Effects of nitrobenzene concentrations and hydraulic retention time on the treatment of nitrobenzene in sequential anaerobic baffled reactor and continuously stirred tank reactor system

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Abstract The effects of increasing nitrobenzene (NB) concentrations and hydraulic retention time (HRT) on the performance of anaerobic baffled reactor (ABR) and aerobic completely stirred tank reactor (CSTR) were studied. In the first step the NB concentration was increased from 30 to 700 mg/L at constant COD and flowrates. Maximum COD removal efficiencies in ABR varied between 88–92% as NB concentrations increased from 30 to 210 mg/L. After this dose, COD removal efficiency decreased to 85 and 79% at NB concentrations of 550 and 700 mg/L, respectively. Removal efficiencies of NB were nearly 100% for all NB concentrations in ABR reactor effluent. In the second step, COD and NB concentrations were kept constant while HRT decreased from 10.38 days to 1 day. As HRT decreased from 10.38 to 2.5 days the COD removal efficiencies in the anaerobic and anaerobic/aerobic reactor effluents were 92–94% and 97–98%, respectively. As HRT decreased from 2.5 days to 1 day COD removal efficiencies in the anaerobic and anaerobic/aerobic reactor effluents decreased to 83 and 95%, respectively. This study showed that HRT is a more important operation parameter than increasing NB concentration in ABR/CSTR sequential reactor system. Although ABR/CSTR system exhibited good COD and NB removal efficiencies, the lower HRTs slightly decreased the removal efficiencies compared to increasing NB concentration.

Keywords ABR; aniline; CSTR; nitrobenzene; sequential

Introduction Nitrobenzene (NB) is widely used in the manufacture of explosives, of aniline, as an industrial solvent and as a precursor of dyes and other synthetic products such as herbicides. NB is relatively toxic and persistent in the environment and is listed as a priority pollutant by the US EPA (US EPA, 1994). If the concentration exceeds 2 mg/l in the wastewaters it is declared hazardous waste (US EPA, 1994; Peres et al., 1998; He and Spain, 1999; Majumder and Gupta, 2003). Among various physical, chemical and biological methods for removal of nitrobenzene, the biological treatment option is most cost effective (Majumder and Gupta, 2003). Limited studies have been reported on the COD removal efficiency and biodegradation of the NB under anaerobic conditions. The degradation of nitrobenzene and COD was investigated in a hybrid reactor. Sixty to ninety-six percent COD removal and 80–90% nitrobenzene removal efficiencies were observed (Majumder and Gupta, 2003). In another study carried out by Aziz et al. (1994), the NB removal efficiency was 67% in an anaerobic batch reactor.

One of the many types of high-rate anaerobic reactors presently attracting growing interests is the anaerobic baffled reactor (ABR). The advantages of the ABR reactor includes higher resilience to hydraulic and organic shock loads, longer biomass retention times and lower sludge yields than many other high rate anaerobic treatment systems (Barber and Stuckey, 2000). Differing populations of bacteria across the compartments has been shown to increase resistance to variations in feed load, temperature and pH.
The most significant advantage of the ABR is its ability to separate acidogenesis and methanogenesis longitudinally down the reactor.

Combined anaerobic/aerobic processes are a viable alternative for the treatment of nitroaromatic compounds that are difficult to treat by traditional processes. Generally, an aerobic polishing step is needed after the anaerobic system (pretreatment) to meet effluent quality standards (Angenent et al., 2001). The anaerobic stage is presumed to change the molecular structure of the persistent organic compounds. This change affects the treatment of easily degradable organics in the subsequent aerobic stage (Aziz et al., 1994). Anaerobic/aerobic sequential reactor systems have been used successfully for the treatment of NB and COD (Majumder and Gupta, 2003; Aziz et al., 1994). Peres et al. (1998) researched the biodegradation pathway of NB under anaerobic/aerobic conditions. Literature surveys showed that the treatment of NB with sequential ABR/CSTR reactor systems has not been investigated. Furthermore, NB is listed as a toxic compound by the US-EPA (1994) and IC50 value (50% decreases in methane activity compared to control) of NB was found as 124 mg/L (Kuscu and Sponza, 2005). Therefore, in this study the aim was to determine the effects of HRT and increasing NB concentrations on the performance of sequential ABR/CSTR reactor system.

