REVIEW

Biochemical Potential of *Candida tropicalis* in Enzymatic Degradation of Phenol

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The pronounced and serious effects of phenol in the biosphere have attracted worldwide attention to keep its level below threshold in order to preserve the environment quality. The biochemical degradation through micro-organisms is cost effective among various methods to-date. The metabolic process of micro-organisms is governed by specific enzymes. Phenol is biochemically transformed into catechol followed by ring cleavage either at *ortho* or *meta* position incorporating end products in TCA cycle. The present article provides an overview of biochemical pathway adopted by *Candida tropicalis* to metabolize phenol through aerobic degradation.

Keywords: Aerobic biodegradation, *Candida tropicalis*, *Ortho-meta* pathways, Phenol, Thermo-tolerant.

INTRODUCTION

Industrial wastes generate organic and inorganic toxic compounds as effluents having cumulative hazardous effects. Because of their persistent nature, many of these pollutants are capable of accumulation in living bodies and bio magnify in food chain¹. One of such compounds includes phenol, recognized as hazardous to human health. Phenol is a white crystalline solid having molecular weight of 94.14 g/mol and is a basic structural unit for many synthetic compounds (US Environmental Protection Agency 1990). The origin of phenol in the environment is from both natural and industrial sources.

Phenol is an important groups of xenobiotic chemical² extensively used in several economic fields. These includes herbicides, insecticides, fungicides, pharmaceutical industries, petroleum refineries, paper industries, heat transfer media, insulators, lubricants, resins and dyes manufacturers petrochemical industries, petroleum refineries, pharmaceuticals, cooking operations, coal refining, resin industries, ceramic plants, steel plants and pulp mill^{3,4}. As mankind cannot relinquish the use of these chemicals therefore, exploration of biodegradation properties of micro-organism is particularly pertinent. Phenols are highly toxic compounds which persist in water and give an intensive odor to drinking water⁵. In soil, phenol filters easily as the soil particles have no affinity for phenol and phenol percolates to underground water. So as in air, phenol degrades rapidly within

about 15 h. Therefore, the concentration of phenol is found less in soil than water reservoirs⁶.

The United State Environmental Protection Agency (US EPA) and European Union (EU) has declared phenol as first priority pollutant⁷. Maximum permissible concentration of phenol recommended by the US Environmental Protection Agency (EPA) in fresh water is 10,000 mg/L whereas; the final criterion vale for phenol as recommended by EPA is 860,000 mg/L (EPA, 2009 Fact Sheet). Presence of these compounds in water imparts carbolic odor which makes drinking water inconsumable and harmful for aquatic and terrestrial life^{8,9}. Relative low concentration of phenol which could endanger fish life is 5-25 ppm^{10,11} hence the eradication of phenol from environment is important¹². Due to health hazards of phenol World Health Organization (WHO) has set recommended concentration of 1 mg/L to regulate phenol concentration in drinking water¹³.

For the degradation of phenol and phenolic compounds fungi has got much importance than bacteria and algae¹⁴ due to perfuse and extensive hyphal growth and its ability to tolerate stress conditions like low availability of nutrients, changes in pH, temperature and water scarcity¹⁵.

Candida tropicalis has been found to degrade phenol (among all microorganisms) at its best degrading rates. Many researchers have found *C. tropicalis*, also as best pre-treatment tool, prior to anaerobic waste water treatment¹⁶. Biodegradation

of phenol using *C. tropicalis* produces valuable products like ethanol which intensify its use for phenol wastewater treatment¹⁷. The present review focuses on exploring the potential of *C. tropicalis* as a major agent for elimination of phenol from wastewater.

Biological degradation of phenol and phenolic compounds: Micro-organisms have diverse catabolic pathways for breaking down of many types of recalcitrant and xenobiotic pollutants with few by-products¹⁸. Phenol degrading micro-organisms were isolated as early as 1908. Since then, microbial degradation of phenol and phenol derivatives by bacterial and yeast cultures have been studied¹⁹. The micro-organisms even have evolved sophisticated regulatory network to fine tune the biodegradation pathways for phenolic compounds, *e.g.*, quorum sensing system, which is one of the most complex bacterial regulatory networks, was identified to be involved in phenolic compounds biodegradation as first reported by Yong and Zhong²⁰⁻²². It implied that micro-organism should have great potential in phenolic compounds biodegradation.

