and land brought about by man's activities that may harmfully affect living organisms and other resources. Based on the environmental component being polluted, the following types of pollution are commonly addressed: Air pollution is the introduction of chemicals, particulate matter, or biological materials that cause harm or discomfort to humans or other living organisms, or cause damage to the natural environment or built environment, into the atmosphere. Water pollution is the contamination of water bodies (e.g. lakes, rivers, oceans, aquifers and groundwater). It occurs when pollutants are discharged directly or indirectly into water bodies without adequate treatment to remove harmful compounds. Soil pollution is defined as the build-up in soils with persistent toxic compounds, chemicals, salts, radioactive materials, or disease causing agents, which have adverse effects on plant growth and animal health.

The substances that actually cause pollution are called pollutants. They may be the industrial gases, industrial effluents, soap water, domestic wastes, medical wastes, etc. Pollutants may be classified into two types based on microbial activity. They are:

1. Biodegradable pollutants - Which are broken down under natural conditions due to the action of micro-organisms. Therefore they are considerably less harmful. They behave as pollutants only in very large quantities e.g.excreta, sewage, pollen grains, etc.
2. Non-biodegradable pollutants- Which cannot be broken down under natural conditions by the action of micro-organisms or they take an extremely long
time to be broken down *e.g.*, common plastics, pesticides such as DDT, endosulphan *etc.* and metal wastes such as lead, mercury, arsenic *etc.*

There are two main approaches for the production of chemical commodities: a traditional chemical transformation and a new one biotransformation. The chemical transformation of nitriles and amides requires relatively severe reaction conditions such as acidic and basic conditions, high energy inputs, reaction temperature and pressure, and results in the production of racemic mixture of products (Kobayashi *et al*., 1990, Dufour *et al*., 1998, Ramakrishna *et al*., 1999) and also leads to the formation of toxic byproducts like HCN and large amounts of salts. On the other hand, biotransformation is the chemical modification (or modifications) made by an organism on a chemical compound. If this modification ends in mineral compounds like CO$_2$, NH$_4^+$, or H$_2$O, the biotransformation is called mineralisation. Biotransformation means chemical alteration of chemicals such as (but not limited to) nutrients, amino acids, toxins, and drugs in the body. It is also needed to render nonpolar compounds to polar compounds so that they are not reabsorbed in renal tubules and are excreted. Biotransformation of xenobiotics can dominate toxicokinetics and the metabolites may reach higher concentrations in organisms than their parent compounds.

The remediation of pollutants or toxic material using biological entities (organism as a whole or their enzymes) is commonly known as bioremediation. Bioremediation includes biodegradation of complex organic compounds into simpler and less toxic compounds. The complexity of organic compounds is decreased by transforming them into simpler ones using a biocatalyst (Alexander, 1994). This is
based on growth and metabolism of organisms. Microorganisms mainly bacteria are preferred over others as their sole source of carbon and nitrogen and generate energy by assimilating them into their metabolic pathway. Conditions which are pre-requisite for the microorganisms to become successful for biological degradation are identified as follows: Metabolic process essential to establish the contact between microbial cells or their enzymes with the pollutants. Activation of these compounds occurs by key enzymatic reactions involving oxygenase, peroxidases etc. Degradation pathway required to convert the metabolized form into simpler form so that it can easily be integrated with the system. Increase in intermediary compounds such as pyruvic acid, acetyl CoA, citric acid into central metabolic pathways leads to increased metabolism and greater cell mass.

Biodegradation and biodeterioration of polymeric materials affect industries of manufacturing, aviation and space. It is also observed that polymeric materials are degraded by microorganisms. The degree of degradability is determined mostly by the chemical structures of polymeric materials and the presence of degradative microbial population.

Polymers are treated according to their biodegradability. Microorganisms and microbial biofilms are capable of degrading the polymeric material. Microbial biofilms can form on surfaces of synthetic polymers and subsequently material degradation occurs. Fungi seem to be more effective than bacteria for the degradation of polymers. Electrochemical Impedance Spectroscopy (EIS) spectra indicate microbial attack occurs in several steps depending on the chemical composition of polymers. In the initial step a decrease in impedance has been
detected which is due to the transport of water and solutes into the polymeric matrices and in a second step decrease in impedance occurred as a result of polymer degradation.

Man made polymers are biodegradable but essentially inert. Microbial degradation has been found to be more effective for low molecular weight polymers than for macromolecular matrices. The degradation rate of synthetic polymers is slower than natural polymer. Polymers are useful because of their strength and durability in many applications. However, after the useful life of a polymer is over, society desires that it degrade in the environment back to natural materials.

Degradation changes the properties such as tensile strength, colour, shape, etc. The influence of one or more environmental factors such as heat, light or chemicals (acids, alkalis and some salts) also effect the degradation of polymers. Usually these changes are undesirable, such as cracking and chemical disintegration of products. Some changes are desirable such as lowering the molecular weight of a polymer for recycling. These are rarely occur. The changes in properties are often termed "aging".