Materials and methods

Pilot system

A continuously fed stainless steel anaerobic baffled reactor (ABR) and an aerobic CSTR reactor were used in sequence for the experimentation. The ABR reactor with an effective volume of 38.4 L was divided into four compartments by vertical baffles. The general information related to ABR reactor configuration used in this study was presented elsewhere (Kuscu and Sponza, 2005). The produced gas was collected via a porthole in the top of reactor. The operating temperature of the ABR reactor was maintained constantly at 37 ± 1°C using an electronic heater located in the lower part of the reactor. The CSTR reactor consisted of an aerobic (effective volume = 9 L) and a settling compartment (effective volume = 1.32 L). During the aerobic phase, air was diffused from the bottom of the reactor (Figure 1).

Composition of synthetic wastewater

The COD and NB concentrations in synthetic wastewater were 3,000 mg/L and 30–700 mg/L during the continuous operations of ABR. Glucose was used as carbon source. Vanderbilt mineral medium was prepared in distilled water by dissolving some trace heavy metals and cations (Speece, 1996). Vanderbilt mineral medium was used together with NB and 3,000 mg/L of glucose-COD as feed. To prevent the accumulation of total volatile fatty acid (TVFA) and provide a neutral pH (7.0–7.8), 5,000 mg/L of NaHCO3 was added to the feed.

Analytical methods

Total suspended solid (TSS) and mixed liquor suspended solid (MLSS) in anaerobic granulated and activated sludge were measured by the membrane filtration technique (Standard Methods, 1992). The soluble COD concentration in samples was measured using the closed reflux colorimetric method following Standard Methods (1992). Biogas production was measured with liquid displacement method. Total gas was measured by passing the gas through distilled water containing 2% (v/v) H2SO4 and 10% (w/v) NaCl (Beydilli et al., 1998) Methane gas was detected by using distilled water containing 3% NaOH (w/v) (Razo-Flores et al., 1997). Methane percentage in biogas was determined by a Dräger (Stuttgart, Germany) Pac-Ex methane gas analyser. Total volatile fatty acid (TVFA)
concentration was measured using a titrimetric method (Anderson and Yang, 1992). pH was measured with a pH meter (WWT pH 330). NH$_3$ was measured using spectroquant Merck kits numbered 14752 in a spectrophotometer. Nitrobenzene, catechol and aniline concentrations in the samples were determined by high pressure liquid chromatography (HPLC) using an Ace 5-C$_{18}$ reversed phase column (25 cm × 4.6 mm ID). All the samples were initially centrifuged to remove any particulate matter and then filtered through a 0.45 µm teflon filter using a disposable syringe (Agilent 5185–5835) prior to HPLC analysis. The quantification was accomplished with single wavelengths of 202 and 234 nm for nitrobenzene and aniline, respectively. Elution was prepared with isocratic solvent system consisting of 50% methanol and 50% deionised-water at a flow-rate of 1.4 mL/min (US EPA, 1994). N$_2$ gas was measured in a GC with a thermoionic FID detector. A fox model 10 A thermal glass mass flowmeter was used, while helium was the carrier gas at 20 °C.

