Detoxification of Cyanide Wastewater by *Phanerochaete Chrysosporium* and *Trametes Versicolor*

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Abstract

One of the harmful leaching reagents typically found in metallurgical waste is cyanide (CN^-). The damaging effects of cyanide, as well as its potential environmental consequences, make it a major environmental problem, necessitating the management of its toxicity prior to release into the environment. To avoid cyanide poisoning deaths, which occurs when cyanide attaches to critical iron-containing enzymes and prevents them from providing oxygen-rich blood to the tissues, industries that release cyanide-laden effluents are required to keep concentrations below 0.2 ppm. Chemical, physical, and biological approaches are utilised to reduce the amount of cyanide in wastewater. Biotechnological methods that use cyanotrophic bacteria to clean up cyanide-contaminated surroundings have received a lot of attention recently. The aim of this research is to see whether *Phanerochaete chrysosporium* and *Trametes Vesicolor* could decompose cyanide under various conditions, such as cyanide concentration, culture mass and time. A control experiment with a 100 ppm cyanide solution and no fungal interaction demonstrated that the cyanide in solution did not degrade naturally or self-degrade. When the effect of biomass (0.2 g, 0.4 g, and 0.6 g) on cyanide degradation was evaluated, the 0.6 g culture mass of *P. Chrysosporium* and *T. Vesicolor* resulted in the best myco-detoxification of 90 % and 85 %, respectively, after 48 hours.

Keywords: Cyanide wastewater, Cyanotrophic Organism, Detoxification, *P. chrysosporium*, *T. Vesicolor*

1 Introduction

There are several poisonous compounds that cause environmental issues around the world, and cyanide is one of them. If handled appropriately and wisely, cyanide has a wonderful history of respectful and constructive use by mankind (Ackil, 2003; Potivichayanon and Kitleartpornpairoat, 2010). The cyanide ion CN^-, the most fundamental cyanide species, is a highly adaptable and strong metal binder in aqueous solution, a feature that has been utilised in inventive ways for commercial activities that have benefited humanity. It is, nevertheless, an extremely deadly substance that, if inhaled, swallowed, or absorbed through the skin, can result in death (Young and Jordan, 1995; Dash *et al.,* 2006).

Cyanide comes in a variety of chemical forms, including solid, gaseous, and aqueous species, as well as inorganic and organic species. Weak-Acid

Dissociable (WAD), Strong-Acid Dissociable (SAD), and free cyanide (cyanide anion and hydrogen cyanide), which is the most poisonous form of cyanide, are the three types of cyanide (Young and Jordan, 1995; Luque-Almagro *et al.*, 2005; 2016; Kumar *et al.,* 2017). SAD cyanide is cyanide complexed with metals like gold, silver, iron, and cobalt, whereas WAD cyanide is cyanide complexed with metals like nickel, copper, cadmium, and zinc. Even though it is often placed in its own category, thiocyanate (SCN^-) is a WAD. Cassava starch production, electroplating, steel tempering, nuclear processing, photography, pharmaceuticals, pesticides, fumigants, petrochemicals, resins production, refining, coal and metal mining, processing and finishing industries are just a few of the industries that use cyanide (Akcil, 2003; Potivichayanon and Kitleartpornpairoat, 2010; Luque-Almagro *et al.*, 2016; Kumar *et al.,* 2017; Ofori-Sarpong *et al*., 2020). Though cyanide has use in a variety of industries, it is most commonly used in the extraction of gold and silver from enormous quantities of low-grade ores (Huiatt, 1984; Akcil, 2003; Bushey *et al.,* 2006; Kuyucak and Akcil, 2013).

Some of the lixiviants used in the mining and metallurgical industries to extract gold and silver from their ores include cyanide, halides, thiosulphate, thiourea, and thiocyanate (Coppock, 2009; Asamoah *et al.,* 2014). Because it is less expensive than the lixiviants listed above, cyanide remains the universal ligand for gold extraction (Asamoah *et al.,* 2014). There is also an ease of adsorption of the mineral of interest onto activated carbon after leaching it with cyanide (Ofori-Sarpong and Osseo-Asare, 2013). Cyanide has been the preferred reagent for decades due to the reasons stated above.

