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Evaluation of Aspergillus fumigatus for degradation of Resorcinol.

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Abstract

Resorcinol, a common phenolic compound is associated with many industries like, dye, resins and rubber industries. It has often been linked to serious water pollution, on its discharge into industrial effluents generated. Reports suggest that exposure to resorcinol may lead to health hazards and thus, ways to remove resorcinol from industrial effluents is important. The primary objective of the given investigation was to evaluate Aspergillus fumigatus NTCC1222 for degradation of resorcinol under liquid state fermentation. The best degradation was observed on 8th day of incubation at pH 8 and an incubation temperature of 37oC with 2 g/L concentration of resorcinol as carbon source. The current study provides a preliminary background to further evaluate and characterize Aspergillus fumigatus NTCC1222 for its ability to possibly mitigate resorcinol pollution. This would add to the search for environment compatible solution to removal of related to phenol derivative pollution.

Keywords: Resorcinol; Biodegradation; Aspergillus fumigatus; Environment pollution

1. Introduction

In today's world as we make efforts to find sustainable ways to clean up contaminated environments, interest in the microbial biodegradation of pollutants has intensified manifolds. Biotransformation and bioremediation endeavor to harness the astonishing, natural ability of microbial xenobiotic metabolism to transform, degrade, or accumulate a huge range of compounds including aromatic compounds like resorcinol [1].

Resorcinol (C6H4(OH)2 is a dihydroxy benzene, with the IUPAC name, Benzene 1,3- diol. It is naturally found in Argon oil as main phenols. Resorcinol is a phenol derivative in which a hydrogen atom is substituted by a hydroxyl group in the meta position to the OH. The molecular weight of resorcinol is 110.1g/mol. The appearance of resorcinol is white solid having the melting point 110oC and boiling point of 277oC [2].

Various resorcinol compounds are naturally produced in plants as secondary plant products [3]. Resorcinol is used primarily in the production of special adhesives and/ or improvers of tires and wood products, because of its resistance to high temperature and durability under mechanical stress and then is introduced into the environment through refineries, pulp mills, wood preservation plants and various chemical industries, as well as their wastewaters [4].
Despite the fact that phenolic compounds are present in most soils and sediments, only a few aerobic resorcinol degrading microorganisms have been isolated and characterized [5]. The evaluation of filamentous fungal cultures in degradation of phenolic derivatives like, resorcinol, is further limited [6, 7, 8]. In the current study, we attempt evaluating Aspergillus fumigatus NTCC1222, a known valuable source of amylases [9, 10, 11, 12, 13, 14, 15] with promising applications in conventional chemical industries [16], for their possible use in mitigating resorcinol pollution in industrial waste waters.

2. Materials and Methods

All the chemicals and reagents were of analytical grade and were procured from Himedia Pvt. Ltd, India and Lobachemie Pvt Ltd, India.

2.1 Microorganism

Aspergillus fumigatus NTCC1222 was maintained on potato dextrose agar (PDA) medium under refrigerated (4^{o}C) conditions. For long time preservation the fungal culture was suspended in (15%v/v) glycerol and stored at -20^{o}C [13].

2.2 Effect of culture conditions on resorcinol degradation by Aspergillus fumigatus NTCC 1222

2.0 g/L of resorcinol was added as the sole carbon source to the basal fermentation medium which contained sodium nitrate (2.0 g/L), dibasic potassium phosphate (1.0 g/L), potassium chloride (0.5g/L), magnesium sulfate heptahydrate (0.5g/L), ferrous sulfate heptahydrate (0.01g/L) [17]. The initial (before autoclaving, with no pH control after autoclaving) pH of the medium was adjusted to 6.0. The prepared medium was inoculated with 2 discs (each of 5 mm diameter). The inoculated flasks were kept at 28±2^{o}C in a shaking incubator, maintained at 150 rpm. The flasks were harvested every 24 hours and the fermented medium was evaluated for biomass content and resorcinol degradation each time. For biomass determination, the content of each flask was filtered through whatmann filter 1. The retentate (fungal mycelium) was then used for biomass determination. The filtrate was used to determine catechol 1,2-dioxygenase activity and resorcinol degradation [1, 13, 18, 19, 20, 21] using standard procedures.
The rate of resorcinol was calculated by the given formula:-
% Resorcinol= M*D/W
M= resorcinol (grams) in 100ml solution.
D= dilution factor
W= sample weight (grams)

