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A novel two stage bioreactor involving processed orange peel bed and Acinetobacterradioresistens for the treatment of aniline containing synthetic waste water

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Abstract- Biodegradation is a promising tool to get many of the polluting organic compounds degraded to a safe limit. Besides being a green technology, Biodegradation is capable of contributing complete mineralisation of the target organic compound. The process often takes place at ambient situation and no drastic conditions are generally required. But many of the existing methods for the decontamination of toxic organic pollutants are physical or chemical. Aniline is used in the manufacture of polyurethane, rubber processing chemicals, fibre, dyes, pigments, pharmaceuticals, herbicide andfungicide. The effluents from these industriescontribute significantly to environmental pollution. In the present study we have isolated a novel microorganism Acinetobacterradioresistens strain A1 form soil capable of bringing 80% degradation of aniline within 48 hours of incubation time. We could explore the mechanism of aniline biodegradation and could also design a novel two stage bioreactor system for the effective treatment of aniline containing effluent. The study involved the combination of grounded orange peel powder of size between 500 to 700 microns asthe first stage treatment andbiodegradation with the selected organism as the second stage ina two stage bioreactor system. Treatment of aniline containing effluent was performed with synthetic aniline containing waste water. The experiment was carried out with the bioreactor at different flow rates of 5, 7,9,11 ml/min. The treatment was done with orange peel bed alone, with the selected organism and by both the methods. The percentage reduction in aniline and reduction in COD were done at different flow rates. A Maximum reduction in the chemical oxygen demand could be effected as 99.17% at a flow rate of 5ml/min during 24hr experiment process using the combined treatment with the two stage process. The FT/IR analysis performed to evaluate the structuralchange in aniline in the wastewater could confirm the bioconversion of the pollutant.The designed bioreactorand its performance suggested that the strategies are not only green technologies but also promising for the effective treatment of the aniline containing wastewater.
Keywords- Acinetobacterradioresistens,Aniline wastewater,Chemical Oxygen Demand,FT/IR, Bioreactor

I. INTRODUCTION

Environmental pollution is a serious challenging threat to the health of millions of inhabitants in the global ecosystem. Bioremediation involves the application of microorganism or microbial process or products in degrading contaminants from a defined area.

Industrial effluents should be treated before discharging it into the nearby water bodies. There are a number of unit processes and operations involved in the treatment of industrial effluents[1].The treatment involves primary treatment for the removal of suspended solids and secondary treatment with biological methods for the complete mineralization of the pollutant followed by tertiary treatment. Primary treatment involves separating portion of the suspended solids from the wastewater. Screening and sedimentation usually accomplish with this separation process. The effluent from primary treatment will ordinarily contain considerable organic material which is to be removed by biological methods. Tertiary treatments generally involve the application of reverse osmosis, electrodialysis, desalinisation etc. Various methods such as photo degradation, advanced oxidation process and biodegradation have been used in the removal of pollutants from industrial effluents [2].The present work primarily focuses on the development of an integrated system for aniline carrying industrial wastewater.

The world consumption of aniline was increasing at a higher rate with demand for the production of Methylene diphenyldi-isocyanate for making of poly urethane. Aniline was extensively used for the production of herbicide and fungicide, rubber industry and pharmaceutical industry. The effluent generated from these industries has been directly discharged into the nearby water bodies without any treatment. The use of biological process offer green technologies for the effective removal of these aromatic amines.

Biological treatment systems were emerged as cost effective and eco- friendly techniques for treating waste water from process industries at various levels [3].A two-stage methanotrophic bioreactor system was developed for remediation of water contaminated with trichloroethylene and other chlorinated, volatile, aliphatic hydrocarbons[4].In a three-reactor system a nitroreducing consortium and an aniline-degrading Comamonasacidovorans were used in mixed population andwere able to mineralize the nitroaromatic compound nitrobenzene via aniline, its corresponding aminoaromatic compound [5].A sequencing batch reactor was used to treat aniline-containing wastewater and a simultaneous removal of aniline, nitrogen and phosphorus was achieved using a single sequencing batch reactor of anaerobic/aerobic/anoxic operational process [6].

