BIODEGRADATION OF TOXIC MONOMER ACRYLAMIDE USING MICROORGANISM

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Abstract

Acrylamide can be produced at high level in high carbohydrate heat treated foods. It is a well-known neurotoxicant, Carcinogen and teratogen. In the present Investigation, soil samples were collected and enriched with potato chips and 100 PPM acrylamide for 15 days. The enriched samples were serially diluted, and spread plated on nutrient agar medium, amended with 100 PPM acrylamide. Three dominant isolates of bacteria were obtained. Minimum inhibitory concentrations of the selected isolates were carried out against increasing concentration of acrylamide, ranging from 200 PPM to 900 PPM. All the three isolates (AC1, AC2 & AC 3) exhibited tolerance up to 600 PPM. The strain AC1 displayed maximum tolerance of acrylamide up to 900 PPM. On the basis of the AC1 was selected for further characterization and degradation studies. The effective acrylamide tolerant isolate was identified as *Bacillus subtilis*. Biodegradation of acrylamide was performed at various concentrations 200 PPM and 500 PPM. To study of FTIR and HPLC analysis was also performed with 500 PPM. B*acillus subtilis* grew optimally at pH 7 and temperature 30 and 35^{0}C. The carbon and nitrogen sources were tested. Acrylamide degrading strains were analyzed plasmid DNA by Alkaline Lysis method.

Keywords:- Acrylamide, *Bacillus subtilis*, Biodegradation, FTIR and HPLC.

Introduction

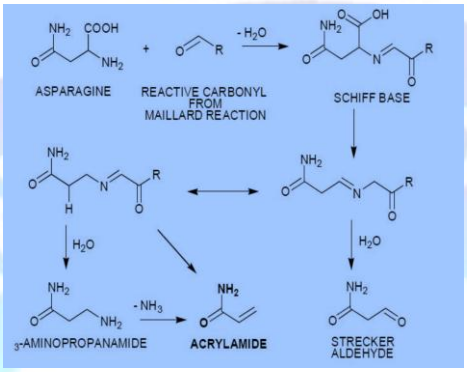
Acrylamide in food is largely derived from heat-induced reactions between the amino group of the free amino acid asparagine and the carbonyl group of reducing sugars such as glucose during baking and frying. The kinetics of acrylamide formation as influenced by processing conditions such as time, temperature, pH, water activity, surface area, and food composition, that the initial rate-determining step in heat induced formation of acrylamide involves a second-order reaction between the R-NH2 of asparagine and the carbonyl group of glucose to form an *N*-glycoside. (Friedman, 2003).

Acrylamide, is an industrially produced α,β unsaturated (conjugated) reactive molecule, is used worldwide to synthesize polyacrylamide. Because of constant exposure to acrylamide, effects of acrylamide in cells, tissues, animals and humans have been extensively studied. Acrylamide (CH2=CH–CO–NH2) or 2-propenamide (IUPAC); various other names are also used, including propenamide, acrylic acid amide, acrylic amide, acrylamide monomer (Kanno 1985).

The Enhanced level of acrylamide in the workplace has been shown in coal preparation (Mullory 1996). Acrylamide is produced industrially for use in products such as plastics and cosmetics. It is intensity used as water soluble thickeners (Sax and Lewis 1987), in gel electrophoresis (USEPA 1981), paper making stabilizers, binders for seed coating foundry sand (Mannsuille 1993) etc.

In spite of the general toxicity of acrylamide in monomer form, some microorganisms can use acrylamide as their sole carbon source for growth. In its catabolism, acrylamide an aliphatic amide, is deaminated to ammonia and acrylic acid (acrylate), a process catalyzed by amidase or amidohydrolase (Zabaznaya *et al*., 1998).

Several microorganisms are known to degrade an array of aliphatic and aromatic amides. Generally, this does not include acrylamide due to its inhibitory effect on sulfhydryl proteins and hence growth of microorganisms. Successful bioremediation depends on the introduction of specific microorganisms capable of degrading toxic pollutants (Mallori *et al*., 1983; Murakami and Alexander, 1989).



