Respirometric assessment of vegetable tanning process wastewater generated from tanneries

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Abstract
Registration, Evaluation, and Authorization of CHEmicals (REACH) regulations insist on ready biodegradability information for organic chemicals that are used on a large scale in industries. Leather industry being one such, involves addition of organic chemicals that are obtained from plant extracts for vegetable tanning process. In view of the fact that biodegradability assessment is of paramount importance now for industrial wastewaters, a study was conducted on vegetable tanning process wastewater generated from tanneries. As per the Organization for Economic Cooperation and Development (OECD) methods for ready biodegradability (301 C), respirometer studies were conducted on wastewater containing different tannin concentrations of 540 mg/L (T1), 900 mg/L (T2), and 1350 mg/L (T3). The oxygen uptake rate had shown a sharp initial peak at 74, 102, and 164 mg O₂/d for the three samples T1, T2, and T3, respectively. The primary degradation % of tannins was found to be 78%, 50%, and 14% for T1, T2, and T3 respectively. The Chemical Oxygen Demand and Biochemical Oxygen Demand removal efficiency also decreased with increase in tannin concentration. The t½ values of tannin, calculated using the first-order kinetic equation, was 8.8, 10.9, and 12.4 days and the experimentally observed t½ values were 9.8, 11.5, and 12.6 days for T1, T2, and T3 respectively. This study establishes that presence of tannins in the aerobic treatment significantly affect the oxygen uptake when the tannin concentration is increased. This study emphasizes that ready biodegradability assessment is essential to establish biodegradability of all the organic chemicals used during leather making process.

KEYWORDS
aerobic treatment, biodegradability, first-order kinetics, oxygen uptake, Respirometry, tannins

1 | INTRODUCTION

The vegetable tanning process during leather making involves the addition of vegetable tannins obtained predominantly from wattle and quebracho. The tanners prefer wattle and quebracho containing condensed tannins in vegetable tanning process because of their strong binding property and polyphenolic nature. The other tannins obtained from Chestnut and
Tara are hydrolysable tannins, that have moderate biodegradability. Pyrogallol, which belongs to hydrolysable tannin category, is also usually present in vegetable tannin extracts that are biodegradable.

During vegetable tanning process, an excess of vegetable tannin extracts are added to ensure full penetration and reaction of tannins into the skin/hides. The wastewater emanating from vegetable tanning bath is a mixture of condensed tannins, hydrolysable tannins, and pyrogallol. For processing 1 kg of skins/hides, around 16-20 L of wastewater is generated, including washings during raw to vegetable tanning process. The concentration of tannins present in the vegetable tanning process wastewater (VTW) alone will be around 19 000 mg/L. It is mixed with other sectional streams and present in composite wastewater generated from raw to vegetable tanning process. The composite wastewater is treated in Common Effluent Treatment Plants (CETPs). The composition and combination of tannin extracts will vary depending on the end product of leather and also on the quantity of water used during the vegetable tanning process.

Due to the presence of tannins, the Chemical Oxygen Demand (COD) of VTW was found to be very high with low biodegradability. Due to variations in tanning practices, the conventional biological treatment systems in use for treating tannery wastewater are less effective in removing COD and Biochemical Oxygen Demand (BOD). The effect of a readily biodegradable fraction in activated sludge has been extensively investigated, but only minor information can be found about the evaluation and behavior of a slowly biodegradable substrate on the oxygen uptake rate (OUR) and COD removal in activated sludge systems.

Dissolved oxygen (DO) consumption or OUR is a significant parameter to determine biodegradation kinetics of aerobic treatment process. The OUR is a physiological characteristic of culture growth, which can be used to evaluate the performance of aerobic activated sludge process (ASP). OUR measurements are used to determine the hydrolysis rate constants of slowly biodegradable organic compounds present in municipal wastewater treatment plants. The oxygen consumed during aerobic treatment is used for respiration of the microorganisms as well as for the degradation of organic substances present in the wastewater. OUR provides a hint on the general metabolic rate of activated sludge microorganisms and hence it can be used as a criterion for assessing the inhibitory effect of certain substances to biological treatment of wastewater. In the presence of toxic compounds or inhibitory substances, the OUR of microbes present in the activated sludge will be affected.