The effluent is a major source of harmful contaminants in the environment, and cyanide is a poisonous reagent (Dash *et al.,* 2006). Because of its toxicity and the potential for severe environmental repercussions, it must be detoxified before being discharged into the environment.

Chemical, physical, and biological treatments are the most common ways to degrade cyanide. Strong oxidants including ferrous sulphate, hydrogen peroxide, alkaline chlorination, sulphur dioxide-air oxidation, potassium hypochlorite, and electrolytic oxidation are all used in chemical processes (Akcil, 2003; Kuyucak and Akcil, 2013; Kitis *et al.,* 2005; Dwivedi *et al.,* 2011). Physical procedures for cyanide degradation include dilution, natural attenuation, activated carbon adsorption, reverse osmosis, electrowining, and hydrolysis/distillation (Dwivedi *et al.,* 2011). Aside from failing to produce results that meet effluent quality standards, chemical methods typically have a high operating cost (Ebbs, 2004; Kumar *et al.,* 2017; Cabello *et al.,* 2018). As a result, using microorganisms to destroy cyanide in tailings solutions and other mining-related effluent is a proven alternative to the current chemical and physical processes (Raybuck 1992; Akcil, 2003; Dwivedi Naveen *et al.,* 2011; Kumar *et al.,* 2017).

Several investigations have shown that various fungi, such as *Phanerochaete chrysosporium*, *Trametes versicolor*, *Rhizopus oryzae*, *Pleurotus sajpr-caju*, *Trichoderma koninjii*, *Gliocladium virens*, *Trichoderma spp.*, and *Fusarium spp*., have the ability to degrade cyanide (Padmaja and Balagopal, 1985; Ezzi and Lynch, 2005; Cabuk *et al.,* 2006; Ofori-Sarpong *et al*., 2020). For a variety of practical reasons, the white-rot fungus, *Phanerochaete chrysosporium*, and *Trametes versicolor* are popular among these fungal strains. Their capacity to break down lignin and a number

of complicated compounds have been reported (Bumpus et al, 1985; Cameron *et al.,* 2000; Li *et al.,* 2011; Xu *et al.,* 2015). These qualities have drawn attention to them in areas of environmental concern, such as pulp bioleaching and bioremediation.

The focus of this paper was thus to access the ability of *Phanerochaete chrysosporium* and *Trametes versicolor* to degrade cyanide using millet as the growth media. Although some works have been done using these fungi to degrade cyanide but other growth media such as glucose and yeast extract were used (Cabuk *et al*., 2006). Millet is simple to use and requires no expertise for its preparation.

2 Materials and Methods Used

2.1 Materials Used

The fungal spores of *Phanerochaete chrysosporium, Trametes versicolor* and millet were obtained from Prof. Grace Ofori-Sarpong of the Minerals Engineering Department, University of Mines and Technology, UMaT, Tarkwa. Cyanide solutions of different concentrations, rhodamine and silver nitrate solutions were available at the Minerals Laboratory, University of Mines and Technology UMaT, Tarkwa.

2.2 Method Used

The experimental work was carried out in the Minerals laboratory, University of Mines and Technology, Ghana. The work was divided into stages, which included medium preparation and fungus culturing, cyanide solution preparation, and contact with the fungal culture. pH, residence time, cyanide concentration, and dosage of fungal biomass were all utilised in the detoxification process.

