CHAPTER 4

RESULTS AND DISCUSSION

4.1 CHARACTERISTICS OF DISTILLERY WASTEWATERS

The typical characteristics of the raw spentwash and anaerobically digested distillery wastewater (ANDDW) collected from a local distillery near Chennai are presented in Tables 4.1 and 4.2, respectively. The anaerobically digested distillery wastewater (ANDDW) was used as an influent for sequencing batch reactor in the present study. The spentwash is characterised by the presence of low pH, high percentage of dissolved organic and inorganic matter and dark brown colour.

The chemical oxygen demand (COD) and high biochemical oxygen demand (BOD) typically range between 90,000 – 1,40,000 mg/L and 27,000 – 38,600 mg/L, respectively. The wastewater is characterized by high organic content, of which 50% may be present as reducing sugars. Cane molasses distillery spentwash contains all the dissolved impurities present in the cane juice and the nutrients added during the molasses fermentation. The water soluble hemicelluloses, proteins, gums, organic non-sugars and minerals present in the cane juice present in the spentwash in original or converted forms exert a high chemical oxygen demand (Chandra and Gupta 1997, Thompi 2000).

The high COD and BOD of the distillery spentwash and biodigester effluent are due to the presence of a number of organic compounds, such as,
polysaccharides, reduced sugars, lignin, proteins, melanoidin, waxes, etc. and also due to the presence of organic compounds like phenols and polyphenols (Chaudhari et al 2007).

The raw spentwash is acidic in nature, with a pH in the range of 3.9 - 4.3, the low pH of the spentwash might be due to the formation of organic acids during fermentation process wherein the reducing sugars are broken down to ethyl alcohol and carbon dioxide (Chandra and Gupta 1997). In addition, spentwash contains compounds such as lactic acid, oxalic acid, ethanol and acetic acid that contributes low pH to the effluent (Wilkie et al 2000). If disposed untreated on land, it reduces the alkalinity of the soil, and crops may be destroyed.

Besides high organic load and low pH, the spentwash contains a high nitrogen and phosphorus content of 2,560 – 2,900 mg/L and 76 – 94 mg/L, which has the potential of eutrophication of natural water bodies. The high nitrogen, phosphorus and potassium content of the wastewater have been further reported to be capable of replacing the application of inorganic fertilizers under controlled conditions.

The dark brown colour is primarily attributed to a dark brown pigment (melanoidins) as well as the presence of phenolics, caramel and melanin (Godshall 1999, Kalavathi et al 2001). Melanoidins are high molecular weight polymers and they are formed from the reaction of reducing sugars and amino compounds. The source of the coloured compounds in the distillery spentwash is due to the thermal degradation of sucrose in the solution, which produces various compounds such as 5-hydroxy methyl furfural and coloured condensation products. The highly coloured nature of spentwash can block out sunlight from rivers and streams, thus reducing oxygenation of the water by photosynthesis.
The characteristics of spentwash are highly variable and dependent on the feed stock and various aspects of ethanol production process. Spentwash generated from distilleries is the main threat to environment due to its very high organic and inorganic loading, low pH and high colour. As per the CPCB standards if all the wastewater is treated, the treated effluent from distillery industry should have a pH between 5.5 - 9; suspended solids 100 mg/L; and maximum BOD level of 30 mg/L for disposal into water courses and 100 mg/L for disposal on land (Table 4.3).

**Table 4.1 Characteristics of raw spentwash**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Parameters*</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Sample 4</th>
<th>Sample 5</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pH</td>
<td>4.0</td>
<td>4.3</td>
<td>4.2</td>
<td>3.9</td>
<td>4.0</td>
<td>4.1</td>
</tr>
<tr>
<td>2</td>
<td>BOD</td>
<td>27,000</td>
<td>36,000</td>
<td>38,600</td>
<td>34,000</td>
<td>31,000</td>
<td>33,320</td>
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<tr>
<td>3</td>
<td>COD</td>
<td>90,000</td>
<td>1,20,000</td>
<td>1,40,000</td>
<td>1,10,000</td>
<td>98,000</td>
<td>1,11,600</td>
</tr>
<tr>
<td>4</td>
<td>BOD/COD</td>
<td>0.3</td>
<td>0.3</td>
<td>0.28</td>
<td>0.3</td>
<td>0.31</td>
<td>0.3</td>
</tr>
<tr>
<td>4</td>
<td>TSS</td>
<td>16,016</td>
<td>16,250</td>
<td>18,870</td>
<td>17,000</td>
<td>15,600</td>
<td>16,747</td>
</tr>
<tr>
<td>5</td>
<td>TDS</td>
<td>22,800</td>
<td>30,500</td>
<td>20,800</td>
<td>24,000</td>
<td>29,000</td>
<td>25,420</td>
</tr>
<tr>
<td>6</td>
<td>TKN</td>
<td>2,564</td>
<td>2,600</td>
<td>2,900</td>
<td>2,700</td>
<td>2,560</td>
<td>2,665</td>
</tr>
<tr>
<td>7</td>
<td>Phosphorus</td>
<td>76</td>
<td>94</td>
<td>88</td>
<td>86</td>
<td>90</td>
<td>87</td>
</tr>
<tr>
<td>8</td>
<td>Potassium</td>
<td>9,600</td>
<td>12,000</td>
<td>10,030</td>
<td>12,000</td>
<td>12,600</td>
<td>11,246</td>
</tr>
<tr>
<td>9</td>
<td>Alkalinity</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>Colour (A475)</td>
<td>0.909</td>
<td>0.899</td>
<td>0.836</td>
<td>0.802</td>
<td>0.891</td>
<td>0.867</td>
</tr>
</tbody>
</table>

*except pH and Colour all the parameters in mg/L
Table 4.2 Characteristics of anaerobically digested distillery wastewater

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Parameters*</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Sample 4</th>
<th>Sample 5</th>
<th>Mean</th>
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<tbody>
<tr>
<td>1</td>
<td>pH</td>
<td>8</td>
<td>7.9</td>
<td>8.1</td>
<td>8.1</td>
<td>8.3</td>
<td>8.1</td>
</tr>
<tr>
<td>2</td>
<td>BOD</td>
<td>8,000</td>
<td>8,450</td>
<td>8,000</td>
<td>9,200</td>
<td>8,150</td>
<td>8,360</td>
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<tr>
<td>3</td>
<td>COD</td>
<td>36,600</td>
<td>37,400</td>
<td>37,000</td>
<td>38,600</td>
<td>38,200</td>
<td>37,560</td>
</tr>
<tr>
<td>4</td>
<td>BOD/COD</td>
<td>0.21</td>
<td>0.22</td>
<td>0.21</td>
<td>0.23</td>
<td>0.21</td>
<td>0.21</td>
</tr>
<tr>
<td>5</td>
<td>TSS</td>
<td>12,675</td>
<td>17,000</td>
<td>17,260</td>
<td>20,000</td>
<td>15,600</td>
<td>16,507</td>
</tr>
<tr>
<td>6</td>
<td>TDS</td>
<td>20,800</td>
<td>27,300</td>
<td>29,500</td>
<td>27,340</td>
<td>26,360</td>
<td>26,260</td>
</tr>
<tr>
<td>7</td>
<td>TKN</td>
<td>1,332</td>
<td>1,047</td>
<td>1,277</td>
<td>1,338</td>
<td>1,297</td>
<td>1,258</td>
</tr>
<tr>
<td>8</td>
<td>Phosphorus</td>
<td>56</td>
<td>63</td>
<td>70</td>
<td>69</td>
<td>67</td>
<td>65</td>
</tr>
<tr>
<td>9</td>
<td>Alkalinity</td>
<td>2,960</td>
<td>2,760</td>
<td>3,200</td>
<td>2,900</td>
<td>2,820</td>
<td>2,928</td>
</tr>
<tr>
<td>10</td>
<td>Colour (A&lt;sub&gt;475&lt;/sub&gt;)</td>
<td>0.911</td>
<td>0.900</td>
<td>0.876</td>
<td>0.924</td>
<td>0.918</td>
<td>0.905</td>
</tr>
</tbody>
</table>

*except pH and colour all the parameters in mg/L

Anaerobic digestion of spentwash is the best possible technical option for treatment at the first step. However, spentwash even after anaerobic treatment does not meet the stringent effluent standards laid down by CPCB, India, in terms of very high levels of BOD, COD, Solids etc. (Table 4.3).

Spentwash even after anaerobic treatment has a high pollution potential, with high chemical oxygen demand (COD) of 36,600 – 38,600 mg/L showing a removal efficiency of 60 – 63% in the system. Anaerobic treatment system reduces the organic pollution load and brings down BOD to 75 – 80% of the original value; however the anaerobically digested distillery wastewater (ANDDW) still contains BOD in the range of 8,000 – 9,200 mg/L. Pathade (2001) also reported similar values of BOD in the range of 8,000 – 10,000 mg/L in the anaerobically digested distillery effluent.
The anaerobically digested distillery wastewater (ANDDW) is darker in colour compared to the raw spentwash. In addition, the problem of colour associated with this effluent not only remains unsolved but actually gets aggravated since the colour causing melanoidin pigment intensifies under anaerobic conditions (Pena et al 2003). Melanoidins are recalcitrant compounds; thus, the conventional biological treatment methods are not effective for complete colour removal from this stream.

The pH of wastewater increases from 4.0 to 8.0 after anaerobic digestion and this might be due to the oxidation of organic acids to CO₂ and the reaction between the CO₂ and basic compounds to form carbonates and bicarbonates as illustrated by Beltran et al 1999. Sangave et al (2007 a) also stated that the increase in the pH can also be attributed to the accumulation of bicarbonate (i.e., mineralization of organic matter with the formation of CO₂ leading to the shifting of the acid-base equilibrium to HCO₃⁻).

