Ozonation of Activated Sludge of Pulp and Paper Industry for Reduction in Quantity and Contamination

A Thesis

Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

By

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This thesis is dedicated to my Grand Father and my late Grand Mother for their wonderful affection, encouragement and support that they gave me. They are my inspiration to reach this academic height.

Sanjeev

CERTIFICATE

This is to certify that the thesis entitled **"Ozonation of Activated Sludge of Pulp and Paper Industry for Reduction in Quantity and Contamination"** which is submitted by **Mr. Sanjeev Gupta,** in fulfillment of the requirement for the award of the degree of **Doctor of Philosophy** to the School of Chemistry and Biochemistry, Thapar University, Patiala, is a bona fide research work carried out by him under our supervision and guidance. The matter embodied in this thesis has not been submitted in part or in full to any other University or Institute for the award of any degree.

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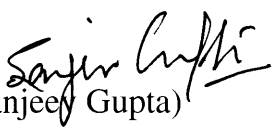

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Abstract

Aerobic biological treatment with activated sludge process is predominantly applied for the treatment of wastewater in integrated pulp and paper mill around the world. 50-70% of the biodegradable organic material is mineralized with generation of additional biological cells, typically termed as excess sludge or waste activated sludge (WAS). Handling and disposal of WAS is confronted with several difficulties; regulatory stringency due to contamination of organochlorine compounds is the greatest hurdle. The objectives of the present research are to reduce the net disposable biomass and organochlorine compounds in the integrated pulp and paper industry. The research was carried out on the ozonation of filamentous biosludge of both laboratory reactor and integrated pulp and paper mill, and flocculating biosludge in the batch mode in the laboratory set-up. Ozonated biosludge was post treated in laboratory scale activated sludge process. In the initial phase of the research, biosludge was characterized for different parameters and sludge yield was determined in the laboratory reactors.

The concentration of adsorbable organic halogens (AOX) and extractable organic halogens (EOX) compounds in dewatered biosludge from integrated pulp and paper mill was 2119 and 641 mg/kg respectively. Ten chlorophenolic compounds out of 12 compounds identified for regulation by USEPA were detected in the biosludge and the combined concentration was 2043 µg/kg. Toxicity equivalency (TEQ) due to chlorophenolic compounds in the biosludge was 717 µg/kg. The concentration of 2,3,7,8-TCDD and 2,3,7,8-TCDF was 16 and 210 pg/g of dry sludge respectively; the upper bound I-TEQ from PCDD/Fs was 54 pg/g DS. The concentration of organochlorine compounds in sludge was dependent on the concentration of the same in influent wastewater.

The application of ozone on biosludge altered its physical, chemical and biological characteristics. With increase in ozone dosage and subsequent dissolution of biosludge, the CODs content in the aqueous phase was increased. The solubilised material was highly biodegradable. The BOD to CODs ratio which was 6.2 in the untreated wastewater increased to 69.6% at 92.3 mg O_3/g DS dosage. Due to desorption of lignin from biosludge, increase in lignin and subsequent colour in the aqueous phase was observed. During ozonation of biomass, the AOX and EOX compounds were exposed for oxidation, which resulted in 41

and 67% removal of these compounds respectively at 92.3 mg O_3/g DS dosage. The toxicity equivalency of total chlorophenolic compounds was decreased by 52.7% after ozone treatment; none of these compounds was detected in the aqueous phase after ozone treatment. Similarly, 92-100% removal of PCDD and PCDF compounds was observed at an ozone dosage of 46 mg/g DS. The upper bound I-TEQ from PCDD/Fs was reduced to 3.7 pg/g DS. The lysis of filamentous organisms and reduction in zeta potential made the biosludge settling in nature. The compact and dense flocs were found resistant towards ozone attack, whereas the diffused and pinpoint flocs were prone to ozonation.

Ozonation of three times of excess sludge at an average ozone dosage of 46.4 mg/g DS followed by biological oxidation was capable to reduce significantly the disposal of waste activated sludge. The sludge yield in control and ozone bioreactors was 0.31 and 0.059 g/g of CODs removal. The recycling of ozonated biosludge to activated sludge process and thereby extra load of pollutants did not affect the performance of the process and removal efficiency of pollutants like COD, colour, AOX etc. There was no additional build-up of AOX, EOX and chlorophenolic compounds in the biosludge due to recycling of ozonated biosludge. The two stage process comprising of ozonation of biosludge coupled with biological treatment in activated sludge process resulted in 80.1, 81.2 and 79.9% lower discharge of AOX, EOX and chlorophenolic compounds with sludge respectively without affecting the concentration of these parameters in the treated wastewater. The process was effective for controlling the proliferation of filamentous organisms in the biological reactor which resulted in good settling characteristics of biosludge. The good viable count and better oxygen uptake rate in the bioreactor revealed that the activity of organism was better in the bioreactor. Ozonation can be a potential oxidative pretreatment process for reducing the WAS and paving the way for cost effective overall treatment of hazardous WAS in pulp and paper industry. Few recommendations have also been proposed based on the outcome of the research work.

