2.1 Nitrogenous Pollutants
Ammonium (NH₄⁺), nitrite (NO₂⁻) and nitrate constitute the most common nitrogenous compounds found in wastewaters. These ions can naturally be present in water bodies as a result of atmospheric deposition, surface and ground water runoff, dissolution of nitrogen rich geological deposits, nitrogen fixation by certain bacteria and biological degradation organic matter. Several industrial sectors requires different N-substituted aromatic compounds such as nitro-aromatics, azo dyes and aromatic amines, for intensive production of dyes, explosives, pesticides and pharmaceuticals products. These chemicals are intensively designed to remain unaffected under conventional product service condition and it is this property, linked with their toxicity to microorganism, which make their biodegradation difficult. The release of these recalcitrant N-pollutants into the water streams may create serious health and environmental problems. Many organic nitrogen pollutants have been shown to be toxic for different aquatic species and to have mutagenic and carcinogenic activity. Moreover due to their hydrophobic nature many recalcitrant N-pollutants have the risk to bio-accumulate in the food chain. (Pinheriro et al., 2004, Dos Santos et al., 2007).

The primary sources of nutrient pollution includes runoff of fertilizers, animal manure, sewage treatment plant discharges, storm water runoff, car and power plant emissions, and leaked septic tanks also nutrients from row crops and concentrated animal feeding operations are contributors in most nutrient pollution. (US EPA 2016)

2.2 Impact of Nitrogen Pollution
The three major environmental problems caused by nitrogen pollution in aquatic ecosystems are; (1) it can drastically decrease the pH of fresh water ecosystems without much acid-neutralization capacity, leading to acidification of these water bodies; (2) it stimulate or enhances the development and maintenance of primary producers, resulting in eutrophication of aquatic ecosystem and (3) it can also impair the ability of aquatic animals to survive, grow and reproduce as a consequence of direct toxicity of nitrogenous compounds. In addition nitrogen pollution of ground and surface waters can induce adverse effects on human health. (Camargo and Alonso
Several organic nitrogenous contaminants generated by different industrial sectors are associated with toxicity, carcinogenesis, mutagenesis and allergies in living organism.

2.3 CONVENTIONAL NITROGEN REMOVAL PROCESS

Conventional biological nitrogen removal from municipal and industrial wastewater comprises of two biological steps i.e the nitrification, the oxidation of ammonium to nitrite or nitrate, and the denitrification—the reduction of nitrite or nitrate to nitrogen gas. But, many wastewaters contains the low level of organic matter which is not sufficient for complete denitrification step and addition of an external carbon source, such as methanol, is often required to attain complete denitrification (van Loosdrecht et al., 1998, Jetten et al., 1999). The cost of the chemicals addition and the treatment of the additional sludge that is generated increases the overall operating costs in the wastewater treatment plant.

2.3.1 Nitrification

Nitrification is the biological oxidation of ammonia to nitrite then to nitrate. It is carried out by two types of chemolithoautotrophic bacteria. Ammonia oxidation is catalysed by ammonia oxidizing bacteria (AOB) whereas nitrite oxidation is catalysed by nitrite oxidizing bacteria (NOB). Compared with heterotrophic organism, growth of nitrifying bacteria is slow and scarce, even in the optimal conditions. Nitrification is a process which occurs in natural environment like soils, continental and marine waters in which it plays fundamental role in nitrogen cycle. This process is the first step of nitrogen removal in biological wastewater treatment.

2.3.1.a Ammonia Oxidation

It is generally accepted that ammonia (NH3) rather than ammonium (NH4+) is the substrate for AOB Ammonia is oxidized according to following reactions. (Kowalchuk and Stephen 2001)

\[
\begin{align*}
\text{NH}_3 + 2\text{H}^+ + 2\text{e}^- + \text{O}_2 & \rightarrow \text{NH}_2\text{OH} + \text{H}_2\text{O} \quad (2.1) \\
\text{NH}_2\text{OH} + \text{H}_2\text{O} & \rightarrow \text{HNO}_2 + 4\text{H}^+ + 4\text{e}^- \quad (2.2)
\end{align*}
\]
The first reaction is catalysed by an ammonia monooxygenase (AMO) and second one by a hydroxylamine oxidoreductase (HAO).

Ammonia is used as an electron donor by the AOB and the final electron acceptor is oxygen. Two of the electrons produce in second reaction are used to compensate for the electron input for the first reaction, whereas the other are passed via electron transport chain to the terminal oxidase, there by generating a proton motive force (Kowalchuk and Stephen 2001). This proton motive force is used as the energy source for ATP production.

The following reaction gives the sum reaction of ammonia oxidation to nitrite.

\[
\text{NH}_3 + 1.5 \text{O}_2 \rightarrow \text{HNO}_2 + \text{H}_2\text{O} \quad (2.4)
\]

\[
\text{NH}_3 + 1.5 \text{O}_2 \rightarrow \text{NO}_2^- + \text{H}_3\text{O}^+ \quad (2.5)
\]

The standard free energy yield (\(\Delta G^*\)) from the oxidation of ammonia is \(-275 \text{ kJ mole}^{-1}\). It can be seen from reaction (2.5) that ammonia oxidation produce acidity and that this reaction is highly oxygen consuming 1.5 mole oxygen per mole of ammonia which 3.43 g oxygen per g of ammonia nitrogen.

**2.3.1.b Nitrite oxidation**

Nitrite is oxidized to nitrate by NOB in one single step

\[
\text{NO}_2^- + 0.5\text{O}_2 \rightarrow \text{NO}_3^- \quad (2.6)
\]

This reaction catalyzed by a nitrite oxidoreductase (NOR). Nitrite is the electron donor of NOB respiration while oxygen is final electron acceptor.

The free energy yield from the oxidation of nitrite is only \(-74 \text{ kM mole}^{-1}\). The consequence is low growth yield, even when compare with AOB.
2.3.2 Microbiology of Nitrification

2.3.2.a Ammonia Oxidizers (AOB)

AOB form two monophyletic groups, one within the beta-and one within the gamma-proteobacteria (Purkhold et al. 2000). Most of the AOB are beta-proteobacteria: *Nitrosomonas, Nitrospira* and *Nitrococcus mobilis* (that is related to *Nitrosomonas*) whereas the other Nitrococcus species are gamma-proteobacteria (Schmidt et al. 2003). It is generally accepted that nitrosomonads (including *Nitrococcus mobilis*) and not nitrosospiras (including the genera *Nitrospira, Nitrosolobus* and *Nitrosovibrio*) are important for ammonium oxidation in wastewater treatment plants (Wagner et al., 2002). Moreover, (Konneka et al. 2005) were the first in isolating an ammonia oxidizing archeon.

2.3.2.b Nitrite oxidizers (NOB)

NOB includes *Nitrobacter, Nitroccocus*, both being part of the alpha proteobacteria, and *Nitrospira* that forms separate division (Schmidt et al., 2003). A fourth genus, *Nitrospina* has only be found in marine environment. The use of molecular tools showed that uncultured *Nitrospira*-like bacteria are the most commonly found NOB in wastewater treatment plant (Daims et al., 2001). This predominance of *Nitrospira*-like bacteria over Nitrobacter could be due to their different survival strategies. *Nitrospira*-like NOB are K-stratigists, which means they are well adapted to low nitrite and oxygen concentration because of their low ks (high affinity to substrates) even if they may possess a low µmax. On the other hand, *Nitrobacter* is supposed to be fast growing r-strategists with low affinity to nitrite and oxygen which gives a compitative advantage in environments with high substrate concentration. Nitrite concentration in reactors are generally low, therefore *Nitrobacter* are outcompeted by *Nitrospira*-like bacteria. (Schramm 1999).

In systems with temporally or spatially elevated nitrite concentrations such as sequencing batch reactor or biofilm reactors, both nitrite oxidizers should be able to coexist (Wagner et al., 2002). Thus, *Nitrobacter* and *Nitrospira*-like bacteria have been observed simultaneously by florescent in situ hybridization (FISH) in a nitrifying sequencing batch biofilm reactor (Daims 2001).
2.3.3 Denitrification

Denitrification is biological reduction of nitrate to nitric oxide, nitrous oxide and nitrogen gas. The nitrate reduction reaction involves the following reduction steps from nitrate to nitrite to nitric oxide, to nitrous oxide and to nitrogen gas

\[
\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2
\]  

(2.7)

Biological denitrification is an integral part of biological nitrogen removal which involves both nitrification and denitrification.

2.4 ANAMMOX PROCESS

The Anammox process (Anaerobic Ammonia Oxidation) removes ammonium without oxygen and with nitrite as an electron acceptor. The cost of conventional nitrogen removal process via nitrification denitrification is mainly associated with aeration cost and addition of expensive electron donor (e.g. methanol). The anammox process provides an alternative to nitrification, with no requirement of extra electron donor.

Figure 2.1. Conventional Nitrification/Denitrification and Anammox Process.
Since the discovery of anammox process in Delft (Netherlands) evidence for anammox activity has been obtained in a variety of laboratories and engineered systems (Schmid et al., 2005), pilot plants for ammonium removal (van Dongen et al. 2001; Fux 2002), and even marine sediments (Thamdrup and Dalsgaard 2002) as well as the sub-oxic zone of the Black Sea (Kuypers et al., 2003). Thereby, other anammox organism different to Candidatus “Brocadia Anammoxidans” were identified: Candidatus “Kuenenia stuttgartiensis”, Candidatus “Scalandua Brodae”, Candidatus Scalandua wagneri”, Candidatus “Brocadia fulgida” and Candidatus “Anammoxoglobus Propionicus” were discovered and their 165rRNA sequences were determined (Schmid et al., 2000; Schmid et al., 2003; Kuypers et al., 2003; Kartal et al., 2007,2008)

The studies about enrichment or operation of anammox process at laboratory or Pilot scale reactors up to date were performed mainly with bacteria of genus C. “Brocadia” or C. “Kuenenia”, so the major part of the available information on anammox is about these types of bacteria.

Nitrogen removal is one of the most desirable aspects of wastewater treatment at present and is usually performed through sequential nitrification and denitrification processes. Ammonium NH$_4^+$ is oxidized to nitrate (NO$_3^-$) followed by a reduction of (NO$_3^-$) to dinitrogen (N$_2$). The ANAMMOX process is a novel and promising alternative to conventional denitrification systems to remove nitrogenous compounds at lower cost (Dong et al., 2003; Pathak et al., 2006).