Similarly, the influence of cultural conditions fermentation medium pH, temperature and incubation temperature on resorcinol breakdown and biomass content was also determined by varying the resorcinol concentration (1 to 2.5 gm/L), temperature (27-47^{o}C), and pH (5.0 to 8.5).

2.4 Statistical analysis

The experiments were carried out in triplicates. The biodegradation and microbial growth (biomass) were reported as an average of the values. The enzyme activity was reported as mean '±' standard deviation of the values.

3 Results and Discussion

3.1 Effect of culture conditions on resorcinol degradation by *Aspergillus fumigatus* NTCC 1222

The test fungus *Aspergillus fumigatus* NTCC 1222 was inoculated in the fermentation medium containing the resorcinol as sole carbon source. The biodegradation activity was analyzed every 24 hrs for upto 12 days (Table 1). The biodegradation was found to be the highest (23.4%) on 8th day of incubation, which was also the day of maximum biomass accumulation indicating resorcinol uptake by the test fungus for growth and energy. At the same time, though the enzyme activity increased with incubation time, the highest enzyme activity was reported on 7th day of incubation. As resorcinol degradation is reported to be catabolized via multiple metabolic pathways, different enzymes may be involved in the breakdown of the given substrate [3, 22, 23]. As we tested just catechol 1,2 dioxygenase for supplementing resorcinol (phenol derivative) uptake and breakdown, we did not proceed with detailed evaluation and characterization of phenol/phenol derivative degrading enzymes. The resorcinol degradation coincided with biomass accumulation, as indicating by the same incubation time required for highest resorcinol degradation as well as biomass accumulation. Slight drop in enzyme activity on 8th day of incubation indicate possible involvement of other biodegradation enzymes in the degradation of resorcinol. Further decrease in biomass accumulation may be associated with the depletion of nutrients and accumulation of waste products [1].

Table 1: Effect of incubation period on resorcinol degradation

Day	Resorcinol Degradation (%)	Biomass determination rate (g/ml)	Enzyme Activity(U/ml)
1	5.7	0.2	14.87±0.5
2	6.4	0.5	58.48±1.0
3	6.8	0.8	72.95±1.1

4	8.0	1.1	89.24±0.9
5	10.5	1.4	105.82±0.9
6	16.2	1.7	130.24±0.6
7	18.6	1.9	131.44±0.7
8	23.4	2.1	124.81±1.1
9	22.3	2.0	119.38±1.9
10	22.0	1.8	105.82±0.6
11	20.0	1.7	94.06±0.2
12	19.7	1.7	89.84±1.0
Fermentation conditions: **Temperature: 28º C**; **pH**: **6**			

Resorcinol added at a concentration of 2 gm/L was found to support resorcinol degradation (18.8%) at 28ºC temperature and a slightly acidic pH (6.0) while the degradation reduced at a higher concentration (2.5 gm/L) by around 39% than the maximum. Five different incubation temperatures were used to see the effect of temperature on biodegradation ability of the test fungus on resorcinol. The flasks were kept incubated for optimized time at optimized pH using 2 gm/L resorcinol as the carbon source. The biomass accumulation as well as resorcinol degradation was both best (3.1 gm/mL and 19%, respectively) observed at 37ºC. At 27º C, the resorcinol degradation rate was around 12.6%., indicating a 7% increase in resorcinol degradation at an increase of 10ºC in incubation temperature. The highest (29.6 %) degradation of resorcinol was observed at mildly alkaline pH (8) when the test microbial strain was grown in the resorcinol-containing fermentation medium at variable pH under optimized incubation time and desired temperature with 2 gm/L resorcinol concentration. At pH 6, the degradation was the lowest. The pH 8 was the most favored with the resorcinol degradation being the highest (29.3 %) at the given pH. The growth was also the best at pH 8 (biomass content 2.5 g/L). Different microorganisms have been reported to show ability to use resorcinol and resorcylic acids [24] including, uptake of resorcinol as sole source of nitrogen and carbon [1] with *Pseudomonas putida* being one of those capable to use resorcinol as carbon source [18] even with orcinol as substrate indicating, existence of alternative pathway for resorcinol degradation [3].