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List of Abbreviations

Abbreviation		Full Name
AOX	:	Adsorbable organic halogens
ASP	:	Activated sludge process
ATP	:	Adenosine triphosphate
BOD	:	Biochemical oxygen demand
C	:	Chlorination stage
C_D	:	Chlorine dioxide substituted chlorination stage
CFU	:	Colony forming unit
COD	:	Chemical oxygen demand
CODs	:	Soluble chemical oxygen demand
COD_T	:	Total chemical oxygen demand
CPs	:	Chlorophenols
D	:	Chlorine dioxide stage
DBP	:	Dibromophenol
DCM	:	Dichloromethane
DCP	:	Dichlorophenol
DS	:	Dry sludge
E_{OP}	:	Alkali extraction stage with oxygen and peroxide reinforcement
ECD	:	Electron capture detector
ECF	:	Elementary chlorine free
EOX	:	Extractable organic halogens
ETP	:	Effluent treatment plant
F/M ratio	:	Food to microorganism ratio

GC	:	Gas chromatography
HRT	:	Hydraulic retention time
IS	:	Internal standard
I-TEQ	:	International toxicity equivalency
MCP	:	Monochlorophenol
MLSS	:	Mixed liquor suspended solids
MLVSS	:	Mixed liquor volatile suspended solids
N	:	Nitrogen
NTP	:	Normal temperature and pressure
OD	:	Oven dried
OUR	:	Oxygen uptake rate
P	:	Phosphorous
PCP	:	Pentachlorophenol
PCDD	:	Polychlorinated dibenzodioxin
PCDF	:	Polychlorinated dibenzofuran
PID	:	Proportional Integral Derivative
POX	:	Purgeable organic halogens
Rc	:	Control bioreactor
Ro	:	Ozone bioreactor
Rhl	:	Bioreactor at higher AOX load
Rrl	:	Bioreactor at reduced AOX load
RAS	:	Returned activated sludge
rpm	:	Revolution per minute
SAR	:	Sodium absorption ratio
SBR	:	Sequencing batch reactor

SOUR	:	Specific oxygen uptake rate
SRT	:	Sludge retention time
SS	:	Suspended solids
SVI	:	Sludge volume index
TCC	:	Trichlorocatecol
TCDD	:	Tetrachlorodibenzodioxin
TCDF	:	Tetrachlorodibenzofuran
TCG	:	Trichloroguaiacol
TCS	:	Trichlorosyringol
TCP	:	Trichlorophenol
TDS	:	Total dissolved solids
TeCC	:	Tetrachlorocatecol
TeCG	:	Tetrachloroguaiacol
TeCP	:	Tetrachlorophenol
TEF	:	Toxicity equivalency factors
TEQ	:	Toxicity equivalency
TOC	:	Total organic carbon
tpa	:	Tonne per annum
TSS	:	Total suspended solids
TVC	:	Total viable count
WAS	:	Waste activated sludge
WBL	:	Weak black liquor
WWTP	:	Wastewater treatment plant

Chapter-I

1.0 Introduction

The manufacture of pulp, paper, and paper products is one of the largest industries in the world; the major producing countries being the United States of America, Canada, Japan, Sweden, Finland and China. Presently USA is the largest producer of paper material (90 million tonne/year) followed by China (78 million tonne/year). More than 83% of US annual pulp output is produced by the kraft pulping or sulfate process (USEPA, 2002). The pulp and paper manufacturing is one of the core industrial sectors in India. India produces about 8 million tonne of paper /year (Dugal H.S., 2009; Industry guide, 2011). It plays a vital role in socio-economic development, while it is associated with significant environmental concerns due to its large footprints on environmental resources. While manufacturing pulp from wood, bleaching is carried out which generates toxic substances. Aerobic biological treatment by activated sludge process has widely been used to treat pulp and paper mill wastewater. During biological treatment, microorganisms oxidize dissolved and particulate organic matter into simple end products with generation of biomass. The excess biomass is separated from the treated wastewater and disposed of in concentrated form called excess sludge or waste activated sludge (WAS). Due to the very nature and operation of the industry which are described in the following sections, the biosludge originating from pulp and paper industry contains various organic, inorganic and microbiological contaminants. Organochlorines viz. chlorophenols, dioxins and dibenzofurans are some of the toxicants. Due to the presence of organochlorine compounds, sludge from pulp and paper industry has been classified as hazardous waste (MoEF, 2008). Being slimy in nature, it is difficult to dewater. The disposal of biosludge is a challenging issue in pulp and paper industry.

1.1 Scientific aspects of pulp and paper manufacturing process

Processes of the manufacture of paper in an integrated kraft pulp and paper mill can be divided into four stages viz. pulping, bleaching, papermaking and chemical recovery.

1.1.1 Pulping process

At the pulping stage, wood or other fiber material is treated chemically to separate out the fiber fraction from other constituents of wood. Chemical pulping using kraft process is the most prevalent throughout the world. It uses sodium sulfide (Na_2S) and sodium hydroxide (NaOH) as pulping chemicals. The liquor (white liquor) comprising of these two chemicals is mixed with the wood chips in a reaction vessel (digester), the products consist of wood fibers (pulp) and liquor that contains the dissolved lignin; typically termed as black liquor. The chemical process results in approximately 50 percent production of pulp.

1.1.2 Bleaching process

The bleaching of unbleached chemical pulp is carried out with elemental chlorine or its derivatives like chlorine dioxide (ClO_2) or hypochlorite (NaOCl, and $Ca(OCl)_2$). Upto the end of 20^{th} century, nearly every chemical pulp mill was using elemental chlorine as the first bleaching stage in India. Because of environmental concerns arising out of formation of organochlorine compounds, pulp mills now use partially ClO_2 substituted chlorination or elemental chlorine free bleaching technologies. During bleaching, pulp is processed through three to five stages of chemical reaction and water washing. Bleaching stages generally alternate between acid and alkaline conditions. Chemical reactions take place with lignin during the acid stage. The alkaline stages extract the reaction products of lignin through dissolution. At the washing stages, reaction products are removed. The most common bleaching sequences followed by mills in India are $CE_{OP}D_1D_2$ or $C_DE_{OP}D_1D_2$ or $D_0E_{OP}D_1D_2$.

