PERFORMANCE OF AEROBIC BIOREACTORS DURING TREATMENT OF TANNING INDUSTRY WASTEWATER

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ABSTRACT

The performance of aerobic bioreactors loaded with novel biomass support (the KMT® support particles) during the treatment of tanning industry wastewater is presented in this paper. The wastewater samples were collected from TANWAT Company in Njombe, which extracts tannins from barks of the wattle tree. The wastewater generated from TANWAT is very acidic with high loading of organic compounds, which are very complex and difficult to biodegrade using conventional methods. The paper introduces a new technology for treatment of heavily loaded industrial wastewater, like tannins, that is the Three-Phase Fluidized Bed Bioreactor (TPFBB) which utilizes the KMT® support particles. Bench scale bioreactors (BSBs) were used to establish the suitable inoculum for use in the TPFBB. To establish the effect of the KMT® support particles, the BSBs were operated with and without the biomass support particles (BSPs). The BSBs were also inoculated with different types of inoculum (waste stabilization ponds (WSP) and rumen fluid (RF) inoculums) to establish the suitable source of microorganisms. Control samples were also implemented accordingly. The results show that the WSP was a suitable inoculum for which high chemical oxygen demand (COD) removal efficiency and tannins concentration reduction efficiency (TCRE) were observed. Tests conducted in the TPFBB with initial COD of about 13,200 ppm shows that the reactor is capable of biodegrading the tannins (at 85% efficiency) after aeration times of up to 7 days in recirculation mode. The final COD values of 1,000 ppm were reached in most cases. For final discharge, however, such a final COD necessitates the use of sub-surface flow constructed wetland (SSFCW) coupled to the TPFBB for final smoothing of the effluent. The TPFBB is recommended for treatment of tannin wastewater to minimize chemical pollution in the receiving water bodies.

Key words: Tannins; biomass support particles; wastewater treatment; aerobic bioreactors; aeration.

INTRODUCTION

The final disposal of wastewater generated by industrial processes (such as the milk industry, slaughter houses, tanneries, breweries, food processing industries, etc.) and that from domestic sewage is a serious problem. In this study, tannins wastewater treatment in biological reactors was investigated using the TPFBB and BSBs loaded with the KMT® support particles and inoculated with different sources of microorganisms. The main objectives of the study were: characterizing tannins wastewater in terms of chemical and physical properties (like pH, COD, TDS, tannins concentration), and evaluating the performance of the bench scale bioreactors (BSB) and the TPFBB in treating tannins wastewater in terms of the effluent quality parameters.

The wide variety and large quantity of chemical compounds found in the industrial wastewaters are responsible for most of the environmental pollution when discharged untreated into rivers open ponds, lakes and oceans (Manyele, 1996). Similar to other industrial wastewaters, tannins extraction wastewater must be treated before discharging to the environment, since tannins are serious oxygen scavengers, with high pollution capacity.

Industrial wastewater discharges threaten human health and the environment because such activities have compelled the use of even more toxic and harmful substances. Treatment of industrial and domestic wastewater as means of reducing environmental pollution has gained importance as the only means of solving the above-mentioned problems.

Biological treatment methods, using either aerobic (Cunha-Santiro *et al.*, 2002; Frigon *et al.*, 2003; Cooper, 1985; Chatib *et al.*, 1981;) or anaerobic microorganisms (Cunha-Santiro *et al.*, 2002; Field, 1989), have been used to degrade the large organic loads carried by the industrial wastewater. During aerobic processes, cell respiration is the main mechanism involved in the biodegradation carried out by microorganisms. It transforms complex molecules into simpler and more stable molecules, liberating the necessary energy for maintenance and growth of the cells. While total oxidation of organic compounds like poly-phenols can be achieved in the aerobic process, oxidation in the anaerobic process is only partial as it occurs in the absence of oxygen.

The TPFBB is a new alternative for biological treatment of industrial wastewaters. In this type of reactor a high concentration of biomass is maintained because microorganisms are attached to the support particles (called biomass). Polysaccharides represent around 65%, while proteins represent 10 to 15% of the extracellular products within the biomass. Dense particles were traditionally used in fluidized bed biological reactors as support for the biofilm. Entrainment of support particles from the reactor was a problem that appeared as the biofilm grew which decreases the particle density. In order to achieve homogeneous fluidization and effective aerobic degradation in this reactor, support particles are fluidized by the wastewater flowing upwards (which must be strongly oxygenated) or flowing co-currently with air.

In the TPFBB, lower density support particles are used fluidized only in the presence of air. Several authors suggested use of polymeric material particles, which allows prior treatment to improve biofilm adhesion and growth on particle surfaces. In order to degrade higher concentrations of COD, it is necessary to have enough viable biomass available for degradation to occur within the reactor. The TPFBB is more efficient due to its ability to provide large specific surface area of biofilm, high biomass concentration, high resistance to shock loads and higher volumetric degradation capacity (Manyele *et al.*, 1998). Its use in industrial wastewater treatment has proved fruitful results (Manyele *et al.*, 2007; 2004). The main advantages of the TPFBB are the lower hydraulic retention time (HRT) compared to other types of biological reactors and the small size of equipment required. In this study, the performance of the TPFBB and BSBs on tannins wastewater biodegradation is studies under prolonged aeration time (Manyele *et al.*, 2007; 2004).

The TPFBB is a single-stage column with a gas distributor. The gas (air) is distributed at the reactor bottom by means of a perforated plate distributor, and rises upwards in form of bubbles concurrently with the liquid. Enough oxygen is added and dissolved by aeration to maintain adequate dissolved oxygen in the reactor (Manyele *et al.*, 2007; 2004). The aeration process homogenizes the phases and supplies the required oxygen (Manyele *et al.*, 2007; 2004; Manyele *et al.*, 1997) for microbial growth. With a proper distributor design (Manyele *et al.*, 2004; Manyele, 1998), the gas-liquid flow is equally distributed across the reactor, resulting in uniform fluidization and bed expansion (Manyele *et al.*, 2004). With large size particles like the KMT® support, large bubbles are broken into smaller size and into large numbers of fine bubbles, enhancing mass transfer rate (Manyele, 1998).