It has been regarded as an innovative, low cost method to treat wastewater containing high ammonium concentrations (Jetten et al., 1997). By coupling anammox with other processes like sharon and partial nitrification is a smart way of application for municipal treatment and existing wastewater treatment plants could be converted from energy-consuming into energy-producing systems (Kartal et al., 2010). It is a most popular topic of researches in the fields of microbiology and environmental science and engineering due to its advantages of effective removal of both ammonium and nitrite under anoxic conditions with high removal rate, lesser sludge production.
TABLE 2.1 Review of different anammox reactors

<table>
<thead>
<tr>
<th>Reactor Type</th>
<th>Sludge Type</th>
<th>HRT (h)</th>
<th>Influent $\text{NH}_4^+$ (mg/L)</th>
<th>Influent $\text{NO}_2^-$ (mg/L)</th>
<th>Nitrogen Removal efficiency (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLR</td>
<td>Anammox sludge</td>
<td>10</td>
<td>1545</td>
<td>-</td>
<td>42</td>
<td>Sliekers et al. (2003)</td>
</tr>
<tr>
<td>SBR</td>
<td>Anammox granules</td>
<td>24</td>
<td>150</td>
<td>150</td>
<td>98</td>
<td>Arrojo et al. (2006)</td>
</tr>
<tr>
<td>MSBR</td>
<td>Anammox sludge</td>
<td>24</td>
<td>390</td>
<td>390</td>
<td>&lt;80%</td>
<td>Trigo et al. (2006)</td>
</tr>
<tr>
<td>UFFBB</td>
<td>Denitrifying sludge</td>
<td>0.2-8</td>
<td>20-550</td>
<td>20-460</td>
<td>50-63</td>
<td>Tsushima et al. (2007b)</td>
</tr>
<tr>
<td>SBR</td>
<td>Anammox sludge</td>
<td>-</td>
<td>700</td>
<td>0</td>
<td>60</td>
<td>Vazquez-Padin et al. (2009)</td>
</tr>
<tr>
<td>SBR</td>
<td>Anammox sludge</td>
<td>-</td>
<td>200-250</td>
<td>0</td>
<td>77</td>
<td>Vazquez-Padin et al. (2009)</td>
</tr>
<tr>
<td>UASB</td>
<td>Anammox sludge</td>
<td>&lt;24</td>
<td>100-458</td>
<td>100-575</td>
<td>99%</td>
<td>Ni et al. (2011)</td>
</tr>
</tbody>
</table>

GLR, gas-lift reactor; SBR, sequencing batch reactor; MSBR, membrane sequencing batch reactor; UFFBB, up-flow fixed-bed biofilm reactor; UASB, upflow anaerobic sludge blanket

Trigo et al., (2006) studied the start-up of an Anammox process in a membrane sequencing batch reactor (MSBR) fitted with submerged hollow fibre membrane module for biomass retention. The Anammox biomass reactor was used seeding the reactor and fed with synthetic wastewater. It was observed that during initial operating stage, the activity of microorganism was disturbed by salt precipitation that results in drop of the nitrogen removal rate from 100 to only 10 mg/L per day. To avoid salt precipitation the calcium and phosphorus concentrations were decreased in the synthetic medium during the last operating stage due to which the activity of the system regained and nitrogen removal rate up to 710 mg/L per day could be achieved with almost full nitrogen removal. It was reported that anammox biomass grow as granules and not in the form of flocs which showed that these microorganisms have a tendency to grow in the form of aggregates. The use of the membrane results in biomass retention in the system. Results also demonstrated that
the use of the membrane sequencing batch reactor could be a suitable option for nitrogen removal by using the Anammox process.

Yang et al., (2011) investigated the nitrogen removal performance of the anaerobic ammonium oxidation (Anammox) process and the microbial cultures that are capable of performing well at ambient temperatures in Anammox systems. A reactor fixed with spiral structure was used as the gas–solid separator. The synthetic inorganic feed mainly composed ammonium and nitrite was supplied to reactor. The reactor was operated for about 92 days. At operating temperatures of 33°C and 23°C, nitrogen removal rates obtained were 16.3 and 17.5 kg-N m⁻³ d⁻¹ respectively. At ambient temperatures such a high nitrogen removal rates has not been reported earlier studies. It was also observed during the study that the Anammox activity did not inhibited by high influent nitrite concentration of 460 mg/L. The freshwater Anammox bacterium KU2, was identified as the dominant bacterial culture is considered to be responsible for the stable nitrogen removal performance at ambient temperatures.

Jin et al., (2008) performed the quantitative analysis of stability of anammox process in different reactor configurations and the stability of anammox process in three different reactor configurations was evaluated. The analysis was performed using three laboratory scale anammox reactors namely upflow anaerobic sludge blanket (UASB) reactor, upflow stationary fixed film (USFF) reactor and anaerobic sequencing batch reactor (ASBR). The reactors performance was compared for their stability based on hydraulic and substrate concentration shocks. The performance of anammox reactors displayed differential stability towards varying substrate and flow rate shocks. It was observed that instability indices for substrate shock were higher than that for flow rate shock. The anammox reactors were more tolerant to flow rate shock than to substrate shock. The stability of the three reactors was ranked as UASB reactor > USFF reactor > ASBR to substrate concentration shock and ASBR > UASB reactor > USFF reactor to flow rate shock, respectively. In terms of stability, UASB reactor could be declared as more suitable configuration than USFF reactor.

Kieling et al., (2007) performed experimental study on stat-up of Anammox process based on sludge wash-out strategy in order to attain it in a shortest possible duration. The study was carried out in two anaerobic sequencing batch reactors (ASBR) RI and
RII. Seeding of the SBRs was done using a sludge obtained from domestic wastewater treatment plant. During the start-up phase RI was operated as a continuous stirred tank reactor (CSTR) having dilution rate of 0.2 d\(^{-1}\), which leads the sludge washout from the system. After this period, the remaining sludge was retained in the reactor. The reactor RII was operated as an ASBR for the entire study period with a high biomass retention. For 380 days of operation the performance of the two SBRs was compared with respect to nitrogen removal. It was observed that that during the last RI operation phase there was an exponential increment in specific nitrogen removal rate with achieved values of 85 mg N/g TSS d. Whereas the removal rate value obtained in a batch test was 190 mg N/g TSS. The specific nitrogen removal rate was found to be almost constant for RII with an average value of 6 mg N/g TSS d being observed during the operation period. The rate reported in batch test was 20 mg N/g TSS d for RII. These results of the study showed that reactor RII with higher total suspended solids means reactor with high biomass retention was not effective in terms of nitrogen removal improvement. In addition, the observed nitrogen removal rate for RI was comparable to those reported in the literature for the same process, and this could be improved in the future by optimizing the ASBR operation. Anammox-like bacteria were found using fluorescence in situ hybridization (FISH) in reactor RI after 225 days and a new Anammox species was identified.

Zhengyong et al., (2007) studied the nitrogen removal mechanism while treating the ammonium-rich landfill leachate by a set of sequencing batch biofilm reactors (SBBRs) system. Initially the 58 days acclimatization period 33 days stabilization period was provided for effective nitrogen removal. The liquid temperature during operation was found to be 32°C, after stabilization period. The ammonium removal in the SBBR was found to be 95%. Intermittent aeration was done frequently which leads to suppression in activity of nitrate bacteria, and also eliminated the influence on the activity of anaerobic ammonium oxidation (ANAMMOX) bacteria and nitrite bacteria. This influence was caused due accumulation of nitrous acid and the lowering of pH. During the aeration stage, the concentration of dissolved oxygen was maintained between 1.2–1.4 mg/L. The nitrite bacteria became dominant and nitrite start accumulating in the system. During the anoxic stage, with decreasing concentrations of the dissolved oxygen, anammox bacteria became dominant in the
system and the nitrite that was accumulated in the aeration stage removed along with ammonium simultaneously.

2.5 CANON PROCESS
The CANON system (Completely Autotrophic Nitrogen Removal Over Nitrite) can potentially remove ammonium from wastewater in a single, oxygen-limited treatment step. The usefulness of CANON as an industrial process will be determined by the ability of the system to recover from major disturbances in feed composition. The CANON process relies on the stable interaction between only two bacterial populations: *Nitrosomonas*-like aerobic and *Planctomycete*-like anaerobic ammonium oxidising bacteria. These autotrophic cultures convert ammonia directly to dinitrogen gas with nitrite as an intermediate. Application of this concept to wastewaters can potentially lead to complete ammonia removal in a single autotrophic reactor. The two groups of microorganisms interact and perform the two sequential reactions simultaneously. Subsequently, anaerobic ammonium oxidizers *Planctomycete*-like ANAMMOX bacteria convert ammonium with the produced nitrite to dinitrogen gas and trace amounts of nitrate (Strous *et al.*, 1999b).

The Canon process is the combination of partial nitrification and Anammox processes within a single reactor Third *et al.*, 2001. The Canon process has been found to be a cost-effective autotrophic nitrogen removal process, as it requires less oxygen, has no need for a carbon source, and yields a lower amount of sludge, compared to conventional nitrification/denitrification processes. (Ahn *et al.*, 2006).

Chang *et al.*, (2013) study the performance of a completely autotrophic nitrogen removal over nitrite (CANON) process in bio-ceramic filter for treating wastewater with different substrates (ammonia) at ambient temperature. The completely autotrophic nitrogen removal over Nitrite (CANON) reactor was fed with various concentrations of ammonia (400, 300, and 200mg N/L) keeping influent ammonia loading rate constant. The results showed that the CANON system can achieve good treatment performance at ambient temperature of 15-23°C. The average removal rate and removal loading of ammonia and total nitrogen was 83.90%, 1.26 kg N/(m3-day), 70.14% and 1.09 kg N/(m3-day) respectively. Among the various parameters affecting the CANON process such as pH, DO and alkalinity, it was found that the pH
was the key parameter of the performance of the CANON system. Monitoring the variation of pH would provide better control of the CANON system. Denaturing gradient gel electrophoresis was used for analysis of microorganisms. The results of DGGE analysis showed that there were some major changes in the community structure of ammonium oxidizing bacteria (AOB), which had low diversity in different stages, while the species of anaerobic ammonium oxidizing bacteria (anammox) were fewer and the community composition was relatively stable. These observations showed that anaerobic ammonia oxidation was more stable than the aerobic ammonia oxidation, which could explain why the CANON system maintained good removal efficiency under the changing substrate conditions.

Ahn et al., (2006), studied the feasibility and performance of upflow granular sludge bed reactor for nitrogen removal by canon process. Both synthetic wastewater and sludge digester reject water were used for the study. The first experiment was conducted using the synthetic wastewater (up to 110 mg NH$_4$+–N / L), hydraulic retention time was kept 5 days and air supply was provided in external aeration chamber as a source of oxygen with flow recirculation. The ammonium removal was about 95% was achieved. The second experiment was conducted using wastewater from sludge digester having 438± 26 mg NH$_4$+–N / L, the hydraulic retention time was kept 7 days and 5 days, small amount of nitrite and nitrate were reported in the effluent of both experiments. The process showed lower oxygen uptake 0.29–0.59 g O$_2$ /g N and less alkalinity 3.1–3.4 g CaCO$_3$ /g N consumption as compared to other new technology in biological nitrogen removal. The process also offers the feasible, economical and compact reactor configuration with excellent biomass retention, resulting in low investment and maintenance cost.

Third et al., (2005) reported a successful enrichment of Anammox bacteria from activated sludge under anaerobic conditions in a sequencing batch reactor (SBR) and were able to successfully initiate a Canon process in an intermittently fed chemostat. In the oxygen-limited conditions, nitrite oxidizers have to compete for oxygen with the aerobic ammonia oxidizers and for nitrite with anaerobic ammonia oxidizers. Possible inhibition of nitrite oxidizers by free ammonia has been suggested (Abeling et al., 1992). Considering this, ANAMMOX processes are feasible at low bulk oxygen concentrations. ANAMMOX bacteria are reversibly inhibited by low (0.5%
air saturation) concentration of oxygen (Strous et al., 1997b). The combined process can occur under oxygen-limited conditions.