2.3 Preparation of cultures

Two 250 mL Erlenmeyer flasks were rinsed with water and allowed to dry to eliminate any physically attached substance. The washed flasks were then filled with 20 g millet as the culture media, followed by 20 ml water in a 1:1 ratio. To prevent unwanted material from entering the flasks, the contents were thoroughly mixed and covered with aluminum foils. The flasks containing the combination were then autoclaved for 30 minutes at 121°c to destroy any remaining microorganisms and soften the media for the fungi to feed on. To accommodate the fungal spores, the samples were allowed to cool to about 40°c

P. chrysosporium spores were combined with distilled water and used to inoculate the millet mixture after cooling. To provide the fungi enough air, holes were punched in the aluminum foil. After that, the cultures were incubated in an MRC orbital shaker for one week at 37°C to achieve mature fungus species. The same procedure was repeated for the culturing of *T. versicolor*.

At the end of the incubation period, the fungal biomass was separated from the extract by sieving through quadruple-layered cheesecloth.

2.4 Preparation of Cyanide Solution and Contacting with Fungal Culture

Sodium hydroxide was used to adjust the pH of distilled water to 11.5 before mixing it with various amounts of solid sodium cyanide to achieve solution concentrations of 10, 20, 50, 100, and 200 ppm of cyanide. The prepared cyanide solution was contained in a number of 250 ml Elenmeyer flasks. 100 ml cyanide solution was poured into each of the 250 ml Elenmeyer flasks; 3 cultures of 0.2 g, 0.4 g, and 0.6 g were added and capped with aluminum foil for each concentration of 10 ppm, 20

ppm, 50 ppm, 100 ppm, and 200 ppm. Control tests were also set up under the same conditions as the above, with the exception that no fungus was added. The experiment lasted 48 hours. To prevent cyanide volatilisation, the pH of the cyanide solutions was measured and kept at 11.5 in a basic medium.

2.5 Determination of pH and Cyanide Strength

The pH of the residual solutions was measured with a Metrohm 913 pH meter at the end of the residence time, and any drops were recorded. To determine the concentration of free cyanide in the residual solution samples, a silver nitrate-cyanide titration was used. The silver nitrate solution was made by dissolving 1.733 g of silver nitrate in one liter of deionized water, while the rhodamine solution was made by dissolving 0.1 g of rhodamine in 20 ml of ethanol and topping up to the 1-liter mark. Before titrating each cyanide sample with the silver-nitrate solution, two drops of rhodamine indicator were added and shaken well.

2.6 Analysis of Data

Titration with silver nitrate was used to measure the extent to which cyanide was reduced. Equation 1 was used to calculate the residual cyanide strength, where [CN^-] represents the concentration of residual cyanide solution after the degradation experiment and [AgNO3] represents the concentration of silver nitrate solution used.

$$[CN^-] = \frac{\text{Volume of } AgNO_3}{\text{Volume of } CN^-} \times [AgNO_3] \quad (1)$$

$$Q = \frac{(C_i - C_f)V}{M} \times 100 \quad (2)$$

The data collected was evaluated. Equation 2 was used to calculate the degradation capacity;

The amount of sodium cyanide biodegraded by a given fungal biomass is represented by Q. The initial and final cyanide solution concentrations in ppm are Ci and Cf; M represents the fungal biomass in grams, and V is the experimental volume of cyanide solution in L. (Patil and Paknikar, 1999; Sethuramasamy *et al.*, 2016; Ofori-Sarpong *et al.*, 2020)

3 Results and Discussions

In order to control the toxicity of cyanurated wastewater, this paper evaluated the ability of *P. chrysosporium* and *T. versicolor* to metabolise cyanide at basic pH. Various concentrations of cyanide solutions (10, 20, 50, 100, and 200 ppm) at pH 11.5 were interacted for up to 48 hours with 0, 0.2, 0.4, and 0.6 g of fungal biomass. Natural cyanide attenuation, cyanotrophic ability of *P. chrysosporium* and *T. versicolor*, and effect of cyanide concentration and biomass on cyanide degradation are the four main sections in which the findings and discussions are given.