Phenol and its derivatives damages the cytoplasmic membrane, causes the inactivation of microbial enzymes systems thus damaging the whole living cell²³. But there are certain micro-organisms which have mechanism to withstand the lethal effect of phenol²⁴.

The efficiency of biodegradation process depends on the nature of organic compound to be degraded, the type of micro-organism, nature of enzymes produced by the micro-organism, the degradation mechanism and favorable environmental conditions²⁵. Various micro-organisms utilize phenol as sole carbon and energy source despite phenol is toxic and causes cell and growth inhibition at high concentration²⁶.

A number of studies have been conducted for isolation and identification of micro-organisms which are involved in phenol degradation²⁷ and these includes members belonging to bacteria, fungi and algae. Some of these micro-organisms are mentioned in Table-1.

***Candida tropicalis*: An efficient biodegrading species for phenol:** Fungi contributes substantial role in recycling and degradation of aromatic compounds. Various yeasts have been studied for the degradation of phenols and phenolic compounds^{57,58}. Among them member of *Candida* genus *i.e.*, *Candida tropicalis* have been investigated most⁵⁹. It is hydrocarbonoclastic yeast with ability to degrade aliphatic and cyclic compounds like phenol at relatively high concentrations^{60,61}. It has wide existence in the places with phenol^{62,63}. *C. tropicalis* is capable of consuming phenol as carbon source by enzymatic mechanisms.

C. tropicalis is found as thermo-tolerant yeast which can degrade phenol at wide temperature range from 29-42 °C⁵⁹. *Candida* includes about 154 species among which only 4 are specifically important for phenol degradation. These species degrade phenol at different concentrations. Most dominating species in phenol degradation includes *C. maltosa*, *C. tropicalis*, *C. albicans* and *C. rugosa*. All these species biodegrade phenol through the similar pathway yet their degradation rate varies. This variation is due to certain external or environmental factors which limits their activity. According to the available literature it has been found that *C. tropicalis* has greater potential for phenol degradation by optimizing growth factors.

TABLE-1
PHENOL DEGRADING MICRO-ORGANISMS

Group	Microorganism	Ref.
Bacteria	<i>Bacillus stearothermophilus</i>	28
	<i>Pseudomonas fluorescens</i>	29
	<i>Alcaligenes sp. A7-2</i>	30
	<i>Flavobacterium</i>	31
	<i>Moraxella</i>	32
	<i>Arthrobacter aurescens</i>	33
	<i>Acinetobacter johnsonii</i>	34
	<i>Bacillus laterosporus</i>	35
	<i>Agrobacterium radiobacter</i>	36
	<i>Klebsiella pneumoniae</i>	37
	<i>Pseudomonas putida</i>	38
	<i>Pseudomonas syringae</i>	39
	<i>Nocardia sp.</i>	40
	<i>Rhodococcus erythropolis</i>	41
	<i>Bacillus brevis</i>	42
	<i>Acetobacter</i>	43
Fungi	<i>Pseudomonas membranaefaciens</i>	44
	<i>Pseudomonas aeruginosa</i>	22
	<i>Rhizoctonia praticola</i>	12
	<i>Agaricus bisporus</i>	45
	<i>Geotrichum candidum</i>	46
	<i>Trametes trogii</i>	47
	<i>Acinetobacter calcoaceticus</i>	48
	<i>Aspergillus niger</i>	49
	<i>Phanerochaete chrysosporium</i>	49
	<i>Pleurotus ostreatus</i>	50
	<i>Coprinus cinereus</i>	51
	<i>Neurospora crassa</i>	52
	<i>Trichosporon cutaneum</i>	53
	<i>Fusarium sp. HJ01</i>	54
	<i>Candida tropicalis</i>	18 & 19
Algae/cyanobacteria	<i>Phormidium valderianum</i>	55, 56
	<i>Ankistrodesmus braunii</i>	56