The major acrylamide or aliphatic amides-degrading bacteria is the genus *Pseudomonas.* Strains of *Pseudomonas* known to degrade acrylamide or aliphatic amides are *Pseudomonas aeruginosa* (Brown and Clarke, 1970; Prabu and Thatheyus, 2007), Although acrylamide is degraded rapidly at low concentrations (10 ppm) in surface water, higher concentrations are slower to degrade (Cherry *et al*., 1956), remaining stable for more than 60 days in tap water (Brown *et al*., 1980). Acrylamide is readily biodegraded by microorganisms in soil and water systems under aerobic conditions by deamination to acrylic acid and ammonia (Nawaz *et al*., 1998).

In the present study, an attempt has been made to isolate and characterize a bacterium capable of degrading higher concentrations of acrylamide and also detecting the degradation products by High Performance Liquid Chromatography and Fourier Transform Infrared Spectroscopy.

Material and Methods:

Collection of Sample

Soil sample was collected from agricultural location in Madurai, with a previous history of polyacrylamide applications in the form of the herbicide glyphosate commercial formulation, and is transported into the laboratory at room temperature.

Enrichment Culture Technique

Soil sample was enriched with potato chips for 15 days, by enrichment culture using basal medium containing 0.5% (W/V) acrylamide as the sole nitrogen source.

Isolation of Acrylamide Tolerant Microorganisms

Acrylamide degrading microorganisms were isolated from enrichment culture by spreading the turbid culture onto medium agar containing acrylamide (100 PPM) and incubated at 28^0C for 24 hrs. The microorganisms with different morphological characteristics were selected and purified to a single colony by repeated streaking under the same conditions and maintained at 4^0C for further analysis.

Determination of Minimum Inhibitory Concentration (MIC) for Acrylamide Tolerant Microorganisms

Minimum inhibitory concentration (MIC) was carried out to determine the resistant capacity of acrylamide degrading bacteria, at different concentrations of acrylamide. The nutrient agar medium was prepared with various concentrations of acrylamide ranging from 200 to 900 PPM, and the isolates were streaked. After 24hrs results were monitored.

Biochemical and Morphological Characterization

The selected acrylamide degrading bacterium was identified using morphological and biochemical characterization methods. A standardized colorimetric identification system utilizing eight conventional biochemical tests and four carbohydrate utilization tests were studied.

Optimization studies

Effect of Initial pH and Temperature

The optimum pH for acrylamide degradation was determined by culturing the microorganisms in basal medium with different pH values 5, 6, 7, 8 and 9. The medium was supplemented with 100mg^{-1} of acrylamide and kept at 30^{0}C with rotatory shaking. Bacterial growths were measured after 48 hours to evaluate the effect of initial pH in colorimeter at 540 nm. The optimum temperature for acrylamide degradation was determined by culturing the microorganisms in basal medium with different temperature values. The medium was supplemented with 100mg^{-1} of acrylamide and kept at 30^{0}C with rotatory shaking. Bacterial growths were measured after 48 hours to evaluate the effect of initial pH in colorimeter at 540 nm.

Effect of Carbon and Nitrogen Sources

The effect of carbon sources for acrylamide degradation was determined by the microorganisms in basal medium with different sources includes Glucose, Fructose, Lactose, Mannitol and Sucrose.The medium was supplemented with 100mg^{-1} of acrylamide and 1% carbon sources and kept at 30^{0}C with rotatory shaking. Bacterial growths were measured after 48 hrs to evaluate the effect of carbon sources in colorimeter at 540 nm. The effect of nitrogen sources for acrylamide degradation was determined by the microorganisms in basal medium with different sources include Peptone, Beef extract, Yeast extract, Casein and Gelatin. The medium was supplemented with 100mg^{-1} of acrylamide and 1% nitrogen sources and kept at 30^{0}C with rotatory shaking. Bacterial growths were measured after 48 hrs to evaluate the effect of nitrogen sources in colorimeter at 540 nm.

Acrylamide Degradation by Bacteria

Acrylamide degradation was performed in 50 ml of basal medium, supplemented with 100 PPM acrylamide. The medium was inoculated with 5 ml bacterial inoculum and incubated at 28^{0}C with rotatory shaking. Reaction mixtures were sampled at regular intervals and analyzed.

Analysis of Acrylamide Degradation by Flask Culture Method

The efficiency of the acrylamide degrading microorganisms was monitored by estimating the ratio between acrylic acid and residual acrylamide. The growth medium containing 200 PPM and 500 PPM acrylamide as sole carbon source, was inoculated with 5 ml of 24hrs bacterial suspension. Cultures were cultivated at room temperature for 20 days with continuous shaking. The acrylamide degradation was detected by Fourier Transform Infrared Spectroscopy and High Performance Liquid Chromatography.