The use of low-cost and eco-friendly adsorbents has been an ideal alternative for aniline removal from effluents. Many agricultural residues are extensively used for removal of toxic components from waste water especially in dye industry. The application of the agricultural residues as adsorbents can provide a suitable alternative for the removal of aniline or aniline containing dye industry from the industrial effluents [7].Orange peel is mainly composed of cellulose, pectin, hemi-cellulose, lignin and other low molecular weight compounds.Orange peel was being reported as an efficient and cost effective bio-adsorbent for removing dyes, metals and organic pollutants from industrial wastewater[8]. In the present study an attempt was made to remove aniline from synthetic waste water using a newly designed two stage bioreactor, with orange peel powder acting as an adsorbent for the initial treatment and subsequently with the selected bacteria for the specific organic conversion of aniline.

II. MATERIALS AND METHODS

2.1. Adsorption with powdered orange peel

The orange peels were cleansed with distilled water to remove the dust particles and water soluble impurities. After washing, the peels were cut in to small pieces, dried in sunlight for two days until the peel becomes crisp. Using a grinder the peels were powdered and using a molecular sieve the powder was sieved to obtain a size between 500 and 700 micron size .The powder was then treated with diethyl ether to remove the pigmentation. The powder was again washed with hot water at 800C and dried in oven at 600C for 1 hr. This processed powder was used for the aniline removal.

Five gram of orange peel powder was added to 100ml MSAM (pH 6.0) containing 5mM aniline and was incubated on a rotary shaker for 30, 60, 90 minutes at 120rpm. After different intervals, the medium was centrifuged at 5000rpm to remove the orange powder and the extract was assayed. One ml of supernatant was taken for Spectrophotometric analysis by Diazocoupling method[9].Composition of MSAM is as follows.KH_2PO_4 -1g, $(NH_4)_2SO_4$ -1g, $MgSO_4\ 7H_2O$ - 0.5g, $CaCl_2$ anhydrous - 0.001g in one litre of the medium[10].

2.2. The aniline containing synthetic wastewater

The composition of initial synthetic wastewater was prepared with analytical-grade chemicals as follows: aniline 125 mg, NH_4Cl 50.2 mg, $NaNO_3$ 79.7 mg, KH_2PO_4 21.9 mg, $K_2HPO_4.3H_2O$ 36.8 mg, CH_3COONa 192.3 mg and 1 ml of trace element solution in per litre. The trace element solution contained the following ingredients are per litre - $FeCl_3.\ 6H_2O$ 5.0 g, H_3BO_3 0.10 g, $CuSO_4.5H_2O$ 0.10 g, KI 0.20 g, $MnCl_2.4H_2O$ 0.50 g, $Na_2MO_4.2H_2O$ 0.20 g, $ZnSO_4.7H_2O$ 0.30 g, $COCl_2.6H_2O$ 0.15 g, EDTA-2Na 10.0 g [6].

2.3. Designing of Bioreactor

Treatment of aniline containing effluent was performed with synthetic aniline containing wastewater using the newly designed two stage bioreactor. The composition of initial synthetic wastewater was prepared with analytical-grade chemicals [6].The two stage bioreactor for the treatment of aniline wastewater was designed with Perspex glass with 1.5 litre capacity reservoir, first stage reactor and second stage reactor.The overview of the two stage bioreactor system with the reservoir was provided in the Figure 1.The reservoir contained an inlet for the sample entry and one out let with stopper/ flow controller to the next stage. The diameter of the pipe was 4 cm .The storage capacity of the reservoir was 1500ml and the height of the reservoir was 50cm from the ground level. The passage from first level to second level was about 30cm distance (Figure.2).

The first stage reactor served the purpose of primary treatment and the second stage reactor served the purpose of biological treatment (Figure 3).The first stage of the reactor meant for orange peel treatment was of storage capacity 1400ml and was above 30cm height from the ground level. An orange bed was formed of 2cm height from the base of the reservoir; a mesh of 12cm X 12cm was placed above the bed to prevent the disturbance when the water samples enter the bed. At the centre of the chamber an inlet tube of 2.2 cm height was placed where the sample from the first level reservoir entered into the bed from the reservoir. The treated sample was passed to third level.