Bio-degradation capability of *C. tropicalis* for phenol has been the subject of several investigations. Yan *et al.*⁶⁴ and Stoilova *et al.*⁶⁵ have reported work related to isolation of different strains of *Candida* from various sources like industrial effluents and waste water. Many techniques used by researchers in respect to phenol removal from wastewater are; substrate inhibition of phenol oxidation⁶⁶, physiological changes caused in cell population by phenol degradation⁶⁷, protoplast fusion technique^{60,68}, modeling of airlift bioreactors for biodegradation^{69,70}, immobilization of the cells in various matrixes^{71,72}. All this work indicates the importance of *C. tropicalis* as major agent for the degradation of phenol and phenolic compounds. But still there is need of further research regarding molecular and genetic aspects of *Candida spp.*

It is obvious from Table-2 that percentage degradation of phenol by *C. tropicalis* is higher than other known species of genus *Candida*. According to studies made by Tsai *et al.*⁷⁴ *C. albicans* can degrade phenol at higher concentration as compared to any of the other known micro-organism. The concentration of phenol which can be degraded by *C. albicans* is 1000 ppm at 30 °C within 3 days but the efficiency of *C. albicans* decreases at higher temperature 35 or 40 °C. The cell density and cell culture reaches a stationary phase at 40 °C and reduce to about 7 %. Whereas, *C. tropicalis* can tolerate 42 °C and can easily degrade phenol up to a concentration of 2000 ppm at pH 5-7⁷³.

TABLE-2
Candida SPECIES IN DEGRADATION OF PHENOL

<i>Candida</i> species	Initial conc.	Substrate	Reference
<i>Candida tropicalis</i>	2000 ppm	Phenol	73
<i>Candida albicans</i>	1000 ppm	Phenol	74
<i>Candida maltosa</i>	1700 ppm	Phenol	3
<i>Candida rugosa</i>	500 ppm	Phenol	44

Candida maltosa has practical use in bioremediation to remove phenol and other aromatic compounds from soil. But less data is available regarding different parameters that affect its bio-degrading ability. Comprehensive studies are required for manipulation of potential of *C. maltosa* in biodegradation of phenolic containments from environment³.

Comparative studies made between *C. rugosa* and *C. tropicalis* indicates that *C. rugosa* can only degrade phenol at concentration of 500 ppm. When level of phenol increases above 500 ppm it inhibits degradation process and *C. rugosa* cannot tolerate the toxic effect of phenol⁴⁴ whereas, *C. tropicalis* NCIM 3556 can easily tolerate toxic effect of phenol and biodegrade 2000 ppm of phenol⁷³. This shows that *C. tropicalis* is active biodegrading agent for phenol elimination from waste water and industrial effluents. Overall contribution of genus *Candida* in phenol biodegradation has been shown in Fig. 1. The pie chart is constructed from the data given in Table-2.

Fig. 1. *Candida* species in phenol degradation: 58 % phenol degradation is showing high efficiency of *Candida tropicalis*

Metabolic pathway of phenol bio-degradation: The aerobic catabolic pathway of phenol comprises of three steps *i.e.*, formation of catechol from growth substrate, ring cleavage and finally the metabolism of ring cleavage product either by *ortho*^{75,76} or *meta*-pathway⁷⁴ to central metabolism. Among yeast *Trichosporon cutaneum* is most studied with reference to phenol degradation pathway and it was thought that same pathway should be adopted by *Candida tropicalis*⁷⁷.

In the first step hydroxylation of phenol occurs and phenol is converted into catechol. This step requires molecular oxygen to initiate enzymatic activity. In *C. tropicalis* this step is considered to be catalyzed by three enzymes⁷⁷. These includes (i) mixed function mono-oxygenase system which are located in the membrane of endoplasmic reticulum and containing cytochrome P450 (EC 1.14.15.1)⁷⁸ (ii) cytosolic NADPH-dependent phenol hydroxylase (EC 1.14.13.7)⁷⁹ (iii) membrane-bound or soluble peroxidases (EC 1.11.1.7)⁷⁸. Recently it was found that cytochrome P450 expressed in *C. tropicalis* and has the ability of hydroxylation of phenol into catechol⁷⁷. So it can be said that *Candida* has a number of

enzymatic systems which helps in efficient degradation of phenol and its conversion into catechol.