1.1.3 Paper making

At the final stage, the stock is prepared by refining the pulp, and the paper is manufactured. Stock preparation includes dispersion of pulp in water, refining to increase surface area and addition of wet end chemicals. Wet-end operation includes the formation of paper sheet from wet pulp stock, whereas dry-end operation includes drying of paper, surface treatment and spooling for storage.

1.1.4 Kraft chemical recovery process

During chemical recovery, process chemicals from the spent cooking liquor (black liquor) are recovered for reuse. The chemical recovery process has important financial and environmental benefits for pulp and paper mills. The black liquor generated during pulping contains 15-17% solids, which is concentrated to 65-80% solid level which is burnt in a recovery boiler; the recovered chemicals are dissolved in weak white liquor. The resultant liquor is called green liquor. The impurities like dregs are separated and dissolved sodium carbonate is converted into active sodium hydroxide (white liquor) during causticization process. White liquor is again used in the pulping process.

1.2 Wastewater characteristics

In comparison to the global best specific water consumption of 28.7 m^3/ tonne for wood based pulp and paper mill, water consumption in pulp and paper mills in India is 43-150 m^3/ tonne of product (NPC, 2006; MoEF, 2010). High consumption of water is largely attributed to the use of old technology/ equipment and poor water management practices. Most of the pulp and paper mills are onshore and use surface water. The discharge of wastewater with additional evaporation loss is generally used to give a fair picture of water consumption. Each pulp and paper making process utilizes large amounts of water, which reappears in the form of wastewater.

Among the processes, pulping generates a high strength wastewater especially in chemical pulping. Pulp bleaching process generates toxic substances as it utilizes chlorine and its derivatives for brightening the pulp. Various substrates like resin acids, unsaturated fatty acids, diterpene alcohols, chlorinated resin acids, and others are generated depending upon the pulping process used. The wastewater, which contains filler, fines and starch, from paper machine is generally recycled to process. The pollutants generated at various stages of the pulping and paper making process are given in Table 1.1.

Wastewater from the pulp and paper mill contains a broad spectrum of organic and inorganic substances. It typically consists of fibrous suspended solids and dissolved organic compounds in high concentration. Both low and high molecular weight compounds are present in dissolved form. Generally small organic molecules exert biochemical oxygen

demand (BOD), whereas lignin and its derivatives cause a chemical oxygen demand and attribute colour. Characteristics of wastewater from Indian pulp and paper mills are given in Table 1.2. The time bound reduction of water use as per CREP has resulted in reduction in water consumption in paper production. The average water consumption for large paper mills (wood based), agro residue based mills and waste paper based mills is 40-120, 75-100 and 35-50 m^3/ tonne of product respectively (Chinnaraj et al., 2011; Endlay et al., 2011). The proposed norms for chemical pulp and waste paper based pulp and paper mills are 80 and 20 m^3/ tonne of product (CPCB, 2011).

Table 1.1: Potential water pollutants from pulp and paper making processes

Source	Pollutant
Wood handling/debarking and chip washing	Solids, BOD, COD, color
Chip digester and liquor evaporator condensate	BOD, COD, colour, reduced sulfur compounds
Wastewater from pulp screening, thickening, and cleaning	Suspended solids, BOD, COD
Bleach plant filtrates	BOD, COD, color, TDS, organochlorine compounds
Paper machine water	Suspended solids, TDS, BOD, COD
Fiber and liquor spills	Solids, BOD, COD, color

USEPA, 2002

Formation of organochlorine compounds in natural eco-system is well documented. More than 1500 organohalogen compounds have been identified (Biester et al., 2004). The pulp and paper mill is one of the artificial or manmade sources of organochlorine compounds in recipient waterways (Zheng and Allen, 1996; Ali and Sreekrishnan, 2000). Organochlorine compounds are formed during the bleaching of wood pulp with chlorine (Cl_2) and chlorine derivatives such as hypochlorite and chlorine dioxide (ClO_2) (Roy et al., 2004). The reactions between chlorine and lignin are substitution, oxidation and addition.

Table 1.2: Characteristics of wastewater from Indian pulp and paper mills

Parameter	Large paper mill	Small paper mill	
		Agro mill	Waste paper mill
Flow (m^3/t paper)	197-280	187-383	72-159
pH	6.6-10	6.0-8.5	7.1-7.7
TSS (mg/l)	620-1120	600-1115	350-885
BOD_5 (mg/l)	240-380	220-1067	100-273
COD (mg/l)	840-1660	2120-4763	472-876
COD/BOD ratio	2.95-4.37	2.49-5.40	2.7-5.7
Colour (Pt-Co unit)	300-655	15000-24000	-
Lignin (mg/l)	-	320-700	-
SAR	2.0-6.3	4.7-7.6	-

Ray, 2006

Organochlorine compounds in water and wastewater can be monitored by several techniques; among them, the one based on adsorbable organic halogens (AOX) is the most commonly used (Zheng and Allen, 1996; Barroca et al., 2001). During the production of one tonne of paper, 100 kg of colour imparting substances and 2-4 kg of organochlorines are generated in the bleach plant effluent (Kansal et al., 2008). A physical-chemical classification of this chlorinated organic material from conventionally pulped and bleached softwood kraft pulp is shown in Figure 1.1. Among the organochlorine compounds derived from lignin, 80% are of high molecular weight and rest 20% compounds are of low molecular weight (MW<1000). A tiny fraction (1-3% of the total organochlorines) is lipophilic and is the cause of environmental concern. Collectively these are termed as extractable organic halogen (EOX) compounds. Chlorophenolic compounds, dioxins and dibenzofurans fall in this category (Berry et al., 1991; Bajpai and Bajpai, 1997).

