Application of *Aspergillus awamori* Grown on Citrus Peel Supplemented Growth Medium for Cyanide Bioremediation

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Abstract: Solid waste and wastewater effluents emanating from many industries contain free cyanide. The environmental risk of this compound stems from its emission as component of the hydrocyanic gas, which is toxic. Cyanide and its complexes have been shown to have bioaccumulative properties which in turn result in ecological deterioration with both human and animal health implications. In this study, bioremediation of cyanide by *A. awamori*isolates was carried using Citrus peel (CP) supplemented growth medium, refined pectin (RP) and Czapek Yeast Agar (CYA) with water as control in shake flask cultures (180 rpm) at 30 °C for 48 h. *A. awamori*from the various media demonstrated cyanide bioremediation potential with the CP medium showing considerably higher cyanide reduction. The presence of quantifiable NH_3/NH^+ in the media solutions during the biodegradation was indicative of cyanide reduction. Use of CP adjunct-grown *A. awamori*to bioremediate cyanide contaminated wastewaters is an alternative environmentally friendly catalytic process for cleaning up the environment and improving water quality.

Key words: Cyanide, *Aspergillus awamori*, bioremediation, catalysis, Citrus peel.

Introduction

Solid waste and wastewater effluents emanating from many industries contain free cyanide. The environmental risk of this compound stems from its emission as component of the hydrocyanic gas, which is toxic. The gas is a product of a combination of free cyanide and cyanide complexes released into the environment by various industries mostly especially as wastewater (Patil and Paknikar, 2000). Cyanide is often found in organic, hydrocarbon chains or asinorganic, transition, alkali and alkali earth metalcomplexes. Many cyanide complexes are highly unstable, thus temperature, pH and light can degrade the components to form free cyanidewhich is the most toxic form of cyanide (Stamatialis*et al.,* 2008; Rao *et al.,* 2010).

Chemical methods employed to remediate cyanide contaminated wastewaters are not only economically hostile or ineffective for certain heavy metals, they also increase the chemical oxygen demand (COD) of the wastewater due to the excessive quantities of chlorine used in the treatment processes (Patil and Paknikar, 1999). This renders the water undesirable for reuse, toxic to aquatic life and may produce organic substances.

A number of microorganisms including *Nocardia*sp. and *Rhodococcus*sp. (bacteria), *Aspergillus* sp.,*Fusarium*sp. (fungi) *a*s well as *Arthrospira maxima* and *Scenedesmusobliquus*(algae) have been reported to possess enzymatic mechanisms able to bioremediate free cyanide and cyanide complexes (Akcil*et al.,* 2003; Rao *et al.,* 2010). However, despite the availability of organic waste and fungal strains, there are limited reports on their application in the bioremediation of cyanide.

Also, cyanide and its complexes have been shown to have bioaccumulative properties which in turn result in ecological deterioration with both human and animal health implications (Patil and Paknikar 2000). In view of the foregoing, there is need to investigate the potentials other fungi and some agricultural wastes in the bioremediation of cyanide. This study thus investigated the bioremediation of cyanide using *Aspergillus awamori*grown on citrus peel supplemented growth medium.

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Methodology

Pulverized *C. sinensis*peels (800 ml w/v) was hydrolyzed with 98% H_2SO_4 (5ml), sterilized (116°C, 13 min) then pH adjusted to 4.5 (1 M NaOH) and then filtered. After filtration, the filter cake was gently mixed with KOH (1:2 w/w) in 32 ml of distilled water with magnetic stirring and then heated to gentle boiling in a fume cupboard. Bromobenzene (12 ml) was then added to the boiling mixture, boiled for an additional 10 min, allowed to cool to 10 min then adjusted to pH 4.5 with 32 % HCl in an ice bath. The mixture was then filtered and the cake dried at ambient conditions for 24 h and used as substrate media.

Isolation and Harvesting of Fungal Spores

Swab samples of cyanide contaminated municipal wastewater discharge drain located in the Western Cape, South Africa collected at various points along the drain were inoculated onto 1 % (w/v) Citrus Pectin Agar (CPA) plates and incubated at 37 °C for 5 days. After incubation, the black mycelia of filamentous mould that grew were transferred onto Potato Dextrose Agar (PDA) plates with 0.2% (v/v) Penicillin-Streptomycin (PEN-STREP; (10000 units/L of Penicillin and 10 mg of Streptomycin/ml) anti-biotic solution and further incubated for 5 days at 37 ˚C. Isolates were sub-cultured onto Malt Extract Agar (MEA) and Czapek Yeast Agar (CYA) and further incubated at 26 °C for 7 days. After incubation, *A. Awamori*was identified based on morphological characteristics on MEA and CYA.

Preparation and Harvest of Fungal Biomass

Zero point one percent (0.1%) (w/v) Tween 80 solution was added to each culture plate and a spatula used to harvest the spores and mycelium to form a spore-mycelium suspension. The suspension (20 ml) was then filtered by entrapping the mycellium onto glass wool to obtain a spore suspension which was stored at 4 ˚C.