The second stage reactor was fitted with provision for adding inoculum and also provision for mixing (Figure4). All the reservoir, first stage reactor and second stage reactor were interconnected with finely tuned valves for regulating the flow of the effluent.The second stage involving the treatment with the selected organism was having a height of 15 cm from the ground level and carried a capacity of 1400ml.When the wastewater after the first stage of treatment reached ¾ th level of the reservoir, the inoculum was added from the sterile inlet at the top right corner of the

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reservoir. It also contained a sparger for the proper mixing of the sample. This was connected with a DC power supply was attached with 9V battery.

.The sample for the treatment was collected in the reservoir from which it was drop wise added to the first stage reactor carrying the orange bed (with stopper/ flow controller).The second stage treatment with the selected organism was provided with the provision for inoculum addition and online sparger for mixing the sample. The whole system was arranged in stand with three separate height levels.

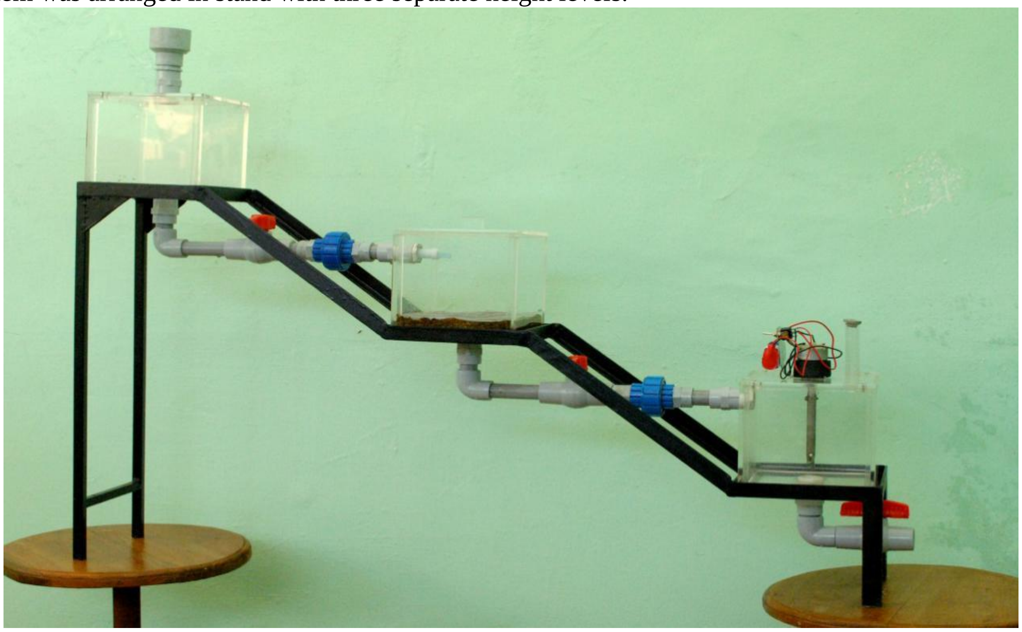

Figure 1. Overview Two stage bioreactor with the reservoir for aniline biodegradation