Respirometric techniques are based on either O₂ consumption or CO₂ production by biomass under aerobic conditions. However, oxygen consumption-based methods are widely used to establish the biological activity of a compound. Respirometry is also used as a tool to study the kinetics of ASP and to study the effect of initial substrate to biomass (S₀/X₀) ratio on biodegradability. The respirometric batch tests constitute a widely accepted method of evaluating the consumption of biodegradable substrates and the OUR. A new respirometer-based approach was developed and designed to characterize the biofilm stoichiometry and kinetics in a biofilm-based ASP.

The Organization for Economic Cooperation and Development (OECD) guidelines are widely used to assess the biodegradability of test chemicals. As per OECD 301 C guidelines, the pass level of 60% Theoretical Oxygen Demand (ThOD) removal has to be reached in 10 days within the 28 days of total test period. European Union REACH (Registration, Evaluation, Authorization of Chemicals) regulations insist on evaluation of biodegradability of chemical substances produced by industry. With this in view, the chemicals used in leather processing operations have to be given special attention since most of them are polyphenolic and difficult to biodegrade. Hence the objective of this study was to assess the effect of tannins generated from vegetable tanning process in the treatment of the process wastewater, using respirometry as a tool. The biodegradability assessment was based on the oxygen uptake for three different concentrations of tannins. First order kinetics was employed to find half-life period of tannins from the initial and final concentration of tannins after the 28 day period of respirometer study.

2 | MATERIALS AND METHODS

2.1 | Characterization of wastewater

The VTW used for the study was collected from an operating Tannery in India. Tannin content (tannin and lignin; Part 5550 method B), COD (COD; Part 5220 method C), BOD₅ (BOD as BOD₅ at 20°C; Part 5210 method B), sulphates (sulphate as SO₄²⁻; Part 4550 method E), and suspended solids (Solids; Part 2450 method D), were measured as described in Standard methods 20th edition. Pyrogallol was measured by the procedure described by Swain and Goldstein.

The respirometric tests were conducted with sludge obtained from a municipal wastewater treatment plant. The sludge was aerated for about 1 day. Thirty minutes after stopping aeration, about one-third of the whole volume of
supernatant was discarded and an equal volume of a solution containing 0.1% each of glucose, peptone, and potassium orthophosphate, was added to the settled sludge and aeration was re-commenced. This procedure was repeated once on a day for a period of 7 days.

2.2 | Respirometer set-up

Respirometry study was carried out for VTW collected from vegetable tanning process. Respirometer used in the study was procured from Challenge Technology, USA with AER-800-4 V20A software for monitoring oxygen uptake. The respirometer set up is depicted in Figure 1. The respirometer consisted of four 0.6 L capacity reactors with a working volume of 0.5 L. The reactors were provided with CO₂ traps in the head space, filled with 30% potassium hydroxide. Modified plastic caps containing rubber top were used for inserting needles for oxygen supply. The reactors were kept in constant stirring at 430 rpm and a constant temperature of 25°C was maintained using Polyscience make temperature controller. Oxygen was supplied from oxygen cylinders at 5 psi pressure continuously throughout the study and distributed to the reactors through the respirometer. The DO concentration inside the reactors was at 2.2 ± 0.2 mg/L. The oxygen consumed inside the reactors was monitored and plotted as Cumulative Oxygen Uptake in terms of milligram of oxygen consumed (mg O₂) vs time in minutes. The oxygen uptake was recorded continuously at 10 minutes interval for a period of 28 days as per OECD protocol.

2.2.1 | OUR measurement

OUR was calculated every day from the cumulative oxygen uptake plot obtained from respirometer. The OUR was calculated using Equation (1).

\[
\text{OUR}_n = \text{OU}_n - \text{OU}_{n-1}
\]

where \(n\)—day (0 to 28); \(\text{OU}_n\)—Oxygen Uptake on \(n\)th day; \(\text{OU}_{n-1}\)—Oxygen Uptake on \(n - 1\)th day.

