Estimation and Biodegradation of Acetylsalicylic Acid in Contaminated Waste Water using ASP and UASB

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Abstract: ***The main Aim of this work was to study the degradation and removal of acetyl salicylic acid using aerobic and anaerobic wastewater treatment and optimization of the conditions to improve the removal of pharmaceuticals. The result of the studies showed that ASP and UASB reactors treated acetyl salicylic acid (300 mg/litre) effectively giving more than 90% of removal efficiency, if sufficient acclimatization period is provided.***

Keywords: Activated sludge process (ASP), Up flow Anaerobic Sludge Blanket (UASB) reactor, Bio degradation, Acetyl salicylic acid, Removal efficiency

1. Introduction

Indian Pharmaceutical industry

The Indian pharmaceutical industry is globally the 3rd largest in terms of volume and 13th largest in terms of value. The Indian pharmaceutical market increased at CAGR of 17.46% in 2015 from U.S. $ 6 Billion in 2005 and is expected to expand at a CAGR of 15.92% to U.S. $55 bn by 2020.By 2020 India is likely to be among top three pharmaceutical markets by incremental growth and sixth largest market globally in absolute size.

In India there are about 250 large units and about 8000 small scale units and 20000 registered units. The Indian Pharma industry has around 70% of the country's demand for bulk drugs, drug intermediates, pharmaceutical formulations, chemicals, tablets, capsules, orals and injectable. Indian pharmaceutical industry is the largest exporter of drugs in terms of volume. Active Pharmaceutical Ingredients from the pharmaceutical companies are emerging pollutants in the aquatic environment, because of their dangerous effect on aquatic environment and humans. The wastewater from pharmaceutical industry consists of high TSS, COD, salts and other harmful chemicals. The chemicals present in the wastewater will depend on the product being processed by the industry. Pharmaceutical companies are ready to consider putting the present treatment technologies on hold and moving towards new cutting edge technology that can treat the effluent in complete at lesser cost.

Traditionally, wastewater treatment technology has been designed to combat the problems of nutrient concentration and microbial population, but recent studies have concentrated on nitrogen and phosphorus removal (Jones *et al.* 1998; Randall and Sen 1996; Rogalla *et al.* 2006; Sriwiriyarat and Randall 2005). However, there are many natural and synthetic compounds, such as pharmaceuticals, which are unable to remove by traditional systems, leading to chemical wastewater releases into the aquatic environment. (Giger *et al.* 2003; Hirsch *et al.* 1999; Sacher *et al.* 2001). In recent years, water companies and regulators have become more concerned about reports of concentrations of pharmaceuticals in sewage, as well as in the aquatic environment, such as lakes, rivers, groundwater, and drinking water (Ayscough *et al.* 2000; Hilton *et al.* 2004; Kanda *et al.* 2003; Stokes and Churchley 2006; Thompson *et al.* 2005). With improving analytical growth sense that more compounds can be detected at lower concentrations. Majority of the pharmaceuticals were removed at the secondary biological treatment processes (Boyd *et al.* 2005; Carballa *et al.* 2005; Jones *et al.* 2005; Miao *et al.* 2005; Nakada *et al.* 2006; Perez *et al.* 2005; Ternes *et al.* 2004; Verenitch *et al.* 2006).

Two types of secondary biological treatment systems were taken for the present study, aerobic treatment (Activated sludge process) and anaerobic treatment (Upflow Anaerobic Sludge Blanket reactor). Different types of biomass were used for these two systems. Composition and stability, and these two treatment methods interact with pharmaceuticals in different ways, particularly with biodegradation. Quantitative removal of pharmaceuticals by each type of biomass, and investigation of different reasons could lead to enhancing pharmaceutical removal using present wastewater treatment technologies.