Catechol can undergo fission either by an intradiol or an extradiol type of cleavage (*ortho-meta*-fission). The *ortho* cleavage of catechol converts it into *cis, cis*-muconic acid. This step is also called as β -keto adipate pathway which is catalyzed by catechol 1,2-dioxygenase⁷⁵ *cis, cis*-muconic acid, then converted into 3-oxoadipate to succinyl-CoA and finally into acetyl-CoA. In case of *meta*-fission 2 hydroxymuconic semialdehyde is formed which is catalyzed by catechol 2,3-dioxygenase (C2, 3D) (EC 1.13.11.2)⁸⁰ which is then converted to formate, acetaldehyde and pyruvate. Finally the products of both pathways will incorporate into the tricarboxylic acid cycle (TCA) or Krebs cycle⁸¹. So *Candida* metabolizes phenol by utilizing its enzymes.

Most of the research scientists agree that *C. tropicalis* degrades phenol using the *ortho*-cleavage pathway. The catabolism of phenol is confirmed by the intermediate products most commonly the formation of catechol whereas, the further pathway is confirmed by analyzing the products which are formed at later stages of phenol metabolism. Many researchers have worked on finding the actual pathway of phenol degradation in *C. tropicalis*. The HPLC chromatography and enzymatic results affirms that *C. tropicalis* degrade phenol by *ortho*-cleavage pathway. This conclusion was made on the basis of formation of *cis cis*-muconic acid^{58,75}. This pathway is illustrated in Figs. 2 and 3.

Fig. 2. Metabolic pathway for aerobic biodegradation of phenol by *Candida tropicalis* via *ortho* cleavage

Fig. 3. Metabolic pathway for aerobic biodegradation of phenol by *Candida tropicalis* via meta cleavage

Factors affecting the degradation efficiency of *Candida tropicalis*:

Every micro-organism has its own unique physio-chemical requirements for growth. Basak *et al.*⁸² have studied nine factors that have noteworthy effect on the degradation process. These include temperature, pH, nutrients (NaCl, phosphate ion, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and yeast extracts), agitation, incubation time and inoculum size (Fig. 4). Understanding of such parameters is important for optimizing the effective removal of deleterious compounds like phenol from the environment⁸³.

Fig. 4. Factors affecting *Candida tropicalis* proficiency

Temperature is an important factor in degradation process for phenol degradation by *C. tropicalis*. Different studies reported number of strains which are mesophilic and can tolerate temperature ranging from 29-30 °C^{43,58}. Isolation and identification of such thermo tolerant strains is important so that these strains can be utilized for waste water treatment in countries with an average temperature range of 35-40 °C. Kuntiya *et al.*⁵⁹ reported that *C. tropicalis* strain can tolerate temperature up to 42 °C. Temperature changes have direct effect on enzymatic activity of organisms which eventually affect the biodegradation process. The possibility for tolerating such high temperature might be genetic makeup of *C. tropicalis* but rather more data is required regarding heat tolerance.

C. tropicalis tolerate a broad pH range⁵⁸ and the optimal pH range is 5-7^{57,84} of phenol containing media. So it can be used as major bio-degrading agent of phenol. But cause behind this broad range tolerance is still unknown.

Kuntiya *et al.*⁵⁹ observed that *C. tropicalis* degrades phenol without the requirement of any additional nutrient supplements whereas, other microbes requires certain nutrient supplements *e.g.*, yeast extract and peptone even to degrade phenol at high concentration (> 500 mg/L). These nutrient supplements are supposed to be required to lessen the toxic effect of phenol on such microbial cell^{84,85}.

Certain ions and inorganic salts plays critical role in microbial growth. The presence of higher level of inorganic salts (sulfate ions and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) inhibits the phenol bio degradation^{82,86}. However phosphate ions promote the bio degradation of aromatic compounds as it is a major cellular component in the form of phospholipids, nucleic acids and ATP. It also helps to stabilize the pH of the medium by acting as buffering agent⁸⁷. Similarly Lakshmi *et al.*⁸⁸ documented that yeast extract is good source of nitrogen which is required for different cellular components synthesis.