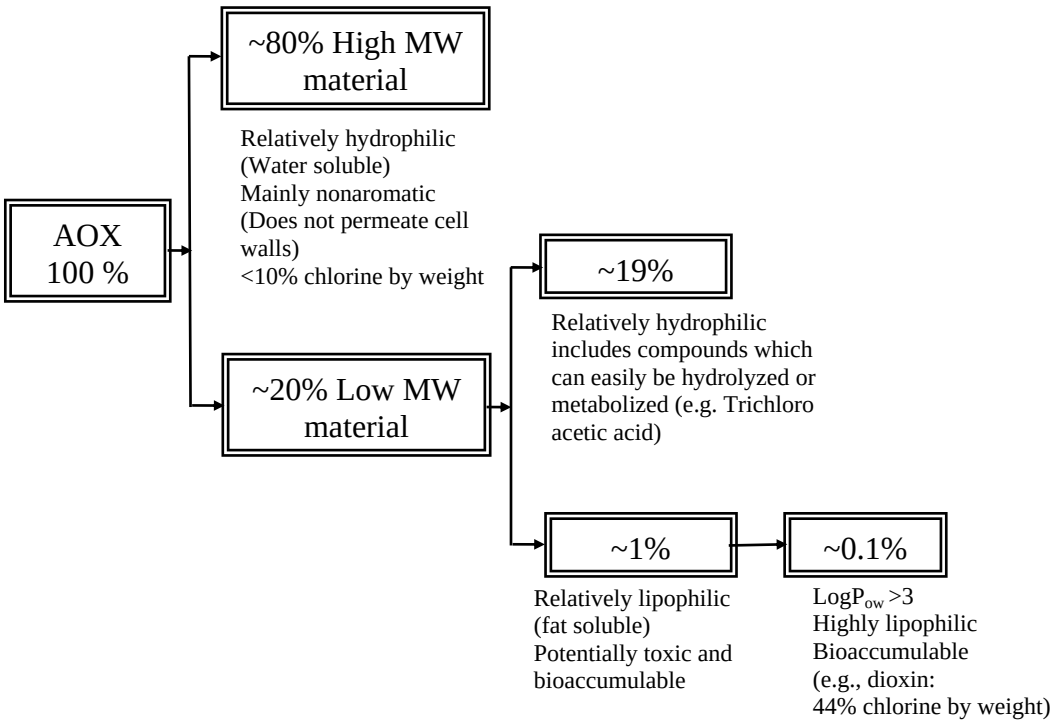


Figure 1.1: Characteristics of AOX compounds in the effluent (Berry et al., 1991)

Chlorophenolic compounds include phenols, guaiacols, catechols, syringol and vanillins substituted with one to five chlorine atoms per molecule. Typically, bleaching processes that result in the formation of 2,3,7,8-tetrachlorodibenzodioxin (TCDD) and 2,3,7,8-tetrachlorodibenzofuran (TCDF) also generate the higher substituted tri-, tetra-, and penta-chlorinated compounds (Freire et al., 2003; Roy et al., 2004).

USEPA has identified 12 organochlorine compounds for regulation in the effluent of pulp and paper mills and has established effluent limitations guidelines and pretreatment standards (USEPA, 1999) for these chlorinated phenolic compounds. This is in response to environmental concerns and government regulations on the limits of these discharges following recognition of their potential adverse biological effects. From the point of compliance, maximum permissible concentration of 12 chlorophenolics is given in Table 1.3 (USEPA, 2000).

Table 1.3: Chlorophenolic compounds under regulation

Compound	Maximum concentration (µg/l)
2,4,6-trichlorophenol	2.5
2,4,5-trichlorophenol	2.5
2,3,4,5-tetrachlorophenol	2.5
3,4,6-trichloroguaiacol	2.5
3,4,5-trichloroguaiacol	2.5
4,5,6-trichloroguaiacol	2.5
3,4,6-trichlorocatecol	5.0
Pentachlorophenol	5.0
3,4,5-trichlorocatecol	5.0
Tetrachloroguaiacol	5.0
Trichlorosyringol	2.5
Tetrachlorocatecol	5.0

USEPA, 2000

In both scientific and industrial communities, there has been a growing interest in the best available technologies for the bleaching of chemical pulps in order to reduce the discharge of organochlorine compounds in the liquid effluents.

1.3 Wastewater treatment

Improved fiber retention, better in-plant utilization of raw materials, and use of efficient and environment friendly processes/ technologies are effective means of reducing pollution at site. In last decade, the amount of water consumption in pulp and paper mills has been drastically reduced. External treatment of effluent is usually carried out by means of screening and sedimentation to remove suspended solids (i.e. primary treatment) followed by biological oxidation to remove suspended and dissolved organic material (i.e. secondary treatment). Any treatment beyond primary and secondary treatment is usually termed as tertiary treatment. The sludge generated during different stages is thickened and dewatered prior to disposal. The schematic diagram of typical effluent treatment process is illustrated in Figure 1.2.