The role of the biomass support particles (BSP) in the bioreactor is to increase the rate of mass transfer by offering large surface area for mass transfer. Another important role of the BSP is to support microorganisms, which adhere to the surface of the former as biomass. The BSPs play another role of increasing the rate of oxygen mass transfer and utilization by breaking large air bubbles to smaller size (Manyele, 1998; Manyele *et al.*, 1998; Jolly-Voillemin *et al.*, 1996; Atkinson, *et al.*, 1981). Therefore, the bioreactor is operated at higher gas velocity to enhance the availability of oxygen for microbial activities (Manyele *et al.*, 1997). In this study, polyethylene BSPs with a density of 950 kg/m^3 was used (Sukanya *and Rima,* 2001; Manyele, 1998; Manyele *et al.*, 1998).

Tannins are defined as compounds present in water-soluble plant extracts capable of converting (tanning) animal hide to leather. They fall into large groups of complex polyphenolic compounds with molecular weight ranging between 500 and 20,000 (Paul *et* *al.*, 2002). When they exist in plants, tannins are widely distributed in its parts, often in the bark of roots and stem or outer layer of roots. There are two main groups of tannins regardless their sources. Catechols (or condensed tannins) are more stringent and tan more quickly than pyrogallols (or hydrolysable tannins). They make leathers pink, red or dark brown hues that are more solid. Mimosa, birch, hemlock, quebrache, alder and fir barks contain catechol while oak barks contain both types. When deposited on the leather, pyrogallols improve its solidarity, wearing properties and resistance to water. Hence, they are favored for sole leather, bookbinding, upholster, etc. Uses of tannins products include: manufacture of inks; in medical settings as astringent and for treatment of burns; in animal skins to make leather and it transforms certain proteins of animal tissue into compounds that resist decomposition.

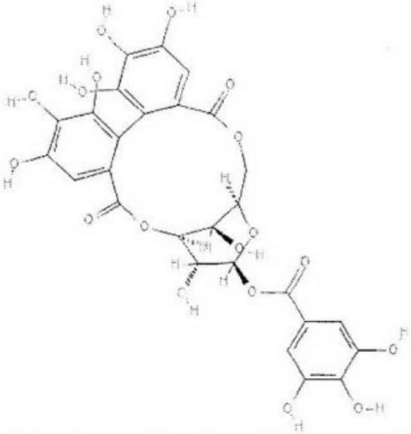
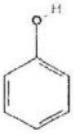
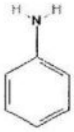
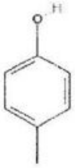
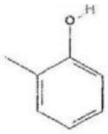
Tannins wastewater is produced as the effluent when materials containing tannins are used. Some of the wastewater from agro industrial processes includes: vegetable tanneries effluent, olive oil mill effluent, wine vinasse, coffee pulp water, debarking wastewater, pulp and paper wastewater and fiberboard processing wastewater (Field, 1989). Similarly, tannins wastewater is obtained in tannery industries during the tanning process. Depending on the process, the wastewater can comprise of different organic compounds as shown in Table 1. Meanwhile, Table 2 shows the molecular formula and structures of important compounds in tanning industry wastewater treatment.

Table 1: Organic compounds frequently encountered in the treatment of tannins related industrial wastewater (Frigon *et al.*, 2003; Wokoli *et al.*, 1999)

Source of waste	**Organic compound**
Debarking wastewater	Phenols, Oil, Fat, Phosphate, Nitrates
Bark leachate wastewater	Phenols, p-cresol, o-cresol, acetate, propionate, butyrate, phosphates, nitrates
Vinasse wastewater	Phenols, m-cresol, o-cresol, Catechol, aniline, phosphates, Nitrates
Wood wastewater	Phenols, phosphates, nitrates, aniline, acetate, o-cresol, propionate

The ability of tannins to form complex with protein and amino acids leads to the inhibition of organic matter biodegradation. At right concentration, tannins are toxic to the environment (Bone, 1998). At 15 ppm tannins have been known to cause fish kills. Concentration ranging from 325 to 3000 ppm has been reported to be inhibitory to methanogenic bacteria. Concentration of 1 – 2% tannins have been shown to reduce the overall decomposition of organic materials applied to soil. However, biodegradation is confirmed as an important mechanism of organic chemical removal in natural systems. The fate of chemicals and their partitioning over environmental systems should include complex evaluation of microbial ability to degrade tannins (Cunha-Santino *et al.*, 2002).

Table 2: Molecular formulae/structures of important compounds associated with tannin industry wastewater treatment

Compound	Molecular structure
Tannin: $C_{27}H_{22}O_{18}$	
Phenol: C_6H_6O and Aniline: C_6H_7N	 
p- and o-cresols: C_7H_8O	 

The direct aerobic degradation of polyphenolic compounds into carbon dioxide and water is not possible. However the degradation can be achieved by transformation into other organic compound. Aerobic degradation of organic pollutant in the receiving water bodies requires high levels of dissolved oxygen concentration (DOC), lack of which is also a measure of pollution level (Manyele, 1996). Among the factors that influence the dissolved oxygen concentration in wastewater treatment plants are mechanism and the rate of aeration, cell concentration, temperature and content of impurities (Manyele, 1998). The effect of oxygen concentration on its up take rates by the microorganisms has been shown to increase linearly with the dissolved oxygen concentration up to critical value, beyond which oxygen uptake rate remains constant (Cunha-Santino *et al.*, 2002).

