Feasibility of hybrid bioreactor in the treatment of wastewater containing slowly biodegradable substances
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ABSTRACT

Slowly biodegradable substances and recalcitrant pose a serious threat to the environment owing to their bio-resistant nature. With the increase in industrial activities, more and more quantity of these substances is being discharged through wastewater. The biodegradability potential of such substances can be anticipated from low BOD/COD ratio. Traditionally, the wastewater containing recalcitrant/slowly biodegradable materials is treated by the chemical oxidation followed by biological method. Although chemical oxidation enhances the BOD/COD ratio appreciably in the first step, it has a drawback of forming chemical sludge. In view of that a unique hybrid bioreactor is thought for so that its biofilm segment can perform under inhibitory environment. The present paper reviewed the background and the experiences of treatment of the slowly biodegradable substances and explores the scope of the hybrid bioreactor in this regard. Specific applications of hybrid bioreactor in treating aforesaid wastewater are also highlighted.

Key words: Hybrid bioreactor, past experience, recalcitrant, slowly biodegradable substance, Specific application.

1. Introduction

Slowly biodegradable substances in the wastewater are generally the aliphatic and aromatic organic compounds (e.g. Phenol, Benzene, Toluene, Xylene, Chloroform etc.), and their salts with sulphate, chloride etc. They pose serious problems in wastewater treatment, due to their resistance to biodegradation and potential toxicity to the environment and human health. These compounds are difficult to treat in conventional biological treatment system and are designated as refractory or recalcitrant. Presence of recalcitrant compounds in wastewater and high concentration of total dissolved inorganic solids associated with low BOD/COD ratio (<0.35) is generally considered as complex or slowly biodegradable and it results inhibitory effect on the biological process. The wastewater containing slowly biodegradable substances are generated from petroleum refineries, fine chemical based industries, pharmaceutical industries, oil refineries, manufacturing of synthetic resins, agricultural industries, coke oven unit, steel plants, paper and pulps industries, tannery units etc.

In the past, combined biological-chemical method was widely adopted for the treatment of wastewater containing refractory substances. Under this system, the refractory substances, not degradable in biological treatment were transformed through chemical treatment like oxidation with ozone and hydroxyl radical into biodegradable ones for further treatment in a biological process (Karrer et al., 1997). In progress of development of the treatment system
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for slowly biodegradable substance advanced oxidation was applied prior to any biological treatment for transforming the recalcitrant into biodegradable one. Pre-ozonation and lifted moving bed biological activated carbon treatment was also accomplished in treating petrochemical wastewater (Lin et al., 2001). The improvement in this system compared to earlier is that the toxicity generated by Ozone in the advanced oxidation process (AOP) is detoxified in the biological treatment. Another research on the treatment of 'pentachlorophenol (PCP)', a highly chlorinated aromatic compound was conducted in a sequential chemical–biological treatment where degradation of PCP was performed by oxidation with ozone and finally treated in the trickling filter (Hong and Zeng, 2002).

Likewise textile wastewater containing recalcitrant was also treated by advanced oxidation process by combining O$_3$, H$_2$O$_2$ and UV properly (Al-Kdasi et al., 2004). Thereafter, with progress of time combined ozone-GAC adsorption was also performed as a pretreatment of recalcitrant before biological treatment (Kurniawan et al., 2006). Apart from the ozonation, advanced oxidation like fenton (H$_2$O$_2$, Fe$^{2+}$), photo-fenton oxidation was also applied in treating antibiotics wastewater to make the refractory compounds into biodegradable one (Elmolla and Chaudhuri, 2008). Similarly, various oxidation and combined processes such as UV/H$_2$O$_2$./Hypochlorites, fenton and electro-oxidation, photo-chemical, photo-catalytic, electro-catalytic oxidation, wet air oxidation, ozonation, biological method followed by ozone/UV/H$_2$O$_2$, coagulation or electro-coagulation and catalytic treatments have been practiced in the treatment of tannery wastewater. In this case, with sulphide as main sources of pollutant, electro-coagulation was the most efficient method. For chromium removal, photo catalytic oxidation process using nano-TiO$_2$ and wet air oxidation in the presence of manganese sulphate and activated carbon as a catalyst were observed to be more efficient (Rameshraja, and Suresh 2011). It was also reported that bio-recalcitrant α-methylphenylglycine was treated by photo-fenton oxidation under solar irradiation coupled with the activated sludge process (Serra et al., 2011).