Spentwash has limited biodegradability (60-70%) through anaerobic route since the organic removal by anaerobic system was only 60 – 70%; anaerobically digested spentwash has low biodegradability due to high inorganic matter. The BOD/COD ratio was 0.3 for the raw spentwash, and 0.2 for the anaerobically treated effluent. This denotes that the biodegradable matters could not be almost removed by the anaerobic system. And therefore, aerobic biological treatment could be employed for the treatment of anaerobically digested distillery wastewater for the complete organic removal from the wastewater stream.
Table 4.3 Effluent standards for distilleries, maltries and breweries

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Parameter</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pH</td>
<td>5.5-9.0</td>
</tr>
<tr>
<td>2</td>
<td>Colour and odour</td>
<td>Absent</td>
</tr>
<tr>
<td>3</td>
<td>Suspended solids (mg/L)</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>BOD (3 day) (mg/L)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(a) Disposal into inland surface water/river/stream</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>(b) Disposal on land for irrigation</td>
<td>100</td>
</tr>
</tbody>
</table>

(Source: CPCB, 2003)

4.2 TREATMENT OF ANDDW: BATCH STUDY

The preliminary study on the treatment of anaerobically digested distillery wastewater (ANDDW) was investigated prior to laboratory scale sequencing batch reactor study. The preliminary study was conducted as batch study in shake flasks with anaerobically digested distillery wastewater supplemented with mineral medium (1:1 v/v) along with enriched bacterial consortium for further use of the bacterial consortium in the laboratory scale sequencing batch reactor. The results of the batch experiments in shake flasks are shown in Figures 4.1 and 4.2.

The initial COD and BOD concentration were around 18,000 mg/L and 6,800 mg/L, respectively. The maximum COD and BOD removal efficiencies were 83% and 90%, respectively with an effluent COD and BOD concentrations of 3,040 and 710 mg/L, after 5 days of incubation. The maximum BOD reduction of 90% was achieved within 3 days of incubation, whereas the COD reduction of 83% was achieved only after 5 days of incubation.
Figure 4.1 Colour, COD and BOD reduction during the batch studies in the treatment of ANDDW
Figure 4.2 Colour, COD and BOD removal efficiencies during the batch studies in the treatment of ANDDW

A significant increase in COD and BOD reduction (>80%) was observed (Figure 4.2), whereas colour reduction was comparatively less (50%). COD reduction was as low as 45% in 2 d and the removal efficiency gradually increased to 80% after 3 days of incubation and maximum COD removal efficiency (83%) was achieved at 5th day and remained static thereafter. It is clear from the figure that the COD, BOD reduction starts during the logarithmic growth phase and the COD, BOD reduction was maximum during the stationary phase. The colour removal was around 50% even after 7 days of incubation and this might be due to the presence of recalcitrant compounds (non biodegradable) that contribute to the colour.

The enriched culture gave a maximum decolourisation yield of 50%, which was achieved only after 6 days of incubation (Figure 4.2). In this study, no decolourisation activity was observed in the anaerobic treatment
system, while under the aerobic condition the decolourisation was moderate for the bacterial consortium when the incubation period was extended to 6 days. The results obtained in the batch treatment of anaerobically digested distillery wastewater by the enriched bacterial culture have shown the feasibility of high COD and BOD removal whereas not as much of decolourisation was observed with distillery effluent as a sole source of carbon and nitrogen. This bacterial culture was selected for further investigation in sequencing batch reactor studies.

This batch treatment system seems to exploit the full potential of the enriched bacteria in the reduction of COD and BOD. Therefore further study was conducted in a sequencing batch reactor system using these enriched bacterial consortium to facilitate further reduction of COD and BOD of the anaerobically digested distillery wastewater before its disposal into the environment.

The metabolic pathways for the utilization of complex compounds that remain undegraded in the spentwash may not exist in bacterial communities involved in the conventional treatment (Johanides 1983). This might be responsible for their recalcitrance (Leisinger 1983). Therefore, it might be important to exploit the biodegradation potential of soil microorganisms from the sites chronically exposed to recalcitrant compounds of distillery spentwash. Such soils may facilitate evolution of biodegradative capability in the microorganisms, and hence may act as reservoirs of bacterial consortia capable of degrading pollutants (Focht 1994).

Therefore, after successful experiment with the batch system, sufficient quantity of enriched bacterial consortium was developed and transferred to the lab-scale sequencing batch reactor for the treatment of anaerobically digested distillery wastewater and wherein the combined use of activated sludge and the enriched bacterial consortium was investigated.
The effect of hydraulic retention time on the aerobic treatment of anaerobically digested distillery wastewater was investigated in a laboratory scale sequencing batch reactor. The performance of SBR at different HRTs of 12 h, 24 h and 48 h was investigated. HRT was optimized by varying the retention time (12 h, 24 h and 48 h) alone with a fixed influent volume of 1,500 mL. The influent COD and BOD was 17,000 and 2,400 mg/L and a constant OLR of 9.0 kg COD/m$^3$/d was maintained. The performance of the SBR was assessed in terms of BOD, COD, TSS, TKN and phosphate removal and the HRT with maximum removal efficiency was chosen to be the optimum HRT for operation of the SBR. The results obtained are presented and discussed in this section.

### 4.3.1 pH and DO

The pH of the SBR was in the range of 7.5 – 8.0 throughout the experiment. This pH range serves to be the optimum for proper growth of microorganisms (Gaudy and Gaudy 1981, Metcalf and Eddy 2003). The pH value gradually decreased from the initial value of 7.5 to 7.0 and then the pH gradually resumes to 7.5 at the end of aerobic oxidation. This trend can be possibly attributed to the conversion of the parent molecules in the wastewater, initially into acidic intermediates, which were further consumed to carryout various metabolic processes of the cell. This view was also supported by Sangave et al (2006) in their study on enzyme assisted biodegradation of distillery wastewater reported that a pH of 7.5 decreased to 6.9 in 8 h of oxidation. They also stated that pH below 6.4 and above 9.4 is detrimental to the organisms and limits the activity of the enzymes of that organisms.

The variation in DO during the experiment at different HRT illustrated in Figure 4.3 indicates that the DO at the end of react phase was
maintained between 2.5 to 4.5 mg/L. The DO in the SBR decreased significantly with decrease in HRT.

![Figure 4.3 Variation of DO in the SBR at different HRTs](image)

**Figure 4.3 Variation of DO in the SBR at different HRTs**

The average DO at 12 h, 24 h and 48 h HRT was 2.8, 3.7 and 4.6 mg/L respectively. At HRT of 24 h and 48 h the variation in DO was not much prominent but at HRT of 12 h the DO was as low as 2.8 mg/L. This rapid variation and drop in DO may be due to higher consumption of oxygen at high organic loading caused by higher flow rates and low HRT (Samarakoon et al 2005).

### 4.3.2 Removal of Total Suspended Solids

The variation in TSS in the SBR effluent at different HRTs is shown in Figure 4.4. The SBR showed a moderate removal of suspended solids. The TSS removal efficiency was almost 62% with marginal difference in the variation of HRTs of 24 h and 48 h and the TSS removal efficiency was only 40% for the HRT of 12 h. The average TSS in the effluent was 6,200 mg/L for HRT of 24 h and 48 h and 9,400 mg/L for HRT of 12 h. The total
suspended solids removal is attributed to the organics (COD and BOD) reduction in the biological treatment system. It implies that the reduction in the suspended solids contributes to the reduction in the COD and BOD of the wastewater.

![Figure 4.4 TSS in the SBR effluent at different HRTs](image)

**Figure 4.4 TSS in the SBR effluent at different HRTs**

### 4.3.3 Removal of Organics

The variation of BOD and COD in the SBR effluent at different HRTs (12 h, 24 h and 48 h) at an organic loading rate of 9 kg COD/m$^3$/d is shown in Figure 4.5 and 4.6. During startup, the BOD was around 2,400 mg/L and the BOD gradually decreased to 990 mg/L showing the removal efficiency of 58% at 12 h HRT within a period of 5-6 days. No further increase in removal efficiency was observed in 12 h HRT and the effluent BOD attained steady state being in the range of 970 - 990 mg/L, respectively. However, HRTs of 24 h and 48 h showed a removal efficiency of 84% and 86%, respectively. This implies that 24 h and 48 h HRT has only a marginal difference in their removal efficiencies. The average effluent BOD at 24 h and 48 h HRT was around 370 and 340 mg/L, respectively. It is evident from Figure 4.5 that an increase in the HRT results in increase in BOD reduction.
The COD of the influent wastewater was around 17,333 mg/L. At 12 h HRT, the COD removal efficiency was as low as 34% (less than 50%) and took around 21 to 24 days to attain such removal efficiency. The COD removal of around 54 and 56% was achieved at HRT of 24 h and 48 h with effluent COD being 7,950 mg/L and 7,680 mg/L, respectively. COD removal efficiency was low when compared to BOD removal efficiency which was 84 – 86%.

Figure 4.5 Removal of BOD in the SBR at different HRTs

Figure 4.6 Removal of COD in the SBR at different HRTs
Moreover, unlike BOD, the COD removal took longer time (20-25 days) to achieve better removal efficiency and this might be due to the presence of high molecular weight substances like polyphenols and melanoidins in the distillery wastewater. The molecular weight distribution of organics plays a key role in the biological treatment process. Low molecular weight compounds like amino acids, VFA and glucose in the wastewater are easily assimilable therefore the BOD removal starts earlier than COD. Polymers in the distillery wastewater such as starch, cellulose and proteins cannot be directly transported across the bacterial membranes. Therefore, some bacteria excrete hydrolases (amylase, cellulase, protease) that degrade the polymers to small assimilable molecules. The hydrolysis of polymers to monomers is rate determining step that requires sufficient time for enzyme production and therefore the removal of COD is getting delayed (Sangave et al 2006).