Operation conditions
The ABR reactor was continuously operated until reaching constant COD removal efficiencies and methane gas percentages through 60 days when operated without NB in the start-up period. In this research two studies were performed: in the first step, the ABR reactor was operated through 240 days while increasing the NB concentrations from 30 to 700 mg/L. The influent COD concentrations and COD loading rate were kept constant at approximately 3,000 mg/L and 0.289 g/m$^3$day, respectively, through anaerobic operation. Glucose, giving a COD concentration of 3,000 mg/L was used as co-substrate to provide the electrons for the reduction of NB. Hydraulic retention time (HRT) was kept constant at 10.38 days. In the second step, the influent COD and nitrobenzene concentrations were kept constant at 3,000 mg/L and 100 mg/L, respectively, during continuous operation (Table 1). The ABR reactor was operated at six different HRTs (10.38, 5.14, 3.5, 2.4, 1.5 and 1 days). The ABR and CSTR reactors were operated at steady state conditions for approximately 15–25 days at every HRT and NB concentration. The HRTs were not decreased before reaching steady-state conditions. All the data was collected under steady-state conditions. The steady state conditions were defined with COD removal efficiencies and methane gas productions higher than 90 and 40%, respectively, for 5 consecutive days. The results given in the figures and tables are the mean values of
ANOVA test was applied to the experimental data in order to determine the regression coefficients and the significance of the results.

Results and discussion

Effect of increasing NB concentrations on the performance of ABR, CSTR and total reactor system

COD and NB removal efficiencies in ABR/CSTR reactor at increasing NB concentrations. The maximum COD removal efficiencies in ABR varied between 88–92% as the NB concentration was increased from 30 to 210 mg/L (Figure 2) ($R^2 = 0.98$, $F = 12.9$). After these doses the COD removal efficiency decreased to 85 and 79% at NB concentrations of 550 and 700 mg/L, respectively. The removal efficiencies of NB were nearly 100% for all NB concentrations in ABR reactor effluent. The COD removal efficiency was 98% in the sequential anaerobic/aerobic system. In a study carried out by Aziz et al. (1994) a CSTR reactor was operated at various HRTs (8, 12, 16 and 24 days) and influent NB concentrations (50, 100, 150 and 200 mg/L). The removal efficiency of nitrobenzene was between 75 and 85% when the wastewater was treated by the aerobic process alone. However, the removal efficiency of NB was 95% in the acidogenic-aerobic process and the COD removal efficiency was 98% in the anaerobic/aerobic total system. In our study the NB removals in ABR were higher while the total COD removal exhibited similar results to the study performed by Aziz et al. (1994). The treatment performance of a wastewater containing NB was investigated in a hybrid reactor by Majumder and Gupta, (2003). The hybrid reactor consisted of a trickling filter and an aeration tank giving a combination of

![Figure 2](https://iwaponline.com/wst/article-pdf/55/10/227/439089/227.pdf)
attached and suspended growth systems. The study of the hybrid reactor’s performance was obtained at five different HRTs (4.4, 5.6, 8.5, 9.25 and 11.9 h). Maximum COD and NB removal efficiencies were 95.83 and 97.93%, respectively, at HRT of 29.55 h. These results exhibit lower removal efficiencies than this study.

Gas production in ABR reactor at increasing NB concentrations. The total gas production remained at between 3,200–3,400 mL/day until a NB concentration of 210 mg/L (Figure 3) ($R^2 = 0.99$, $F = 13.4$). Similarly, the methane gas production and the methane gas percentage remained stable (1,500 mL/day and 48–50%, respectively) as the NB concentration was increased from 30 to 210 mg/L ($R^2 = 0.98$, $F = 12.5$). As the NB concentration increased from 210 to 700 mg/L the total, methane gas productions and methane percentage decreased to 1,700 mL/day, 600 mL/day and 38%, respectively. This could be attributed to a reduction of the activity of methane gas forming methanogen archae at high NB. Furthermore, the gas composition varied at high NB concentrations. 120 mg/L NH$_3$ and 10% N$_2$ gas was measured (data not shown) at high NB concentrations such as 550–700 mg/L. The optimum NB concentrations for maximum total, methane gas production and methane gas percentage varied between 30–210 mg/L.

