TREATMENT OF TOXIC WASTEWATER BY USE OF BIOLOGICAL PROCESSES

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Declaration Form

DECLARATION

I hereby, declare that this manuscript, entitled “Treatment of Toxic Wastewater by Use of Biological Processes”, is the result of my own work except for quotations and citations which have been duly acknowledged.

I also declare that, to the best of my knowledge and belief, it has not been previously or concurrently submitted, in whole or in part, for any other degree or diploma at Nazarbayev University or any other national or international institution.

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Date: 25-Jan-2018

Abstract

The present work evaluates the capacity of suspended-growth un-acclimated sludge in treating 2-chorophenol (2-CP) and 2,4,6 – trichlorophenol (2,4,6 – TCP) containing synthetic wastewater in Continuous Flow Reactor (CFR) and in Sequencing Batch Reactor (SBR). In CFR, 2-CP concentrations were 103 and 163 ppm; 2,4,6 – TCP concentrations were 71 and 72 ppm. Under these 2-CP and 2,4,6 –TCP loadings, TSS growth was halted. Also, continuous decrease in nitrification was observed characterized by increasing effluent ammonium and decreasing nitrate production. 2-CP and 2,4,6 – TCP removals were decreasing under all their concentrations. In SBR, 2-CP inlet concentrations were 17 and 51 ppm; 2,4,6 – TCP inlet concentrations were 20 and 26 ppm. TSS demonstrated continued growth under all chlorophenol concentrations. 2-CP 17 and 51 ppm inhibited nitrification process. 2,4,6 – TCP 20 and 26 ppm did not cause nitrification inhibition. 100% removal of all 2-CP and 2,4,6 – TCP feed concentrations was achieved. Aeration in SBR played a significant role removing 43.9 % of Total Carbon (TC) and 39.9% of Total Nitrogen during the 1st day of experiment.

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1. Introduction

1.1 General

Chlorophenols represent the group of chemicals where number of chlorines atoms between one and five are attached to the phenol. There are 19 types of chlorophenols existing. [*1*]**.** The most common ones having industrial origin and commercial importance are 2,4-dichlorophenol(2,4-DCP), pentachlorophenol (PCP), 2,4,5 - trichlorophenol (2,4,5 - 2,4,6 - TCP), 2-chlorophenol (2-CP), and 4-chlorophenol (4-CP) as shown in Figure 1.1 below [*2*].

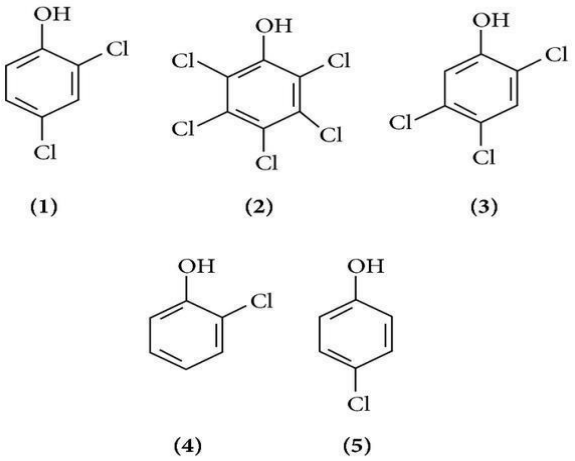

Figure 1.1: (1) 2,4-dichlorophenol(2,4-DCP), (2) pentachlorophenol (PCP), (3) 2,4,5 - trichlorophenol (2,4,5 - 2,4,6 - TCP), (4) 2-chlorophenol (2-CP), (5) 4-chlorophenol (4-CP).

Chlorophenols are found in the effluents of the following industries such as pulp, paper and textile productions [*3*]. Higher chlorophenol production levels arise from heavy industries such as oil refinery (6 - 500 mg/L), coal furnace (9 -

6800 mg/L), petrochemical plants (2 - 1220 mg/L), and coking (28 - 3900 mg/L) [*4*]. Chlorophenols also originate from agricultural sources [*5*].

Chlorophenols contained in the industrial wastewater as well as municipal water must be treated to meet pollutants concentration limits issued by local legislation. Chlorophenols, if not treated properly, pose a serious hazard to the environment including aquatic life, animals, and human health when discharged to the rivers or lakes. For example, 2,4,6 - TCP, according to International Agency for Research on Cancer, can be cancerogenic to the humans [*6*]. Acute poisoning with chlorophenols leads to necrotic structural changes of mouth, esophagus, and stomach which can lead to death. According to Ref. *7*, the adjective "necrotic" originates from the word "necrosis" which means premature death of living cells or tissues. Chronic exposure to chlorophenols leads to hypotension, low body temperature and weakness. As for effects on human organs, chlorophenols can lead to damage of kidney, lungs, liver, and digestive tract [*8*]. 2-CP and 2,4,6 - TCP, which are the target pollutants for removal in this experiment, are defined as top priority contaminants by European Union (EU) and US Environmental Protection Agency (EPA) [*2*] as shown in Table 1 below.

Table 1.1:. Priority contaminants set by EU and US EPA.

EU	**US EPA**
2 - Amino - 4 - chlorophenol	Phenol
2-Chlorophenol	2-Chlorophenol

3- Chlorophenol	2,4 - Dichlorophenol
4 - Chlorophenol	4 - Chloro - 3 -methylphenol
4 - Chlorophenol - 3 - methylphenol	2,4,6 - Trichlorophenol
2,3,4 - Trichlorophenol	Pentachlorophenol
2,4,5 - Trichlorophenol	
2,4,6 - Trichlorophenol	
3,4,5 - Trichlorophenol	
3,5,6 - Trichlorophenol	
Pentachlorophenol	

Additional incentive to properly treat wastewater is to remove nitrogen. Nitrogen in high concentrations could cause eutrophication in water, thus, posing a threat to an aquatic life. Eutrophication is the uncontrolled supply of growth-limiting element such as nitrogen; such tendency leads to overgrowth of algae. One of main threats of eutrophication is the oxygen depletion taking place during the biodegradation of large surface area covering algae. Another nitrogen-containing compound is ammonium which at pH above 8 could transform to toxic free ammonia. [9]**.** Having above in mind, it is of high importance for society and environment to have chlorophenols and nutrients properly treated in wastewater. Furthermore, the outcome of this work will be a foundation for future biological wastewater treatment researches at Nazarbayev University Environment Science & Technology Group (ESTg). Encountered in this work limitations and assumptions

could be overcome, and more sophisticated approach could then be taken in devising future experiments.

Method deployed in this Master Thesis involves experimental approach. The experiments were run for CFR and SBR using the suspended-growth un-acclimated sludge from the same local municipal wastewater treatment plant. Per CFR and SBR, two reactors were deployed: baseline and inhibitor. The difference among them was the presence of inhibiting compound such as 2 - CP or 2,4,6 - TCP in inhibitor reactor. The baseline reactor did not contain any inhibitors and served as control reactor to evaluate the inhibiting impact of 2-CP and 2,4,6 - TCP on the sludge. The durations of CFR and SBR experiments were about 2.5d (HRT of 24h) and 8d (HRT of 1.43d) respectively. The sampling was carried out each day to monitor the trends in sludge growth, nitrate oxidation (nitrification), and removals of chlorophenols , total carbon and nitrogen. Also, sludge activity regulating operating conditions were monitored and, if necessary, adjusted once per day.

1.2 Aims & Objectives

The aim of this Master Thesis is to assess the impacts of 2-CP and 2,4,6 - TCP on suspended-growth un-acclimated sludge performance in CFR and SBR. Also, sludge performance depends on reactors configuration. Thus, the efficiencies of current CFR and SBR configurations are to be evaluated.

To achieve this aim, the following questions have to be answered:

- Is un-acclimated sludge capable of completely removing varying 2-CP and 2,4,6 - TCP concentrations? What are the chlorophenols removal dynamics throughout the experiments?
- How do varying 2-CP and 2,4,6 - TCP concentrations affect carbon oxidation and nitrification processes? What are the differences between CFR and SBR experiments?
- Do varying 2-CP and 2,4,6 - TCP concentrations inhibit bacteria growth rate throughout the experiment?
- How are the operation parameters such as pH and DO affected by bacterial activity in experiments? What information do we get from these results?
- What is residence time distribution in CFR? How ideal is the behavior of CFR?
- What is the role of air stripping in compounds removal rate in SBR? How does the compounds removal rate by air compare to that by bio-reaction? Are the chlorophenols resistant to removal by air stripping?

2. Literature Review

Activated sludge consists of consortium of different microorganisms such as bacteria, fungi, rotifiers, and protozoa. Bacteria are responsible for degrading

organic compounds in wastewater. Depending on availability of oxygen, bacteria could be aerobic, facultative, and anaerobic. Aerobic bacteria are those which cannot exist without the dissolved oxygen. Facultative bacteria are adaptable to the both presence and absence of oxygen. Anaerobic bacteria exist at zones without the oxygen. The following reactions involving bacteria are observed in this Master Thesis experiments:

- Organic matter (glucose, sodium acetate, chlorophenols) biodegradation by heterotrophic bacteria.
- Ammonium oxidation by chemotrohic nitrifiers to generated nitrate as final product.
- Nitrate conversion to nitrogen gas by heterotrophic bacteria.

2.1. Organic matter oxidation

Heterotrophic bacteria need carbon from organic matters for cell synthesis and energy generation. Thus, biodegradation of organic matter by heterotrophic bacteria takes place per following reaction [*10*]**:**

$$Organic\ matter + O_2 \text{ ----------} \rightarrow New\ Cells + CO_2 + H_2O \qquad R\text{-}1$$

Amount of energy yield from oxidation of organic compound depends on type of terminal electron acceptor. The higher is the electron acceptor on energetic level, more energy is generated from organic matter. Aerobic bacteria using

oxygen as electron acceptor obtain the highest energy from biodegradation compared to facultative and anaerobic bacteria. Thus, aerobic bacteria could be characterized by higher growth rate. Higher growth rate is one of main critical advantages of preferring aerobic bacteria over anaerobic in chlorophenol removal [*11*]. The types of electron acceptors from higher to lower energetic levels include O_2, NO_3^-, and SO_4^2 [*12*].

2.2. Ammonium oxidation

Ammonium is oxidized in a 2-step reaction called nitrification by chemoautotrophic bacteria (or nitrifiers) yielding nitrate (NO_3^-) and energy as final products. Chemoautotrophic bacteria obtain their energy from oxidizing inorganic ions. The nitrification process takes place by the following reaction schemes [*13*]**:**

$$2\,NH_4^+ + 3O_2 \longrightarrow 2NO_2^-\ (nitrite) + 2H_2O + 4H^+ \qquad R\text{-}2$$

$$2\,NO_2^- + O_2 \longrightarrow 2\,NO_3^-\ (nitrate) \qquad R\text{-}3$$

The overall nitrification reaction is:

$$NH_4^+ + 2O_2 \longrightarrow NO_3^- + 2H^+ + H_2O \qquad R\text{-}4$$

Conversion of ammonium to nitrite is carried out by *Nitrosomonas, Nitrosococcus, Nitrosolobus and Nitrosovirbrio* bacteria genera. Conversion of nitrite to nitrate is performed by *Nitrobacter*, *Nitrococcus*, and *Nitrospira* bacteria genera [*14*]. As with the case of heterotrophic bacteria, nitrification bacteria generate energy and

new bacterial cells during nitrite and nitrate formations. Ammonium conversion is slower compared to nitrite conversion.

2.3. Denitrification

The process of nitrate conversion to nitrogen gas is called denitrification. Denitrification is carried out by heterotrophic bacteria at the presence of carbon source as substrate. As reaction takes place at anoxic conditions, nitrate is used as bound-oxygen receiver in organic matter biodegradation. The complete nitrate removal path is the following: [*14*]

$$NO_3^- \rightarrow NO_2 \rightarrow NO \rightarrow NO_2 \rightarrow N_2 \qquad R\text{-}5$$

2.4. Optimum pH for bacteria activity

The bacteria living media characteristics such as pH and DO are crucial to the sustainment of microbial activities. pH between 6.5 and 8.5 was reported to be best for bacterial growth of sludge in treatment plants [*15*]. [*11*] also confirmed that optimal pH range for carbonaceous oxidation is between 6.5 and 8.5. Growth outside this range can take place at reduced rate. Especially below lower boundary, the filamentous bacteria could grow excessively. Filamentous bacteria lead to poor sludge settling negatively affecting sedimentation. Other conditions for thriving of filamentous bacteria are low Food to Microorganism ratio, low dissolved oxygen, and limited nutrients. The optimal pH range for nitrification is between 7.5 and 8.5. Ammonia oxidation rates diminish considerably at pH below 7.0. Optimum

oxygen uptake is considered at pH 7.0 and 7.4 [*16*], [*10*]. [*17*] reported that at temperature of 15 deg C and DO of 5.0 mg O_2/L sludge settleabiltiy index (SVI) increased from 70 mL/g at pH of 5.8 to 37 mL/g at pH of 9. SVI indicates the volume taken per sludge mass of 1 gram. The lower SVI is, the better is the sludge settleability.

2.5. Optimum DO on bacteria activity

The desired DO for biodegradation and nitrification is usually between 0.6 and 2.0 mg O_2/L [*11*]. However, it is recommended to establish aeration rates above given range to account for uneven aeration and for continuous growth of bacteria. Continuous growth of bacteria leads to increasing oxygen consumption. Denitrification takes place at anoxic conditions where DO must be less than 0.5 mg

2.6. Role of aeration in wastewater compounds removal

As aeration is an inherent part of aerobic degradation, it is role in wastewater compounds removal can be significant. To the best of my knowledge, no separate work has been reported on the effect of air in aerobic biodegradation. That might be due to the moderate level of aeration rate used. Moreover, aeration at higher rate in other researches was not used to support sludge suspended in contrast to the current work in CFR and SBR. Instead, removal of compounds by factors other than bacteria has been accounted for by volatilization and photo degradation losses. Volatilization is the evaporation of volatile compounds in solution and

photo degradation is the loss of compounds due to ambient light. [*18*] reported that losses due to volatilization and photo degradation were less than 3%. [*19*] observed that 4-chlorophenol losses due to volatilization were less than 4-5%. According to [*20*], 2-CP losses due to volatilization and photo degradation were less than 2%. Moreover, aeration can also contribute to the removal of carbons other than toxic compounds and nitrogen which have not been also reported previously.

2.7. Continuous-type reactors

2.7.1. Fluidized-Bed Biofilm Reactor (FBBR).

Fluidized bed biofilm reactor (FBBR) is one of highly efficient wastewater treatment technologies. In FBBR, wastewater treating bacteria are attached to small-sized particles called media and grow on it forming biofilm. Thanks to concentrated and efficient distribution of bacteria, active biomass concentration in FBBR can be in the range of 8000 - 40000 mg/L compared to 3000 - 6000 mg/L in conventional activated sludge process [*21*]. FBBR working principle consists of influent wastewater flowing up through bed of particles with attached and immobilized bacteria. The influent wastewater velocity is set to allow to fluidize or to induce motion to particles and to avoid their washout. Bacterial biofilm is resistant to washout under reasonable flow rate conditions. Thanks to denser active biomass concentration and particles larger contact surface area, higher BOD, COD, toxic compounds, and nitrogen removals can be achieved. Longer sludge retention

time (SRT) due to biomass immobilization is important in removal of toxic and xenobiotic compounds. Dense distribution of bacteria allows them to handle shock loading. From an economic point of view, FBBR takes less space and time in achieving desired wastewater treatment levels. The disadvantages of FBBR are higher power consumption for upward influent pumping and necessity for proper inlet and outlet configurations to achieve desired flow distribution within the reactor [*22*]. FBBR has been widely used in removal of xenobiotic compounds. Complete removal of 2,4,6 - trichlorophenol (2,4,6 - TCP) and phenol (Phe) mixture was reported by [*23*] . In this experiment, consisting of two stages, almost complete removal of 2,4,6 - trichlorophenol and phenol was observed throughout both stages. During stage one, 120 mg/L of 2,4,6 - TCP and 30 mg/L of Phe with the presence of 1000 mg/L COD sucrose were fed to the bioreactor , while during second stage, feeding of sucrose was stopped. Sucrose serves as easily degradable carbon source which enhances bacteria growth rate, thus, contributing enormously to higher removal rate of toxic compounds. [*24*] also reported the use of similar co-substrate such as glucose for developing aerobic granules to achieve the 94% removal of 2,4 - dichlorophenol. In [*23*], large supply of sucrose accelerated intense bacterial colony growth on biofilm carriers. Such approach during the first stage helped to achieve full degradation of 2,4,6 - TCP and Phe. It was expected that already increased bacterial population after first stage

would demonstrate the same performance during the second stage where the supply of sucrose was stopped. Thus, during second stage, full degradation of 2,4,6 - TCP and Phe was achieved at the expense of large bacterial population. As was mentioned before, active biomass concentration in FBBR can reach high levels; especially, under very favorable condition such as large supply of easily degradable substrate. In contrast to supplementing bacteria with co-substrate, in this experiment [*25*], bacteria were acclimated to toxic compounds to be treated such as 2-chlorophenol (2-CP), phenol, and m-cresol before being introduced into FBBR. Prior acclimation of bacteria to tolerable amount of toxic compounds under favorable conditions leads to development and growth of specific bacteria aimed at degrading the exposed toxic compounds [*26*]. Use of acclimated bacteria helped to achieve 99.8% removal of phenolic compounds. 2- CP, phenol, and m-cresol inlet concentrations were 100 mg/L, 50 mg/L, and 50 mg/L respectively. Prior augmentation of bacteria by easily degradable substrate or their adaptation to toxic compounds under comfortable conditions can both help to achieve higher toxic compounds removal.

2.7.2. Packed-Bed Biofilm Reactor (FBBR).

Another type of frequently used continuously operated reactor is Packed-Bed Biofilm Reactor (PBBR). Compared to FBBR, immobilized bacteria residing particles are fixed, thus, forming a permanent bed throughout the cross-sectional

area of the reactor. Because of densely packed particles and, thus, higher contact surface per volume of reactor, PBBR provides with intense interaction between bacteria and wastewater. This results in a higher treatment efficiency compared to FBBR. However, densely packed distribution of particles pose serious disadvantage such as clogging. As bacteria-formed biofilm grows and fills limited porous area between particles, clogging takes place restricting the flow of influent wastewater through treatment media [*27*]. An important feature of PBBR reactor is the hydraulic residence time (HRT). Due to close packing of biofilm carrying particles, increasing contact time between bacteria and wastewater is critical for achieving higher toxic effluent removal. Such PBBR structural feature is leveraged by increasing HRT during the wastewater treatment. By increasing HRT from 2.5 hours to 14.4 hours it was possible to increase the removal of mixture of 2-CP, 4-CP, and 2,4,6 - TCP from 85.6 % to 100% at 23 deg C [*28*]. 2-CP, 4-CP, and 2,4,6 - TCP inlet concentrations were the same and varied from 25 to 37.5 mg/L. Increasing HRT from 8 hours to 24 hours allowed to boost 4-bromophenol removal from 30% to 98% [*29*]**.** However, higher removal rate is achieved at the expense of treatment duration. Higher HRT demands longer operational period to process all inlet wastewater. Moreover, extended and intensified treatment due to high HRT can lead to early clogging of pores within fixed bed structure. Inspite of

potential drawbacks of PBBR, it is recognized for higher treatment efficiencies especially if used for short lasting operations.

2.8. Batch-type reactors

Sequential Batch Reactor (SBR) is frequently used contemporary reactor of batch-type. SBR has different modifications based on the way the bacteria are utilized. Bacteria can exist in the form of suspended sludge and granular form. SBR operation consists of sequential operational steps such as filling, reaction, settle, and draw. The main advantages of SBR are elimination of clarifiers and absence of shock loading. As settling is part of SBR cycle, clarifiers are not needed resulting in capital cost savings. SBRs are not subject to unexpected spike in influent flow rate fluctuations as they operate batch-wise.

2.8.1. SBR with Suspended Growth sludge (SG-SBR)

Suspended sludge in SBR is more susceptible to toxicity of xenobiotic compounds compared to granular and immobilized sludge. [*30*] compared the performance of SBR suspended sludge in treating phenol from 100 mg/L to 1000 mg/L with aerated and unaerated fill stages. In one reactor, phenol was fed during fill stage with aeration on, while in another reactor, aerator was switched off during filling. It was reported that both methods did not affect the phenol removal efficiency of both reactors demonstrating almost complete removal of phenols. However, use of unaerated fill is not desired when used in aerobic biodegradation.

Toxic compounds, during unaerated fill stage, will accumulate. When react stage begins with aeration on , accumulated toxic compounds can induce shock load on suspended sludge. The higher is the accumulation, more inhibitive the shock load can be to suspended sludge. [*31*] reported than phenol removal efficiency started decreasing below 90% when its inlet concentration exceeded 800 mg/L. Moreover, if sludge is not pre-acclimated to toxic compounds, not supplied with co-substrate, or if higher concentration of more toxic compounds such as 2-CP or 2,4,6 - TCP to be used, the shock load on suspended activated sludge can cause permanent inhibition of bacterial activity. Thus, aerated fill stage is possibly a relieving factor in successful treatment of 2-CP and 2,4,6 - TCP. For 2-CP and 2,4,6 - TCP, difference between aerated and unaerated filling in terms of toxic compounds removal has not been reported to date. With aerated react stage, the activated sludge will already have degraded some toxic compounds when reaction stage begins leading to shorter reaction time. Such approach allows avoiding unnecessary shock loading. Thus, use of aerated fill stage is recommended for SBR involving suspended sludge.