As evident from the Figure 4.6, nearly 40 to 45 % of COD remains undegraded in the effluent contributing to a residual effluent COD of 7,950 and 7,680 mg/L with 24 h and 48 h HRT, respectively. This higher undegraded COD is attributed to the fact that presence of humic substances and refractory compounds in the distillery wastewater, which are relatively stable under the normal treatment conditions because of the limits of microbial action. Wang et al (2007) reported that the delay in COD reduction might be due to the presence of slowly biodegradable substrate contained in the brewery wastewater.

Haldane and Logan (1994) reported in a macromolecular (polymer) degradation study that the polysaccharides can be released back into the solution before being completely utilised by suspended microbes, thus producing a large molecular weight distribution with only minimal changes in the total COD. The organic matter concentration decreased in relation to the initial COD concentration as a consequence of the bacterial growth in the
reactor i.e. due to the increase in MLSS concentration (Benitez et al 2003). Benitez et al (2003) stated that the Hydraulic retention time in the reactor has a direct influence in the removal of the organic matter and an increase in the HRT results in an increase in removal efficiency of BOD ranging from 31.3 to 84.8% with HRT between 24 and 72 h during the degradation of wine vinasses by activated sludge system.

In another study, Jain et al (2001) achieved a maximum COD removal of 57.5% for an HRT of 3 days for the aerobic treatment of distillery spentwash with an influent COD of 12,048 mg/L, respectively.

4.3.4 Removal of TKN

Figure 4.7 presents the removal of TKN in the SBR effluent at different HRTs. The influent TKN was 500 mg/L. During the HRT of 12 h, the average TKN in the effluent was 180 mg/L, showing a removal efficiency of 64%. Similarly, HRT of 24 h and 48 h showed the TKN removal of 77% and 81% with the effluent TKN of 115 mg/L and 95 mg/L, respectively.

![Figure 4.7 Removal of TKN in the SBR at different HRTs](image-url)
During the startup, the nitrogen removal was slow and the effluent TKN was high. It is evident that only after 12 days, the nitrogen reduced from 500 mg/L to 200 mg/L at 12 h HRT. This might be due to the slow growing nitrifiers that delays the nitrification process. Nitrogen is removed from the system by nitrification and denitrification process. Nitrification is evidenced by the presence of nitrate in the effluent at the end of aerate phase. The average nitrate concentration in the effluent at HRT of 12 h, 24 h and 48 h was 50 mg/L, 62 mg/L and 78 mg/L, respectively at the end of aerate phase. Also, the average nitrite concentration in the effluent at HRT of 12 h, 24 h and 48 h was 2.3 mg/L, 1.9 mg/L and 1.7 mg/L, respectively. With an increase in the HRT, the DO concentration increases therefore the nitrate concentration also increases whereas, the nitrite concentration decreased. Also, denitrification in the system is evidenced by the decreased level of nitrate at the end of settling phase. At the end of settling phase, the nitrate level decreased from 50 mg/L, 62 mg/L and 78 mg/L to 43 mg/L, 51 mg/L and 60 mg/L for the HRTs of 12 h, 24 h and 48 h, respectively. Also an increase in the biomass concentration would have contributed to the TKN removal efficiency. The quantity of N removed for bacterial growth is calculated to be 65 mg/L, 85 mg/L and 100 mg/L for 12 h, 24 h and 48 h HRT with the hypothesis that N used for bacterial growth is 5 % of the total BOD used.

4.3.5 Removal of Phosphate

Figure 4.8 shows the phosphate removal at different HRTs. The SBR showed a phosphate removal of only 8% at 12 h HRT with an average phosphate concentration in the effluent being 44 mg/L. At HRT of 24 h and 48 h, the phosphate removal was comparatively higher with 18 and 19 %, respectively. Similar to nitrogen, phosphate is also removed by assimilation mechanism for the growth of biomass. The reason for less phosphate removal is that the required phosphate is assimilated by the biomass and the remaining phosphate is left into the effluent. Phosphate removal in wastewater usually
occurs at anaerobic conditions. In the present study, the SBR undergoes only oxic and anoxic phase alone therefore there is no significant phosphate removal. The quantity of phosphate removed for bacterial growth is calculated to be 6 mg/L, 8 mg/L and 9 mg/L for 12 h, 24 h and 48 h HRT with the hypothesis that phosphate used for bacterial growth is 1 % of the total BOD used.

![Figure 4.8 Removal of phosphate in the SBR at different HRTs](image)

**Figure 4.8 Removal of phosphate in the SBR at different HRTs**

### 4.3.6 Comparison of Removal Efficiency at Different HRTs

The performance of sequencing batch reactor treating anaerobically digested distillery wastewater at the hydraulic retention times of 12 h, 24 h and 48 h were compared and illustrated in the Figure 4.9. It is clear from the Figure, the BOD removal efficiencies (84% and 86%) were higher at HRTs of 24 h and 48 h. These HRTs also shows relatively good removal efficiencies in terms of TKN removal (77 and 81%) showing very marginal increase at 48 h compared to 24 h. Therefore, HRT of 24 h was selected as optimum HRT and was used further for the treatment of ANDDW combined with domestic wastewater.
Figure 4.9 Comparison of removal efficiency of different HRTs

4.4 TREATMENT OF ANDDW IN SBR: EFFECT OF OLR

The effect of the organic loading rate on the aerobic treatment of anaerobically digested distillery wastewater was investigated in a laboratory scale sequencing batch reactor. The reactor was operated at different organic loading rates of 1.8, 3.6, 5.4 and 9.0 kg COD/m$^3$/d by varying the influent COD of 3,600, 9,000, 12,000 and 17,300 mg/L respectively and the reactor was operated at an optimized HRT of 24 h.

4.4.1 Removal of Organics

The COD and BOD removal in the SBR at different OLRs are depicted in Figure 4.10. The figure shows that when the influent COD concentration was varied from 3,600 mg/L to 17,300 mg/L, the COD removal efficiency was more than 43% for all the OLRs tested and eventually the effluent COD was in the range of 1,420 – 9,500 mg/L.
Increasing the OLR from 1.8 to 3.6 kg COD/m$^3$/d, increased the COD removal efficiency from 60% to 74%, respectively. An increase in substrate concentration increases the growth of microorganisms, thereby enhancing substrate removal along with the biomass growth therefore, the COD removal efficiency increases to 74% at 3.6 kg COD/m$^3$/d. It was also observed that the COD removal efficiency decreased from 74% to 67% at an organic loading rate of 5.4 kg COD/m$^3$/d. A further increase in the OLR to 9 kg COD/m$^3$/d, results in further drop in the COD removal efficiency to 43%. This deleterious response might be due to the fact that high organic loadings greater than 3.6 kg COD/m$^3$/d brought about a decrease in the biomass concentration (MLVSS – 3,600 mg/L) and accumulation of inorganics in the reactor (high MLSS – 12,000 mg/L) causing destabilization of the reactor and process failure and thereby significantly affects the reactor performance in terms of organic removal. COD removal efficiencies in the range of 43 to 74% were achieved for organic loading rates in the ranges of 1.8 – 9.0 kg COD/m$^3$/d. The eliminated OLRs were calculated to be 1.08, 2.66, 3.6 and 3.87 kg COD/m$^3$/d for the various OLRs of 1.8, 3.6, 5.4 and 9.0 kg COD/m$^3$/d showing that the maximum capacity of the reactor is less than 4 kg COD eliminated/m$^3$/d. Therefore the system is effective for the treatment of high strength wastewater at organic loading rate of 3.6 kg COD/m$^3$/d with the potential of rapid startup.

The BOD removal efficiency during each of the organic loadings from 1.8 to 9.0 kg COD/m$^3$/d was in the range of 84 to 98%, respectively. However, at higher organic loads, the effluent quality deteriorated showing effluent BOD of 340 mg/L at 9.0 kg COD/m$^3$/d for an influent BOD concentration of 2,400 mg/L.
Figure 4.10  Removal of BOD and COD in the SBR effluent at different organic loading rates
4.4.1.1 Substrate utilization rate

The relationship between the substrate utilization rate and the COD loading rate was evidenced by a straight line with a high correlation coefficient ($R^2 = 0.9558$) (Figure 4.11). This implies that the substrate utilization rate increases when the COD loading rate is increased; although the increase in COD loading rate leads to decreased percentage of COD removed. This shows that the process can utilize more organics at higher organic loading rates. Mohan et al (2007) also reported that substrate utilization rate increased at increasing organic loads.

Figure 4.12 shows a linear relationship between the effluent COD and specific organic loading rate and it implies that for an effluent COD of 9,000 mg/L, an applied food to biomass ratio of up to 1 is quite reasonable for satisfactory operation.

