Treatment of Slaughterhouse Wastewater by Integrated Anaerobic/Aerobic Bioreactors Loaded with Immobilized Nanoporous Activated Carbon

by

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Abstract

Slaughterhouse wastewater consists of moderate to high strength complex wastewater comprising about 45% soluble and 55% coarse suspended organics exhibiting high COD and BOD levels. Conventional wastewater treatment methods cannot effectively treat slaughterhouse wastewater. Thus, a four-stage sequential anaerobic/aerobic immobilized bio reactor system comprising a two stage Fluidized Anaerobic immobilized Reactor (FAIR – I and FAIR – II), a Fluidized Immobilized Cell Carbon Oxidation (FICCO) reactor and a Chemo Autotrophic Activated Carbon Oxidation (CAACO) reactor was tested in a slaughterhouse treating wastewater between 3 m^3 /day to 17 m^3 /day. Nanoporous activated carbon (NPAC) was used for the immobilization of microorganisms in all of the reactors. The NPAC BET surface area was found to be 291 m^2/g with the average pore diameter of 28 Å. Spin density (free electrons) in the NPAC, was calculated to be 16×10^{18} spins/g using ESR spectroscopy. The overall NH_3-N, TKN, COD and BOD removal efficiency was 64%, 71%, 82% and 85% respectively. Multivariate analysis (PCA and cluster analysis) found that the COD removal by the FICCO and CAACO reactors is more efficient than the FAIR reactors. The treatment was confirmed through UV-visible and UV-fluorescence spectroscopic analysis.

Introduction

India has the largest population of livestock in the world (509 million), with 191 million cattle, 135 million goats, 109 million buffaloes, 64 million sheep, and 10 million pigs and over 729 million poultry referenced in the Department of Animal Husbandry, Dairying & Fisheries report, 2012. There are more than 3600 authorized slaughterhouses in the country. About 32.5% of sheep, 36.5% of goats, 1.9% of buffaloes, 28% of pigs, and 0.9% of cattle are slaughtered every year.[1,2] Slaughterhouse produces wastewater containing about 45% soluble and 55% coarse suspended organics exhibiting high Chemical oxygen demand (COD) and Biochemical oxygen demand (BOD) levels.[3] Moreover, the slaughterhouse wastewater is highly proteinaceous in nature and thus it has a high putrefaction rate which leads to environmental pollution problems.[4,5] Further, it also contains blood, undigested food, suspended solids due to rumen contents, flesh pieces, feathers, and pieces of bone.[6] Thus, it may cause many diseases such as tuberculosis, Salmonellosis and Helminthosis if not properly treated before disposal. Improperly treated slaughterhouse wastewater results in de-oxygenation of the water bodies and leads to ground water contamination.[1,7]

Conventionally, anaerobic treatment systems were found to be suitable for the slaughterhouse wastewater treatment.[8-10] Later the Dissolved Air Flotation (DAF) process,[11,12] the Up-flow Sludge Blanket Reactor (USAB) process,[1,13-14] and an anaerobic up-flow contact process[15,16] were used for the treatment. Due to the inefficiency of a single process alone for the treatment of complex slaughterhouse wastewater, many hybrid systems were subsequently explored. Manjunath et. al. (2000) studied the slaughterhouse wastewater treatment by DAF-UASB hybrid reactors.[17] Chen and Lo (2003) studied the slaughterhouse wastewater in treatment plant using two-phase biological system of activated sludge/contact aeration process.[18] Rajkumar et. al. (2012) used Anaerobic Hybrid Reactor System packed with pleated polyvinylchloride rings to treat poultry slaughterhouse waste water.[19] Later Sunder and Satyanarayanan (2013) investigated an Anaerobic Hybrid Reactor System packed with special floating media to treat poultry slaughterhouse wastewater.[1] Though many lab scale hybrid reactors system were successful, only a few processes were proven in the field scale studies. Hence, there has been a constant search for the optimal hybrid reactor system for effective oxidation with minimal sludge production. Thus, this present research was designed to study the efficacy of a four-stage sequential hybrid anaerobic and aerobic bioreactor system containing NPAC, as a catalyst to immobilize and enhance microorganism participation, at field scale.

Specifically, this research evaluates the efficiency of sequential bio reactors comprising of a two stage Fluidized Anaerobic Immobilized

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Reactor (FAIR – I and FAIR – II) followed by a FICCO (Fluidized Immobilized Cell Carbon Oxidation) reactor and a CAACO (Chemo Autotrophic Activated Carbon Oxidation) reactor installed in a slaughterhouse.

Materials and methods

Materials

All the chemicals used in the study were purchased from Merck, India. The mixed consortia used for the degradation of the organics present in the slaughterhouse waste water was cultivated from the acclimatized slaughterhouse wastewater.