Degradation can be useful for recycling/reusing the polymer waste to prevent or reduce environmental pollution. Degradation can also be induced deliberately to assist structure determination. Polymeric molecules are very large (on the molecular scale), and their unique and useful properties are mainly a result of their size. Any loss in chain length lowers tensile strength and is a primary cause of premature cracking. Biotransformation have some advantages viz. mild reaction conditions, substrate and product specificity, formation of products with a very high level of
purity, potential for conducting stereo- and region-selective transformation that are difficult to achieve through conventional chemical methods (Ramakrishna et al., 1999).

Acrylamide is used as a building block in the making of polyacrylamide. The chemical formula of acrylamide is C₃H₅NO. It is soluble in water, ethanol, ether and chloroform. Acrylamide is used in waste water treatment, gel electrophoresis, paper making, printing ink emulsion stabilizers etc. Potato chips and French fries have been found to contain relatively high levels of acrylamide compared to other foods; lower levels are also present in bread and cereals.

Acrylamide forms in some food during high temperature cooking processes such as frying, roasting and baking (Mottram et al., 2002). It does not come from food packaging or the environment, it forms from sugars and an amino acid (asparagine) that is naturally present in food [Malliard rex] (Tareke et al., 2002). It is found in foods made from plants such as grain products, potato products or coffee. When cooking is done for longer periods or at high temperatures 120°C (248°F), acrylamide accumulates (Mottram et al., 2002). Acrylamide do not form from boiling and steaming (Tareke et al., 2002).

The average content of acrylamide in potato chips is approximately 1000 µg/kg and in French fries approximately 500 µg/kg. Fried bread products also contain acrylamide, e.g. doughnuts, breakfast cereals, fried potato products, biscuits, cookies and snacks, such as tortilla chips and popcorn. It was reported in December 2002, that the acrylamide content rises dramatically in French fries, from approximately 300 micrograms per kilogram at a cooking temperature of 175°C to
1,100 micrograms per kilogram at 180°C by The German Federal Institute for Risk Assessment. It was also studied that the type of potato and the storage method may also influence acrylamide formation and the acrylamide content in the deep-fried end product, because the sugar composition varies from potato to potato.

At a conference of the American Chemical Society in Boston in August 2007, a team of Swiss researchers reported that they have found acrylamide in dried fruits. They also suggest that acrylamide may be capable of being formed at relatively mild conditions though these reactions that are still not fully understood. It was found that high levels of this compound is found in dried pears and prunes by researchers. The Swedish National Food Administrations conducted a survey in 1997-98 on 1200 individuals, aged 17 to 70 and found that an average intake of approximately 25 µg per day (maximum intake is approximately six times higher) of acrylamide.

The percentage contribution based on an intake of 40 micrograms of acrylamide per day results in potato products: 36% (French fries 16%, fried potatoes 10%, potato chips 10%), bread: 16%, biscuits, cookies and wafers: 5%, breakfast cereals: 3% remaining foodstuffs groups, basically not investigated yet 40%. The University of Stockholm and Sweden’s National Food Administration reported that the high levels of acrylamide are formed in carbohydrate rich plant products cooked at high temperature in pan frying, deep frying, grilling, roasting and baking.

Acrylamide is toxic but its polymer polyacrylamide is non-toxic. Indiscriminate discharge of acrylamide and polyacrylamide has led to their presence in terrestrial and aquatic ecosystems causes water and soil pollution (Cherry et al.,
High level of acrylamide is known to cause neurological damage in workers using acrylamide polymers to clarify water in coal preparation plants (Mullay, 1996). In 2002, Scientists in the Swedish Government estimated that acrylamide could be responsible for 45,000 cancer cases in the country each year. Several type of cancer can be caused by exposure to acrylamide (Dearfield et al., 1988). Haemoglobin adducts and induction of abnormalities can generate in daughter cells of animals and plants by acrylamide and glycidamide (Shairashi, 1978; Shanker et al., 1987; Bergmark et al., 1991).

The National Toxicology Program (NTP) and the International Agency for Research on Cancer considers acrylamide to be a “probable human carcinogen” based on studies in laboratory animals (Fuhr et al., 2006). The risk of renal cell carcinoma (RCC) increased by using refined cereals, and breads in diet. Cigarette smoke and fried food are a source of exposure of acrylamide (Urban et al., 2006). In spite of the general toxicity of acrylamide in monomer form, some microorganisms can use acrylamide as their sole carbon source for growth.

When foods are overheated or burnt carcinogenic substances such as polycyclic aromatic hydrocarbons (PAHs) are produced. After ingestion of PAHs, these can be absorbed in the intestine and distributed to other organs through blood circulation. Besides causing cancer, PAH exposure is also associated with many adverse effects in laboratory animals, including reproductive toxicity, cardiovascular toxicity, bone marrow toxicity, immune system suppression, and liver toxicity. PAHs are formed when fat from grilled meats falls down on flames or hot coals,
which causes cancer. These PAHs rise as vapour and permeate the meat. Heavy charring of meat also produces high concentrations of PAHs carcinogens, such as benzopyrene.