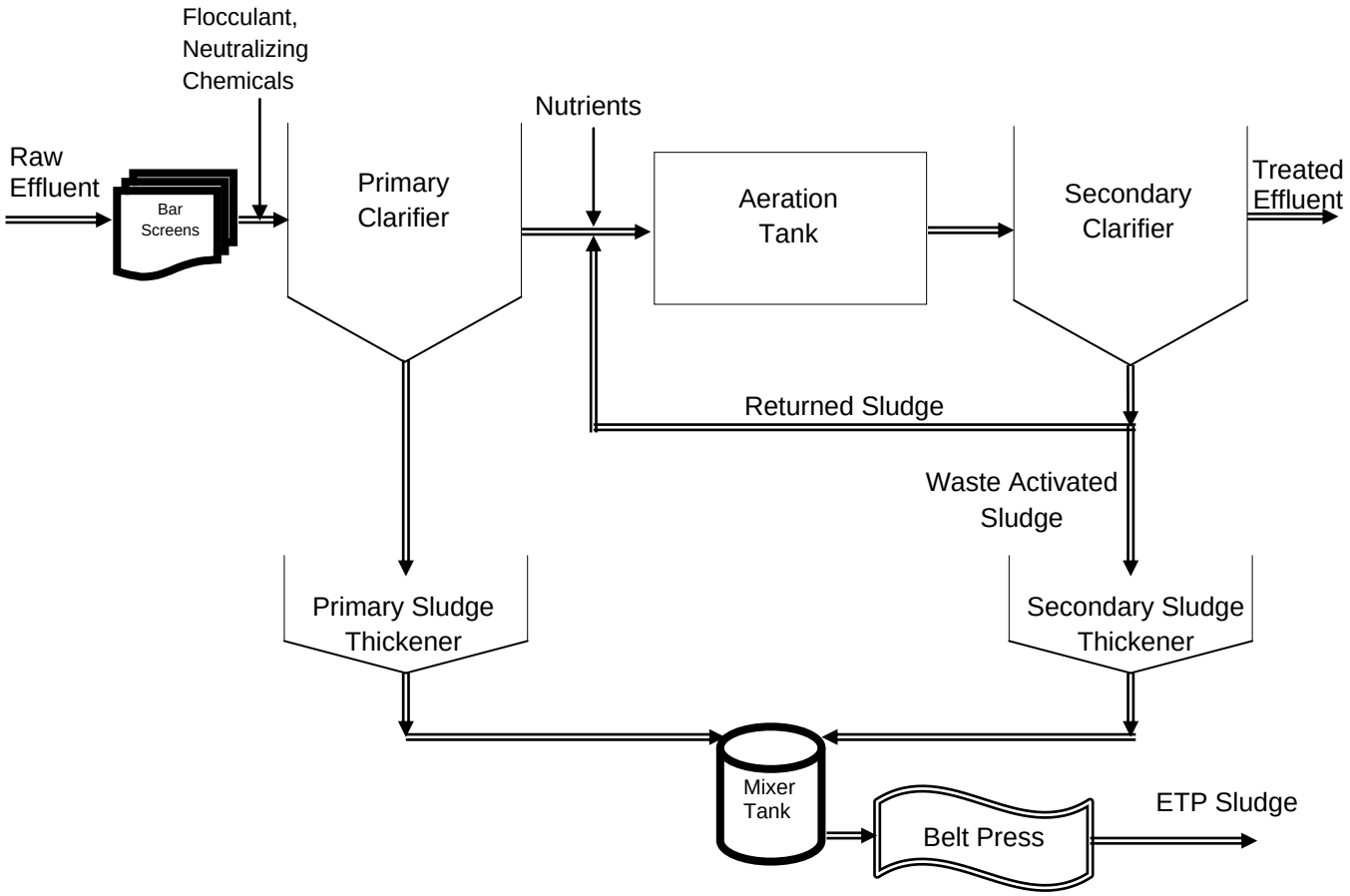


Figure 1.2: Schematic diagram of typical ETP in pulp and paper mills

1.3.1 Primary treatment

Primary treatment generally refers to the methodology for removing suspended solids from effluents. In the pulp and paper mill, solids removal is always accompanied by some reduction in COD, BOD and toxicity. Screening is often used as a preliminary step to remove relatively large floating or suspended particles from an effluent stream. Generally screening is performed by bar screens.

Sedimentation or gravity settling is the most common process used to separate the fibrous material from raw wastewater. Sedimentation is carried out in a holding pond or basin with sufficient retention time for settling the solid particles. Ideal sedimentation unit provides quiescent flow to allow the settlable solids to move to the bottom. Solids are pushed to the center sump with a sludge scraper. Depending on the characteristics of the solids, the concentration of underflow may vary between 1.5 to 6% (Smook, 1992). In addition to the

basic function of separation of solids, primary clarifier acts as an equalization basin cum shock absorber.