MATERIALS AND METHODS

In this study, two main performance tests were conducted, that is, bench scale bioreactor (BSBs) and the TPFBB tests. Specific experiments conducted include determination of chemical oxygen demand (COD) and tannins concentration in the TPFBB and BSBs.

Determination of Tannins Concentration

About 20 ml acetone was used to extract tannins from 1 gram of tannins powder. The suspension was shaken for 15 min using a vortex mixer followed by centrifuge at 3000 rpm for 15 minutes. The supernatant was then diluted into clean vials, and added to 70% aqueous acetone to make the total volume of supernatant and acetone to be 1.2 ml sample. This mixture was transferred into clean vials into which 6 ml acid butanol and 0.2 ml iron reagent were added and heated at 90°C for 50 minutes. The absorbance of the sample and standard solutions were obtained after reactions between reagents and tannins. Using this data, the standard curve was plotted from which the linear equation constants were established and used to calculate tannins concentration from sample absorbance Values.

The acid butanol was made by mixing 950 ml of n-butanol with 50 ml concentrated HCl. The iron reagent was prepared by mixing 2% ferric ammonium sulfate with 2N HCl (obtained by filling 16.6 ml of concentrated HCl up to 100 ml with distilled water). Approximately 0.5 g $FeNH_4(SO_4)_2.12H_2O$ were dissolve in 25 ml of 2N HCl and store in a dark bottle.

In a 13 x 100 mm screw cap culture tube 6.0 ml of the acid butanol were added to a 1.0 ml aliquot of the sample (or a smaller volume of the sample made up to 1.0 ml with the sample solvent). After cooling the tube, the absorbance was read at 550 nm. The absorbance of a blank containing only sample solvent, acid butanol and iron was subtracted from the sample absorbance. Because water decreases the color yield in this reaction, the reaction must be standardized with standard dissolved in exactly the amount of solvent that the samples are to be dissolved in. Non-aqueous samples will give the greatest colour, but the assay is sensitive enough to give results even with substantial amounts of water present.

All incubations were performed at room temperature. The standard samples were prepared by taking from 50 µl to 300 µl of standard Catechin solution and adjusting the volume to 875 µl with a buffer solution, followed by adding 125 µl of the ferric chloride reagent and mixing. The zero tannins samples were made with 875 µl buffer solution and 125 µl of ferric chloride reagent followed by reading the absorbance at 550 nm. This absorbance must be subtracted from the absorbance obtained from the standard tannins samples and the wine samples. The standard samples and the zero tannins concentration were incubated for 10 minutes after which the absorbance was read also at 550 nm.

Determination of COD by Closed Reflux Titrimetric Method

Using this method, a sample is remixed in a strong acid solution with a known excess of potassium dichromate ($K_2Cr_2O_7$). After digestion, the remaining unreduced dichromate is titrated against ferrous ammonium sulfate to determine the amount of dichromate consumed. The amount of oxidizable matter is calculated in terms of oxygen equivalent. The standard 2-h reflux time may be reduced if a shorter period yields the same results. Some samples with very low COD or with highly heterogeneous solid content were analyzed in replicate to yield reliable data. Culture tubes and caps were washed with 20% sulfuric acid before first use to prevent contamination. The most critical volumes were those of the sample and digestion solution.

Bench Scale Bioreactors (BSB)

The aim of bench scale bioreactor experiments was to select the suitable microorganisms to be used in wastewater treatment in the TPFBB for minimal final COD and minimal tannins concentration. The growth characteristic of microorganism was also developed based on bench scale bioreactor performance. Bacteria strains used in this experiment were rumen fluid (RF) and inoculums from waste stabilized ponds (WSP). Moreover, the BSB experiments were used to determine the operation conditions like biomass support loading, pH and initial concentrations. Figure 1 shows the experimental setup for the BSBs, comprising of

1000-ml conical flasks, air rotameter, bacteria strains, rumen fluid and inoculums.

Bacteria strains were prepared by addition of the following nutrients: 25 ml of D–glucose; 0.5 ml of trace solution; 250 ml of mineral medium; and 20 ml of rumen fluid. The optimum solids loading in each BSB was established to be 0.05 kg solids/liter (Manyele *et al.*, 2004). All BSBs were aerated at equal rates to eliminate the effect of air flow rate.

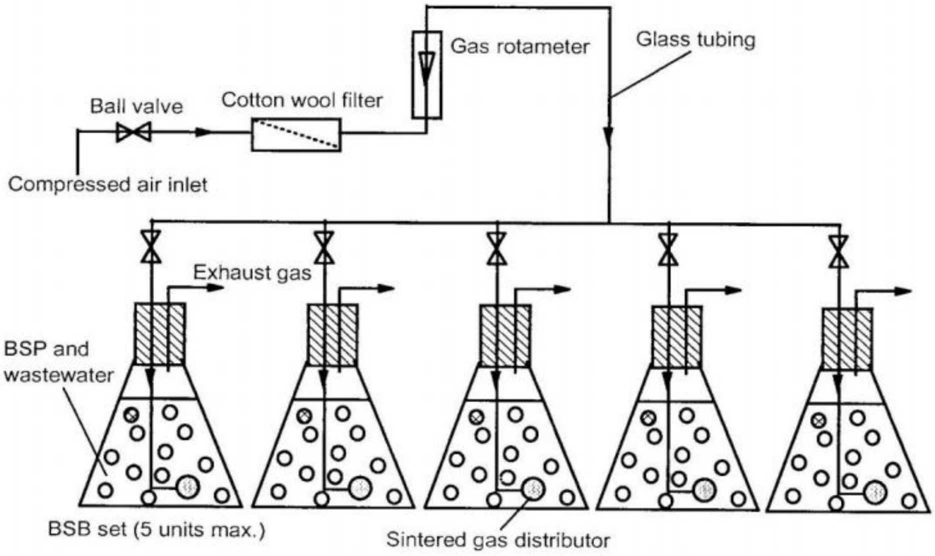


Figure 1: Experimental setup for the bench scale bioreactors (BSBs)