3.1 Effect of Natural attenuation

Natural degradation, also known as attenuation, refers to any process that reduces cyanide levels in a waste without requiring human involvement. Natural degradation of cyanide solutions in shallow ponds occurs as a result of increased interaction with ambient carbon dioxide, which lowers the pH and speeds up the conversion to HCN and so volatilization (Adams, 1992; Oudjehani *et al.*, 2002).

To test the effect of natural attenuation and to serve as a control, cyanide concentrations of 10 ppm, 20

ppm, 50 ppm, 100 ppm and 200 ppm were set up and covered to limit exposure to the atmosphere, as well as without fungal biomass. At 2 hours, 4 hours, 8 hours, 12 hours, 24 hours and 48 hours, samples were obtained and the free cyanide content was determined. Fig. 1 depicts the outcome.

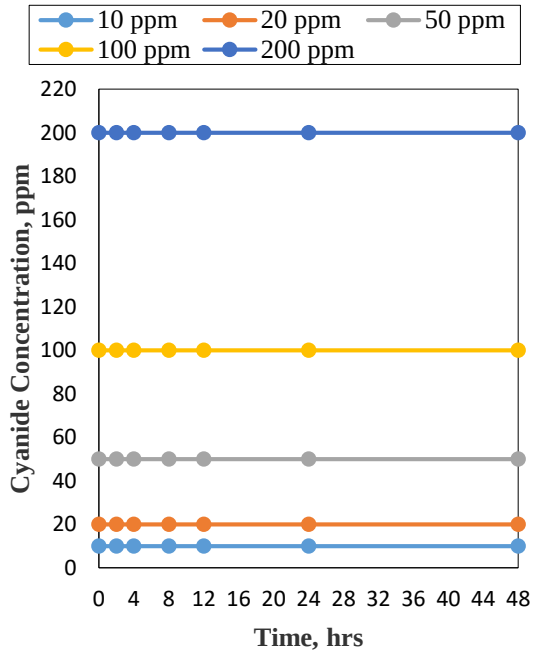

Fig. 1 Effect of Natural Attenuation on Cyanide Degradation

The concentrations of 10 ppm, 20 ppm, 50 ppm, 100 ppm and 200 ppm were stable over the whole 48-hour period, as shown in Fig. 1. This means that in the closed Elenmeyer flasks used for the experiment, there was no cyanide oxidation or hydrogen cyanide volatilization. This could be because there was no reaction of cyanide with ambient carbon to lower the pH, resulting in hydrogen cyanide volatilisation or cyanide oxidation. This was done to see if the fungal biomass has the ability to break down cyanide.

3.2 Effect of Concentration on Cyanide Degradation

Because the extent of cyanide elimination is controlled by its initial concentrations loading for the hymenonycete *P. chrysosporium* and *T. versicolor*, different quantities of cyanide are known to degrade at different rates (Hossain *et al.*, 2005). The effect of cyanide concentration on degradation by *P. chrysosporium* and *T. versicolor* were determined in this research over a 48-hour contact duration, and the results are presented in Fig. 2a and 2b.

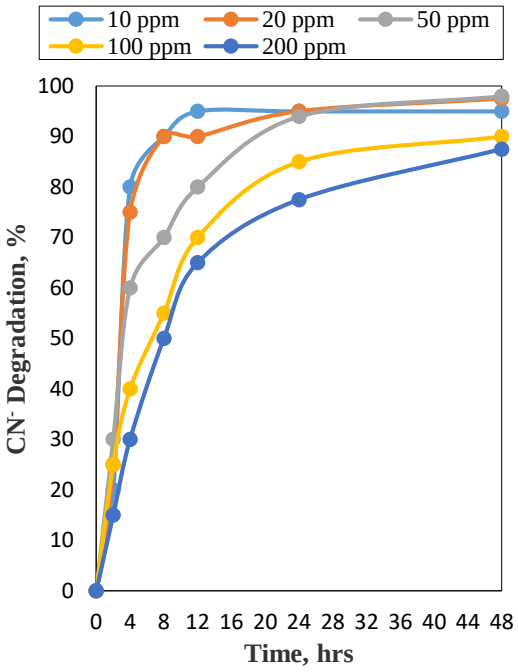

Fig. 2a. The Effect of Cyanide Concentration on Degradation by *P. Chrysosporium*