In a separate study, a sequential Fenton-biological oxidation for the treatment of recalcitrant components of metal working fluid (MWF) wastewater was investigated. Fenton pre-treatment of the MWF effluent greatly improved biodegradability index (BOD$_5$/COD increased in the range of 0.160-0.538) with a synchronous lowering in the toxicity of the wastewater, making the recalcitrant component more amenable to subsequent biological treatment. Consequently, chemical oxidation process like catalytic wet oxidation (CWO) under mild condition was employed for pretreatment of low biodegradable coking wastewater with a view to enhance their biodegradability (Chen et. al., 2012). There are several instances, where biological processes have been employed for the treatment of a wide variety of slowly biodegradable wastes. Many process configurations and treatment schemes are proposed in literatures, among which biological fluidized bed (BFB) reactor is unique one. In this system, the formation of biofilms on fluidized media particles allows retaining of a high reactor biomass and maintaining a long mean cell residence time, so accomplishing high substrate utilization in the reactor. It has been observed that the transport of an inhibitory substrate through a biofilm gets retarded, so reducing the impact of substrate inhibition on microorganism cells. The interactions between mass transfer and bacterial rate processes in bio particles are important for increasing the efficiency of a BFB reactor. In this regard, a process kinetic model considering the interactions between intra biofilm mass transfer and bacterial rate process was developed based on Haldane inhibition kinetics (Lai and Shieh , 1997).

In a separate study, Phenolic wastewater was also treated in a fixed biofilm reactor (FBR) using polyurethane foam sponge cube with plastic ballast rings for attaching microorganisms.
Haldane’s Model was fitted to describe the kinetics of Phenol degradation (Hsien and Yen-Hui, 2005). Venkatmohan et al. (2005) performed biological treatment of slowly biodegradable composite chemical wastewater having BOD/COD ratio close to 0.32 with high concentrations of sulphate and total dissolved inorganic solids using upflow anaerobic sludge blanket reactor (UASB). Effective biological treatment of the composite chemical wastewater upon introduction of appropriate inoculums to the reactor was confirmed from substrate and sulfate removal data and non-accumulation of VFA. Similarly, applicability of UASB for treating Tannery wastewater was also reviewed for the sake of better performance compared to the conventional systems (Durai and Rajasimman, 2011).

In the last three decades, Hybrid Bioreactor comprising of suspended and attached growth biomass has been adopted widely for the treatment of slowly biodegradable substances. Conceiving this idea, slowly biodegradable volatile organic compound like benzene present in wastewater was also treated in a special hybrid bioreactor. This bioreactor had two segments - biofilter section and a bubble column bioreactor section. Influent benzene was removed by immobilized cells in a bubble column bioreactor, whereas gas phase benzene stripped by air injection was removed in a biofilter. Thus the organic load on the hybrid bioreactor could be shared by the biofilter and bubble column bioreactors and thereby fluctuation of total load could be absorbed by varying the distribution of benzene between those two segments (Yeom and Yoo, 1998).

Regarding the kinetics of removal of slowly biodegradable substances, it has been confirmed that the Haldane’s model is more applicable than that of Monod. However, there are various other models proposed by Contois, Powell, Han levenspiel, Luong, Edward, Aiba, Teisser, Yano-Koga and so on for inhibitory substrates. It is already observed that most of available kinetic models have been derived by assuming single growth i.e. either suspended or attached and not complying with the concept of the hybrid bioreactor where two competitive growths take place simultaneously. However, there is one unique model developed by Chi-Yuan Lee (1992) for the hybrid bioreactor having suspended and attached growth biomass. But, unfortunately analytical solution of the real problem using this model is found to be cumbersome and also inconsistent.

Hybrid bioreactor carrying both suspended-growth and attached-growth microorganisms has been regarded as a novel and excellent bioreactor system for treating the wastewater containing inhibitory substances. The hybrid bioreactor process has a potential to significantly improve the efficiency of biological treatment of slowly biodegradable substance. Compared with the conventional activated sludge process, it features high quality effluent and low sludge production rate. The present review paper is aimed to assess the performance of various methods in treating the slowly biodegradable substances and also to explore the scope of hybrid bioreactor in doing so. Past experiences on laboratory scale study and full-scale application of treatment methodologies for slowly biodegradable substances are illustrated. A comparative evaluation of hybrid bioreactor for treating slowly biodegradable substances has also been accomplished with respect to other traditional processes.

2. Slowly biodegradable substance and its degradation potential

Recalcitrant compounds are highly stable, highly toxic, and also carcinogenic in nature, along with odor problem even at very low concentration. These generally belong to volatile organic aromatic and aliphatic compounds and are mainly the products of petroleum and fine chemical industries. Common recalcitrant substances are phenol, benzene, toluene, xylene,
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halogenated aromatic compounds, chloroform, trichloroethylene etc. The typical characteristics of industrial wastewater sample containing recalcitrant substances are furnished in table 1.