2.8.2. SBR with granular sludge (GSBR)

Conventional suspended sludge in SBR can be vulnerable to poor settling. Poor settling leads to low effluent quality as more unsettled sludge is removed with effluent. Also, poorly settleable sludge are difficult to replace resulting in

decreased treatment efficiency. The solution comes with dense activated sludge in the form of granules. Formation of granules takes place in five steps: microbial population increase step, floc appearance step, floc cohesion step, mature floc formation step, and aerobic granules formation step [*32*]**.** Granular shape facilitates easier sludge settling when necessary. Other important advantages of granular sludge over suspended growth sludge are higher biomass retention and capacity to bear higher organic loading rates [*32*]. The successful implementation of granular sludge in toxic compounds removal depend on how fast granules form throughout the reaction. Granules formation depends on many factors. **[***33*] reported above 93% removal of 2,4,6 - TCP with its inlet stepwise concentration from 10 to 100 mg/L. In **[**33**]**, glucose and sodium acetate were fed as easy carbon sources for bacterial growth and granules formation. Granules formed from glucose has irregular surface texture in the form of folds or crevice that facilitate better substrates diffusion and mass transfer rates [*34*]. Granules formed from sodium acetate have spherical surface that is less efficient in substrate diffusion. [*34*] reported higher COD removal by sludge granules formed when fed with glucose compared to sodium acetate. Settling phase was chosen to be 3 min. During the granule development, the settling duration plays a crucial role in filtering out sludge flocs that will form future granules. Thus, short settling time is preferred. When settling time is short, granule forming flocs settle quicker

compared to rest of dispersed sludge. The process is continued every cycle as the diameter of settling floc increases leading to granule formation. Optimum settling time was reported to be between 2 to 10 minutes [*35*]. Another key parameter affecting granule formation is HRT. In [*35***]**, HRT was 12 hours. Smaller HRT is preferred. When HRT is small, influent flow rate is higher. Growth substrate containing influent, which flows at higher rate past growing granule, induces faster mass or substrate transfer rate to granules. As a result, granules grow at higher rate. **[***36*] reported that for HRTs of 2,6,12, and 24 hours resulting mean granules diameters were 3.5, 1.2, 1.1, and 0.7 mm respectively. However, care must be taken at designing HRT as too short value of it can result in wash out sludge.

2.9. Organic carbon and ammonia removal

Many reseraches have been dedicated to the removal of toxic compounds such as 2-CP and 2,4,6 - TCP, but very few works have been concerned with the nutrients removal such as organic carbon and ammonia as part of Total Carbon (TC) and Total Nitrogen (TN) under the effect of these toxic compounds. [37] reported over 45% TN removal in batch-type experiment using single bacteria species called Chlorella vulgaris at 50 mg/L 2,4,6 - TCP concentration. Use of mixed consortia of bacteria is preferred over using single species in treating toxic compound containing wastewater. In mixed culture, several types of bacteria aimed at degrading particular toxic compound can develop when exposed to these

toxic compounds. Their combined effort can help to achieve higher TN removal rate. Mixed bacteria cultures exist in the activated sludge used in current CFR and SBR experiments. **[***38***]** reported 13% removal of ammonia under 50 mg/L inlet 2-CP concentrations. However, no report has been found on 2-CP concentration at which ammonia removal inhibition starts. Also, to date, no information has been found on the effect of 2,4,6 - TCP on ammonia removal. Effect of 2,4,6 - TCP on organic carbon removal in terms of COD was reported by [*39*] for combination of PBBR and aeration tank. COD removal decreased from 97% to 90% when influent 2,4,6 - TCP feed increased from 57 to 390 mg/L. Bearing in mind the robustness of FBBR in toxic compounds removal such as 2,4,6 - TCP, concetration of 2,4,6 - TCP up to 390 mg/L was not inhibitive on carbon removing bacteria. It is interesting then to observe how less advanced CFR and SBR will remove organic carbon under the effect of 2,4,6 - TCP.

As was mentioned from [*32*] and [*25*], supply of co-substrate parallel to toxic compounds treatment in FBBR and prior acclimation of sludge before introducing to PBBR without parallel supply of co-substrate can help to achieve higher 2,4,6 - TCP and 2-CP removals. Moreover, FBBR and PBBR due to their inherent design features such as higher biomass concentrations and large contact surface area provide with superior treatment. Thus, it is interesting to observe how addition of co-substrate such as glucose and using un-acclimated sludge can help

to handle 2-CP and 2,4,6 - TCP in less advanced reactors such as CFR and SBR. Implementing aerated filling can help to handle 2-CP and 2,4,6 - TCP loads in SBR. There is a knowledge gap in evaluating the effect of 2,4,6 - TCP on ammonia removal. Moreover, more information needs to be obtained on the role of 2-CP and 2,4,6 - TCP in organic carbon and ammonia removal in simple and basic CFR and SBR. As effect of aeration on removal of toxic compounds, carbon, and nitrogen has not been reported, it has to be evaluated to truly evaluate the biodegradation capacity of activated sludge in SBR in the presence of aeration. Aeration impact in SBR is severe compared to CFR as in the former reactor twice as high aeration rate was used in addition to external aerator operated at maximum rate.

3. Materials and Methods

W-11 aerobic reactor from Armfield was used for both CFR and SBR experiments. For CFR experiments, white cylindrical filter with 16 microns pore size was used to filtrate treated effluent (Figure 3.1). For SBR experiments, filter was not used in reactor as shown in Figure 3.2.

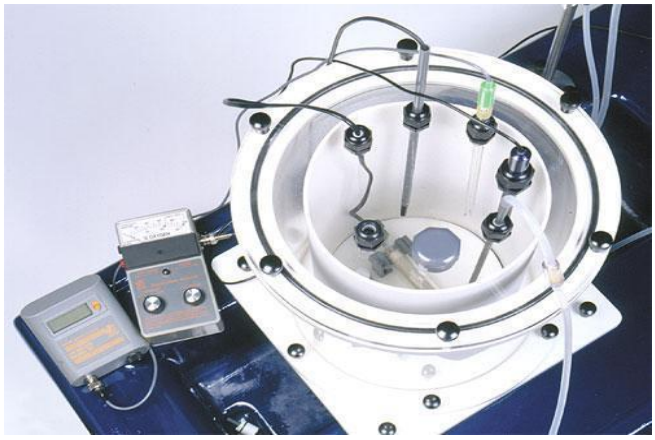

Figure 3.1: W-11 reactor with filter (white cylinder inside the reactor) for CFR experiment

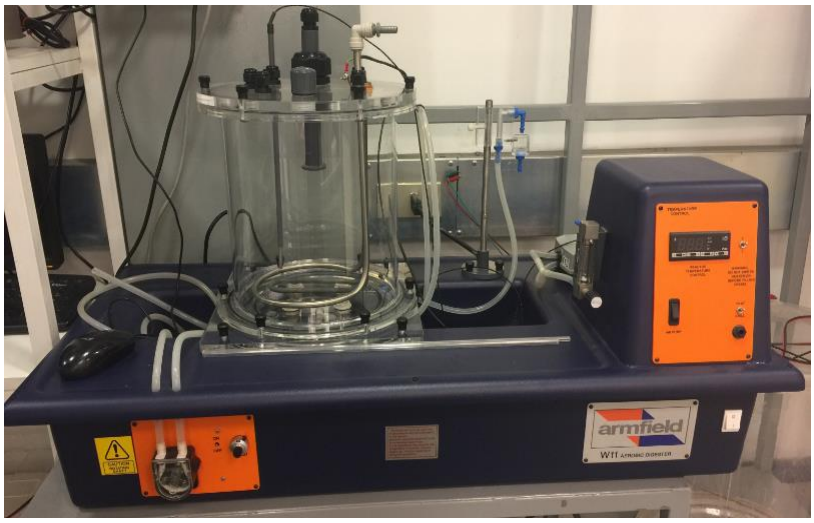

Figure 3.2: W-11 reactor without filter for SBR experiment

3.1 Continuous Flow Reactor (CFR) Experiment

The reactor configuration plays an important role in the performance of the activated sludge process. In order to compare with the literature data it is important for the configurations to be similar. CFR is used to simulate the operation of Continuously Stirred Tank Reactors (CSTR) which is used in other researches in

the similar field. The main difference of CFR from CSTR is the absence of stirrer. Stirrer functions to keep the sludge suspended and to properly mix it with the feed. The mixing is, thus, vital in achieving proper reaction within the reactor. In CFR, aeration was used instead of stirrer to carry out above mentioned functions. Nevertheless, aeration delivers sufficient mixing in CFR. Residence Time Distribution (RTD) experiment was , thus, carried out to verify that CFR simulate the CSTR and , also , to check if non-ideal behavior patterns present in CSTR also exist in CFR.

3.1.1. Residence Time Distribution (RTD) Experiment

3.1.1.1. General

Physical layout and complex fluid dynamics imposes a non-ideal flow in continuous reactors. For example, common non-idealities present in CSTRs are feed bypassing and presence of dead-zone. Bypassing is characterized by the tendency of feed solution molecules to take shorter path through nearby located outlet to exit the reacting system. As a result, bypassing reactants do not participate in reaction within the stirred region. Dead zones are the regions within reactor where no reaction between reactants takes place due to the absence of mixing; mixing is one of reaction driving forces. Both bypassing and dead zone lead to lower exit conversions of reactants and, thus, to lower products concentrations.

Both drawbacks of CSTRs such as bypassing and dead zones are illustrated on Figure 3.3 below:

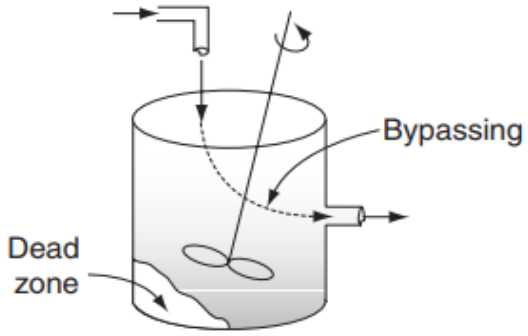

Figure 3.3. Bypassing and dead zone in CSTR

In our CFR experiment, such non-ideal behavior could negatively affect the interaction between activated sludge and feed wastewater leading to lower pollutants removal. Thus, before proceeding to CFR experiment itself, it is vital to check that experiment method in CFR is less prone to non-ideal behavior.

3.1.1.2. Tracer Injection Methods

Tracer experiment is based on the injection of chemical called tracer, with concentration C_0, at the reactor inlet and observing the final concentration, $C_{f,}$, of injected chemical at the reactor outlet. There are two types of tracer experiment: pulse and step modes. Pulse mode is based on the one-time injection of tracer within specific time frame. Step mode experiment is in opposite to pulse mode; tracer is injected continuously with constant concentration. Step mode is preferable over pulse mode as latter has disadvantages. Firstly, according to [*40*], pulse mode tracer injection time into reactor must be very short compared to residence times in

various reactor segments. Secondly, the dispersion between point of injection and reactor entry must be negligible. Dispersion disturbs the concentration of inlet tracer, thus, affecting residence time calculations. One-time tracer injection in pulse mode must have constant concentration at the time of entry into the reactor. Advantage of step mode is that the inlet tracer concentration stays constant and does not cause disturbance to residence time distributions in reactor.

3.1.1.3. Feed Solution

For step mode tracer experiment, sodium chloride (NaCl) was used as a tracing agent in feed solution. Instead of concentration, conductivity of dissolved sodium and chlorine ions was measured. Use of NaCl as a tracing agent was justified by the fact that it is very quick to obtain conductivity values using conductivity meter. If non-reactive compound was to be used to monitor its concentration, then, it would be time-consuming to sample the solution and to analyze it for chosen compound concentrations. Thus, the use of NaCl as tracing agent and the measurement of its conductivity are justified for its flexibility.

2500 ppm NaCl solution was prepared to be fed into the reactor. To obtain such solution, 78.75 g of NaCl was dissolved in 31.5 L of tap water. Expected stock solution conductivity was about 5100 microS/cm or 5.1 mS/cm according to the following table [*41*]:

Table 3.1: Conductivity of NaCl as a function of concentration.

Salt	Conductivity equivalent	TDS/conductivity
Sodium chloride	1.00 ppm TDS* = 2.04 uS/cm	0.49
Sodium sulfate	1.00 ppm TDS = 1.49 us/cm	0.67
Calcium sulfate	1.00 ppm TDS = 1.36 us/cm	0.74
Sodium bicarbonate	1.00 ppm TDS = 1.06 us/cm	0.91

TDS* - Total Dissolved Solids

Mettler Toledo FEP-30 conductivity meter was used to measure conductivities during this experiment. Average NaCl feed solution conductivity throughout the experiment was around 5.10 – 5.14 mS/cm which is pretty close to predicted value from Table 1, thus, proving that our conductivity meter works properly.

3.1.1.4. Experiment Run

Tracer experiments was conducted for operating volume of 7.4 L which is close to the one (7.5 L) used in actual CFR experiment. It was assumed that volume difference between 7.4 L and 7.5 L would not significantly affect actual HRTs for these two volumes.

Starting from t=0, the feed solution was pumped into reactor at average flow rate of between 3.63 ml/min by embedded peristaltic 24 V DC pump. 3 times per day feed solution, reactor, and outlet conductivities were measured. Feed solution conductivity was measured to verify the constant conductivity as feed solution was renewed. The experiment was to be stopped when outlet reactor conductivity ratio to feed conductivity was about 0.98 at about t=85.5 hours.

3.1.2. CFR Experiment Procedures

First two experiments were run for 2-CP at average inlet concentrations of about 104 and 165 mg/L. 3rd and 4th experiments were run for 2,4,6 - TCP at average inlet concentrations of 70 and 73 ppm.

3.1.2.1 Reactor Setup

Two Armfield W-11 aerobic reactors (Figure 3.4) with membrane filters were used. Membrane filters retain sludge allowing only treated wastewater to pass. The reactor is made of plastic glass. Full capacity of reactor without filter is 12.5 L according to the manufacturer [*42*]. The operating volume for this experiment was 7.5 L that consists of 2 L of sludge and 5.5 L of tap water.

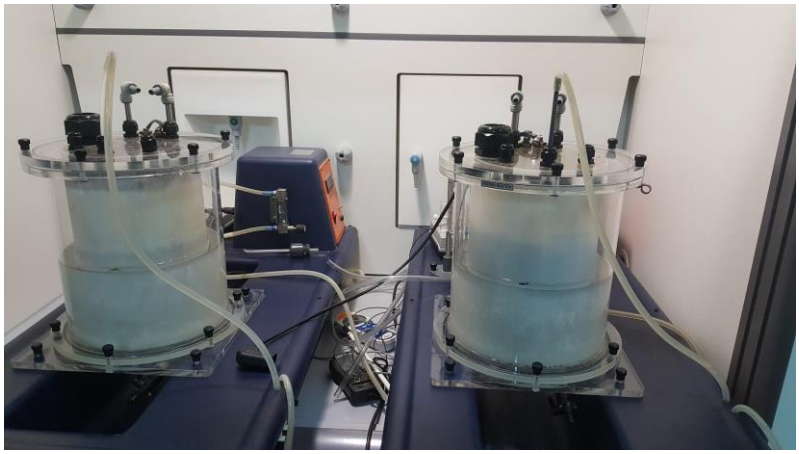

Figure 3.4: Baseline and inhibitor reactors for CFR experiment

3.1.2.2 Feed Solution

The 3.5 L of synthetic wastewater stock solution based on deionized water was prepared as part of feed solution. The composition of stock solution is listed in Table 3.2:

Table 3.2: Stock solution composition

Compound	Concentration (g/L)
Glucose	8
Bacterial Peptone	2.4
Lab Lemco	1.6
Ammonia hydrogen carbonate	0.8
Potassium hydrogen carbonate	0.4
Sodium hydrogen carbonate	0.4

Peptone, as a source of amino acids, peptides, and proteins, act as nutrient to support the bacterial reproduction. Lab lemco contains vitamins, carbohydrates, organic nitrogen compounds, and salts. These compounds in lab lemco also contribute to bacterial growth. Ammonia hydrogen carbonate is added as a source of ammonia for nitrification and as a buffer agent to regulate feed solution pH. Potassium and sodium hydrogen carbonate act to sustain neutral pH for bacterial growth. Glucose and sodium acetate are added as carbon sources for heterotrophic bacteria cell generation. Sodium acetate used as co-substrate (additional substrate) might take part in reduction of toxicity of xenobiotic compounds and of cell growth inhibition. Easily degradable carbon sources such as sodium acetate and

glucose can accelerate the bacterial growth, which, in turn, helps to achieve higher chlorophenols biodegradation rate [*18*].

Trace metals were added to the stock solution with the following composition as listed in Table 3.3.

Table 3.3: Stock solution metals content

Chemical	Concentration (g/L)
$FeCl_3$	5
$CaCl_3$	5
KCL	1

Trace metal solution was added to the feed in the ratio of 1 ml/15L of feed solution. The wastewater and trace metal solution compositions were taken from W - 11 aerobic reactor manufacturer [*42*].

Prepared 3.5 L of stock solution was then diluted to 17.5 L of final feed solution by addition of 14 L of deionized water. Thus, the stock solution components concentrations shown in Tables 3.2 and 3.3 have the following concentrations in final feed solution shown in Table 3.4:

Table 3.4. Synthetic wastewater composition in final feed

Compound	Concentration (g/L)
Glucose	1.60
Bacterial Peptone	0.48
Lab Lemco	0.32

Ammonia hydrogen carbonate	0.16
Potassium hydrogen carbonate	0.08
Sodium hydrogen carbonate	0.08
$FeCl_3$	1
$CaCl_3$	1
KCL	0.2

Components listed in Table 3.5 and 3.6 represent initial TC, TN, and NH_4^+ in baseline and inhibitor reactors. Also, initial pH and DO ranges are listed in the tables below. There is no NO_3^- present in feed solution of both reactors. Average values and standard deviations of each parameter were obtained from all experiments.

Table 3.5: Baseline reactor feed solution parameters

Parameter	**Average Value**	**Standard Deviation**
TC (mg/L)	927.02	20.67
TN (mg/L)	113.84	2.31
NH_4^+ (mg/L)	44.37	2.92
pH	7.36 – 7.90	NA
DO	6.66 – 8.26	NA

Table 3.6: Inhibitor reactor feed solution parameters

Parameter	Average Value	Standard Deviation
TC (mg/L)	943.28	19.81
TN (mg/L)	116.91	3.07
NH_4^+ (mg/L)	43.15	2.26
pH	7.60 – 8.04	NA
DO	5.22 – 8.36	NA

To balance the amount of carbon from chlorophenol in inhibitor reactor, sodium acetate was added to baseline reactor feed solution. Added sodium acetate concentration depended on type of chlorophenol and its concentration as shown on Table 3.7 below.

Table 3.7: Sodium acetate amount added.

	Exp.1	**Exp. 2**	**Exp. 3**	**Exp.4**
Type of chlorophenol	2-CP	2-CP	2,4,6- TCP	2,4,6- TCP
Chlorophenol, mg/L	103	163	71	72
Sodium acetate, g	3.35	3.35	2.18	2.18

3.1.2.3 Activated Sludge

10 L of recycle activated sludge was taken from local municipal water treatment plant. The sludge was then left for 24-hour aeration to reach endogeneous conditions. Endogeneous condition is characterized by the state where the food to microorganisms ratio is very low. This is to ensure that any food substrate from wastewater treatment plant is eaten out by bacteria. Thus, after 24 hours, there should not be any food that could affect sludge behaviour in reactor. Doing so external uncertainty could be eliminated. For continuous experiment, the sludge was not removed and renewed. Also, sludge was not acclimated to 2-CP and 2,4,6 – TCP before start of experiment. Initial TSS value varied between 2.25 – 3.42 mg/L and 2.24 – 3.66 mg/L for baseline and inhibitor reactors respectively.

3.1.2.4 Experiment Run

Ready 17.5 L feed solution was fed to the reactors by peristaltic pump at flow rate around 4 ml/min. Pumping rate was checked twice per day during samplings. Experiments lasted for approximately 65 hours.

3.1.2.5 Reactor monitoring

At each sampling, reactor pH and Dissolved Oxygen (DO) were checked. Reactor pH was supported at around 7.0 and 8.3. It was measured by a Mettler Toledo FE pH meter. Aeration was constant at 1.5 L/min for both reactors. DO in

both reactors were within 4.2 - 8 mg O_2/ L range and was measured by WTW Oxi 7310 DO meter.

3.1.2.6 Liquid Sampling

At each sampling, 50 ml of sample was taken from feed and reactor outlet from both baseline and inhibitor reactors. These samples were used for TC, TN, and IC analyses. Another 15 ml of sample from inhibitor reactor feed and outlet were taken for High-Pressure Liquid Chromatography (HPLC) analysis. Samples for HPLC were preserved with sulfuric acid (H_2SO_4) to prevent degradation of chlorophenols. pH was for HPLC samples was kept below pH 2. Acidification of samples for HPLC allows holding them for 14 days [*43*]. All samples for TC, TN, IC, and HPLC had been filtered through 0.45 micrometer pore-size regenerated cellulose syringe filters from Agilent. All filtered samples were kept in fridge at 4^oC.

During each sampling, two 10 mL of mixed liquor suspended solids (MLSS) were taken from both reactors to obtain TSS. Due to uneven mixing in reactor, having two samples per reactor provide with more representative value of TSS after averaging. 10 mL of MLSS was filtered through GE Whatman GF/C filter paper using vacuum suction pump. Dehydrated MLSS was then dried at 105 deg C for 24 hours. Difference between pre-weighed clear and dried filter weights gave TSS concentration [*16*].