Agitation speed is another important factor that contributes to aerobic bio-degradation of phenolic compounds⁸⁹. Agitation speed up to 150 rpm increases the microbial growth and degradation rate possibly due to the enhanced oxygen mass transfer⁹⁰.

***Candida tropicalis*: Aerobic biodegradation and detoxification of waste water phenolics:** Phenols are organic micro pollutant with toxic properties; can inhibit anaerobic biodegradation⁹³. It may be due to having a toxic effect on micro biota. *C. tropicalis* (mutant) has ability to degrade phenol (aerobically) up to 2500 ppm⁶⁹. Phenolics quantity in municipal waste waters is low as compared to industrial waste waters⁹². However municipal solid wastes are polluting soil with phenol. At high concentrations phenol causes inhibition of microbial activity and thus stops activity of biodegradation⁹³. Overall inhibition or incomplete degradation of phenols causes serious effects on soil biota, human health and environment⁹¹.

C. tropicalis can remove 62-65.6 % phenolic compounds from waste waters by aerobic biodegradation while anaerobic biodegradation can do only 51 %^{16,94}. Anaerobic biodegradation has an advantage of producing methane gas, can further be used as energy sources. If these (aerobic and anaerobic) applied together for phenol removal, can fully degrade phenol from waste waters. Martinez-Garcia *et al.*¹⁶ used *C. tropicalis* for

aerobic treatment of olive mill waste water (as *C. tropicalis* do not degrade phenol by anaerobic) together with anaerobic co-digestion. By using this method, phenol can be degraded completely. Moreover addition of organic source (*i.e.*, pig slurry *etc.*) with *C. tropicalis* can also reduce organic compounds in water.

Recently biological sequencing batch reactors (SBR) and membrane biological reactors (MBR) have been practiced efficiently. However MBR is emerging technology; using activated sludge bio-reactor and microfiltration membrane with varying phenol removal rates of 8-16 mg/L in expected 500 days^{92,95}. By using the same technology acclimatizes with *C. tropicalis* can degrade phenol 50,000 mg/L per day⁹⁶.

Techniques enhancing potential of *C. tropicalis* in biodegradation: Scientists worked on improving the efficiency of micro-organisms by using modern technology. The over view of these technologies is given in Fig. 5. Chang *et al.*⁶⁸ worked on *C. tropicalis* M4 and irradiate this strain mutated with UV light and N-methyl-N'-nitro-N-nitrosoguanidine (NTG). The studies revealed that mutant strain have higher growth and phenol degradation rate than wild strain, also the phenol hydroxylase activity was found to enhance about 1.9 and 2.2 folds higher.

Fig. 5. Ways to enhance *Candida tropicalis* efficiency to degrade phenolic compounds

Low-power laser irradiation: Low-power laser irradiation is an emerging technology for the biological strains mutation. Jiang *et al.*⁹⁷ used this technology and studied the effect of low-power laser on *C. tropicalis* and prepared a strain CTM 2. Enzyme assay of strain CTM 2 showed that CTM 2 has an increased activity of phenol hydroxylase from 46.49-62.96 U g⁻¹ cells. When the amino acid sequence of enzyme was compared both in wild *C. tropicalis* and mutant strain CTM 2 changes was found in four amino acids (172D3A, 233L3F, 268N3F and 296V3D) which might be the reason for the increased phenol hydroxylase activity. These technologies bring change in the enzymes promoters and even a change in amino acid sequence itself and intensify the activity of phenol hydroxylase enzyme.

Immobilization technique: Several attempts have been made to degrade phenol by free *C. tropicalis* cells for last two decades. It has been demonstrated already that higher concentration of phenols disrupts microbial cells and eventually inhibits the metabolism so immobilization technology for phenol degradation has gained much attention. The immobilization technique has many advantages over free cells as the gel provides protection to microbial cells from temperature, pH and toxic effect of phenols^{98,99} so facilitates the continuous degradation of phenols.