**FIGURE 1** Respirometer setup. (1—Respirometer, 2—reactor bottle holding container provided with RPM control, 3—temperature controller, 4—excess oxygen collection bottle, 5—online monitoring system, 6—oxygen cylinder)
Aerobic reactor studies to monitor COD, BOD$_5$, tannin and pyrogallol concentration

Four lab scale aerobic reactors were also run simultaneously with the same conditions, as maintained in respirometer, to check the degradation levels of COD, BOD$_5$, tannin, and pyrogallol during the 28 days period. Samples were collected every 4 days and analyzed for COD, BOD$_5$, tannin, and pyrogallol. The COD, BOD$_5$, tannin, and pyrogallol removal data from the aerobic reactors were correlated with the oxygen uptake curve obtained from respirometer. A control reactor termed “C” was also run in parallel containing only the inoculum (sludge), which served as blank.

Theoretical oxygen demand (OD$_{Th}$) and observed oxygen demand (OD$_{Obs}$)

The theoretical oxygen demand for any chemical can be calculated based on its molecular weight and elemental composition. According to OECD 301 methods, for a compound with the chemical composition C$_c$H$_h$O$_o$, the theoretical oxygen demand can be calculated using Equation (2a) given below:

$$OD_{Th} = \frac{16(2c + \frac{1}{2}h - o)}{MW}$$ (2a)

where, MW = molecular weight, c—moles of carbon, h—moles of hydrogen, o—moles of oxygen.

The molecular formula for tannin is C$_{76}$H$_{52}$O$_{46}$ and the molecular weight is 1701 g/mol. The ThOD was calculated to be 1.24 mg O$_2$/mg tannin. Theoretical oxygen uptake (OU$_{Th}$) can be calculated by multiplying OD$_{Th}$ with milligram of tannin present in the wastewater. Observed oxygen demand (OD$_{Obs}$) is calculated by dividing observed oxygen uptake (OU$_{Obs}$) from the respirometer by milligram of tannin concentration present in the wastewater as given in Equation (2b).

$$OD_{obs} = \frac{OU_{obs}}{mg \text{ of tannin concentration}}$$ (2b)

Primary degradation of tannins

Primary degradation of tannins was derived at the end of respirometer studies that is, 28 days period. According to OECD 301 C protocol, the primary degradation of a specific substance can be calculated using Equation (3) and the specific substance considered was tannin in the present study.

$$D_t = \frac{S_t - S_b}{S_t} \times 100$$ (3)

where, $D_t$—primary degradation %; $S_b$—concentration of tannin in the control reactor (blank) after 28 days; $S_t$—concentration of tannin in the test reactors after 28 days.

Half-life period determination

The biodegradation of tannin containing wastewater with different tannin concentrations was derived with the help of first order kinetics. The first order kinetic parameter $k$ was calculated using Equation (4). The half-life period of tannins were arrived from the first order kinetic equation using the initial and final tannin concentration data obtained from respirometer.

$$C_{rem} = C_0e^{-kt}$$ (4)

where $C_{rem}$—remaining tannin concentration after test period of 28 days (mg/L); $C_0$—initial tannin concentration (mg/L); $k$—first order rate constant.
From the $k$ value, calculated $t_{1/2}$ was calculated using Equation (5).

$$\text{Calculated } t_{1/2} = \frac{\ln 2}{k}$$

(5)

Observed half-life period (Observed $t_{1/2}$) was calculated using the tannin concentration data obtained from the aerobic reactors.

3 | RESULTS AND DISCUSSION

3.1 | Wastewater characterization

The VTW was characterized for pH, COD, BOD$_5$, Sulphates, and Solids content and is presented in Table 1.

