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# Fungal Diversity from Solid and Liquid Waste of Paper Industry and their Cyanide Degrading Activity

**M. Kannahi* and R. Arulmozhi**

Department of Microbiology and A Division of Biotechnology, STET Women's College, Sundarakkottai, Mannargudi – 614 001, India.

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**Microbial diversity constitutes the most extraordinary reservoir of life in the biosphere that we have only just begun to explore and understand. A preliminary investigation on the microbial diversity of paper mill effluents organic wastes from the landfill site of Solamkampatti Paper mill Thanjore Dt., Tamil Nadu. The isolation and characterization of native microbes on different media may generate information on the nature, characteristics, and degrading efficiency of various hazardous wastes by the micro-organisms. The qualitative analysis of species in microbial community was undertaken. The study revealed that fungal isolate mostly belong to *Aspergillus* and *Penicillium spp. Aspergillus niger,Trichoderma harzianum* and *Fusarium oxysporum* isolated from the paper industry liquid and solid waste were examined for cyanide degradation. *A niger* showed 100% cyanide degradation of 100 ppm cyanide (KCN) concentration within 25 h where as *Trichoderma harzianum* showed 100% degradation in 35 h and *Fusarium oxysporum* showed 100% degradation in 45 h of incubation by forming ammonia and $CO_2$ through the formation of formamide. A five times increase was observed in the biomass of *A. niger* and four times increase in that of *Trichoderma* and two times increase *Fusarium oxysporum* in cyanide containing minimal medium. The study has suggested that fungi in the wastes thus reduces cyanide toxicity.**

**Key words**: Paper industry, Waste, Cyanide, Fungal sps.

Diversity has been estimated that our planet is about 4.6 billion years old. Fossilized remains of prokaryotic cells around 3.5 to 3.8 billion years old have been discovered in stromatolites and sedimentary rocks[1]. Microbial diversity increased greatly as oxygen became more plentiful[2]. There are many thousands of species are all unique, each species are beautiful in its own way. It is usual for a particular fungus to produce a visible fruiting body only under a precise combination of conditions, including geographic locations, elevation, temperature, humidity, light level and surrounding flora.

Liquid waste is the waterborne human, domestic and farm wastes. It may include industrial effluent, subsoil or surface waters. There are two basic sources of solid wastes: non municipal and municipal. Non municipal solid waste is the discarded solid material from industry, agriculture, mining oil some solid wastes are unsafe to the health and well – beings of humans. Generally, the most common waste product is paper (about 40% of the total)[3].

The Solakampatti paper mill is the only major industry in Tamil Nadu. The paper production process produced large amount of effluents, both liquid wastes generally discharged into water bodies and solid wastes such as waste water treatment sludge, unused bamboo chips, lime sludge and coal ash generally disposed as land fills. Microorganisms in the site, use the waste constituents as nutrients, thus detoxifying the materials and their digestive processes breakdown complex organic molecules into simpler less toxic molecules. Different types of fungus present in liquid and solid waste disposal of paper industry.

* To whom all correspondence should be addressed.
E-mail: kannahisri79@gmail.com

The fungi include *Aspergillus niger, Aspergillus flavus, Aspergillus oryzae, Trichoderma viride, T. harzianum, T. reesii, Fusarium oxysporum, Fusarium lateritium, Penicillium chrysogenum, Geotrichium spp, Gliocladium* spp, *Rhizopus, Alternaria, Mucor, Stemphylium loti, Fusarium solani, Cryptococcus humicolous* etc., [4].

Biodegradation is defined as stimulation of microorganisms to degrade rapidly hazardous organic contaminants to environmentally safe levels in soils, waters, sludge and residues. The use of micro organisms is one such technology to degrade environmental contaminants[3]. Cyanide, a toxic substance is produced as a by product in many industries such as gold mining, metal – plating, steel – tempering, electroplating and other mining industries. Cyanide containing effluent from above these factories polluted in soil, water and threatens the environment and quality of life in rural areas [5].

