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DESIGNING A COST EFFECTIVE AIR-LIFT REACTOR FOR THE BIOREMEDIATION OF COMPOSITE TANNERY EFFLUENTS

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Key words : Tannery effluents, Tannins, COD, TDS, Air Flow Rates (AFR).

ABSTRACT

Tannin degrading microbes *Bacillus laterosporus* MTCC 6017, *Klebsiella pnuemoniae* MTCC 7162 and *Pseudomonas aeruginosa* isolated from tannery effluents were tested for their ability to degrade vegetable tannins in tannery effluents, along with other polluting parameters in the effluents like COD and Total Dissolved Solids (TDS). An inexpensive Air-Lift Reactor (ALR) was designed to carry out effluent treatment (32 l) at a pilot scale. The efficiency of the ALR on the reduction of COD, tannin and TDS by the consortium of microorganisms was assessed at different air flow rates (0.1 l / s to 1.5 l / s) and three diluted loading rates (1: 5 to 1: 15). The optimum AFR rate was found to be between 0.5 and 1.0 l / s, to get significant result in 48 h. Reduction in the loading rates by means of dilution (1: 5) enhanced the performance of the isolates under ALR, reduction increased to 87%, 89% and 54% for COD, tannin and TDS respectively at the end of 48 hours.

INTRODUCTION

Leather Industry is one of the major foreign exchange earners of India, accounting for 2.5% of the global leather-related trade and provides employment to 2.5 million people (CII report, 2006). In Tamil Nadu there are 750 tannery units accounting for 65% of Indian tanning capacity (www. aishtma.com, March 2007). Though tanneries are revenue and job generating sector, the pollution

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from their effluents is of major concern. The objectionable constituents present in large amounts in the effluents are suspended solids, chlorides, sulfides, chromium, tannins and organic wastes (Buljan et. al, UNIDO 2000). Although no tannery in Tamil Nadu operates without access to Effluent Treatment Plants (or) Combined Effluent Treatment Plants (CETP) (www. aishtma.com, 2007), the treatment plants are not able to meet the discharge norms pertaining to TDS. To achieve zero discharge and bring down the TDS level within the permissible discharge norms, a Reverse Osmosis (RO) plant is being set up at CETP Ranipet at a total cost of Rs 40 crores (Balaji, 2006). RO is an expensive technology not amenable for small units in terms of both cost and technical management. The need for cheaper and efficient handling methods has to be envisaged to tackle TDS along with other tannery effluent parameters like (BOD, COD and the most toxic pollutants tannins. Bacteria are considered highly sensitive to tannins but some isolates are resistant and effectively degrade tannins (Deschamps*et al.* 1983; Field *et al.* 1989 and Bhat *et al.* 1998). Tannins are toxic to aquatic organisms and rumens; they affect the efficiency of methanogenic bacteria affecting the anaerobic treatment processes (Field et. al, 1989). Tannins are degraded by microorganisms by the production of enzyme tannase. (Lekha and Lonsane, 1997 and Bhat *et al.* 1998). A bacterial strain has been reported for effective removal of TDS from tannery waste water (US patent 6905863 B2, 2005).

Air Lift Reactors (ALR) has become a technically and economically attractive alternative for many chemical, biological and environmental control processes. ALR offers the possibility of very simple and effective fluidization even in case of high solids loading, which would stratify in conventional mechanically agitated reactors, requires the power only to inject the aeration gas and simplicity of reactor design (Tobajas *et al.* 1999).

In this current study the effect of bacterial strains isolated from tannery sources on the composite tannery effluent was taken up by designing an Air-Lift Reactor and generating polynomial equations for understanding the relation between the parameters, the loading rates and time (Hydraulic Retention Time - HRT) so that they can be used as a model for further scale up treatment studies.