Source and collection of slaughterhouse wastewater

The common facility center in Chennai used to slaughter goat and sheep was selected to validate the treatment scheme. The slaughterhouse was to slaughter 100 to 600 animals per day with the maximum number of 600 animals on Sundays. The facility center has a provision to collect blood, animal organs and skin in a scientific way. The water consumption of the facility center varied from 2 m^3 /day to 19 m^3 /day at an average of 30 L per animal. The large and small intestines of the animals are washed to remove the ruminal contents. The wastewater along with ruminal fluids and cattle dung is collected in a collection tank.

NPAC preparation

Rice husk was pre-carbonized at 400°C, then activated at 800°C using phosphoric acid, washed several times with hot water, dried in a hot air oven and stored in desiccators. The NPAC prepared in this manner was used as the base catalyst in the four stage reactors.

NPAC characterization

N_2 adsorption–desorption isotherms were used to determine the surface area and pore size distribution. The NPAC N_2 adsorption–desorption isotherm was measured using an automatic adsorption instrument (Quantachrome Corp. Nova-1000 gas sorption analyzer). Electron spin resonance (ESR) spectra and spin density was obtained using a Bruker-IFS spectrometer. TEMPOL was used as the reference spin probe compound to carry out preliminary experiments. The detailed ESR methodology was referenced from Swarnalatha et al. (2009).[20] The C, H, and N content for NPAC produced at varying heat treatments was determined using a CHNS 1108 model Carlo Erba analyzer. The NPAC surface morphology was determined, using a Leo-Jeol scanning electron microscope (SEM). The NPAC sample was coated with gold by a gold sputtering device to enhance surface morphology visibility.

Treatment scheme for slaughterhouse wastewater

The wastewater from the slaughterhouse was screened two times, first through a coarse 25 mm screen followed by a fine 10mm screen. The screened slaughterhouse wastewater was treated through four-unit operation in series comprising of a two stage FAIR– I and FAIR – II reactor set followed by a FICCO and CAACO reactor (Figure 1). The slaughterhouse wastewater was tested through the above treatment sequence continuously for 30 days.

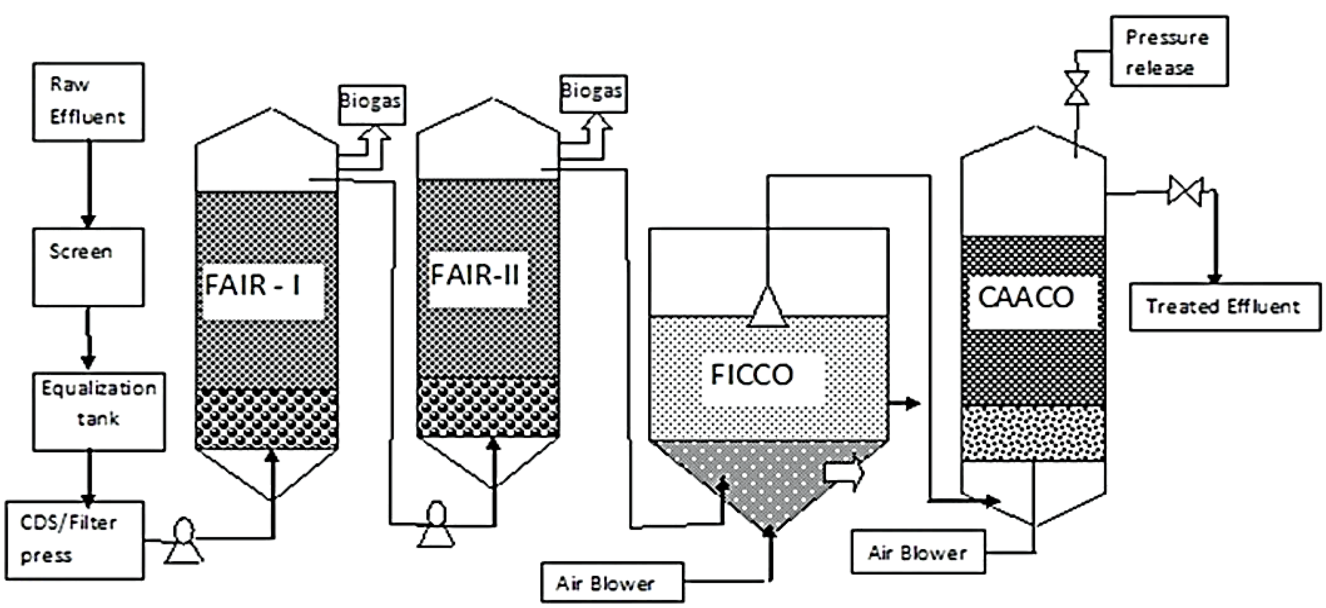

Figure 1. Schematic diagram of the four stage integrated treatment processes for the slaughterhouse wastewater

Description of the reactors

FAIR reactor details

The FAIR reactor is operated in an up-flow direction. The reactor was filled with NPAC to immobilize the microbes in the pores of the carbon matrix. The reactor has a provision to collect biogas generated during soluble and insoluble anaerobic mineralization. The settling zone in the reactor is filled with polypropylene contact medium to separate out suspended solids from the treated wastewater.