3.2 Sequencing Batch Reactor (SBR) Experiment

SBR experiment was carried out in W-11 Armfield aerobic reactor shown on Figure 3.5 without filter and agitator.

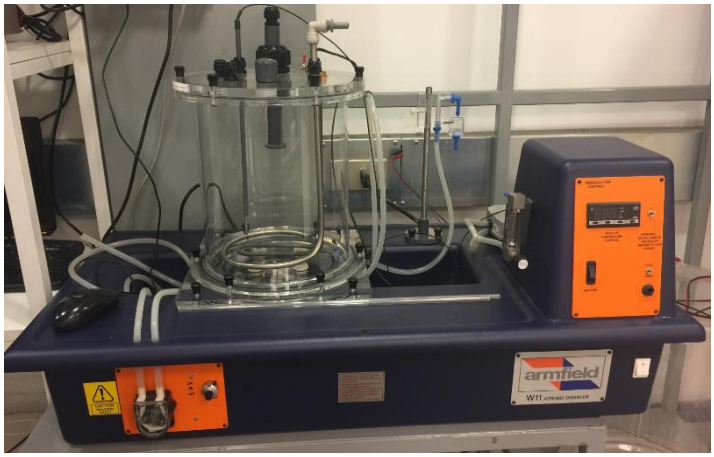

Figure 3.5: W-11 reactor without filter and stirrer for SBR experiment

Agitator plays an important role in helping to suspend sludge for more efficient interaction with feed solution. However, the reactor did not have the stirrer. To compensate for the absence of stirrer, reactor aerator was put on maximum. Throughout the experiment, it was observed in Baseline and Inhibitor reactors that majority of sludge had tendency to settle at the floor of the reactor during the experiment. Undesired settling could reduce biodegradation potential of sludge. Aquarium diffuser (external aerator) was then added to each reactor's aeration system. The aeration rate was also at maximum level. The diffuser was installed along the circumferential heating spiral at the bottom of the reactor to cover as much area along the reactor walls as possible.

It was suspecte suspected that very intense aeration could cause stripping leading to additional removal of TC, TN, and chlorophenols by air. As a result, the true biodegradation potential of sludge could be masked. Therefore, air stripping experiment was conducted to evaluate how air stripping contributed to inlet wastewater compounds revmoal.

3.2.1 Air Stripping Experiment Procedures

Air stripping experiment was conducted at the following regimes as shown on Table 3.8.

Table 3.8: Air stripping experiment regimes.

Regime #	Description	Aeration mode; magnitude	Purpose
1	Without sludge; synthetic wastewater; no chlorophenol	Internal+ external; maximum (both)	To evaluate the role of sole aeration in TC and TN removal.
2	With sludge; synthetic wastewater; no chlorophenol	Internal+ external; maximum (both)	To evaluate the combined role of sludge and aerators in TC and TN removal.
3	With sludge; synthetic wastewater;2-chlorophenol	Internal+ external; maximum (both)	To evaluate the performance of sludge under the influence of aeration and pollutants on TC , TN, and 2-chlorophenol removal.
4	With sludge; synthetic wastewater;2,4,6-trichlorophenol	Internal+ external; maximum (both)	To evaluate the performance of sludge under the influence of aeration and pollutants on TC, TN, and 2,4,6 - trichlorophenol removal.

5	Without sludge;synthetic wastewater; no chlorophenols	Internal; maximum	To evaluate the role of reactor aerator in TC and TN removal.
6	Without sludge; synthetic wastewater; no chlorophenols	Internal; half	To evaluate the role of reactor aerator in TC and TN removal.

3.2.1.1. Feed Solution

The inlet stock solution composition was that shown in Table 3.2. Only 2.2 L of feed solution was processed to analyze the effect of stripping after one day since the beginning of SBR experiment. The reactor volume corrected final inlet solution concentration is shown on Table 3.9. Dilution volume of 3.03 was applied.

Table 3.9. Synthetic wastewater composition in final feed (reactor corrected)

Compound	Concentration (g/L)
Glucose	0.53
Bacterial Peptone	0.16
Lab Lemco	0.31
Ammonia hydrogen carbonate	0.05
Potassium hydrogen carbonate	0.03
Sodium hydrogen carbonate	0.03
$FeCl_3$	0.33
$CaCl_3$	0.33
KCL	0.06

3.2.1.2. Experiment Run

The feed solution was added to the reactor working under one of regimes listed in Table 3.9. The experiment lasted for 21 hours to represent one reaction cycle of SBR experiment. In SBR, we had 1 hour of FILL and 20 hours of REACT stages. During 1 hour of FILL stage of SBR experiment some reactions between sludge and feed was taking place as the reactor was aerated. Thus, FILL stage, to some degree, is considered as part of REACT stage.

3.2.1.3. Sampling

Data sampling was carried out at t=0 and t=21 hours. pH of both working reactors were adjusted by use of H_2SO_4 to initial values around 7.5-7.7 present in actual SBR experiment.

3.2.2 SBR Experiment Procedures

3.2.2.1 Reactor Setup

For SBR experiment, the operating volume was 6.7 L. This was achieved by mixing 2 L of sludge, 2.5 L of tap water, and 2.2 L of feed solution. Such proportion of operating volume constituents had not changed till the end of SBR experiments. Aeration was set at 3 L/min compared to that of 1.5 L/min in CFR experiment. The reason was to apply higher aeration rate to adequately keep sludge in suspension in the reactor.

3.2.2.2 Feed Solution

Stock solution, as in CFR experiment, had the same concentration of components as shown in Tables 3.2 and 3.3. As it was necessary to process 17.5 L of feed solution in batch mode, it required then 8 days with about 2.2 L of feed solution added each day. However, allowing 17.5 L to last for 8 days could lead to feed solution quality deterioration. Thus, two separate feed solutions with 8.75 L each were prepared one after another. Each batch solution was consumed within 4 days. To prepare 8.75 L batch feed solution, 1.75 L of stock solution was diluted to 8.75 L by deionized water.

The elemental composition of the feed solution is the same as in CFR reactor. However, to represent feed solution at reactor conditions due to dilution at t=0, the feed solution concentration for baseline and inhibitor reactors were modified as shown in Table.3.10 applying dilution factor of 3.03.

Table 3.10. Synthetic wastewater composition in final feed (reactor corrected)

Compound	Concentration (g/L)
Glucose	0.53
Bacterial Peptone	0.16
Lab Lemco	0.31
Ammonia hydrogen carbonate	0.05
Potassium hydrogen carbonate	0.03
Sodium hydrogen carbonate	0.03
$FeCl_3$	0.33

$CaCl_3$	0.33
KCL	0.06

Feed solution TC, TN, and NH_4^+ concentrations are also corrected for dilution as shown in Tables 3.11 and 3.12 for baseline and inhibitor reactors.

Table 3.11: Baseline reactor feed solution parameters

Parameter	**Average Value**	**Standard Deviation**
TC (mg/L)	306.66	13.81
TN (mg/L)	40.07	1.43
NH_4^+ (mg/L)	11.7	0.75
pH	7.62-7.81	NA

Table 3.12: Inhibitor reactor feed solution parameters

Parameter	**Average Value**	**Standard Deviation**
TC (mg/L)	307.62	17.12
TN (mg/L)	39.96	0.89
NH_4^+ (mg/L)	11.82	0.46
pH	7.51 – 7.98	

For SBR, 4 experiments involving 2-chlorophenol and 2 experiments involving 2,4,6- trichlorophenol were conducted. Table 3.13 presents the

chlorophenols concentrations and sodium acetate amounts used. Compared to CFR, here chlorophenols conentrations in feed solution were adjusted by 3.03 Dilution Factor to account for reactor volume and to have consistent comparison with outlet data.

Table 3.13. Chlorophenol types and concentrations used in SBR experiments

	Exp. 1	**Exp. 2**	**Exp.3**	**Exp. 4**	**Exp.5**	**Exp. 6**
	2-CP	2-CP	2-CP	2-CP	2,4,6-TCP	2,4,6-TCP
Conc. in Feed, mg/L	148.5	165	51	51	62.5	78.5
Reactor Volume Corr. Conc., mg/L	49	54.5	17	17	20	26
Sodium acetate, g	1.68	1.68	1	1	0.68	0.86

To ensure better data quality, Experiments 1-2 and 3-4 data were combined. Thus, all involved parameters average values were taken for Experiments 1-2 and 3-4. It was assumed that, in Experiment 1 and 2, 2-CP concentrations Standard Deviation of 2.75 is negligible. Experiments 3 and 4 have the same 2-CP concentrations. Table 3.14 presents updated chlorophenols concentrations and sodium acetate used after averaging Experiments 1-2 and 3-4 data.

Table 3.14: 2-CP and 2,4,6 – TCP concentrations in feed solutions.

	Exp. 1	Exp. 2	Exp.3	Exp. 4
	2-CP	**2-CP**	**2,4,6-TCP**	**2,4,6-TCP**
Average Reactor Volume Corr. Conc., mg/L	51.5	17	20	26
Standard Deviation	2.75	0	NA	NA
Sodium acetate, g	1.68	1.68	0.68	0.86

3.2.2.3 Activated Sludge

Fresh recycled activated sludge was taken from local municipal treatment plant. The sludge was then aerated for 24 hours to reach endogeneous conditions. During batch experiment, the sludge was not removed and renewed. Prior to start of experiments, sludge was not acclimated to 2-CP and 2,4,6 – TCP. Initial TSS ranged from 1.92 to 2.39 for baseline and from 1.92 to 2.28 for inhibitor reactors.

3.2.2.4 Experiment Run

SBR experiment consisted of 8 cycles lasting 8 days. One cycle, lasting 24 hours, consisted of aerated FILL, aerated REACT, SETTLE, and DRAW stages. During aerated FILL stage, 2.2 L of feed solution from 8.75 L was pumped at about 36 ml/min. Aeration from reactor aerators was at 3 L/min (maximum).

REACT stage facilitated biodegradation by sludge to take place. SETTLE stage was used to achieve separation between treated supernatant clear liquid at the top and sludge at the bottom with aeration off. Supernatant clear liquid of 2.2 L was pumped out at the DRAW stage; the same fed volume of inlet wastewater was thus withdrawn from the reactor. Cycle repeated until all feed solution was processed. The schematic of the process is shown in Figure 3.6.

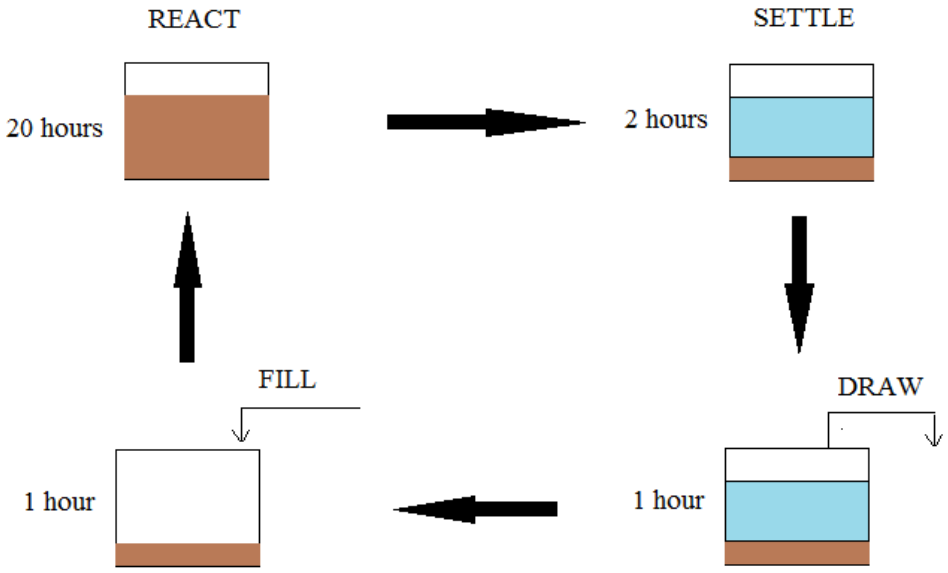

Figure 3.6: SBR experiment layout.

HRT was 1.43 days. The duration of each stage were 1, 20, 2, and 1 hours respectively. The time distribution for stages was adapted from [*44*] as this work almost had almost the same operating (7 L) and sludge volumes (3 L). Such selection of time distribution by [*44*] seems viable. 1 hour fill time for feed resulting in 36.7 ml/min rate had to be enough to avoid chlorophenol shock

loading. Shock loading may cause the sludge to take longer time to acclimatize. Thus, for example, if feed was pumped in 30 min at a rate of 73.3 ml/min, it could cause, to some degree, the temporary inhibition of bacterial activity. Moreover, as the FILL stage is aerated, the biodegradation of feed water should have already started reducing feed toxicity before REACT stage starts. REACT stage of 20 hours was considered to be long enough to reduce TC, TN, and chlorophenol concentrations in synthetic wastewater considerably. SETTLE stage of 2 hours should be enough to allow clear separation between supernatant liquid and sludge. Moreover, at the last 3 days of experiment, sludge grew so much that there was very little space for supernatant liquid. 2 hours of settling was sufficient to carefully withdraw treated feed and not to disturb the sludge. During SETTLE stage, the sludge stays without oxygen for 2 hours. According to [*45*] absence of oxygen for less than 4 hours does not affect negatively nitrifying microorganisms. Pump's capacity was sufficient to withdraw 2.2 L of treated synthetic wastewater during DRAW stage.

3.2.2.5 Reactor monitoring

pH and DO were checked at the end of previous REACT and at the beginning of next REACT stage. Starting pH at the beginning of next REACT stage was controlled and maintained within 7.5 – 7.7 range. 10% v/v H_2SO_4 was added to the reactor to control pH.

Mettler Toledo FE-20 was used to measure pH and WTW Oxi 7310 was used to measure DO in the reactor. Such approach allowed to completely evaluate the behaviour of these variables from one REACT stage to another.

3.2.2.5 Liquid Sampling

40 mL samples for TC, TN, IC, and HPLC analyses from both feed and 2.2 L outlet solutions were taken at t=0 and at t=21. t=21 samples were not actually taken after 21 hours since the start of experiment, but after 24 hours when SETTLE and DRAW stages were complete. t=21 was used to denote the end of REACT stage where biodegradation took place. At SETTLE and DRAW stages the quality of feed in terms of biodegradation was assumed not to change as there was no contact with sludge.

Samples were filtered through General Electric (GE) Whatman Regenerated Cellulose and IsoLab PVDF 0.45 micrometer pore size syringe filters for further analysis. As experiment lasted for 8 days and all samples had to be processed all at once, the samples were preserved with 30% v/v solution of H_2SO_4 at pH below 2 and placed in the fridge. This allowed preventing sample quality deterioration. 2 MLSS samples per reactor were taken once per cycle at the end of REACT stage. Samples were taken at the middle between the top of aerators and below the liquid level at the zone where most sludge was concentrated during REACT stage. GE Whatman GF/F glass microfibre filter papers were used to filter MLSS for TSS.

Dehydrated MLSS was kept in 105 deg C oven for 24 hours. Difference between pre-weighed and heated filter papers gave TSS data. TSS data was of 2 measurements per reactor.

Due to absence of stirrer in order to keep the sludge in suspension high aeration rate was used throughout the REACT stage. For this reason, the stripping experiments were necessary. Sludge suspension and settling depends, apart from reactor mixing conditions, on the properties of activated sludge which in turn depend on the operation of the wastewater treatment plant. Thus, inevitably, suspension and settling quality was varying during the experiments.

3.3. Materials Brands and Purity

Various chemicals used throughout CFR and SBR experiments have own purity provided by manufacturer. Table 3.15 presents such chemicals with associated purity and manufacturer.

Table 3.15: Purity and brand of chemicals used.

Chemical	Purpose	Purity	Manufacturer
Glucose	Synthetic wastewater constituent	≥ 97.5 %	Sigma Aldrich
Bacterial Peptone	Synthetic wastewater constituent	Information not available	Sigma Aldrich
Lab Lemco	Synthetic wastewater constituent	Information not available	Oxoid
Ammonia hydrogen carbonate	Synthetic wastewater constituent	≥ 99 %	Sigma Aldrich
		≥ 99.7 %	Sigma Aldrich

Potassium hydrogen carbonate	Synthetic wastewater constituent		
Sodium hydrogen carbonate	Synthetic wastewater constituent	≥ 99 %	Sigma Aldrich
2-chlorophenol	Inhibitor	≥ 99 %	Sigma Aldrich
2,4,6 – Trichlorophenol	Inhibitor	98%	Sigma Aldrich
Sulfuric Acid	Samples Acidification	98%	Sigma Aldrich
Potassium Hydroxide	Samples alkalisation for IC	≥85%	Sigma Aldrich
Sodim Chloride	Feed solution constituent in RTD Experiment	≥ 99 %	Sigma Aldrich

3.4. Equipment Used

- 930 Compact IC Flex Ion Chromotograph equipment was used to analyze inlet and outlet samples in CFR and SBR experiments for ammonium (NH_4^+) and nitrate (NO_3^-). Samples for analysis were prepared by diluting 5 mL of filtered sample to final volume of 25 mL adding ultra-pure water.
- Multi N/C 3100 analyzer from Analytik Jena AG was used to analyze inlet and outlet samples in CFR and SBR experiments for Total Carbon (TC) and Total Nitrogen (TN). Samples for analysis were prepared by diluting 1 mL of filtered sample to final volume of 20 mL adding ultra-pure water.
- Agilent 1200 Ultra High-Performance Liquid Chromatography (UHPLC) was used to analyze inlet and outlet samples in CFR and SBR experiments for

chlorophenol concentrations. Samples for analysis were prepared by using only 1mL of filtered sample.

4. Results and Discussion

For parameters such as inlet TC, TN, and chlorophenol concentrations in CFR experiments no corrections have been done due to dilution. The real residence time for CFR experiment at 7.4 L operating volume was found to be about 24 hours according to RTD experiment. From RTD experiment, experimental cumulative distribution function F(t) curve (Figure 4.1) show that , for dilution to occur at about 24 hours , ratio of inlet and outlet concentrations should be about 0.5. In RTD experiment, there was no reaction but dilution. For all CFR parameters, at 24 hours, inlet and outlet concentrations for mentioned parameters were not higher than 0.2 on averages. So we assume that the reaction is predominant over dilution, and no dilution correction, thus, has been applied.

For SBR experiments, dilution correction or factor had been applied to inlet TC, TN, NH_4^+ and chlorophenol concentrations by the following equation:

$$D.F. = \frac{V_F}{V_I} \qquad (4.1)$$

In the sections and graphs below, the letter “E” and accompanying “number” stands for “Experiment” and “number of Experiment”. Also, Table 4.1. below

summarizes all the parameters examined throughout RTD, Air stripping, CFR, and SBR Experiments.

Table 4.1: Parameters to be examined in experiments

RTD Experiment	
Parameters To Be Examined:	1. Actual HRT 2. Cumulative Distribution Profile for Inlet 3. Exit-age Distribution Profile for Inlet
Air Stripping Experiment	
Parameters To Be Examined:	1. TC and TN removal by internal and external aeration 2. TC and TN removal by internal/external aerations and sludge 3. TC and TN removal by internal aeration at maximum rate 4. TC and TN removal by internal aeration at half-maximum rate 5. 2-CP removal by internal and external aerations 6. 2,4,6 - TCP removal by internal and external aerations
CFR and SBR Experiments	
Parameters To Be Examined:	1. pH and DO 2. Total Suspended Solids (TSS) 3. TC Removal per TSS/ Absolute TC Removal 4. TN Removal per TSS/ Absolute TN Removal 5. Ammonium Removal 6. Nitrates Production 7.Chlorophenols Removal

4.1. RTD Experiment

HRT value obtained from RTD experiment for 7.4 operating volume of CFR is about 24 hours. HRT is the average time spent by the feed molecules in the reactor. The closeness of CFR at 7.4 L operating volume to ideal CSTR is well demonstrated by cumulative distribution curve, F(t), shown on Figure 4.1.

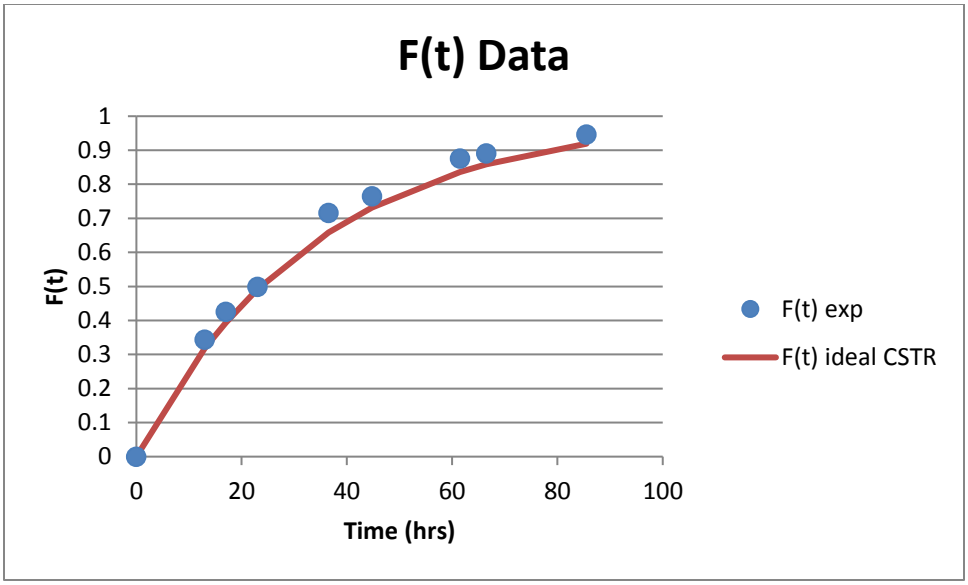

Figure 4.1: F(t) data from RTD experiment.