METHODS

Microorganisms isolated from soil and tannery effluents, were screened for tannase production by using simple plate assay method using tannic acid as sole carbon source (Bradoo *et al.* 1996). Isolated organisms were grown in minimal media (Tannic acid 2 % w/v, $NaNO_3$ 0.3 % w/v, KCl 0.05 % w/v, $MgSO_4.5H_2O$ 0.05 % w/v, KH_2PO_4 0.1 % w/v) containing tannic acid as the sole carbon source. The three isolates later identified as *Bacillus laterosporus* MTCC 6017, *Klebsiella pnuemoniae* MTCC 7162 and *Pseudomonas aeruginosa* were maintained on nutrient agar plates, slants and in tannic acid minimal media plates as well as glycerol stocks. For effluent treatment, initially cultures were grown in nutrient broth for 24 hours. The grown culture, in necessary aliquots, was centrifuged and the pellet was suspended in normal saline. The suspensions were further used to inoculate 1 l shake flasks containing 300 ml minimal medium. The flasks were incubated in shakers at 37° C and 150 rpm for 24 hours. 3% (v/v) of 600 x 10^6 /mL (MacFarland scale 2) inoculums of the isolates were used for ALR studies. The effluent samples were analyzed in triplicates as per the standard method described by American Water Work Association (AWWA) (Arnold *et al.* 1998)

Chemical Oxygen Demand (COD) (AWWA 5220 C)

Most types of organic matter are oxidized by a boiling mixture of chromic and sulfuric acids. The sample was refluxed in strong acid solution with a known excess of potassium dichromate ($K_2Cr_2O_7$). After digestion in a digester (Lovibond ET125, Germany), the remaining unreduced $K_2Cr_2O_7$ was titrated with ferrous ammonium sulfate to determine the amount of $K_2Cr_2O_7$ consumed and the oxidizable matter was calculated as measurment of oxygen equivalent.

Tannin (AWWA 5550 B)

Tannins contain aromatic hydroxyl groups that react with Folin phenol reagent (tungstophosphoric and molybdophosphoric acids) to form a purple color. The absorbance was estimated using a spectrophotometer (Shimadzu, Japan) at 700 nm. The unknown tannin was estimated by plotting standard graph for tannic acid and optical density obtained was used for calculating the tannin concentration.

Total Dissolved Solids (TDS) (AWWA 2540 C)

A well mixed sample was filtered (glass-fiber Whatman grade 934AH) and the filtrate was evaporated to dryness in a weighed dish and dried to constant weight at 180° C. The TDS observed from the sample was rechecked with digital TDS meter (Utech TD scan, India. 1990 ppm range) with appropriate dilutions.

Design aspects

Important reactor's design parameters were dependent on AFR, so the design parameters like gas holdup (å), aspect ratios, liquid circulation velocity U_L (m / s), circulation time t_C (s), average liquid linear velocity V_L (m / s), gas superficial velocity U_G (m / s), mixing time t_m (s), linear liquid velocity U_L (m / s) – theoretical and k_La values were taken up and estimated according to ALR constraints of Gouveia *et al.* 2003 and Doran, 2006.

Mathematical Modeling

For obtaining a relation between the effect of AFR on loading rate and retention time, five different air flow rates from 0.1 l/ s, 0.25 l/ s, 0.5 l/ s, 1.0 l/s and 1.5 l/ s were applied for 48 hours and the reduction in COD, TDS and tannin were analyzed at every 12th hour.

In the next setup, the effluent was diluted to three dilution rates (1: 5, 1: 10 and 1: 15) and each diluted effluent was treated for 48 hours with the reduction rate being noted at every 12th hour. AFR was maintained at a constant rate of 1.0 l /s for the three runs. The results obtained were used to derive correlation coefficient, liner regression and finally a polynomial equation was formulated using STATISTICA® 6.0 software.

RESULTS

Figure 1 illustrates an internal loop Air-Lift reactor with the riser and downcomer separated by a draft tube. Air is sparged from the bottom in to the draft tube, where d_1 (bottom clearance) is 5 cm and d_2 (top clearance) which varies according to the volume and air flow rates. H_D (draft tube height) is 40 cm, H_R (reactor height) is 60 cm. D_R (riser diameter or reactor diameter) is 30 cm and D_D (draft tube diameter) is 15 cm. The results obtained for the five AFR were The Gas holdup (G) values were 0.0063, 0.012, 0.025, 0.05 and 0.1 respectively. The aspect ratios (H_r / D_r) were 2.68, 2.7, 2.733, 2.8 and 2.933. The experimental values obtained for liquid circulation velocity U_L (m / s) were 0.1, 0.101, 0.117, 0.21 and 0.293 respectively.