Table 2: Effect of substrate concentration, incubation temperature and pH on resorcinol degradation

S.No.	Optimization parameter	Value range	Biodegradation, %	Biomass, g/L

1	**Initial resorcinol concentration, g/L**	**1.0**	9.2	0.2
		1.5	11.0	0.6
		2.0	18.8	1.4
		2.5	11.5	0.7
Incubation conditions: Incubation pH: 6.0 Incubation time, days: 8 Incubation temperature, ^{o}C: 28				
2.	**Incubation temperature, ^{o}C**	**27**	12.6	1.2
		32	15.2	2.0
		37	19.0	3.1
		42	11.9	1.7
		47	10.1	1.3
Incubation conditions: Incubation pH: 6.0 Incubation time, days: 8 Resorcinol concentration: 2 g/L				
3.	**Incubation pH**	**5.0**	11.6	0.2
		5.5	15.0	0.7
		6.0	17.6	1.1
		6.5	19.5	1.4
		7.0	21.2	1.9
		7.5	19.3	2.1

		8.0	29.6	2.5
		8.5	21.3	2.1
Incubation conditions: Incubation temperature, ^{o}C: 37 Incubation time, days: 8 Resorcinol concentration: 2 g/L				

Microbial degradation of a mix of different phenolics was evaluated and it was found that cross feeding led to poor phenol degradation in resorcinol acclimated Up-flow-Anaerobic Sludge Blanket (UASB) reactor while it was readily degraded in catechol acclimated reactor. When both catechol and resorcinol were fed to the resorcinol acclimated reactor, resorcinol degradation was inhibited by catechol [25]. Darley et al. [26] attempted identification of genes involved in anaerobic breakdown of resorcinol by *Azoarcus anaerobius.* The co-degradation of resorcinol and catechol in a catechol acclimated UASB has also been reported [27]. The efficiency of fungal cultures like, *Penicillium chrysogenum* [28, 29] in degradation of phenol/phenol derivatives have been seen. Philipp and Schink [30] observed anaerobic breakdown of resorcinol as the sole source of carbon and energy using denitrifying bacterium *Azoarcus anaerobius* LuFRes1. Schink et al. [31] reported resorcinol pathway to be one of the three involved in anaerobic degradation of mononuclear aromatic compounds. Groseclose et al. [23] reported resorcinol breakdown pathways in *Azotobacter vinelandii.* The degradation of resorcinol, catechol and hydroquinone under anaerobic conditions has been investigated by Latkar et al. [32]. [33] Lepik and Tenno [33] showed aerobic breakdown of resorcinol, phenol, and 5-methylresorcinol using activated sludge. [34] Ngugi et al., [34] studied resorcinol degrading bacteria associated with intestinal tract of *Macrotermes michaelseni*.

4. Conclusion

The test strain degraded resorcinol best in 8 days at temperature 37^{o} C and pH 8 at a resorcinol concentration of 2 g/L and can well be explored for minimizing waste water pollution owing to release of phenol derivatives like resorcinol, into the natural environment. Further enzyme profiling and characterization of the fungal strain for action against different phenol derivatives will help in evaluating the test strain to solve phenol/phenol derivative related pollution in contaminated waste waters.

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