1.3.2 Secondary treatment

Secondary or biological treatment is the heart of the wastewater purification process. Under aerobic conditions, microorganisms (mostly bacteria) consume oxygen to convert organic waste into the ultimate end products, carbon dioxide and water. An important aspect of biological oxidation process is to provide adequate aeration and mixing for intimate contact between large concentration of microorganisms and the substrate. Aerobic biological oxidation can be accomplished by various means, depending on the characteristics of the wastewater, the area available for external treatment, and the required degree of BOD removal. Aerobic process is divided into three categories viz. suspended growth process, attached growth process and hybrid process.

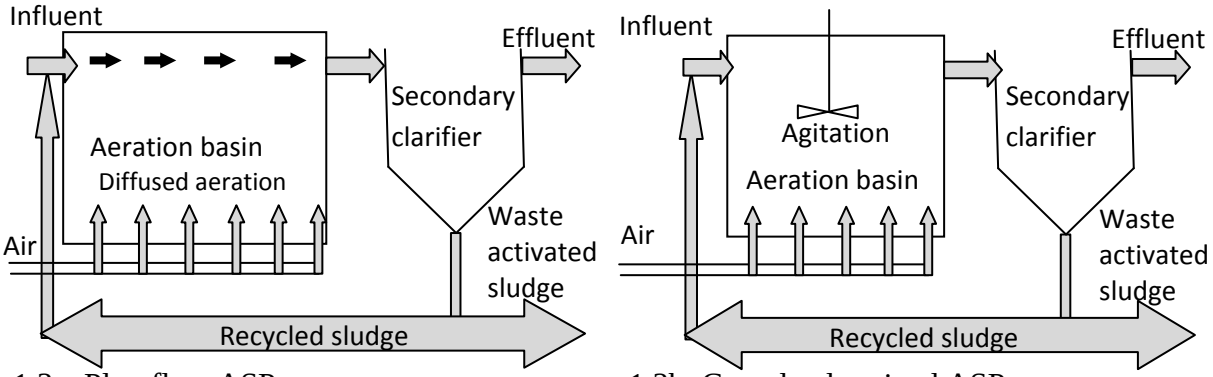
The activated sludge process (ASP) is the most popular, well adapted, high-rate suspended growth biological process for treatment of industrial and municipal wastewaters. The process was named activated sludge by Ardern and Lockett, 1913 as it involved the production of an activated mass of microorganisms capable of aerobic stabilization of organic material in wastewater. ASP is differentiated based on mixing arrangement as ‘plug flow’ and ‘completely mixed’ (Tchobanoglous et al., 2003; Seviour & Nelsen, 2010).

Plug flow system

The plug flow system generates less filamentous bacterial mass and produces settling sludge than completely mixed one. This configuration often runs inefficiently from uneven load distribution along the reactors. Thus, demand of oxygen at the inlet of the aeration basin is high. Here DO remains near to zero, whereas the same remains higher at discharge end. The mixed liquor is kept in suspension by aeration only (Figure 1.3a).

Completely mixed system

The aeration deficiency in the plug flow system is overcome through the completely mixed system. However, this configuration is susceptible to bulking by filamentous bacteria. Returned activated sludge (RAS) and incoming wastewater are mixed rapidly with the biomass, reducing the risk of toxic shock (Figure 1.3b).



1.3a: Plug flow ASP 1.3b: Completely mixed ASP

Figure 1.3: Flow diagram of plug flow and completely mixed activated sludge process

The essential feature in the suspended growth configurations is the development of a microbial floc in suspension in the aeration tank. Clarified wastewater is fed continuously in this tank and the organisms multiply as dissolved organic waste is metabolized. After the treatment, wastewater is passed through a clarifier where separation of solid–liquid takes place. A certain portion of this biomass is recycled to the aeration tank to maintain desired microorganism concentration and food to microorganism ratio. The excess biomass is concentrated and disposed of. Compared to the aeration lagoon, the activated sludge process has some disadvantages; it is sensitive to changes in the characteristics of the wastewater, the requirement of nutrients is relatively higher, and settling aids are sometimes required for proper clarification.

Activated sludge process is widely used to treat pulp and paper mill effluent. The treatment can generally achieve relatively higher reduction of BOD and toxicity (Diez et al., 2002). Partial removal of AOX compounds in the biological system has also been recognized (Reeve, 1991; Taghipour and Evans, 1996). The removal of these compounds is achieved through volatilization, biosorption, and biological dechlorination (Leuenberger et al., 1985). Microorganisms hydrolyze the dissolved and particulate organic matter into simple end products and subsequently oxidize those with generation of additional biomass.

Cell yield or sludge yield coefficient is dependent on the nature of substrate, various environmental and process conditions viz. pH, temperature, dissolved oxygen, nutrients, hydraulic retention time (HRT), sludge retention time (SRT), food to microorganism (F/M) ratio etc. which influence the metabolization of the organic carbon (Gaudy & Gaudy, 1981). Excess cells are separated from the purified water in a concentrated form called waste

activated sludge which is contaminated with various substances spilled from or generated in the pulp and paper making process.