Sample Preparation for FTIR

The degraded metabolites were collected after 20 days of incubation. The degraded residue was air dried and used for FTIR analysis. Sample for FTIR can be prepared in a number of ways. For liquid samples, the easiest is to place one drop of sample between two plates of sodium chloride (salt). Salt is transparent to infrared light. The drop forms a thin film between the plates. Solid samples were milled with potassium bromide (KBr) to form a very fine powder. This powder is then compressed into a thin pellet which can be analyzed. KBr is also transparent in the IR. Alternatively, solid samples can be dissolved in a solvent such as methylene chloride and the solution placed onto a single salt plate. The solvent was then evaporated off, leaving a thin film of the residue on the plate.

HPLC Analysis

The high performance liquid chromatographic separation of the degraded compound was carried out on a LC – 10 AT Vp model HPLC using 250 x 4.60nm Rheodysne column (c-18). The solvent system methanol and water (HPLC Grade) was used in the ratio of 88:12. The operating pressure was 114 kgf, at a flow rate 0.8ml/minutes and the temperature was set at 30oC. The UV –vis (SPD -10AVp) detector was set at 210nm. The sample was mixed with the solvent in the ratio of 50:50 and filtered using Millipore filter before injection. About 25µl of the sample filtrate was injected into the column. The sample was run for 10 minutes and the retention time was noted. The elution time was compound with the standard and the compound was identified.

Plasmid DNA Isolation by Alkaline Lysis Method

Isolation of plasmid DNA was performed by using alkaline lysis. The plasmid DNA obtained was run on 0.8%agarose gel electrophoresis using ethidium bromide.

Results

Enrichment and Isolation of Acrylamide Degrading Microorganism

Soil samples were collected and enriched with potato chips and 100 PPM acrylamide for 15 days. After 15 days, the enriched samples were serially diluted and the suitable diluted culture was spread plated on nutrient agar medium, amended with 100 PPM acrylamide, in order to isolate resistant acrylamide degrading microorganisms (Plate:1 and 1.1).

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations of the selected isolates were carried out against increasing concentration of acrylamide. Nutrient agar plates were supplemented with different concentrations of acrylamide ranging from 200 PPMto 900 PPM in order to perform minimum inhibitory concentration of selected isolates. All the three isolates (AC1, AC2 & AC 3) exhibited tolerance up to 600 PPM. The strain AC1 displayed maximum tolerance of acrylamide up to 900 PPM (Table:1, Plate:2 and 3). On the basis of the results, AC1 was selected for further characterization and degradation studies.

Morphological, Physiological and Biochemical Characterization of the Resistant Acrylamide Degrading Microorganisms

The isolate AC1 was found to be a gram positive short rod shaped bacterium. The organism produced endospores and motile in nature. The colonies on nutrient agar was found opaque, flat and dry with undulated margins after 24 hrs of incubation at 37^0C. (Table: 2).

The biochemical characterization of the isolate AC1 was presented in (Table: 3). The organisms from the result observed AC1 was identified as *Bacillus subtilis*. The results were compared in accordance with the Bergey's manual of determinative bacteriology.

Optimization Studies

Effect of pH and Temperature on the Acrylamide Tolerant Bacteria (AC1) in the Basal Medium

The effect of initial pH (5, 6, 7, 8 & 9) on the growth of the strain AC1 was studied at room temperature. The measurement of growth was carried out after 72 hrs of incubation. The result of this study was depicted in (Figure:1). The strain B*acillus subtilis*grew optimally at pH 7. The growth of the isolate declined dramatically outside this range. The effect of incubation temperature on the growth of acrylamide degradating bacteria *Bacillus subtilis* was studied at different temperatures ranging from 20-40^0C (20, 25, 30, 35 and 40^0C). The results were presented in (Figure: 2). The results revealed that, an optimum growth and acrylamide degradation was observed in between 30 and 35^0C. A drastic reduction

in growth was seen at 45^0C. Similarly at temperatures lower than 30^0C, the growth of microorganisms was reduced.

