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Biotransformation of 2- Chlorophenol by an Alkaliphile isolated from Salt Lake Lonar (MS). India.

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Abstract: *2- Chlorophenol degradation was studied by using an Alkaliphile isolated from Salt Lake Lonar after adaptation to three months. 2- Chlorophenol degradation study was extended to observe the effect of additional carbon and nitrogen source on percent degradation. Marginal difference in percent degradation was noted. Metabolites of the degradation pathway were trapped by solvent extraction and characterized by GCMS. 2, 3,dihydroxy benzoic acid was confirmed by GCMS solution. Almost all experimental concentration [5 mM] of 2 Chlorophenol was found degraded at the end of 24 hr. Enzyme induction study showed activation of CYP450, Cat 1,2 dioxygenase and Cat 2,3 dioxygenase.*

Keywords: *Biotransformation, 2- chlorophenol, PCP, Bacillus badius D1.*

I. Introduction

Chlorophenols are widespreaded pollutants observed in soil and water. These are released in environmental lakes or river bodies by industrial operations like paper and pulp industries, pesticides, disinfectants, bleaching agents, surfactants, dyes and drugs; etc. It has been found that these can also appear as degradation products of other chlorinated xenobiotics[1]. Chlorophenols accumulate in water, soil and air due to high stability. It gives unpleasant taste and odor to drinking water exerting negative effects on different biological processes [2]. Chlorophenols are carcinogenic and toxic to environment. These pollutants are massively discharged into the environment from uncontrolled industrial activities [3]. Different chlorinated products or chlorolignin derivatives formed during Kraft pulp bleaching. Chloroguacols, chlorophenols, chlorocatecols are extremely toxic. The analysis of pulp paper mill effluent and its degradation products by GC–MS analysis revealed the formation of low molecular weight compound like 2-chlorophenol and tetrachlorohydroquinone from polychlorinated phenols [4]. These recalcitrant compounds generated by various processes accumulate in sediments or at different levels and affects the aquatic life processes [5]. 2, 4 dichlorophenol and 2, 4, 6 trichlorophenol are used as wood preservatives [6]. Its degradation product might be mnochlrophenol. The concentrations of chlorophenols in oceanic waters observed of 5-10 ng/L and river waters about 2-2000 µg/L was noted. Chlorophenols were also observed in drinking water due to substitution of organic matter and low molecular weight compounds due to chlorine atoms derived from inorganic chlorine oxidants [7].

2 -chlorophenol has MP 9.8 ^{0}C, BP 174.9 ^{0}C. It is very hazardous in case of skin contact, ingestion, and inhalation. It is responsible for itching, scaling, reddening, or, occasionally, blistering of skin. Inhalation of dust contaminated with it produces irritation to gastro-intestinal or respiratory tract. Severe over-exposure can produce lung damage, choking, unconsciousness or death [8].

The oxidative bio-degradation is supposed as cost effective, versatile technique to avoid secondary pollution [9].

Chemicals: 2 chlorophenol by SRL, peptone yeast extract, buffers were used by Hi-Media.

II. Methodology

Six 500 ml conical flasks with 250 ml alkaline broth media were used for bio-degradation study.1% inoculum of 24 hr. grown culture was added and allowed to grow for 24 hr in shaking incubator at 37 ^{0}C, 110 rpm. At the end of 24 hr 1 to 5 mM concentration of 2- chlorophenol was added in each flask keeping one as an abiotic control among them saperately. The experimental flask was monitored after six hr. intervals. All experiments were carrid in triplicate.

The analysis was carried by using spectrophotometer Jasco Varian 630; monitoring the aliquots absorbance at 280 nm on centrifugation at 10000g. Metabolites were captured by solvent extraction and characterized by GCMS.

Cell free extract and enzyme activities: 24 hr grown bacterial culture was induced by 5mM 2 chlorophenol and isolation of cell mass, cell free extract was carried by Du Pont Soverall cold centrifuge at 10,000g. The protein content was determined by Lawry method [10]. CYP – 450 content was estimated by Omura and Sato [11, 12],

The cat 1, 2 dioxygenase activity was determined by Urszula Guzik [13]; and the cat 2, 3 dioxygenase by Grekova [14] respectivly.