The Three Phase Fluidized Bed Bioreactor (TPFBB)

Compressed air was used as the gas-phase in all experiments. The gas superficial velocity was varied from 3 mm/s to 42 mm/s. The gas rotameter was used to measure the gas velocity. Tannins wastewater was used as the liquid-phase. The KMT® biomass support made up of polyethylene plastic was used as BSP, that is, as the solid phase (Manyele *et al.*, 2004; 1998; 1997; Manyele, 1998; 1996). Aeration of tannins wastewater was performed at optimum condition to study the effect of aeration on the tannins wastewater. The optimum conditions selected for the aeration were: solids loading = 5.0 kg; liquid flow velocity = 1 – 4 mm/s; and air flow velocity = 20 – 33 mm/s (Manyele *et al.*, 2004). Experiments were conducted by determining the COD and the tannins concentration in the TPFBB at different aeration times. Figure 2 shows the experimental set-up for the TPFBB (Manyele *et al.*, 1997).

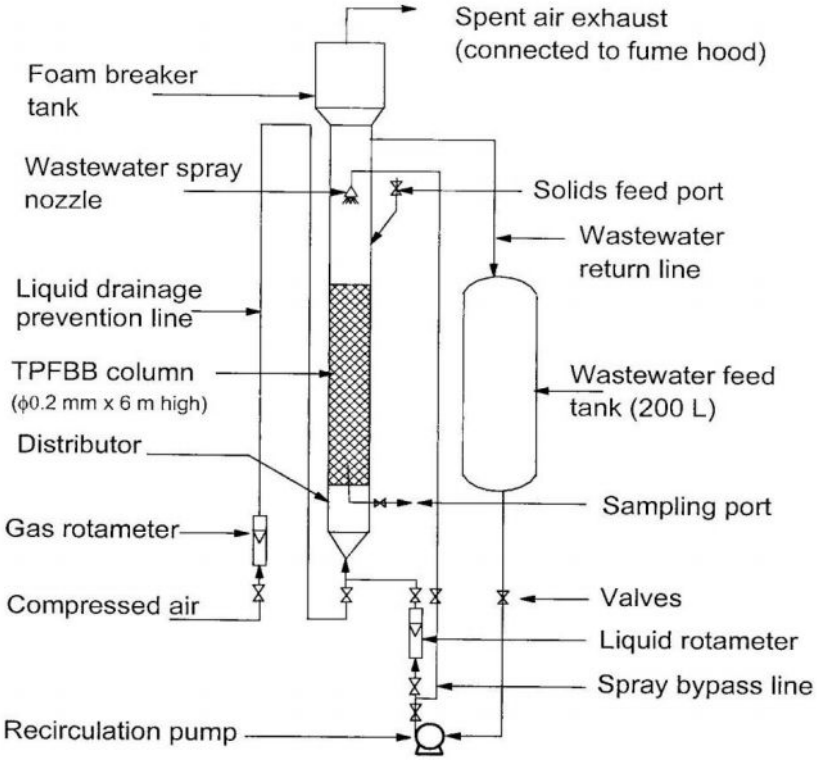


Figure 2: Experimental set up for the TPFBB

RESULTS AND DISCUSSION

Variation of pH, Temperature and TDS with Aeration Time

During aeration of the BSBs and the TPFBB, the variation of temperature, pH and TDS with time were studied for 7 days as summarized in Figure 3. The temperature of the BSBs decreased slightly with time, but constantly within a 26-28°C range for the first 6 days, but decreased thereafter to 22-25°C range. In the TPFBB, however, higher temperatures were observed which ranged between 30 and 32°C. Results show that, during the whole aeration period, the temperature remained in the mesophilic range (i.e., 20 to 45°C), which gives optimal performance for most microbial processes (Edwards, 1995).

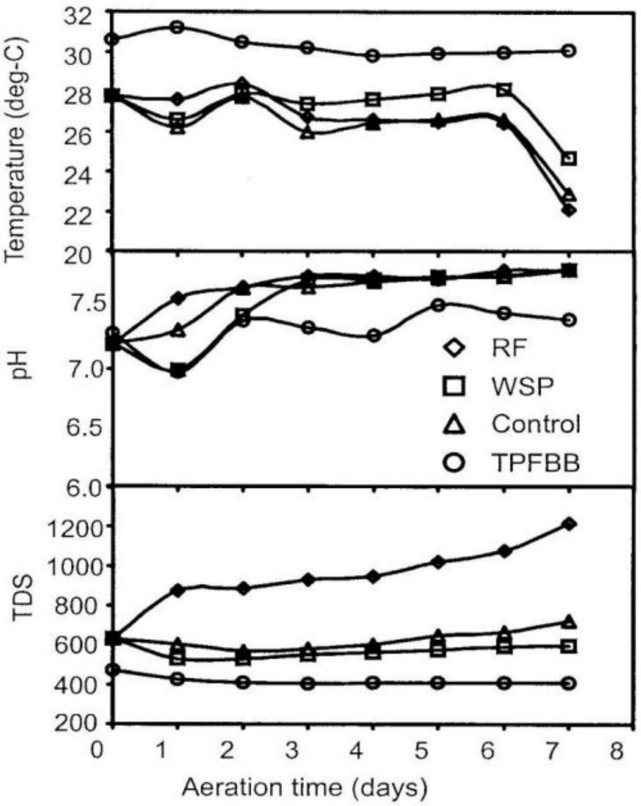


Figure 3: Temperature, pH and TDS profiles during aeration of tannins wastewater samples in bench scale bioreactors

The pH of the BSBs contents increased from 7 to 8 within the first 3 days and remained constant for the rest of the aeration time. All BSBs reached the same pH values for the rest of the aeration time. Initially, the BSB contents were kept at the same pH of 7.2, but after starting aeration, each BSB behaved differently up to the end of day 3. However, the BSB inoculated with RF has slightly higher values of pH at all times except day 7 when all the three BSBs reached the same pH value. In the TPFBB, lower pH values were observed throughout the aeration time and ranged between 7.0 and 7.5. Compared to beverage wastewater which reached the ultimate pH of 9.0 from an initial value of 11.0 (Manyele *et al.*, 2004), the ultimate pH is lower in this case. This can be attributed to the large amounts of acidic compounds in the tannins wastewater.