Sequential nitrobenzene reduction and aniline biodegradation. This study indicated that, in the first step, nitrobenzene was reduced to aniline under anaerobic conditions, and then in the second step, aniline was mineralised under aerobic conditions. These findings agree with studies performed by Aziz et al. (1994) and Peres et al. (1998). Dang (1989) found that aniline is more readily biodegradable than NB in aerobic process. Three different mechanisms have been described for the mineralisation of NB. The first involves its degradation via catechol to 2-hydroxymuconic semialdehyde in an oxidative pathway (He and Spain, 1999). In the second mechanism, the Nitrobenzene is degraded to 2-aminomuconic semialdehyde via 2-aminophenol, which is further degraded to pyruvate and acetaldehyde (He and Spain, 1999). The third mechanism is reported under anaerobic conditions. Nitrobenzene is reduced to aniline in an anaerobic or acidogenic reactor (Aziz et al., 1994; Peres et al., 1998). Aziz et al. (1994) investigated the biodegradability of nitrobenzene using sequential acidogenic-aerobic process. The results of nitrobenzene degradation show that 1 mol of nitrobenzene was reduced to 1 mol of aniline in the acidogenic process. Aniline was rapidly biodegraded in aerobic process. Peres et al. (1998)
investigated the biodegradation of nitrobenzene to aniline. It was observed that nitrobenzene was reduced to aniline by the reductive consortium in first step, and, in the second, oxidative step, aniline was mineralised to catechol with meta cleavage.

Figure 4a, b shows the HPLC chromatograms of anaerobic and aerobic effluents. Aniline peak was obtained at a retention time of 3.44 min at 234 nm wavelength in the effluent of anaerobic stage. Similar peaks were shown on the chromatograms at the same retention times in the studied samples. The presence of aniline peak in ABR effluent indicated that the nitrobenzene converted to aniline. No aniline peak was determined in the aerobic effluent. This indicated that the complete degradation of aniline occurred in the aerobic stage. No catechol was observed in the oxidative step as reported by Peres et al. (1998).

Aniline variation in ABR/CSTR reactor at increasing NB concentrations. Aniline concentrations in the effluent of ABR increased from 13 to 290 mg/L as the NB concentrations were increased from 30 to 700 mg/L (Figure 5). Aniline was removed with 100% removal efficiency in the aerobic reactor until an influent NB concentration of 300 mg/L. After this influent NB concentration, aniline removal efficiencies decreased from 100 to 89% in the aerobic reactor.

The high aniline concentrations produced in anaerobic reactor could not removed by the aerobic microorganisms due to aniline toxicity (Aziz et al., 1994). The HPLC chromatograms in effluent of aerobic reactor showed that no other intermediate product was measured. The optimum NB concentrations varied between 30–210 mg/L for maximum aniline removal efficiencies (E = 100%) in the aerobic CSTR reactor effluents.

Effect of HRT on COD and NB removal efficiencies

COD and NB variations in ABR/CSTR reactor at decreasing HRTs. As the HRT decreased from 10.38 to 2.5 days, the COD removal efficiencies in the anaerobic and anaerobic/aerobic reactor effluents were 92–94 and 97–98%, respectively (R² = 0.90, F = 9.8). As the HRT decreased from 2.5 to 1 days the COD removals efficiencies in the anaerobic and anaerobic/aerobic reactor effluents decreased to 83 and 95%, respectively) (R² = 0.88, F = 10.1). The same results were obtained by Aziz et al. (1994) and Majumder and Gupta (2003). One hundred percent NB removal efficiencies

![Figure 4](https://iwaponline.com/wst/article-pdf/55/10/227/439089/227.pdf)
were obtained in anaerobic reactor at all HRTs (see Figure 6). NB was completely
removed even with a very short HRT such as 1 day in the ABR reactor. The reason for
low COD removal efficiencies compared to high NB removals could be explained by the
fact that the intermediate products which originated from the degradation of NB were
measured as COD. The NB was biodegraded under reductive anaerobic conditions in
very short HRTs, as reported by Majumder and Gupta (2003). The residual COD
remaining from the anaerobic reactor was removed in the aerobic reactor. The optimum
HRT for maximum COD and NB removals in anaerobic and total system varied between
1.5 and 10.38 days. Majumder and Gupta (2003) found a maximum removal efficiency of
NB (93%) at an influent NB concentration of 90 mg/L and a HRT of 29.55 h in a hybrid
reactor. In this study, the ABR reactor exhibited higher NB removals at high initial NB
and low HRTs.