Effect of Carbon and Nitrogen Sources on the Acrylamide Tolerant Bacteria (AC1) in the Basal Medium

In order to improve the efficacy of acrylamide degradation, different carbon sources such as glucose, fructose, lactose, mannitol and sucrose were used at an initial concentration of 1% (W/V), with 100 PPM acrylamide as the sole nitrogen source. All of the tested carbon sources supported cellular growth, when compared to control. Among the carbon sources tested, glucose gave the enhanced cellular growth with the optical density of 1.85, after 72 hrs of incubation (Figure: 3).

Glucose with the supplementation of fructose in the basal medium exhibited the highest cellular growth with OD 1.49. The other carbon sources tested like lactose exhibited (1.12), followed by mannitol (0.94) and sucrose (0.90) slightly enhanced the cellular growth rate. The nitrogen sources were secondary energy sources of the microorganisms, which play an important role in the growth of the organism and acrylamide degradation. The supplementation of additional nitrogen sources like beef extract, yeast extract, casein and gelatin were studied (Figure: 4). The results revealed that addition of yeast extract in the basal medium enhanced the cellular growth (OD 0.55) and acrylamide degradation, followed by beef extract (0.46).

Degradation Studies

After 20 days, biodegradation of acrylamide at various concentrations 200 PPM and 500 PPM Plate: 4 and 5 were monitored by Fourier Transform Infrared Spectroscopy and High Performance Liquid Chromatography.

FTIR Analysis

FTIR is a reliable rapid Economical technique. The present study is conserved with biodegradation of acrylamide infrared region in mid IR (400-4000 cm^{-1}) is the most commonly used region for analysis. Since the molecule characteristic absorbance frequencies and primary molecules in the ranges, the chemical change in the functional group of acrylamide were monitored using FTIR. The functional groups present in molecules tend to absorb IR radiation to the wave number less of the other structure in the molecules. Hence, there is a correlation between IR band position and chemical structure in the molecules.

The result of FTIR study indicated a pronounce difference between control and experimental treated acrylamide samples. The IR absorbance of the experimental treated acrylamide concentration of 200 PPM revealed a prominent peak of 3431.36 cm^{-1} stretch and vibration of the functional group exhibited O-H stretch carry in a functional alcohol groups. Simultaneously another prominent peak indicated 2927.4 cm^{-1} indicated the presence of C-H stretching groups which is obtained due to the degradation of enzymatic transformation of acrylamide monomer designated to be alkane groups.

The FTIR spectra revealed another peak values of 1637.53 cm^{-1} suggested the presence of amide groups with the functional expression f C=O. The FTIR spectra revealed another important peak value of 1429.25 cm^{-1} indicated the presence of C-H bending of alkane group, which was gave to enzymatic breakdown of monomer acrylamide by the microorganisms. Another spectral analysis revealed a prominent peak of 1066.64 cm^{-1} indicated C-O stretches of alcohol groups.

The FTIR analysis exhibited an important peak of 1278.81 cm^{-1} found to be C-O stretching of acid groups which is due to degradation of acrylamide in to acrylic acid monomer and metabolites acrylic acid and ammonia can be utilized as carbon and nitrogen sources for the synthesis of cell materials.

The results of FTIR study concerned with 500 PPM revealed a prominent peak with varied difference between control and experimental treated acrylamide sample. The FTIR spectra revealed a prominent

peak of 3398.57 cm^{-1} indicate O-H group was one of the functional group responsible for adsorption. Simultaneously another peak with 3331.07 cm^{-1} indicated primary amines one secondary band considered to be very weak, another one primary amine with N-H band stretches.

On analysis with a higher concentration of acrylamide with 500 PPM indicated another prominent peak 2935.66 cm^{-1} indicated strong alkane C-H stretches suggest functional group changes from acrylamide monomer into a metabolite. Similarly, a FTIR spectra also revealed a band value of 1847.81 cm^{-1} indicated C=O band considered as strong acid presumed to be functional group changes from acrylamide to acrylic acid.

The FTIR spectra of biotransformation of acrylamide with 1647.21 cm^{-1} revealed amide structures C=O occurred due to enzymatic cleavage of microorganisms in the process of degradation. Similar peak of FTIR spectra denoted at 1413.82 cm^{-1} indicated a peak of C=C stretch with weak band. Another broad spectrum with a peak of 1074.35 cm^{-1} indicated C-N stretch suggested to be an amine, responsible for adsorption of acrylamide by microorganism *Bacillus subtilis.*

HPLC Analysis

The analysis of HPLC or monitoring the formation or disappearance of peak obtained due to the degradation of acrylamide was analyzed with the peak value and retention time.