With the observed pH range during the BSB operation, there is no possibility of upsetting the TPFBB. In general, a pH below 6.0 or above 9.0 can upset the wastewater treatment plants. Because the pH of the wastewater is usually easier to drop by addition of acid than it is to increase with the addition of a base (buffered on the basic side), then acidic wastewater is detrimental to wastewater treatment than is basic wastewater (Edwards, 1995).

The variation of TDS with aeration time is also presented in Figure 3. In all cases, the initial TDS was 600 ppm. The BSB inoculated with RF indicated an increase in TDS from 600 to 1200 ppm, while other reactors remained at TDS values closer to the initial value. The TDS values in the other two reactors (RF and control) decreased slightly for the first 3 days before increasing again. The BSB inoculated with RF indicates that more biomass was being produced, leading to increased TDS values compared to other reactors. The values of TDS observed in the TPFBB were lower than those in the BSBs, and remained constant at 400 ppm.

Bench Scale Aeration Results

The effect of the selected BSP (that is, KMT® support) on the TCRE and COD removal efficiency was studied by inoculating two reactors with WSP and two reactors with RF, but keeping each one of the two reactors without BSP. One control BSB was also implemented without BSP and no inoculation. Figure 4 summarizes the variation of TCRE with aeration time for all five BSBs.

High values of TCRE were observed for BSBs with (RF + BSP) and (WSP + BSP). For these bioreactors, the reduction efficiencies reached 65% within the first day of aeration, and then increased linearly to 80% maximum on day 7. In the rest of the BSBs, the TCRE increased slowly to 60% in 7 days. The KMT® support strongly improves the TCRE due to increased aeration efficiency and surface area for biomass support (Manyele *et al.*, 2004; Manyele, 1996).

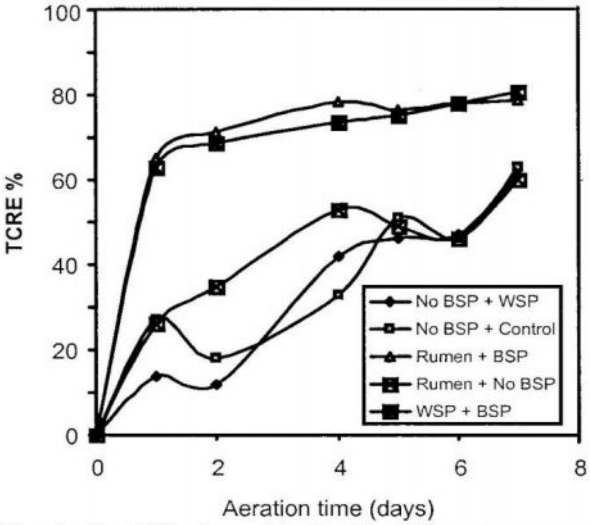


Figure 4: The TCRE profiles in the BSBs inoculated with different sources of microorganism with and without BSP

Figure 5 shows the results on the effect of KMT® support on the COD removal efficiency in the BSBs. Higher values of COD removal efficiency were also observed for (RF + BSP) and (WSP + BSP) which reached 65% in the first day of aeration and increased slowly to 80% on the 7th day.

In this case, the control BSB was also provided with BSP leading to improved COD removal efficiency compared to the TCRE in the Figure 4. However, because no microorganisms were initially inoculated in the control BSB, the COD removal efficiency decreased from a highest of 65% to 55% on the 7th day. In spite of inoculating a BSB with RF, aerating without BSP was observed to have lower values of both TCRE (Figure 4) and COD removal efficiency (Figure 5). Results show that KMT® support plays a big role in improving biodegradation efficiency of tannins wastewater.

The Variation of COD with aeration time in the BSBs loaded with KMT® support is shown in Figure 6. Treatment of tannins wastewater in a biological reactor requires a set of known parameters like types of biomass support, type of inoculum and the optimum air flow rate. Figure 6 shows the variation of COD with aeration time for 4 days with an initial COD load of 11,000 ppm. The BSB inoculated with WSP shows fast decrease in COD compared to RF and the control BSBs. In this set of tests, all the reactors were loaded with the KMT® support particles. The BSB with WSP inoculum reached 1000 ppm COD level after 3 days only, while the BSB inoculated with RF reached a minimum of 2000 ppm COD level. This shows that the WSP inoculum is more effective than the RF, as it brings COD to the lowest level of 1000 ppm.

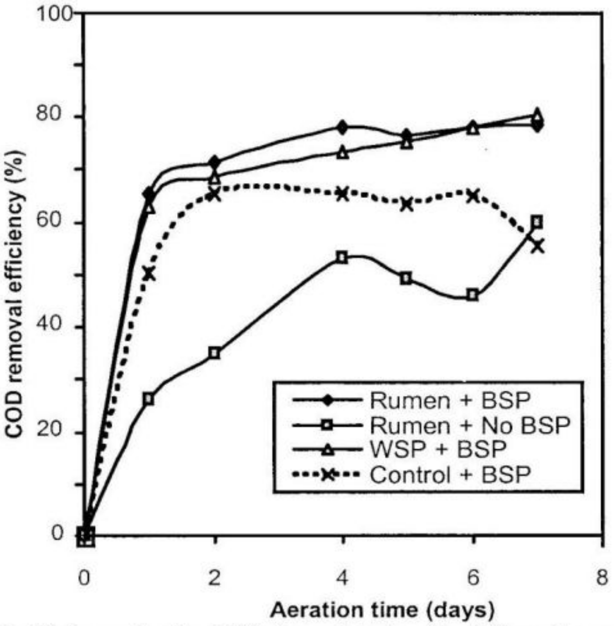


Figure 5: COD Removal efficiency in the BSBs inoculated with different sources of microorganism with and without BSP.