### Table 2.2 Review of different canon reactors

<table>
<thead>
<tr>
<th>Reactor type</th>
<th>Sludge type</th>
<th>HRT (h)</th>
<th>Influent $\text{NH}_4^+$ (mg/L)</th>
<th>Nitrogen Removal efficiency (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBR</td>
<td>Anammox+ AOB</td>
<td>24</td>
<td>-</td>
<td>36-92</td>
<td>Third et al. (2001)</td>
</tr>
<tr>
<td>SBR</td>
<td>Anammox</td>
<td>24</td>
<td>131</td>
<td>42</td>
<td>Sliekers et al. (2002)</td>
</tr>
<tr>
<td>RBC</td>
<td>OLAND sludge</td>
<td>24</td>
<td>840</td>
<td>89</td>
<td>Pynaert et al. (2003)</td>
</tr>
<tr>
<td>UP-FLOW</td>
<td>Anaerobic granules and anoxic activated sludge</td>
<td>120</td>
<td>438</td>
<td>56.7</td>
<td>Ahn and Choi (2006)</td>
</tr>
<tr>
<td>MABR</td>
<td>Nitrifying+ Anammox</td>
<td>-</td>
<td>200</td>
<td>84</td>
<td>Gong et al. (2008)</td>
</tr>
<tr>
<td>RBC</td>
<td>OLAND sludge</td>
<td>32</td>
<td>1215</td>
<td>76</td>
<td>Vlaeminck et al. (2009)</td>
</tr>
<tr>
<td>NRBC</td>
<td>Anammox+ partial nitrifying</td>
<td>5-6</td>
<td>200</td>
<td>70</td>
<td>Chen et al. (2009)</td>
</tr>
<tr>
<td>SBR</td>
<td>Nitrifying+ Anammox</td>
<td>12</td>
<td>180-330</td>
<td>78</td>
<td>Vazquez-Padín et al. (2009)</td>
</tr>
<tr>
<td>UP-FLOW</td>
<td>Nitrifying</td>
<td>5</td>
<td>130-300</td>
<td>22</td>
<td>Cho et al. (2011)</td>
</tr>
<tr>
<td>UP-FLOW</td>
<td>Anammox</td>
<td>3-5</td>
<td>206</td>
<td>16</td>
<td>Cho et al. (2011)</td>
</tr>
</tbody>
</table>

OLAND sludge, aerobic nitrifiers + heterotrophic denitrifiers + anammox biomass; MABR, membrane aerated biofilm reactor; SBR, sequencing batch reactor; RBC, rotation biological contactor; NRBC, non-woven RBC

Sliekers et al., 2002 studied completely autotrophic nitrogen over nitrite in one single reactor. They investigated the microbiology and the feasibility of this new, single-stage, reactor for completely autotrophic ammonia removal. The reactor was inoculated with biomass obtained from a working anaerobic ammonia oxidation
(Anammox) reactor and operation started anoxically. But later on oxygen was supplied to the reactor to develop nitrifying population. Oxygen was kept as the limiting factor. The development of a nitrifying population was analyzed using Fluorescence In Situ Hybridization and off-line activity measurements. These methods also showed that during steady state, anaerobic ammonium-oxidizing bacteria remained present and active. In the reactor, no aerobic nitrite-oxidizers were detected. The denitrifying potential of the biomass was below the detection limit. About 85% of Ammonia was converted to N\textsubscript{2} and the remaining 15% was recovered as NO\textsubscript{3}. N\textsubscript{2}O production was negligible (less than 0.1%). Addition of an external carbon source was not required to bring about the autotrophic denitrification to N\textsubscript{2}.

Third et al., 2001 investigated the effect of ammonia limitation on CANON process. The study was carried out in two different reactors one was SBR and the other was a chemostat. Synthetic media was fed to the reactors and it was found that the changes that occurred in CANON system due to Ammonia limitation condition that continues for about one month are completely reversible and system regained its original form as soon as the ammonia limitation removed. It showed that CANON system can withstand the major disturbances re-established itself without any irreversible changes to system.

2.6 SHARON PROCESS
The SHARON process (single reactor system for high ammonia removal over nitrite process) is a new process for biological nitrification. This process is operated without any biomass retention in a single aerated reactor at a relatively high temperature about 35 °C and pH above 7 (Hellinga et al., 1997). The process involves partial nitrification of ammonium to nitrite, and this greatly reduces the expense of aeration. The SHARON process can be carried out in a simple continuous stirred tank reactor (Thamdrup et al., 2002) and is ideally suited for removing nitrogen from waste stream with a high ammonium concentration (>500 mg N/l) (Jetten et al., 1997; van Dongen et al., 2001). This process was developed at the Technical University Delft, the Netherlands (Thamdrup et al., 2002) and full-scale experience has recently been gained in its operation. Typical applications are treatment of reject water from dewatering of digested sewage sludge (Mulder et al., 2001; van Kempen et al., 2001) and wastewater from sludge drying or incineration plants. Other applications are
treatment of landfill leachate and wastewater from digestion of organic waste and manure (Notenboom et al., 2002). SHARON is the first successful process in which nitrification/denitrification with nitrite as an intermediate has been achieved under stable conditions (van Kempen et al., 2001). To obtain the stable partial nitrification, the operating variables (temperature, pH, hydraulic retention time, substrate concentration, dissolved oxygen) are controlled in a chemostat operation (Thamdrup et al., 2002). Unfortunately, control of these process variables can be difficult in large-scale operations.

2.7 GRANULAR SLUDGE TECHNOLOGY
The new aerobic granular sludge technology has the ability to contribute to and improve the biological treatment of wastewater. Compared to present wastewater treatment plants, similar efficiencies at lower costs can be achieved with the compact aerobic granular sludge technology. Aerobic granules are self-immobilized microbial aggregates that are developed in sequencing batch reactors (SBR) without adding a carrier material. Aerobic granules can be described as compact and dense microbial aggregates of different bacterial species with an approximately spherical external appearance (Beun et al., 1999; Tay et al., 2001). The large microbial diversity found in aerobic granules has led researchers to hypothesize that granulation is not a function of specific microbiological groups but of reactor operating conditions (Beun et al., 1999). Compared to conventional flocs, aerobic granules have a wide range of beneficial properties, including: a regular, dense and compact structure, high biomass retention in the bioreactor, good settleability, and the ability to withstand high flows rate and high organic loading rates. These properties explain the recent aerobic granular technology has been the subject of extensive studies (Liu and Tay, 2004).

A conventional BNR process achieves nitrogen removal through a continuous two-stage treatment process, aerobic nitrification and anoxic denitrification (Metcalf & Eddy, 2004). In granular sludge SBR it has been observed that these two processes can occur simultaneously in a single sludge, single-stage process under low dissolved oxygen (DO) conditions called simultaneous nitrification and denitrification (SND) (Fuerhacker et al., 2000). SND relies on the formation of anoxic zones in the central part of the aerobic granule caused by the mass transfer limitation of oxygen (Figure 2.1). In the aerobic zone on the edge of the aggregate, autotrophic bacteria can nitrify.
using oxygen, whereas in the anoxic zone at the centre of the aggregate, heterotrophic bacteria can denitrify.

Fig 2.2 Oxygen profile inside a microbial aggregate under low oxygen Concentration (Lemaire 2007)

Due to its unique granule characteristic lots of researches are going on focusing on aerobic granular sludge development for the treatment of a wide variety of practical wastewater, including high strength wastewater containing organics, nitrogen, phosphorus and heavy metals (Adav et al., 2009;). Nowadays, aerobic granule-based technology has been considered to be most efficient, environment-friendly and cost effective biological method of wastewater treatment.

Devlin et al., (2016) studied the Granulation of activated sludge under low hydrodynamic shear and different wastewater characteristics. The study was carried out in five reactors operated with low up-flow superficial air velocities of 0.41 cm/min. Three reactors were fed with low strength synthetic wastewater having COD 340mg/L. Fourth reactor was fed with medium strength wastewater and fifth one was fed with high strength wastewater having COD 630mg/L and 1300 mg/L respectively. It was observed that under low hydrodynamic shear the granulation was only achieved with low-strength wastewater. During sludge granulation process about 55 to 70% of soluble chemical oxygen demand (COD) was used before aeration. and 91% COD, 62% total nitrogen (TN), and 96% total phosphorus (TP) removal was achieved for low-strength wastewater. The granulation was also reported in reactor operated at
medium-strength wastewater but granules formed quickly acquired a filamentous surface layer that resulted in decreased performance and loss of nitrification, about 94% COD, 30% TN, and 85% TP removal was achieved from medium strength wastewater. No sludge granulation was reported with high-strength wastewater and 85% removal was reported. Results of the study showed that high shear force was not desirable for sludge granulation and granulation process depended on multiple factors.

Wang et al., (2016) studied the effect of organic matter interference on ammonia removal in autotrophic anammox granular sludge (AGS) bed reactor and found that the bioreactor showed the high resistance to organic matter interference for a longer period of 33 days with 89.7% of the total nitrite removal in by anammox. After that AGS gradually shifted to MGS mixotrophic anammox granular sludge (MGS) and results in 51.9% of the total nitrite removal. The anammox remain dominant below COD/NO₂-N ratio 1.71 and contribute to about 68.9% of nitrite removal but above that the anammox was inhibited results in less than 34.1% of total nitrite removal. The results of study proved the robustness of AGS to organic matter interference provides a better option for the development of anammox technology suitable for the treatment of ammonia rich wastewaters with coexistence of organic matter.

Bindu and Madhu (2013) studied the influence of organic loading rates on aerobic granulation process. The synthetic wastewater was fed to the laboratory scale SBR at different organic loading of 3, 6 and 9 kg COD/m³/d. The SVI values obtained at these loading rates were 31, 25.1 and 30.6 ml/g respectively while the SVI of seed sludge was 245 ml/g. The sludge volume index is an important characteristic for testing the sludge settleability. The sludge in the form of flocs possesses low density and small particle size which results in low settling rates and higher SVI values. In SBR selection between poor settling and good settling sludge floc could be achieve by providing lower settling time, this separates very small suspended particles from the system that are hard to settle and only the particles that settle within the given settling time will remain in the reactor. (Qin and Liu 2008).

Aerobic granular sludge was successfully developed in a pilot-scale sequencing batch reactor (SBR) with the effluent of internal circulation reactor and using activated sludge for reactor seeding. Nitrogen removal was determined in the start-up period of
aerobic granulation process. The process of partial nitrification was observed and nitrite accumulation rates between 84.6 and 99.1 % obtained. It was mainly caused by ammonium oxidizing bacteria in the seeding activated sludge, high ambient temperature (32°C) and free ammonia concentration. After 50 days of operations the aerobic granular sludge reactor showed good performance in simultaneous Ammonia and organic matter removal. The maximum nitrogen removal efficiency of 83.1 % was achieved after the formation of aerobic granules. The average diameter of mature aerobic granular sludge found to be in the range from 0.5 to 1.0 mm. It was also depicted that, the pH and DO concentrations could be used as effective parameters for biological reactions occurring in the aerobic/anoxic process. The results could provide further information on the cultivation of aerobic granular sludge with real wastewater, especially nitrogen-rich industrial wastewater (Wei et al., 2012).

The autotrophic nitrogen removal process in the granular sludge bed reactor (GSB-ANR process) is a new and promising biotechnology for nitrogen removal from wastewater, which requires single reactor, simple operation and inorganic Carbon The predominant functional microorganisms were from Planctomycetes and Nitrosomonas. The excellent performance of GSB-ANR process was ascribed to: (a) The high activities of aerobic ammonia-oxidizing bacteria (AOB) and anaerobic ammonium oxidation (ANAMMOX) bacteria; (b) the good settlability of the granular sludge; (c) the suitable DO concentration that satisfied the oxygen requirement of AOB and prevented ANAMMOX bacteria from oxygen inhibition (Wang et al., 2012).