It was observed from the characteristic of VTW that the COD was in the range of $22000 \pm 1350$ mg/L and tannin concentration was around $19800 \pm 2200$ mg/L. This showed that around 85% of COD was contributed by tannins. But BOD$_5$ was observed to be very less, around $3800 \pm 520$ mg/L. BOD$_5$/COD ratio was in the range of $0.17 \pm 0.03$, which pointed to a low biodegradability index. This sectional stream of VTW is mixed with other sectional streams of wastewater generated during pre-tanning process and reaches the CETPs. Due to dilution with other sectional streams, the concentration of tannins observed in the composite wastewater generated from raw to vegetable tanning process was calculated to be around 850-1350 mg/L. Hence, to study the effect of tannins on aerobic treatment, concentrations of 540, 900, and 1350 mg/L were selected, considering the concentration of tannins present in the composite wastewater generated from raw to vegetable tanning process.

3.2 | OUR w.r.t. tannin concentration

Cumulative oxygen uptake data was obtained from AER-800-4 V20A software coupled with the respirometer. The OUR derived from Equation (1) is presented in Figure 2. From Figure 2 in the control reactor C, the first peak of OUR that is, 14 mg O$_2$/day was observed. According to OECD methods and ISO standards, the control reactor value should not exceed cumulative oxygen uptake of 60 mg O$_2$ for 28 days.$^{25}$ As per data obtained from the present study, cumulative oxygen uptake was 58 mg O$_2$ for 28 days, which confirmed that only endogenous respiration was observed in the inoculum.

The OUR profile reached the initial sharp peak at 74, 102, and 164 mg O$_2$/L for the sample T1, T2, and T3 respectively at the end of 24 hours. The initial pyrogallol content present in the wastewater was found to be 150, 220, and 290 mg/L for the three reactors T1, T2, and T3, respectively. The rapid consumption of oxygen uptake at the end of 24 hours was attributed due to the pyrogallol present in T1, T2, and T3 reactors, respectively. Similar observations were reported by Lobo et al.$^{26}$ that a sharp peak of respiration was observed when pyrogallol was injected to the respirometer study. Similar OUR peak and the observations reported from other studies are listed in Table 2. All these previous studies reported first peak in the OUR curve within hours of the experiment for easily degradable substrates. In the present study, the pyrogallol content is the hydrolysable tannin portion present in the wastewater, which is biodegradable, but the first peak in the present study was delayed up to 24 hours. The delay in the OUR peak was attributed to the presence of tannins in the wastewater, which are difficult to degrade.

| S. no | Parameter         | Range               |
|-------|-------------------|---------------------|
| 1     | pH                | $3.8 \pm 0.4$       |
| 2     | COD, mg/L         | $22000 \pm 1350$    |
| 3     | BOD$_5$, mg/L     | $3800 \pm 520$      |
| 4     | BOD$_5$/COD ratio | $0.17 \pm 0.03$     |
| 5     | Tannin, mg/L      | $19800 \pm 2200$    |
| 6     | Pyrogallol, mg/L  | $2400 \pm 280$      |
| 7     | Suspended solids, mg/L | $3600 \pm 340$ |
In sample T1, a smaller second peak was observed on the fifth day, which represented the breakdown of tannins and subsequent formation of pyrogallol. In samples T2 and T3, the second peak was observed on the seventh and ninth day, respectively. The pyrogallol concentration corresponding to the second peak observed in the aerobic reactor studies was found to be 139, 296, and 413 mg/L for T1, T2, and T3, respectively.

### 3.2.1 Comparison of COD, BOD<sub>5</sub>, and tannin removal w.r.t oxygen consumption

The aerobic reactor studies were conducted simultaneously to monitor the removal of COD, BOD<sub>5</sub>, tannin, and pyrogallol concentration during the 28 days of study period. The composition and initial characteristics of the parameters in the aerobic reactors are presented in Table 3.