Cumulative distribution curve states what proportion of all molecules spends time less than *t* in reactor. Experimental and theoretical F(t) curves show almost close agreement with slight deviation above 40 hours. Slight deviation shows that F (t) is above that for ideal CSTR. Such behavior is characterized by the fact that feed molecules leave the reactor quicker implying the presence of bypassing. F(t) function in terms of solution conductivity was calculated according to the following equation:

$$F(t) = \frac{\sigma_{ro(t)} - \sigma_{r(0)}}{\sigma_{f(avg)} - \sigma_{r(0)}} \qquad \text{Eqn.4.2}$$

where $\sigma_{ro(t)}$ (mS/cm) is the conductivity at reactor outlet at time *t*, $\sigma_{r(0)}$ is the conductivity in reactor at t=0, and $\sigma_{f(avg)}$ is the average feed solution conductivity. F(t) curve for ideal CSTR was calculated by the following equation:

$$F(t)_{CSTR} = 1 - e^{(-\frac{t}{\tau})} \qquad \text{Eqn. 4.3}$$

where t is the experiment time and τ is the theoretical Hydraulic Residence Time (HRT). Theoretical HRT was calculated by:

$$\tau = \frac{V_r}{Q} \qquad \text{Eqn. 4.4}$$

V_r is the reactor operating volume and Q is the average volumetric flow rate.

Another curve called exit-age distribution function, E (t), is used to characterize the CFR reactor performance in tracer experiment as shown in Figure 4.2. E(t) describes the fraction of influent molecules that stay in the reactor longer than time t . The trend shows downward decline of the curve. The spikes indicate the backflow of molecules through the filter back to the reactor. This indicates higher proportion of feed molecules staying within the reactor. The backflow is assumed to be negligible as F(t) curve behavior is close to ideal.

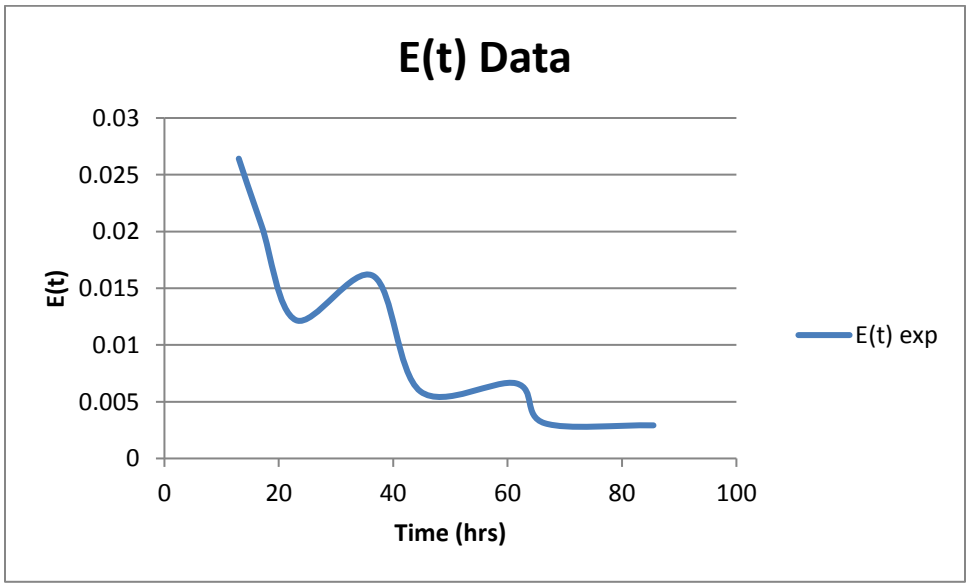

Figure 4.2: Exit-age distribution curve, E(t), from RTD experiment.

E(t) curve was calculated by the following equation:

$$E(t) = \frac{dF(t)}{dt} \qquad \text{Eqn.4.5}$$

where $F(t)$ is the cumulative distribution function and t is the time of experiment. Running RTD experiment helped to calculate actual HRT and to evaluate the reactor's extent of ideal behavior. Close agreement with ideal behavior indicates that such reactor configuration mimics the CSTR and the results from this reactor could be compared with other related works.

4.2. Air Stripping Experiment

Table 4.2 below displays obtained percentage TC, TN, and chlorophenols removals between t=0 and t=21 hours. Obtained removal percentages are averages of duplicated experiments.

Table 4.2: Air stripping experiment results

Run #	Description	Aeration mode; magnitude	TC % removal (between t=0 and t=21)	TN % removal (between t=0 and t=21)	Chlorophenol % removal (between t=0 and t=21)
1	Without sludge; synthetic wastewater; no chlorophenol	Internal+ external; maximum (both)	43.9	39.9	-
2	With sludge; synthetic wastewater; no chlorophenol	Internal+ external; maximum (both)	85.1	96.2	-

3	Without sludge; synthetic wastewater; 2-CP (14 ppm)	Internal+ external; maximum (both)	39.2	34.2	29.4
4	Without sludge; synthetic wastewater; 2,4,6-TCP (18 ppm)	Internal+ external; maximum (both)	33.6	40.9	10.5
5	Without sludge;synthetic wastewater; no chlorophenol	Internal; maximum	34.9	16.3	-
6	Without sludge; synthetic wastewater; no chlorophenol	Internal; half	28.6	10.4	-

As can be seen from Table 4.1, at the 1st Run, just pure aeration (internal+external) used during the SBR experiments removed about 43.9 % of TC and 39.9 % of TN. At the same time, presence of sludge in addition to both internal and external aeration led to 85.1 % of TC and 96.2 % of TN removals. It can be concluded that during the 1st day of experiment air contributed to almost half of TC and TN removal. However, such trend is not expected to be constant throughout the experiment. Sludge has higher removal rate compared to maximum internal and external aerations. From Figure 4.3, it can be observed that TC removal rate curve with presence of sludge has steeper slope compared to that without sludge.

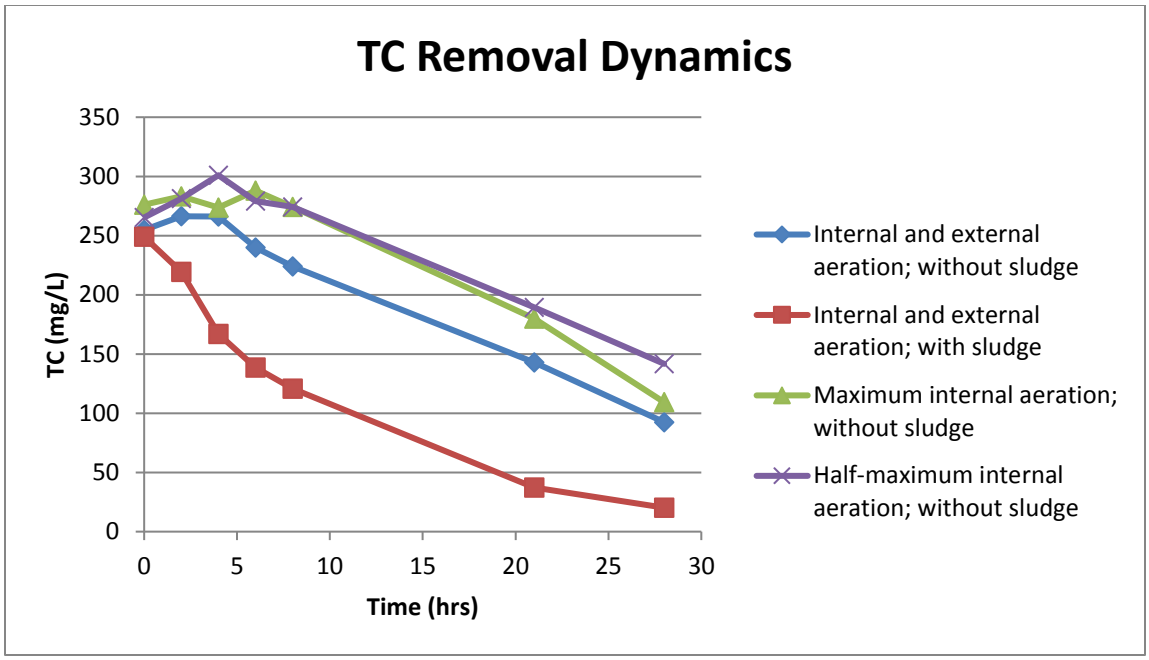

Figure 4.3: TC removal data by aeration and sludge

Moreover, as the experiment proceeds, sludge will keep growing. This will lead to sludge domination in TC removal. The aeration affects the sludge performance most during the first day of experiment. As experiment proceeds, aeration effect on sludge performance will get weaker. It is assumed that the TC removal rate by aeration stays constant throughout the experiment.

Figure 4.3 also presents TC removal rate by full and half internal aerations without sludge. These aeration modes exhibit the smallest TC removal when compared to that with full aeration. Sole use of internal aeration (without external one and sludge; 5th run) at maximum rate (3 L/min) reduced % TC and %TN removals by air from 43.9% and 39.9% (actual SBR experiment) to 34.9 % and to 16.3% respectively. Use of internal aeration (without external one and sludge; 6th

Run) at half-capacity (1.5 L/min) reduced % TC and % TN removals by air from 43.9% and 39.9% (from actual SBR experiment) to 28.6% and to 10.4% respectively. Half-aeration rate (1.5 L/min) without external one was used during CFR experiment. DO at such aeration mode varied between 4.2 - 8.0 mg O_2/L being more than enough for efficient aerobic biodegradation by sludge [*12*]. Consequently, the impact of internal aeration at half-rate on TC removal in CFR was minimal. Half-internal aeration for CFR was enough to keep the sludge suspended.

Aeration also affected 2-CP and 2, 4, 6 – TCP removals. 2-CP (14 ppm inlet concentration) removed by sole aeration (internal+external; 3rd Run) was about 29.1 %. 2,4,6 – TCP removal by the same aeration mode was 7.5 %. 2-CP and 2,4,6 – TCP removal rates by sole aeration after the 1st day are smaller compared to TC and TN removals. As will be observed later on, the 2-CP and 2,4,6 - TCP removal rates will increase as the sludge keeps growing. It is assumed that removal rate of chlorophenols by aeration is constant and the sludge will dominate later on in chlorophenols removal.

The importance of this experiment in a qualitative manner conveys the information that the SBR reactor design has deficiency. Such deficiency could be relieved by use of stirrer and by reducing the air supply rate. Stirrer provides with adequate mixing of sludge and feed solution, thus, eliminating the need in very

high air supply used for sludge suspension in SBR experiments. An efficient approach would be to reduce aeration to the level that gives sufficient margin for successful aerobic bacteria performance and to use stirrer as the main supporting media for sludge during reaction. As a result, use of stirrer and half-aeration (1.5 L/min) is expected to better represent the biodegradation capacity of activated sludge.

4.3. CFR and SBR Experiments

4.3.1 pH and Dissolved Oxygen (DO)

pH and DO results are to be analyzed together. These two parameters are critical for the sludge growth, carbon degradation, and nitrification processes. Figures 4.4 and 4.5 present pH data for CFR E1, E2, E3, and E4. For all 4 experiments pH range was from 6.9 to 8.3. Up to 30 hours in E1 and E2 and up to 20 hours in E3 and E4 decrease of pH takes place for all reactors except E1 and E3 Baseline. Decrease of pH is associated by bacterial activity where organic matter biodegradation and nitrification takes place.

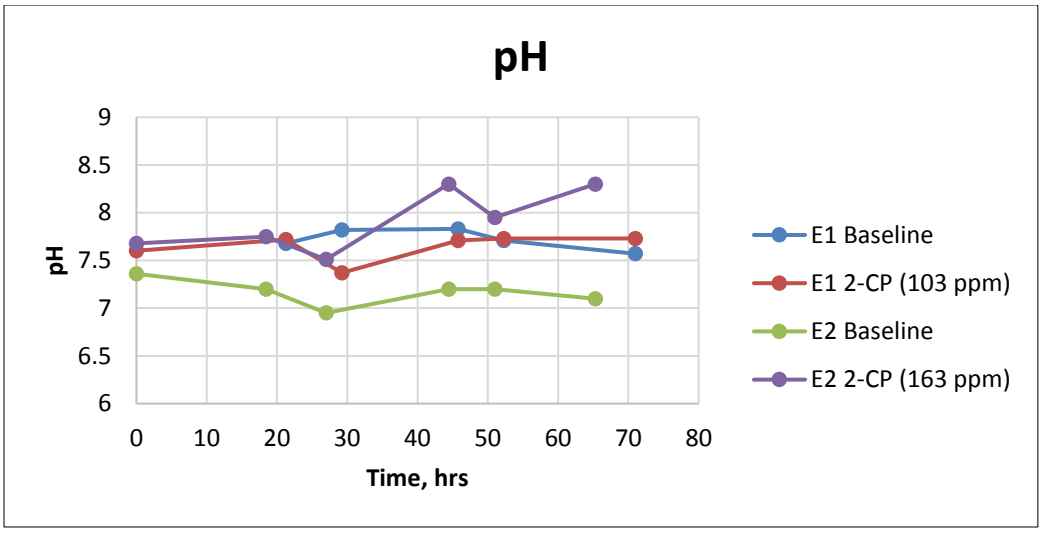

Figure 4.4: pH data for CFR E1 and E2

Former and latter processes produce CO_2 and H^+ ions respectively which lead to solution alkalinity reduction. Following increase of pH in these reactors could be caused by volume rise within them. Volume rise by basic feed solution leads to rise in pH. Such ongoing volume change could mask the biodegradation processes. For CFR, pH is within normal range for of

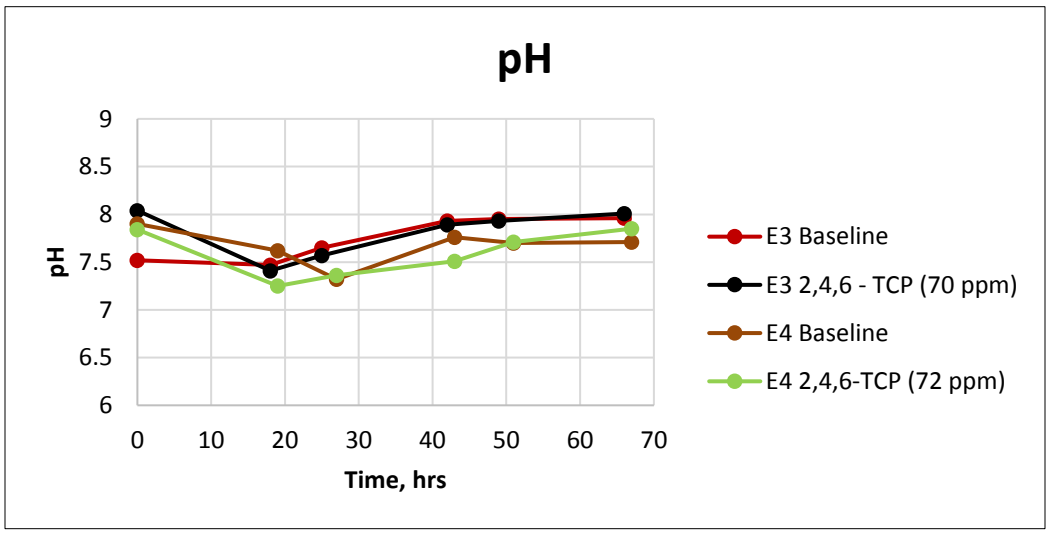

Figure 4.5: pH data for CFR E3 and E4

Figures 4.6, 4.7, 4.8, and 4.9 present SBR pH data for E1, E2, E3, and E4. pH for all these experiments ranged between 6.5 and 8.6. The cyclical behavior of pH could be observed. At the beginning of REACT stage pH is low and high at the end of REACT stage.

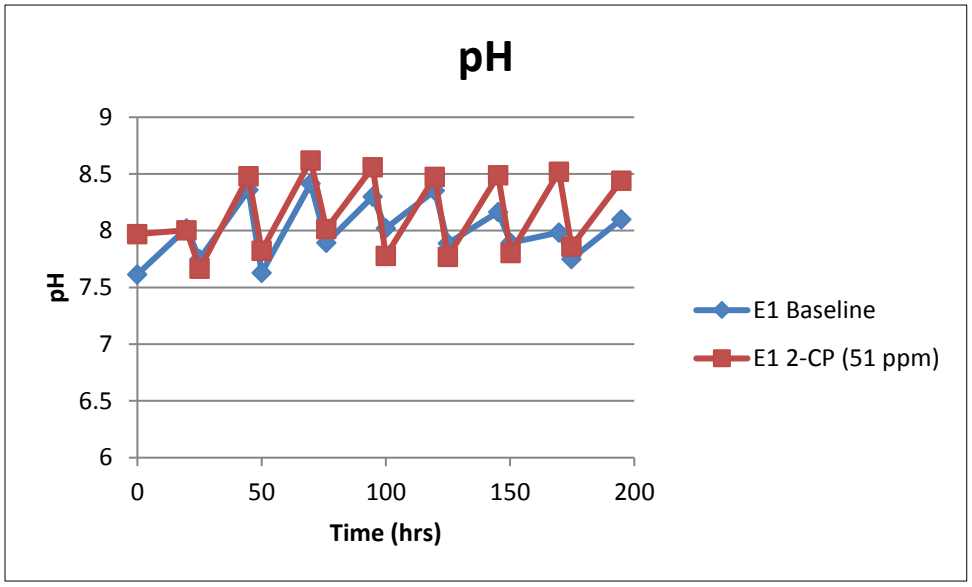

Figure 4.6: pH data for SBR E1

Low pH is due to biodegradation and nitrification activities. As was mentioned above, biodegradation results in the release of CO_2 which is acidic according to reaction R-1 on page 16. Also, during ammonium oxidation in nitrification process, hydrogen ions are produced according to the reaction R-2 on page 16, thus, reducing the reactor solution alkalinity. Increase of pH at the end of REACT stage is associated with decrease in bacterial activity. Additional evidence of bacterial activity cessation is also shown by the increase of DO at the end of

REACT stage. Oxygen is consumed by bacteria to facilitate metabolic processes such as biodegradation and nitrification. Rise of pH can also be due to intermediates produced during the biodegradation of organic matter.

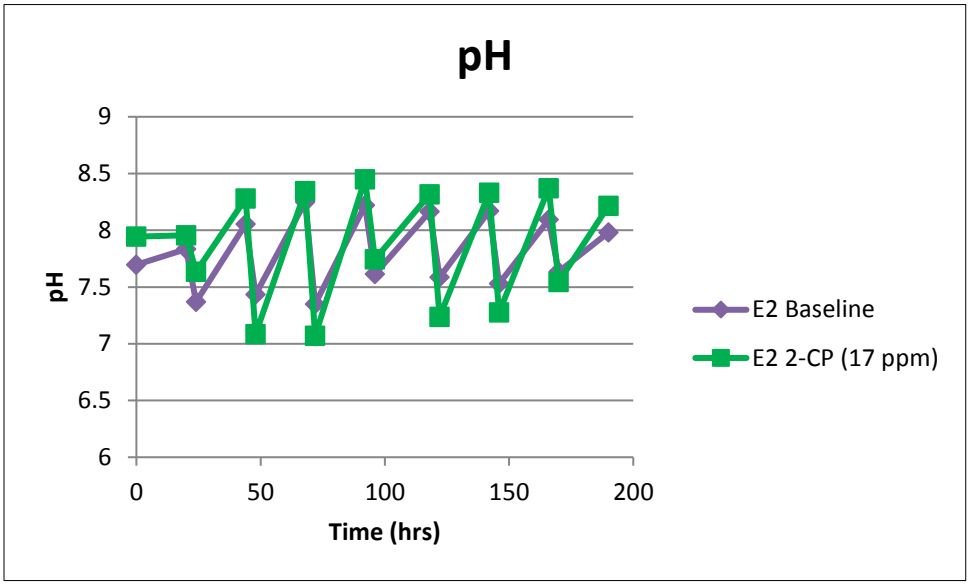

Figure 4.7: pH data for SBR E2

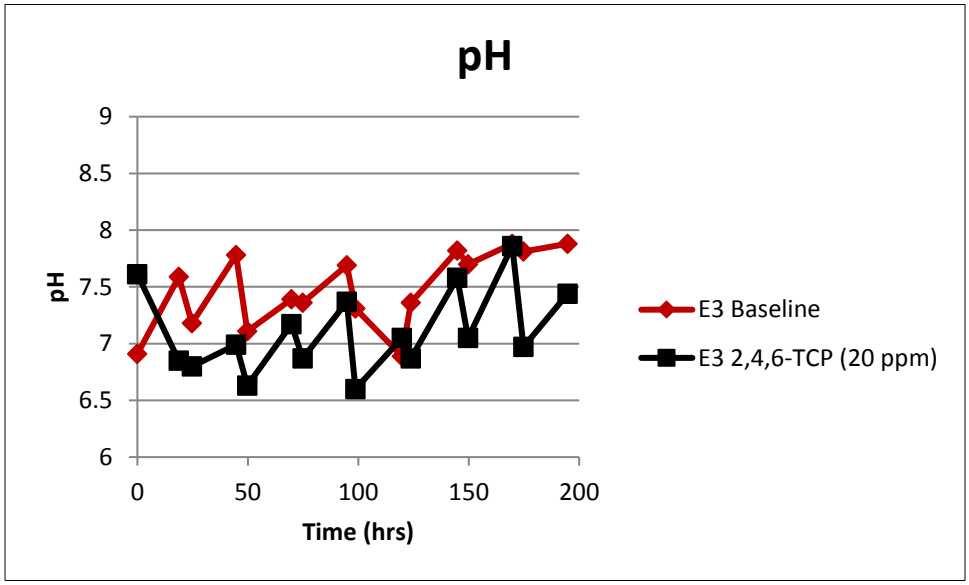

Figure 4.8: pH data for SBR E3

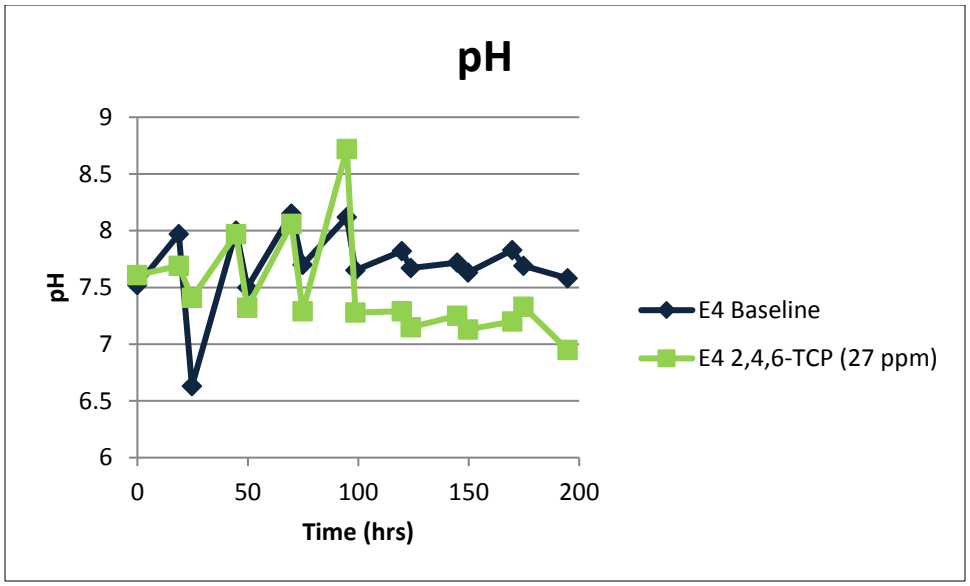

Figure 4.9: pH data for SBR E4

The optimum pH for bacteria activity is between 6.5 and 8.5 [*10*], [11]. For nitrification, pH values between 7.5 and 8.5 are reported to be most suitable. Nitrification seriously inhibited at pH below 6 [*16*], [*10*]. pH data for all CFR and SBR experiments are sufficiently high being above 6.5. That means that observed pH for all experiments was within optimum operation range for bacteria and did not negatively affect their performance.