Xiaoming et al., (2011) investigated the granulation of simultaneous partial nitrification and anaerobic ammonium oxidation (Anammox) in a single, oxygen-limited, sequencing batch reactor. The reactor was seeded with methanogenic granular sludge started anaerobically and synthetic medium fed to reactor proposed by Van de Graaf et al. to cultivate Anammox biomass. Later on, mixture gas (air and nitrogen gas) was supplied to the reactor and a nitrifying population developed. The results showed that autotrophic granules were developed successfully by maintaining the dissolved oxygen concentration in the reactor between 0.3 and 0.5 mg/L, and a total inorganic nitrogen removal efficiency of 63.7% was achieved with a higher nitrogen load increased by reducing HRT to 3 days. It was also observed that the calcium and
phosphorus concentrations in the feeding medium are important factors that influence the autotrophic granules performance. When the calcium and phosphorus concentrations were exceeded the desired limit, salt precipitation was occurred that interfered with microbial activity, and caused a decrease of the nitrogen removal rate of the system. By decreasing calcium and phosphorus concentrations in feed, salt precipitation was avoided and the activity of the system restored quickly. The scanning electron microscopy was used for observing and analyzing the process of sludge evolution and inner structure of the granules.

The aerobic granular sludge was successfully developed in a pilot-scale sequencing batch reactor (SBR) installed on site to treat real wastewater. The reactor was inoculated using traditional activated sludge. Duration of about 1 or 2 months required by lab-scale reactor for aerobic granulation, but it took relatively long period of about 400 days for activated sludge to transform into granule-dominant sludge in the pilot-scale SBR on site. After 400-day operation the sludge inside the reactor was a mixture of flocs and granules with floc ratio ranged from 5 to 30%, sludge volume index with 5 min settling (SVI5) always maintained at around 30 mL/g. The results denaturing gradient gel electrophoresis (DGGE) revealed that similar microbial community structures represented by between coexisted flocs and granules in the reactor indicated no strong microbial selection after the granules were dominant in the reactor. Chemical oxygen demand (COD) and ammonium removal efficiencies were above 80 and 98%, respectively, after 50 days operation, and the total inorganic nitrogen removal efficiency was about 50%. The results in this study demonstrate that it is feasible to form aerobic granules in pilot scale SBR reactor and maintain the long-term stability of granular sludge with a high influent quality fluctuation. Meanwhile, stable COD and ammonium removal efficiencies can be obtained in the reactor (Liu et al., 2010).

Sumino et al., (2006) conducted a study to find the effects of carbon to nitrogen ratio and total organic carbon loading on nitrogen removal via simultaneous nitrate reduction and anaerobic ammonium oxidation in a single reactor system. An upflow reactor was seeded with the granular sludge obtained from a methane fermentation reactor and fed with synthetic wastewater containing nitrate keeping carbon to nitrogen ratio of 1 to cultivate the heterotrophic denitrifying bacteria. When 30%,
nitrogen removal efficiency was achieved by attaching anammox sludge to nonwoven-carrier and then ammonia was supplied to the reactor. Nitrogen removal efficiency significantly increased to 80–94%. The result of the study demonstrated that the nitrogen removal ratio are not dependent on the amount of granular sludge, but greatly affected by altering carbon to nitrogen ratio and total organic carbon load. It was detected by performing stable isotopic analysis using nitrate showed that anammox reaction results in nitrogen gas formation.

De Kreuk et al. (2005) investigated that aerobic granular sludge technology that present a possibility to design compact wastewater treatment plants based on simultaneous chemical oxygen demand (COD), nitrogen and phosphate removal in one sequencing batch reactor. In earlier studies, it was shown that aerobic granules, cultivated with an aerobic pulse-feeding pattern, were not stable at low dissolved oxygen concentrations. Selection for slow-growing organisms such as phosphate-accumulating organisms (PAO) was shown to be a measure for improved granule stability, particularly at low oxygen concentrations. Moreover, this allows long feeding periods needed for economically feasible full scale applications. Simultaneous nutrient removal was possible, because of heterotrophic growth inside the granules (denitrifying PAO). At low oxygen saturation (20%) high removal efficiencies were obtained; 100% COD removal, 94% phosphate (P) removal and 94% total nitrogen (N) removal (with 100% partly by (biologically induced) precipitation. Monitoring the laboratory scale reactors for a long period showed that N-removal efficiency highly depends on the diameter of the granules.

Rikke et al. (2005) investigated the microbial community composition and activity in aggregates from a lab-scale bioreactor, in which nitrification, denitrification and phosphorus removal occurred simultaneously. The biomass was highly enriched for polyphosphate accumulating organisms facilitating complete removal of phosphorus from the bulk liquid; however, some inorganic nitrogen still remained at the end of the reactor cycle. This was ascribed to incomplete coupling of nitrification and denitrification causing nitrate accumulation. After 2 hours of aeration, denitrification was dependent on the activity of nitrifying bacteria facilitating the formation of anoxic zones in the aggregates; hence, denitrification could not occur without simultaneous nitrification towards the end of the reactor cycle. Nitrous oxide was identified as a product of denitrification, when based on stored PHA as carbon source.
This observation is of critical importance for application of PHA-driven denitrification in activated sludge processes.

Yang et al., (2003) developed microbial granules at different substrate N/COD ratios in sequencing batch reactors (SBR). Results showed that heterotrophic, nitrifying, and denitrifying populations could co-exist in microbial granules, while increased substrate N/COD ratio led to significant shifts among three populations in granules. Enhanced activities of nitrifying and denitrifying populations were obtained in microbial granules developed at high substrate N/COD ratios, however, heterotrophic populations in granules tended to decrease with the increase of substrate N/COD ratio. It was found that dissolved oxygen (DO) concentration had a pronounced effect on the efficiency of denitrification by microbial granules, however, the results also indicated that a certain mixing power should be provided to ensure mass transfer between liquid and granules during denitrification. It was demonstrated that complete organics and nitrogen removal can be achieved in single granule-based SBR with high efficiency and stable performance.

The effects of C/N ratio and total organic carbon (TOC) loading on nitrogen removal through simultaneous nitrate reduction and anaerobic ammonium oxidation in a single reactor were examined. Granular sludge taken from a methane fermentation reactor was placed in an upflow reactor and supplied with synthetic wastewater containing nitrate at a C/N ratio of 1 to grow heterotrophic denitrifying bacteria. When nitrogen removal ratio reached 30%, anammox sludge attached to nonwoven-carrier was added into the same reactor and then ammonia was added to the synthetic wastewater. Nitrogen removal ratio was markedly increased to 80–94%. In this system, nitrogen removal ratio was affected by C/N ratio and TOC loading, not by the amount of granular sludge. A stable isotopic analysis using 15N-labeled nitrate showed that N₂ gas was formed by anammox reaction (Sumino et al., 2006).

2.8 SEQUENCING BATCH REACTOR
Sequencing batch reactor (SBR) is a fill-and-draw activated sludge treatment system. Although the processes involved in SBR are identical to the conventional activated sludge process. SBR is compact and time oriented system, and all the processes are
carried out sequentially in the same tank. SBR system is the upgraded version of the conventional activated sludge process, and is capable of removing nutrients from the wastewater.

SBRs are used all over the world and have been around since the 1920s. With their growing popularity in India, Europe and China as well as United States, they are being used successfully to treat both municipal and industrial wastewaters, particularly in areas characterized by low or varying flow patterns. Municipalities, resorts, and a number of industries, including dairy, pulp and paper, tanneries and textiles are using SBRs as practical wastewater treatment alternatives.

Improvements in equipment and technology, especially in aeration devices and computer control systems, have made SBRs a viable choice over the conventional activated-sludge system. These plants are very practical for a number of reasons:

• In areas where there is a limited amount of space, treatment takes place in a single basin instead of multiple basins, allowing for a smaller footprint. Low total-suspended-solid values of less than 10 milligrams per liter (mg/L) can be achieved consistently through the use of effective decanters that eliminate the need for a separate clarifier.

• The treatment cycle can be adjusted to undergo aerobic, anaerobic, and anoxic conditions in order to achieve biological nutrient removal, including nitrification, denitrification, and some phosphorus removal. Biochemical oxygen demand (BOD) levels of less than 5 mg/L can be achieved consistently. Total nitrogen limits of less than 5 mg/L can also be achieved by aerobic conversion of ammonia to nitrates (nitrification) and anoxic conversion of nitrates to nitrogen gas (denitrification) within the same tank. Low phosphorus limits of less than 2 mg/L can be attained by using a combination of biological treatment (anaerobic phosphorus absorbing organisms) and chemical agents (aluminum or iron salts) within the vessel and treatment cycle.

• Older wastewater treatment facilities can be retrofitted to an SBR because the basins are already present.

• Wastewater discharge permits are becoming more stringent and SBRs offer a cost-effective way to achieve lower effluent limits. Note that discharge limits that require a greater degree of treatment may necessitate the addition of a tertiary filtration unit following the SBR treatment phase. This consideration should be an important part of the design process.
Activated sludge process, oxidation ponds, aerated lagoons and oxidation ditches are the commonly adopted suspended growth biological treatment systems. Compared to the pond and lagoon systems, activated sludge systems also lend themselves for a number of design and operational control measures to improve performance and achieve desired treated wastewater quality. However, the flexibility in design and process control for these systems comes at the cost of high external energy inputs and skilled operation requirements.

Conventional activated sludge process (ASP) is not designed to remove nitrogen. Further, due to its short detention time, the sludge produced is not well digested warranting an additional sludge digestion treatment. Since the 1970s, a modification of the conventional activated sludge process has made the emergence of the sequencing batch reactor (SBR) process. Conventional ASP systems are space oriented. Wastewater flow moves from one tank into the next on a continuous basis and virtually all tanks have a predetermined liquid volume. The SBR, on the other hand, is a time-oriented system, with flow, energy input, and tank volume varying according to some predetermined, periodic operating strategy. Hence, SBR is best defined as a time-oriented, batch process, falling under the broad category of an unsteady-state activated sludge system (Irvine et al., 1978).

Current interest in sequencing batch treatment of wastewater would appear to be a return to the original notion of the activated sludge process. The first notable, but short lived, resurgence of interest in batch biological treatment occurred in the early 1950s. The second resurgence occurred in the 1970s with the efforts of Irvine and his co-workers investigating the suitability of batch biological processes (Irvine et al., 1978). Around the same period, interest in the batch operated biological treatment systems surfaced also in Australia (Goronszy, 1979). The system developed in Australia was based on the original Pasveer oxidation ditch concept, where a single reaction vessel took the form of an endless loop of shallow ditch in which inflow, aeration, settlement and discharge followed a specific cycle.
2.8.1 SBR Technology for Wastewater Treatment

In its most basic form, the SBR system is simply a set of tanks that operate on a fill-and-draw basis. The tanks may be an earthen or oxidation ditch, a rectangular basin, or any other concrete/metal type structure. Each tank in the SBR system is filled during a discrete period of time and then operated as a batch reactor. After desired treatment, the mixed liquor is allowed to settle and the clarified supernatant is drawn from the tank. The essential difference between the SBR and the conventional continuous flow activated sludge system is that SBR carries out functions such as equalization, aeration and sedimentation in a time rather than in a space sequence.