The analysis with acrylamide monomer *Bacillus subtilis* treated at 200 PPM revealed a prominent chromatogram 1.327 and 1.457 respectively. Similarly another peak with retention time 2.3 mins with the peak of 2.213 was observed. The acrylamide monomer was readily hydrolyzed by the enzymes of *Bacillus subtilis.* The chromatogram of HPLC exhibited another peak of 2.560, which denotes polymeric samples with the presence of acrylic acid monomers and the metabolites of acrylic acids and ammonia. The detection of acrylic acid and it's metabolites with low polarity were found in the reaction. HPLC analysis was also performed with *Bacillus subtilis* with an increased concentration of 500 PPM. The chromatogram revealed a prominent peak of 2.233 and 2.513 with rotation time of 2.5 mins.

The degradation products and the standard retention time was found to be 3.1 mins. The change in rotation time denotes the formation of intermediants of acrylamide monomer degradation. Similarly, analysis of HPLC for the control treated sample was also denoted.The prominent peaks of 2.740, 2.173, 1.420 and the retention time of 2.2, 2.8 which presume that acrylamide as a conjugative molecule exhibiting aliphatic compounds.

Plasmid DNA Isolation by Alkaline Lysis Method

Bacillus subtilis was found to be has a good degree of resistance for acrylamide. In the present investigation, plasmid DNA from acrylamide tolerant strain was isolated by alkaline lysis method. Agarose gel electrophoresis was performed to get plasmid profile of the acerylamide tolerant isolate *Bacillus subtilis.* It was observed that the strain exhibited single band plasmid DNA with different molecular weight with 12 and 15 kb. (Plate:6).

Table 1: Determination of minimum inhibitory concentration (MIC)

S.No	Concentration (PPM)	Isolated organisms		
		AC1	AC2	AC3
1	200	+	+	+
2	300	+	+	+
3	400	+	+	+
4	500	+	+	+
5	600	+	+	+
6	700	+	-	-
7	800	+	-	-
8	900	+	-	-

Table 2: Depicts morphological, physiological & cultural characteristics studies of Isolate AC1

S.No	Characteristics	AC-1
1	Gram's staining	Gram positive
2	Colony morphology	Cream colored, opaque, flat dry colonies with undulate margins.
3	Motility	Motile
4	Spore staining	Present

Table 3: The Biochemical Characterization of the Isolate AC1

S.No	Biochemical characterization	Observation
1	Indole production test	Negative
2	Methyl red test	Negative
3	Citrate utilization test	Positive
4	Triple sugar iron test	Alkaline slant
5	Voges proskauer test	Negative
6	Nitrate utilization test	Negative
7	Gelatin hydrolysis test	Positive
8	Starch hydrolysis test	Positive
Carbohydrate fermentation test		
1	Dextrose	Positive
2	Fructose	Positive
3	Mannitol	Positive
4	Sucrose	Positive

Plate:1

Plate:1.1

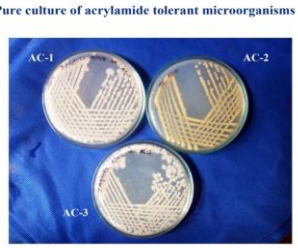
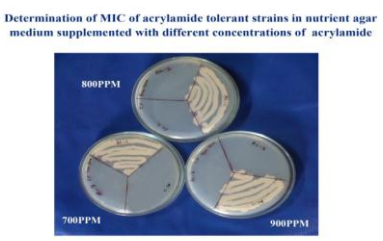


Plate:2

Plate:3



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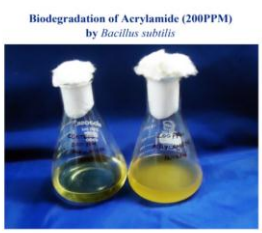

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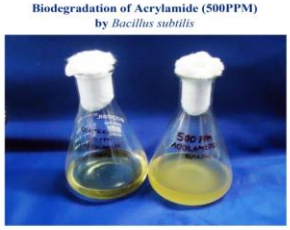


Plate:6

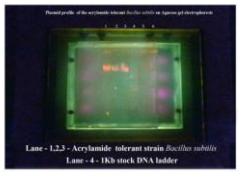


Figure 1: Effect of pH on the Acrylamide tolerant bacteria (AC-1) in the basal medium