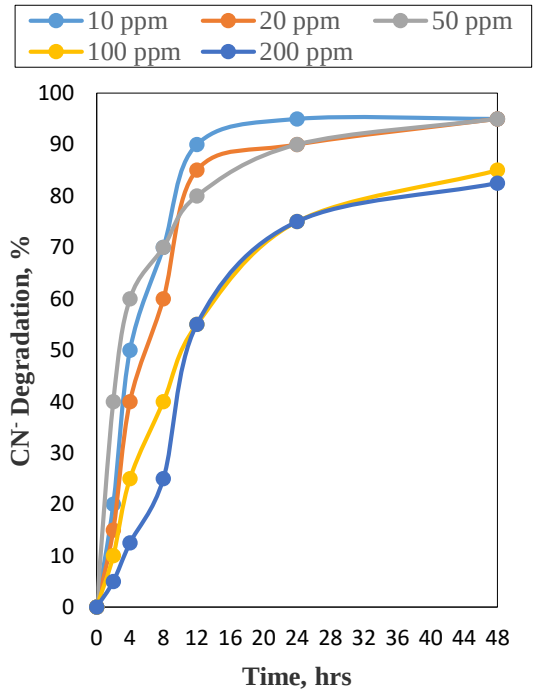

Fig. 2b. The Effect of Cyanide Concentration on Degradation by *T. versicolor*

Cyanide concentrations of 10 ppm, 20 ppm, 50 ppm, 100 ppm, and 200 ppm were prepared and contacted with a dry mass of 0.6 g culture, which was covered to prevent exposure to the atmosphere. The amount of cyanide degraded increased with contact time in all situations, as shown in the graphs above. The high number of active sites for adsorption results in higher removal at the beginning. Other researches have confirmed these findings (Shah and Aust, 1993; Singh *et al.*, 2017). In Fig. 2a, the efficiency of cyanide degradation was 20%, 25%, 30%, 35 %, and 15% for 10 ppm, 20 ppm, 50 ppm, 100 ppm, and 200 ppm, respectively, after 2 hours. In the next two hours, the reverse occurred, with 10 ppm recording the maximum degradation of 80 % and 200 ppm recording the lowest degradation of 30 %. The optimal concentration for the experiment was 50 ppm, which degraded at 98% after 48 hours, followed by 20 ppm, which degraded at 97.5%. On the basis of this tendency, it may be stated that cyanide was destroyed faster at lower concentrations than at a greater concentration of 200 pm with *P. chrysosporium*. According to Hossain *et al.* (2005), studies on cyanide biodegradation utilising *Phanerochaete chrysosporium* found that the fungus could tolerate up to 500 ppm initial silver cyanide concentration and a maximum of roughly 94% degradation was reached after 96 hours of treatment. The results in Fig. 2b reveal that at lower cyanide concentrations of 10 ppm, 20 ppm, and 50 ppm, greater percentages of cyanide were destroyed. Contact with 100 ppm and 200 ppm, which are the highest in concentration among the solutions, resulted in smaller percentages of decomposed cyanide. This could indicate that the fungus was more effective at lower concentrations than at higher ones.