FICCO reactor details

The FICCO reactor comprises of three zones. The first zone is recognized as the "react zone" and is comprised of the immobilized carbon which is fluidized by air and wastewater at an up-flow velocity of 5 m/min. The quantity of air needed for the oxidation of organic compounds in wastewater is decided by the organic oxidation kinetics. The air required for the fluidization and oxidation of organics is supplied through the perforated pipe lines provided at the bottom of the reactor. The pressure of air required is a function of up-flow velocity, viscosity of medium, total solids content of the medium, temperature and height of the reactor. The second zone is the fluid separation zone. The unspent oxygen and nitrogen in air are separated using a triangular septum provided at the optimum height of the reactor. The separated air is collected through the perforated chamber. The third zone is the settling zone. The treated wastewater enters through the aperture and is allowed to settle on the inclined baffle plate. The angle of the plate is determined by the suspended solids settling velocity. The settling tendency was enhanced by extending the surface area to capture the particles by including a polypropylene plastic media of defined geometry. The screened suspended solids are sloughed off from the media upon exceeding a critical thickness. The sloughed suspended solids slide back into the reactor through the aperture. The sludge accumulated in the reactor is withdrawn daily through a sludge withdrawal pipe line provided in the reactor.

CAACO reactor details

The CAACO reactor contains a bacterial cell (chemo autotroph) immobilized packed bed filled with NPAC immobilized with *Bacillus sp.,* which is isolated from a facultative lagoon.[21] The air required for the oxidation is provided through a packed bed at two levels passing through perforated pipelines. The wastewater to be treated is transferred to the bottom of the reactor and in an upward flow direction. The treated wastewater is collected from the top of the reactor. The air required for the oxidation of organics is determined by the COD load in the wastewater. The pressure of air is determined by the head loss encountered during the oxidation of the organics in wastewater.

Chemical analysis and instrumental methods

In accordance with standard methods,[22] parameters such as pH, Total Kjeldahl nitrogen (TKN), chemical oxygen demand (COD), biological oxygen demand (BOD), ammoniacal nitrogen (NH_4–N), and volatile fatty acids (VFA) were characterized in triplicate and average of the results were calculated. The attachment of microbes in NPAC used in FAIR-I, FAIR-II, FICCO and CAACO reactors was examined by JEOL JM 5600 Scanning Electron Microscope at 20 kV (JEOL, Japan) accelerating voltage with an electron beam of 5-6 nm.

Statistical analysis

SPSS software (version 18) was used to evaluate the descriptive statistics and correlation analysis. Principal component analysis (PCA) was executed with Varimax rotation (Kaiser Normalization). By applying Ward's method, Square Euclidian distances of standardized median values (Z scores) were used for cluster analysis (CA).

Results and Discussion

Characteristics of NPAC

The complete details of the characterization of the NPAC were elaborated in our previous studies[20, 22-25] and it was used for the effective immobilization of bio catalysts for the treatment of wastewater.[26-28] The carbon, hydrogen and nitrogen percentages were 48.45, 0.70 and 0.10, respectively. Spin density, which is equal to the free electrons, was calculated as 16×10^{18} spins/g by using ESR spectroscopy. The specific surface area of NPAC was calculated using the BET model. The mesoporous surface area was calculated by the *t*-plot method. The results are presented in Table I.

Table I

Surface areas, pore volume and pore dimension of ECS

Parameters	Values
S_{BET} (m^2/g)	291
S_{mes} (m^2/g)	83
S_{mic} (m^2/g)	208
Mesopore volume, V_{meso} (cm^3/g)	0.08801
Micropore volume, V_{micro} (cm^3/g)	0.10035
Total pore volume, V_{tot} (cm^3/g)	0.18836
V_{meso}/V_{total} (%)	46.72
Average pore diameter (Å)	28