DO for all CFR experiments stayed between 3 and 8 as shown in Figures 4.10 and 4.11.

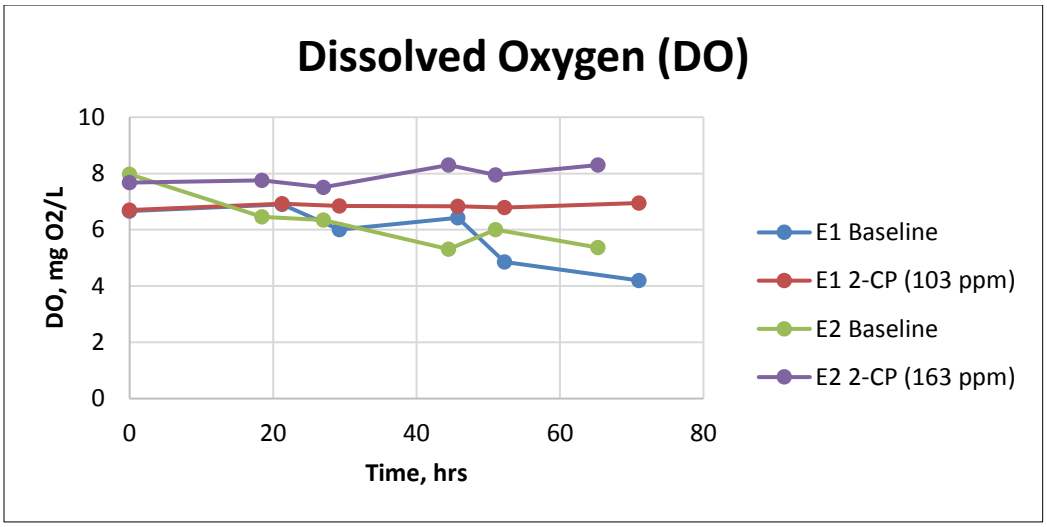

Figure 4.10: DO data for E1 and E2

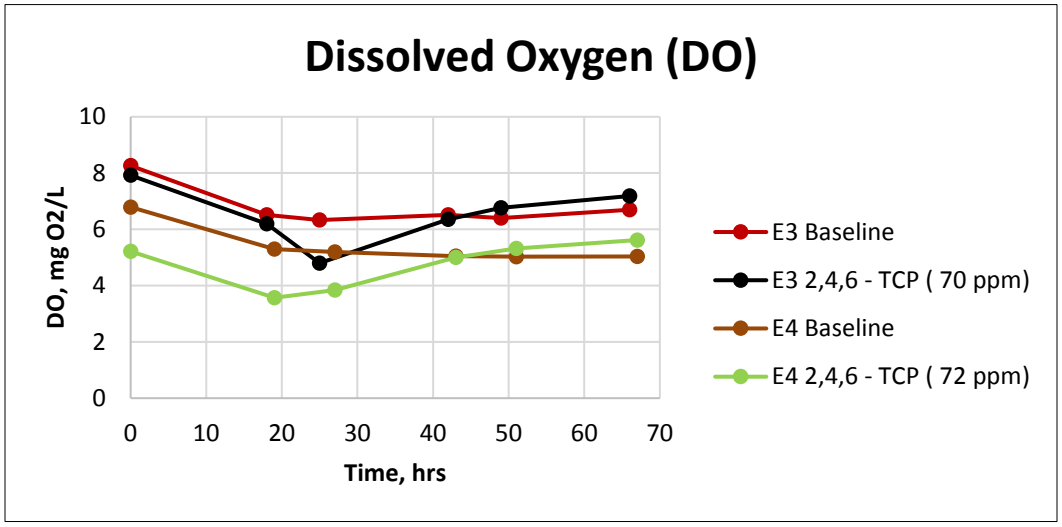

Figure 4.11: DO data for E3 and E4

For SBR, DO has cyclical behavior as pH. DO is low at the beginning of and high at the end of REACT stage. Figures 4.12, 4.13, 4.14, and 4.15 present DO data for SBR E1, E2, E3, E4. DO in E1,E2, E3, and E4 ranged between 0.2 and 7.5. DO at the beginning of REACT stage is low because sludge starts immediately biodegrading and nitrifying feed solution. By the end of REACT

stage, DO increases signaling the decrease in microbial activity. In Figure 4.14, DO reaches almost zero level at the beginning of several REACT stages. At this condition, denitrification takes place. Optimum DO for normal microbial activity is between 0.5 and 2.0 mg/L [*11*]. Thus, DO range in all CFR and SBR experiment provided bacteria with abundant air source. Established DO conditions in SBR and CFR experiments favored the optimum activated sludge activity.

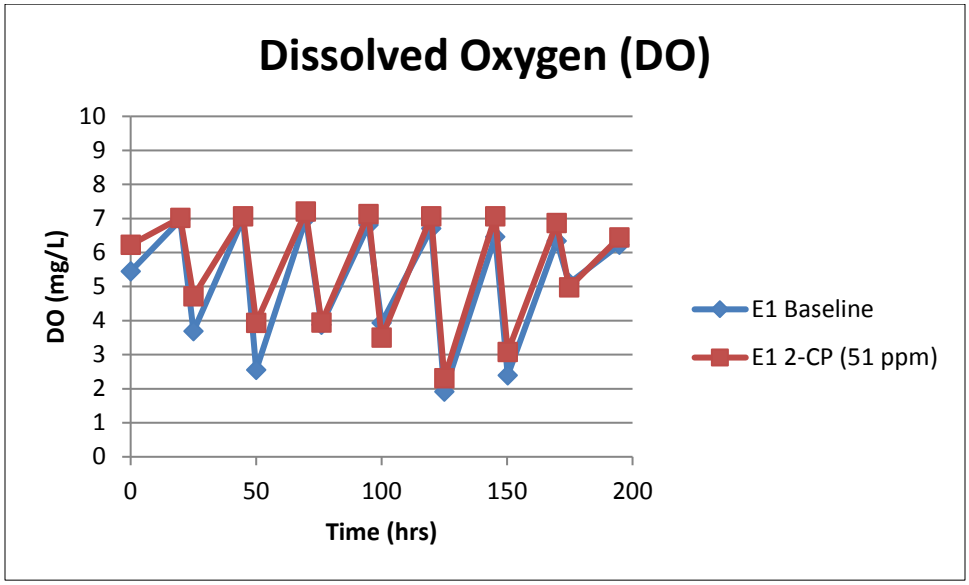

Figure 4.12: DO data for SBR E1

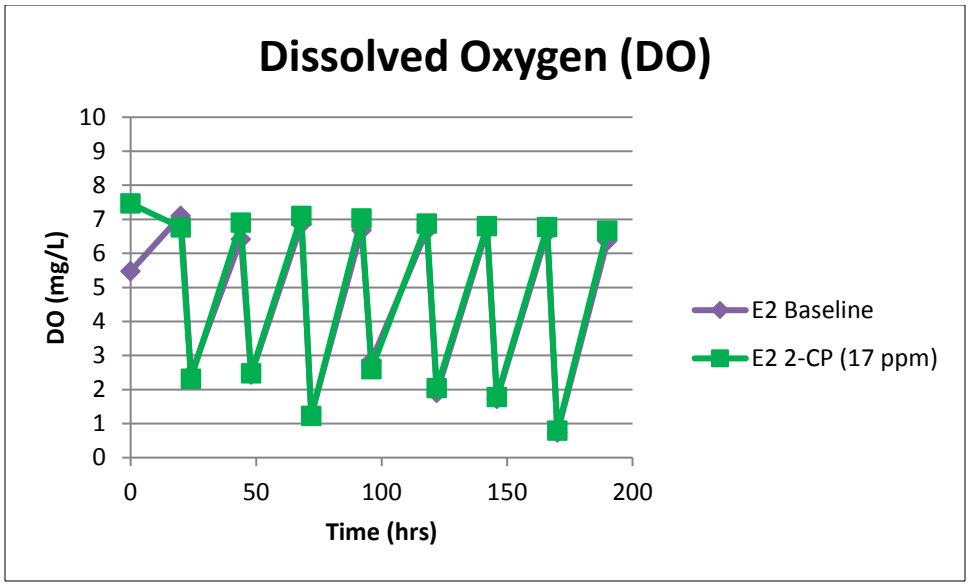

Figure 4.13: DO data for SBR E2

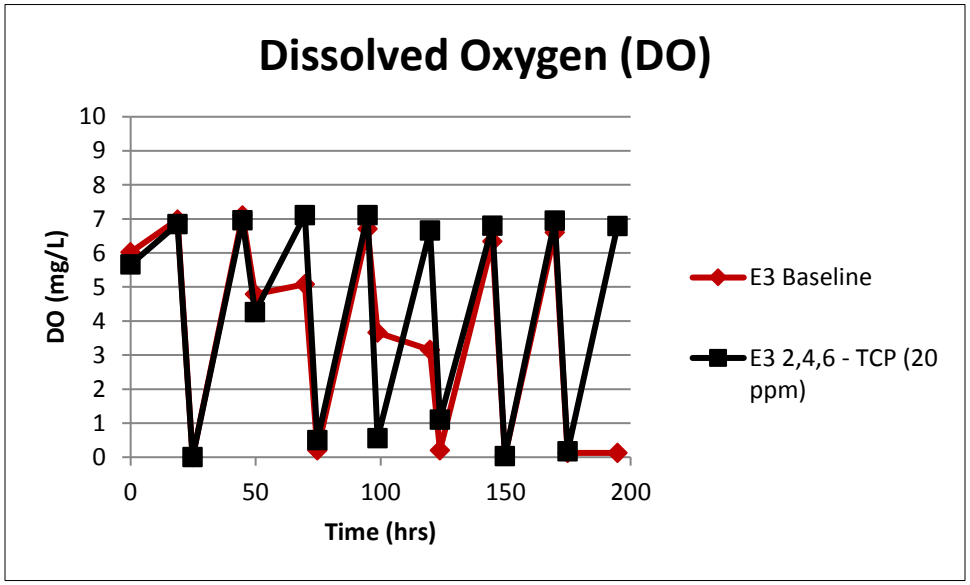

Figure 4.14: DO data for SBR E3

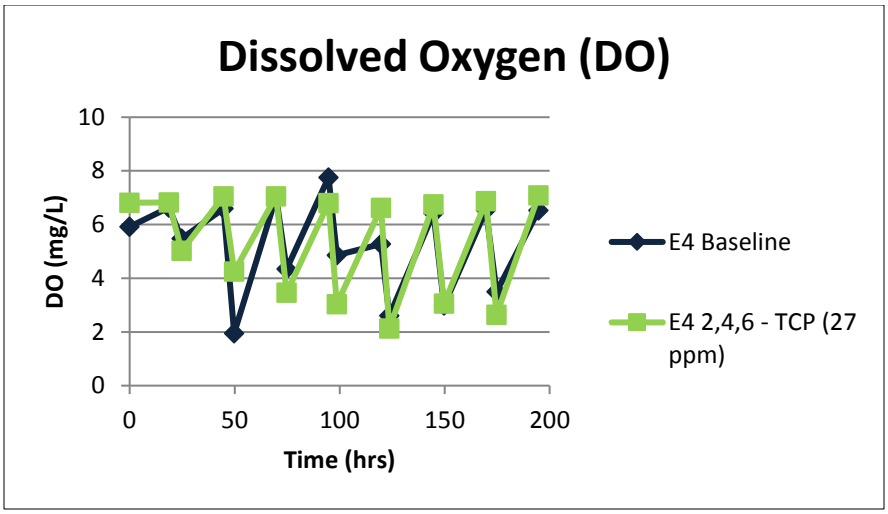

Figure 4.15: DO data for SBR E4

4.3.2. Total Suspended Solids (TSS)

The inhibiting impact of 2-CP and 2,4,6 – CP on sludge performance could be evaluated through the bacteria growth or TSS. If inhibition is permanent, TSS will have decreasing trend. Otherwise, inspite of possible initial decrease, TSS can recover and keep increasing.

For CFR, TSS data are shown in Figures 4.16 and 4.17 for E1, E2, E3, and E4.

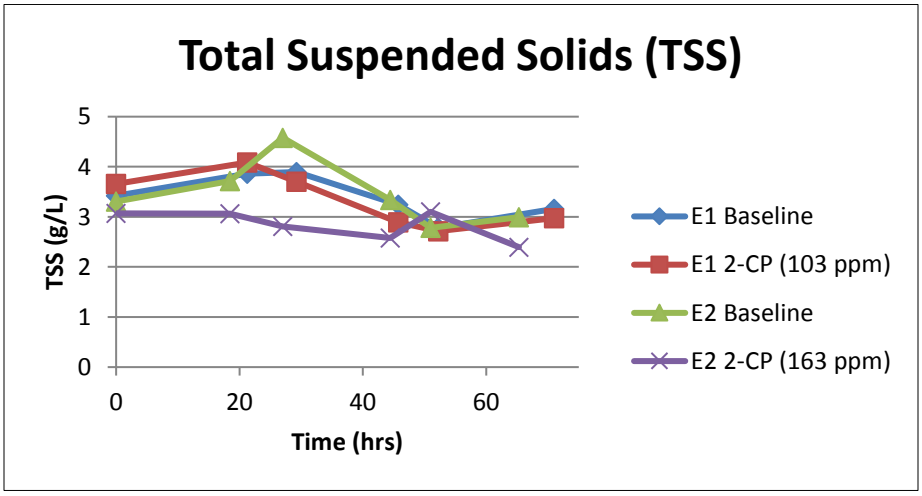

Figure 4.16: TSS data for CFR E1 and E2.

In Figures 4.16 and 4.17, it could be observed that in E1, E2, E3, E4 TSS decreases steadily throughout the experiment, although with some fluctuations. It means that 2-CP concentrations from 103 – 163 ppm are toxic to sludge. 2,4,6 – TCP concentration of 71-72 ppm is also toxic. However, based on relative decrease of TSS, the bacteria growth inhibition is not severe.

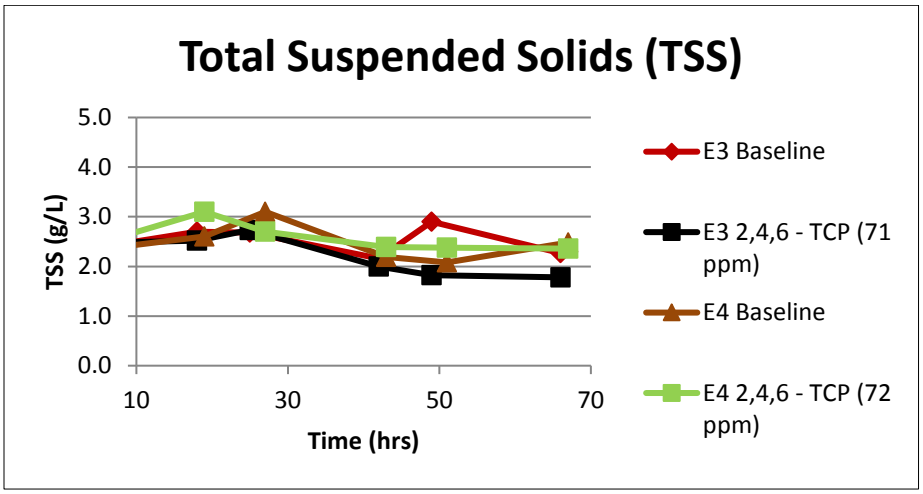

Figure 4.17: TSS data for CFR E3 and E4.

In contrary to CFR, TSS in SBR have increasing trend inspite of some fluctuations. Fluctuations are due to improper mixing at the zone of TSS sampling. Figures 4.18 and 4.19 present TSS data for SBR E1, E2, E3, and E4. Both Baseline and Inhibitor reactors TSS, in spite of some fluctuation at the beginning, steadily increase throughout the experiment. Some difference in TSS level could be observed between E1 Baseline and Inhibitor reactors. Inhibitor TSS is higher than that of Baseline. Such behavior is counter-intuitive as Inhibitor reactor contains 2-chlorophenol which is capable of halting bacterial growth compared to Baseline reactor.

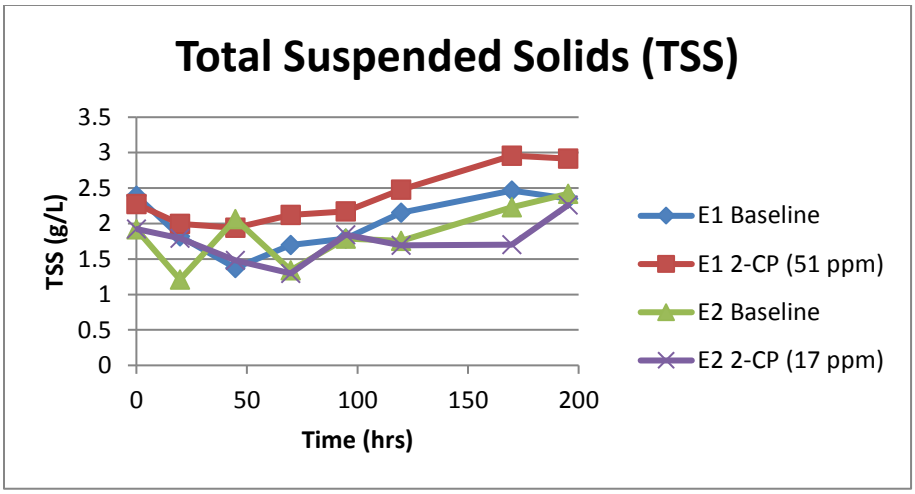

Figure 4.18: TSS data for SBR E1 and E2.

Moreover, Baseline reactors feed contains more growth-promoting easily degradable co-substrates such as glucose and sodium acetate compared to Inhibitor reactor feed. Inhibitor reactor feed contains only glucose as easily biodegradable co-substrate. Thus, such discrepancy could be attributed to non-ideal mixing during TSS sampling.

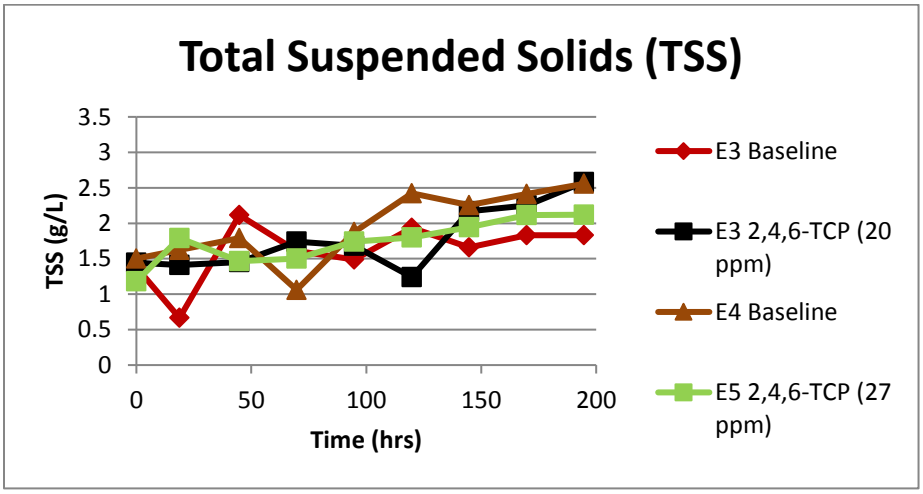

Figure 4.19: TSS data for SBR E3 and E4.