2. Materials and Methods

Analytical methods

Analysis of Alkalinity, phosphates, sulphates, BOD, Ash content of the sludge, Sludge Volume Index (SVI), Chlorides, Total suspended solids (TSS), nitrates and COD were conducted in accordance with Standard Method (APHA 2000). Volatile fatty acid (VFA) concentration was measured after centrifuging the samples to remove the suspended solids. A gas chromatography (AIMIL-NUCON, India, Series 7500) equipped with a Flame Ionization

Detector (FID) and Chromasorb 101 column was used for the analysis of VFA. The detector, injector and oven temperature were 200, 195 and 180˚C, respectively. The carrier gas used was nitrogen, and a mixture of hydrogen and air was used to sustain the flame in the detector. The hazardous compounds were identified by acidifying aqueous samples to pH < 2, extracting the compounds with dichloromethane, filtering using a MDI Teflon syringe filters (SY25NN, 0.2 µm pore size) and injecting 1µl of the filtrate into a gas chromatograph (6890N, Agilent) with a mass selective detector (5973). A HP 5 MS, capillary column, (0.25mm, 30m, 0.25µm), was used for sample elution. All the experiments were carried out in triplicates and the values presented in this paper are a mean of three readings for reproducibility of the results.

The pharmaceutical wastewater, aerobic and anaerobic sludge used in the present study was brought from PETL. (Patancheru effluent plant, Hyderabad). The ASP and UASB reactors were operated continuously with varying organic loading rate of 0.5kg COD/m3/day. To access the suitability of the reactor for treating complex pharmaceutical wastewater under study. One day HRT is maintained in all the three reactors during the start-up period. The stable study state in the present study was defined based on the almost constant COD removal supported with constant operational parameters profile in all the three reactors.

Inoculum

The sludge, which is used for this study, was collected from common effluent treatment plant, Patancheru, Hyderabad. Physic-chemical analysis of pharmaceutical wastewater was done using standard methods (APHA, 2005). The characteristic of wastewater was shown in table. 1.0.

Characteristics of Bulk drug pharmaceutical industrial wastewater

Table 1: Characteristics of pharma effluent

S.NO	*Parameters*	*Concentration (mg/l)*
1	pH	7.65 ± 0.7
2	Electrical Conductivity	6550 ± 32 µ Siemens
3	Alkalinity	950 ± 12
4	Chlorides	1200 ± 46
5	Chemical oxygen demand	4000 ± 76
6	Biochemical oxygen demand	2000 ± 64
7	Color (O.D at 610nm)	0.267 ± 0.01
8	Ammonical nitrogen	25 ± 3
9	Total solids	1500 ± 30
10	Total dissolved solids	1100 ± 45
11	Total suspended solids	400 ± 40
12	Volatile suspended solids	200 ± 18

The stock solution of 1ml was added for a litre of feed. The ratio of COD: N: P was adjusted around at 350:6:2. The feed pH of the reactor system is continuously sustained at neutral by adding up essential quantity of orthophosphoric acid in the reactor. The pH of outlet is also always maintained between 7.5–7.6. This indicates the active methanogens metabolism in the reactor. The operation of anaerobic reactors regarding the pH was best studied Rajeswari et al., 2000.

3. Identification of Acetyl Salicylic Acid using HPLC

Apparatus and chromatographic conditions: We used an shimadzu HPLC equipped with a degasser, LC-2010CHT series, column Inertsil C18, 5m, 150 mm x 4.6 mm (shimadzu corporation, Japan) and we operated at 50 ^{0}C. The mobile phase was 2.5 pH buffer solution and isopropyl alcohol in the ratio of 14:85:1. The flow rate was 1.5ml/min and the injection volume was 5µL. The detector at 275nm as excitation wavelength. Salicylic acid was identified by the external standard method and by comparing of the retention times (RT) with the ones of the standards, in the same chromatographic conditions and quantified by the external standard method. (Duff et al., 1995).

The wavelengths for detection were chosen in order to give an optimal sensitivity and selectivity to this method. Standards of salicylic acid (Sigma, Germany) was used in order to perform quantitative determinations in distilled water solutions with known concentrations. The HPLC chromatogram for standards is presented in Figure 1.