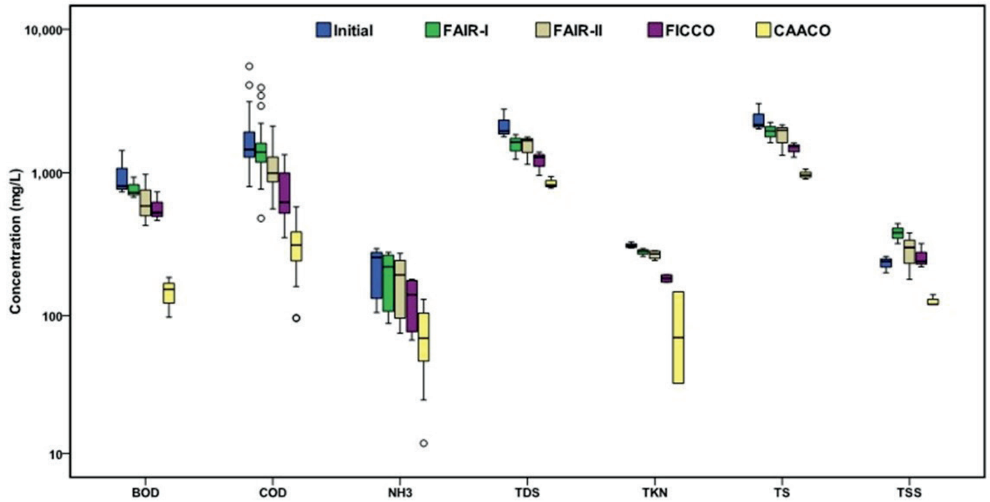

Figure 2. Four stage integrated treatment Box-and-whisker plots

Table II

Slaughterhouse wastewater treatment efficacy at the various stages

Parameters	Values	Initial	FAIR-I	FAIR-II	FICCO	CACCO
BOD, mg/L	Mean	855.00	777.33	662.33	575.00	145.17
	Median	735.00	727.00	585.00	525.00	153.00
	Std. Deviation	520.48	134.75	282.07	141.77	44.27
COD, mg/L	Mean	1832.24	1552.36	1084.40	741.92	314.88
	Median	1452.00	1392.00	992.00	621.50	312.00
	Std. Deviation	1061.35	803.52	413.84	302.27	125.70
NH_3-N, mg/L	Mean	216.17	198.17	181.75	130.67	72.50
	Median	256.00	220.00	193.00	140.50	69.50
	Std. Deviation	76.26	76.11	76.32	46.46	38.57
TDS, mg/L	Mean	2166.67	1570.00	1530.00	1210.00	846.67
	Median	1950.00	1630.00	1680.00	1280.00	820.00
	Std. Deviation	529.37	304.47	340.73	223.38	83.27
TKN, mg/L	Mean	309.25	280.00	267.25	182.50	90.00
	Median	306.00	283.00	270.50	182.50	90.00
	Std. Deviation	12.84	14.40	19.53	12.12	65.82
TS, mg/L	Mean	2400.00	1936.67	1816.67	1470.00	973.33
	Median	2150.00	1950.00	1980.00	1520.00	960.00
	Std. Deviation	549.45	310.21	438.44	170.59	80.83
TSS, mg/L	Mean	233.33	380.00	286.67	260.00	126.67
	Median	240.00	380.00	300.00	240.00	120.00
	Std. Deviation	30.55	60.00	100.66	52.92	11.55
Total Bacterial Count, cfu/mL	Mean	16×10^6	12×10^7	18×10^6	13×10^5	6.9×10^4
	Median	14×10^6	11×10^7	17×10^6	16×10^5	2.5×10^4
	Std. Deviation	9.9×10^6	7.1×10^6	7×10^6	7.7×10^5	11×10^4
Total Coliforms, cfu/mL	Mean	13×10^5	11×10^6	17×10^5	3.5×10^5	2.6×10^4
	Median	12×10^5	10×10^6	13×10^5	2.1×10^4	2×10^3
	Std. Deviation	8.6×10^5	7.8×10^5	8×10^5	7.8×10^5	9.1×10^4

Performance evaluation of the four stage reactors

The multiple box and whisker plots for parameters such as BOD, COD, NH_3-N, TDS, TKN, TS and TSS for the initial wastewater, outlet of FAIR-I, FAIR-II, FICCO and CAACO are given in Figure 2. The normality of the data for each parameter in each reactor was elucidated through skewness and kurtosis along with Shapiro-Wilk test which is more appropriate for the sample size of less than 50.[29] The mean, median and Std. Dev. of initial wastewater, outlet of FAIR-I, FAIR-II, FICCO and CAACO for parameters such as BOD, COD, NH_3-N, TDS, TKN, TS and TSS is presented in Table II.

The COD and BOD median values present in the initial slaughterhouse wastewater were 1452 mg/L and 735 mg/L respectively with a biodegradable index of 0.50 indicating that the wastewater has significant biodegradable organics. The organic nitrogen content was only 50 mg/L derived from animal protein. The remaining part of biodegradable organics is contributed by polysaccharides and fatty substances. The wastewater characteristics suggest that it is a good candidate for anaerobic treatment by the FAIR reactor pairing.