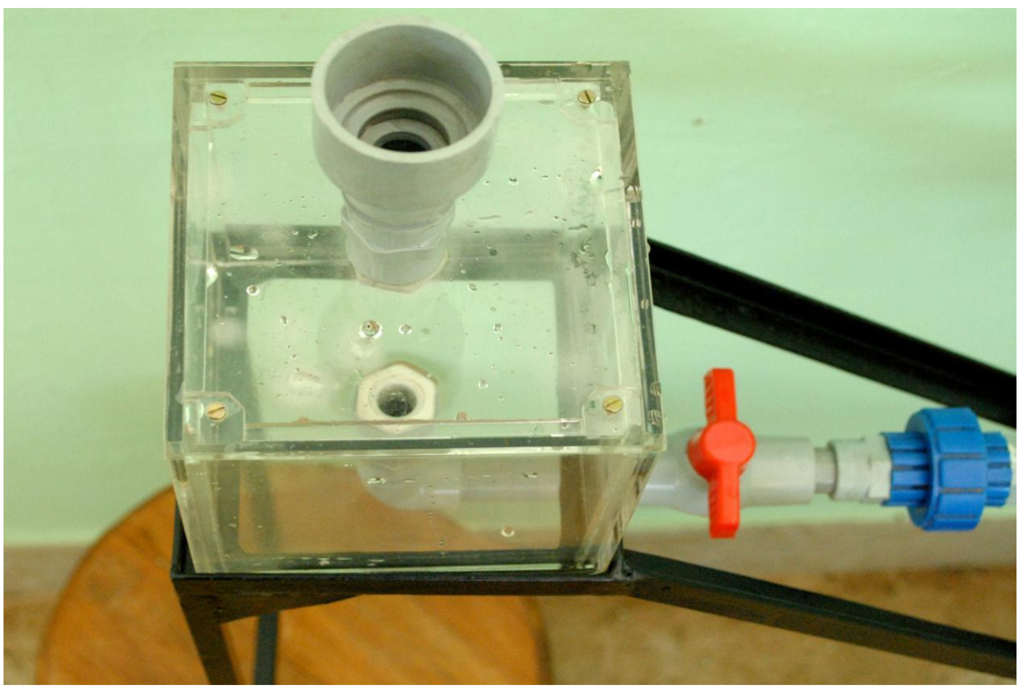

Figure 2.Reservoir which store aniline containing waste water for degradation

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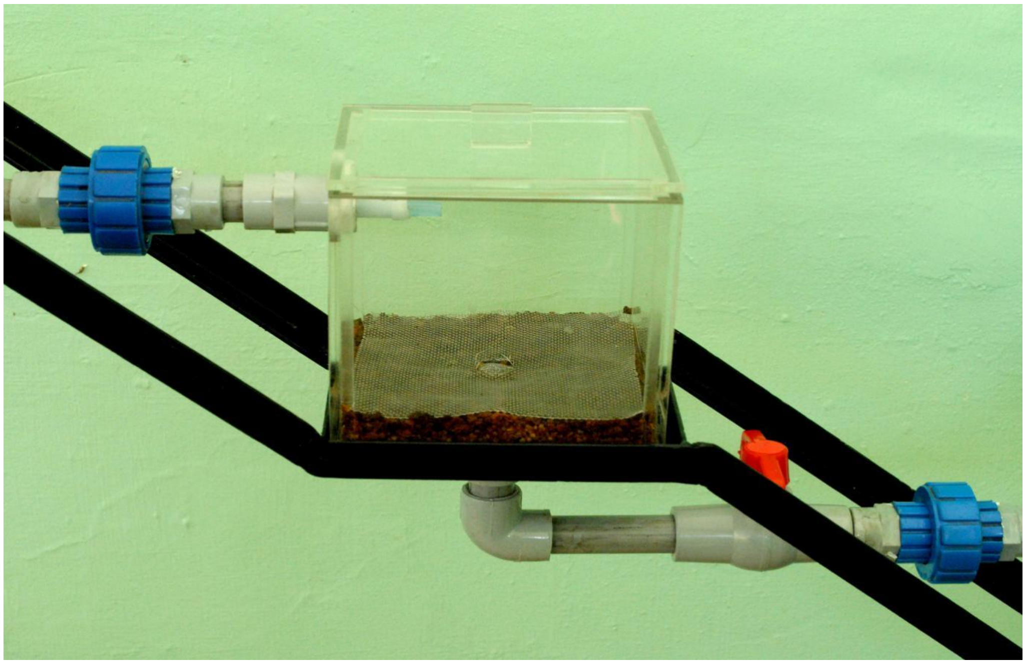