On contrary, no significant difference is seen between E2 Baseline and Inhibitor TSS data despite some fluctuations. At 17 ppm then there is no sludge activity inhibition. 2,4,6 – Trichlorphenol concentrations of 20 and 26 ppm did not also inhibit TSS growth as shown in Figure 4.19. Baseline and Inhibitor reactors TSS in E3 and E4 keep growing at the same rate without any significant differences. Minor level fluctuations could be accounted for non-ideal mixing during sampling. Lower 2-CP concentrations of 51 and 17 ppm in SBR did not cause TSS inhibition compared higher 2-CP concentrations in CFR. The same applies to 2,4,6 – TCP concentrations of 20 and 27 ppm which are being lower than those in CFR did not also cause TSS growth inhibition in SBR.

4.3.3. Effluent ammonium / Nitrate production

Nitrification process, which starts from ammonium oxidation, is highly susceptible to the effects of chlorophenols. Analysis of effluent ammonium data in CFR and SBR will allow evaluating the inhibitive impact of chlorophenols on nitrifying bacteria such as *Nitrosomonas* which is responsible for ammonium oxidation. Analysis of nitrates production data will allow perceiving the inhibition impact on nitrite oxidizing bacteria such as *Nitrobacter*. Figures 4.20 and 4.21 present effluent ammonium data for CFR experiments. For CFR experiments E1, E2, E3, and E4, clear data segregation between Baseline and Inhibitor reactors could be observed.

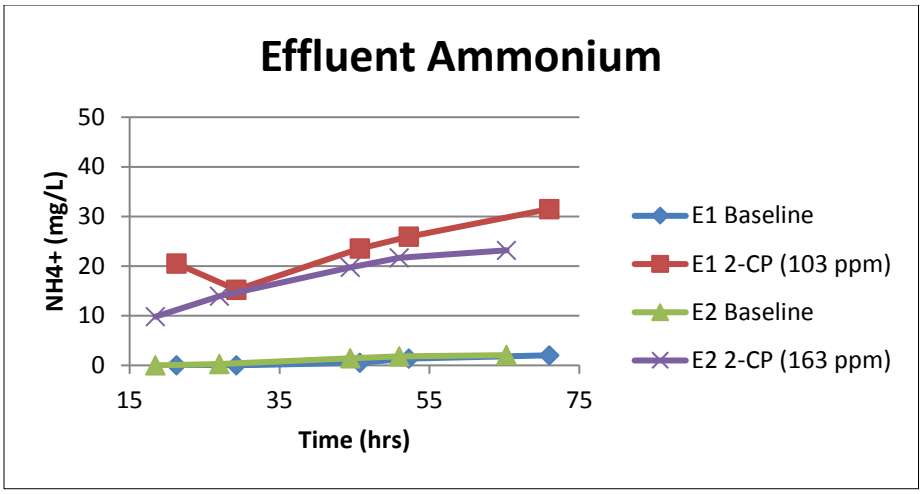

Figure 4.20: Effluent ammonium data for CFR E1 and E2.

Baseline reactors removed almost all ammonium with residual ammonium in effluent being typically below 5 mg/L. In contrast to this, effluent ammonium concentration increases throughout the reaction for all Inhibitor reactors.

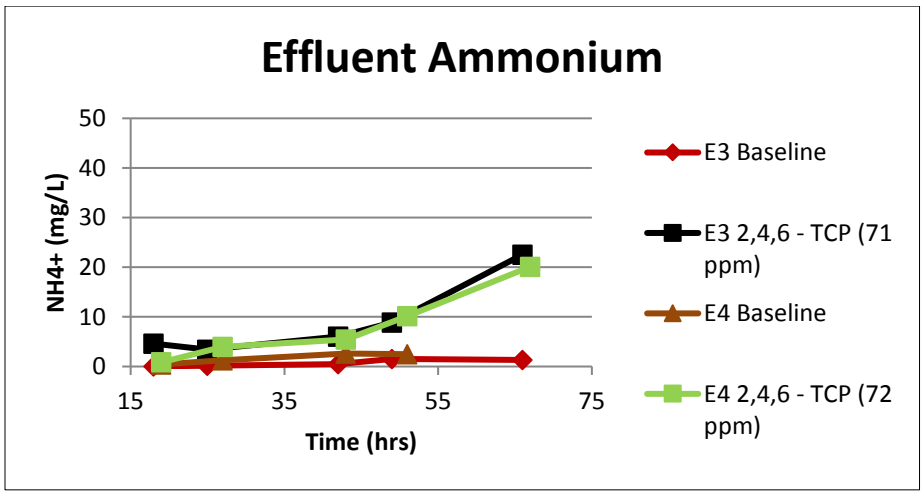

Figure 4.21: Effluent ammonium data for CFR E3 and E4.

2-chlorophenol concentrations between 103 and 163 ppm and 2,4,6 – trichlorophenol concentrations at about 70 ppm inhibit the activity of *Nitrosomonas* which is responsible for ammonium oxidation. As the experiment

proceeds, more *Nitrosomonas* bacteria are inhibited. The inhibiting effect of these two types of chlorophenols could also be observed during the nitrates production.

Figure 4.22 and 4.23 presents nitrates production data for CFR E1, E2, E3, and E4. In the same manner, nitrates production decline in E1 and E2 Inhibitor reactors indicate the inhibition the activity of *Nitrobacter*. As the experiment proceeds, the nitrates production decline is progressive for E1 and E2 Inhibitor reactors as shown in Figure 4.22.

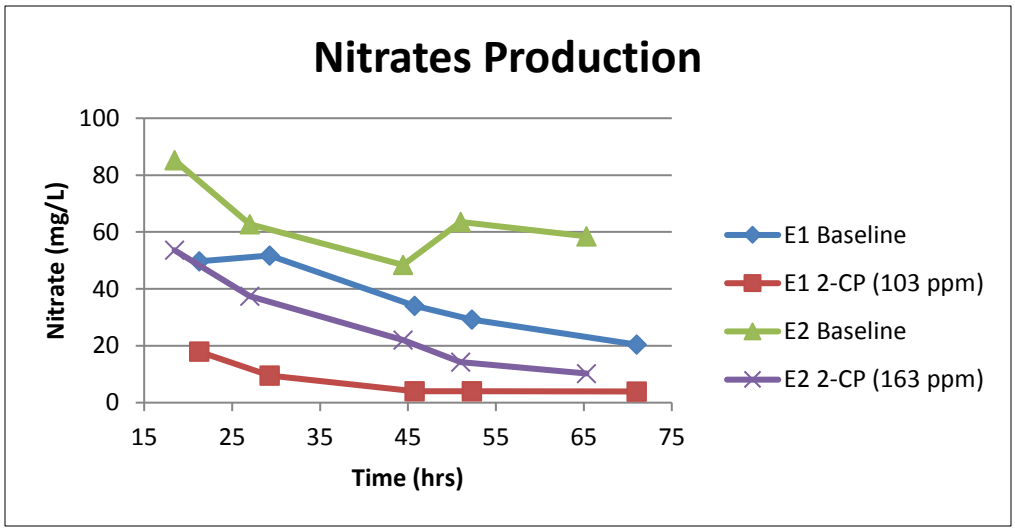

Figure 4.22: Nitrates production data for CFR E1 and E2.

For E3 and E4 Inhibitor reactors, nitrates production rate are at the lower level compared to E2 Inhibitor reactor. Also, progressive nitrification inhibition under 2,4,6 – TCP 71 and 72 ppm concentrations is observable in E3 and E4 Inhibitor reactors as seen in Figure 4.23. Higher initial nitrates at about t=20 could be the residual amount present in the sludge when it was taken from wastewater

treatment plant. Remaining from previous batch nitrates can also contribute to the results of the next data point.

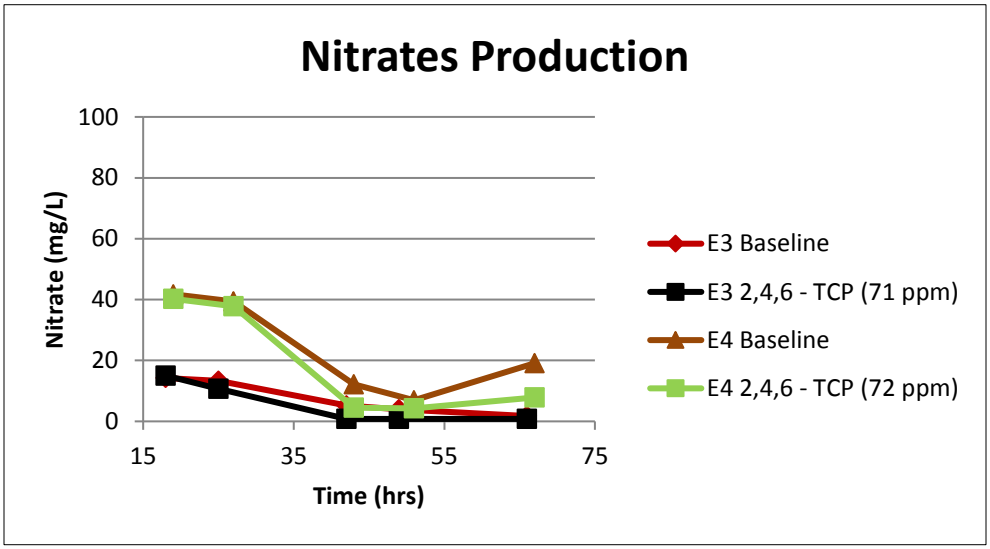

Figure 4.23: Nitrates production data for CFR E3 and E4.

Nitrate production data has some unexpected results for baseline reactors in E1,E2, and E3. As in Inhibitor reactors, nitrates production has declining trend in all Baseline reactors as shown in Figures 4.22 and 4.23. Nitrates reduction could be due to denitrification process where nitrates at anoxic condition are converted to nitrogen gas. The sampling point is located at the bottom of reactor where oxygen concentration is very low creating anoxic conditions. Thus, such reactor configuration could create a zone for denitrification process. Analyzing nitrates production data, it could be observed that *Nitrobacter* bacteria are highly sensitive to chlorophenols. 2-CP concentrations from 103 ppm to 163 ppm and 2,4,6 – TCP concentrations at about 70 ppm are thus inhibitive for nitrification processes.

The same effluent ammonia trend can be observed in SBR Inhibitor E1 and E2 as shown in Figure 4.24. Similar to CFR, ammonium concentration at the exit of reactor increases steadily signaling about progressive inhibition of ammonium oxidizing bacteria. Thus, 2-CP concentrations between 17 ppm and 51 ppm are also inhibitive to ammonium oxidation process.

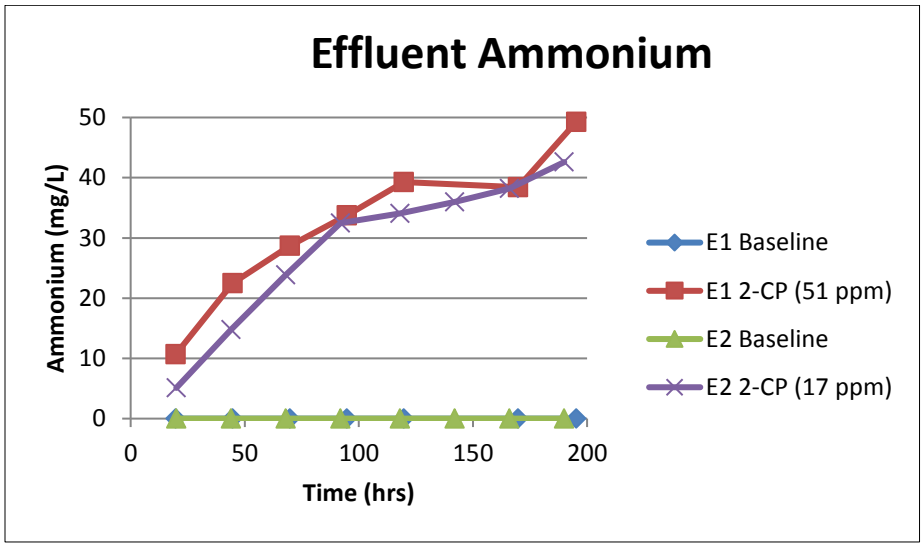

Figure 4.24: Effluent ammonium data for SBR E1 and E2.

Completely unexpected results are observed at E3 and E4 Inhibitor reactors as in Figure 4.25. Inhibitor reactors demonstrate the same behavior as with Baseline ones yielding almost zero ammonium in reactor outlet. Thus, 2,4,6 – TCP concentrations between 20 and 27 ppm are not inhibitive to ammonium oxidizing bacteria. Compared to CFR Baseline reactors, those from SBR Baseline show complete ammonium removal.

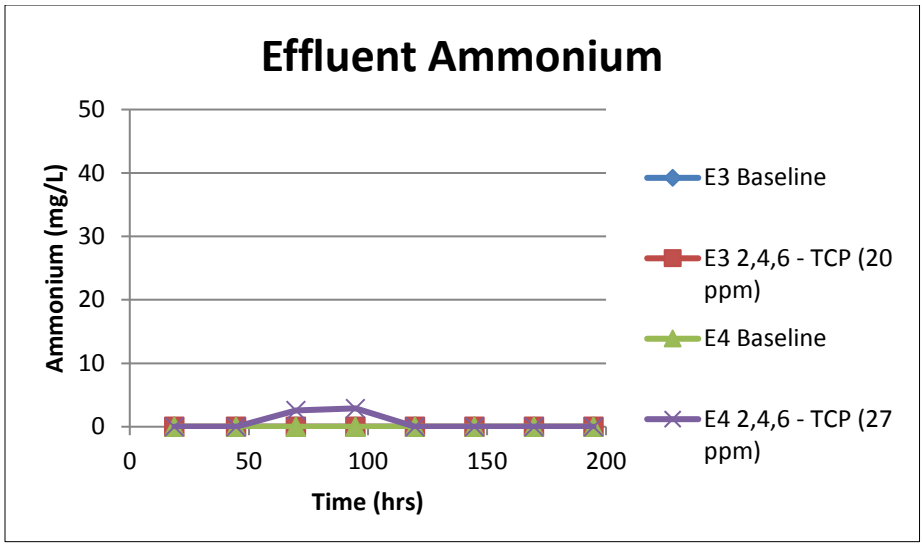

Figure 4.25: Effluent ammonium data for SBR E3 and E4.

Nitrate production data in SBR E1 and E2 Inhibitor Reactor are at almost zero level as shown by Figure 4.26. Nitrates production is severely inhibited at 2-CP concentrations of 17 and 51 ppm. Nitrates producing bacteria such as *Nitrobacter* is, thus, highly vulnerable to 2-CP. Baseline reactors demonstrate increasing nitrates production compared to CFR Baseline reactors.

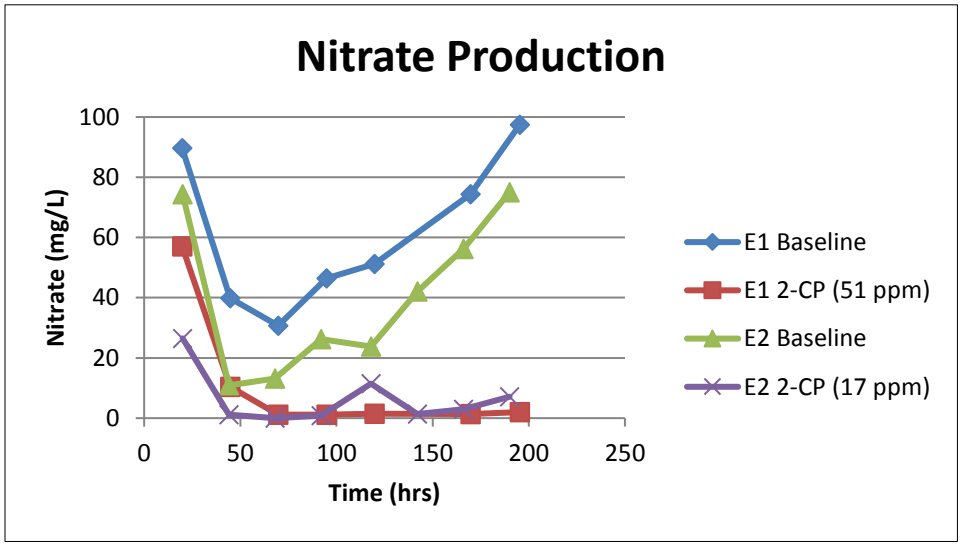

Figure 4.26: Nitrate production data for SBR E1 and E2.

It indicates the absence of anoxic conditions leading to denitrification. High initial nitrates at t=20 could be residual amount present in sludge when taken from wastewater treatment plant. Also, remaining from previous batch nitrates contribute to the results in the next batch.

As with the case of effluent ammonium production in SBR, nitrates production data graph in Figure 4.27 show non-inhibiting nature of 2,4,6 – TCP on nitrate producing bacteria.

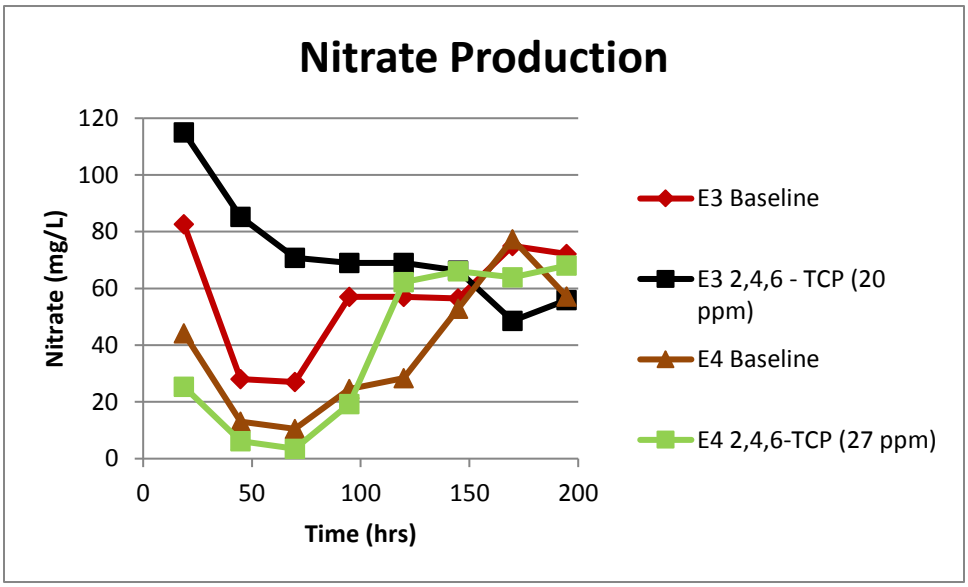

Figure 4.27: Nitrate production data for SBR E1 and E3.

Compared to E1 and E2 Inhibitor reactors, nitrate productions in E3 and E4 Inhibitor reactors involving 2,4,6 - TCP are not inhibited. Initially, nitrate production rate in these reactors is decreased. This could be due to acclimation of nitrifying bacteria to 2,4,6 - TCP or their temporal inhibition. As experiment continues then nitrates production increases. By the end of experiment, nitrate

production in E3 and E4 Baseline and Inhibitor reactors stabilize. This might not be due to inhibition as in E3 and E4 Baseline reactors the same nitrate production level is observed at the end of experiments. Additional indication of absence of nitrate production inhibition in E4 Inhibitor reactor is the similar nitrate production trend observed in E3 and E4 Baseline and in E4 Inhibitor reactors. Nitrate production in E3 Inhibitor reactor is stable throughout the experiment indicating also the absence of inhibition of nitrate producing bacteria. Initial high nitrates level in E1, E2, E3, and E4 reactors could be due to presence of residual nitrate in sludge when taken from local municipal wastewater treatment plant.

4.3.4. TC and TN Removal per TSS/ TC and TN Absolute Removal

Analysis of TC and TN removal per TSS allows evaluation of sludge activities in TC and TN removal which can also be linked to bacteria activity inhibition. TC and TN absolute removals help to assess the overall performance of Baseline and Inhibitor reactors not taking into account bacteria population.

TC and TN removals were calculated per the following equations:

$$\%\, TC\ removal = \frac{(TC_{inlet} - TC_{outlet})}{TC_{inlet}} \times 100 \qquad (4.6)$$

$$\%\, TN\ removal = \frac{(TN_{inlet} - TN_{outlet})}{TN_{inlet}} \times 100 \qquad (4.7)$$

Figure 4.28 presents TC removal per TSS for E1 and E2 reactors in CFR. No significant differences except for E2 Inhibitor reactor could be observed. The TC removal trend goes up till 50 hours indicating higher bacterial activity.