Figure 5 Effect of NB concentration on Aniline removal efficiencies

Figure 6 Effect of HRT on COD and NB removal efficiencies
Gas production in ABR reactor at decreasing HRTs. When the HRT was decreased from 10.38 days to 1 day, the methane percentage decreased from 42 to 30% (see Figure 7) ($R^2 = 0.92$, $F = 10.2$). However, the daily total gas and methane gas productions in ABR increased from 3 to 18 L/day and from 1.5 to 5 L/day, respectively, as the HRT was decreased from 10.38 days to 1 day) ($R^2 = 0.91$, $F = 9.91$). The decline of methane percentage could be attributed to the variation of the total gas composition by the inhibition of activity of the methanogen archae at low HRTs. The total and methane gas productions increased significantly as the HRT decreased, since high organic loading rates applied to the ABR reactor at low HRTs. The optimum HRT for maximum total, methane gas productions and methane percentage varied between 1 and 3.5 days.

Aniline variation in ABR/CSTR reactor at decreasing HRTs. The aniline concentration in the effluent of ABR was approximately 70 mg/L at all HRTs. Aniline removal efficiencies
were 100% until a HRT of 5.19 days in aerobic reactor effluent (Figure 8). After that, HRT aniline removal efficiencies decreased to 70 and 43% at HRTs of 2.5 days and 1 day, respectively, in aerobic reactor (see Figure 8). This could be attributed to the high NB loading rates at low HRTs inactivating the methanogens, which then converted the NB to aniline. The optimum HRT for maximum aniline removal varied between 5.19–10.38 days and 3.5 and 6.9 days in anaerobic and aerobic reactors, respectively.

Conclusions
The results of the study showed that high COD and NB removal efficiencies and methane gas percentages were obtained in an ABR reactor. The following results can be drawn from this study: In the first step, the maximum COD removal efficiencies in ABR varied between 88–92% as the NB concentrations were increased from 30 to 210 mg/L. The removal efficiencies of NB were nearly 100% at all NB concentrations in the ABR reactor effluent. The COD and NB removal efficiencies were 98 and 100%, respectively, in the whole system. The methane gas production and the methane gas percentage remained stable (1500 ml/day and 48–50%, respectively) as the NB concentration was increased from 30 to 210 mg/L. NB was converted into aniline under anaerobic conditions in ABR reactor. This gratuitous reaction was followed by a complete productive break-down of aniline in the aerobic part of sequential total system. Aniline was removed with 100% removal efficiency in the aerobic reactor until an influent NB concentration of 300 mg/L. In the second step, as the HRT decreased from 10.38 days to 2.5 days, the COD removal efficiencies in the anaerobic and anaerobic/aerobic reactor effluents were 92–94% and 97–98%, respectively. One hundred percent NB removal efficiencies were obtained in anaerobic reactor at all HRTs. When the HRT was decreased from 10.38 days to 1 day, the methane percentage decreased from 42 to 30%. However, the daily total gas and methane gas productions in ABR increased from 3 to 18 L/day and from 1.5 to 5 L/day, respectively, as the HRT was decreased from 10.38 days to 1 day. Aniline removal efficiencies were 100% until an HRT of 5.19 days was reached in aerobic reactor effluent. The COD and NB removals and gas percentage in ABR and anaerobic/aerobic reactors system was slightly lower when the HRT concentrations were decreased compared to increasing NB concentrations. The COD, NB and aniline removals in ABR and anaerobic/aerobic reactors system was slightly higher when the NB concentrations were increased. This shows that HRT is not a very important operation parameter for the ABR reactor compared to increasing NB concentrations. In other words, the ABR and whole system are only slightly influenced by the HRT compared to increasing NB concentrations.

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References