Table 1: Characteristics of typical industrial wastewater containing recalcitrant

<table>
<thead>
<tr>
<th>Type of Industry</th>
<th>BOD (mg/l)</th>
<th>COD (mg/l)</th>
<th>Ammonium nitrogen (mg/l)</th>
<th>Total Suspended Solid (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannery</td>
<td>5000</td>
<td>16000</td>
<td>450</td>
<td>-</td>
</tr>
<tr>
<td>Composite</td>
<td>80-</td>
<td>150-</td>
<td>-</td>
<td>15.000</td>
</tr>
<tr>
<td>Textile</td>
<td>6000</td>
<td>12000</td>
<td>-</td>
<td>200</td>
</tr>
<tr>
<td>Dyeing</td>
<td>250</td>
<td>1300</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Dairy</td>
<td>2200</td>
<td>3500</td>
<td>120</td>
<td>-</td>
</tr>
<tr>
<td>Palm oil</td>
<td>34000</td>
<td>67000</td>
<td>50</td>
<td>24000</td>
</tr>
<tr>
<td>Pet food</td>
<td>10000</td>
<td>21000</td>
<td>110</td>
<td>54000</td>
</tr>
<tr>
<td>Pharmaceutical</td>
<td>3225</td>
<td>6300</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Coking wastewater</td>
<td>2334</td>
<td>8092</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

[Ref. Noor Sabrina et al. (2012), Adel Al-Kdasi et al. (2004), Honglin Chen et al. (2012)]

Man-made organic compounds or xenobiotics possess complex ring structures or constituents that make them resistant to and therefore persistent in the environment. Generally, such organics can be grouped into four main categories - (a) the saturated hydrocarbon compounds having no double or triple bonds. The degradability of such compounds decreases with the increase in molecular weight and degree of branching (b) the aromatic hydrocarbon compounds containing conjugated double bonds. The aromatics holding one or two rings degrade easily, whereas higher molecular weight aromatics are less prone to biodegradation. (c) the asphaltenes that include the phenols, fatty acids, ketones, esters and phorphyrins, and lastly (d) the resins that include the pyridines, quinolines, carbozoles, sulfoxides and amides (Maszenan et al., 2010). A list of typical recalcitrants and their characteristic features are shown in table 2.

Table 2: Characteristics of typical Recalcitrant in wastewater

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Recalcitrant</th>
<th>Types</th>
<th>COD/Theoretical Oxygen Demand</th>
<th>Solubility in Water (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Benzene</td>
<td>Hydrocarbon</td>
<td>0.65</td>
<td>0.8</td>
</tr>
<tr>
<td>2</td>
<td>Toluene</td>
<td>Hydrocarbon</td>
<td>0.40</td>
<td>0.47</td>
</tr>
<tr>
<td>3</td>
<td>Ethyl-benzene</td>
<td>Hydrocarbon</td>
<td>0.75</td>
<td>0.15</td>
</tr>
<tr>
<td>4</td>
<td>Chlorobenzene</td>
<td>Halogenated hydrocarbon</td>
<td>0.58</td>
<td>0.5</td>
</tr>
<tr>
<td>5</td>
<td>1,4 dichlorobenzene</td>
<td>Halogenated hydrocarbon</td>
<td>0.34</td>
<td>0.08</td>
</tr>
<tr>
<td>6</td>
<td>Dethyl ether</td>
<td>Ether</td>
<td>0.81</td>
<td>69</td>
</tr>
<tr>
<td>7</td>
<td>Dichloromethane</td>
<td>Halogen alkane and alkene</td>
<td>0.06</td>
<td>13</td>
</tr>
<tr>
<td>8</td>
<td>Trichloromethane</td>
<td>Halogen alkane and alkene</td>
<td>0.08</td>
<td>8</td>
</tr>
<tr>
<td>9</td>
<td>Tetrachloromethane</td>
<td>Halogen alkane and alkene</td>
<td>0.08</td>
<td>0.8</td>
</tr>
<tr>
<td>10</td>
<td>Carbon disulfide</td>
<td>Thiocarbon acid derivative</td>
<td>0.21</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>Dimethylamine</td>
<td>Amines</td>
<td>0.03</td>
<td>Soluble</td>
</tr>
<tr>
<td>12</td>
<td>Trimethylamine</td>
<td>Amines</td>
<td>0.01</td>
<td>Soluble</td>
</tr>
</tbody>
</table>
Recalcitrant usually possess a synthetic origin complex aromatic molecular structures which make them more stable and more difficult to get biodegraded. Recalcitrant are resistant to degradation on account of their fused aromatic structures and thus remain intact for a longer time in the water environment. They tend to move through conventional treatment systems practically without any change. The inhibition in biodegradation due to the presence of recalcitrant induces antibacterial activity. High concentration of those compounds discharged along wastewater into water bodies may cause ecological intoxication. Toxic chemicals in the wastewater can enter the living cell and inhibit one or more enzymes in the metabolic pathways involving either anabolism or catabolism. When the catabolic reactions of respiration get affected, the rate of respiration and energy production are reduced and thereby the rate of growth is also reduced. On the other hand, when the anabolic pathways of biosynthesis are inhibited, the rate of growth is also reduced, and as a result the rate of respiration falls, because the requirement for energy is reduced.