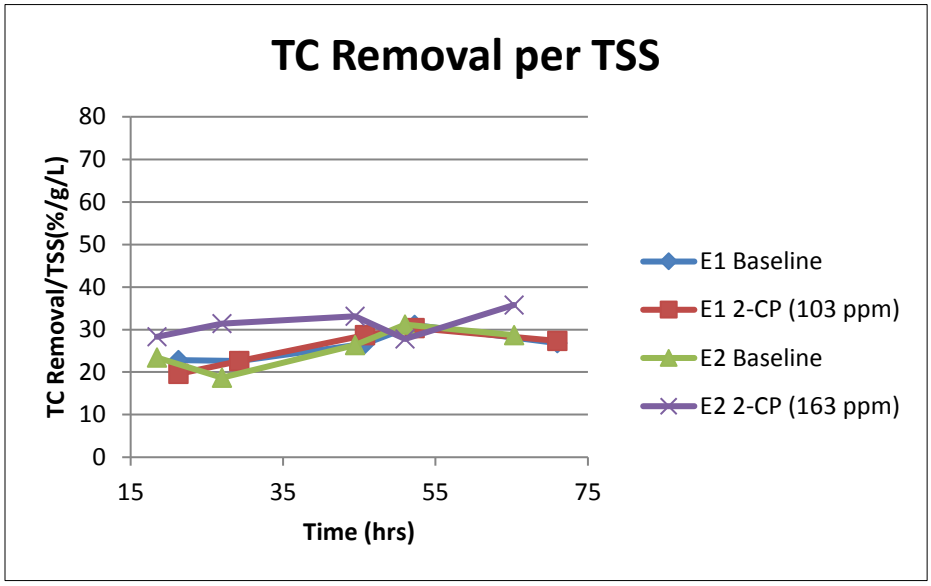

Figure 4.28: TC Removal per TSS data for CFR E1 and E2.

After 50 hours decrease in removal takes place indicating also decrease in bacterial activity. TC removal per TSS in Baseline E1 and E2 as well as in Inhibitor E1 reactors fluctuate between 20 and 30 % /g/L. Fluctuations observed do not indicate significant decrease in bacteria activity. Bacteria activity in E2 Inhibitor reactor also does not show much fluctuation TC Removal per TSS being around 29 – 36 %/g/L. Figure 4.29 shows overall removal trend for TC in CFR E1 and E2. The TC removal rate is high staying above 80 % for all E1 and E2 reactors. The lowest TC removal in E2 Inhibitor among all E1 and E2 reactors indicate decrease of bacteria activity. This can be due to sludge inhibition by 2-CP of 103 ppm.

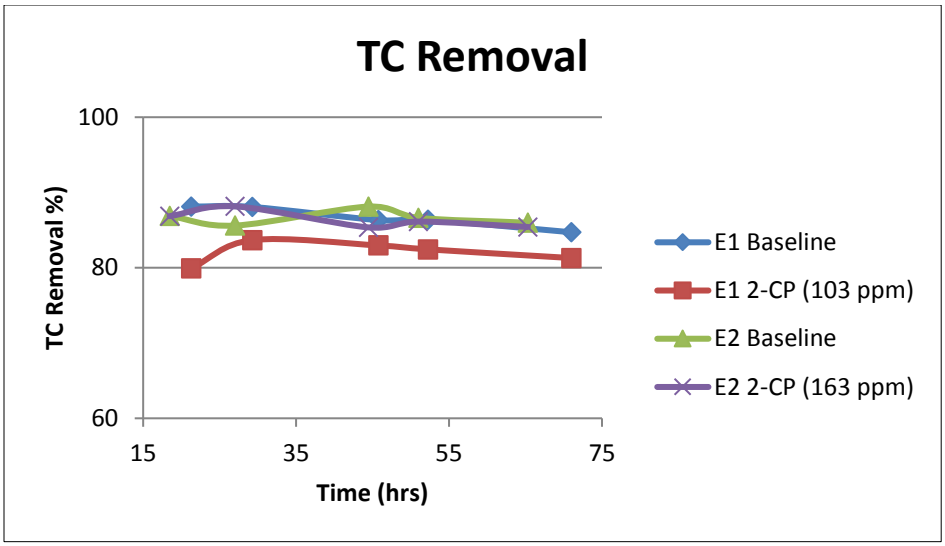

Figure 4.29: TC Removal data for CFR E1 and E2.

In CFR E3 and E4 reactors, increasing TC removal per TSS could be observed in Figure 4.30.

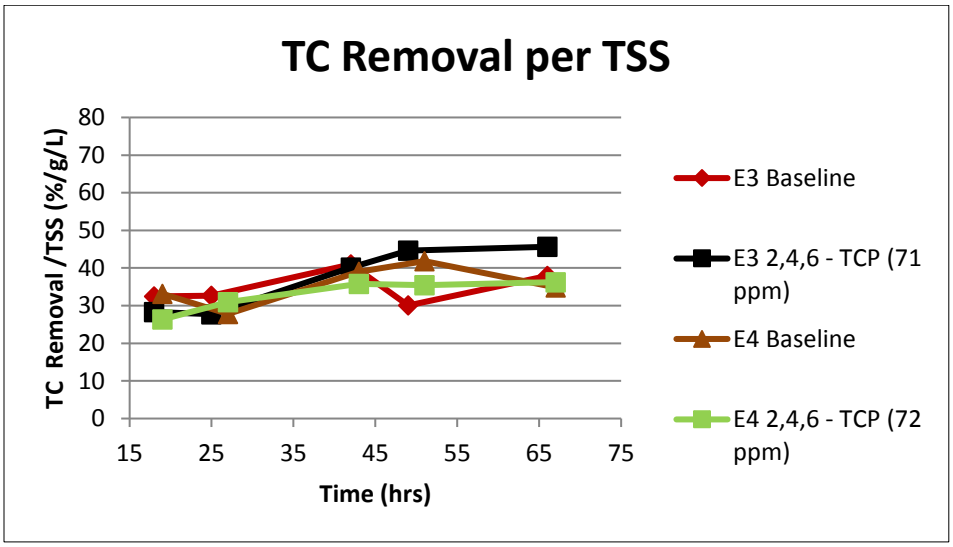

Figure 4.30: TC Removal per TSS data for CFR E3 and E4.

For E3 and E4 experiments, TC overall removal is also high staying above 80% except for E4 Inhibitor reactor as shown in Figure 4.31. Sludge activity inhibition takes place at 2,4,6- TCP concentration of 70 ppm.

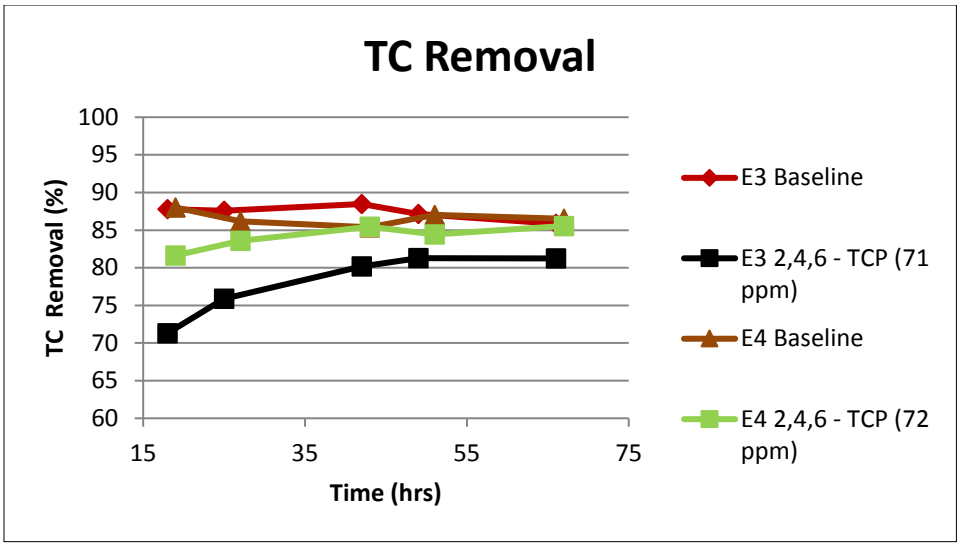

Figure 4.31: TC Removal data for CFR E3 and E4.

In SBR, E1 and E2 show decreasing TC removal rate per TSS as shown in Figure 4.32. Removal rate of TC is lower than rate of TSS increase. Such trend could indicate that bacterial activity in degrading carbon reduced or not all bacteria are taking part in carbon degradation. In E1 and E2 Inhibitor reactors, decrease in bacterial activity could be due to toxic 2-chlorophenol.

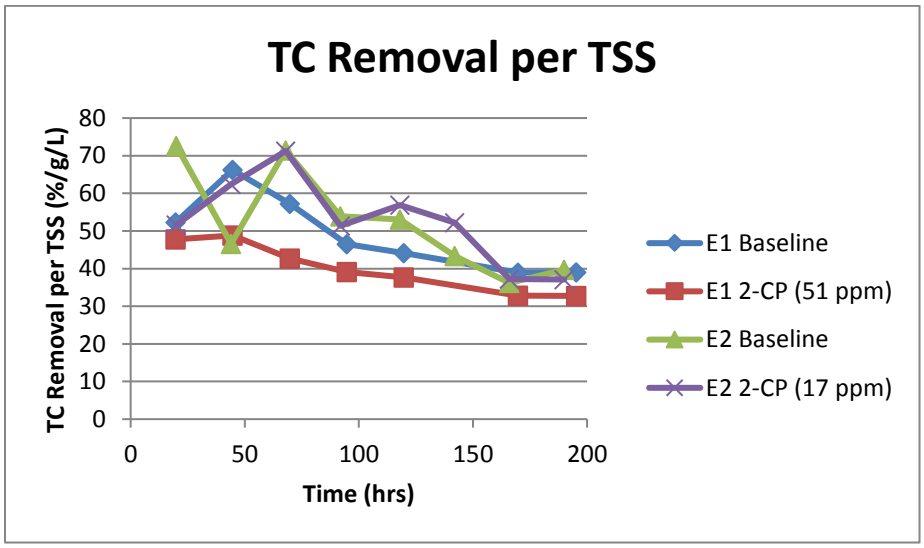

Figure 4.32: TC Removal per TSS data for SBR E1 and E2.

However, initial and final TC removal per TSS values for E1 and E2 Inhibitor reactors have small difference indicating the toxic effect of 2-CP was not significant.

Overall, sludge in E1 and E2 Baseline and Inhibitor reactors demonstrated high TC removal above 90% with some fluctuations as shown in Figure 4.33.

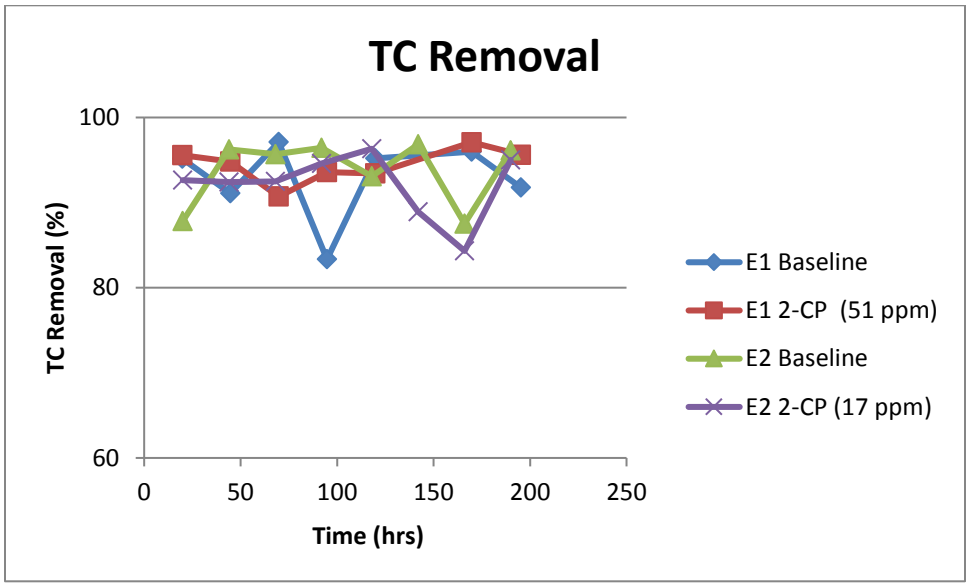

Figure 4.33: TC Removal data for SBR E1 and E2.

TC removal per TSS in SBR E3 and E4 reactors has also decreasing trend as in E1 and E2 reactors as shown by Figure 4.34 except for E4 Inhibitor data. Inhibitor E4 data show relatively stable trend. It could indicate that the presence of 2,4,6 -TCP is not inhibitive to bacteria and more of them are involved in TC removal. E3 and E4 Baseline reactors show decreasing trend. It can indicate that not all of microorganisms are active in biodegrading organic carbon.

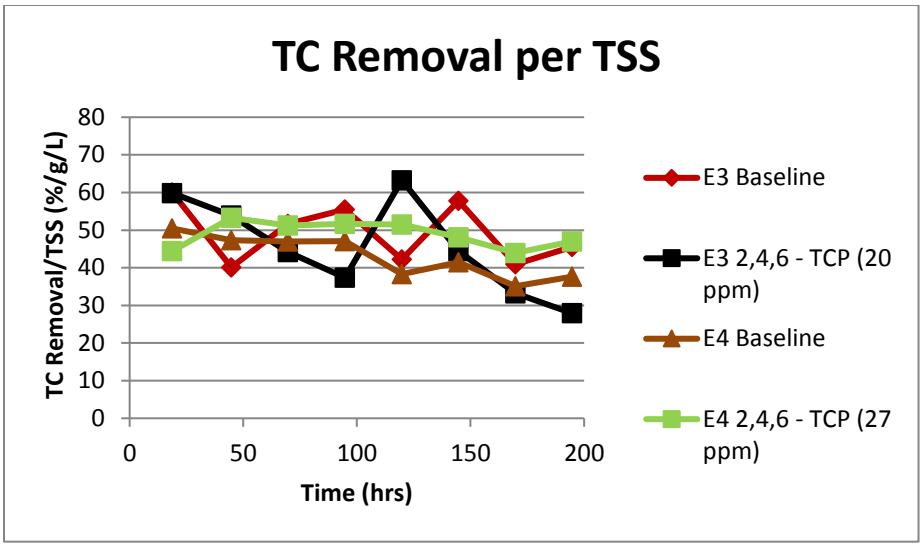

Figure 4.34: TC Removal per TSS data for SBR E3 and E4

E3 and E4 Baseline reactors show high TC removal above 80% inspite of fluctuations as shown on Figure 4.35. E3 and E4 Inhibitor reactors start showing lower TC removal being above 70%. However, as the experiment proceeds, E4 Inhibitor reactor performance increases reaching 100% at the end. Data for E3 Inhibitor reactor is unstable.

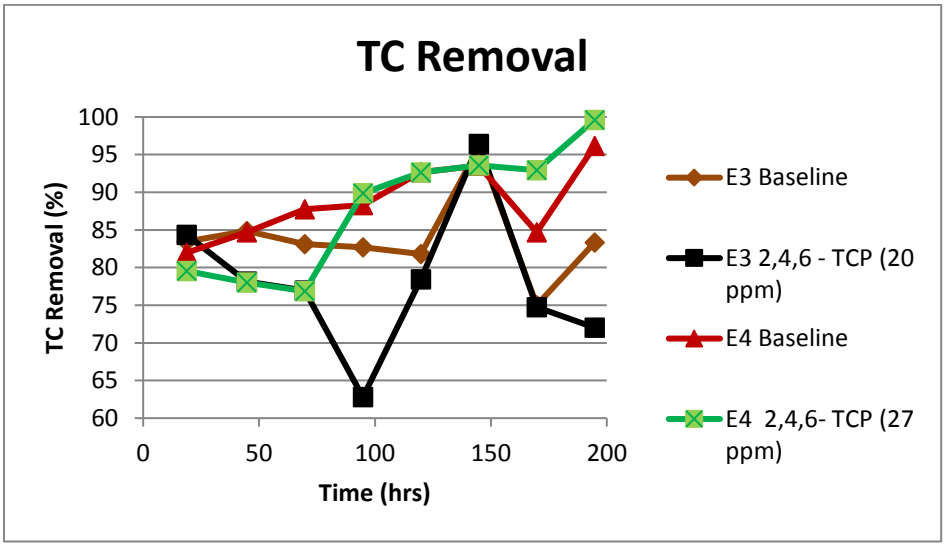

Figure 4.35: TC Removal data for SBR E3 and E4

Taking into account significant fluctuations, it can be assumed that TC removal for this reactor is between 70% and 90%.

As for TN removal per TSS in CFR, the same trend as in TC case could be observed in Figure 4.36 at all E1 and E2 reactors. The bacterial activity increases till 60 hours then decreases. For Inhibitor E1 and E2 after 60 hours, the effect of inhibition could take place.

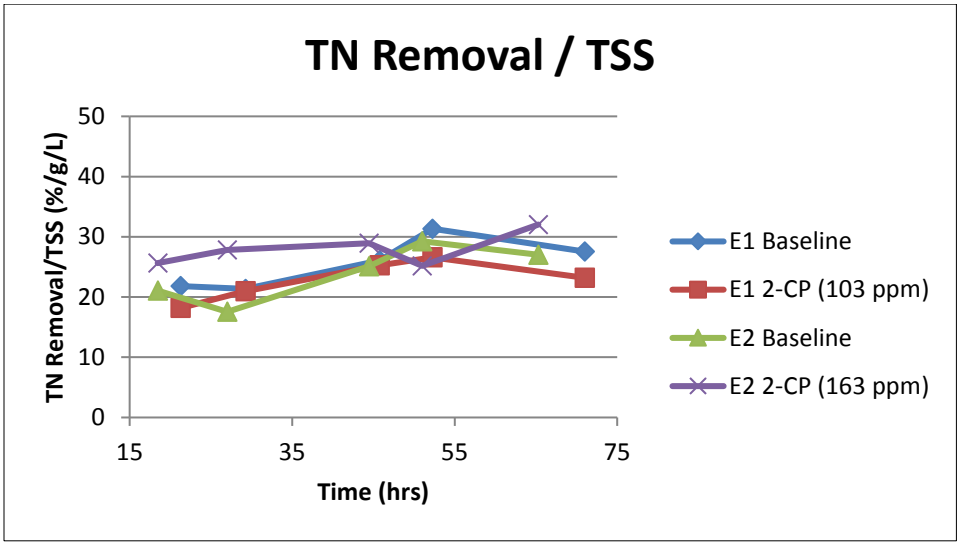

Figure 4.36: TN Removal per TSS data for CFR E1 and E2

The absolute removal of TN in CFR E1 and E2 reactor is high for Baseline reactors being above 80% as shown in Figure 4.37. For E1 and E2 Inhibitor reactors, similar decreasing trend can be observed. The removal of TN progressively decreases being lower being below 80% and 70% for E1 and E2 Inhibitor reactors. Such results imply the inhibition of nitrifying bacteria as was observed in higher ammonium effluent and lower nitrates production.

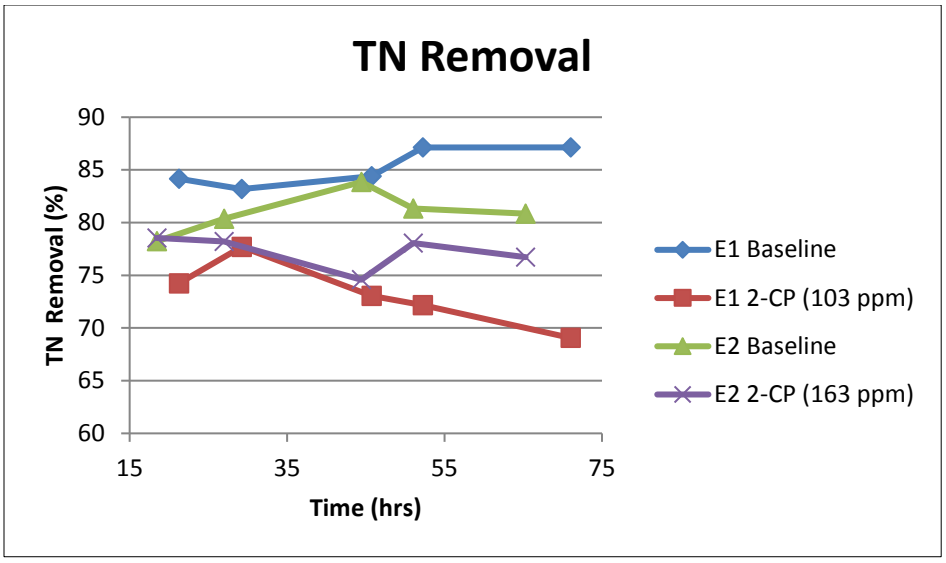

Figure 4.37: TN Removal data for CFR E1 and E2

TN removal per TSS trend in CFR E3 and E4 has overall upward going trend as seen in Figure 4.38. Increasing TN removal can take place under decreasing TSS condition. It means remaining bacteria degrade TN at higher rate. No significant difference is observable between E3 and E4 Inhibitor reactors until t=45 hours. After t=45 hours, TN removal per TSS in E4 Inhibitor decreases.