![Graph showing the relationship between COD loading rate and substrate utilization rate](image)

\[ y = 0.1463x + 0.9306 \]

\[ R^2 = 0.9558 \]

**Figure 4.11** Relationship between the COD loading rate and the specific substrate utilization rate
Figure 4.12 Effect of specific organic loading rate on the effluent COD

4.4.2 Removal of Nitrogen

The variation of TKN in the SBR effluent during different organic loading rates is depicted in Figure 4.13. The figure shows that when the influent TKN concentrations were 100, 250, 330 and 500 mg/L for the OLRs of 1.8, 3.6, 5.4 and 9.0 kg COD/m$^3$/d, the effluent TKN were 2, 36, 68 and 128 mg/L for the respective OLRs. An increase of organic loading rate from 1.8 – 9.0 kg COD/m$^3$/d showed a drop in removal efficiency from 99 to 66%. The TKN removal efficiency varied between 94 – 99% for an organic loading rate of 1.8 kg COD/m$^3$/d. Further increase in organic loading rate to 3.6 and 5.4 kg COD/m$^3$/d resulted in a drop in TKN removal efficiency to 85 and 71%, respectively. Highest loading rate of 9.0 kg COD/m$^3$/d showed a further drop in removal efficiency to 66%. The effluent quality was poor during the higher organic load and wash out of biomass that was reflected from the effluent TSS would have contributed to increase of TKN in the effluent. Such deleterious response might be due to loss of nitrifying activity at higher
organic loads which is clearly evident from the decrease in nitrate level in the effluent at higher organic loads. The nitrate level of the effluent at organic loadings rate of 3.6 kg COD/m$^3$/d was 42 mg/L, whereas nitrate level decreased at the higher loading rates of 5.4 and 9.0 kg COD/m$^3$/d with the effluent nitrate being 30 mg/L and 24 mg/L, respectively. At higher organic loads, the effluent TKN ranged from 120 to 200 mg/L. Such a response might be due to several reasons like washout of biomass, cell death and autorelease (secondary release) of nitrogen and also inhibition of nitrification.

Higher organic load have been reported to inhibit nitrification decreasing the ammonia and nitrogen removal rates. This view was also supported by Tawfik et al (2002) and Lyssenko and Wheaton (2006). According to He et al (2007) high loading of organic material results in lower nitrification efficiency because of loss of ammonium through assimilation by heterotrophs.

The system showed a significant nitrogen removal of 81 to 99% at organic loadings between 1.8 to 5.4 kg COD/m$^3$/d. The continuous and faster increase in biomass reveals that a fraction of nitrogen was assimilated and used for the growth of biomass.

In the present study apart from assimilation, nitrification and denitrification also occurs in the system that was evidenced by the presence of potential denitrifying bacteria identified from the SBR system namely, *Pseudomonas, Bacillus, Flavobacterium* and *Alcaligenes species* and nitrification is evidenced by the presence of nitrifiers like *Nitrosomonas*.

Patureau et al (1994) also reported that the presence of heterotrophic nitrifying and denitrifying bacteria accomplishes simultaneous nitrification and denitrification with the conversion of ammonia to gaseous nitrogen products. The pH of the system in the range of 7.5 to 8.0 is again favourable for optimal nitrification rates in the system.
Figure 4.13  Removal of TKN and Phosphate in the SBR effluent at different organic loading rates
In this SBR study, nitrification is favoured by the presence of high DO in the aerate phase (DO: 3 – 4 mg/L) for the conversion of ammonia into nitrate. During the aeration off period (anoxic fill), denitrification is favoured wherein nitrate is used as electron acceptor in lieu of DO for BOD removal. Nitrate removal is also accomplished during non aerated settle, decant and nonaerated fill period. Thereby SBR provides simultaneous nitrification-denitrification processes in this study with a high nitrogen removal of 70 – 99% even at higher loading rates.

### 4.4.3 Removal of Phosphate

The variation in phosphate removal in the SBR effluent at different organic loading rates is illustrated in Figure 4.13. The phosphate removal showed varied response pattern at different organic loading rates. Initially at an organic loading rate of 1.8 kg COD/m$^3$/d, the phosphate in the effluent was 12 mg/L showing a lower phosphate removal efficiency of 10 – 14%. When the OLR was increased to 3.6 and 5.4 kg COD/m$^3$/d, the phosphate removal efficiency was 36% and 39% respectively with effluent phosphate in the range of 33 and 37 mg/L, respectively. The performance of the reactor improved showing an increase in phosphate removal with increase in organic loadings. The reason for such increased phosphate removal at higher organic loadings might be due to the assimilation of phosphate for the growth of biomass. The growth of biomass is evidenced from the MLSS and MLVSS concentration (Figure 4.14). Secondly, with an increase in the organic loading rate from 1.8 to 5.4 kg COD/ m$^3$/d, the solid retention time decreased from 20 to 5 days and low SRT values has been reported to increase phosphate removal efficiency. However, the phosphate removal efficiency dropped to 16 % at higher organic loading of 9.0 kg COD/m$^3$/d even at very low SRT of 5 days and the effluent quality was poor with phosphate in the effluent being 41 mg/L. The reason for lower phosphate removal at higher organic loading
with low SRT might be due to the washout of biomass in the effluent resulting in an increase in the concentration of phosphate in the effluent. This is also evident from the higher effluent TSS concentration. The reason for low phosphate removal at comparatively lower organic loading rate of 1.8 kg COD/m$^3$/d with longer SRT (20 days) could be due to high rate of nitrification in the react phase of the SBR that resulted in a significant amount of nitrate transfer to the anaerobic phase (Settling phase) which is critical for phosphorus removal. Secondly, the low phosphorus removal efficiency might be due to the longer SRTs. The long SRT process is associated with lower sludge wasting, thereby the production of phosphate storing bacteria in the system is probably less therefore less phosphorus is removed from the system as evidenced from the phosphate level in the effluent. The adverse effect of long SRTs is that the phosphorus-storing bacteria are in a more extended endogenous phase, which will deplete more of their intracellular storage products. If the intracellular glycogen is depleted, the phosphate removal process becomes less efficient (Stephens and Stensel 1998).

When the organic loading rate was increased to 3.6 and 5.4 kg COD/m$^3$/d, the SRT of the system reduced to 7 days and provides phosphate removal of 36 – 39% higher than the systems with long SRT (1.8 kg COD/m$^3$/d). The uptake phosphate might be used for the biomass production. During the phosphorus removal mechanism, the phosphorus in the influent wastewater is incorporated into cell biomass, which subsequently is removed from the process as a result of sludge wasting. Numerous bacteria are capable of storing excess amounts of phosphorus as polyphosphates in their cells.
4.4.4 MLSS and MLVSS

MLSS and MLVSS of the SBR at different OLRs is depicted in the Figure 4.14. During the startup of the reactor at 1.8 kg COD/m$^3$/d OLR, the MLSS was 2,300 mg/L and MLVSS was 1,950 mg/L with the MLVSS to MLSS ratio of 0.84 indicating good quality of sludge. At 1.8 kg COD/m$^3$/d OLR, the average increase in the MLVSS was 150 mg/d. The MLVSS increase was relatively slow when compared to the higher OLRs. The MLSS and MLVSS attained a maximum of 3,100 mg/L and 2,300 mg/L, respectively over a period of 50 days of operation.

During 3.6 kg COD/m$^3$/d OLR, the average MLSS and MLVSS was 5,300 and 4,200 mg/L, respectively and attained a maximum of 9,100 mg/L and 6,100 mg/L in a period of 20 days. The MLVSS to MLSS ratio being 0.67 was very low that affects the performance of the system. The reason might be due to the accumulation of inorganics which has led to an increase in MLSS thereby limiting the growth of biomass.

In contrast to other OLRs, 9.0 kg COD/m$^3$/d OLR had detrimental effect on the SBR because all the OLRs showed an increase in MLSS and MLVSS, whereas 9.0 OLR showed a decrease in MLSS and MLVSS during the initial stages of operation. Once the reactor attained steady state, a rapid increase in MLSS was observed whereas the MLVSS remains low. The MLVSS/MLSS dropped up to 0.5, indicating more accumulation of inorganics in the reactor that affects the performance of the treatment system.
Figure 4.14 Concentration of MLSS and MLVSS at different OLRs (kg COD/m$^3$/d)
4.4.5 Sludge Volume Index

SVI is an important parameter to determine the settleability and dewaterability of the sludge (Metcalf and Eddy 2003, Lamine et al 2007). In the present study, SVI at the 1.8 kgCOD/m$^3$/d OLR was 60 mL/g indicating good settleability of the sludge (Figure 4.15). The increase in organic loading rate to 3.6 kg COD/m$^3$/d results in SVI of 75 mL/g. The increase in the SVI was due to increase in the settled volume of the sludge. SVI < 100 mL/g indicate good settleability characteristics of the sludge yielding high biomass concentration in the aeration tank (Lamine et al 2007). The sludge quality was also excellent with complete flocculation of the sludge particles leaving clear supernatant. The increase in the OLR to 5.4 kgCOD/m$^3$/d results in the SVI of 128 mL/g that shows poor settling along with sludge foaming that carries sludge flocs floating in the supernatant of the mixed liquor.

The sudden increase in the OLR to 9 kg COD/m$^3$/d results in a SVI of 168 mL/g. The increase in SVI after 9 kg COD/m$^3$/d was due to bulking sludge that was difficult to settle. Once, it is beyond 150 mL/g it is suspected to have filamentous growth (Metcalf and Eddy 2003) as in the case of 9 kg COD/m$^3$/d. Since SVI is directly proportional to settled volume of sludge, increase in settled volume results in increase in SVI. The sludge quality was also disturbed with sludge foaming. The SBR sludge showed good settleability with 1.8 and 3.6 kg COD/m$^3$/d. The scanning electron microscope study of the SBR sludge shows Rotifer like species. Rotifers are generally found only in well aerated systems with good biological floc structure (Metcalf and Eddy, 2003).
Figure 4.15 SVI of the SBR sludge at different OLRs

Figure 4.16 SEM photograph of the SBR sludge showing Rotifer like species
4.4.6 Comparison of SBR Performance at Different OLRs

The performance of sequencing batch reactor treating anaerobically digested distillery wastewater at organic loading rates of 1.8, 3.6, 5.4 and 9.0 kg COD/m\(^3\)/d were compared and illustrated in the Figure 4.17. It is clear from the figure, the COD and BOD removal efficiencies (74% and 97%) were higher at an organic loading rate of 3.6 kg COD/m\(^3\)/d compared to other loading rates. This loading rate also shows relatively good removal efficiencies in terms of TKN and Phosphate removal (85 and 37%). The biomass growth was higher at 3.6 and 5.4 kg COD/m\(^3\)/d with MLVSS concentration of 4,200 and 4,000 mg/L and sludge quality at 3.6 kg COD/m\(^3\)/d loading rate was quite satisfactory with SVI of 75 mL/g showing good settleable properties. Therefore, OLR of 3.6 kg COD/m\(^3\)/d was selected as optimum OLR and was used further for the treatment of ANDDW combined with domestic wastewater.