The median COD and BOD values after treatment by the FAIR reactor pairing were 992 mg/L and 585 mg/L respectively. The median COD values after FICCO and CAACO was 622 mg/L and 312 mg/L respectively. The median BOD values after FICCO and CAACO was 525 mg/L and 153 mg/L. The organic nitrogen content was reduced to 20 mg/L whereas NH_3-N was finally reduced to 69.5 mg/L. ORP of wastewater was -47 mV indicating that the FICCO treated wastewater contained oxidized or stabilized products. In FICCO, the dissolved organics adsorb onto the carbon matrix and diffuse into the immobilized microbes and metabolized. The metabolized products are diffused back to the bulk medium. The oxidation of organics is facilitated by hydroxyl radicals generated from molecular oxygen (in the form of air).

Though the overall NH_3-N, TKN, COD and BOD removal efficiency was 64%, 71%, 82% and 85% respectively, a major removal of these parameters takes place at the CAACO reactor (COD, 63%; BOD, 77%; NH_3-N, 43% and TKN, 51%). The overall removal efficiency of TS and TDS is 61% and 62% respectively. Though the overall removal efficiency of TSS was 43%, increases in the TSS is observed in the anaerobic processes and FICCO when compared to the initial wastewater. This may be due to the suspended growth of anaerobic organism in the FAIR process which is also evident by the Total viable count.

Multivariate statistical analysis (PCA and cluster analysis)

Multivariate analysis was performed to find out the relation between several treatment systems[30,31] to study the trend of the COD and NH_3-N removal. Principal components (PC) with Eigen values higher than 1 were extracted by introducing a Varimax rotation along with Kaiser Normalization in the PCA analysis. Figure 3a signifies the relation between the treatment systems in COD removal while Figure 4a represents the relations of treatment systems in NH_3-N removal. PC1, PC2 and PC3 explained 48.8, 27.4 and 21.3 % (total of 97.5 %) of the variance in Figure 3a, whereas PC1 and PC2 in Figure 4b explained 73.7 and 24.7 % (total of 98.4 %) of variance in Figure 4a. To confirm the PCA associations, a comparison with cluster analysis can be made.[30] By applying Ward's method, Square Euclidean distances of standardized median values (Z scores) were used for clustering. Hierarchical clustering was performed and presented as a dendrogram by applying variables such as COD removal (Figure 3b) and NH_3-N removal (Figure 4b).

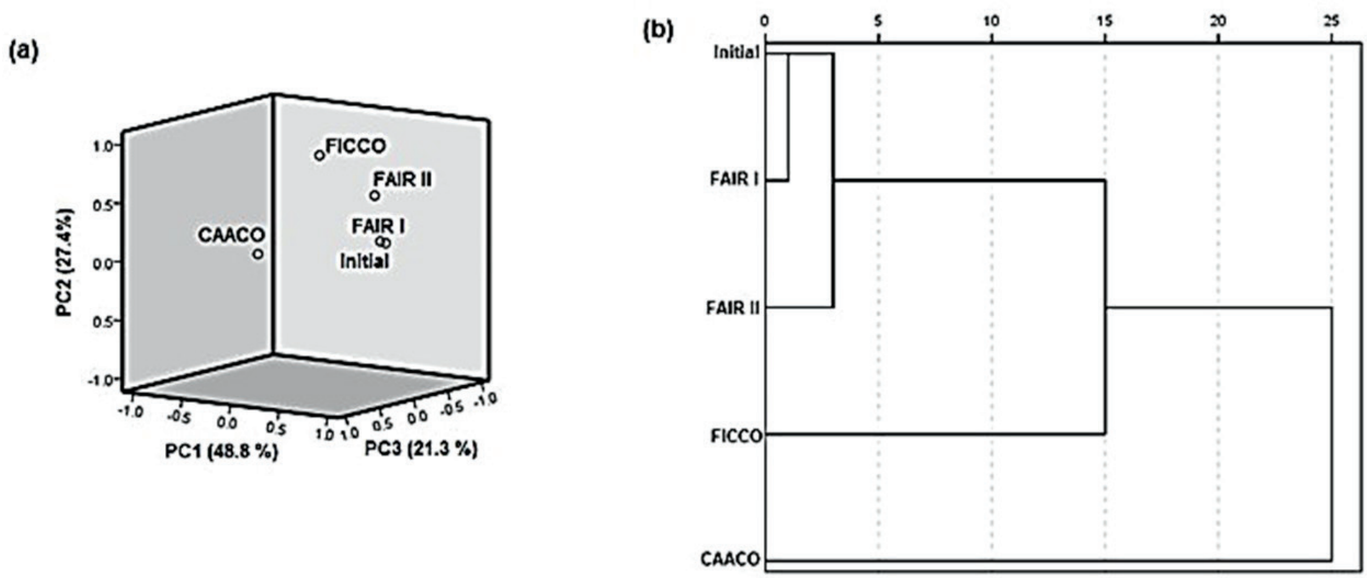

Figure 3. Trend comparison of various reactors in COD removal **(a)** Principal component analysis and **(b)** hierarchical cluster analysis