The data obtained from the aerobic reactor studies and the cumulative oxygen uptake obtained from respirometer were compared and depicted in Figure 3A-C for the three tannin concentrations studied. It can be observed from Figure 3A-C that the oxygen uptake increased, and correspondingly the tannin concentration decreased. The initial COD and BOD<sub>5</sub> of T1 were found to be 1080 and 270 mg/L, which gradually decreased up to 431 and 191 mg/L, respectively till the eighth
### Table 3  Sample composition in the reactors

| S.no. | Reactor ID | Initial concentration (mg/L) |  |
|-------|------------|------------------------------|---|
|       |            | COD                          | BOD<sub>5</sub> | Tannin | Pyrogallol |
| 1     | T1         | 1080 ± 210                   | 270 ± 95       | 540 ± 115 | 150 ± 35  |
| 2     | T2         | 1860 ± 180                   | 470 ± 120      | 900 ± 140 | 220 ± 50  |
| 3     | T3         | 2720 ± 310                   | 735 ± 145      | 1350 ± 185| 290 ± 80  |

**Figure 3** Changes in COD, BOD<sub>5</sub>, tannin, and pyrogallol concentration with respect to oxygen uptake (A) T1–540 mg/L tannin (B) T2–900 mg/L tannin (C) T3–1350 mg/L tannin
day. The COD removal was comparatively higher than BOD$_5$ removal in the 8 day period, which showed that tannins were difficult to biodegrade. Similar trend of gradual decrease in pyrogallol concentration was observed for the samples T1, T2 and T3.

The sample T1 and T2 took 4 and 8 days to reach an oxygen uptake level to 482 and 463 mg O$_2$, respectively, but the sample T3 showed an increase in oxygen uptake up to 510 mg O$_2$ on the fourth day itself. It showed that increasing the concentration of tannins also increased the pyrogallol presence in the sample. In T3, the COD removal was significant for the first 4 days from 2720 to 1380 mg/L, but BOD$_5$ removal was found to be far less from 736 to 690 mg/L till the fourth day. Correspondingly, the pyrogallol concentration reduced from the first to the eighth day. But after the eighth day, it was found to be increase from 136 to 149 mg/L on the 12th day for T3. This increase in pyrogallol is reflected in the OUR curve with a peak at ninth day to 45 mg O$_2$ from 42 mg O$_2$ at ninth day for T3. The COD and BOD$_5$ reached 763 and 195 mg/L after 28 days for the sample T3 as shown in Figure 3C, which is very high when compared to T1 and T2.

### 3.3  Theoretical oxygen demand (OD$_{Th}$) and observed oxygen demand (OD$_{Obs}$)

The details of the calculated OD$_{Th}$ and the observed oxygen demand OD$_{Obs}$ for each tannin concentration studied is given in Table 4. The actual theoretical oxygen demand (OD$_{Th}$) of a tannin molecule was found to be 1.241 mg O$_2$/mg tannin. From this OD$_{Th}$ value, for each concentration of tannin, the theoretical oxygen uptake could be calculated. For example, for 540 mg of tannin, the theoretical oxygen uptake will be 648 mg O$_2$ for the 28 days study period. It was found that the OU$_{Th}$ was 648, 1080, and 1620 mg O$_2$ for T1, T2, and T3, respectively. But the experimentally observed Oxygen Uptake OU$_{Obs}$ from the respirometer studies at the end of 28 days gave a cumulative oxygen uptake of 747, 900, and 1037 mg O$_2$ for T1, T2, and T3, respectively. It was observed that, for T1 alone, the OU$_{Obs}$ was higher than the OU$_{Th}$, but for T2 and T3 OU$_{Obs}$ it was lower than the OU$_{Th}$.

From the OU$_{Obs}$ values, the observed oxygen demand OD$_{Obs}$ could be calculated using Equation (2b). The OD$_{Obs}$ value was found to be 1.38, 1.00, and 0.76 for T1, T2, and T3, respectively. For T1, the OD$_{Obs}$ (1.38) was higher than the OD$_{Th}$ (1.24), which depicted that tannins had not significantly affected the oxygen uptake. But for T2 and T3, the OD$_{Obs}$ was lower than OD$_{Th}$ that is, 1.00 and 0.76, respectively. This showed that when the tannin concentration was higher at 900 and 1350 mg/L, the oxygen uptake was significantly affected. This effect was reflected in the primary degradation % of tannins and also in the COD and BOD$_5$ removal efficiencies.