The magnitude of liquid circulation is one of the most important design and scale-up parameters for ALR, liquid circulation influences the gas hold up in the reactor and mixing. For aerobic systems, the liquid velocity should be sufficient to ensure gas recirculation to achieve maximum aeration efficiency (Nicolella *et al.* 2000). Heijnen *et al.* 1996 have developed a model to predict hydrodynamic behavior in an internal airlift reactor for waste water treatment at a pilot-scale and full-scale reactor.

The liquid mixing time depends on the flow regime. Doran, 2006 has proposed a theoretical linear liquid velocity for a heterogeneous flow where the upward liquid velocity at the center of the reactor should be such that it fulfills the criteria $0.1 < D < 7.5$ m and $0 < U_G < 0.4$ m / s. where D is the diameter of the reactor (D_R) which is 0.3.

The experimental average liquid linear velocity V_L (m / s) was obtained as 0.1066, 0.1069, 0.1228, 0.2175 and 0.2967 respectively. Liquid circulation time t_C (s) was experimentally obtained as 14.6, 16.6, 6.6, 5.4 and 3.6. The gas superficial velocity U_G (m / s) was found to be 0.0142, 0.0057, 0.0283, 0.0566 and 0.0849 respectively. Mixing time t_m (s) obtained for each AFR was 25.9, 19.1, 15.2, 12.1 and 10.6 respectively. Linear liquid velocity U_L (m / s) was calculated as 0.233, 0.3156, 0.3963, 0.4981 and 0.5694. The volumetric mass-transfer coefficient k_La was arrived at for each AFR as 0.0085, 0.0162, 0.026, 0.0428, and 0.0569.

The values for gas-liquid mass transfer coefficient in reactors depend largely on bubble diameter and gas holdup. The exact bubble sizes and liquid circulation pattern are impossible to predict in Air Lift Reactors, therefore the accurate estimation of mass transfer coefficient will be difficult. Hence Doran 2006 has proposed a correlation $k_La < 0.32 \times U_G^{0.7}$ where k_La is the combined volumetric mass-transfer coefficient, the correlation is valid if the criteria of gas hold up were with in the range of $0 < U_G < 0.3$ m / s.

Linear equations for loading rate under different AFR

Composite effluent (32 L) was biologically treated with three potential tannin degraders *Bacillus laterosporus* MTCC 6017, *Klebsiella pnuemoniae* MTCC 7162 and *Pseudomonas aeruginosa* in an ALR, with the initial biomass of 600 x 10^6 / ml (MacFarland scale 2). Eight experimental runs were carried out (five runs for different AFR and three runs with diluted effluent). The results obtained from each experimental run for all Air Flow Rates and dilution runs were fitted with the help of statistical software STATISTICA® 6.0 to find correlation among the variables and to generate quadratic (polynomial) equations for the parameters.

Quadratic equation for loading rate under different AFR

The reduction in COD, tannin and TDS analyzed (triplicate) every 12 h are given in tables 1 for all the experimental runs involving five different AFR. Fresh effluents were used for each run and hence the variation in the concentration level during each run.
The quadratic equation obtained for all AFR were

1. $\text{COD} = 6384.6082 - 1691.8838 \times \text{AFR} + 10.2988 \times \text{Time} + 1048.8409 \times \text{AFR}^2 - 32.9131 \times \text{AFR} \times \text{Time} - 0.7846 \times \text{Time}^2$ (1)
2. $\text{Tannin} = 1255.3829 - 96.4901 \times \text{AFR} - 0.9998 \times \text{Time} + 58.5501\ \text{AFR}^2 - 7.2798 \times \text{AFR} \times \text{Time} - 0.0648 \times \text{Time}^2$ (2)
3. $\text{TDS} = 13600.6339 - 3366.6035 \times \text{AFR} - 0.6718 \times \text{Time} + 1989.1898 \times \text{AFR}^2 - 34.5162\ \text{AFR} \times \text{Time} - 0.749 \times \text{Time}^2$ (3)

The optimum AFR rate was found to be between 0.5 and 1.0 l / s, fort maximum reduction in COD, tannin and TDS at lesser retention time (Figure 2, 3 and 4). The correlation values obtained show there was a relation between loading rates COD, TDS and tannin.