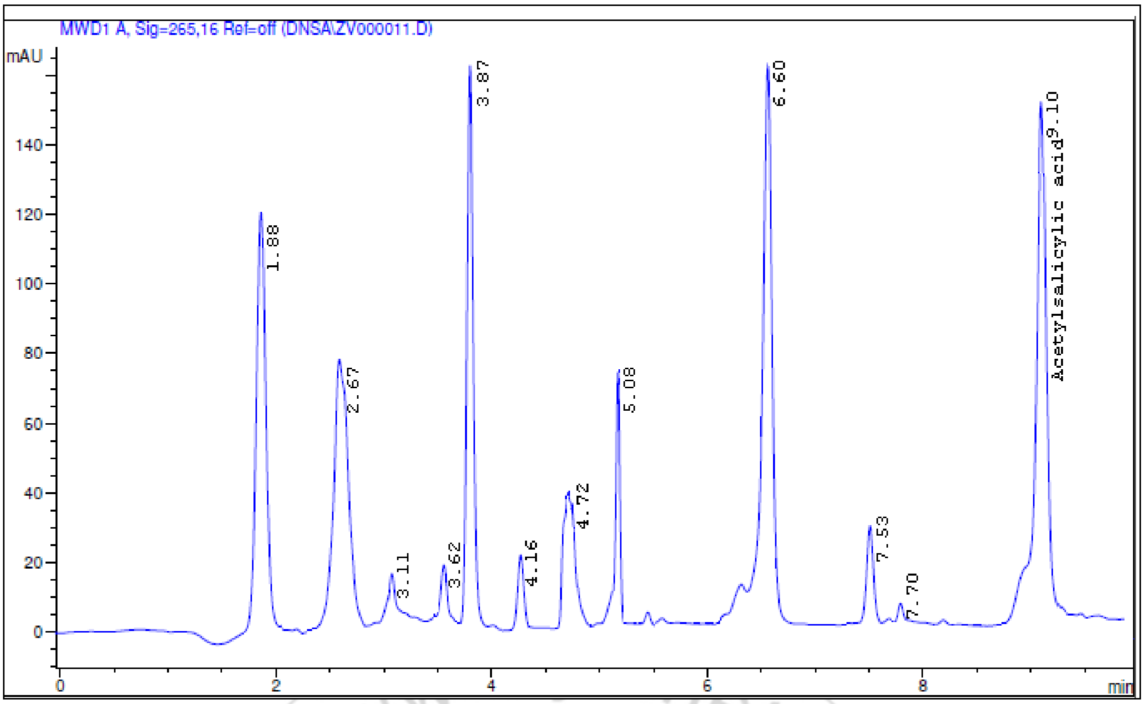

Figure: Identification of Acetyl Salicylic Acid

Table 2: HPLC Optimized conditions for Acetyl Salicylic Acid

Parameter	*Optimized condition*
Chromatograph	shimadzu HPLC
Column	Inertsil C18, 5m , 150 mm x 4.6 mm
Mobile phase*	2.5 pH buffer solution and isopropyl alcohol in the ratio of 14:85:1
Flow rate	1.5ml/min
Detection	275nm
Injection volume	5µl
Temperature	50^0C

4. Results and Discussion

Biodegradation of Acetylsalicylic acid using Activated Sludge Process

Table 3: Acetyl salicylic acid and COD removal with Activated sludge process

		Acetylsalicylic Acid mg/L		TSS mg/l	
HRT (hrs)	COD Removal %	Influent	Effluent	Influent	Effluent
30	36±2	30±1.5	19.2±1.5	65±3	28±2
25	35±3	30.1±1	19.6±2	65±4.5	27±3
20	35±4	30±2.3	19.5±2	65±5	26±2
15	34±2	30±1.5	19.8±1.5	64±3	27±1.5
10	34±1	30.2±2	20±1	64±4	28±1
5	33±2	30±1	20.1±2	65±2	27±2

The above table shows the variation of COD due to aeration after a certain interval. From the results it was observed that COD % reduction was increased with an increase in HRT from 33 to 36% at 5 to 30 hrs respectively, whereas the compound removal also followed same trend and the initial compound of 30mg/l was brought down to 19.2mg/l at 30 hrs HRT. Under optimum conditions along with the COD, concentration of TSS was also reduced, where it decreased from 65mg/l to 26mg/l at. After settling, the TSS in the settling tank the maximum TSS will be removed and thus an improved water quality will have achieved. The optimum HRT was found to be 30hrs for the treatment of Acetylsalicylic Acid.