Figure 3. First stage of degradation – orange bed was formed of 2cm height

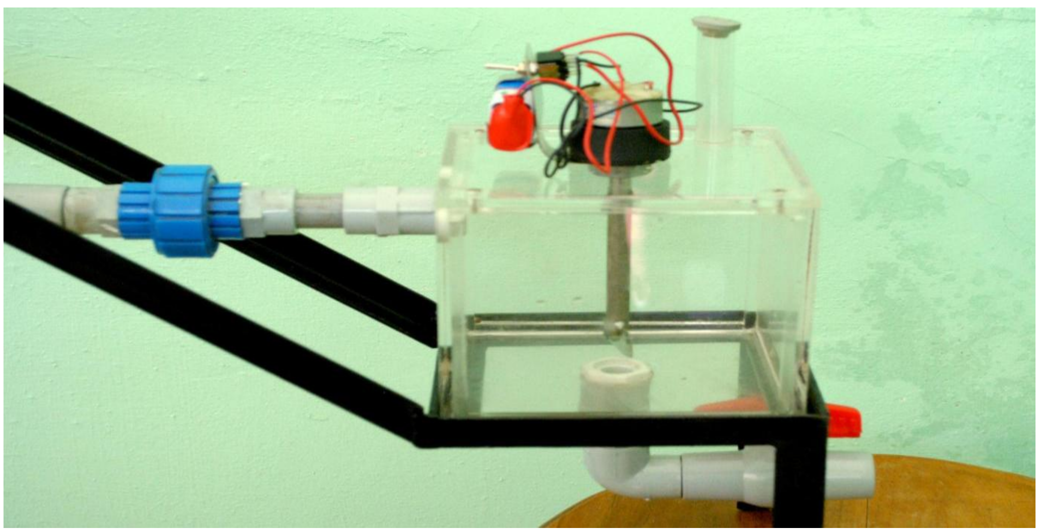


Figure. 4. Second stage of thereactor withagitator
wherethe selected organism was used for the aniline degradation

2.4. Treatment of waste water with the designed reactor

Synthetic waste water was prepared and was added to the storage reservoir. The wastewater was allowed to pass through to the second level with various flow rates. Threedifferent treatment strategies were adopted in which the waste water was treated with (1) Orange peel powder alone (2) Treatment with the selected Bacteria(3) With both Orange peel powder and Selected bacterium. Biological treatment was done with Acinetobacterradioresistens A1,a novel aniline degrading microorganism isolated from the soil near dye industry, Coimbatore, Tamil Nadu, India. The strain was isolated through enrichment culture technique. The selected isolate was inoculated into nutrient broth of 5mM aniline concentration and the overnight culture was harvested by centrifugation. The pellets were collected and suspended in physiological saline (0.85% NaCl) to obtain the concentration at OD -1. This was used as inoculum in the biodegradation studies. When the wastewater after the first stage of treatment reached ¾ th level of the reservoir, the inoculum was added from the sterile inlet at the top right corner of the reservoir. The sample was checked for the aniline assay on 24 hr treatment with diazocoupling method, FT/IR, COD.

III. RESULTS

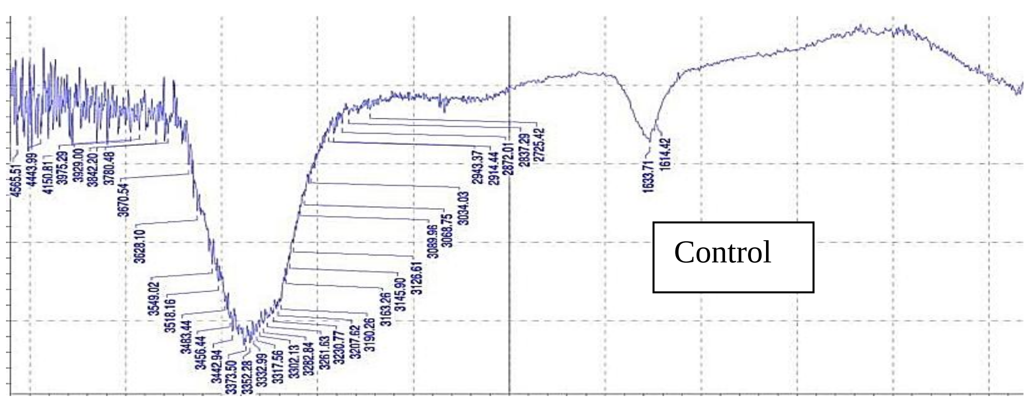
Treatment of aniline containing wastewater was carried out with the newly designed bioreactor at different flow rates of 5, 7,9,11 ml /min. The treatment was done with orange peel bed alone, with the selected organism alone and by both the methods. The percentage reduction in aniline (Table 2) and also the percentage reduction in COD (Table 3) were estimated at various flow rates. The maximum performance could be attained at a flow rate of 5 ml/hour