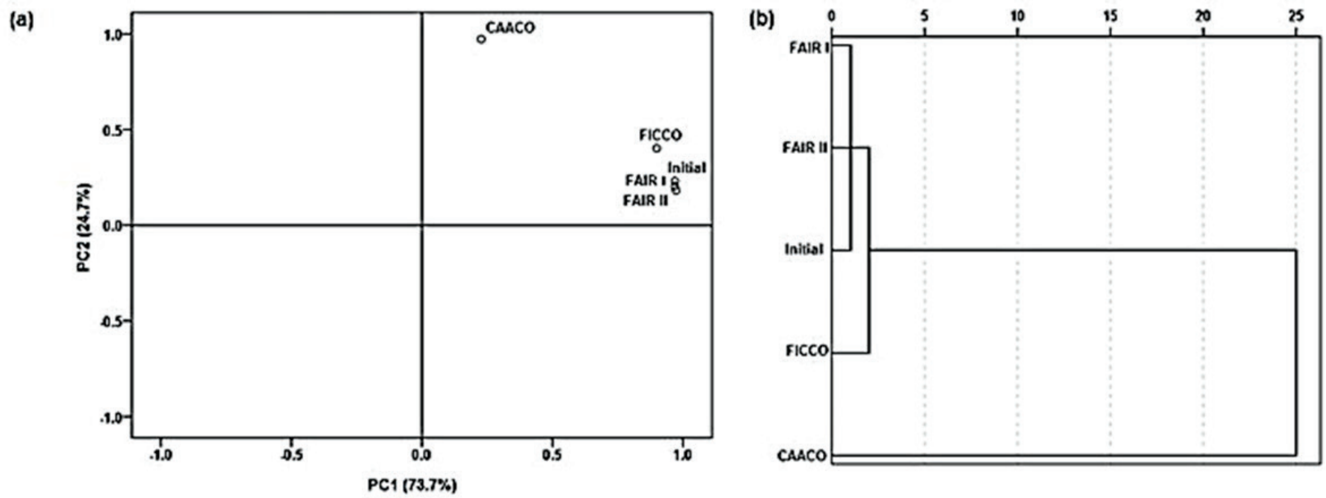

Figure 4. Trend comparison of various reactors in NH_3-N removal **(a)** Principal component analysis and **(b)** hierarchical cluster analysis for the integrated treatment processes for the slaughterhouse wastewater

The results indicated in Figure 3a and Figure 3b compared and can be elucidated as follows: PC3 in Figure 3a represented the treatment CAACO as a high positive score whereas FICCO represented in PC2 as high positive score. PC1 represented by Initial slaughterhouse water, FAIR I and FAIR II treatment process. In cluster analysis, the extreme distance among two clusters signifies the two most dissimilar groups.

The cluster analysis (Figure 3b) also showed the Initial, FAIR I and FAIR II as Group – I, whereas FICCO and CAACO as other individual groups. Thus, the trend of COD removal efficiency in FAIR reactors resembles each other and also to the initial slaughterhouse water whereas the trend in removal efficiency of CAACO and FICCO reactors are more efficient compared to the FAIR reactors. Figure 4b represents the CAACO as Group II which are all placed in a high positive score in PC1 of Figure 4a. Group I in Figure 4b is further sectioned into two groups in which group Ib represents FICCO which is placed in PC2 (Figure 4a) with a little lesser positive value when compared to Initial slaughterhouse water, FAIR I and FAIR II. Thus, it shows that the NH_3-N removal is more efficient in CAACO than other reactors.

Catalyst NPAC organic cleavage mechanistic view

NPAC use for organic compound degradation in slaughterhouse wastewater follows two pathways:

Can be used as the supporting matrix providing the space for the immobilization of microorganisms on its surface to increase the contact time between the organics and the organisms at aerobic/anaerobic conditions to enhance degradation.