Biodegradation of pharmaceutical compounds by using UASB reactor

Acclimation of UASB reactors with Pharmaceutical Compounds

The stabilization of reactor for treating pharmaceutical chemicals containing wastewater took almost 45 days with 30mg/l of influent chemical compounds respectively. During this period, the compound reduction and COD reduction and gas production was monitored and given in the results. From these results we had observed that at each increment of the chemical compound concentration, the compound reduction was decreased from 90% to 60% and at the end of the acclimatization period gradually it was increased to 90%. (Datar et al. 2001). The biogas production has been increased from 0.7 l/d to 4.5 l/d. This indicates that the compound is converted in to biogas. The methane percentage in biogas was varied between 65- 72% in all the UASB reactors.

Degradation of Acetyl Salicylic Acid in UASB reactor

Acclimation of UASB reactor with Acetyl Salicylic Acid

The effluent of the UASB reactor contains Acetyl Salisylic Acid with 5mg/l concentration. When the reactors reached to end of the acclimatization period, the removal efficiency of Acetyl Salicylic Acid was 94%.

HRT study on Acetyl Salicylic Acid

In case of Acetyl Salicylic Acid, the COD removal was decreased from 94 % to 79% with decreasing the HRT from 30 to 5h and the compound removal efficiency also decreased from 93 % to 82% and also found that 20h HRT was the optimum HRT for the degradation of Acetyl Salicylic acid.

Table 4: The effect of HRT on the biodegradation of Acetylsalicylic Acid

HRT	COD Removal %	Acetylsalicylic Acid mg/L		Effluent VFA mg/L	Biogas L/day
		Influent	Effluent		
30	94±1.2	30±1.2	6.6±0.8	110±3.5	2.3±0.1
25	91±2.1	30.1±1.5	6.321±0.5	106±4.2	2.3±0.2
20	88±2.5	30±1	5.1±0.6	102±3.5	2.1±0.1
15	85±3	30±2	6.3±0.3	104±2.5	2±0.1
10	82±2	30.2±1.3	6.04±0.4	98±2	1.9±0.2
5	79±1.5	30±1.6	5.4±0.2	98±3.4	1.7±0.1

The performance of the UASB reactors with respect to operational period in terms of COD removal percentage and Acetylsalicylic acid biodegradation were shown in the table 4.0. Each increment of HRT during acclimation period caused decrease in concentration of Acetylsalicylic acid in the effluent for first few days and dropped gradually before reaching a steady concentration. It was observed that stepwise increase in HRT from 5 to 30 hours caused increase in the removal efficiencies of COD and Acetylsalicylic acid degradation from 79% to 94% and 30 to 5 mg/L in UASB reactor. But increase in HRT to 30 hours showed decreasing performance in the removal efficiencies of Acetylsalicylic acid to 5.1 mg/L to 6.6 mg/L and took 35 days. In addition to the above parameters effluent VFA and Biogas production was monitored simultaneously and the same was presented in the same table, it was fallowing the same trend of the COD percentage removal and showing the best biogas yield at 30 days of 2.3 L/day and 110 VFA mg/L. The UASB reactor took more time than ASP reactor for acclimation and this may be due to slow growing anaerobic microorganisms than aerobes.

It can be concluded from the study that Acetylsalicylic acid degradation and COD removal percentage increased in UASB by providing the sufficient time to microorganisms to adjust to new feed composition.