3.3 Free Cyanide Degrading Ability of *P. chrysosporium* and *T. Versicolor*

The decrease in cyanide concentration was achieved by preparing two 100 ppm cyanide solutions, one of which was contacted with a dry mass of 0.6 g *P. chrysosporium* of the culture mass and the other was kept as a control by denying it of a culture mass and both were covered with aluminum foil. The same set up was repeated for *T. Versicolor*

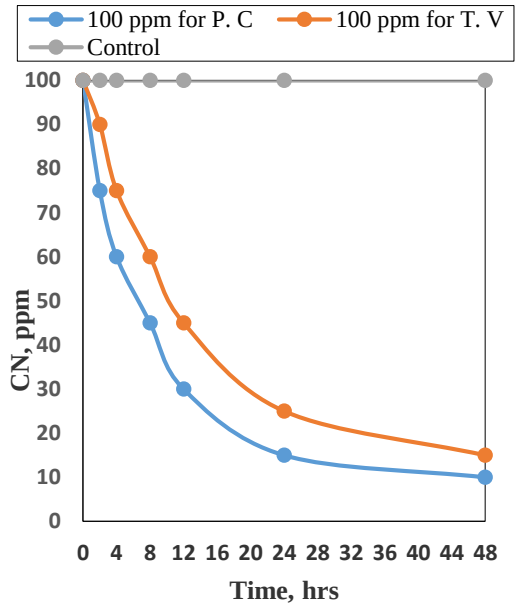

Fig. 3 Degradation of Free Cyanide Ability of *P. Chrysosporium and T. Versicolor*

After a 48-hour encounter, the concentration of the 100 ppm cyanide solution with no fungal contact remained the same throughout the period. This means that there was no natural/self-degradation of the solution. However, the cyanide concentration in *P. chrysosporium* and *T. versicolor* dropped from 100 ppm to 10 ppm and 15 ppm, respectively, indicating that the fungi degraded 90% and 85% of the free cyanide. By secreting lignin peroxidase, manganese peroxidase, and hydrogen peroxide, the fungus was able to break down the free cyanide (Tien and Kirk, 1983; Hossain *et al.*, 2005; Cabuk *et al.*, 2006). The free cyanide concentrations were 10 and 15 ppm, which surpassed the EPA and WHO's allowed cyanide discharge limit of 0.2 ppm. However, this does not establish the fungus' inefficiency, as the trends in Fig. 3 suggest that increasing culture mass and contact duration to around 96 hours will allow for additional degradation. As a result, once these measures are modified, the maximum allowable concentration may be achieved.

3.4 Effect of Culture mass on Cyanide Degradation

The effect of fungal biomass on cyanide breakdown as a function of contact time is shown in Fig. 4a and 4b. At a pH of 11.5, 100 mL of 100 ppm CN was contacted for up to 48 hours with different fungal biomass (0.2, 0.4, and 0.6 g). With increasing doses of fungal biomass and contact time, the capacity to degrade cyanide increased. According to Fig. 4a, 0.6 g of fungal biomass eliminated 90% within 48 hours of contact, while 0.2 g of biomass removed 80% and 85% within 24 and 48 hours, respectively. In 24 and 48 hours, the 0.2 g biomass eliminated 50% and 60% of the cyanide.