3. Background of the treatment of slowly biodegradable substances

Either solely chemical treatment or combined chemical-biological methods were traditionally used for the removal of slowly biodegradable substances present in wastewater. Ozonation or Chlorination is still mostly practiced to convert the refractory substances into simple biodegradable compounds and then to treat the same by means of biological method. This approach of treatment has also been designated as chemical oxidation followed by biological removal of refractory substances. The process design of such Advanced Wastewater Treatment necessitates kinetic analysis of each method and finally their interfacing. Although there is little variation in the configuration of the chemical reactors, a wide range of biological reactors have been developed so far. Out of different biological reactors, attached growth system played an important role in the final removal of refractory substances. In the light of this practice, Pre-ozonation and lifted moving-bed biological activated carbon (BAC) was used as an advanced treatment process treating phenol, benzoic acid, amino benzoic acid and petrochemical wastewater containing acrylonitrile butadiene styrene (ABS) (Lin et al., 2000).

Combined biological-chemical process (Karrer et al., 1996) was also adopted for the treatment of wastewater containing bio-refractory compounds which were transformed into low molecular weight acid like acetic acid, oxalic acid etc. Biological treatment and chemical oxidation were consecutively carried out using ozone as a chemical oxidant until a desired removal was obtained. A substrate inhibition model was presented using a fluidized bio-particle, which considered the interactions between intra-biofilm mass transfer and bacterial rate process based on Haldane inhibition kinetics (Lai and Shieh, 1997). Apart from the Biological Fluidized Bed (BFB) reactor the hybrid bioreactor composed of a bio-filter and a bubble column bioreactor (Yeom and Yoo, 1999) was also employed to remove liquid and gas phase Benzene. In this case, liquid and gas phase benzene were removed by immobilized cells in bubble column bioreactor and the biofilter respectively. The classical Monod kinetics was applied to analyze the removal of Benzene.

A sequential chemical-biological treatment was performed for highly chlorinated aromatic compound Pentachlorophenol (PCP) (Hong and Zeng, 2002). Chemical oxidation was accomplished by both ozone and secondary hydroxyl radical and finally the degradation was attained by biological unit like trickling filter. Apart from that, Advanced oxidation process (AOP) using O₃, H₂O₂ and UV as oxidizing reagent was adopted to treat textile wastewater.
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containing recalcitrant (Al-Kdasi et al., 2004). Kurniawan et al., (2006) reported a study on the treatment of recalcitrant contaminants by ozonation followed by GAC adsorption. UV-H₂O₂ as an advanced treatment was also applied to degrade recalcitrant and potentially carcinogenic tri-alkyl phosphates in wastewater. Different advanced oxidation processes using Fenton, Photo-Fenton and ozonation were also applied as pretreatment of antibiotics wastewater (Watts and Linden, 2008, Elmolla and Chowdhuri 2008). In a later study, Photo-Fenton process under solar irradiation was coupled to an aerobic activated sludge system in form of sequence batch reactor for treatment of aqueous solution polluted with non-biodegradable alpha-methyl phenyl glyoxime (Serra et al., 2011). At the same time, the catalytic wet oxidation under mild condition was applied as pre-treatment of low biodegradable coking wastewater with a view to improve its biodegradability prior to biological treatment (Chen et al., 2011).

Effort was also made to remove the organic recalcitrant substance from wastewater by the microbial mass via the process of biosorption, where passive uptake of pollutants from aqueous solutions was obtained by the use of selected live and dead microbial mass (Aksu, 2005). Consequently, a fixed biofilm process using polyurethane foam sponge cube with plastic ballast rings to attach microorganisms was analyzed to treat phenolic wastewater (Hsien and Lin, 2005). Meanwhile, two-stage fixed bed biofilm reactor was employed for the treatment of winery wastewater containing recalcitrant for the sake of discharge into sewerage system (Andreottola et al., 2005). Among biological processes, Upflow anaerobic sludge blanket treatment was performed for low biodegradable composite chemical wastewater containing sulphate (Venkata Mohan et al, 2005) and also for high strength tannery wastewater (Durai and Rajasimman, 2011).

In an unique approach, Juang and Tsai (2006) studied the biodegradation of equimolar phenol and sodium salicylate (SA) by Pseudomonas putida in a microporous polypropylene (PP) hollow fiber module, which was pre-wetted by contacting with ethanol to make them more hydrophilic. Besides, aerobic granules were developed in laboratory by self aggregation of microorganisms in absence of support carrier for the treatment of wastewaters containing potentially inhibitory substance, toxic heavy metals and radioactive material (Maszenan et al, 2011). In this case concentration gradient created by the layered structure of the granules protected microorganisms inside the granule from the impact of direct toxicity of the recalcitrant. A brief overview on different methods already adopted for the treatment of slowly biodegradable substances / recalcitrant is presented in Table 3.