![Figure 4.17 Comparison of SBR performance at different organic loading rates](image)

Figure 4.17 Comparison of SBR performance at different organic loading rates
4.5 PERFORMANCE OF SBR IN THE TREATMENT OF ANDDW COMBINED WITH DOMESTIC WASTEWATER

The performance of Sequencing batch reactor for the treatment of Anaerobically digested distillery wastewater combined with domestic wastewater (1:4 v/v) are presented and discussed in this section.

4.5.1 Removal of Organics

Figure 4.18 illustrates the reduction of BOD and COD in the SBR effluent during the treatment of combined wastewater. The average concentration of COD and BOD in the influent wastewater were 10,000 and 1,350 mg/L, respectively. A significant amount of COD reduction (73%) was observed at 24 h HRT. The COD concentration of effluent was 2,740 mg/L. Whereas, during the treatment of ANDDW diluted with tap water (1:4 v/v) the COD reduction was relatively lower (70%) under the same operating conditions. This increase in removal efficiency might be due to the presence of sufficient nutrients in the domestic wastewater that might have supported for the growth of biomass and COD reduction. The high percentage of organic removal could also be attributed to the fact that the ANDDW along with domestic wastewater gave a relatively high biomass yield. Zhang et al (2003) reported and it is well known that high biomass concentration will provide higher organic removal. The system provides good settleability of sludge which implies that wash out of microorganism does not took place.

The BOD reduction was relatively high (98%) compared to the COD reduction. The final effluent BOD was 30 – 45 mg/L. The data showed that the initial BOD/COD ratio of the influent wastewater was 0.135, which decreased to 0.0009 after aerobic treatment with SBR. Therefore, SBR treatment system seems to utilize full potential of the microorganisms in the
reduction of BOD and COD. The residual organics (2,740 mg/L of COD) in the treated effluent might be the recalcitrant/non biodegradable organics.

Figure 4.18 Removal of COD and BOD during the treatment of ANDDW combined with domestic wastewater
Pathade et al (2001) were able to achieve 88% COD reduction for an influent COD of 3,000 mg/L only at 6 d HRT during the treatment of anaerobically digested distillery wastewater under aerobic conditions using developed mixed microbial seed culture. Wherein the present study attains 70% COD removal at 1 d HRT itself for a higher organic loading rate of 10,000 mg/L of COD.

Kanetkar et al (1990) reported 89% reduction of BOD at 6.6 d HRT in the activated sludge treatment of anaerobic lagoon distillery effluent using acclimatized biomass. Whereas in the present study, the SBR with acclimatized biomass achieved 98% BOD removal.

4.5.2 Removal of TKN

Figure 4.19 presents the reduction of TKN in SBR effluent during the treatment of ANDDW combined with domestic wastewater. At 24 h HRT, the average TKN in the effluent was 53 mg/L, showing a removal efficiency of 86%. In comparison, the treatment of ANDDW diluted with tap water showed a TKN removal of 85 – 86 %.

Nitrogen can be removed by assimilation into biomass or by nitrification process. Nitrogen removal by assimilation was evidenced by continuous increase in MLVSS (biomass) concentration. During start up, the nitrogen assimilation was slow and this might be due to slow growing nitrifying bacteria which needs long generation time to establish and reach sufficient population to nitrify ammonia in the wastewater. Also, high biomass concentration within the reactor would have enabled high TKN removal efficiency.
Figure 4.19 Removal of TKN and Phosphate during the treatment of ANDDW combined with domestic wastewater

The nitrification in SBR was evidenced by the presence of nitrite and nitrate in the effluent. The average nitrate concentration in the effluent was 40 mg/L, respectively. Similarly, the average nitrite concentration was 1.9 mg/L, respectively. The higher nitrate concentration in the effluent show
that the sufficient dissolved oxygen in the reactor converts ammonia to nitrate. The low nitrite concentration in the effluent also indicated the complete occurrence of nitrification process.

### 4.5.3 Phosphate Removal

Figure 4.19 illustrates the reduction of phosphate in the SBR effluent during the treatment of ANDDW combined with domestic wastewater.

The SBR showed a maximum phosphate removal of 31% at an OLR of 3.6 kg COD/m$^3$/d with the average phosphate concentration in the effluent being 46 mg/L. In case of the treatment of ANDW diluted with tap water, the phosphate removal was 33%.

Similar to nitrogen, phosphate was also removed by assimilation mechanism for the growth of biomass. The uptake of phosphate occurs when the wastewater passes through aerobic and anaerobic phase in the Sequencing batch reactor. In the present study, the reason for less phosphate removal is due to the presence of nitrate in the settling phase that serves as an electron acceptor in lieu of DO. The presence of nitrate has been reported to inhibit the phosphate removal in the system. Only the required phosphate was assimilated by the biomass and the remaining is just washed out in the effluent.

### 4.5.4 MLSS and MLVSS

The average MLSS and MLVSS concentration of the sequencing batch reactor treating ANDDW combined with domestic sewage at 3.6 kg COD/m$^3$/d was 5,600 and 4,500 mg/L, respectively (Figure 4.20). The MLSS during the start up of SBR was 4,500 mg/L. Thereafter, the biomass increased gradually and reached a MLSS of 7,000 mg/L in 15 days. Once the
MLSS of 7,000 mg/L was attained, the sludge was wasted from the reactor to maintain the MLSS concentration between 4,000 and 5,000 mg/L. Sludge wasting enables the growth of new biomass. The MLVSS/MLSS ratio varied in the range of 0.75 – 0.85. Initially, the MLVSS/MLSS ratio was 0.85 which later decreased to 0.72 with increase in the MLSS concentration whereas the MLVSS concentration remained the same. The accumulation of inorganics might be contributed to the decrease in MLVSS/MLSS ratio.

Figure 4.20 MLSS and MLVSS of the SBR for the treatment of ANDDW combined with domestic wastewater

4.5.5 Sludge Volume Index

In the present study, the SVI was in the range of 60 – 110 mL/g (Figure 4.21). Initially, the SVI was around 60 mL/g which indicates good settleability characteristics of the sludge with high biomass concentration. The SVI increased to 110 mL/g in 15 days. The increase in SVI was due to the increase in the settled volume of the sludge. The sludge quality declined during this time period. Therefore, sludge wasting was carried out to maintain the MLSS concentration and the MLSS reduced from 7,000 to 4,000
SVI < 100 mL/g indicate good settleability characteristics of the sludge yielding high biomass concentration in the aeration tank (Lamine et al 2007).

Figure 4.21 Sludge Volume Index of the SBR during the treatment of ANDDW combined with domestic wastewater

4.5.6 Scanning Electron Microscopy

Detailed microscopic view of activated sludge flocs of the SBR are depicted in the SEM photograph (Figure 4.22). Numerous flocs comprising of dense and compact bacterial structure with rod and cocci like species embedded in extracellular polymeric substances (EPS) matrix was observed. The bacteria were intimately associated with one another by means of EPS. EPS is a hydrated gel layer that is essential for the formation of flocs (Wang et al 2007).

Figure 4.22 presents the SEM photograph of the SBR sludge showing the floc structure at different resolutions. Floc comprises a dense matrix of bacteria embedded in the thick hydrated gel like substance, the EPS.
The EPS not only allowed the bacteria to occur in clusters but also as isolate cells within EPS matrix. The SEM photographs evidence good settleability of the SBR sludge.

Figure 4.22  SEM photograph of SBR sludge showing floc structure of size (a) 5 \(\mu\)m (b) 1 \(\mu\)m
4.6 OZONATION OF TREATED DISTILLERY EFFLUENTS

Laboratory scale ozonation experiments were carried out in order to investigate the effect of ozone on the treatment of anaerobically digested distillery wastewater before aerobic treatment (Pre-Ozonation) and after SBR treatment (Post-Ozonation).

4.6.1 Effect of ozone on the ANDDW

Ozonation of anaerobically digested distillery wastewater (ANDDW) was conducted at ambient temperature in the glass bubble column reactor as previously described, by varying the time of reaction and ozone application rate. The results of the study are presented and discussed here.