III. Results

3.1 UV-Vis Spectrophotometric analysis of 2 -Chlorophenol biodegradation:

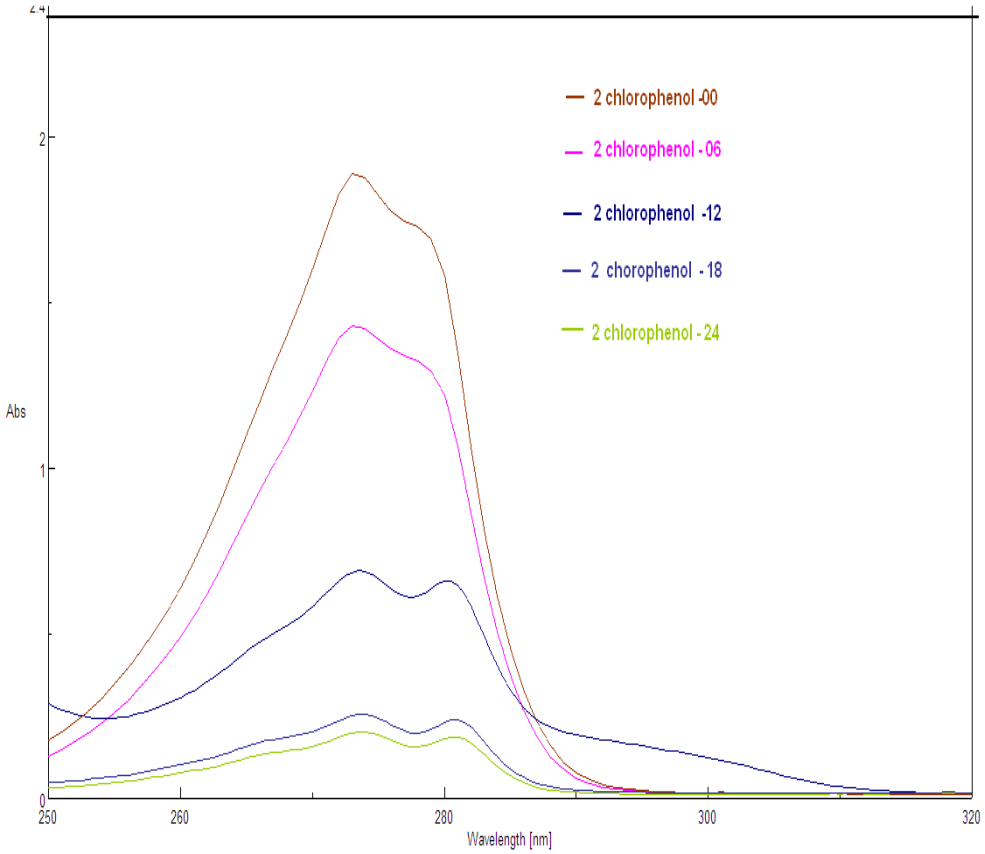

Fig.1. UV-Vis spectrophotometric analysis of 2 -chlorophenol

The [**Fig.1**] clearly indicate the change in spectra from 280 nm to 300 nm at 12hr. It denotes the biotransformation in the catabolite. The spectrum is addative in nature.

3.2 Percent Degradation of 2- Chlorphenol at various Concentration:

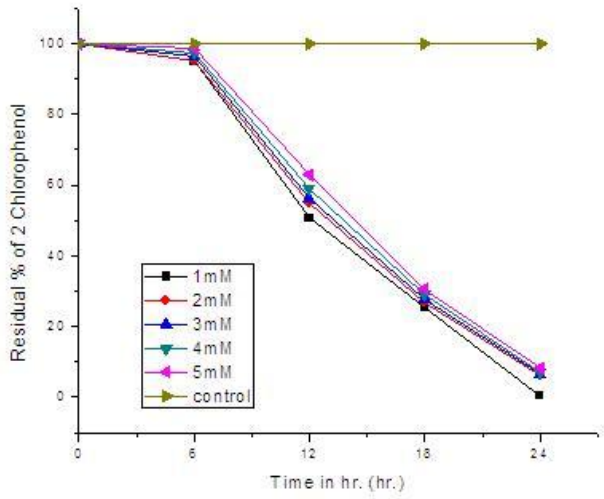

Fig.2. 2 -Chlorophenol degradation at various concentration

Marginal difference in degradation of 2 chlorophenol [**Fig.2**] was noted after the concentration varied from 1 to 5 mM. Within 24 hr the whole concentration was found degraded.