**Table 3:** Overview on different methods already adopted for the treatment of Slowly Biodegradable Substances.

<table>
<thead>
<tr>
<th>Process Identity</th>
<th>Type of wastewater</th>
<th>Slowly biodegradable substances / Recalcitrant</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic granulation</td>
<td>Oil refineries, pharmaceuticals, pesticide</td>
<td>Phenol</td>
<td>Maszenan et al., 2010</td>
</tr>
<tr>
<td>Aerobic granulation</td>
<td>Antibiotic, pesticide, leather manufacturing.</td>
<td>p-nitrophenol</td>
<td>do</td>
</tr>
<tr>
<td>Bioreactor Type</td>
<td>Industry</td>
<td>Contaminant</td>
<td>Reference</td>
</tr>
<tr>
<td>---------------------------------------</td>
<td>---------------------------------</td>
<td>------------------------------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>Fixed biofilm reactor</td>
<td>Petrochemical, Oil refining, polymeric resin production factory, thermal power plant</td>
<td>Phenol</td>
<td>Hsein and Lin, 2005</td>
</tr>
<tr>
<td>Bubble column bioreactor and biofilter</td>
<td>Petroleum and fine chemical industry</td>
<td>Benzene</td>
<td>Yeom and Yoo, 1998</td>
</tr>
<tr>
<td>Pre-Ozonation and lifted moving bed biological activated carbon advanced treatment</td>
<td>Petrochemical</td>
<td>Phenol, Benzoic acid, acrylonitrile butadiene styrene.</td>
<td>Lin et al., 2000</td>
</tr>
<tr>
<td>Sequential chemical-biological treatment</td>
<td>Wood preservative industry</td>
<td>Pentachlorophenol</td>
<td>Andrew and Zeng, 2002</td>
</tr>
<tr>
<td>Advanced oxidation by O₃, H₂O₂ and UV</td>
<td>Textile wastewater</td>
<td>Insoluble dyes</td>
<td>Kdasi and Idris, 2005</td>
</tr>
<tr>
<td>Upflow anaerobic sludge blanket (UAS B)</td>
<td>Tannery wastewater</td>
<td>Chemical oxygen demand (Slowly biodegradable)</td>
<td>Durai and Rajasimman, 2011</td>
</tr>
<tr>
<td>Solar photo fenton coupled with aerobic ASP</td>
<td>Pharmaceutical</td>
<td>α-methyl-phenyl-glycine(α-MPG)</td>
<td>Serra et al., 2011</td>
</tr>
<tr>
<td>Biosorption in Immobilised cell process using batch reactor</td>
<td>Antibiotic wastewater</td>
<td>Sulfamethazine</td>
<td>Yu and Lin, 2011</td>
</tr>
<tr>
<td>Advanced oxidation with fenton, photo fenton and ozonation</td>
<td>Antibiotic wastewater</td>
<td>Chemical oxygen demand (Slowly biodegradable)</td>
<td>Elmolla. and Chaudhuri, 2008</td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th>Photo oxidation</th>
<th>Synthetic</th>
<th>2-chloro ethyl phosphate</th>
<th>Watts and Linden, 2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ozone-GAC adsorption</td>
<td>Synthetic</td>
<td>Chemical oxygen demand (Slowly biodegradable)</td>
<td>Kurniawan et al., 2006</td>
</tr>
<tr>
<td>Hybrid reactor composed of bubble column bioreactor and biofilter</td>
<td>Petroleum and fine chemical industries</td>
<td>Benzene</td>
<td>Yeom, 2006</td>
</tr>
<tr>
<td>Upflow anaerobic sludge blanket (UASB)</td>
<td>Chemical based industry</td>
<td>Organo-chlorine phenolic compound</td>
<td>Venkatamohan and Krishnaprasad, 2005</td>
</tr>
</tbody>
</table>

4. Past Research experiences on the treatment of slowly biodegradable substances

A fast and easy test procedure was developed for efficiently treating the wastewater containing bio-refractory substances in a combined biological-chemical process (Karrer et al., 1996). The laboratory test was performed with a model recalcitrant compound like m-chloronitrobenzene (mCNB). The efficient treatment was characterized by the minimum use of expensive chemical oxidant, e.g. ozone, for a specified removal. Biological treatment and oxidation was consecutively carried out until a desired removal was obtained. In this treatment the refractory compound was transformed into low molecular weight acid like acetic acid, oxalic acid etc. Consequently an analysis of substrate inhibition kinetics in a fluidized bio particle presented a model considering the interactions between intra biofilm mass transfer and bacterial rate process based on Haldane inhibition kinetics (Lai and Shieh, 1997). The model predicted that, under given circumstance a bio-particle effectiveness factor of greater than unity may be attained for a range of biofilm thicknesses indicating that a bio-particle was effective for an inhibitory substrate. The bio particle effectiveness factor (ratio of observed bio-particle reaction rate to bio-particle reaction rate under bulk liquid condition) could be used in conjunction with fluidization correlations to predict the overall efficiency of a biological fluidized bed reactor in presence of substrate inhibition. Later, a research study was performed on removal of benzene (a recalcitrant) by the hybrid bioreactor composed of a bio-filter and a bubble column bio-reactor (Yeom, and Yoo, 1999).