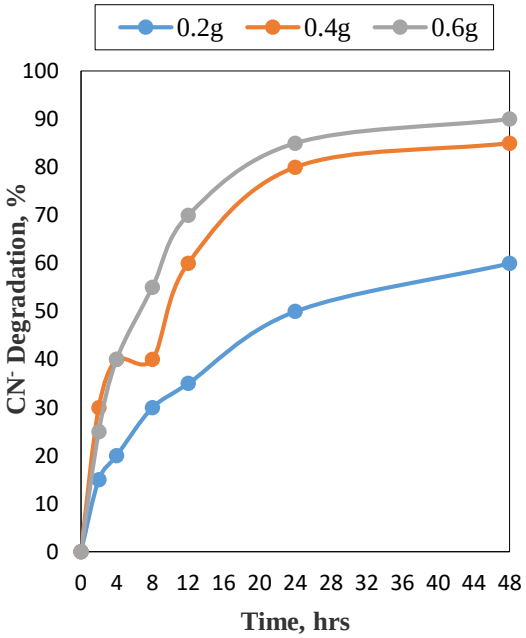

Fig 4a. Effect of Culture Mass (*P. Chrysosporium)* on Cyanide Degradation

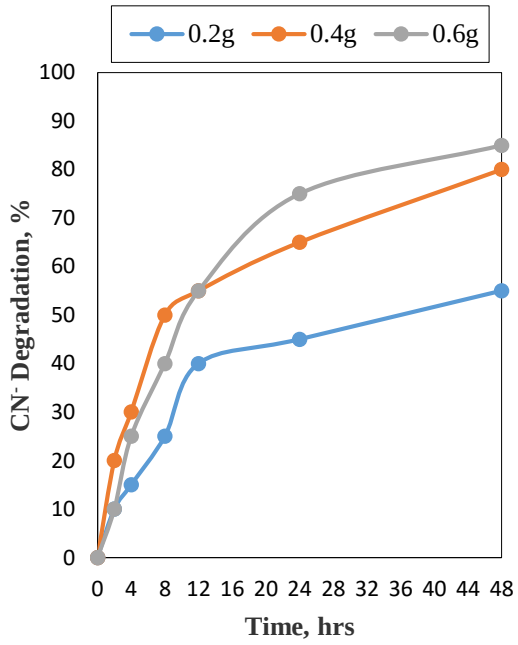

Fig 4b. Effect of Culture mass (*T. Versicolor)* on Cyanide Degradation

Fig. 4b also proves that the culture mass has an impact on the rate of degradation. The 0.6 g, which is the most amount of culture among them, had the highest percentage of cyanide destroyed during the contacting times (85%). The 0.2 g, on the other hand, degraded the least amount of cyanide (55%) at the end of the contacting durations. The cyanide destroyed by the 0.4 g is somewhere between the 0.2 g and the 0.6 g, which is 80%.

According to Ofori-Sarpong *et al.* (2020), even when cyanide in the solution has been nearly depleted, increasing amounts of biomasses are not filled to capacity during biomass degradation. Although not all of the cyanide could be removed as the amount of biomass decrease, the little agitation might expose a larger percentage of the surface to cyanide interaction. As a result, treating the solution with smaller batches of biomass is more effective and efficient. The two alternatives illustrate that if smaller amounts of biomass are dosed periodically into the cyanide solution instead of large amounts at once, the percentage removal may improve.

3.5 Effect of Time

The effect of time on cyanide degradation by *P. chrysosporium* and *T. Versicolor* were investigated, and the findings have been presented above. In this study, the maximum contact time was 48 hours. Degradation efficiency rose over time, as shown in the graphs. This could be because the fungi had more time to secrete more lignin peroxidase, manganese peroxidase, and hydrogen to breakdown the cyanide as contact time increased.

Although, with time, the efficiency of all degrading processes improved. However, in comparison to 100 ppm and 200 ppm, degradations were more effective at 10 ppm, 20 ppm, and 50 ppm.

4 Conclusions

The ability of *P. chrysosporium* and *T. vesicolor* to metabolise cyanide at basic pH was investigated in order to reduce the toxicity of cyanurated wastewater to a level that is environmentally acceptable for disposal. When the cyanide solutions were left for 48 hours in the control experiment, the results revealed that there was no spontaneous attenuation (self-degradation). Concentrations of 200 ppm, 100 ppm, 50 ppm, 20 ppm, and 10 ppm were reduced to 25 ppm, 10 ppm, 1 ppm, 0.5 ppm, and 0.5 ppm, respectively with *P.chrysosporium*, and to 35 ppm, 15 ppm, 2.5 ppm, 1 ppm, and 0.5 ppm with *T. versicolor*. After degradation, the cyanide concentration achieved exceeded the EPA and WHO's authorised cyanide discharge limit of 0.2 ppm. This, however, does not confirm the fungus' inefficiency, as the graphs show that increasing culture mass and contact time allows for more deterioration. As a result, once these

parameters are changed, the maximum permissible concentration may be reached.

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