In comparison with free cell, an immobilized cell culture provides better conditions for biodegradation of phenol. The metabolites secreted by *C. tropicalis* are entrapped by the carrier material used for the immobilization so they are available to cells for longer time making the process of phenol degradation better and more efficient. Immobilization of *C. tropicalis* brings physiological changes such as enhanced enzyme activity and reduces specific cell growth and cell yield⁷².

Another advantage of immobilization technique is that microbial cells can be reused and separated from the reaction mixture. Various types of solid matrices used for immobilization of *C. tropicalis* include polyacrylamide (PAA) gel beads⁷², alginate gels and porous glass^{72,100,101}. The studies made by Chen *et al.*⁷² using PAA gel showed that *C. tropicalis* degrades phenol concentration of 400 and 500 ppm in 5 and 10 days, respectively. So the removal efficiency of immobilized *C. tropicalis* is over 95 % as compared to free cells. Chung *et al.*¹¹ found that free *C. tropicalis* degrades 600 ppm of phenol whereas; the degradation level reaches to 1000 ppm for immobilized cells.

Co-metabolism: As mentioned already that phenolic compounds are toxic and cause uncoupling of microbial cells. The severity of toxicity varies with type of micro-organism and phenolic compound¹⁰¹. There are many reports which describe the co-metabolic removal of aromatic compounds¹⁰². In such system mixture of organic substrate are present in which one substrate acts as (primary substrate) food for micro-organisms and other organic compound (co-substrate) is only partially metabolized in the presence of primary substrate¹⁰³. The process of metabolism of aromatic compounds becomes inefficient and slow as these compounds are recalcitrant and inhibits cell growth but provision of another energy source improves their breakdown. *C. tropicalis* W1 is important as it not only degrades phenol but also 4-chlorophenol (4CP) if present as contaminant in the same medium. Presence of phenol induces the degradation of 4-chlorophenol which might be due to induction of enzymes in *C. tropicalis* W1 which degrades 4-chlorophenol along with phenol¹²⁶.

Mix microbial culture technique: Most of the studies deal with removal of phenolic compounds by using pure culture¹⁰⁴ while mix culture approach has been studied less for chlorophenol^{105,106}. Despite using single microbial culture for phenol bio-degradation several scientists used mixed culture approach. Alcocer *et al.*¹⁹ investigated the degradation of mono-, di- and tri-chlorophenols using mixed culture of *Burkholderia spp.*, *Microbacterium phyllosphaerae* and *C. tropicalis*. It was observed that pure or mixed strains were

impotent to metabolize chlorophenols unless phenol is supplied as primary substrate. The microbial consortium co-metabolically removes contaminants from 95-99.8 %.

Use of surfactants: Chemical compounds that reduce the surface tension between liquids are called as surfactants. They act as wetting agents, detergents, emulsifiers and dispersants. Some micro-organisms (bacteria, yeast, fungi) have ability to produce surfactants which are termed as bio-surfactants or biological surfactants. Bio surfactants are produced either as part of their extracellular material or a part of cell membrane¹⁰⁷. Bio-surfactant is of increasing interest in bio degradation of organic compounds due to their specificity¹⁰⁸. Phenol is hydrophobic in nature so chemical surfactants have been used by several researchers to remove phenol from industrial wastewater^{109,110}. Phenol adsorbed with surfactants vesicles core reducing the amount of free phenol from wastewater¹¹¹. In most studies, bio-surfactant is monorhamnolipid which aids the biodegradation of phenol by *C. tropicalis*. It improves the removal efficiency of phenol from 86-99 % in 30 h. Tween 80 a chemical surfactant also enhances the degradation rate of phenol by improving the cell growth and reducing the toxicity of phenol. Besides the use of surfactants *C. tropicalis* CE017 also produces bio-surfactants which ease the process of phenol metabolism⁴⁴. This bio surfactant contains carbohydrates which help in the emulsification of kerosene-in-water mixture¹¹². Hence, exogenous application of surfactants can increase the efficiency of *C. tropicalis* promoting the process of phenol degradation.