Table 1-Percentage degradation of aniline in the synthetic wastewater samples collected after treatment with the two stage bioreactor system at different flow rates

Si No	Flow rate	System used in Bioreactor	Percentage of Degradation
1	5 ml/minute	Orange	93.68 ± 2
2	7 ml/minute	Orange	91.75 ± 2
3	9ml/ minute	Orange	89.83 ± 2
4	11ml/minute	Orange	88.46 ± 1
5	5 ml /minute	Bacterium	88.73 ± 2
6	7 ml/ minute	Bacterium	87.08 ± 1
7	9ml/ minute	Bacterium	85.16 ± 1
8	11ml/minute	Bacterium	85.98 ± 2
9	5 ml/minute	Orange + Bacterium	99.17 ± 2
10	7 ml/ minute	Orange + Bacterium	98.07± 1
11	9ml/ minute	Orange + Bacterium	95.87 ± 2
12	11ml/ minute	Orange + Bacterium	94.23 ± 3

Table 2- Percentage reduction of the COD of the synthetic waste water samples collected after treatment with the Two Stage Bioreactor system at different flow rates

Si No	Flow rate	System used in Bioreactor	Percentage of reduction in COD
1	5 ml/ minute	Orange	90.42 ± 1
2	7 ml/ minute	Orange	81.29 ± 3
3	9ml/ minute	Orange	74.79 ± 2
4	11ml/ minute	Orange	67.83 ± 1
5	5 ml / minute	Bacterium	91.91 ± 4
6	7 ml/ minute	Bacterium	90.56 ± 2
7	9ml/ minute	Bacterium	88.34 ± 1
8	11ml/ minute	Bacterium	85.41 ± 3
9	5 ml / minute	Orange + Bacterium	97.53 ± 2
10	7 ml/ minute	Orange + Bacterium	93.05 ± 1
11	9ml/ minute	Orange + Bacterium	92.19 ± 2
12	11ml/ minute	Orange + Bacterium	90.90 ± 1



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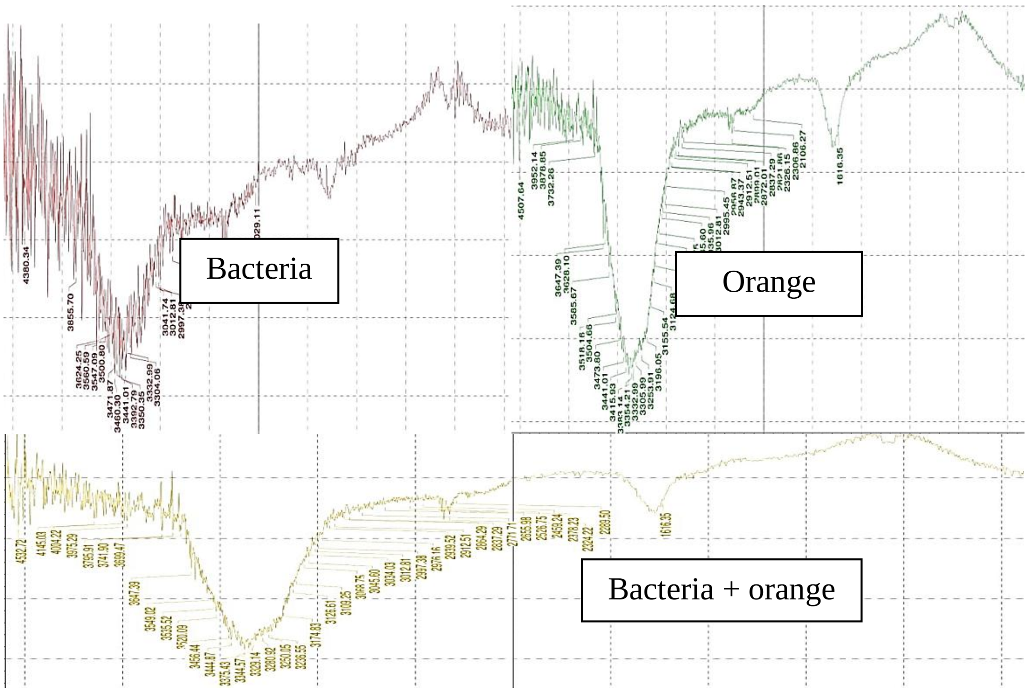