NPAC itself serves as a better catalyst in presence of oxygen than anaerobic conditions. This may be due to the presence of free electrons in the conduction band and positive holes in the valence band. The electrons present in the conduction band initiate reaction (Equation 1) in the presence of oxygen to form reactive oxygen species.

$$-C\,(e^-_{cb}) + O_2 \rightarrow -C\,(O_2^{\bullet})_{ads} \qquad (1)$$

The formation of hydroxyl radicals takes place through the formation of hydroperoxyl radicals (Equation 2),

$$-C\,(O_2^{\bullet})_{ads} + H^+_{(aq)} \rightarrow -C(HO_2^{\bullet})_{ads} \qquad (2)$$

The adsorbed hydro peroxyl radicals are converted into hydroxyl radicals and remain adsorbed on the surface of NPAC (Eq. 3),

$$-C(HO_2^{\bullet})_{ads} + H^+_{aq} \rightarrow -C(2OH^{\bullet})_{ads} \qquad (3)$$

The positive charged centers in NPAC serves to adsorb the organic substrate (OS) and degrades the organics (Equations 4 and 5),

$$-C(h^+_{vb}) + (OS) \rightarrow -C^{+}(OS^{\bullet})_{ads} \qquad (4)$$

$$-C(2OH^{\bullet})_{ads} + -C^{*}(OS^{\bullet})_{ads} \rightarrow H_2O + CO_2 + -C(e^-_{cb}) + -C(h^+_{vb}) \qquad (5)$$

The above-mentioned mechanisms are theorized to explain how organic pollutants can be degraded.

Instrumental evidence

The morphology and attachment of organisms to NPAC used in FAIR-I, FAIR-II, FICCO and CACCO reactors were studied by Scanning electron Microscopy (SEM). The Figure 5(a) indicated the presence of pores on the surface of NPAC with large surface area as mentioned in Table I. The occupancy of aerobic/anaerobic organisms onto NPAC clearly shown in Figure 5 (b) to 5 (d). The figures denoted that the presence of different kinds of organisms

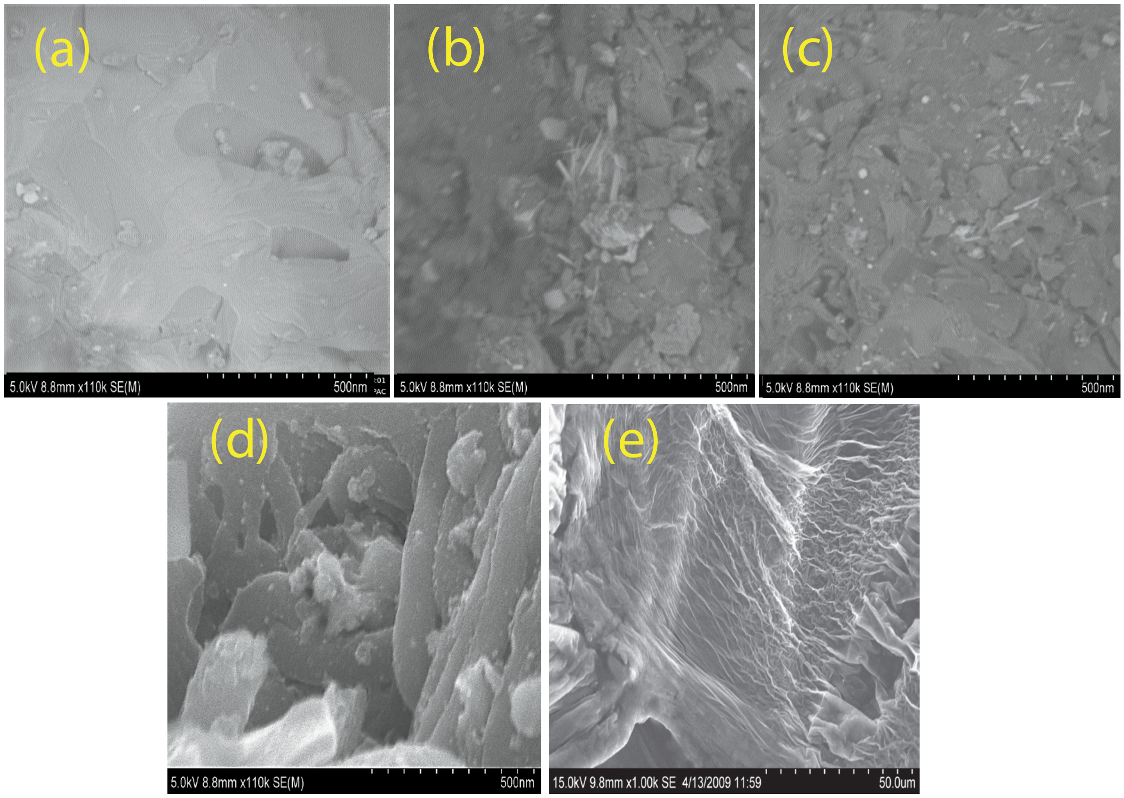

Figure 5. SEM images of **(a)** actual nanoporous activated carbon **(b)** nanoporous activated carbon used in FAIR-I **(c)** FAIR-II **(d)** FICCO and **(e)** CACCO reactors

on the surface of NPAC which were involved in the degradation of organic constituents from slaughterhouse wastewater discharged from tanneries.