Neujahr *et al.*¹¹³ reported that the key enzyme phenol hydroxylase which promotes the degradation of phenol in *C. tropicalis* works efficiently in the presence of NADPH. Later on this finding was investigated by Komarkova *et al.*⁶⁷ that phenol hydroxylase activity is high till the cell uses its reserve nutrients but as soon as the level of these reserve nutrients drops, the production of NADPH also becomes limited which eventually decreases the phenol hydroxylase activity. This happens because in starvation condition *Candida* cells produce extracellular metabolites which inhibit the respiration activity of cell and finally limit phenol hydroxylase activity. So the inhibition of respiration can be avoided by removing extracellular metabolites from the growing medium.

Number of *Candida* strains have been identified till now which efficiently remove phenol from industrial wastewater

(Table-3). Among these strains some are more efficient phenol degrader in less time duration. The strains follow the same pathway for phenol degradation as mentioned above. Although these strains belong to *C. tropicalis* but their degradation rate varies greatly from each other. The difference in their degradation rate can be accounted to their different nutritional and environmental requirements *i.e.*, carbon and nitrogen source, inorganic salts, temperature and pH. This parameter affects the phenol degrading ability of these strains.

Conclusion

Phenol became a major environmental pollutant which is difficult to eradicate. Many physical and chemical methods have been employed but biological method is found to be most promising method for degradation of phenol and phenolic compounds. Microbes can degrade phenols in aerobic and anaerobic conditions and *C. tropicalis* has been demonstrated for effective (aerobic) degradation of phenols. Enzymes such as mono-oxygenase, cytosolic NADPH-dependent phenol hydroxylase and peroxidases facilitate the conversion of phenol to less toxic compounds through various steps. Besides the presence of enzymes *C. tropicalis* has ability to withstand high temperature and pH changes without affecting the degradation process. Hence, it is concluded that *C. tropicalis* has rich enzyme system for the degradation of phenolics so can be employed in bioremediation technologies.

For continuous and efficient biodegradation process optimization of external factors is necessary. Temperature is most influencing factor among all the other external factors. Temperature tolerating ability of *C. tropicalis* strain can be utilized on large scale biodegradation of phenols where temperature changes are abrupt. It can work on broad pH range with less nutrients requirement. *C. tropicalis* can be used for treatment of industrial wastes by enhancing the natural capabilities through process like immobilization, pure and mixed cultures of *C. tropicalis* with other micro-organisms, co-metabolism and laser used technology. Sequencing batch reactors (SBR) and membrane biological reactors (MBR) are considered as most efficient bioreactors for phenol removal. *C. tropicalis* can be seen as attractive alternative for degradation of phenol from waste water. Since less work is done on the *C. tropicalis* with respect to its biodegrading ability so it can provide a new field for researchers to explore the genetic

TABLE-3
Candida tropicalis STRAINS IN PHENOL DEGRADATION

<i>Candida tropicalis</i> strains	Place of Isolation	Phenol conc. (ppm)	Time (h)	Phenol degradation rate (%)	Comments	Ref.
<i>C. tropicalis</i> CE017	Oil-refinery wastewater	500	24	>65	This strain produces bio-surfactant naturally which enhances its phenol degrading ability.	44
<i>C. tropicalis</i> JH8	Activated sludge	1800	62	>99	Strain requires greater time period to degrade phenol from wastewater.	114
<i>C. tropicalis</i> Z-04	Aerobic granules	2000	48	99.10	Time required to degrade phenol is greater than <i>C. tropicalis</i> NCIM 3556	115
<i>C. tropicalis</i> W1	Sludge	900	30	>66	The strain has ability to degrade 4-CP in the presence of phenol by co-metabolism.	26
<i>C. tropicalis</i> NCIM 3556	Industrial wastes	2000	16	99.87	The pre-grown cells have ability to degrade phenol about 95% due to their ability to overcome phenol toxicity.	73
<i>C. tropicalis</i> CTM2	Acclimated activated sludge	2600	70.5	99	Strain mutant by He-Ne laser irradiation and possessed strong capacity to degrade phenol.	97

diversity of the micro-organism including genes involved in biodegradation process which can be manipulated in the development of transgenic plants having biodegrading ability for phenolics.

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