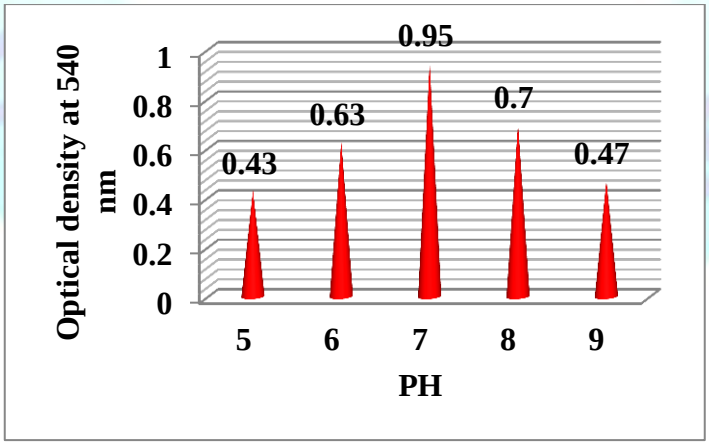


Figure 2: Effect of Temperatures on the Acrylamide tolerant bacteria (AC-1) in the basal medium

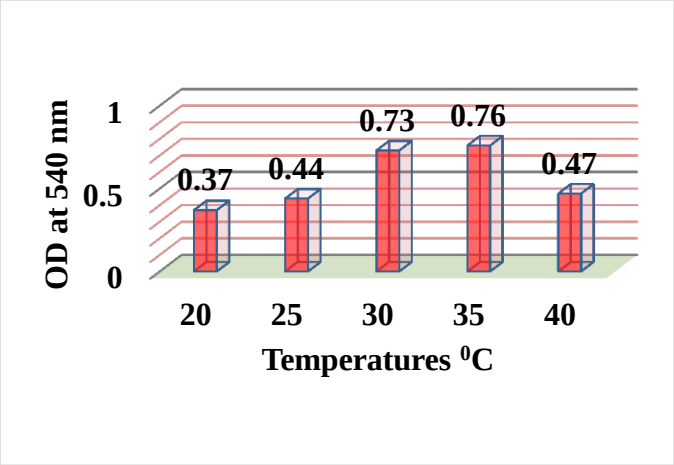


Figure 3: Effect of carbon sources on the Acrylamide tolerant bacteria (AC-1) in the basal medium

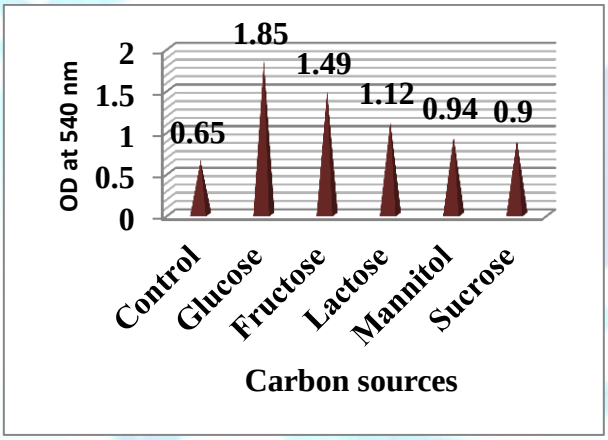
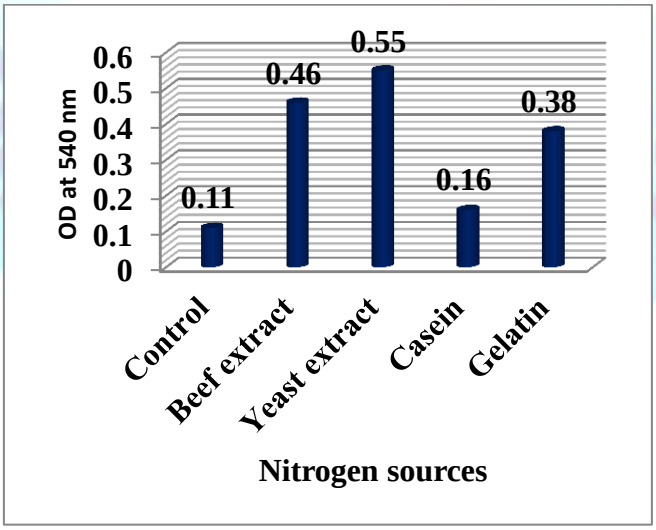


Figure 4: Effect of Nitrogen sources on the Acrylamide tolerant bacteria (AC-1) in the basal medium



Discussion

Acrylamide is a potential cause of a wide spectrum of toxic effects and is classified as probably "carcinogenic to humans". The discovery of acrylamide in human foods has given rise to extensive studies exploring its formation mechanisms and levels of exposure has spurred search into suitable analytical procedures for its determination of food stuffs (Zhu et al, 2008).