Another dangerous carcinogenic compound called PHIP (2-Amino-1-methyl-6-phenylimidazo[4,5b]pyrldine), which is one type of heterocyclic amines (HCAs/HAs) present in some grilled chickens. In September 2006, a doctor’s group sued seven leading fast food chains in the US because their grilled chicken contained PHIP. According to the president of this group, even a grilled chicken salad would increase the risk of breast cancer, prostate cancer and other types of cancer. If the meat are burnt during boiling or pan frying, cancer causing chemicals called heterocyclic amines (HCAs) are created within muscle meats (e.g. beef, pork, fowl, and fish).

Acrylamide, an aliphatic amide, is deaminated to ammonia and acrylic acid (acrylate), catalyzed by amidase or amidohydrolase (Shanker et al., 1990; Nawaz et al., 1994, 1998; Zabaznaya et al., 1998). Acrylamide is oxidized to the epoxide glycidamide through an enzymatic reaction with help of cytochrome P450 2E1 (Besaratinia and Pfeifer, 2004).

Securing and remediation, alone or in combination with each other are the methods for treatment of contaminated sites. In securing, physical barriers are setting up for the protection of the environment, while in remediation decontamination or reduction of pollutants is involved. Securing measures often represent only a time restricted solution because physical barriers are subject to aging and environmental influences. Remediation methods are necessary, when the
source of contamination continues to remain in place. This makes future remediation activities necessary.

Bacterium *Rhodococcus palustris* grows rapidly with acrylamide under photoheterotrophic conditions (doubling time of 10 to 12 h) but poorly under anaerobic dark or aerobic conditions. *Rhodococcus palustris* strain Ac1, deamidates acrylamide to acrylate with the subsequent degradation of acrylate. This was the rate-limiting reaction in cell growth. 13C nuclear magnetic resonance studies of [1,2,3-13C] acrylamide metabolism confirmed the rapid conversion of acrylamide to acrylate by actively growing cultures. However, it failed to detect any subsequent intermediates of acrylate degradation. *R. palustris* strain Ac1 grew well for each of acrylamide, acrylate, and propionate with comparable doubling times. *R. palustris* strain CGA009 was incapable of growth on acrylamide- or acrylate-dependent medium, but grew comparably with propionate over the same time course. These results provide the first demonstration of anaerobic photoheterotrophic bacterial acrylamide catabolism and provide evidence for a new pathway for acrylate catabolism involving propionate as an intermediate (David *et al.*, 2005).

In aerobic acrylate-utilizing bacteria, Acrylate metabolism proceed via hydroxylation to β-hydroxypropionate by aerobic acrylate utilizing bacteria, which is further oxidized to CO₂ (Ansed *et al.*, 1999, Wampler and Ensign, 2005). *Clostridium propionicum* is capable of fermenting acrylate to acetate and propionate under anaerobic condition. Acrylate further serves as a terminal electron acceptor for *Desulfovibrio acrylicus*, producing propionate which is not further metabolized
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as a growth substrate (Van der Maarel et al., 1996). (R)-lactate dehydrates to acrylate by cell extracts of *C. propionicum*, suggesting that the reverse reaction could be another strategy for acrylate catabolism (Schweiger et al., 1985).

Fig 1.1: Summarizes the deamidation reaction that produces acrylic acid from acrylamide and the possible subsequent fates of acrylate.

Amides contain amido (-CONH₂) functional group and they are derived from their parent family called cyanide or nitriles (C N). Amidase catalyzes the hydrolysis of amides to carboxylic acid and ammonia and are wide spread in nature (both in biotic and abiotic components of ecosystem):

Amidase

\[
R-\text{CONH}_2 + H_2O \rightarrow R-\text{COOH} + NH_3
\]

Amidases are mesophilic thermostable enzymes. Amidases exhibit diverse substrate specificity. These are sulfhydryl proteins and mostly dimeric. The active
site of amidases contains Asp191 and Ser195 residues. Amidase also exhibit an acyl transfer activity (acyltransferase) in the presence of hydroxylamine.

\[
\text{Acyltransferase} \\
\text{R-CONH}_2 + \text{NH}_2\text{OH} \rightarrow \text{R-CONHOH} + \text{NH}_3
\]

Amidase have found great potential in the manufacture of various antibiotics, L-carnitine, cephalosprine and various other economically important organic acids. Beside this acyl transferase activity of amidase has been found to be very useful in pharmaceuticals. Amidases also have applications in the biodegradation of toxic amides and nitriles from polluted sites.

In view of the increasing evidence of a significant role played by amidase in bioremediation and food industry, the present study entitled “Isolation and Identification of Acrylamide Degrading Bacteria from Soil” has been designed and undertaken with following major objectives:

- Screening and isolation of acrylamide degrading bacteria from soil.
- Optimization of conditions for the \textit{invitro} degradation of acrylamide.
- Identification of acrylamide degrading bacteria.
- Biochemical characterization of bacteria.
- Molecular identification of bacteria.
- Isolation of amidase enzyme.