4.6.1.1 Removal of COD

The anaerobically digested distillery wastewater with a COD of 9,760 mg/L was subjected to ozonation at different time intervals. Figure 4.23 depicts the results of variation in COD during the ozonation experiments. It can be seen from the figure that COD decreased continuously with reaction time during the initial stage and later it remained almost constant. At an ozone application rate of 0.2 g/h, i.e. for an ozone dosage of 0.006 g ozone/ g COD, the COD reduced from 9,760 mg/L to 5,940 mg/L in 30 minutes of ozonation. Whereas, at an ozone application rate of 1 g/h (i.e. ozone dosage of 0.006 g ozone/ g COD), the COD reduced gradually from 9,760 mg/L to 5,970 mg/L in 20 minutes of ozonation and further reduced to 5,430 mg/L in 30 minutes of ozonation and no further reduction in COD was observed after 30 minutes of ozonation. No significant COD reduction was observed with higher ozone application rate. In both the cases, no further reduction in COD of 5,430 mg/L was achieved as the oxidation proceeds. This might be attributed to the fact that distillery wastewater contains a high concentration of bicarbonate,
which act as hydroxyl radical scavengers thus inhibiting the reaction of hydroxyl radicals with the organic matter. As a result, hydroxyl radicals generated from the decomposition of ozone were not likely to contribute to further reduction in COD level. Therefore the COD reduction in anaerobically digested distillery wastewater is mainly due to direct mechanism (molecular ozone reaction) rather than indirect mechanism (hydroxyl reaction). Carboxylic acids and aldehydes are usually the end products of direct ozonation reactions (Beltran et al 2001 a) and they made ozone based oxidation process less efficient. Therefore, the COD reduction was moderate in the present study.

Kim et al (1985) also reported that carboxylic acids have been indicated as products of melanoidin ozonation and carboxylic acids hardly react with ozone (Hoigne and Bader, 1983) but they contribute to COD. Jammes et al (1994) also supported this view, stating that ozonation of humic substances led to the formation of small molecules, mainly aldehydes (formaldehydes, acetaldehydes, glyoxyl and methylglyoxal) and carboxylic acids (formic, acetic, oxalic, glyoxylic, pyruvic and ketomalonic acids) which accumulate in the solution due to their resistance towards further degradation by ozonation.

The Figure 4.23 also shows that at for an ozone application rate of 0.2 g/h, a maximum COD reduction of 40% was achieved at 30 minutes of ozonation. At that stage, the ozone consumption was 58 mg for an ozone dosage of 65 mg and only 10% of ozone remained unreacted. Whereas, for an ozone application rate of 1g/h, a maximum COD reduction of 44% was achieved in 25 minutes of ozonation. During this period, the ozone consumption for oxidation was 78 mg at an ozone dosage of 160 mg and the remaining 82 mg of ozone i.e. 52% of ozone escapes unreacted.
Figure 4.23 Removal of COD during ozonation of ANDDW
The experimental results clearly reveal that an increase in the flow rate of ozone from 0.2 g/h to 1 g/h had an insignificant effect on the removal efficiencies. It is also evident that the flow rate of ozone up to 0.2 g/h was sufficient to provide enough ozone molecules to the system for effectively oxidizing the existing organic compounds.

Singh et al (2008) achieved a COD removal of 61% for the thin stillage from ethanol plant at an ozone application rate of 7 mg/min and influent COD of 3,750 mg/L. Whereas, the present study attained a COD reduction of 40% for the lowest ozone application rate of 3.3 mg/min (i.e. 0.2 g/h) for the influent COD of 9,760 mg/L, respectively.

Sangave et al (2007 a) have reported that ozonation of thermally pretreated distillery wastewater led to a COD reduction of around 13% at the end of 20 min. of ozonation for an influent COD of 10,500 mg/L and ozonation of anaerobically digested distillery wastewater results in a COD reduction of 7% for an influent COD of 16,200 mg/L. Wherein, the present study achieved a COD removal efficiency of 40% at 0.2 g/h ozone dosage and 44% at 1 g/h ozone dosage.

4.6.1.2 Removal of colour

Figure 4.24 shows the removal of colour at two different ozone application rates. As a result of ozonation, there was a significant decrease in absorbance at 475 nm. It was found that before ozonation, ANDDW (100 times diluted) showed an absorbance of 4.145. At ozone application rate of 0.2 g/h, the ozonated sample (100 times diluted) showed an absorbance value of 1.145. At an ozone application rate of 1 g/h, the absorbance reduced to 0.888. The reduction in absorbance propably indicates the degradation of the coloured pigments (melanoidin) in the effluent. Wastewater
decolourisation occurred mainly at the initial stage of the reaction (20 min of reaction). Lower ozone application rate of 0.2 g/h led to colour reduction of 65% after 30 min of ozonation. Ozone application rate of 1 g/h provided a colour removal of more than 70% in 20 min of reaction and a further increase in reaction time up to 30 min increased the efficiencies from 70% to 78%. Melanoidins have conjugated carbon-carbon double bonds in their structure. The colour removal was probably due to the fact that ozone is able to cleave these conjugated carbon-carbon double bonds (Kim et al 1985).

However in this study, the efficiencies achieved in colour removal were much higher than those in COD removal. It is likely that ozone transforms the visible chromophore groups instead of breaking high molecular weight compounds into lower molecular weight components (Pena et al 2003) and mineralization of compounds do not take place. Therefore, the COD removal was lower compared to the colour removal efficiencies.

Sangave et al (2007 a) achieved a colour removal of 33% during the ozonation of thermally pretreated distillery wastewater. The sample (200 times diluted) showed an initial absorbance of 0.324 at 475 nm and after 20 min ozonation there was colour reduction, which in terms of absorbance was 0.216.

Sreethawong et al (2008), during ozonation of distillery wastewater with iron oxide catalysts achieved a decolourisation efficiency of 80 – 90% for mass ozone flow rates of 2.96, 3.90 and 4.64 g/h respectively. In the present study, low ozone flow rates of 0.2 g/h and 1 g/h itself achieved a significant colour reduction of 65% and 78% in 30 minutes of ozonation.
Figure 4.24 Removal of colour during ozonation of ANDDW
4.6.1.3 Reduction in UV<sub>254</sub> absorbance

Figure 4.25 gives the reduction in UV absorbance of the ozonated effluent. A decrease in UV absorbance of the effluent could be mainly due to loss of aromaticity of the organic compounds by ozonation. It was found that before the ozonation, ANDDW sample (100 times diluted) showed an absorbance value of 4.335 while after the ozonation, the sample (100 times diluted) showed an absorbance value of 2.44 indicating a moderate reduction in UV absorbance of the wastewater for the ozone application rate of 1 g/h. These values follow the same reduction efficiency as that observed for the COD. The moderate UV absorbance may be attributed to the fact that the ozone reacts with the organic substances leading to the depolymerization of the aromatic compounds.

Killops (1986) and Gilbert (1998) reported that ozonation led to quick decolorization and a decreased in UV absorbance of humic substances was due to loss of aromaticity. Sangave et al (2007b) reported that ozonation of ANDDW led to a decrease in the UV<sub>254</sub> absorbance from 0.816 to 0.586 resulting in 28% of aromatics removal in 20 minutes of ozonation.

Benitez et al (2003) have reported a reduction of 51% in UV absorbance during 9 h of ozonation and 40% reduction in absorbance in 6 h of ozonation in the treatment of wine vinasses wastewater. Beltran et al (2001a) have reported a UV<sub>254</sub> reduction of 25% for ozonation of distillery wastewater. Whereas the present study achieved a reduction of 38% and 44% of UV absorbance in 30 minutes for ozone dosage rates of 0.2 g/h and 1 g/h, respectively.
Figure 4.25  Reduction in UV absorbance (at 254nm) during ozonation of ANDDW
4.6.1.4 pH

The initial pH of the wastewater was between 7.5 to 8.0 and pre-
ozonation was carried out without modifying the pH of the ANDDW. Ozonation process led to a slight drop in the pH value of the wastewater (from 8.7 to 8.0) over a treatment period of 20 min, indicating the formation of the acidic degradation products. At basic pH, ozone decomposes to non selective hydroxyl radicals, which, in turn, attack the organic pollutants. However, distillery wastewater contains more of bicarbonates, which acts as inhibitors of hydroxyl radicals. Consequently, direct attack by molecular ozone reaction occurs in the oxidation of distillery effluent rather than hydroxyl radical reaction, resulting in the formation of carboxylic acid groups which contributes to acidic characteristics of the wastewater (Beltran et al 2001 b).

Carboxylic acids have been indicated as products of melanoidin ozonation (Kim et al 1985) and therefore the initial pH of the wastewater decreases with the reaction time due to the formation of carboxylic acids. Sangave et al (2007 b) in their ozonation studies reported that at the end of every 2 min of ozonation, the pH value dropped by 0.1 -0.2 units due to the formation of acidic degradation products.

4.6.1.5 Biodegradability of the ozonated effluent

The biodegradability of the ozone treated ANDDW was tested by subjecting the ozonated effluent to aerobic oxidation. Ozone treated ANDDW sample has an initial COD of 5,400 mg/L, BOD of 900 with the BOD/COD of 0.166. Whereas, ANDDW without ozone pre-treatment has an initial COD of 9,000 mg/L and BOD of 1,500 mg/L with the same BOD/COD ratio of 0.166. Ozone treated ANDDW was subjected to aerobic oxidation in a Sequencing batch reactor. In meanwhile, ANDDW without ozone pre-treatment was subjected to ozonation in another SBR (control) at same operating conditions. As a result, for ozone pretreated samples and for the control sample (without
ozone pre-treatment), the extent of COD reduction in the aerobic oxidation is same with the effluent COD of 2,700 mg/L and BOD of 30 mg/L. This clearly shows that pre-treatment of ANDDW with ozone did not enhance its biodegradability.

4.6.2 Ozonation of SBR Treated Distillery Effluent (Post-Aerobic Ozonation)

The efficiency of ozonation in the removal of organics and colour from the aerobic SBR effluent is discussed here. The operating time to reach the steady state of the ozonation system was first investigated.