Although the end point reached after 4 days goes to similar values in the COD level, aeration costs can be highly significant if RF inoculum is used in the TPFBB because it reached the minimum COD level after a longer aeration time than the BSB inoculated with WSP. Moreover, the lowest COD levels are still above 1,000 ppm which is not acceptable for discharge into rivers or municipal sewers.

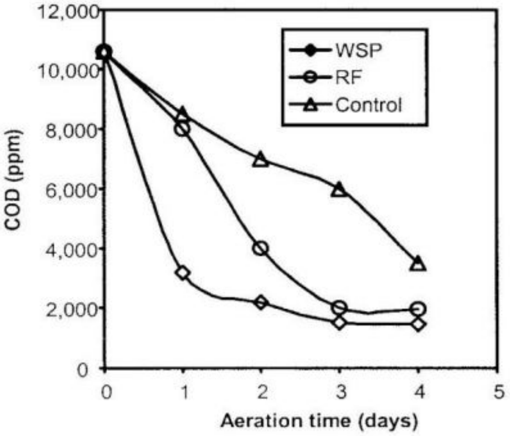


Figure 6: The variation of COD with aeration time for different inoculums types in the BSBs loaded with KMT® particles.

The Variation of the Tannins Concentration with Aeration Time

The effect of inoculum type was further investigated by setting the BSB contents at a lower tannins concentration level of 6,200 ppm (different from the results shown in Figure 6). All bioreactors were operated without the KMT® support particles. The minimum tannins concentration of 2000 ppm was reached after 7 days for rumen and 9 days for control. On the other hand, a minimum of 1000 ppm was reached after 9 days for WSP inoculated BSB.

However, the WSP reached to a minimum of 1,000 ppm, which was the lowest of all cases tested. Moreover, such a minimum was reached after a long time of aeration which cannot be cost effective in a large bioreactor like the TPFBB. The use of BSP speeds up the biodegradation efficiency as shown in Figures 4, 5 and 6. Results further suggest that the KMT® support particles are necessary for effective treatment of tanning industry wastewater.

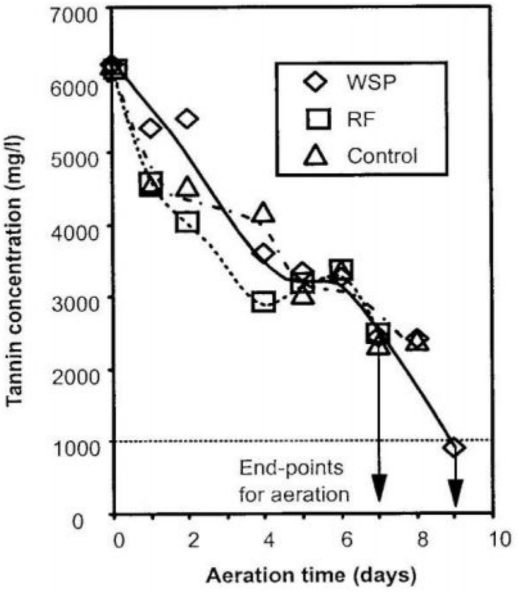


Figure 7: The variation of tannins concentration with aeration time for inoculated bench scale bioreactors without KMT® particles

The effect of BSP was further studied with inoculated BSBs at an initial tannins concentration of 5,000 ppm, as shown in Figure 8. The tannins concentration in both WSP and RF inoculated bioreactors decreased continuously with aeration time but a turning point in tannins concentration was observed for a control bioreactor after 2 days of aeration. Both WSP and RF bioreactors gave a final tannins concentration of 1,000 ppm corresponding to a TCRE of 84%. The increase in tannins concentration in the control BSB after day 3 can be attributed to the evaporation and loss of water.

Aeration Results in the TPFBB

Tannins biodegradation results from the inoculated BSB gave an indication of suitable inoculum for use in the TPFBB. Based on the above results, WSP inoculum was selected for further studies in a large-scale bioreactor. In the TPFBB, the KMT® support particles were used throughout, loaded at an optimum value of 5 kg, with the reactor operated at a height of 2 m, corresponding to the solids loading of 0.04 kg solids/l (Manyele *et al.*, 2004).

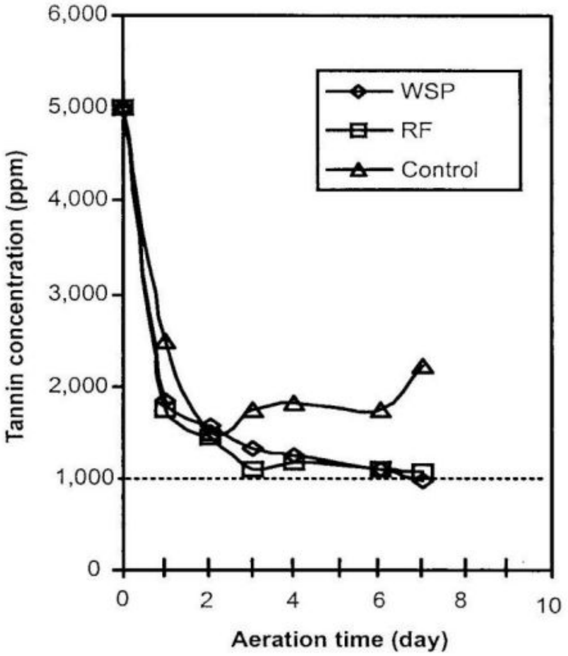


Figure 8: The variation of tannins concentration with aeration time for inoculated BSBs with KMT® particles

Figure 9 shows the variation of COD and tannins concentration with aeration time in the TPFBB inoculated with WSP. Using different measurement methods for the samples from the TPFBB, the initial values of COD and tannins concentration were set at 13,200 and 5,000 ppm, respectively. The COD was observed to fall faster than the tannins concentration reaching similar values of about 1,000 ppm after 7 days. Beyond that, prolonged aeration resulted in a very small change in both COD and tannins concentration. The TPFBB was further aerated for 15 days, but the concentration of tannins and COD values did not change.