3.3 Effect of various carbohydrate sources on biodegradation of 2 -Chlorophenol:

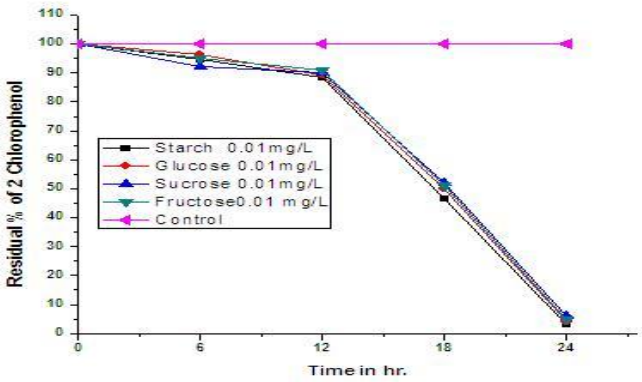

Fig.3 Percent degradation of 2 -Chlorophenol at different Carbon sources

The [**Fig.3**] shows the percent degradation of 2- Chlorophenol. It seems that almost all experimental concentration of 2- chlorophenol had been degraded by *Bacillus badius* D1 within 24 hr. Marginal difference in percent degradation was noted with respect to various carbon sources.

3.4 Effect of various Nitrogen sources on biodegradation of 2- Chlorophenol:

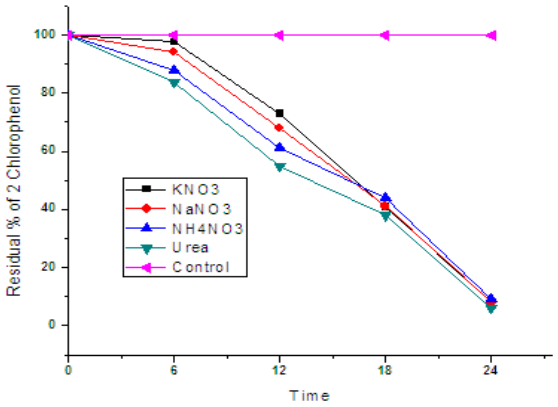

Fig.4 Percent degradation of 2 -Chlorophenol with different Nitrogen sources

The [**Fig.4**] indicate the effect of various additional nitrogen sources on percent degradation of 2 Chlorophenol. Among the additional nitrogen sources provided urea was found more beneficial for degradation. The GCMS analysis revealed that one of the metabolite observed as 2, 4 dihydroxy benzoic acid m/w 154 and the other was 2, 4 dihydroxy benzaldehyde m/w 138.

3.5 Tentative pathway of 2- Chlorophenol biodegradation:

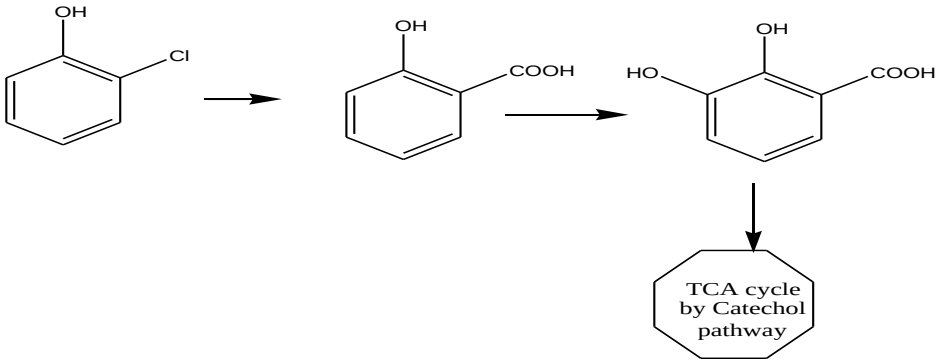

Fig 5 2- Chlorophenol degradation pathway

3.6 Effect on enzymes involved in biodegradation:

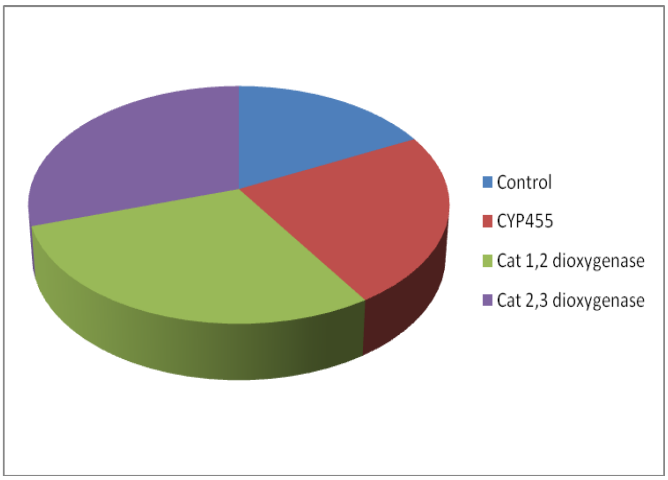

Fig.6 Effect of 2 chlorophenol on enzymes involved in biodegradation.

The [Fig.6] clearly shows the induction in enzymes involved in biotransformation of 2 Chlorophenol compaired to Control.