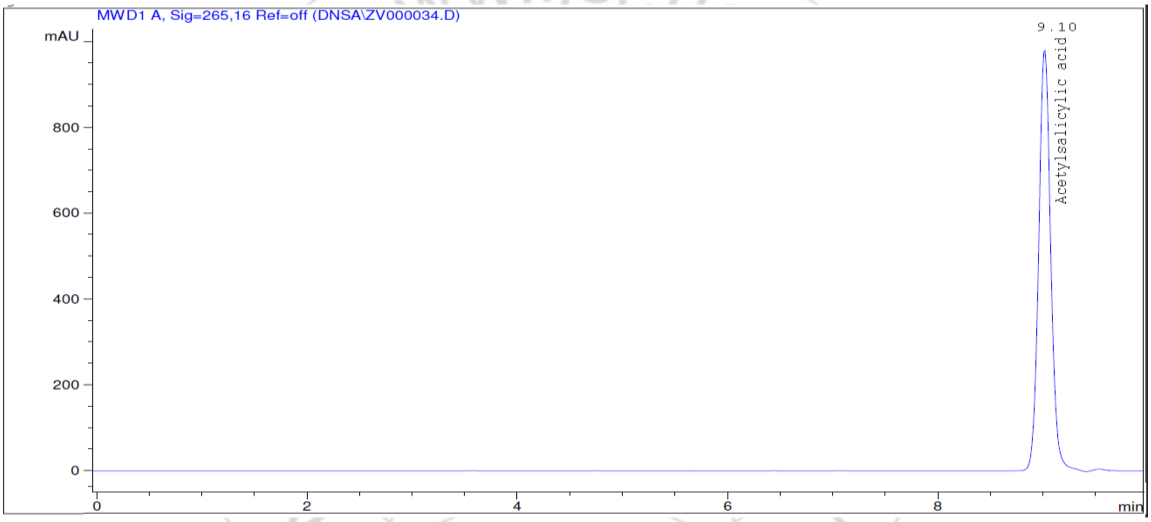

Figure 6: standardization of Acetylsalicylic acid

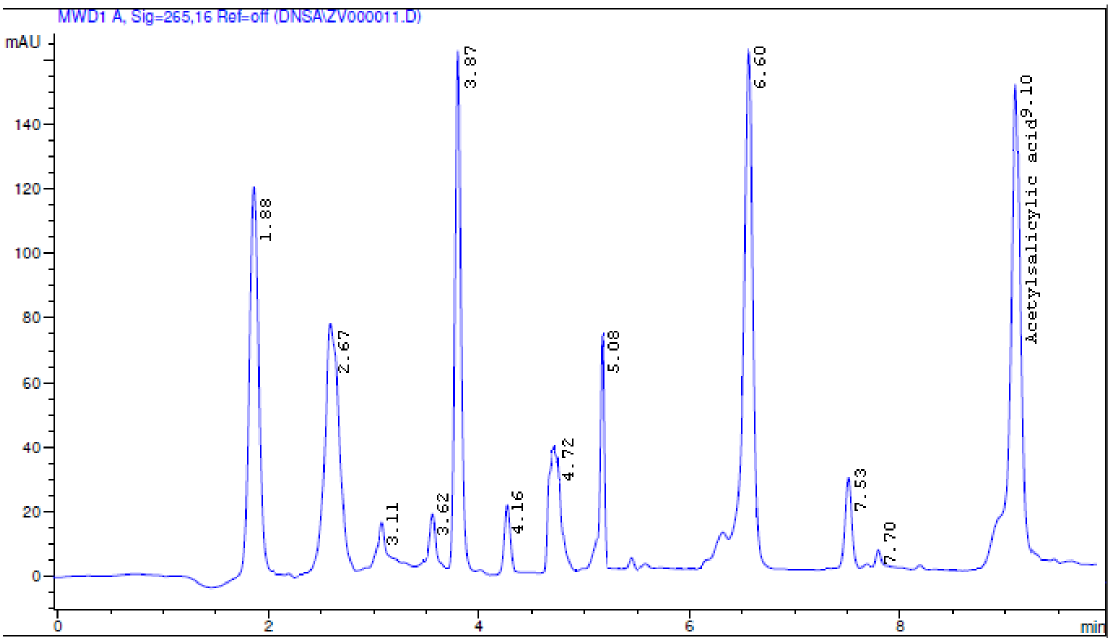

Figure 7: Concentration of Acetylsalicylic acid in Wastewater at 30 HRT

The degradation trend of Acetylsalicylic acid was observed by monitoring the influent and effluent concentrations of the compound using the standard curve (Figure 6.) developed by using optimized conditions of the HPLC. The concentration of the compound in the waste water (Figure 7) was compared with the standard graph obtained by the HPLC with the same optimization condition of the standard curve.

The trend of concentration variation of Acetylsalicylic acid degradation with respective to the number of days were presented in the figure 8 The maximum removal 97% was observed in 19 days and it was gradually dropped to 92% in 25days and it was stabilized at 94% in 30 days of the total acclimatization period 45 days.