Besides, the nature of degradation of bio-refractory substances and the growth of a biofilm were investigated in a laboratory-scale pre-ozonation unit and a lifted moving-bed biological activated carbon (BAC) reactor. The wastewater containing ABS from phenol, benzoic acid, aminobenzoic acid and petrochemical industries was used in this study (Lin et al., 2001). The optimal reaction time and ozone dosage of pre-ozonation for bio-refractory conversion were obtained as 30 min and 100 – 200 mg O₃/hr respectively. After pre-ozonation of 30 min treatment, BODs/COD ratio of influent and effluent got enhanced apparently from 20 to 35%,

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approximately. One sequential treatment process was also performed for recalcitrant industrial wastewater. Firstly, a micro algal treatment removed nutrients from wastewater and oxygen was produced for other organisms (fungi and invertebrates) that consumed the organic matter. Secondly, macrophytes further removed organic matter and eliminated the microalgae from the treated wastewater. This study demonstrated the feasibility of combining microalgae and macrophytes for bioremediation of recalcitrant industrial wastewater (Valderrama et al., 2002).

Hong and Zeng (2002) conducted a sequential chemical-biological treatment for highly chlorinated aromatic compound Pentachlorophenol (PCP). The degradation of PCP under ozonation was firstly initiated, prior to subsequent degradation of intermediates by both ozone and secondary hydroxyl radical and thus its biodegradation potential was examined. Other than single Aerobic system anaerobic digestion coupled with an aerobic/anoxic membrane bioreactor was also utilized for treating agricultural wastewater containing recalcitrant (Cicek, 2003). Later, Zumriye Aksu (2005) reviewed the applications of biosorption for the removal of hazardous organic compounds. It was concluded that biosorption might be a passive route of uptake of organic compounds by the living/non-living biomass. Biosurfactants, surface active agents produced by bacteria and yeast, are potentially useful in wastewater treatment, particularly due to their nature, low toxicity, biodegradability and excellent surface active properties. They are found effective for enhancing metal removal from the wastewater (Mullian and Gibbs, 2003). Besides, algal/microbial biomass was also used in the removal of uranium from mining wastewater (Kalina et al, 2004).

Phenolic wastewater was treated in a fixed biofilm process using polyurethane foam sponge cube with plastic ballast rings to attach microorganisms (Hsien and Lin, 2005). The mathematical model for phenol utilization was developed using Haldane Kinetics and verified by conducting a fixed biofilm column test. At a steady-state condition, the phenol concentration collected from experimental data was slightly greater than that obtained from the model simulation. Apart from that treatment of textile waste water containing recalcitrant was performed by advanced oxidation process (AOP) using O₃, H₂O₂ and UV as oxidizing reagent (Al-Kdasi, 2004).

A sequential anaerobic and aerobic treatment by two step bioreactors was performed for removal of colour in the pulp and paper mill effluent. The percentage of removal of colour, lignin, COD, absorbable organic halides (AOX) and phenol in anaerobic treatment was found 50%, 62%, 29% 25% and 29% respectively. Thereafter anaerobically treated effluent was separately applied in the bioreactor in presence of fungal strain, paecilomyces sp., microbrevis luteum (bacterial strain). The aforesaid percent removals were obtained as 80%, 81%, 93%, 74% and 76% respectively (Singh, 2007). While studying the kinetics of cell growth and p-cresol degradation simultaneously six kinetic models like Haldane, Andrews, Webb, Yano, Aiba, and Teissier were fitted to the experimental growth kinetic data Among these models by Haldane, Andrews, Webb and Yano (correlation coefficient R²>0.99 )were found to be most suitable models (Singh et al 2007). Later UV-H₂O₂ as an advanced treatment was also applied to degrade recalcitrant and potentially carcinogenic tri-alkyl phosphates in water. Different advanced agents like Fenton, Photo-Fenton, ozone have been applied as pretreatment of antibiotics wastewater (Watts and, Linden, 2008). It has been observed that AOP pretreatment can reduce organic concentration and thereby improves biodegradability of recalcitrant.
In a batch reactor phenol biodegrading potential under aerobic condition was studied with mixed microbial culture collected from effluent treatment plant of a coke oven industry. The investigation showed that, after acclimatization, the mixed culture could biodegrade up to 700 mg/l of phenol. The biodegradation kinetics was fitted to different substrate inhibition models by MATLAB 7.1. Among all models, Haldane model was best fitted (Root Mean Square Error = 0.0067) for phenol degradation (Dey and Mukherjee, 2010).

Maszenan et al. (2010) reviewed the scope of using aerobic granules formed by self aggregation of microorganisms in absence of support carrier for treatment of wastewaters containing potentially inhibitory substance, toxic heavy metals and radioactive material. It was investigated that due to compact and dense structure aerobic granules having a regular shape enhanced settleability, higher biomass retention, multi-microbial function, higher tolerance to toxicity, greater tolerance to shock loading and relatively low excess sludge production. Moreover, concentration gradient created by the layered structure of the granules protects microorganisms inside the granule from the impact of direct toxicity of the recalcitrant. Subsequently, Photo-Fenton process under solar irradiation was coupled to a sequence batch reactor for the treatment of bio- recalcitrant alpha-methyl phenyl glycine (Serra et al., 2011).