### 3.4  Primary degradation of tannins

The pass levels for the ready biodegradability tests stipulate that 70% Dissolved Organic Carbon (DOC) removal or 60% ThOD removal should be achieved in 28 days. In this study, raw VTW was used as the substrate. The wastewater predominantly contains tannin and contributes to COD. Hence the primary degradation % of tannin was calculated using Equation (3) as described in OECD guidelines.25

As per OECD guidelines, the pass levels have to be reached in 10-days within the 28-day period of the test. The 10-day window begins when the degree of biodegradation has reached 10% DOC, ThOD, or ThCO$_2$ and must end before day 28 of the test.15 The primary degradation of the tannin samples with respect to their initial concentration of tannin is represented in Figure 4.

It was observed that, the % primary degradation decreased as the initial tannin concentration increased. For sample T1, the highest degradation of 78% was attained, but for T2 and T3, the degradation percent was 50% and 14%, respectively. Xylitol (C$_5$H$_{12}$O$_5$), a food additive compound was biodegraded by 82% after 14 days in OECD 301C test.31 The inhibitory effects of tannins on bacteria have been associated with the binding to extracellular matrix, inhibition of cell membrane

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**Table 4** Comparison of theoretical and observed oxygen demand (OD$_{Th}$ and OD$_{Obs}$)

| Tannin concentration (mg/L) | Theoretical oxygen uptake OU$_{Th}$ (mg O$_2$) | Observed oxygen uptake OU$_{Obs}$ (mg O$_2$) | Observed oxygen demand OD$_{Obs}$, (mg O$_2$/mg tannin) |
|-----------------------------|---------------------------------------------|---------------------------------------------|-------------------------------------------------|
| 540 (T1)                    | 648                                         | 747                                         | 1.38                                            |
| 900 (T2)                    | 1080                                        | 900                                         | 1.00                                            |
| 1350 (T3)                   | 1620                                        | 1037                                        | 0.76                                            |
and enzyme activity and substrate. In this study, it was observed that the degradation levels of tannin in the 28 days period was affected by the increase in tannin concentration.

3.5 Half-life period determination

It is observed from the above results that the initial tannin concentration plays a vital role in degradation of tannins. Calculated $t_{1/2}$ represents the half-life period calculated from first order kinetic equation using the data obtained from respirometer study, that is, initial and final tannin concentration after 28 days. Experimentally observed $t_{1/2}$ represents the time taken for the tannin concentration to reduce into half of its initial concentration from the aerobic reactors. Table 5 shows the initial and final tannin concentration after 28 days study with respirometer, the calculated $k$ value, $t_{1/2}$ value and the experimentally observed $t_{1/2}$ for the three tannin concentrations tested. The calculated $t_{1/2}$ values were approximated to 8.8, 10.9, and 12.9 days for reactors T1, T2, and T3, respectively.

From the aerobic reactor studies, the tannin concentrations were estimated and the time to reach half of the initial concentration was calculated as presented in Figure 5. It was seen that, for sample T1, the time taken to reach half of the

| $C_o$ (initial tannin at $t = 0$) | $C_{rem}$ (final tannin at $t = 28$ days) | $k$ Value | Calculated $t_{1/2}$ (days) | Observed $t_{1/2}$ (days) |
|----------------------------------|----------------------------------------|------------|-----------------------------|---------------------------|
| 540                              | 60                                     | 0.078      | 8.8                         | 9.8                       |
| 900                              | 152                                    | 0.064      | 10.9                        | 11.5                      |
| 1350                             | 283                                    | 0.056      | 12.4                        | 12.6                      |
initial concentration, that is, 270 mg/L was 9.8 days. For sample T2, the time taken to reach half of the initial concentration, that is, 450 mg/L was 11.5 days. For sample T3, the time taken to reach half of the initial concentration, that is, 675 mg/L was 12.6 days.