Figure 5. FT/IR spectrums of ether extract of control sample, treated with selected bacterium, orange peel powder and with both orange peel powder and the selected isolate of aniline synthetic waste water.

On analysing the reactor treated aniline containing waste water with FT/IR to confirm the structural change, promising results were obtained. There was no significant change in the spectrum of the orange peel treated sample as there was no effective degradation retained only temporary removal. However in both the cases of organism treatment (2995 cm-1 and 2821cm-1) and combined treatment there were considerable changes in the FT/IR spectrum (2997cm-1, 2655 cm-1, 2526 cm-1) confirming the structural change in the aniline present in the wastewater.(Figure 5).

IV. DISCUSSION

On an attempt to remove aniline by efficient green protocols, processed and dried orange peel powder was used. The method was found to be effective and quick as it brought the reduction in the aniline content by 70% in just 30minutes of treatment. Further, the removal rate could be increased only to 75% even after 120 hours of treatment (Table 1). The porous nature of the processed orange peel is instrumental in the removal of aniline. During this step only physical adsorption was taking place and no aniline was getting degraded. The maximum sequestration is constant and it could be attained in 30 minutes. As no free sequestration space was available, further removal of aniline was impossible. Hence it followed that aniline removal using orange peel could be used only as a strategy in primary treatment to bring down the higher concentration of aniline to a level where biological treatment was possible.Acinetobacterradioresistens strain A1capable of degrading aniline upto 80% within 48 hr of incubation time was used as the inoculum for the degradation studies in the bioreactor.

The designing was done to utilize the system for continuous mode. The reservoir was fitted with top inlet for introducing the effluent sample. The effluent from the reservoir was slowly passed into the reactor fitted with processed orange powder bed. The inflow rate was kept very slow and the effluent was made to fall into the outer bed which then was allowed to slowly rise and get eluted to the second stage for the biological treatment. The effluent on reaching the third chamber was mixed with the inoculum and was mixed slowly with the inbuilt on line agitator at nearly 150 rpm.

The treatment was done at various flow rates of 5ml/minute, 7ml/minute, 9ml/minute and 11ml/ minute. The efficiency of treating the effluent with orange peel alone, bacterium alone and with both selected bacterial system and orange peel were done and the decrease in both aniline and COD were estimated. A Maximum reduction of 99.17 % could be obtained in the combined treatment resulting in the subsequent COD reduction of97.53. The only organic compound present in the synthetic waste water was aniline alone and hence removal of aniline resulted in the decrease of major part of COD (Table 2 and 3).

V. CONCLUSION

Aniline removal with the help of orange peel powder showed maximum removal of 75 % in 120 minutes contact time was a promising approach of aniline removal using agriculture waste.Acinetobacterradioresistens capable of degrading aniline with maximum efficiency was selected for the biodegradation studies in the newly designed bioreactor.Two stage bioreactor was used for the treatment of aniline containing industrial wastewater with four different flow rate of 5, 7,9,11 ml per minute. Three different sets of experiment were done in the bioreactor with orange peel powder alone, bacterium alone, and the combined treatment with bacterium and orange peel powder. 99.17 % degradation was achieved in the least flow rate of 5ml/min of 24 hr experiment process and the percentage of COD reduction was observed as 97.53 %. The observations of the present study will definitely give way to new trends in the field of bioprocess enabling the designing of novel strategies for the effective treatment of industrial waste water.

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