Cyanide Bioremediationn

To three different sets of the batch cultures each containing 1 ml of spore suspension (standard inoculum of $2x10^6$ spores/ml was prepared for standardization purposes only) in 250 ml flasks, 42.5 ml of 1% (w/v) refined citrus pectin, 1 % (w/v) powered orange peel, and Czapek yeast agar was added respectively followed by 7.5 ml of a 1 g CN-/L cyanide solution. The cultures were then incubated at 30˚C for 180 rpm.

Citrus Peel Media

After incubation, the batch culture samples were centrifuged at 13000 rpm for 5 min after which free cyanide and ammonia/ammonium (NH_3/NH_4^+) concentrations (ppm) in the solution was measured using Merck cyanide (CN-) (09701) and Merck ammonium (NH_4^+) (00683) test kits.
The experiments were run in duplicate in which sampling was every 48 h. Differences in free cyanide concentration among the cultures showed rate of bioremediatoion of the cyanide solution.

Results

The morphological characteristics of *A. awamori*on various media used for isolation are shown in Fig 1. In all the media the fungi exhibited a thick white wooly mycelium in the early stages of growth that develops black spores with further incubation. Results showed that while sporulation was more profuse on Malt extract agar (MEA) (Figs 1a and b), growth was moderate on Czapek yeast agar (CYA) (Figs 1 c and d) and fungal growth was faster with heavier mycelia and spores on the citrus pectin agar (CPA) (Fig 1e). Results of cyanide bioremediation among the various cultures showed that on the orange peel medium, there was higher cyanide reduction compared to the other nutrient media (Fig. 2). The concentration of cyanide in solution reduced from 150 ppm on Day 1 to 76 ppm on Day 6, followed by that of refined pectin from 150 on Day 1 to 130 on Day 6. On the other hand, for isolates grown on other media, concentration of the cyanide increased steadily from Day 1 to Day 6 with the Czapeck medium recording the lowest concentration from 150 to 187 compared to that of water (control) whose cyanide concentration increased steadily from 150 ppm on day 1 to 240 on Day 6.

Discussion

Though *A. awamori*can grow naturally on many substrates including orange peels, in this study, hydrolysis of the peels was carried out in order to improve the release of significant quantities of sugars such as glucose, fructose and sucrose, as well as uronic acids to stimulate the release of hydrolytic enzymes (Maharaja *et al.,* 2010; Mrudulaand Anitharaj2011; Umsza-Guez*et al.,* 2011) by the fungus thereby enhancing their rapid growth (Figs a-e) and possibly, complete breakdown of the peels.

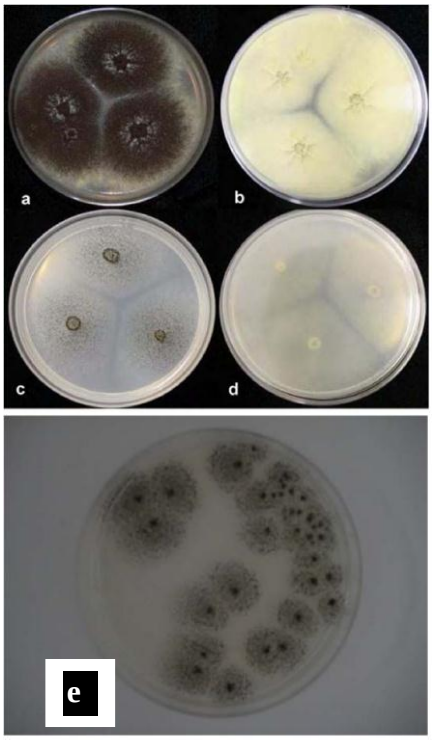

Fig. 1. Growth of *A. awamori*on MEA (a & b) , CYA (c & d) and CPA (e) after incubation at 37 ˚C for 5 days