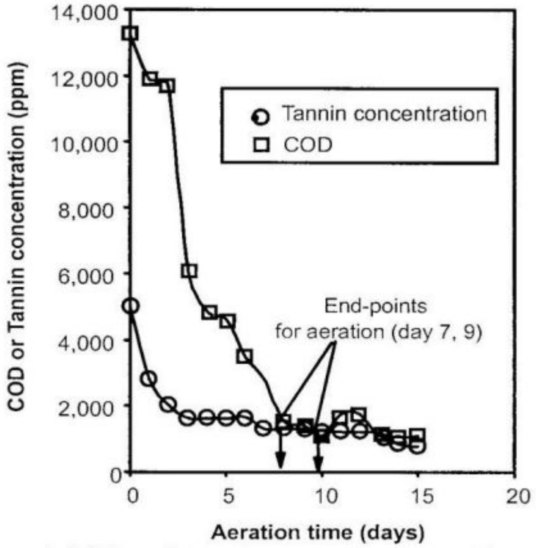


Figure 9: The variation of COD and tannins concentration with aeration time in the TPFBB with inoculum from WSP in the TPFBB

COD Removal Efficiency and TCRE in the TPFBB

Figure 10 shows the variation of COD removal efficiency and TCRE in the TPFBB for tannin wastewater inoculated with WSP. The results from tannins concentration measurements shows a faster rise to 70% but the highest removal efficiency of 85%, reached after 15 days, was lower than the COD removal efficiency, which rises slowly to 90% in 7 days and remains constant.

It should be noted that the data presented in Figure 9 and 10 results from same samples from the TPFBB analyzed using different methods. Figure 10 shows also that the trends of the COD removal efficiency and TCRE are similar but efficiency values are different. Both Methods are suitable for bioreactor performance assessment. However, the TCRE method underestimates the efficiency from day 5 to 15 but shows faster increase before day 5.

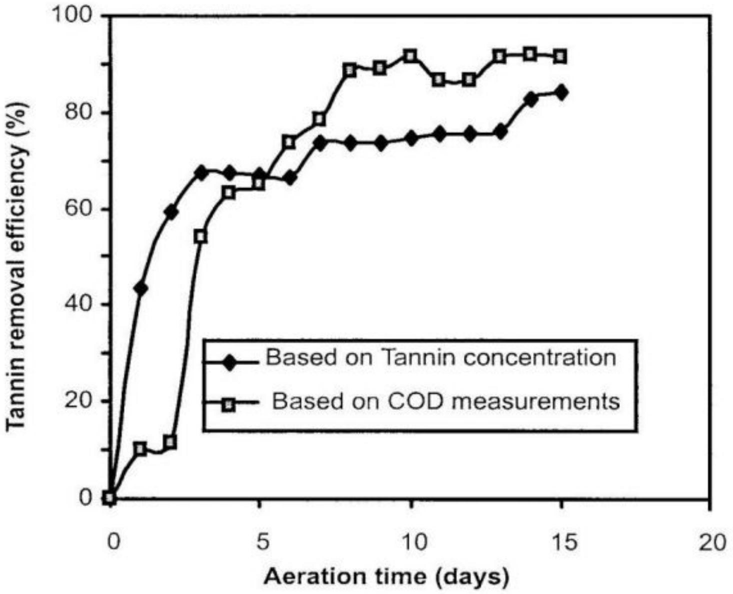


Figure 10: COD removal efficiency in the TPFBB for wastewater inoculated with WSP

General Discussion

Experimental values of COD and tannins concentration obtained in BSB with the KMT® solid loading shows high growth rate of the microorganism and high biodegradation efficiency. The performance of the microorganisms was measured by calculating the efficiency in COD removal using the following relationship (Manyele, 1996):

$$E = \frac{COD_i - COD_f}{COD_i} \times 100\% \qquad (1)$$

On the other hand, the tannins concentration removal efficiency (TCRE) was determined from the relationship:

$$E = \frac{[T]_i - [T]_f}{[T]_i} \times 100\% \qquad (2)$$

As it can be seen from the plots, for the rate of change of COD and tannins concentration is higher in BSBs with solid loading for inoculums from WSP than in other BSBs. The implication of the trend is that the growth of inoculums from WSP was faster compared to the other microorganisms. The variation of the COD and tannins concentration with aeration time has been established. This relationship was later used to show which curve gives the better trends in order to choose the best microorganism for use in the TPFBB. Aerobic treatment of tannins wastewater in the TPFBB with inoculums from WSP gives the largest COD removal.

The TPFBB is capable of biodegrading heavily loaded wastewater to COD levels which need polishing before final discharge. On the other hand, subsurface flow constructed wetlands can accept low loads of COD and polish them to acceptable levels for final discharge. This concept is being tested for tannins wastewater (Manyele *et al.*, 2004).

CONCLUSIONS

The growth characteristics of microorganism developed based on BSB performance proved to be useful in predicting hydrocarbon removal rate in TPFBB. It can be drawn that it is possible for tannins wastewater treatment by using the selected approach. The test of the BSB without KMT® support particles (BSP) for tannins wastewater treatment in BSB has slightly lower performance because there was no attachment of microorganisms and also due to poor aeration efficiency.