4.6.2.1 Removal of COD

The SBR treated effluent having a COD of 2,760 mg/L was subjected to ozonation. Figure 4.26 gives the variation in the COD of the effluent as a result of ozonation of SBR effluent. A comparison of COD removals between two different ozone application rates of 0.2 g/h and 1 g/h, are also shown in figure. The influent COD of 2,760 mg/L reduced to a maximum of 1,800 mg/L and 1,600 mg/L for the ozone application rates of 0.2 g/h and 1 g/h, respectively at the end of 30 min of ozonation and no further decrease in the COD was observed after 30 min. The COD values remained constant after 30 minutes of ozonation. A reduced amount of COD reduction indicates only molecular level transformations (breaking of complex molecules into simpler lower carbon chain molecules) during ozonation. For the lowest ozone application rate of 0.2 g/h (ozone dose of 0.006 g ozone/g COD), COD removal of 33% was observed with 30 minutes of ozonation. While for the highest ozone application rate of 1 g/h (ozone dose of 0.016 g ozone/g COD), 42% COD removal was achieved in 30 minutes of ozonation. No significant difference was observed between the two ozone application rates. Therefore, it is not practical to increase the flow rate in the ozonation system.
Figure 4.26 Removal of COD of during ozonation of SBR treated effluent (post ozonation)
However, the COD removal efficiency during post ozonation (42% and 33%) is comparatively lesser than ozonation of ANDDW (44% and 40%) for the same ozone application rates. Ozonation of SBR effluent led to the reduction in the COD value, in other words, it brought about the mineralization of some of its contents during the aerobic SBR treatment step itself.

From the Figure 4.26 it’s clear that for a given time, an increase in the ozone dosage applied to the wastewater led to an increase in COD removal. For the highest ozone application rate of 1 g/h, the ozone dosage was 180 mg for 30 minutes of ozonation, wherein the ozone reacted with the organics in the wastewater was only 60 mg, i.e. only one-third of the applied ozone was consumed by the wastewater and the remaining unreacted ozone was let out from the reactor without being consumed. Whereas at an ozone application rate of 0.2 g/h, the applied ozone of 60 mg was consumed completely for the reaction and only 4 mg of ozone remained unreacted.

However, due to partial mineralization of the organic compounds by the initial aerobic biodegradation step, the post-ozonation consumes less ozone compared to the pre-ozonation at equivalent ozone doses, as lesser amount of organic carbon has to be oxidized during the post-ozonation. The COD removal trends are nearly identical for both pre-ozonation and post-ozonation.

Coca et al (2003) stated that the ozonation of biologically pretreated molasses wastewater having a COD of 4,580 mg/L results in a COD reduction of 15% to 25% for the ozone application rate of 1.6 g/h to 11.5 g/h.
4.6.2.2 Removal of colour

Ozonation of SBR effluent results in significant colour removal. The colour removal in wastewater during ozonation is shown in Figure 4.27. The colour was effectively reduced from an initial absorbance value of 2.35 to 0.295 and 0.272 for the ozone application rates of 0.2 g/h and 1 g/h in 30 minutes of ozonation showing a significant colour reduction of 89% and 87% respectively. There is a significant decrease in colour during the first 20 minutes of reaction and direct reactions between ozone molecules and colorants are more predominant and a slight decrease during the remaining time of ozonation. Later, after 30 minutes, the A values remain almost constant.

Though ozonation step led to a significant reduction in colour, higher ozonation time did not enhance the colour removal efficiency and ozone left the reactor without being consumed.

Coca et al (2007), in their study on molasses wastewater ozonation have reported colour reduction of about 70 -75% in 20 minutes, increasing up to 85 – 87% after 40 min ozonation for an ozone flow rate of 1.7 g/h for ozonation of aerobic effluent. Whereas, this study achieved colour reduction of 87% and 89% in 30 minutes at ozone application rate of 0.2 g/h and 1 g/h, respectively.

Coca et al (2003) stated that the ozonation of biologically pretreated molasses wastewater results in colour reduction of 71% to 93% at the ozone application rate of 1.6 g/h to 11.5 g/h.

Pena et al (2003) reported that ozone does not oxidize functional groups responsible for colour but it only transforms chromophore groups, and ozone do not break brown polymers into smaller compounds.
**Figure 4.27** Removal of Colour during ozonation of SBR treated effluent (post-ozonation)
4.6.2.3 Reduction in UV$_{254}$ absorbance

The variation in aromatic content during the ozonation of SBR effluent observed during UV absorbance at 254 nm is presented in Figure 4.28. A measure of absorbance at 254 nm indicated that the ozonation of SBR effluent led to decrease in the absorbance value from 2.7 to 1.98 at ozone application rate of 0.2 g/h and 2.7 to 1.9 for ozone application rate of 1 g/h showing a removal efficiency of 26% and 29%, respectively.

Ozonation at low pH favours highest removal of unsaturated and aromatic compounds (i.e. polyphenol and other UV$_{254}$ absorbing compounds). Therefore, the UV absorbance removal was very lower for distillery wastewater ozonated at basic pH.

The experimental results clearly reveal that an increase in the ozone application rate from 0.2 g/h to 1 g/h showed an insignificant effect on the removal of colour. The results obtained were in the same trend.

4.6.2.4 pH

The pH of the effluent was also monitored during the ozonation step. The effect was similar to that observed for ANDDW. A slight decrease in pH was observed with increasing ozone dosage rates. The initial pH of the wastewater was about 7.9 and this value reduced to 7.6 after ozonation. The pH decrease may be attributed to the formation of carboxylic groups with more acidic characteristics.
Figure 4.28 Reduction in absorbance (UV\textsubscript{254}) absorbance during ozonation of SBR treated effluent (post-ozonation)
4.6.3 Comparison of Pre-Ozonation and Post-Ozonation

The performance of ozonation on the treatment of anaerobically digested distillery wastewater and SBR effluent was compared and presented in the Figure 4.29. The figure shows the performance of ozonation in terms of COD, Colour and UV$_{254}$ absorbance reduction for two different ozone application rates (0.2 g/h and 1 g/h).

![Figure 4.29 Comparison of removal efficiencies of pre-ozonation and post-ozonation](image)

From the figure it’s clear that COD and colour removal and UV$_{254}$ absorbance reduction were slightly lower at ozone application rate of 0.2 g/h compared to the higher ozone application rate of 1 g/h. At the same time, no significant difference in the removal efficiencies was observed for the two ozone application rates. The COD and UV$_{254}$ removal efficiencies were better in post-ozonation compared to pre-ozonation in term of COD and Colour removal. This part of the results has been filed for patent with GOI (Vasudevan and Kanimozhi 2009).
According to the results, it can be concluded that ozonation is an effective treatment to remove colour but less effective to remove organic matter that contributes COD. However, ozonation generates organic compounds (like carboxylic acids) that also contribute to COD and rarely produces complete mineralization of organic matter to carbon-dioxide and water. SBR with pre-ozonation produces effluent with COD of 2,700 mg/L, whereas SBR with post-ozonation produces effluent with 1,800 mg/L. Therefore, it is recommended that SBR with post-ozonation can be adopted for effective colour removal and partial oxidation of organic compounds present in the biologically treated distillery wastewater.

4.7 CHARACTERISATION OF BACTERIAL STRAINS IN SBR SLUDGE

The individual bacterial strains were isolated from the activated sludge of the sequencing batch reactor. The isolated bacterial strains were identified using conventional biochemical tests and by molecular technique (16S rRNA sequencing). Twenty two bacterial strains were isolated and identified from the SBR sludge. The results of the conventional and molecular techniques are presented below.

4.7.1 Biochemical Characteristics

The biochemical tests were carried out for 22 different bacterial isolates and Figure 4.30 depicts the photographic view of various biochemical tests. Genus identification of the unknown bacterial culture was accomplished by the use of Bergey’s Manual of Systematic Bacteriology (2005) for each organism. The bacterial species prevailed in SBR sludge is presented in Table 4.4. A total of 22 isolates viz., Lysinibacillus sp. (CESNV1), Flavobacterium sp. (CESNV2), Myroides sp. (CESNV3), Pseudomonas sp. (CESNV4), Nitrobacter sp (CESNV5), Alcaligenes sp.
(CESNV6), *Comamonas sp.* (CESNV7), *Paenibacillus sp.* (CESNV8) *Pseudomonas sp.* (CESNV9), *Bacillus sp.* (CESNV10), *Paenibacillus sp.* (CESNV11), *Bacillus sp.* (CESNV12), *Bacillus sp.* (CESNV13), *Bacillus sp.* (CESNV14), *Bacillus sp.* (CESNV15), *Bacillus sp.* (CESNV16), *Bacillus sp.* (CESNV17), *Pseudomonas sp.* (CESNV18), *Alcaligenes sp.* (CESNV19), *Klebsiella sp.* (CESNV20), *Bacillus sp.* (CESNV21), *Bacillus sp.* (CESNV22) were identified from the biochemical characterisation.

![Biochemical characteristics of Myroides sp.](image)


**Figure 4.30 Biochemical characteristics of Myroides sp.**

In general, all the 22 bacteria identified using biochemical tests belong to five main phylas namely Firmicutes, CFB group, γ-Proteobacteria, β-Proteobacteria and γ-Proteobacteria. These groups of bacteria are found to be commonly present in the water, wastewater and sewage treatment plants.
Table 4.4 Biochemical characteristics

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<td>Bacillus sp.</td>
</tr>
</tbody>
</table>
4.7.2 16S rRNA Sequencing

In this study, the bacterial diversity of sequencing batch reactor treating distillery wastewater was investigated by 16S rRNA sequencing. Twenty two bacterial strains were isolated from activated sludge of the SBR. Out of 22 strains, 15 strains were identified by 16S rRNA sequencing. Genomic DNA was extracted from the fifteen isolated strains and the DNA was amplified by PCR. The PCR amplified products were analysed in 1% agar gel electrophoresis analysis. The PCR amplified DNA bands of the 15 bacterial strains in the electrophoresis gel are presented in the figure 4.31.