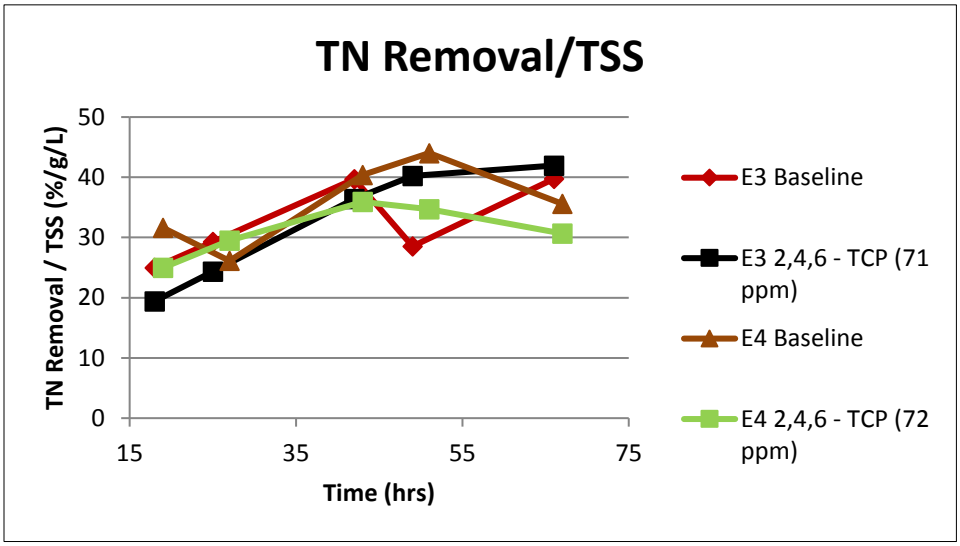

Figure 4.38: TN Removal per TSS data for CFR E3 and E4

TN removal in E3 and E4 Baseline reactors is higher than that in Inhibitor reactors as shown in Figure 4.39. E3 and E4 Baseline reactors achieved overall TN removal above 80%. Inhibitor reactors removal stayed about between 60% and 80%.

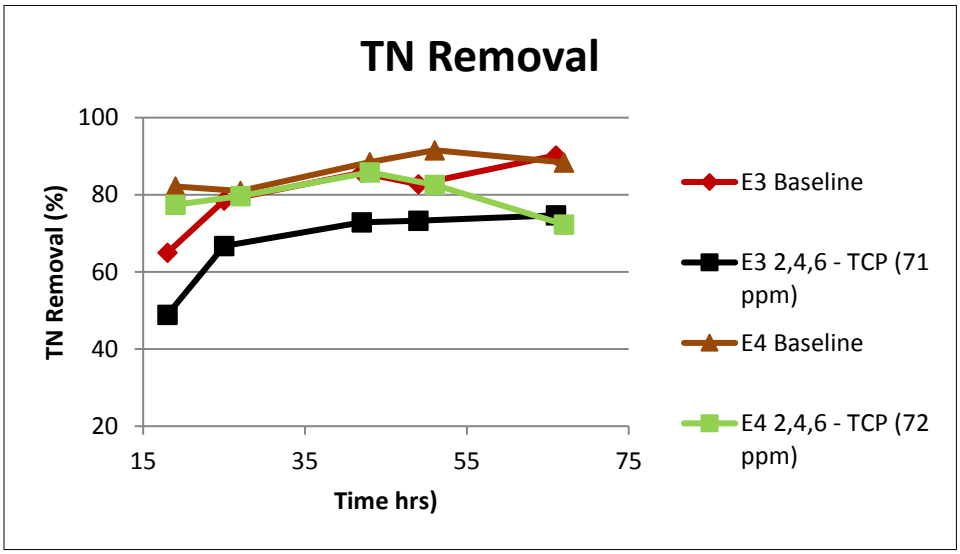

Figure 4.39: TN Removal data for CFR E3 and E4

TN removal per TSS in SBR has decreasing trend for E1 and E2 reactors as shown in Figure 4.40. However, compared to TC removal per TSS, specific TN removal rate is lower. Especially, specific TN removal rate in E1 and E2 Inhibitor reactors approach zero by the end of the experiment. This signals very low activity of bacteria responsible for nitrogen removal. It can be due to inhibiting effect of 2-CP. As was seen before, E1 and E2 Inhibitor reactors almost zero ammonium oxidation and nitrate production data as shown in Figures 4.24 and 4.26. Decrease of TN removal in E1 and E2 Baseline reactors can indicate decreased activity of

nitrifying bacteria. Nitrifying bacteria are less competitive for substrate compared to heterotrophic one.

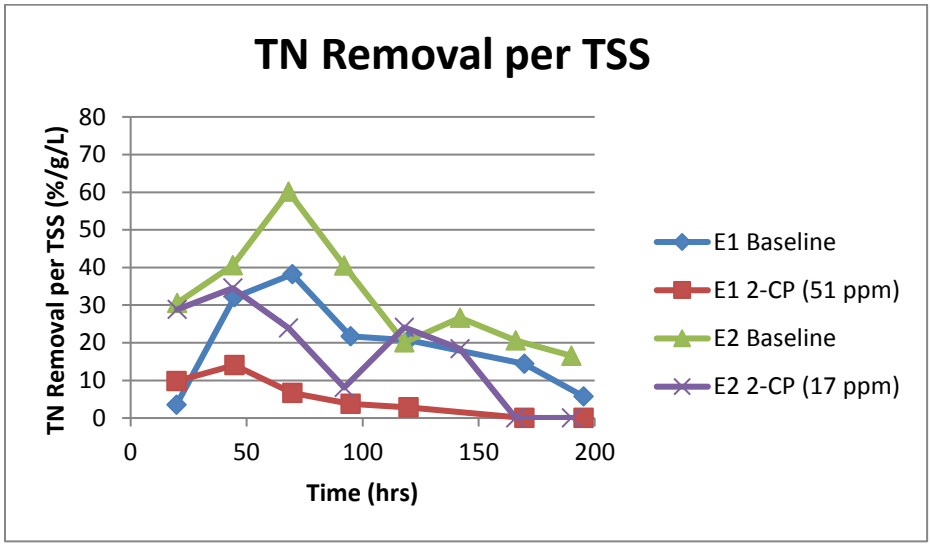

Figure 4.40: TN Removal per TSS data for SBR E1 and E2

As expected, absolute TN removal in E1 and E2 are lower compared to TC removal in same experiment as shown in Figure 4.41.

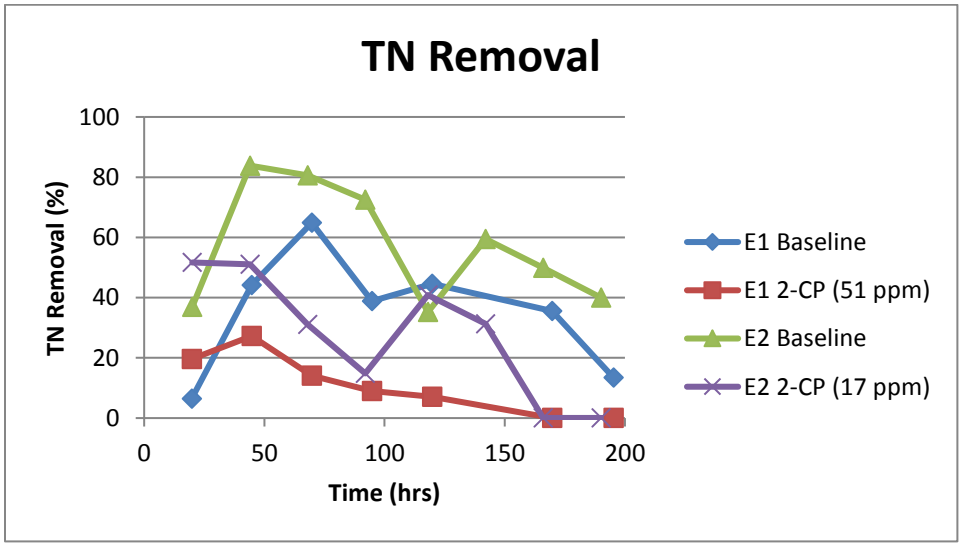

Figure 4.41: TN Removal data for SBR E1 and E2

Removal trend is decreasing with fluctuations coming along.TN removal rate for E1 and E2 did not exceed 80 % at the highest. The lowest removal rates are observed for E1 and E2 Inhibitor reactors reaching zero by the end of experiment.

E3 and E4 Baseline and Inhibitor reactors also demonstrate decreasing specific TN removal rates with some fluctuation as seen on Figure 4.42. However, E3 and E4 Inhibitor reactors involving 2,4,6 - TCP have higher TN removal rate compared to E1 and E2 reactors involving 2-CP.

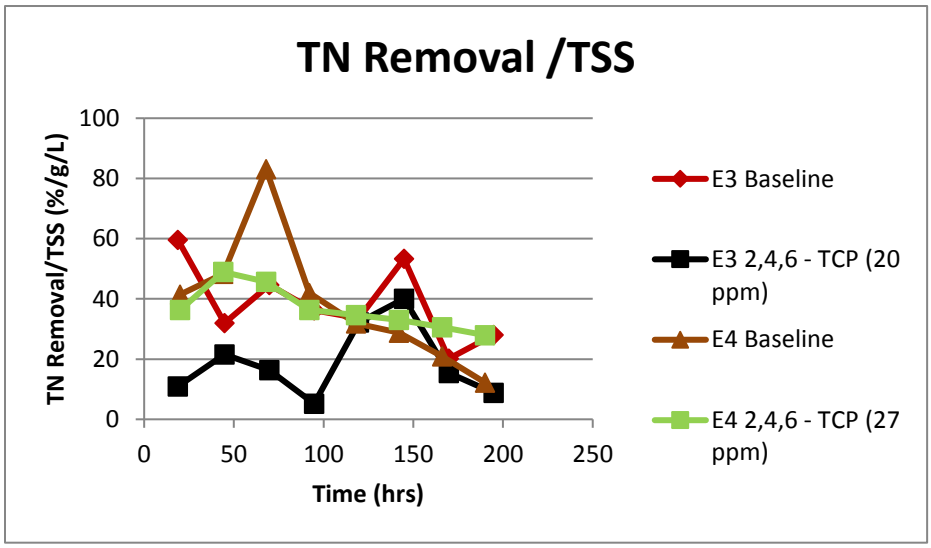

Figure 4.42: TN Removal per TSS data for SBR E3 and E4

TN removal range in E3 and E4 reactors is higher for Baseline reactors compared to Inhibitor reactors as shown on Figure 4.43. The highest TN removal of 90% is demonstrated by E3 and E4 Baseline reactors. Compared to E1 and E2 Inhibitor reactors, E3 and E4 Inhibitor reactors involving 2,4,6 - TCP showed higher TN removal. Comparing overall TN removal dynamics in Baseline reactors with TC removal, it can be concluded that nitrogen removing bacteria are less

active and competitive in comparison to carbon removing bacteria. Lower TN removal in Inhibitor reactor could be explained by toxic effect of chlorophenols on nitrogen removing bacteria.

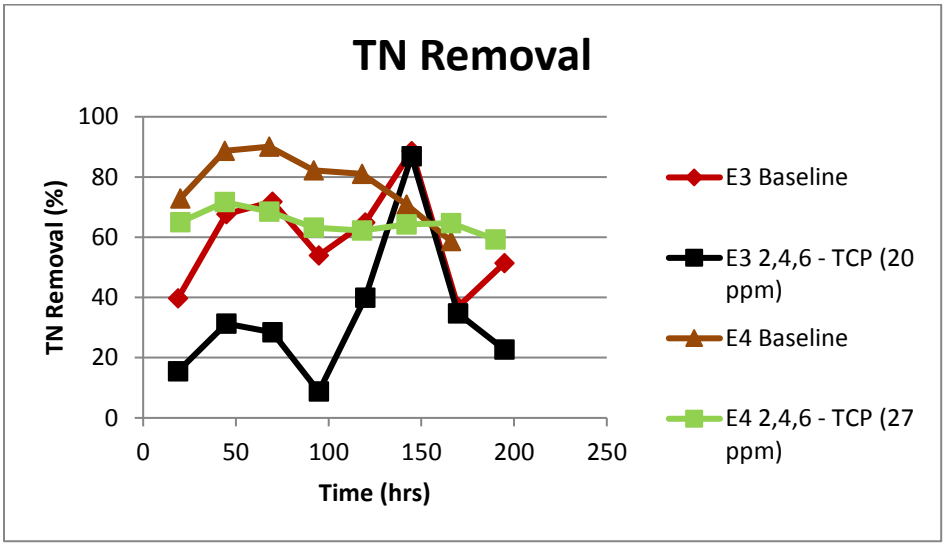

Figure 4.43: TN Removal data for SBR E3 and E4

4.3.5. Chlorophenol Removal

Chlorophenol removal efficiency evaluation is the part of assessing the reactor and sludge performances. Figure 4.44 presents chlorophenol removal efficiency for suspended-growth un-acclimated sludge in CFR E1, E2, E3, and E4 Inhibitor reactors. Downgoing removal trend indicates ongoing sludge inhibition except for E1 Inhibitor reactor. 2-CP concentration at 163 ppm and 2,4,6 – TCP concentrations at 70 ppm are all both inhibitive to sludge activities. Therefore, low TC removal rates were observed before in all E2, E3, and E4 Inhibitor reactors. 2-CP concentration of 103 ppm might not be inhibitive as removal trend in E1 Inhibitor reactor is constant.

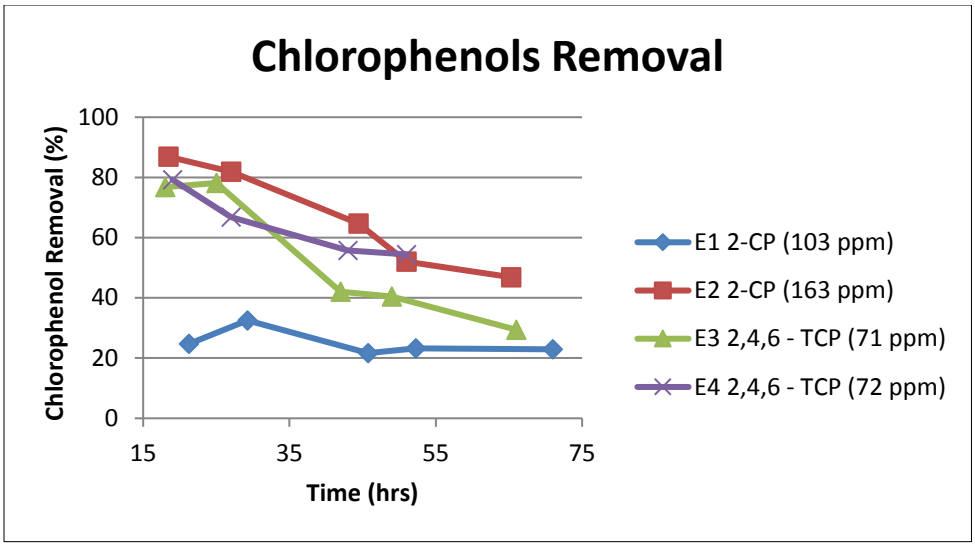

Figure 4.44: Chlorophenol removal data for E1, E2, E3, and E4

Compared to CFR, suspended-growth un-acclimated sludge in SBR shows opposite performance. Activated sludge in SBR demonstrated complete removal of 2-CP and 2,4,6 - TCP as can be seen in Figures 4.45 and 4.46.

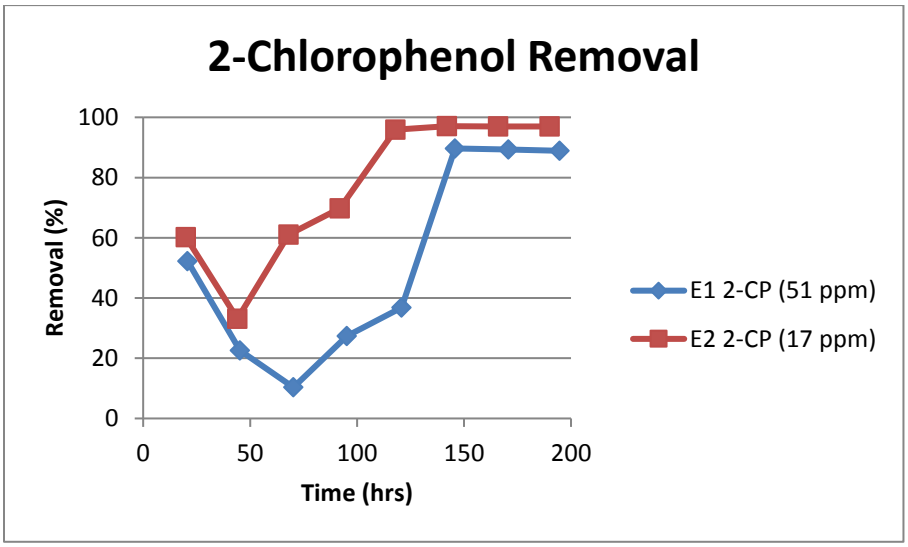

Figure 4.45: 2-CP removal data for E1 and E2

The removal trend in all reactors is the same. Initially high removal rates could be due to air stripping and due to low value of TSS. Also, low sludge activity is due to

acclimation to the toxic comound. As reaction goes on, TSS rises and removal rates of 2-CP and 2,4,6 - TCP increases achieving complete removal by the end of experiment. 17 ppm 2-CP took less time to be fully degraded compared to 51 ppm 2-CP . Thus, SBR needs extended operation period to achieve considerable chlorophenols removal depending on its initial concentration.

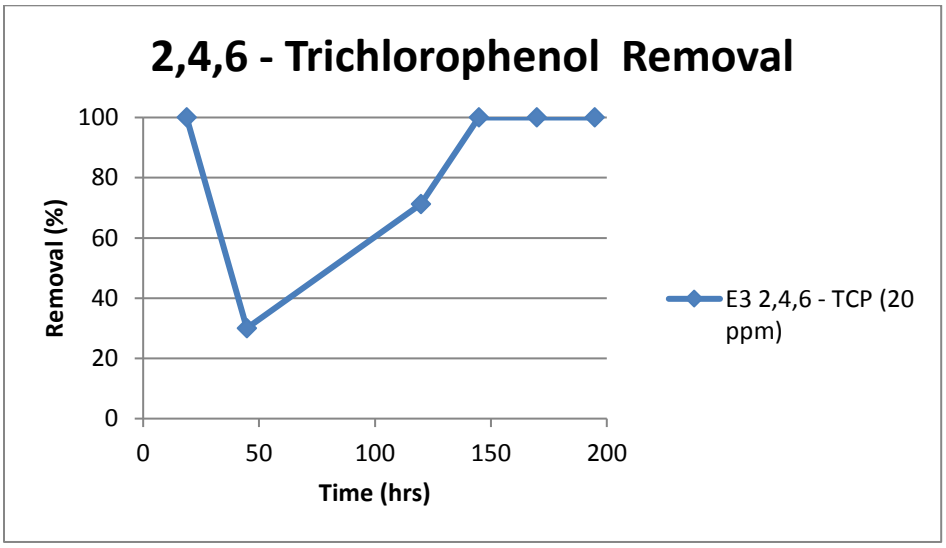

Figure 4.46: 2,4,6-TCP removal data for E3

Also, comparing CFR and SBR performances, it can be concluded that higher concentration levels of 2-CP and 2,4,6 – TCP in CFR are more inhibitive compared to lower chlorophenols concentration level in SBR.

5.Conclusion

Conduction of lab-scale experiments in CFR and SBR to evaluate the performance of suspended-growth un-acclimated sludge has pointed out the following important outcomes:

1. CFR reactor behavior is close to the ideal CSTR. In contrast to CFR, SBR configuration had serious deficiency in terms of excess aeration. Excess aeration contributed to half of TC and TN removals during the first day of experiment. Such problem can be relieved by using stirrer to keep sludge in suspension and to use internal aeration at 1.5 L/min as in CFR. Use of sole internal aeration at 1.5 L/min allowed achieving the lowest TC and TN removals possible.
2. 2-CP concentrations of 103 ppm and 163 ppm in CFR as well as 51 ppm and 17 ppm in SBR caused reduced ammonium degradation and nitrates production. Thus, nitrification inhibition can be observed in CFR and SBR reactors due to 2-CP. 2,4,6 - TCP of 71 and 72 ppm also caused nitrification inhibition in CFR. However, 20 and 26 ppm of 2,4,6 - TCP did not cause nitrification inhibition in SBR.
3. Elevated 2-CP and 2,4,6 - TCP concentrations in CFR halted the TSS growth . In contrast, TSS showed uniform growth trend in SBR at reduced 2-CP and 2,4,6 - TCP concentrations.

4. 2-CP and 2,4,6 - TCP removals also had decreasing trend in CFR. Inspite of adding easily-degradable co-substrate such as glucose in CFR reactor, 2-CP and 2,4,6 - TCP concentrations are too high to be completely degradable by suspended-growth un-acclimated sludge. In contrast to CFR, SBR showed complete removal of 2-CP and 2,4,6 - TCP at lower concentrations. Addition of glucose and use of aerated filling should have promoted TSS growth and less shock-loading, thus, helping to remove 2-CP and 2,4,6 - TCP. Thus, Suspended-growth un-acclimated sludge is efficient at removal of lower 2-CP and 2,4,6 – TCP concentrations.

6. Future Research

The conducted research is the starting place for comprehensive evaluation of activated sludge performance in SBR and CFR. As a result, further researches are necessary to get the full representation of activated sludge treatment process. The following researches are recommended:

- Evaluate 2-CP and 2,4,6 – TCP concentrations to get inhibition profile for suspended-growth un-acclimated sludge.
- Evaluate the impact of 2-CP and 2,4,6 – TCP on sludge by conduct specific tests such as oxygen uptake rate (OUR), ammonium uptake rate (AUR), and nitrogen uptake rate (NUR).

- Evaluate other chlorophenols such as 2,4 – dichlorophenol to identify inhibition profile for their removal, for TC and TN removal as well as for nitrification process.
- Conduct the above mentioned researches in SBR using stirrer and aeration at 1.5 L/min to minimize the effect of air in compounds removal.

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