In a study of kinetics model for winery wastewater, experimental data was correlated to several kinetic models, and Haldane model was found best particularly for lower biomass concentrations. Therefore, initial high biomass concentration was ascertained as a requirement in aerobic treatment of winery wastewater, in order to cope with the large fluctuations in its organic load (Silva et al., 2011). In a separate study, an advanced oxidation using electro generated ferrous ions and H$_2$O$_2$ was investigated to treat phenol containing wastewater (Jiang and Mao, 2012). It was observed that continuous addition of H$_2$O$_2$ was more effective than the addition of H$_2$O$_2$ in a single step to get higher COD removal efficiency.

### 4.1 Field-scale treatment of Slowly Biodegradable Substances

A two stage fixed bed biofilm reactor was applied at full scale for the treatment of winery wastewater containing recalcitrant prior to discharge into sewerage system (Andreottola et al., 2005). Besides, Upflow anaerobic sludge blanket (UASB) treatment was successfully employed for the treatment of low biodegradable composite chemical wastewater having (BOD/COD) ratio of 0.32 and containing sulphate as well as total dissolved inorganic solids (Venkatamohan et al., 2005)

Similarly, the treatment of wastewater containing benzene was demonstrated through a combined model of bubble column reactor (continuously stirred tank reactor) and bio filter (plug flow reactor) (Yeom, 2007). In another study Kurniawan et al. (2006) reported the treatment of recalcitrant contaminants using ozonation followed by GAC adsorption.

In the treatment of toxic industrial wastewater the Michaelis-Menten equation was used to describe thiocyanate degradation, but a Haldane-inhibition model was utilized to satisfactorily describe cyanide degradation. On the other hand, a slightly modified Haldane model with inhibition by free ammonia was used to describe ammonium oxidation in a better way (Drakides and Lay-Son, 2009).
A synthetic effluent containing a binary mixture of synthetic dyes was treated by a combination of a preliminary physicochemical stage followed by a biological stage based on ligninolytic enzymes produced by *Phanerochaete chrysosporium*. This treatment included biosorption onto peat as pretreatment, which decreased the volume and concentration to be treated in the biological reactor, thereby obtaining a completely decolorized effluent. (Palma *et al.*, 2011).

An advance treatment of textile wastewater containing recalcitrant was performed by using activated sludge process followed by column reactor packed with cell immobilized pellets and finally with coagulation-flocculation followed by ozone oxidation (Dukgyu *et al.*, 2013). It was observed that activated sludge process was not satisfactory in removing recalcitrant organic matter. Packed bed column reactor was found effective in removing most of the suspended and soluble recalcitrant organic matter. Ozone oxidation was finally done for removing most of the colour of the wastewater.

Apart from that, Polyphenol oxidase enzyme was used in bioremediation of phenolic contaminants from industrial wastewater. In this enzymatic removal method, the selectivity, controllability and economy of their reactions in comparison with conventional chemical redox reactions was found acceptable (Mukherjee *et al.*, 2013). Consequently treatment of pulp and paper mill wastewater was done with solar photo-Fenton process. It enhanced the biodegradability of wastewater. In this study, the biochemical oxygen demand to chemical oxygen demand ratio (BOD$_3$/COD ratio was found to increase from 0.028 to 0.83. The effluent BOD and total suspended solids concentrations were determined to be 0 and 30 mg/l, respectively (Ginni *et al.*, 2013).

### 4.2 Perspective of the hybrid bioreactor system

The conceptual diagram of a typical hybrid bioreactor comprising of suspended activated sludge and biofilm is shown in figure 1. In this reactor a fraction of substrate is used by suspended biomass and the remaining by attached biomass resulting in competition between the two growths for the substrate as shown in figure 2. The combination of suspended and attached growth provides the system with increased biomass concentration and sludge age more than those in ASP.

![Figure 1: hybrid bioreactor comprising of suspended activated sludge and biofilm](image-url)

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In the past there was a problem in increasing the biomass in a conventional activated sludge reactor due to difficulty in separation of large quantity of biomass in the secondary clarifier. These problems are eliminated in the hybrid bioreactor because of the attachment of the bulk of the biomass to the support medium. It has been established two to five fold increase in biomass concentration is done in hybrid bioreactor compared to that in a conventional activated sludge process (Fadel. et al., 2002). Hybrid bioreactor has been adopted for the treatment of wastewater containing low biodegradable /recalcitrant substances, where it has improved the efficiency of biodegradation for the same. In such a system, Solid retention time (SRT) is higher for the attached biomass than for the suspended biomass (Artiga et al., 2004). Conventional activated sludge process has process flexibility, whereas fixed biofilm processes are inherently stable and resistant to inhibition and hydraulic shock loadings. Therefore, hybrid bioreactor being the integrated process by means of fixed film media into activated sludge basin combines the advantage of both the systems.

4.3 Application of hybrid bioprocess in the treatment of slowly biodegradable substances

A performance study on the removal of benzene from wastewater was conducted using a bioreactor composed of biofilter and bubble column bioreactor. Liquid benzene was removed through bubble column bioreactor, whereas gas phase benzene was stripped in biofilter indicating appreciable removal efficiency of benzene by this hybrid bioprocess (Yeom and Yoo, 1998). Observation was made on the treatment of antibiotic wastewater by an integrated hybrid process combining powdered activated carbons with ultrafiltration and reverse osmosis. In this case removal efficiency of the recalcitrant like terracycline was found 72% in this hybrid bioprocess (Zhang et al, 2005).