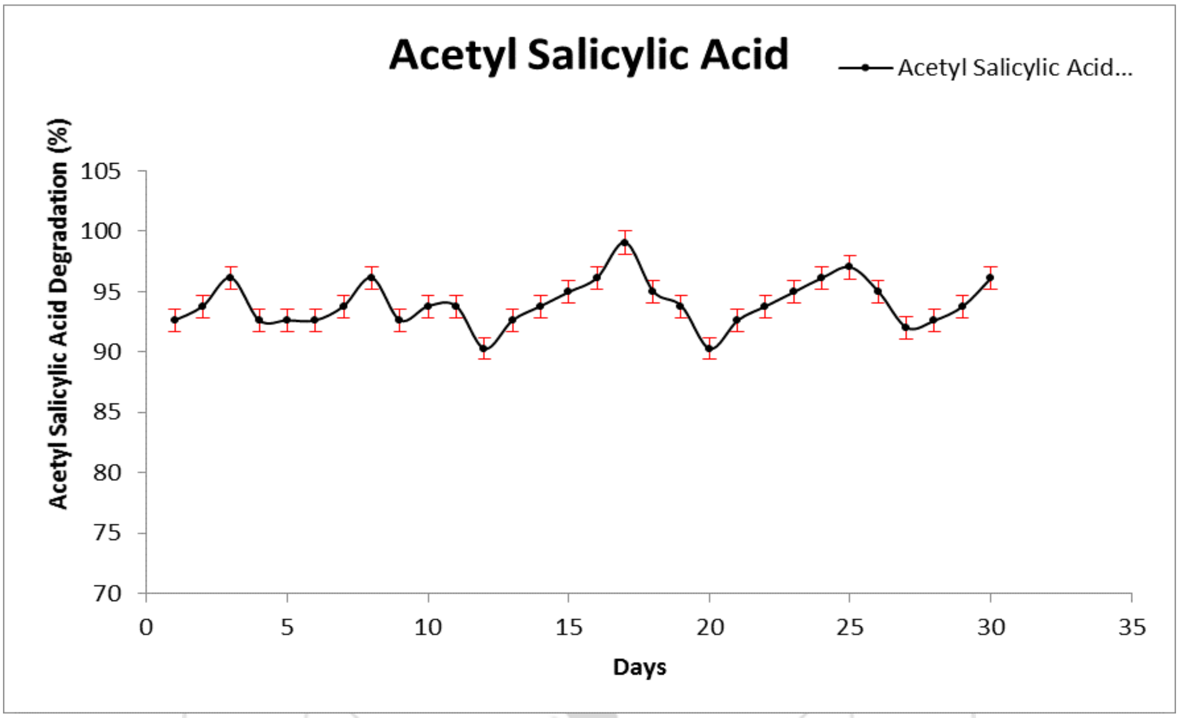

Figure 8: Concentration variation of Acetylsalicylic acid during Wastewater treatment at 30 HRT

Cost estimation

Cost estimation was done for a UASB reactor on the basis of wastewater generation. The overall costs are represented by the sum of the capital costs, the operating and maintenance costs. Cost estimation is used for the evaluation of feasibility to the pilot plant mode so that further this information will help to industry in scale up of the reactors. In the large scale systems these costs all are depend on the type of pollutant and how much concentration was present in that wastewater, and also depend on the quantity of wastewater, flow rate of the wastewater and size of the designed system. Table 5 shows the overall operational cost including electricity and nutrients for the degradation of pharmaceutical compounds. The total estimated operational cost was 2.2 US $ for degrading 50m3/day wastewater contain pharmaceutical compounds. But for reuse of wastewaters this cost is competitive by comparing with treated wastewater.

Table 5: Operational cost for UASB reactor

S. No.	Requirement	cost (US $/day)
1	Electricity	1.7
2	Nutrients	0.5
3	Total	2.2

5. Conclusions

- It can be concluded from the data obtained that the ASP reactor is efficient when compared to UASB in terms of start-up of the reactor (within 30 days where as UASB took 40 days).
- In ASP reactor the maximum COD removal efficiency of 97% at 20 hours HRT.
- The UASB reactor showed relatively efficient performance (97% COD removal at 20 hours HRT) compared to the ASP (96% COD removal at 20 hours HRT) in treating pharmaceutical wastewater but took more time to stabilize.
- The result of the studies showed that ASP and UASB reactors treated Pharmaceutical waste water (300 mg/litre) effectively giving more than 90% of removal efficiency if sufficient acclimatization period is provided.
- Earlier growth and acclimation of biomass was found in the ASP than UASB may be due to the presence of slime producing aerobes in the mixed sludge.

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