One advantage of the time orientation of the SBR is flexibility of operation. The total time in the SBR is used to establish the size of the system and can be related to the total volume of a conventional continuous-flow facility. As a result, the fraction of time devoted to a specific function in the SBR is equivalent to some corresponding tank in a space oriented system. Therefore, the relative tank volumes dedicated to, say, aeration and sedimentation in the SBR can be redistributed easily by adjusting the mechanism which controls the time (and, therefore, share the total volume) planned for either function. In conventional ASP, the relative tank volume is fixed and cannot be shared or redistributed as easily as in SBR.

Because of the flexibility associated with working in time rather than in space, the SBR can be operated either as a labor-intensive, low-energy, high sludge yield systems or as an energy-intensive, low-labor, low sludge yielding system for essentially the same physical plant. Labor, energy and sludge yield can also be traded off with initial capital costs. The operational flexibility also allows designers to use the SBR to meet many different treatment objectives, including one objective at the time of construction (e.g. BOD and suspended solids reduction) and another at a later time (e.g. nitrification/denitrification in addition to BOD and suspended solids removal).

2.8.2 Physical Description of the SBR System

An SBR system may be designed as consisting of a single or multiple reactor tanks operating in parallel. Each operating cycle of a SBR reactor comprises five distinctive phases, referred to as: fill, react, settle, draw and idle phases. Figure-1 illustrates a SBR reactor operation for one cycle (batch) of wastewater treatment. Overall control
of the system is accomplished with level sensors and a timing device or microprocessor. A detailed discussion of each of the phases of the SBR is provided in the following sections:

![Figure 2.3 SBR Cycle (Source EPA 2000)](Image)

**2.8.2.1 Fill Phase**

Fill provides for the addition of influent to the reactor. During Fill, the influent wastewater is added to the biomass (i.e. mixed liquor suspended solids) which remained in the tank from the previous cycle. Depending upon the treatment objective, the fill may be static, mixed or aerated.

(a) **Static Fill** (no mixing or aeration) results in minimum energy input and high substrate concentration at the end of this phase.

(b) **Mixed Fill** (mixing without aeration) results in denitrification, if nitrates are present, a subsequent reduction of BOD and energy input, and in the anoxic or anaerobic conditions required for biological phosphorus removal.
(c) **Aerated Fill** (mixing and aeration) results in starting of aerobic reactions leading to a reduction of cycle time, and holds substrate at lower concentrations, which may be important if biodegradable constituents present in wastewater are toxic at high concentrations.

Studies recommend static Fill with neither aeration nor mechanical mixing, as this helps promote high fermentation rates with allow flocculent bacteria to outcompete filamentous species, hence prevent sludge bulking (Chudoba *et al.*, 1973; Schroeder, 1982).

### 2.8.2.2 React Phase

With the reactor full, the React phase begins. In general, vigorous aeration is the feature of this phase. However, as in fill, the react phase may require to be carried out in high dissolved oxygen concentrations (aerated react), or in low dissolved oxygen concentrations (mixed react). The time allocated for react should be sufficient to achieve the desired level of effluent quality. The time dedicated to react phase can vary from a low of zero to more than 50% of the total cycle time. If only organics removal is desired, the aeration period can be as short as 15 minutes. However, longer aeration periods in the order of 4 hours or more, are normally required for long term stability of the process and nitrification. Where denitrification following nitrification is required, aeration during the react period is interrupted. Anoxic conditions would then prevail over a period of hours followed by a short period of aeration. This will strip away the nitrogen gas bubbles and aid in sedimentation.

### 2.8.2.3 Settle Phase

The settle phase allows for separation of bio-solids from the treated effluent without any inflow or outflow, in the SBR reactor that may have a volume more than ten times that of a secondary clarifier used for conventional continuous-flow activated sludge plant. The major advantage of SBR is its use as a clarifier, which allows for truly quiescent sedimentation conditions. Because all of the biomass remains in the tank until some fraction must be wasted, there is no need for underflow hardware normally found in conventional clarifiers. In contrast, the conventional ASP systems, continuously remove mixed liquor and passes through the clarifier only to return a major portion of the sludge to the aeration tank. Thus in conventional systems,
quiescent conditions are assumed in design, but not achieved in operation as a result of secondary currents.

2.8.2.4 Draw or Decant Phase
This is the withdrawal phase to discharge the clarified effluent from the reactor. There are several withdrawal mechanisms available. It may be as simple as a pipe fixed at some predetermined depth with the flow regulated by an automatic valve or a pump. Alternatively, an adjustable or floating weir at or just beneath the liquid surface can be used. As with the fixed pipe arrangement, discharge from the weir can be regulated by an automatic valve or a pump. In any case the withdrawal mechanism should be designed and operated in a manner that prevents floating matter from being discharged.

The time dedicated for draw phase can range from 5% to more than 30% of the total cycle time. The time for draw should not be overly extended because of possible problems with rising sludge. One hour is the usual time period allowed for this phase of the operation.

2.8.2.6 Idle Phase
Idle is the phase between discharging the treated effluent and before filling the reactor again. This time can be effectively used to waste sludge. The frequency of sludge wasting is determined by the net solids increase in the reactor for each cycle, and the mixing and aeration equipment capacity. After sludge wasting, aeration and/or mixing can be provided, depending upon the overall system objectives. Alternatively, idle can be eliminated altogether. In instances where operation of SBR does not include an idle period, as noted earlier, sludge wasting may be achieved by solid wasting from the mixed liquor during the react phase.

2.9 Advantages and disadvantages
Some advantages and disadvantages of SBRs are listed below:

2.9.1 Advantages;
- Equalization, primary clarification (in most cases), biological treatment, and secondary clarification can be achieved in a single reactor vessel.
- Operating flexibility and control.
- Minimal footprint.
- Potential capital cost savings by eliminating clarifiers and other equipment.

2.9.2 Disadvantages;
- A higher level of sophistication is required (compared to conventional systems), especially for larger systems, of timing units and controls.
- Higher level of maintenance (compared to conventional systems) associated with more sophisticated controls, automated switches, and automated valves.
- Potential of discharging floating or settled sludge during the DRAW or decant phase with some SBR configurations.
- Potential plugging of aeration devices during selected operating cycles, depending on the aeration system used by the manufacturer.
- Potential requirement for equalization after the SBR, depending on the downstream processes.

2.10 Applications of SBR in wastewater treatment

Some of the past studies based on practical SBR applications are reviewed here.

Lochmatter et al., (2014) studied the effect of aeration control for performing nitrogen removal over nitrite with aerobic granular sludge in sequencing batch reactors. The nutrient removal performances for N and P and COD have been analyzed and the population of nitrite-oxidizing bacteria in the granular sludge were also examined. To reduce nitrite-oxidizing bacteria population and hence achieve nitrogen removal over nitrite, intermittent aeration was done, aeration phase length and control alternate high-low dissolve oxygen has proved to be an effective way to washout these microbial cultures. Nitrogen removal efficiencies of up to 95% were achieved for an influent wastewater with COD:N:P ratios of 20:2.5:1. The total nitrogen removal rate was 0.18 kgN/m³/d and 74% nitrogen removal. The nitrite-oxidizing bacteria concentration reduced by over 95% in 60 days at a temperature of 20°C and it was possible to switch from nitrogen removal over nitrite to nitrogen removal over nitrate. At 15 °C, the nitrite-oxidizing bacteria concentration decreased further but not much, and nitrite oxidation could not be completely suppressed. However, the combination of aeration phase length control and high-low DO was also
at 15 °C successfully maintained the nitrite pathway despite the fact that the maximum growth rate of nitrite-oxidizing bacteria at temperatures below 20 °C is in general higher than the one of ammonium-oxidizing bacteria.

Neczaj et al. (2008) conducted a study for co-treatment of leachate and dairy wastewater using sequencing batch reactor Two laboratory scale SBR were operated during the study SBR, one was solely treating dairy wastewater while other was fed with wastewater after 25% dilution of landfill leachate. The experiment were performed for varying aeration period. And it was found that most suitable aeration period for co-treatment of landfill leachate was 19 hrs with anoxic phase of 2 hrs. The COD, BOD and TKN removal efficiencies obtained were 98.4%, 97.3% and 79.2% respectively which shows satisfactory treatment ability of SBR. The experiment was also conducted by varying the HRT along with varied organic loading rate (OLR). The results of experiments showed a significant effect on removal efficiency i.e. efficiency was reduced due to less HRT and more OLR. The best effluent quality was obtained under OLR of 0.8 kg BOD5/m3/d and HRT of 10 days for co-treatment of landfill leachate.

Vetter et. al., (2006), studied the performance of sequencing batch reactor fitted with with media for nitrification and denitrification of high strength wastewater by using tank type reactor of diameter 7.62 m, height 4.3 m with a working volume 177 m3 and glass-lined cylindrical tank with conical bottom, fitted with three banks of manufactured fixed-film synthetic media. The influent biochemical oxygen demand of approximately 1,600 mg/l was reduced to 50 mg/l, consistently less than 100 mg/l. Total Kjeldahl nitrogen concentrations were obtained with TKN removal efficiency 60-90 % depending upon the loading rate and the operating conditions.

Qing et al., (2007), studied advanced nitrogen removal using pilot-scale SBR. The profiles of dissolved oxygen (DO), pH, and oxidation reduction potential (ORP), nitrification and de-nitrification controlled by the automatic process control system built on three layer network for pilot-scale aerobic-anoxic sequencing batch reactor (SBR) with a treatment capacity of 60 m3/d. The outlet effluent chemical oxygen demand (COD) and total nitrogen (TN) were under 50 and 5 mg/l respectively even for low temperature 13°C.
Ileri et al., (2003) studied the treatment of Pharmaceutical Industry wastewater mixed with Domestic Wastewater by Sequencing Batch Reactor. The various parameters monitored include BOD$_5$, NH$_3$, PO$_4^{3-}$, SS, MLVSS, pH, temperature, sludge volume and microorganisms predominance under constant settling time and variable aeration time in the first phase and in the second phase with variable settling time and constant aeration time. The influent concentrations of mixed wastewater were BOD (90–130 mg/L), COD (200–300mg/L), SS (900 mg/L) pH 6.4–6.8, T (20°C), NH$_3$ (26mg/L), PO$_4^{3-}$ (8.5mg/L) and the effluent concentrations from sequencing batch reactor (SBR) are found as BOD (13–18 mg/L), COD (25–37mg/L), SS (9-21 mg/L), pH 7.3-7.6, T (23°C), NH$_3$ (1mg/L), PO$_4^{3-}$ (8.1mg/L). When aeration time was kept 6 hours the highest BOD removal efficiency achieved was 83 % and at 4 hours aeration with 90 minutes of settling time the highest BOD removal efficiency achieved was 81%. 4 hours of aeration time and of 60 minutes of settling time was considered as the optimum condition with 82% BOD$_5$ and 88% COD removal. Average ammonia removal of 96%, suspended solid removal of 98% and phosphorus removal 14 and 4% were found at 90 and 30 min settling times, respectively. SBR was found efficient for the treatment of mixed pharmaceutical industry and domestic wastewater.

Janczukowicz et al., (2000) conducted the study to examine the settling characteristics of activated sludge in sequencing batch reactor. The experiments were performed using bench-scale reactor, fed with wastewater obtained from the University of Olsztyn treatment plant. Concentrations of the activated sludge varied between 2.5 and 6.0 kg SS/m$^3$. The analyses parameters of the sludge includes: sludge concentration, settleability, sedimentation velocity and sludge volume index (SVI). The results of the study showed that activated sludge possesses very good settling properties. The low SVI (30 - 60 ml g-1 SS) values were reported as a result of intensive and rapid sedimentation which shortened the settling period to less than one hour. The low SVI also prevent the sludge bulking in the reactor. The aeration done during the react period increased the dissolved oxygen concentrations in the aeration tank resulted in biomass production.
2.9 LANDFILL LECHATE

Leachate generation is a major problem for municipal solid waste (MSW) landfills and causes significant threat to surface water and groundwater. Leachate can be defined as a liquid that passes through a landfill and has extracted dissolved and suspended matter from it. Leachate results from precipitation entering the landfill from moisture that exists in the waste when it is composed. In order to minimize the negative influence on the environment, leachate must be treated to remove organic compounds and nitrogen before being discharged.