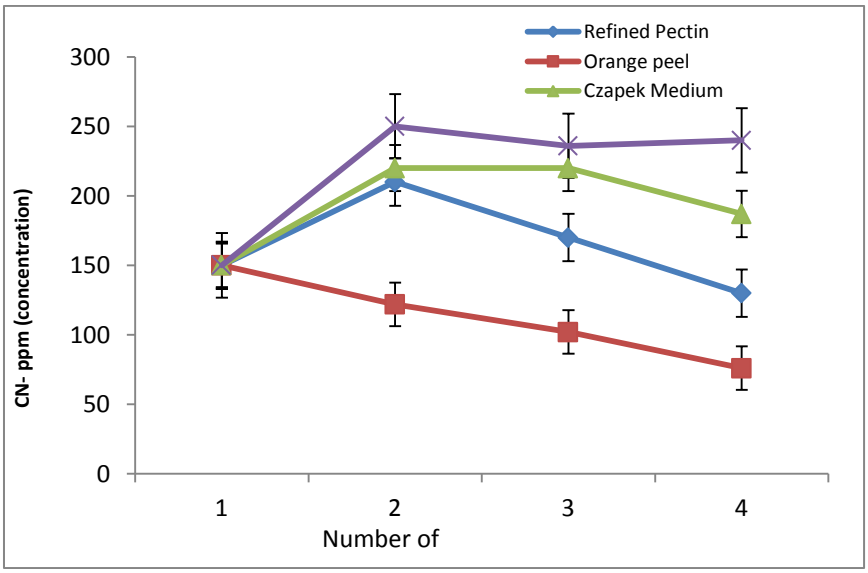

Fig. 2. Cyanide bioremediation by A. awamori grown on various media

Large quantities of organic wastes are generated every year. Many of the wastes, particularly from the agricultural sector, can be utilized as nutrient source for microbial systems. The use of orange peel to cultivate *A. awamori*does not only provide a cheap alternative source of mycological media but also provides a friendlier means of cleanup of the environment. Cyanide remediation by *A. awamori*strains is such that the ammonia produced from the cyanide hydrolysis was utilized as a nitrogen source by the fungus thereby eliminating any chances of further contaminating the environment with any harmful by product or any secondary metabolite.

Higher cyanide reduction is a function of high release and consequently, higher concentration of ammonia/ammonium in solution. The fact that *A. awamori*form orange peels exhibited the highest rate of cyanide remediation means that in the orange peel

medium has the highest concentration of the ammonia/ammonium in solution compared to the other media which is indicative of cyanide reduction(Kaplan*et al.,* 2006; Vejvoda*et al.,* 2010).The rich carbon sources present in the orange peel and the further supplementation by NH_3/NH_4^+, had shown to be an added advantage of using waste orange peel as a potential nutrient source.

The change in the cyanide concentration in the water medium (control) was due to volatilisation. Though NH_3/NH_4^+ was utilized by the growing fungus as source of Nitrogen, the presence of quantifiable NH_3/NH_4^+ in solution during biodegradation is indicative of cyanide reduction (Kaplan*et al.,* 2006; Vejvoda*et al.,* 2010).

Conclusion

The study showed that the use of Citrus peel-adjunct-grown *A. awamori*to bioremediate cyanide contaminated wastewaters is an alternative environmentally friendly catalytic process for cleaning up the environment and improving water quality. However ffurther investigation of other agrowastes and means to enhance the complete hydrolysis of their sugars with a view to completely degrading them thereby enhancing fungal growth should be carried out. The ability of *A. awamori*as well as other fungi to bioremediate other chemical wastes including heavy metals should also be investigated.

References

Akcil, A., Karahan, A.G., Ciftci H. and Sagdic, O. (2003). Biological Treatment of cyanide by natural isolated bacteria (*Pseudomonas* sp.). *Mineral Engineering* 16(7): 643-649.

Kaplan, O., Vejvoda, V., Plíhal, O., Pompach, P., Kavan, D., Bojarová, P., Bezouška, K., Macková, M., Cantarella, M., Jirků, V., Křen, V. and Martínková, L.(2006). Purification and characterization of a nitriles' from *Aspergillus niger*K10. *BMC Biotechnology* 11(2):1-15.

Mrudula, S.andAnitharaj, R. (2011). Pectinase production in solid state fermentation by *Aspergillus niger* using orange peel. *Global Journal of Biotechnology and Biochemistry*6(2): 64-71.

Maharaja S., Cretan, DemandKrishnappa, M. (2010). Effect of temperature on cellulose enzyme activity in crude extracts isolated from solid wastes microbes. *International Journal of Microbiology Research* 2(2): 44-47.

Patil, Y.B. and Paknikar, K.M. (1999). Removal and Recovery of Metal Cyanides using a Combination of Biosorption and Biodegradation Processes. *Biotechnology Letters* 21(10): 913-919.

Patil, Y. B., and Paknikar, K. M. (2000). "Development of a process biodetoxification of metal
cyanides from wastewaters," *Process Biochemistry* 35(10): 1139-1151.

Rao, M.A., Scelza, R., Scotti, R. and Gianfreda, L. (2010). Role of enzymes in the remediation of polluted environments. *Journal of Soil Science and Plant Nutrition* 10(3): 333-353.

Stamatialis, D.F., Papenburg, B.J., Girones, M., Saiful, S., Bettahalli, S.N.M., Schmitmeier, S. and Wessling, M (2008). Medical applications of membranes: drug delivery, artificialorgans and tissue engineering. *Journal of Membrane Science* 308(1-2) 1-34.

Umsza-Guez, M.A., Díaz, A.B., de Ory, I., Blandino, A., Gomes, E. and Caro, I. (2011). Xylanase production by *Aspergillus awamori* under solid state fermentation conditions on tomato pomace. *Brazilian Journal of Microbiology* 42(4) 1585 - 1597.

Vejvoda, V., Kubáč, D., Davidová, A., Kaplan, O., Šulc, M., Šveda, O. R., Chaloupková, R.andMartínková, L. (2010). Purification and characterization of nitrilase from *Fusariumsolani*IMI196840. *Process Biochemistry* 45(7) 1115-1120.