Quadratic equation for loading rate under different dilutions experimental runs

Apart from AFR the loading rates of the polluting parameters are also important as they have the influence on HRT- the retention time. The composite effluent was diluted and tested with three dilution rates 1: 5, 1: 10 and 1: 15, with a constant air flow rate of 1.0 l / s. The quadratic equation obtained for all dilution runs were

1. $\text{COD} = -83.53 + 8006.09 \times \text{Dilution} - 2.98 \times \text{Time} - 14821.14 \times \text{Dilution}^2 - 71.24 \times \text{Dilution} \times \text{Time} + 0.0068 \times \text{Time}^2$ (4)
2. $\text{Tannin} = 53.65 + 1690.47 \times \text{Dilution} - 1.7219 \times \text{Time} - 1104.35 \times \text{Dilution}^2 - 25.7568 \times \text{Dilution} \times \text{Time} + 0.009 \times \text{Time}^2$ (5)
3. $\text{TDS} = -460.8698 + 21467.8745 \times \text{Dilution} - 13.5717 \times \text{Time} - 47015.2654 \times \text{Dilution}^2 - 96.3873 \times \text{Dilution} \times \text{Time} + 0.2191 \times \text{Time}^2$ (6)

Figure 5, 6 and 7 depict the relationship between reductions in the loading rates over time of the parameters for all the dilution. The optimum dilution was observed as 1:5 which was significant comparing the relation between the variables.

DISCUSSION

For optimizing the design and operation under pilot scale studies, design parameters were tested for five different Air Flow Rates and their efficiencies were compared for three different dilution rates at a constant loading rate of 1.0 l / s.

The design aspect values obtained for all five AFR were well within this range. Hence the AFR rates selected for the study was good for the experimental study and has the optimum range to carry out the desired result. The higher the AFR, the lower the sludge formation will be. The liquid linear velocity above 0.4 m / s (Doran 2006) will affect the biomass concentration as there will be more shear stress on the cells resulting in poor reduction rates of the polluting parameters. Although the values obtained for design constraints were well within the optimum range for this ALR, in terms of efficiency of the microbial action on the loads of COD, tannin and TDS with respect to retention time when all AFR were taken in to consideration, the results obtained showed there was a saturation beyond the Air Flow Rate of 1.0 l / s. The optimum AFR should be between 0.5 and 1.0 L / s, there was a positive interaction of loading with AFR and retention time (equation 1, 2 and 3), and AFR had a significant impact on reduction efficiency as it was evident from the results obtained when the Air Flow Rate was increased from 0.5 l/s to 1.0l/s, the COD, tannin and TDS reduction increased form 40 %,

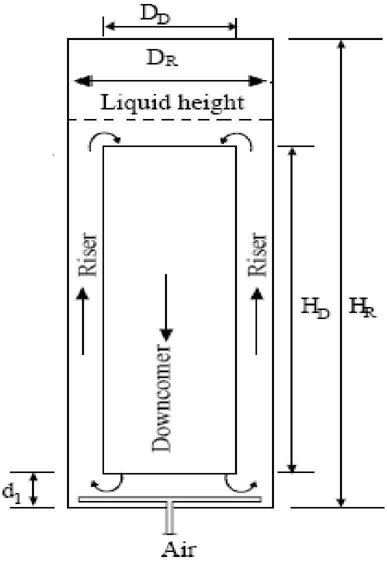

Fig. 1 Schematic view of Air-Lift reactor

Fig. 2 3D surface Contour plot for COD (all Air Flow Rates)

Fig, 3 3D surface Contour plot for tannin (all Air Flow Rates)

Fig. 4 3D surface Contour plot for TDS (all Air Flow Rates)

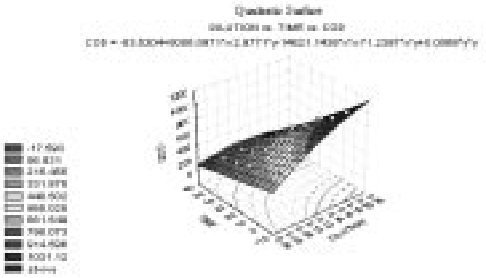

Fig. 5 3D surface Contour plot for COD (all dilution runs)

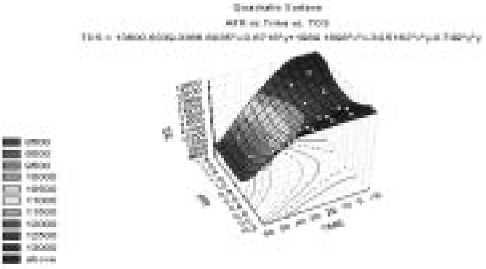

Fig. 6 3D surface Contour plot for tannin (all dilution runs)

Fig. 7 3D surface Contour plot for TDS (all dilution runs)

38 % and 25 % to 56 %, 50 % and 29 % respectively at the end of 48 hours (Table 1). Though there was more percentage reduction when AFR was 1.5 l /s, the increase is less significant when comparing that of reduction results obtained for 0.5 l/s and 1.0l/s. Higher AFR impart more oxidation, cause shear stress on the biomass and not efficient on the basis of power consumption.