Apart from that a synthetic wastewater containing perchloroethylene (PCE) was treated through a continuous flow flat sheet hybrid membrane aerated biofilm reactor (MABR). Appreciable removal efficiency of PCE was attained through this hybrid bioprocess (Ohandja and Stuckey, 2007). Moysa and Zon (2008) reported a performance study on the treatment of rapeseed oil by activated sludge in the membrane bioreactor using ultrafiltration membrane. The use of MBR showed better effectiveness of wastewater treatment than the bioreactor coupled with a secondary settling tank.
Wastewater from nuclear fuel recovery unit containing chlorophenol was treated with advanced oxidation process followed by biological hybrid process. In this study, the maximum removal efficiency of chlorophenol was observed as 98% (Makgato and Chirwa, 2009). After a few years, a synthetic mixture of dyes was treated by physic-chemical process followed by biological process using ligninolytic enzymes. The final treatment generated a completely decolorized effluent (Palma et al 2011). The performance of hybrid bioprocess in treating various wastewater containing slowly biodegradable substances is depicted in Table 4.

Table 4: Performance of hybrid bioprocess in the treatment of wastewater containing slowly biodegradable substances

<table>
<thead>
<tr>
<th>SI No</th>
<th>Identity of wastewater</th>
<th>Constituting Slowly Biodegradable Substances</th>
<th>Name of hybrid bioprocess</th>
<th>Important Findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Petroleum and fine chemical industry wastewater</td>
<td>Benzene</td>
<td>Biofilter and Bubble column bioreactor</td>
<td>At 1 hr residence time, complete benzene removal was done.</td>
<td>Yeom, and Yoo, 1998</td>
</tr>
<tr>
<td>2</td>
<td>Antibiotic wastewater</td>
<td>Tetracycline</td>
<td>Powdered activated carbons (PACs) with ultrafiltration (UF) and reverse osmosis</td>
<td>Tetracycline removal efficiency was 72%</td>
<td>Zhang et al, 2005.</td>
</tr>
<tr>
<td>3</td>
<td>Dry-cleaning and degreasing industries</td>
<td>Perchloroethylene</td>
<td>A continuous flow flat sheet hybrid membrane aerated biofilm reactor</td>
<td>The volumetric rate of PCE removal is the highest so far reported in the literature.</td>
<td>Ohandja and Stuckey, 2007</td>
</tr>
<tr>
<td>4</td>
<td>Oil industry</td>
<td>Edible oil</td>
<td>Activated sludge method in membrane bioreactor</td>
<td>The use of an ultrafiltration membrane Increased the efficiency of wastewater treatment.</td>
<td>Moysa and Zoń, 2008</td>
</tr>
<tr>
<td>5</td>
<td>Wastewater from nuclear fuel recovery unit</td>
<td>chlorophenol Is.</td>
<td>Biological hybrid system</td>
<td>Removal efficiency 98%</td>
<td>Makgato and Chirwa, 2009</td>
</tr>
<tr>
<td>6</td>
<td>Wastewater from dye industry</td>
<td>Dyes</td>
<td>Biological stage based on ligninolytic enzymes</td>
<td>Obtained completely decolorized effluent</td>
<td>Palma et al, 2011</td>
</tr>
</tbody>
</table>
5. Conclusion

Waste water containing recalcitrant or inhibitory substances from various industries like petroleum refineries, mining, textile, pharmaceuticals, synthetic resins, tannery etc are growing very rapidly. In view of that, necessity for developing an eco-friendly method for the treatment of slowly biodegradable substances has become a great challenge now-a-days. So far, the wastewater containing recalcitrant or inhibitory organic compounds was mainly treated by a combination of advanced chemical oxidation and biological process like either suspended growth or attached growth. Advanced chemical oxidizing agents like ozone, H₂O₂, Fenton reagents, ultraviolet irradiation are not only found very costly but also they can produce persistent oxidized compounds. Apart from that, handling and disposal of the chemical sludge arising out of this treatment are the critical issues in the present scenario. Hence, the concept of hybrid bioreactor i.e. the addition of attached biofilm in the conventional Activated Sludge Process may be a viable option for such treatment.

Hybrid bioreactor exhibiting both suspended and attached growth has been already used for treating low biodegradable /recalcitrant substances. The attached phase biomass in such a reactor is capable to function even under toxic and inhibitory environment by virtue of its thickness. Thus the slowly biodegradable aromatic and aliphatic organic compounds can be utilized by the biofilm, whereas the easily degradable substances are susceptible for stabilization by the suspended biomass. Therefore, integration of those two types of biomass in hybrid bioreactor results in satisfactory removal of recalcitrant without any hazard to the environment.

6. References


Feasibility of hybrid bioreactor in the treatment of wastewater containing slowly biodegradable substances


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