The COD removal efficiency with RF, inoculums from WSP and Control BSB with solid loading was 85%, 90% and 50% on the sixth days respectively, while the TCRE was 78%, 80% and 55% on seventh day, respectively. By utilizing available engineering parameters of the TPFBB and properties of microorganisms in tannins wastewater treatment the effective biodegradation of toxic wastewater can be achieved. In TPFBB the COD removal efficiency was observed to be at 75% from sixth day of biodegradation up to fifteenth days. The maximum attained value was 90%. It can then be concluded that hydraulic retention time with inoculums from WSP is ten to fourteen days.

The final COD values of 1,000 ppm necessitates use of sub-surface flow constructed wetlands (SSFCW) coupled to the TPFBB for final smoothing of the effluent. The TPFBB is recommended for use in tannin wastewater treatment as it will reduce the COD loading from very high to as low as 1000 ppm, compared to direct discharge of untreated wastewater from tannin industries.

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ACRONYMS

BSB	Bench scale bioreactor
BSP	Biomass support particles
COD	Chemical oxygen demand
HRT	Hydraulic retention time
RF	Rumen fluid
SSFCW	Subsurface flow constructed wetland
TCRE	Tannins concentration reduction efficiency
TDS	Total dissolved solids
TPFBB	Three-phase fluidized bed bioreactor
WSP	Waste stabilization pond

REFERENCES

Atkinson, B., Black G.M., and Pinches, A., (1981), "The Characteristics of Solid Supports and Biomass Support when Used in Fluidized-Beds". In Cooper P.F. and Atkinson, B., (Eds), *Biological Fluidized-Bed Treatment of Water and Wastewater*. Ellias Horwood Publishers, Chichester, pp. 75-89.

Bone, K., (1998), "Herbal Pharmacokinetics-The Behaviour of Large Molecules". In Proceedings NHAA International Conference, Sydney.

Chatib, B., Grasmic, A., Emaleh, S., and Ben Amin, R., (1981), "Biological Wastewater

Treatment in a Three-Phase Fluidized Bed Reactor", in "Biological Fluidized Bed Treatment of Waster and Wastewater", P.F. Cooper and B. Atkinson, (Eds.), Ellis Horwood Ltd., Chichester, UK, pp. 192 – 204.

Cooper, P.F., (1981), "Biological Fluidized Bed Reactors for Treatment of Sewage and Industrial Effluents", in "Comprehensive Biotechnology", M. Moo-Young, (Ed.), Vol. 1, Pergamon Press, New York, pp. 993-1005.

Cunha-Santino, M.B., Bianchini, Jr., I., and Serrano, L.E.F., (2002), "Aerobic and Anaerobic Degradation of Tannic Acid on Water Samples from Monjolinho Reservoir". *Brazilian Journal Biology*, **62**(4A): 585-590.

Edwards, J.D., (1995), Industrial Wastewater Treatment: A Guide Book, Lewis Publishers, NY.

Field, J.S., (1989), "The effect of tannin compounds on anaerobic wastewater treatment". In WAU Dissertation, No. 1299.

Frigon, J.C., Cimpoia, R., and Guiot, S.R., (2003), "Sequential Aerobic/Aerobic Biotreatment of Bark Leachate". In *Water Science Technology*, **48**(6), pp. 203-209.

Jolly-Voillemin, C., de Bellofoin, C., and Delmas, H., (1996), "Solids Effects on Gas-Liquid Mass Transfer in the Three-Phase Slurry Catalytic Hydrogenation of Diponitrile over Raney Nickel, *Chem. Eng. Sci. J.*, **51**(10), 2149-2158.

Manyele, S.V., (1996), "Investigation of Oxygen Transfer in a Three-Phase Fluidized Bed Bioreactor using KMT® Support in Industrial Wastewater Treatment", MSc Thesis, University of Dar es Salaam, Tanzania.

Manyele, S.V., Halfani, M.R., and Sokol, W., (1997), "Oxygen Transfer Rate in a Three-Phase Fluidized Bed Bioreactor with KMT® Biomass Support, when used in Industrial Wastewater Treatment: Part I, Effect of Air Flow", Tanzania J. of Engineering and Technology, **21**(2) 1-21.

Manyele, S.V., (1998), "Design Consideration for a Three-Phase Fluidized Bed Bioreactor", *Tanzania J. of Engineering and Technology*, **22**(1), pp. 11-22.

Manyele, S.V., Halfani, M.R., and Sokol, W., (1998), "Oxygen Transfer in Three-Phase Fluidized Bed Bioreactor with Novel Biomass Support: Part II, Effect of Support Mass and Liquid Height", *Tanzania J. of Engineering and Technology*, **22**(2), 168-185.

Manyele, S.V., Halfani, M.R., and Benjamin, Y., (2004), "Aeration Efficiency of a Three-Phase Fluidized Bed Bioreactor during Food Industry Wastewater Treatment", *Tanzania J. of Engineering and Technology*, **27**(2) 60-70.

Manyele, S.V., Peay, M., and Halfani, M.R., (2007), "Treatment of Beverage-Processing Wastewater in a Three-Phase Fluidized Bed Biological Reactor", *International J. of Food Science and Technology*, in press.

Paul, W.H., Rebecca, F., Mark, D.H., and Hagerman, H., (2002), "Determination of Hydrolyzable Tannin (gallotannins and Ellagitannins) after Reaction with Potassium Iodate", *Agricultural and Food Chemistry*, **50**, pp. 1785-1790.

Sukanya, J., and Rima, R.M., (2001), "Hydrodynamics in Three-Phase Circulating Fluidized Bed Reactor Using Low-Density Particle with Newtonian and Non-Newtonian Fluids." Anna University, India, paper No. 0847.

Wokoli, M., Etiegni, L., and Ofosu-Asiedu, (1999), "Design of an Effluent, Tannin Extract Effluent Treatment", *Water Science and Technology*, **39**(11), pp. 321-324.