IV. Discussion

The accumulation of halogenated compounds in the rivers, streams and lakes made aware to save the ecosystem. The accumulated chloro-organic compounds created risk to living creatures as well as human beings[15]. Chlrophenols stick to soil and to sediments at the bottom of lakes, rivers, or streams. These can affect the reproductivity of animals, the newborn formed with reduced weight. Lower concentrations in water gives unpleasant taste and odour. They can undergo slow changes resulting from different chemical, physical,biological,or photochemical processes[16,17].

Various physicochemical methods like adsorption, oxidative degradation in presence of air ion[18] or use of ionizing radiation[19] are employed for bioremediation. Even photo-oxidation using nano material or using acidic condition, anaerobic or neutral culture methods were also employed[20]. Bioremidiation of chloroorganic compuds in alkaline condition is least studied aspect and hence attempt was made to study the biodegradation using an Alkaliphile Bacillus badius D1 isolated from Salt Lake Lonar (MS) Buldana.

Bioremediation using chloroorganic metabolizing bacteria is cost effective and sustainable practice for the removal of polyorganopollutants from water bodies. Seven mechanisms of dehalogenation are known, namely, oxygenolytic, hydrolytic, reductive, thiolytic dehalogenation, intermolecular nucleophilic displacement, dehydrohalogenation and hydration[21]. Haloalkane dehalogenases are bacterial enzymes cleaving the carbon – halogen bond of halogenated aliphatic compounds by a hydrolytic mechanism. In this reaction, the water molecule serves as co-substrate during the catalysis and there is no proof indicating the involvement of co-factors or metal ions in the catalytic mechanism [22,23].

Every chemical compound may undergo chemical, biological or photochemical reactions. 2-chlorophenol degrading bacteria were isolated from a natural enrichment that may be adapted to chlorophenols and used for bioremediation [24,25]. Para-substituted halophenols are degraded more readily than meta- and, especially, ortho-substituted ones. Polyhalogenated phenols are less susceptible to microbial attack than monohalogenated phenols[26]. It is known that the position and nature of the halogen atom affect the rate

of decomposition[27,28]. The C-Cl bond and the position of chlorine atoms relative to the hydroxyl group are responsible for their toxicity, carcinogenicity, stability and persistence in the environment [29]. Some-times the intermediates formed can be more toxic and stable than original halophenols [30]. Some of the microbes produce extracellular polymeric substances that can offer a protective barrier under environmental stress for survival [31, 32]. The efficiency of biodegradation of organic compounds is influenced by the type of the organic pollutant, the nature of the organism, the enzyme involved, the mechanism of degradation and edaphic factors [33].

Chlorophenol removal was studied by several researchers [34,35,36,37,38,39]. There are no harmful products formed after completion of enzymatic reaction. Hence, enzymatic treatment is fully eco-friendly treatment [40]. Many of the bacteria and their enzymes were isolated and used for dehalgeating purpose [41, 42, 43].

Higher concentrations of 2-chlorophenol 2.5 mM were reported inhibitory to cell growth [44]. But *Bacillus badius* tolerated and degaradaed higher concentrations [**Fig. 2, Fig.3, Fig. 4**].The additional carbon and nitrogen sources have shown the marginal change in percent degradation; however urea as additional nitrogen source shown positive effect in percent degradation. This might be justified as ammonia liberating in the broth media could be helpful to create media alkaline. On the basis of GCMS study the tentative pathway [**Fig.5]** for biodegradation of 2 Chlorophenol was constructed. One of the metabolite 2,3 dihydroxy benzoic acid was observed in it.

The cytochrome P-450-dependent enzyme systems belong to a class of enzymes which catalyze oxidative reactions. Biological oxidations may involve the insertion of oxygen into the substrate molecule. This oxygen may be derived from Water, as in the case of oxidation of fatty acids. For many substrates, such as nonpolar aliphatic or aromatic compounds, however, this route is energetically unfavourable. These compounds may be oxidized by molecular oxygen with the aid of enzymes called oxygenases. When the reaction requires the incorporation of both atoms of the oxygen molecule, the enzymes are called dioxygenases[45].These enzymes increases the polarity of chloroorganic compounds and ultimately its solubility.

Bacillus badius follow both ortho cleavage pathway as well as meta cleavage pthway of catachol has already reported in our early report. In this study the catechol 2,3 dioxygenase activity observed more [**Fig.6**] than Catechol 1,2 dioxygenase activity. It means that *Bacillus badius* follows the extradiol cleavage pathway preferentially.

V. Conclusion

The microorganism *Bacillus badius* D1 is an alkaliphile isolated from Salt Lake Lonar MS might be useful for bioremediation of organochlorine contaminated sites. It uses the organochlorine compounds as a carbon and mineral source.

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