Acrylamide could be absorbed through unbroken skin, mucous membranes, lungs and the gastro intestinal tract. Free asparagine in combination with reducing sugars generates significant amounts of acrylamide when pyrolysed at temperatures >120^{o}C (Stadler et al., 2002).

Microbial degradation of acrylamide has been explored extensively with a diversity of microbes mainly *Pseudomonas, Bacillus, Arthrobacter, Xanthomonas* and *Rhodococcus*(Cha and Chambliss, 2011).In the present investigation, acrylamide degrading microorganisms were isolated from fried foods such as potato chips. The preliminary study on the enrichment culture technique was performed in order to isolate the acrylamide degrading bacteria. The enriched sample was serially diluted and spread plated on the nutrient agar plates. Five morphologically distinct colonies were isolated. Three acrylamide degrading microorganisms were selected after screening on modified basal medium containing 100 mg/l of acrylamide.

Similar studies were performed by Neni et al, 2008 for screening acrylamide degrading strains from glyphosate contaminated soils. The data from the morphological, physiological and biochemical characterization tests performed for the isolates in accordance with the methods described in the Bergey's manual of Determinative Bacteriology and identified as *Bacillus cereus*, *Bacillus licheniformis* and *Bacillus subtilis*. Several acrylamide degraders use a coupling reaction of nitrile hydratase and amidase for biotransformation of acrylonitrile to acrylic acid via acrylamide as an intermediate. Many aerobic microorganisms utilize acrylamide as their sole source of carbon and energy. Rapid degradation of acrylamide coupled with growth requires not only amidase or microorganism producing amidase i.e., a set of enzymes that are differentially synthesized in the presence of acrylamide (Wang and Lee, 2009).

Acrylamide were found low concentration in soil and it was found stable in the soil, nearly 60 days. Acrylamide even it toxic, it's readily biodegraded by microorganism *Bacillus subtilis,* under aerobic conditions by deamination to acrylic acid and ammonia.

Our results are in total agreement with the work of Nawaz *et al.,* 1993. He reported that *Pseudomonas* Sp, metabolites acrylamide and it's identical conditions with release of intermediate component acrylic acid, and ammonia. In our study, ammonia was not detected on the first day of treatment, which indicates that acrylamide degradation is not begun until 24 hours. Ver Vers., 1999, reported that acrylamide and acrylic acid monomer and metabolites such as ammonia can be utilized as carbon and nitrogen sources for the synthesis of cell material.

The effect of initial pH on the growth of this strain was studied at room temperature using 50 mm phosphate buffer spanning the range from pH 5.0 to 9.0. The measurement of growth was carried out after 72 hr of incubation. This strain grew optimally in the range of pH7.0. The effect of incubation temperature on the growth of acrylamide-degrading bacteria showed an optimum growth in between 30 and 35^{0}C with no significant difference in terms of cell growth between the two temperatures ($p<0.05$). Different carbon sources and nitrogen sources such as glucose, fructose, lactose, mannitol, and sucrose were used at an initial concentration of 1.0% (w/v) to study their effects on acrylamide degradation with acrylamide as the sole nitrogen source.

Conclusion

The effective acrylamide tolerant isolate was identified and characterized as *Bacillus subtilis*. The optimum pH-7, temperature is 30-35^{0}C, good carbon sources as glucose and nitrogen sources as yeast extract. Biodegradation of acrylamide was performed at various concentrations 200 PPM and 500 PPM. The result of FTIR study indicated a pronounce difference between control and experimental treated acrylamide samples. HPLC analysis was also performed with 500 PPM, and the control was compared. *Bacillus subtilis* was found to be has a good degradation resistance for acrylamide. To analyze the plasmid DNA from acrylamide tolerant strain was isolated by alkaline lysis method. It was observed that the strain exhibited single band plasmid DNA with different molecular weight with 12 and 15 kb.