It was observed from the results that the first order kinetics suits well for degradation of tannin. The calculated \( t_{1/2} \) and experimentally observed \( t_{1/2} \) were found to have a good correlation. Stasinakis et al.\(^{33}\) reported the calculated half-lives were 1.3 and 1.8 days, days for bisphenol A and triclosan, respectively. In the presence of acclimatized biomass, half-lives of 5.0 days were calculated for triphenyltin as reported by Stasinakis et al.\(^{34}\) whereas with non-acclimatized biomass the half-lives were higher than 18 days. Wang et al.\(^{35}\) reported that, when the initial tannin concentration was 150 mg/L, the first order kinetic rate constant \( k \) was 0.1919 after 24 hours. In the present study, the \( k \) value was found to be 0.078. For T2 and T3, the half-life period was approximately 11.5 and 12.6 days that is, it took 11.5 and 12.6 days for 50% degradation. This signified that 60% degradation was not achieved in the 10 day window period according to the pass levels for ready biodegradability. Hence, more emphasis should be given to improving the biodegradability of tannins emanating from VTW in tannery effluent treatment plants. The study showed that the presence of tannin adversely affected the aerobic treatment process and resulted in lower removal efficiency of COD, BOD\(_5\) and tannins. This is also reflected in effluent treatment plants treating wastewater containing tannins with lower removal efficiency of pollutants and presence of recalcitrant organics and color in the final treated effluent.

4 | CONCLUSION

This study signifies the effect of tannins from VTW during aerobic treatment, with the help of respirometry as per OECD 301 C. From the results observed, the following conclusions were drawn:

- The VTW was characterized by low BOD\(_5\)/COD ratio of 0.17 ± 0.03, which revealed a low biodegradability index.
- The respirometry test revealed that the initial sharp peak in the OUR profile of tannin containing wastewater was delayed when compared to studies with easily biodegradable substrates.
- Increasing tannin concentration significantly reduced the oxygen uptake as evidenced by the fact that OD\(_{OBS}\) was found to be higher than OD\(_{TH}\) (1.24) for T1 (1.38), but was lower for the samples T2 (1.00) and T3 (0.76).
- Primary percentage degradation of tannins decreased, that is, 78%, 50%, and 14% when tannin concentration was 540, 900, and 1350 mg/L, respectively.
- The half-life period determination from first order kinetic equation and the aerobic reactor studies showed that it took 9.8-12.6 days for 50% degradation for various tannin concentrations, which depicted that tannins were not readily biodegradable.

Hence the study established that emphasis is needed to improve the biodegradability of tannins from vegetable tanning process which are subjected to biological treatment.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHORS CONTRIBUTION

Abirami Balakrishnan contributed to the data curation, writing the original draft, review, and editing. Sri Bala Kameswari Kanchinadham contributed to the funding acquisition, investigation. Chitra Kalyanaraman contributed to the funding acquisition, methodology, supervision, writing the review and editing.
NOMENCLATURE

OUR  oxygen uptake rate (mg O₂/day)  
OD  oxygen demand  
ODₜh  theoretical oxygen demand (mg O₂/mg tannin)  
ODₜo  observed oxygen demand (mg O₂/mg tannin)  
OUₜh  theoretical oxygen uptake (mg O₂)  
OUₜo  observed oxygen uptake (mg O₂)  
MW  molecular weight  
Dₜ  primary degradation percentage (%)  
Cₐₚ  remaining tannin concentration after test period of 28 days (mg/L)  
C₀  initial tannin concentration (mg/L)  
k  first order rate constant  
Observed t₁/₂  observed half-life period from aerobic reactor studies (days)  
Calculated t₁/₂  calculated half-life period from first order kinetics (days)  
COD  chemical oxygen demand (mg/L)  
BOD₅  5 days of biochemical oxygen demand (mg/L)

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