Spagni et al., (2014) studied the partial nitrification for nitrogen removal from sanitary landfill leachate. The aim of the study was to assess the application of the SHARON (Single reactor High activity Ammonium Removal Over Nitrite) process for the partial nitrification of leachate generated in old landfills. Particular attention was given to the start-up phase of the process. This study demonstrated that partial nitrification can be obtained when treating raw leachate after biomass acclimation. Only a fraction (50–70%) of the ammonia present in the leachate can be oxidised due to a limited amount of alkalinity available. Stable nitritation was obtained by applying a hydraulic retention time (HRT) of 4–5 d, which is higher than the values proposed for the effluent of anaerobic digesters. The high HRT might be a result of the very high ammonia concentration present in the leachate which could severely inhibit the growth of the nitrite-oxidising bacteria.

Wang et al., (2013) studied the advanced nitrogen removal from landfill leachate without the addition of external carbon sources, a combine system consists of anaerobic sequencing batch reactor (ASBR) and sequencing batch reactor (SBR) as shown in Figure 2.5 was used for the treatment of landfill leachate having ammonium 1100mg/L and chemical oxygen demand (COD) 6000 mg/L, respectively.
Figure 2.5 Schematic diagram of the ASBR coupled with SBR (source Wang et al., 2013)

It was found that anaerobic sequencing batch reactor, at volumetric loading rate of 5 kg COD/m$^3$/d, was efficiently remove 80% of the influent COD. About 50% total nitrogen removal was achieved in SBR through denitrification and simultaneous nitritation–denitritation under alternate anoxic and aerobic phase. Though combined ASBR and SBR system the advanced nitrogen removal was accounts for total inorganic nitrogen (TIN) removal efficiency of 99% via denitrification process which results in effluent COD and TN concentrations of 550–650 mg/L and 15–25 mg/L, respectively corresponds to COD and total nitrogen efficiencies of 90% and 95% respectively.

Kulikowska and Bernat (2013) studied the treatment of landfill leachate by nitrification-denitrification process in sequencing batch reactor with glycerine as a external carbon source. The effectiveness of nitrification-denitrification in the presence glycerine, effects of limited oxygen concentration (0.7 mg O$_2$/L) in SBR and sludge production in treatment process was monitored. Sodium acetate as sole carbon source and along with glycerine in the proportions of 3:1 and 1:1 were supplemented
to the reactor. It was found that at low dissolved oxygen concentration nitrification was inhibited and nitrites were the main products. Nitritation efficiency of the system was 98–99% while denitritation efficiency was only 61% in the reactor fed with sodium acetate with higher sludge production. With glycerine addition (1:1) there was a increase in efficiency of the process to 75.6% with significant decrease in production of sludge.

Kalka et al., (2012) studied co-treatment of landfill leachate and municipal wastewater for toxicity removal by biological process. The changes in leachate toxicity during co-treatment by biological process was monitored. The study was carried out in three laboratory scale reactors operated at same conditions (flow rate: 8.5–10 L/d; HRT: 1.4–1.6 d; MLSS: 1.6–2.5 g/L) except influent characteristic and load. The influent of reactor I consisted of municipal wastewater mixed with leachate from post closure landfill; influent of reactor II consisted of leachate collected from transient landfill and municipal wastewater; influent of reactor III consisted of municipal wastewater only. Toxicity of raw and treated wastewater was determined by four acute toxicity tests with Daphnia magna, Thamnocephalus platyurus, Vibrio fischeri, and Raphidocelis subcapitata. It was reported that the Landfill leachate increased initial toxicity of wastewater. However, after biological treatment, there was a significant drop in acute toxicity, but still mixture of leachate and wastewater was found harmful to all tested organisms.

Zhu et al., (2013) studied the biological nitrogen removal from landfill leachate. The study was carried out using experimental setup, as shown in figure 2.6 below, consists of an anaerobic SBR and a pulsed SBR. This combined system of ASBR and (PSBR) was established for enhance COD and nitrogen removal from landfill leachate.
The main function of ASBR was to degrade the organics from raw leachate. During combined operation period, three equal feeds mode (i.e. three times of influent filling with the same volume) was supplied to PSBR. The results obtained from the combines operational period of 157 days showed that the COD removal rate of ASBR was 83–88% under the specific loading rate of 0.43–0.62 gCOD /gVSS/day. PSBR’s operation can be divided into four phases according to the different influent NH4+-N which varied from 800–1000 mg/ L. The total nitrogen removal rate of more than 90% with the effluent total nitrogren of less than 40 mg /L was obtained. It was found that PHB and glycogen can act as electron donor for endogenous. It was reported in results that the system achieved COD and total nitrogen removal rate of 89.61–96.73% and 97.03–98.87%, respectively, without the addition of extra carbon source addition.

D. Kulikowska et al., (2004) studied the organics and nitrogen removal from municipal landfill leachate, the efficiency of ammonium nitrogen removal from municipal landfill leachate by activated sludge in two-stage SBR system was investigated. Treated leachate contains low concentrations of organic substances measured as chemical oxygen demand (COD) – 757 mg O2/dm³ and high concentrations of ammonium – 362 mgN-NH4/dm³. Nitrification was studied in two
parallel, aerated SBR reactors with two different hydraulic retention times (HRT), 3 and 2 days, respectively. It was found that 2 days HRT was sufficient to achieve complete nitrification. In the effluent ammonium, nitrite and nitrate nitrogen concentrations were 0.08 mg NH$_4^+$-N /dm$^3$, 0.04 mg NO$_2^-$-N /dm$^3$ and 320 mg NO$_3^-$-N/dm$^3$, respectively. The ammonium nitrogen removal rate was 20.2 mg/mg NH$_4^+$-N/dm$^3$.h. The effluent from aerobic reactors with HRT 2 days was fed to the anoxic SBR reactors. Methanol as an external carbon source was added to promote denitrification. In the anoxic reactor, at a methanol dosage 3.6 mg COD/mg NO$_3^-$-N and HRT of 1 day complete denitrification was achieve with nitrate nitrogen residual concentrations of 0.9 mg NO$_3^-$-N/dm$^3$. The maximum denitrification rate reported was 48.4 mg NOx-N/dm$^3$/h.

A. Spagni et al., (2009) worked on optimization of nitrogen and COD removal from leachate generated in old MSW landfill. The study was conducted in a bench-scale sequencing batch reactor (SBR) that was operated to optimise the nitrogen and COD removal from leachate generated in old landfills. The results confirm that dissolved oxygen, oxidation reduction potential and pH can be used for monitoring the treatment processes in SBR. Using these parameters, a control system was developed and applied to the SBR. Nitrogen removal was optimised via the nitrite route (nitrification to nitrite and nitrite-denitrification). The study confirms the effectiveness of the nitrite route for nitrogen removal optimisation in leachate treatment, in particular when external COD has to be added to improve the denitrification process. With the application of the control system a significant improvement of the process was obtained.

Kargi et al., (2003) studied the Aerobic biological treatment of pre-treated landfill leachate by fed-batch operation. Landfill leachate obtained from the solid waste landfill area contained high chemical oxygen demand (COD) and ammonium ions which resulted in low COD and ammonium removals by direct biological treatment. COD and ammonium ion contents of the leachate were reduced to reasonable levels by chemical precipitation with lime and air stripping of ammonia. The pre-treated leachate was subjected to aerobic biological treatment in an aeration tank by fed-batch operation. The effects of the feed wastewater COD content and flow rate on COD and ammonium ions removal were investigated. About 76% COD and 23% ammonia
removals were obtained after 30 hours of operation with a flow rate of 0.21 L/h and the feed COD content of 7000 mg/L COD. COD removal efficiency decreased with increasing COD loading rates. A kinetic model for COD removal was developed and the kinetic constants were determined by using the experimental data.

Zhong et al. (2009) worked on Ex situ nitrification and sequential in situ denitrification. It was an attempt for nitrogen management at landfills. The study was conducted in continuous stirred tank reactor (CSTR) and was evaluated for both ammonia and organics removal. The maximum nitrogen loading rate (NLR) and the maximum organic loading rate (OLR) was 0.65 g N/L/d and 3.84 g COD/L/d, respectively were reported. The removal efficiency for ammonia and chemical oxygen demand (COD) achieved were 99% and 57%, respectively. In CSTR operation, free ammonia inhibition and low dissolved oxygen (DO) were observed as main factors affecting nitrite accumulation. In situ denitrification was studied in a municipal solid waste (MSW) column by recalculating nitrified leachate from CSTR. The decomposition of MSW was accelerated by the recirculation of nitrified leachate. Complete reduction of total oxidized nitrogen via denitrification was obtained with maximum TON loading of 28.6 g N t\(^{-1}\) TS d\(^{-1}\). Additionally, methanogenesis inhibition was observed while loading was above 11.4 g N t\(^{-1}\) TS d\(^{-1}\) and the inhibition was enhanced with the increase of total oxidized nitrogen loading.

Ibrahimpasic et al. (2010) worked on nitrogen removal from municipal landfill leachate. The batch bioreactor of 3 L seeded with a microbial culture was used to evaluate the efficiency of nitrogen removal from municipal landfill leachate. The microbial culture, originating from landfill leachate, was developed by an enrichment culture technique. Organic compounds measured as chemical oxygen demand (COD) of 400–600 mg L\(^{-1}\) and high concentrations of ammonium nitrogen (270–312 mg /L characterized the landfill leachate as a mature leachate. The rate of ammonium nitrogen removal was 24.5 mg NH\(_4\)+-N /L/h. Nitrification rate was 20.1 mg NOx-N /L/h. Denitrification was performed with the addition of sodium acetate as external carbon source in ratio C/N 2 and 4. Sodium acetate was insufficient for complete denitrification at C/N 2. Complete denitrification at C/N 4 was performed at denitrification rate 8.3 mg NO\(_3\)−N/ L/h.
Lina et al., (2009) worked on Nitrogen removal via nitrite from municipal landfill leachate. The system consisting of a two-stage up-flow anaerobic sludge blanket (UASB), an anoxic/aerobic (A/O) reactor and a sequencing batch reactor (SBR), was used to treat landfill leachate. During operation, denitrification and methanogenesis took place simultaneously in the first stage UASB, and the effluent chemical oxygen demand (COD) was further removed in the second stage UASB. Then the denitrification of nitrite and nitrate in the returned sludge by using the residual COD was accomplished in the A/O reactor, and ammonia was removed via nitrite in it. Last but not least, the residual ammonia was removed in SBR as well as nitrite and nitrate which were produced by nitrification. The results from the study performed for about 120 days showed that when the total nitrogen (TN) concentration of influent leachate was about 2500 mg/L and the ammonia nitrogen concentration was about 2000 mg/L, the nitrification with 85%–90% nitrite accumulation was achieved stably in the A/O reactor. The TN and ammonia nitrogen removal efficiencies of the system were 98% and 97%, respectively. The residual ammonia, nitrite and nitrate produced during nitrification in the A/O reactor could be washed out almost completely in SBR. The total nitrogen and ammonia nitrogen concentrations of final effluent were about 39 mg/L and 12 mg/L, respectively.