The retention time for all the three parameters were reduced when the loading rates were reduced (dilution runs) and the diluted loading rates showed a positive effect on the retention time under the ALR using these bacterial isolates (equation 4, 5 and 6). Diluting the effluent enhances the reduction rate, as in the case of 1: 5 diluted effluents (Table 2) where there was 87 %, 89 % and 54 % reduction observed at the end of the same 48 h respectively for COD, tannin and TDS. But further dilutions (1:10 and 1:15) does not contribute to significant reduction rate as there was only 88 %, 87% and 56% reduction in case of 1: 15 dilution and 86%, 89% and 57% reduction in case of 1: 10 dilution. More dilution reduces the availability of organics for better biomass production and thus showing fluctuations in the reduction rates.

Finding mathematical relationship between the design parameters and polluting parameters for a pilot scale study will help in understanding the factors involved in a biological treatment, enabling the application for further scale up studies.

Table 1

Experimental runs with different AFR of 0.1 l / s to 1.5 l / s

Trail run	AFR	Time	COD[*] (% reduction)	Tannin[*] (% reduction)	TDS[*] (% reduction)
1	0.1	0	6110 ± 19.8 (0)	1210 ± 14.1 (0)	13000 ± 0 (0)
1	0.1	12	6059 ± 21.2 (0.83)	1185 ± 9.9 (2.1)	12900 ± 0 (0.76)
1	0.1	24	5867 ± 21.2 (4)	1158 ± 8.48 (4.3)	12700 ± 0 (2.3)
1	0.1	36	5494 ± 22.6 (10.1)	1116 ± 11.31 (7.8)	12400 ± 0 (4.6)
1	0.1	48	4977 ± 24.1 (18.5)	1047 ± 12.7 (13.5)	11900 ± 0 (8.5)
2	0.25	0	6102 ± 19.8 (0)	1235 ± 7.1 (0)	13000 ± 0 (0)
2	0.25	12	6009 ± 21.2 (1.5)	1188 ± 5.7 (3.8)	12800 ± 0 (1.5)
2	0.25	24	5606 ± 22.6 (8.1)	1129 ± 9.9 (8.6)	12400 ± 0 (4.6)
2	0.25	36	5082 ± 22.6 (16.7)	1059 ± 7.1 (14.3)	11600 ± 0 (10.8)
2	0.25	48	4301 ± 24.1 (29.5)	974 ± 5.7 (21.1)	10400 ± 0 (20)
3	0.5	0	5935 ± 12. 7 (0)	1275 ± 7.1 (0)	12450 ± 0 (0)
3	0.5	12	5590 ± 10.6 (5.8)	1185 ± 4.2 (7.1)	12100 ± 0 (2.8)
3	0.5	24	5166 ± 10.6 (13)	1079 ± 4.2 (15.4)	11500 ± 0 (7.6)
3	0.5	36	4603 ± 12.1 (22.4)	956 ± 2.8 (25)	10550 ± 0 (15.3)
3	0.5	48	3574 ± 7.8 (39.8)	794 ± 2.8 (37.7)	9300 ± 0 (25.3)
4	1	0	5908 ± 39.6 (0)	1245 ± 7.1 (0)	12500 ± 0 (0)
4	1	12	5369 ± 40.3 (9.1)	1107 ± 10.6 (11.1)	11950 ± 0 (4.4)
4	1	24	4743 ± 28.9 (19.7)	974 ± 8.5 (21.8)	11000 ± 0 (12)
4	1	36	3973 ± 8.5 (32.8)	840 ± 2.8 (32.5)	10000 ± 0 (20)
4	1	48	2578 ± 7.1 (56.4)	620 ± 5.7 (50.2)	8850 ± 0 (29.2)
5	1.5	0	6077 ±28.9 (0)	1216 ± 5.7 (0)	12850 ± 0 (0)
5	1.5	12	5483 ± 29.7 (9.8)	1062 ± 8.5 (12.7)	12200 ± 0 (5.1)
5	1.5	24	4845 ± 24.1 (20.3)	930 ± 2.8 (23.5)	11150 ± 0 (13.2)
5	1.5	36	4016 ± 28.3 (33.9)	751 ± 9.9 (38.2)	10150 ± 0 (21)
5	1.5	48	2549 ± 21.2 (58.1)	552 ± 5.7 (54.6)	9050 ± 0 (29.6)