1.4 Solid waste handling in the activated sludge process

Wastewater treatment in the pulp and paper mill generates huge solid waste (Monte et al., 2009). Amount of sludge generation vary widely among mills. For bleached kraft mills, sludge generation ranged from 14 to 140 kg of sludge per tonne of pulp (USEPA, 2002). Corresponding figure for biological sludge in the large and small paper mills in India are 35 and 105 kg/t of paper (Ray, 2006). Treatment and disposal of WAS in the case of pulp and paper industry pose challenges for environmental and regulatory factors. It has been classified as hazardous waste in India (MoEF, 2008) due to presence of the organochlorine compounds. The prevalent disposal methods of WAS such as land filling, incineration and beneficial uses have come under watch and criticism by the public and regulatory agencies around the world. Because of various environmental and technical reasons, the disposal of WAS becomes a costly affair (Low and Chase, 1999b; Wood et al., 2009). Many researchers have reported sludge management costs as high as 40-60% of operating costs of wastewater treatment. It is to the economic advantage to reduce the sludge generation (Wei et al., 2003; Yang et al., 2003; Chakrabarti, 2005; Yoon and Lee, 2005; Mahmood and Elliott, 2006).

Increased attention has been given on minimization of WAS generation from activated sludge process. Different techniques are in use for generation of lesser amount of sludge based on chemical or biological principles. The reduction of WAS are achieved with the following techniques (Figure 1.4):

a) Process changes during biological treatment

b) Post treatment of waste activated sludge

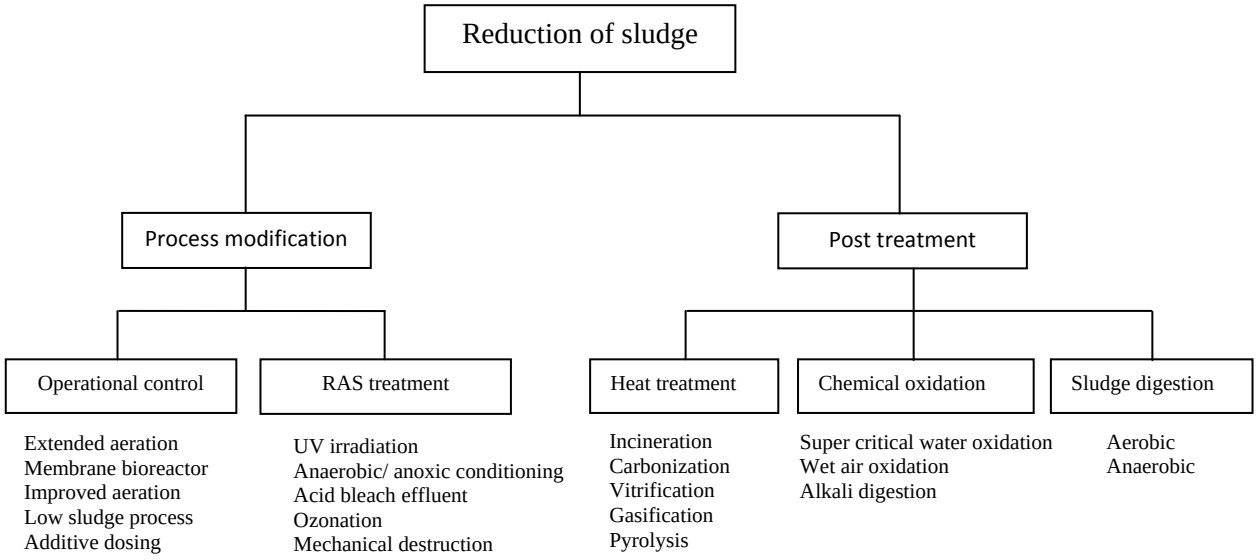


Figure 1.4: Outline of sludge reduction technologies (Mahmood and Elliott, 2006)

Perez-Elvira et al., 2006 have categorized the sludge minimization techniques considering the places in the plant where the treatment is applied viz., i) in the wastewater line, ii) in the sludge line, and iii) in the final waste line (Table 1.4).

Table 1.4: Sludge minimization techniques

In the wastewater line	Reduction of yield coefficient	Lysis cryptic growth	Chemical oxidation	Chlorination	FS
				Ozonation	FS
			Integration of chemical and heat treatment		FS
			Pure oxygen process		FS
			Enzymatic reaction		FS
		Maintenance metabolism	Membrane bioreactor		LS, IN
		Uncoupling metabolism	Chemical uncoupler		LS
			Oxic-settling-anaerobic process		FS
			Anaerobic/aerobic system		LS
		Ecosystem change	Two-stage system		LS
			Oligochaetes		LS
In the sludge line	Pretreatment prior to anaerobic digestion	Physical	Cavitation	High pressure homogenizer	LS
				Ultrasonic homogenizer	LS
			Thermal	Thermal hydrolysis	FS
				Freezing and thawing	LS
			Mechanical	Impact grinding	LS
				Stirred ball mill	LS
				High performance pulse technique	LS
				Lysat-centrifugal technique	LS
			Radiation	Gamma-irradiation	LS
		Chemical	Acid or alkaline hydrolysis		LS
			Pretreatment with ozone		LS
		Biological	Enzymatic pretreatment		LS
		Combined	Combination of thermal, decompression and shear forces		FS
			Chemically enhanced thermal hydrolysis		LS
	Modified anaerobic digestion		Two-stage anaerobic digestion		IN
			Temperature phased anaerobic digestion		IN
			Anoxic gas flotation		FS
In the final waste line			Incineration		FS
			Gasification and pyrolysis		FS
			Wet air oxidation		FS
			Super critical water oxidation		FS

FS: full-scaled, LS: laboratory scale, IN: innovative

Each of the four processes applied in the wastewater line for reduction of sludge yield coefficient is briefly described in the following section:

1.4.1 Lysis-cryptic growth

Cell lysis releases its constituents into the medium, and provides an autochthonous substrate which is reused in microbial metabolism. A portion of the carbon is liberated as

products of respiration resulting in a lower biomass generation. There are two stages in lysis cryptic growth; lysis and biodegradation. The rate limiting step is lysis and an increase of the lysis efficiency is directly linked with reduction in sludge generation.