![Image of PCR amplified DNA bands](image)

**Figure 4.31 PCR amplified DNA bands of different bacterial strains (1 to 15) in Agarose gel: M: Marker**
The PCR amplified products after electrophoresis were analyzed for 16S rRNA sequence. The 15 nucleotide sequences were processed by a Blast search of the GenBank database (www.ncbi.nlm.nih/BLAST) for the identification of the bacterial strains. The results of the 16S rRNA sequencing are given in Table 4.5. All the sequences showed >90% similarity to the 16S rRNA sequences available in Gene Bank. Most of bacteria were phylogenetically associated with five main Phyla or groups, (i.e) \( \alpha \)-Proteobacteria, \( \beta \)-Proteobacteria, \( \gamma \)-Proteobacteria, Firmicutes and CFB group. Most of the phylotypes were related to pollutant degrading bacteria. The phylogenetic tree of the bacterial domain showing the distribution of major sequence styles and giving a rough description of bacterial biodiversity in the aerobic SBR is depicted in Figure 4.32.

The identified strains were CESNV1 (Lysinibacillus fusiformis), CESNV2 (Myroides odoratimimus), CESNV3 (Flavobacterium sp), CESNV4 (Pseudomonas putida), CESNV5 (Brevundimonas sp), CESNV6 (Alcaligenes sp), CESNV7 (Comamonas sp), CESNV8 (Paenibacillus dendritiformis), CESNV9 (Pseudomonas straminea), CESNV10 (Bacillus thuringenesis), CESNV11 (Paenibacillus), CESNV12 (Bacillus cereus), CESNV13 (Bacillus subtilis), CESNV14 (Bacillus firmus), CESNV15 (Bacillus sp.). The nucleotide sequences of the identified strains 15 nos. were submitted to GenBank. The Genbank accession FJ649671- FJ649685 for the 15 different bacterial strains is available in http://www.ncbi.nlm.nih.gov/Genbank.

The phylum that was most represented was Firmicutes, that includes, Lysinibacillus fusiformis, Paenibacillus sp, Paenibacillus dendritiformis, Bacillus firmus, Bacillus cereus, Bacillus subtilis, Bacillus thuringenesis and Bacillus sp. The second most represented phylum was Proteobacteria that includes subclass of \( \gamma \)-proteobacteria, \( \beta \)-proteobacteria and \( \alpha \)-proteobacteria wherein, Pseudomonas putida and Pseudomonas straminea.
belong to γ-proteobacteria, *Alcaligenes denitrificans* and *Comamonas sp.* belong to β-proteobacteria and *Nitrobacter sp.* belongs to α-proteobacteria. The other main phylum was CFB group that includes *Flavobacterium sp.* and *Myroides odoratimimus.*

**Table 4.5  Bacterial strains identified by 16S rRNA Sequence**

<table>
<thead>
<tr>
<th>Isolate No. (Strain no. registered in database)</th>
<th>GENBANK (Accession No.)</th>
<th>Phylotype</th>
<th>Affiliation</th>
<th>Similarity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CESNV1</td>
<td>FJ649671</td>
<td>Firmicutes</td>
<td><em>Lysinibacillus fusiformis</em></td>
<td>99%</td>
</tr>
<tr>
<td>CESNV2</td>
<td>FJ649672</td>
<td>CFB group</td>
<td><em>Myroides odoratimimus</em></td>
<td>95%</td>
</tr>
<tr>
<td>CESNV3</td>
<td>FJ649673</td>
<td>CFB group</td>
<td><em>Flavobacterium sp.</em></td>
<td>96%</td>
</tr>
<tr>
<td>CESNV4</td>
<td>FJ649674</td>
<td>Gammaproteobacteria</td>
<td><em>Pseudomonas putida</em></td>
<td>100%</td>
</tr>
<tr>
<td>CESNV5</td>
<td>FJ649675</td>
<td>Alphaproteobacteria</td>
<td><em>Nitrobacter sp.</em></td>
<td>98%</td>
</tr>
<tr>
<td>CESNV6</td>
<td>FJ649676</td>
<td>Betaproteobacteria</td>
<td><em>Alcaligenes sp.</em></td>
<td>100%</td>
</tr>
<tr>
<td>CESNV7</td>
<td>FJ649677</td>
<td>Betaproteobacteria</td>
<td><em>Comamonas sp.</em></td>
<td>92%</td>
</tr>
<tr>
<td>CESNV8</td>
<td>FJ649678</td>
<td>Firmicutes</td>
<td><em>Paenibacillus dendritiformis</em></td>
<td>91%</td>
</tr>
<tr>
<td>CESNV9</td>
<td>FJ649679</td>
<td>Gammaproteobacteria</td>
<td><em>Pseudomonas straminea</em></td>
<td>94%</td>
</tr>
<tr>
<td>CESNV10</td>
<td>FJ649680</td>
<td>Firmicutes</td>
<td><em>Bacillus thuringensis</em></td>
<td>94%</td>
</tr>
<tr>
<td>CESNV11</td>
<td>FJ649681</td>
<td>Firmicutes</td>
<td><em>Paenibacillus sp.</em></td>
<td>93%</td>
</tr>
<tr>
<td>CESNV12</td>
<td>FJ649682</td>
<td>Firmicutes</td>
<td><em>Bacillus cereus</em></td>
<td>91%</td>
</tr>
<tr>
<td>CESNV13</td>
<td>FJ649683</td>
<td>Firmicutes</td>
<td><em>Bacillus subtilis</em></td>
<td>95%</td>
</tr>
<tr>
<td>CESNV14</td>
<td>FJ649684</td>
<td>Firmicutes</td>
<td><em>Bacillus firmus</em></td>
<td>96%</td>
</tr>
<tr>
<td>CESNV15</td>
<td>FJ649685</td>
<td>Firmicutes</td>
<td><em>Bacillus sp.</em></td>
<td>95%</td>
</tr>
</tbody>
</table>
Figure 4.32 Phylogenetic tree constructed using bacterial 16S rRNA sequences of SBR sludge
α-Proteobacteria includes *Nitrobacter sp.*. *Nitrobacter* is strictly aerobic, chemolithoautotrophic and slow growing bacteria. They play an essential role in nitrification process where they convert nitrite to nitrate in wastewater treatment plants (Wang and Reed 1983, Lefebvre et al 2006). Schramm et al (2000) reported that *Nitrobacter* are favoured at high oxygen concentration. They use organic energy sources beside the major source nitrite. Being facultative autotrophs, they are able to grow on heterotrophic substrates such as pyruvate (Schimdt et al 2003). Paul et al (2008) also reported that *Nitrobacter sp.* is the dominating nitrite oxidizers in most wastewater treatment plants.

β-Proteobacteria includes *Alcaligenes denitrificans* and *Comamonas sp.*. They are the most common group of bacteria present in activated sludge process. Kirchman et al (2001) reported that β-proteobacteria is believed to share the ability to degrade complex organic macromolecules with the bacteriodetes. *Alcaligenes* exhibits nitrifying and denitrifying functions during nitrogen removal in activated sludge process (Park et al 2007). *Comamonas* and *Alcaligenes* are reported as potential denitrifiers in the denitrification treatment system (Wang et al 2007). Most of these bacteria are facultative aerobic organisms with the ability to use oxygen as well as nitrate as electron acceptors for the degradation of organics. Aerobic denitrifier has distinct advantages over traditional anoxic denitrification and promotes simultaneous nitrification and denitrification in the same reactor. Thus, nitrogen removal efficiency can be improved markedly by identifying aerobic denitrifiers (Wang et al 2007).

Gamma-proteobacteria includes heterotrophs like *Pseudomonas putida* and *Pseudomonas straminea*, which are important in the degradation of organic matter and in maintenance of the biofilm structure. Proteobacteria group reported to show high degradation capacity, assimilating acetate, and
other organic acids under oxic and anoxic conditions (Bramucci et al 2003). The bacterial sequences assigned to Proteobacteria and Bacilli also possess hydrocarbon degrading activity. *Pseudomonas* species are the most common and widely distributed of all the denitrifiers and they are identified as having phosphorus removal ability (Wang et al 2008).

**Cytophaga - Flexibacter - Bacteriodetes (CFB) group includes** *Myroides sp.* and *Flavobacterium sp.* They are generally characterized by phosphate accumulating organisms and they are found to dominate Enhanced Biological Phosphate Removal (EBPR) sludge (Liu et al 2005). Besides these, members of the CFB group are also recognized to have a potentially unique role in the utilization of complex organic molecules like pectin and cellulose (Forsberg et al 1981).

**Firmicutes form a most abundant group of bacterial diversity in** SBR sludge belonging to genera Bacilli which suggests that this group of heterotrophic bacteria contribute a significant fraction in degradation of organics in the wastewater. They play a major in denitrification process of nitrogen removal in wastewater treatment by converting nitrate to nitrogen gas. *Bacillus sp.* seems to be the most dominant clone in the SBR sludge treating distillery wastewater. The bacterial strains identified by 16S rRNA sequencing complies with the results of the bacterial strains identified by biochemical characterisation.

Thus, the high strength distillery wastewater due to excess organic matter serves as a rich medium to initiate profuse growth of aerobic and facultatively aerobic microorganisms belonging to the phyla Firmicutes, Proteobacteria and the CFB group. The bacterial consortium used in this study has the full potential to tolerate the distillery wastewater and was able to treat the wastewater containing high COD to the extent of 74% and BOD to the extent of 96%, respectively.