UV-visible and fluorescence spectrum of initial and sequentially treated slaughterhouse wastewater was shown in Figure 6a and 6b. The results indicate that the peak around λ_{295} nm with high intensity due to the presence of π to π* transition and n to n* transition is responsible for unsaturated, reduced sulphur and nitrogen compounds present in slaughterhouse wastewater. After processing, a hypochromic shift was observed which indicates the removal of chemical population present in the wastewater and also a hypsochromic shift which indicates a breakdown of unsaturated compounds into simpler stable compounds which requires more energy to excite when compared with unsaturated compounds. UV-visible supports the data of Table II where the removal of both ammonia and TKN (n to n* transition) takes place with simultaneous removal of COD responsible of unsaturated compounds (π to π* transition). The UV-fluorescence spectrum supports the UV-visible results where both shifts reflect wastewater treatability.

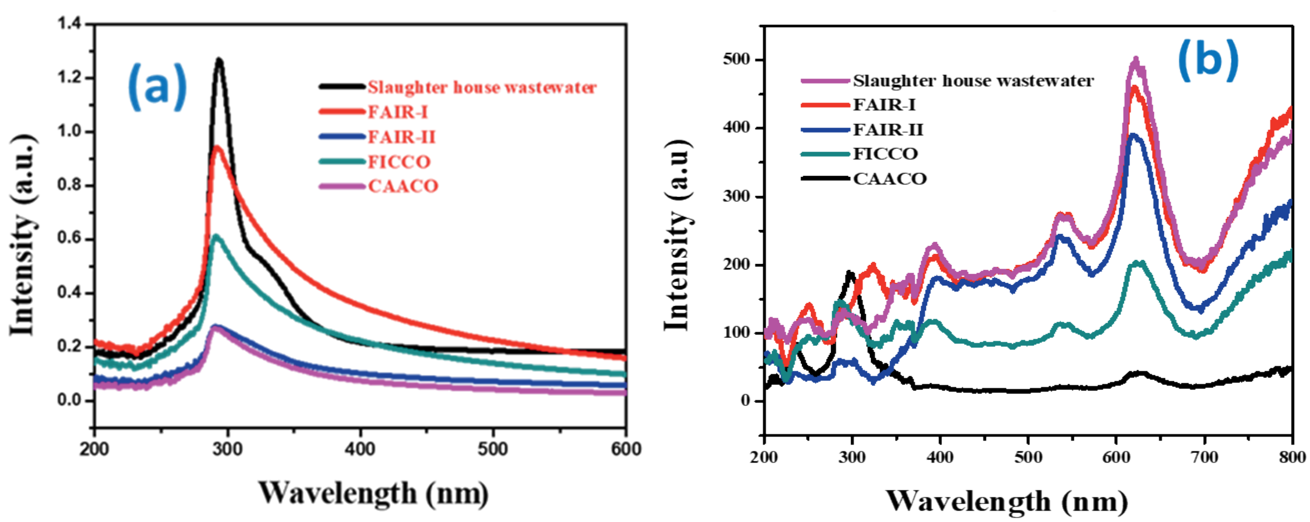

Figure 6. **(a)** UV-Visible and **(b)** UV-fluorescence spectrum for the treatment of slaughterhouse wastewater using integrated sequential anaerobic/aerobic reactor system

Conclusions

The wastewater generated from the slaughterhouse sector fluctuates extensively and thus the efficiency of conventional treatment systems is very much limited for the removal of organics. Hence, the treatment using sequential anaerobic/aerobic immobilized bioreactors comprised of FAIR, FICCO and CAACO was attempted in this study to meet the fluctuating organic load. The median values of the BOD and COD present in the initial slaughterhouse wastewater were 735 mg/L and 1452 mg/L respectively with a biodegradable index of 0.50 indicating the wastewater has biodegradable organics. The median COD values present in the FICCO and CAACO outlets was 622 mg/L and 312 mg/L respectively. The BOD present in the FICCO and CAACO outlets was 525 mg/L and 153 mg/L. The organic nitrogen content was reduced to 20 mg/L whereas NH_3-N was finally reduced to 69.5 mg/L. The efficiency of the reactors was statistically validated by way of a multivariate analysis (PCA and cluster analysis) for COD and NH_3-N removal which shows the trend observed in the FAIR reactors are almost equal, whereas the trend observed in CAACO is different from any other. The treatment was confirmed through UV-visible and UV-fluorescence spectroscopic analysis.

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