Among the biological treatments processes, AS, SBR and UASB are the most frequently applied. These treatments are effective not only to remove over 90% of COD with a concentration ranging from 3500–26000 mg/L, but also to achieve 80% of ammonia removal with a concentration ranging from 100–1000 mg/L. A combination of physico-chemical and biological treatment into an integrated process is effective for leachate treatment. Almost complete removal of COD and ammonia was reported for combined reverse osmosis (RO) and UASB with an initial COD concentration of 35000 mg/L and Ammonia concentration of 1600 mg/L. Integrated Fenton’s oxidation and AS could achieve about 98% and 99% of COD and ammonia removal, respectively, with initial COD and ammonia concentrations of 7000 mg/L and 1800 mg/L. Overall, the selection of the most suitable treatment for leachate depends on its characteristics, technical applicability and potential constraints, effluent limit required, cost-effectiveness, regulatory requirements and long-term environmental impacts.
The major fraction of raw or biologically treated leachate was large recalcitrant organic compounds that are not easily removed during biological treatment. In order to meet strict quality standards for direct discharge of leachate into the surface water, integrated methods of treatment, and combination of biological, chemical, physical and membrane process must be applied. Nowadays the use of membrane technologies, more such Reverse Osmosis (RO), either as pre treatment step in a landfill leachate treatment chain or as single post-treatment step is good available option for enhancing the treatment efficiency.

2.10 Sludge Digester Effluent

Vazquez Padin et al., (2009) studied the application of the Anammox process for two different possibilities for the post-treatment of anaerobic digester supernatants, the combined SHARON-Anammox system, performed in a chemostate and a SBR, respectively, and, a single unit system composed by an air pulsing SBR to carry out the CANON process. The technology based on the combination of the SHARON-Anammox process was used to treat the effluent of an anaerobic digester from a fish canning industry. The presence of organic matter in the influent caused fluctuations in the efficiency of the SHARON unit and an optimal nitrite to ammonium ratio was not achieved in this system to feed the Anammox reactor. Nevertheless an overall percentage of nitrogen removal of 40 – 80% was obtained when the Anammox reactor operated at nitrite limited conditions. In those periods when the effluent from the SHARON unit contained a NO₂-N/NH₄+-N molar ratio higher than 1.3 the Anammox process lost its stability due to nitrite accumulation. The effluent from an anaerobic digester placed at a WWTP was treated by a CANON system operated at room temperature (20 – 24°C). This system was developed from a nitrifying air pulsing reactor working at limiting dissolved oxygen conditions which was inoculated with Anammox biomass. A quick start-up of the system was observed and the reactor reached a nitrogen removal rate of 0.25 g N/L/d after 40 days of inoculation. The maximum nitrogen removal rate reached 0.5 g N/L/d. These results indicate the feasibility of the treatment of effluents from anaerobic digesters using the Anammox process.
Gali et al., 2007 conducted a study on comparison of reject water treatment with Nitrification/denitrification via nitrite in SBR and Sharon chemostat process. They concluded that for the treatment of reject water SHARON would be the most economical solution because it would use half of the oxygen and no external organic carbon source would be required. But the Ammonia removal efficiency achieved was only 56% from SHARON chemostat 97% from SBR by nitrification/denitrification when influent Ammonia concentration was 800 mg/L, so it was suggested that this technology may be complemented by an Anammox process in order to achieve the complete nitrogen removal.

2.11 SLAUGHTER HOUSE WASTEWATER
Slaughterhouse wastewater comes under the category of high strength wastewater as it contains high concentrations of organic matter, suspended solids, soluble and insoluble organics and nutrients also. Thus the slaughter house wastewaters are among one of the major contributors to environmental pollution. Slaughterhouse wastes are a threat to the environment requiring particular attention. Many investigators in the past have conducted with the slaughterhouse wastes in an attempt to biodegrade it, and it was found that Anaerobic treatment methods are more suitable choice for the treatment of slaughter house wastewaters. Various anaerobic processes were successfully applied for treatment of slaughter house wastewaters such as Anaerobic Hybrid Reactor system combining UASB and Filter for the treatment of slaughterhouse. (Ruiz et al., 1997). When anaerobic treatment processes were used to treat slaughter house wastewaters it appreciably removed COD, BOD and SS, and also producing recoverable source of energy in form of methane gas. The anaerobic treatment generates a very low quantity of sludge and also does not require chemical pre-treatment (Sunder et al., 2013).

Pan et al., (2014) studied the Nitrogen removal from slaughterhouse wastewater through partial nitrification followed by denitrification in intermittently aerated sequencing batch reactors at 11°C. The slaughterhouse wastewater contained chemical oxygen demand (COD) of 6068 mg/L, total nitrogen (TN) of 571 mg/L, total phosphorus (TP) of 51 mg/L and suspended solids of 1.8 g/L, on average. The laboratory-scale IASBR reactors had a working volume of 8 L and was operated at an average organic loading rate of 0.61 COD/L/d. At the cycle duration of 12 h, COD
was efficiently removed under three aeration rates of 0.4, 0.6 and 0.8 L air/min. Among the three aeration rates, the optimum aeration rate was found to be 0.6 L air/min with COD, TN, and TP removals of 98%, 98%, and 96%, respectively. The treated wastewater met the emission standards. The microbial community analysis by fluorescence in situ hybridization detect about 12% of ammonium oxidizing bacteria, and 7.2 of nitrite oxidizing bacteria in the activated sludge at the aeration rate of 0.6 L air/min, lead to efficient partial nitrification. PND effectively removed nitrogen from slaughterhouse wastewater at 11°C, but PND efficiency was dependent on the aeration rate applied. PND efficiencies were up to 75.8%, 70.1% and only 25.4% at the aeration rates of 0.4, 0.6, and 0.8 L air/min were reported.

Sunder et al., (2013) conducted the study on Efficient Treatment of Slaughter House Wastewater by Anaerobic Hybrid Reactor Packed with Special Floating Media. The study was performed with 50 litres hybrid reactor. Reactor was packed with light weight floating media. The advantage of media was its light weight and shape which provides 100 m²/m³ area on which the microorganism are immobilized making it more stable to shock loadings. Because the media is always in motion, clogging on the surface due excess biomass deposition was prevented. Effective contact could be made between the substrate and the microorganism by floating nature of media. Studies on different organic loadings ranging between 1.0-6.0 kg COD/m3/Day and two HRT's of one and two days were conducted. The wastewater treatment efficiency of the system were found very good with COD and BOD removal in the range of 86.0% - 93.58% and 88.9% - 95.71% respectively, at one day HRT. The reduction was observed to increase marginally at two day HRT and organic loading rates between 1.0-6.0 kg COD/ m³/d. It was very clear from the results that the special media provided good treatment efficiency.

Nardi et al., (2011) conducted a research on advanced wastewater treatment of poultry slaughterhouse for its reclamation. The advanced treatment comprises of sequencing batch reactor SBR operation, chemical-DAF and UV disinfection. The wastewater was pretreatment anaerobically in UASB reactor and. SBR operation was mainly done to achieve denitrification. The total denitrification efficiency was more than 90%, COD removal 54±24% and total phosphorus removal 43% was achieved. The sludge also possesses good settling characteristics with SVI (118 ± 35 mL/g). It was
suggested that the SBR system along with chemical-DAF and UV disinfection is suitable to use for anaerobically pretreated poultry wastewater.

Sombatsompop et al., (2011) performed a comparative study on sequencing batch reactor and moving bed sequencing batch reactor for piggery wastewater treatment. The organic loading was varied from 0.59 to 2.36 kgCOD/m3.d. The COD treatment efficiency of the SBR and moving-bed SBR was above 60% at an organic load of 0.59 kgCOD/m3.d and above 80% at the organic loads of 1.18-2.36 kgCOD/m3.d. The BOD removal efficiency more than 90% at high organic loads of 1.18-2.36 kgCOD/m3.d was observed. The moving-bed SBR gave 86-93% TKN removal efficiency, whereas the SBR system exhibited the TKN removal efficiency of 75-87% at all organic loads. The effluent suspended solids concentration for SBR systems exceeded the piggery wastewater limit of 200 mg/L at the organic load of 2.36 kgCOD/m3.d while that for the moving-bed SBR system it was under permissible limit. When the organic load was increased, the moving-bed SBR system yielded better treatment efficiency than that of the SBR system. The wastewater treated by the moving-bed SBR system met the criteria of wastewater standard for pig farms at all organic loads, while the treated by the SBR system was not found satisfactory at a high organic load of 2.36 kgCOD/m3.d.

Rahimi et al., (2011) investigated simultaneous nitrification-denitrification and phosphorus removal in a fixed bed sequencing batch reactor (FBSBR) by using polypropylene carriers instead of activated sludge. COD, total nitrogen, and phosphorus removal efficiencies were in the range of 90–96%, 60–88%, and 76–90% respectively, while these values for SBR reactor were 85–95%, 38–60%, and 20–79% respectively. These results show that the simultaneous nitrification–denitrification (SND) is significantly higher in fixed bed sequencing batch reactor (FBSBR) than in conventional SBR reactor.

Li et al., (2008) evaluated the effect of aeration rate on nutrient removal from slaughterhouse wastewater in intermittently aerated sequencing batch reactors. Two 10-L laboratory-scale sequencing batch reactors (SBR₁ and SBR₂) operated at ambient temperature. The pollutants present in the slaughterhouse wastewater had average concentrations of 4,000 mg/L chemical oxygen demand (COD), 350 mg/L
total nitrogen (TN) and 26 mg/L total phosphorus (TP). The duration of a complete SBR operation cycle was 8 h and comprised four operational phases: fill (7 min), react (393 min), settle (30 min) and draw/idle (50 min). During the react phase, the reactors were intermittently aerated four times at 50-min intervals, 50 min each time. DO, pH and oxidation–reduction potential (ORP) in the reactors were real-time monitored. The reactors were operated at four aeration rates for different time durations (0.2 L air/min in SBR\textsubscript{1} for 70 days, 0.4 L air/min in SBR\textsubscript{1} for 50 days, 0.8 L air/min in SBR\textsubscript{2} for 120 days and 1.2 L air/min in SBR\textsubscript{1} for 110 days). When the aeration rate was 0.2 L air/min, the SBR\textsubscript{1} was continuously anaerobic. When the aeration rate was 0.4 L air/min, COD and TP removals were 90% but TN removal was only 34%. When the aeration rates were 0.8 and 1.2 L air/min, average effluent concentrations were 115 mg/L COD, 19 mg/L TN and 0.7 mg/L TP, results in COD, TN and TP removals of 97%, 95% and 97%, respectively. It was found that partial nitrification followed by denitrification can effectively achieved in the intermittently aerated SBR systems.

Kundu et al., (2013) studied the performance evaluation and biodegradation kinetics for the treatment of slaughter house waste in sequencing batch reactor. The performance of a laboratory-scale Sequencing Batch Reactor (SBR) has been investigated in aerobic-anoxic sequential mode for simultaneous removal of organic carbon and nitrogen from slaughterhouse wastewater. The reactor was operated under three different variations of aerobic-anoxic sequence, namely, (4+4), (5+3), and (3+5) hours of total react period with two different sets of influent soluble COD (SCOD) and ammonia nitrogen (NH\textsubscript{4}+–N) level 1000±50 mg/L, and 90 ± 10 mg/L, 1000 ± 50 mg/L and 180 ± 10mg/L, respectively. It was observed that from 86 to 95% of SCOD removal is accomplished at the end of 8.0 hour of total react period. In case of (4+4) aerobic-anoxic operating cycle, a reasonable degree of nitrification 90.12 and 74.75% corresponding to initial NH\textsubscript{4}+–N value of 96.58 and 176.85mg/L, respectively, were achieved. The biokinetic coefficients were also determined for performance evaluation of SBR for scaling full-scale reactor in future operation.