Reference

- Brown, J.E. and P.H. Clarke (1970). Mutations in a regulator gene allowing Pseudomonas aeruginosa 8602 to grow on butyramide. J. Gen. Microbiol., Vol.No: 64 , PP:329-342.
- Brown, J.E. and P.H. Clarke (1980). Mutations in a regulator gene allowing *Pseudomonas aeruginosa* 8602 to grow on butyramide. J. Gen. Microbiol., Vol.No:64, PP:329-342.
- Cha, M, and Chambliss, G. H.(2011). Characterization of acrylamidase isolated from a newly isolated acrylamide-utilizing bacterium, Rastonia eutropha AUM-01. Current Microbi- ology, Vol.No: 62(2), PP:671-678.
- Cherry, A.B., Gabaccia, A.J. and Senn, H.W. (1956). The assimilation behavior of certain toxic organic compounds in natural water. Sewage Ind. Wastes, Vol.No: 28, 1137-1146.
- Friedman, M (2003). Chemistry, biochemistry, and safety of acrylamide.A review. Journal of Agric. Food Chem, Vol.No: 51:PP:4504-4526.
- Kanno and Sei-ichiro., (1985). An estimation of the sublimation rate of acrylamide: J-Stage Home. Vol.No: 23, PP:167-170.
- Mallory, L.M., Yuk, C.S., and Alexander, M. (1983). Alternative prey: a mechanism for elimination of bacterial species by protozoa. APPlied and Environmental Microbiology Vol.No: 46, PP:1073–1079.
- Mannsville, M. (1993).Chemical Products Synopsis, Acrylamide. Mannsville Chemical Products Corporation.
- Mullory, L.M., andAlexander, M. (1996). Alternative prey: amechanism for elimination of bacterial species by protozoa. APPlied and Environmental Microbiology Vol.No: 46, PP:1073–1079.
- Murakami, Y., and Alexander, M. (1989). Destruction and formation of toxins by one bacterial species affect biodegradation by a second species. Biotechnology and Bioenergetics Vol.No: 33,PP: 832–838.
- Nawaz M S, Franklin W, and Cerniglia C E, (1993). Degradation of acrylamide by immobilized cells of a Pseudomonas sp. and Xanthomonas maltophilia. Canadian Journal of Microbiolo- gy, Vol.No: 39:PP: 207–212.

- Nawaz, M. S., Billedeau, S. M., and Cerniglia, C. E (1998). Influence of selected physical parameters on the biodegradation of acrylamide by immobilized cells of *Rhodococcus* sp. Biodegradation,Vol.No: 9, PP:381-387.
- Prabu, C. S.,and Thatheyus, A. J., (2007). Biodegradation of acrylamideemploying free and immobilized cells of *Pseudomonasaeruginosa*. International Biodeterioration and Biodegradation, Vol.No: 60: PP:69–73.
- Sax, N. I, and Lewis, R. J. Hawley's Condensed Chemical Dictionary (11th ed.). NewYork: Van Nostrand Reinhold; (1987).
- Stadler RH, Blank I, Varga N, Robert F, Hau J, et al. (2002) Acrylamide fromMaillard reaction products. Nature.PP: 449-450. Vol.No: 66.
- Ver Vers, M.L., 1999. Determination of acrylamide monomer in polyacrylamide degradation studies by high perfor mance liquid chromatography. Journal of Chromosome Science Vol.No: 37, PP:486–494.
- Wang, C.C. and C.M. Lee (2009). Denitrification with acrylamide in pure culture of bacteria isolated from acrylonitrile-butadiene-styrene resin manufactured for wastewater treatment. Chemosphere, Vol.No: 52, 1118-1123.
- Zabaznaya E V, Kozulin S V, and Voronin S P, (1998). Selection of strains transforming acrylonitrile and acrylamide into acrylic acid. Applied Biochemistry and Microbiology, Vol.No: 34: PP: 341–345.
- Zhu YJ, Zeng T, Zhu YB, Yu SF, Wang QS, Zhang LP, Guo X, Xie KQ.(2008). Effects of acrylamide on the nervous tissue antioxidant system and sciatic nerve electrophysiology in the rat. 3(11):2310-7.