## MATERIALS AND METHODS

**Description of the site**

The liquid and solid wastes dumping sites of Solakampatti paper mill, located in Thanjore district in Tamil Nadu was selected for study purpose. The climate of the area is tropical, warm and humid. The minimum and maximum annual temperature varies from 28ºC to 40ºC. The relative humidity varies from 43 to 87 percentage.

**Sample Collection**

Two different (liquid and solid waste) types of sample collected randomly from different locations of paper mill wastes disposal dumping sites at monthly (Nov-Feb) interval.

Solid waste sample collected from the two different places[6].

(i) Surface solid waste sample above ( 0 c m)
(ii) Solid waste sample from 10 cm depth.

Liquid waste sample collected from paper industry at two different places.

(i) Waste bin
(ii) Sewage sample

Above these two samples were analyzed for the various physico-chemical parameters such as the pH, temperature, dissolved oxygen,$CO_2$ ,phosphate, nitrate, ammonia, magnesium, BOD, chloride and calcium were determined.

**Isolation of fungi** [7]

**From solid waste**

About one gram of soil sample was serially dilluted up to $10^{-1}$ to $10^{-7}$ dillution.

**From liquid waste**

About 1 ml of liquid sample was serially diluted up to $10^{-1}$ to $10^{-7}$ dilution. Appropriately diluted samples were plated initially on potato dextrose agar (PDA) plates. The plates were incubated at 37°C for 3 days. After incubation, colonies were isolated and identified by microscopic observation.

**Fungal inoculum preparation**[8]

Mycelia of *Aspergillus niger, Trichoderma harzianum* and *Fusarium oxysporum* were suspended in 10 ml potato dextrose broth (pH 7.5) taken in 250 ml cotton – plugged flasks. The three cultures were incubated at 0°C on a rotary shaker (1200 rpm) for 3 days. To induce cyanide degrading enzyme activity, 50 ppm filter sterilized KCN solution was added to the medium handled homogenizer.

12 hours before harvesting. Mycelia were harvested by centrifugation (12,000 rpm for 30 min at 4°C), washed twice in physiological water and transferred in manually

**Cyanide degradation assay** [8]

Three sets of 250 ml Erlenmeyer flasks containing 50 ml minimal medium (pH 7.5) with 0.35% Na2 $HPO_4$, 0.15 $KH_2PO_4$, 0.001 % $MgSO_4$ $7H_2O$ and $FeCl_3$ $6H_2O$, 0.01 % yeast extract, 0.1% glucose and 100 % yeast extract, 0.1% glucose and 100 ppm filter sterilized KCN solution were added as carbon source and nitrogen sources respectively. Mycelia of *Aspergillus niger, Trichoderma harzianum* and *Fusarium* o*xysporum* were inoculated the medium and incubated at 30°C on a rotary shaker (2500 rpm). Control flasks with uninoculated medium were also maintained. During the degradation process samples were removed with a syringe at 5 h intervals and quickly centrifuged for 3 min at 12,000 rpm in a micro centrifuge. The supernatant were analyzed for cyanide reduction and for the presence of corresponding transformation products. Analytical[9] and dry cell[10] weight also determined.

## RESULTS AND DISCUSSION

The more recalcitrant organic, substrates, e.g. cellulose and lignin, both competitive and

mutualistic fungi to evolved[11]. The isolated fungal species were identified as *Mucor, Rhizopus, Aspergillus, Penicillium, Fusarium, Gliocladium, Alternaria, Geotrichium spp, etc*[12]. (Gilman, 1957) *Aspergillus* (36 3%) *Penicillium* (22.7%) are probably due to their diverse and extensive enzyme system that protect them from other soil organisms[12]. Of the 22 fungal species isolated, 13 (59.09%) fungal isolates grows on the carbony methyl cellulose – Rose Bengal Agar (CMC – RBA) Based on the screening results on CMC are plates, these isolates with higher zone of activity were selected for further morphological, biochemical and physiological identification and charactization studies (Table 1).