[*] concentrations in mg/L (or) parts per million

Table 2

Experimental runs with dilutions of 1: 5, 1: 10 and 1: 15

Trail run	DiR	Time	COD[*] (% reduction)	Tannin[*] (% reduction)	TDS[*] (% reduction)
1	0.2	0	927 ± 8.5 (0)	349 ± 1.4 (0)	1990 ± 14.1 (0)
1	0.2	12	698 ± 5.7 (24.7)	257 ± 4.2 (26.4)	1550 ± 14.1 (22.1)
1	0.2	24	538 ± 7.1 (42)	198 ± 2.8 (43.3)	1235 ± 7.1 (37.9)
1	0.2	36	303 ± 8.5 (67.3)	109 ± 1.4 (68.8)	1075 ± 7.1 (46)
1	0.2	48	120.5 ± 3.5 (87)	39 ± 1.4 (88.8)	915 ± 7.1 (54)
2	0.1	0	584.5 ± 4.9 (0)	218 ± 2.8 (0)	1255 ± 7.1 (0)
2	0.1	12	445 ± 4.2 (24)	159 ± 1.4 (27.1)	985 ± 7.1 (21.5)
2	0.1	24	353 ± 7.1 (39.6)	117 ± 4.2 (46.3)	790 ± 14.1 (37.1)
2	0.1	36	197.5 ± 7.8 (66.2)	63.5 ± 2.1 (71)	670 ± 14.1 (46.6)
2	0.1	48	81.5 ± 2.1 (86)	24 ± 1.4 (89)	540 ± 14.1 (57)
3	0.067	0	379.5 ± 3.5 (0)	159 ± 1.4 (0)	710 ± 14.1 (0)
3	0.067	12	280.5 ± 2.1 (26.1)	118 ± 2.8 (25.8)	550 ± 14.1 (22.5)
3	0.067	24	209 ± 4.2 (45)	89 ± 1.4 (44)	430 ± 14.1 (39.4)
3	0.067	36	115 ± 4.2 (69.7)	49 ± 1.4 (69.2)	375 ± 7.1 (47.2)
3	0.067	48	47 ± 1.4 (87.6)	20 ± 0 (87.4)	310 ±14.1 (56.3)

[*] concentrations in mg/L (or) parts per million

Table 3

Correlation between AFR, Time, COD, Tannin and TDS

Variable	AFR	Time	COD	Tannin	TDS
AFR	1.000	0.000	-0.405[*]	-0.477[*]	-0.411[*]
Time	0.000	1.000	-0.819[+]	-0.795[+]	-0.796[+]
COD	-0.405[*]	-0.819[+]	1.000	0.977[+]	0.981[+]
Tannin	-0.477[*]	-0.795[+]	0.977[+]	1.000	0.951[+]
TDS	-0.411[*]	-0.796[+]	0.981[+]	0.951[+]	1.000

[*] Significant at $p > 0.1$, [+] significant at $p > 0.05$

Table 4

Correlations for Dilution, Time, COD, Tannin and TDS

Variable	Dilution	Time	COD	Tannin	TDS
Dilution	1.000	-0.000	0.521[+]	0.479[*]	0.778[+]
Time	-0.000	1.000	-0.798[+]	-0.832[+]	-0.556[+]
COD	0.521[+]	-0.798[+]	1.000	0.995[x]	0.923[+]
Tannin	0.479[*]	-0.832[+]	0.995[x]	1.000	0.898[+]
TDS	0.778[+]	-0.556[+]	0.923[+]	0.898[+]	1.000

[*] significant at $p > 0.1$, [+] significant at $p > 0.05$, [x] significant at $p < 0.01$

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