Paul (2009) investigated the performance of the laboratory scale sequencing batch reactor (SBR) for combined soluble organics and nutrients (nitrogen and phosphorus) removal from slaughter-house wastewater by varying dissolve oxygen concentration
and other parameters like dilution factor, filling time, cycle time, anoxic/anaerobic sequence and anoxic/anaerobic time phase. Appreciable denitrification was observed in all the runs performed. The SBR was also performed well for EBPR (enhanced biological phosphorus removal) for slaughter-house waste-water upon adding sodium acetate as carbon source, thus increasing the SCOD/TKN (soluble-COD/Total Kjeldahl Nitrogen) ratio.

Masse et al., (2000) worked on Treatment of slaughterhouse wastewater in anaerobic sequencing batch reactors. Slaughterhouse wastewater was treated in four 42-L anaerobic sequencing batch reactors (ASBRs) operated at 30LC. Two ASBRs were seeded with anaerobic granular sludge from a milk processing plant (MPP) reactor and two ASBRs received anaerobic non-granulated sludge from a municipal wastewater treatment plant. Influent total chemical oxygen demand (TCOD) ranged from 6908 to 11500 mg/L, of which approximately 50% were in the form of suspended solids (SS). Total COD was reduced by 90% to 96% at organic loading rates (OLRs) ranging from 2.07 to 4.93 kg m-3 d-1 and a hydraulic retention time of 2 days. Soluble COD was reduced by over 95% in most samples. During the start-up period, high concentrations biomass was lost in the effluent, but under steady state operation, at OLRs above 3 kg m-3 d-1, biomass retention was adequate and effluent SS averaged 364 mg/L. It was observed that reactors seeded with municipal sludge performed fairly well than those containing the milk processing plant sludge, during start-up, but this difference decreased with time. The biogas produced composed of 75% methane gas. This high degree of methanization showed that most soluble and suspended organics were degraded during treatment in ASBRs operated at 30°C.

2.12 Advance Oxidation Processes
Advanced oxidation processes (AOPs), may be defined as those technologies that utilize the hydroxyl radical (·OH) for oxidation. It has received increasing attention in the field of wastewater treatment in the last decades. These processes have been successfully used as a pre-treatment operation to wastewater for the removal or degradation of harmful pollutants or used as to convert recalcitrant pollutants into biodegradable compounds that can then be further treated by conventional biological processes. The efficacy of AOPs depends on the generation of reactive free radicals, the most important of which is the hydroxyl radical (·OH).
2.12.1 Ozonation

Ozonation of water is a well-known technology and the Ozone possesses strong oxidative properties which allow it to efficiently oxidize many organic compounds in aqueous medium. Oxidation with O3 leaves no harmful residues that have to be removed or disposed like other oxidizing agents such as Cl2, Sarayu et al., 2007, Ulson de Souza et al., 2010. Ozonation is one of the AOP processes used for wastewater treatment, Sarayu et al., 2007. Ozonation can remove harmful substances, increase the biodegradability of organic pollutants and highly effective in colour removal Zayas et al., 2007. Due to these advantages use of ozone in wastewater treatment has gained interest in last few years despite of the high cost of ozone production Merić et al., 2005, Ulson de Souza et al., 2010.

Ozone can react with solutes in two ways, one is by direct oxidation reaction between molecular ozone and organic pollutant (Eq. 2.8) other is by indirect reactions with hydroxyl radicals resulting from ozone decomposition (Eq. 2.9, 2.10) Merić et al., 2005, Coca et al., 2007, Rizzo 2011.

\[
\begin{align*}
\text{O}_3 + \text{R} & \rightarrow \text{RO} + \text{O}_2 \quad \text{(2.8)} \\
\text{O}_3 & \leftrightarrow \text{O} + \text{O}_2 \quad \text{(2.9)} \\
\text{O} + \text{H}_2\text{O} & \rightarrow 2 \text{HO}^* \quad \text{(2.10)}
\end{align*}
\]

The pH plays important role in ozonation process as it affects the double action of ozone on the organic matter which may be a direct or an indirect (free radical) oxidation pathway. At low pH, ozone only reacts with compounds with specific functional groups through selective reactions, such as electrophilic, nucleophilic or dipolar addition reactions (i.e. direct pathway) where as at high pH, ozone decomposes releasing hydroxyl radicals, which is highly oxidizing in nature and reacts non selectively with a wide range of organic and inorganic compounds in wastewater pounds in water (i.e., indirect ozonation pathway) Merić et al., 2005, Turhan & Turgut 2009.

Advance oxidation with ozone has various advantages that are beneficial in the treatment of the wastewater such as no residual sludge degradation and colour
removal occur in single step, easily to performed, little space is required, all residual ozone can be easily converted to oxygen and water. (Rizzo 2011).

2.12.2 Fenton process
Advance oxidation using Fenton’s reagent is accomplished with a mixture of ferrous ions and hydrogen peroxide, in which the ferrous or ferric ions act as a catalyst to decompose the hydrogen peroxide (Canizaresh et al., 2009).

The factors affecting Fenton process includes pH of system, concentration of ferrous ions, concentration of hydrogen peroxide, initial concentration of the pollutant and presence of other ions (Stasinakis 2008, de Sena et al. 2009, Mert et al. 2010). The major advantage of the fenton process is degradation of both organic and inorganic pollutants that results in higher mineralization levels. (Martins et al. 2011, Canizares et al., 2009, de Sena et al., 2009, Stasinakis 2008). Among the various advanced oxidation processes available fenton process is easy to use and proved efficient in term of pollutant removal rate as well as operating cost compared to other processes.

There are various other advance oxidation process also such as Electrochemical oxidation that involves the formation of hydroxyl radicals at the active sites of anode and has been used for the removal of various inorganic and organic pollutants. Rizzo 2011. Other is photocatalytic or photochemical degradation processes in which semiconductor material is excited by electromagnetic radiation possessing energy of sufficient magnitude, to produce conduction band electrons and valence band holes, Stasinakis 2008, Żmudziński 2010. The surface area and the number of active sites offered by the catalyst for the adsorption of pollutants plays an important role in the process. Gogate & Pandit 2004. In past studies the treatment by combination of advance oxidation processes were investigated to effectively remove various contaminants present in wastewaters, which lead to removal of a specific pollutant, reduction in the reaction time and economic cost. (Oller et al., 2011, Stasinakis 2008, de Sena et al., 2009, Zayas et al., 2007).

2.12.3 Applications of Advance Oxidation Process in wastewater treatment
Advance Oxidation process has been successfully applied in the field wastewater treatment either as pre-treatment or post treatment. Some of the studies based on Advance oxidation process in the the field of wastewater treatment are reviewed here.
Aziz et al., (2015) conducted a study for the treatment of landfill leachate based on combined ozone and Fenton process. The performance of combined ozone-fenton system (O$_3$/H$_2$O$_2$/Fe$_{2+}$) in the advanced oxidation was evaluated for COD, color, and ammonia removal at different concentrations of partially aerobic stabilized leachate. The leachate with different concentration was subjected to treatment, with varying initial COD between 250 and 2360 mg/L, color between 470 Pt. Co. to 4530 Pt. Co., and NH$_3$-N between 150 mg/l to 1170 mg/l. With respect to these values the removal efficiencies obtained between 60% and 87% for COD, 95% to 100% for color, and 12% to 22% for NH$_3$–N. The ozone consumption for COD removal at different concentration was calculated, and it was found that the minimum amount of ozone (1.3 KgO3/ Kg COD) consumed at highest initial concentration of COD (2360 mg/L), with 60% COD removal for 60 minutes ozonation. In addition to this the biodegradability (BOD$_s$/COD) ratio improved from 0.09 in raw leachate to 0.27 at 5 initial COD value of 500mg/L. The ozone/Fenton process found efficient for treatment of stabilized leachate and also for improving biodegradability at natural pH, which allows the application of biological treatment of leachate without pH adjustment of the treated effluent after ozonation.

Cortez et al., (2011) performed Fenton and ozone based Advanced Oxidation Processes as a pre-treatment of a mature landfill leachate, to improve the biodegradability of various organic recalcitrant present in the leachate before subjected to biological treatment. The leachate was diluted two times before performing advance oxidation and operating condition during studies were, pH 3, H$_2$O$_2$ to Fe$_{2+}$ molar ratio 3 with 4 mmole /L Fe$_{2+}$ dose and 40 minutes of reaction time. After Fenton treatment BOD$_s$/COD ratio was increased from 0.01 to 0.15 with 46% of COD removal. But the highest removal efficiency and biodegradability was achieved after treatment by ozone at higher pH values, either by ozone only or in combination with hydrogen peroxide. The results showed the enhanced production of hydroxyl radical under these conditions. Operating conditions during ozone treatment were, pH 7. 60 minute contact with ozone at the rate 5.6 g O$_3$/ h and 400 mg/L of hydrogen peroxide, after treatment with ozone the BOD$_s$/COD ratio increased from 0.01 to 0.24 and highest COD removal efficiency achieved was 72%. Fe$_{2+}$/H$_2$O$_2$ was
found the most economical system to treat the landfill leachate on calculating the estimated operating cost.

Kocak et al., (2013) conducted the study, to find effectiveness of Fenton process for leachate treatment. The biologically treated and untreated leachate both were subjected to treatment by Fenton process. Biological treatment of leachate was done by using upflow anaerobic sludge blanket (UASB) reactor and membrane bio-reactor (MBR) processes respectively before subjected to treatment by Advance oxidation. The biologically treated leachate was also subjected to the combined AOPs such as Ultraviolet (UV)/Fenton and Ultrasound (US)/Fenton processes to further improve the efficiency of Fenton process. From results of the study, Fenton process found to be effective for treatment of landfill leachate. It is an efficient oxidation method for the treatment of organic matter and color in leachate. The highest COD removal efficiency of 70% was achieved for untreated leachate and 50 % for biologically treated leachate . In addition 90 % and 98% color removal was also reported for untreated and treated leachate respectively. It was also reported that the treatment efficiency of Fenton process did not show any improvement by other additional treatment methods used in combination with it.

Yasar et al., (2007) conducted the study on Advance Oxidation process using ozone for treatment of biologically treated mixed industrial effluent mainly consist of textile industry wastewater. Ozonation was done achieve effective COD and colour removal from raw and anaerobically treated effluent UASB combined industrial effluent in a laboratory scale bubble column reactor. Ozonation of anaerobic biotreated effluent at ozone dose of 300 mg/h for 10 min resulted in 81% color and 75% COD removal (100 mgO₃/80 mg COD) while for raw wastewater 25 min ozonation resulted 51% color and 67% CO D removal (250 mgO₃/345 mg CO D). For biotreated effluent the optimal operation conditions (pH = 8 and temperature = 25°C) resulted in 100% color and 96% COD removal for 10 min ozonation (100 mgO₃/104 mg CO D). Electrical energy comparison showed that higher electrical energy is required for pre ozonation as compared to post ozonation.