The physico-chemical characteristics of waste soil, liquid and their fungal diversity are presented. The present study revealed that moisture content, soil pH and liquid pH played significant role on the fungal population of the waste dumping site. The Fungal community and their activity in the liquid and solid waste increased with decreased in pH and increased in moisture content[13]. Fungal population compete for somple plant derived substrates and might have developed antagonistic relationship (Table 2).

*A. niger, Trichoderma harzianum* and *Fusarium oxysporum* isolated from the paper industry. Among these three strains, *Aspergills niger* had more cyanide degrading potency than *Trichoderma harzianum* and *Fusarium oxysporum*. *A niger* was able to degrade KCN (100 ppm) completely with in 25 hours of incubation where as *Trichoderma harzianum* needed 35 hours and *Fusarium oxysporum* needed 30 hours . The reduction and disappearance of cyanide in the medium is associated with the cyanide degrading enzyme activity of the fungi. Ammonia and $CO_2$ were then and products of degradation.

During cyanide degradation *Aspergillus niger* showed sudden increase in the biomass after 10 of incubation while *Trichoderma harzianum* and *Fusarium oxysporum* showed constant increase throught out the incubation period due to cyanide toxicity. The biomass of *A. niger* increased from 0.24g ; -1 to 1.15 gl -1 biomass of *Trichoderma harzianum* increased from 0.24g l- 1 to 0.98 gl -1 and that of *Fusarium oxysporum* increased from 0.24 gl – 1 to 0.88 gl-1 with in 30 hours of incubation. The present study highlights

**Table 1.** Physico-Chemical Characteristics of Liquid and Solid waste disposal of paper industry

| Types of Wastes | | Nitrate | Phosphate | Ammonia | Calcium | Magnesium | Chloride | Alkalinity | $CO_2$ | $O_2$ | BOD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Liquid waste mg/l | A | 16.3±0.2 | 19.5±1.0 | 20.8±0.3 | 13.0±0.6 | 15.5±0.0 | 12.6±1.5 | 33.4±0.4 | 16.6±0.0 | 41.7±6.8 | 41.7±6.8 |
| | B | 16.1±0.1 | 19.9±0.0 | 23.2±0.2 | 13.5±1.0 | 15.6±0.0 | 12.5±0.0 | 33.5±0.5 | 16.1±0.1 | 57.5±0.1 | 57.5±0.1 |
| Solid Waste kg/ha | A | 17.6±1.1 | 19.2±1.0 | 25.0±0.8 | 13.9±0.9 | 15.5±0.3 | 13.0±1.5 | 31.6±0.0 | 17.6±1.1 | 48.4±1.5 | 48.4±1.5 |
| | B | 17.5±0.1 | 21.5±1.0 | 24.8±1.9 | 13.0±0.0 | 15.6±0.6 | 13.2±0.0 | 33.5±2.7 | 17.2±0.2 | 44.4±3.1 | 44.4±3.1 |

* A – Waste bin, B – Sewage sample, C – Surface solid waste (0cm) D – 10cm depth solid waste
Values are expressed as Mean ± SD

**Table 2.** Characterization of isolated cyanide degrading fungi

| Fungal isolates | Identification | Physiological Parameters | | | | | | | | | | Time (hours) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | pH | | | | | Temperature (ºC) | | | | | | | |
| | | 5.0 | 6.0 | 7.0 | 8.0 | 9.0 | 20 | 30 | 40 | 50 | 60 | 10 | 20 | 30 |
| $F_1$ | *A.niger* | - | 2+ | 3+ | 3+ | - | - | 3+ | 3+ | 2+ | - | 60% | 80% | 100% |
| $F_6$ | *T.harzianum* | - | 2+ | 3+ | 3+ | - | - | 3+ | 3+ | 2+ | - | 50% | 70% | 90% |
| $F_9$ | *F.oxysporum* | - | 2+ | 3+ | 3+ | - | - | 3+ | 3+ | 2+ | - | 30% | 50% | 70% |

* - Means no growth, 2+ means of moderate growth, 3+ means of abundant growth.

the merits of the fungi, *Aspergillus niger, Trichoderma harzianum* and *Fusarium oxysporum* for their utilization in cyanide degradation.

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