The lysis of sludge can be achieved in several ways (Muller, 2000; Wei et al., 2003; Bougrier et al., 2006):

i. Thermal treatment in the temperature range from 40 to 180 °C
ii. Chemical treatment using acids or alkali
iii. Mechanical disintegration using ultrasound/ mill/ homogenizer
iv. Freezing and thawing
v. Hydrolysis with enzyme
vi. Pure oxygen
vii. Advanced oxidation processes with H_2O_2 and ozone
viii. Combination of two or more techniques

1.4.2 Maintenance metabolism

Microorganisms satisfy first the maintenance energy and then produce new cells. By increasing biomass concentration (e.g. membrane bioreactor) it would theoretically be possible to reach a situation where the amount of energy supplied equals the maintenance demand (Low and Chase, 1999a; Wei et al., 2003).

1.4.3 Uncoupling metabolism

Catabolism is the reaction that reduces the complexity of organic compounds and produces free energy. Anabolic pathways use the free energy to build the molecules required by cell. Anabolism is coupled with catabolism through the rate limiting respiration. Uncoupled metabolism would occur if respiratory control does not exist and instead the biosynthetic processes are rate limiting. Excess free energy would be directed away from anabolism so that the production of biomass is reduced. The uncoupling metabolism is achieved with chemical uncoupler, high initial substrate to biomass ratio, oxic-settling-anaerobic process (Liu et al., 1998; Low et al., 2000; Yang et al., 2003).

1.4.4 Ecosystem change

Activated sludge is an ideal habitat for several organisms other than bacteria. One way to reduce sludge production is to exploit higher organisms such as protozoa and metazoan, in the activated sludge process, that predate on the bacteria while unaffecting the decomposition of substrate (Wei et al., 2003).

1.5 Ozone properties and oxidation treatment

Ozonation is one of the most effective treatments for reducing the production of activated sludge in wastewater treatment plant (Dziurla et al., 2005). The following section will depict in brief different aspects of ozone, its chemistry and reaction with different substrates:

Ozone is a molecule that consists of three oxygen atoms. The ozone molecule is very unstable and has a short half-live, causing it to fall back into oxygen after a while. The properties of ozone are given in Table 1.5.

Table 1.5: Properties of pure ozone

Parameter	Data
Melting point (°C)	-192.5±0.4
Boiling point (°C)	-111.1±0.3
Critical temperature (°C)	-12.1
Critical pressure (atm.)	54.6
Critical volume (cm^3/mole)	111
Density of ozone gas (g/cm^3) @ NTP	1.95

Ozone is more soluble in water than oxygen (Table 1.6), but because of very low partial pressure, it is difficult to obtain a high concentration of ozone dissolved in water at NTP.

Table 1.6: Solubility of oxygen and ozone in water at different temperatures

Gas	Solubility in water (mg/l) at different temperature			
	0 °C	10 °C	20 °C	30 °C
Oxygen				
100%	70.5	54.9	44.9	38.2
21%	14.8	11.5	9.4	8
Ozone				
100%	1374.3	1114.9	789	499.6
4%	55	44.6	31.6	20

(Wang et al., 2007)

Ozone is a strong chemical oxidant, allotropic, highly reactive and has the properties of a dipole. As a result, it has a great capacity to attack organic compounds and metals, with the exception of gold, platinum and iridium. It reacts in two different ways: (1) direct reactions of ozone and (2) indirect reaction of secondary oxidators, such as free OH radicals. Both reactions occur simultaneously, the indirect reaction is based on the high reactivity of hydroxyl radical, which does not react specifically, whereas the direct reaction depends more on the structure of the reactant. The way of the reaction of ozone depends on various factors, such as temperature, pH and chemical composition. This is consequential to the disintegration of ozone into OH radical in water (Cesbron et al., 2003; Gunten, 2003a, b; Salsabil, 2008).

Considering the necessity of reducing the disposable sludge from pulp and paper mill and the complexity of both the wastewater and sludge, the oxidation power of ozone has been exploited in the current research to see how ozone reacts with a complex substrate like waste activated sludge of pulp and paper mill. Following chapter will present an overview of the work that different researchers have reported on minimization of sludge including that of pulp and paper mill.

Chapter-II

2. Review of Literature

The present chapter presents a summary of the work, which the researchers across the world have undertaken, to minimize the sludge including that of pulp and paper mill with different techniques. Ozonation, being potential treatment process for minimizing the biosludge, has been given due importance.

2.1 Minimization of activated sludge from pulp and paper industry

Knudsen et al., 1994 studied two stage activated sludge process for paper mill effluent; first stage with high load (2-4 kg COD/kg mixed liquor suspended solids (MLSS)/d) and second stage with low load (0.2-0.4 kg COD/kg MLSS/d) . The average yield in first stage was estimated to be 0.2 kg SS/kg COD removed which was 20-40 % lower than that in ASP with high loading. In second stage it was 0.1, comparable to empirical yield in low load ASP (0.15-0.2 kg SS/kg COD). The study on change of ecosystem was carried out on different pulp and paper industry wastewaters by Lee and Welander, 1996. The wastewater was first subjected to a treatment in a completely mixed aerobic reactor without biomass recirculation, favoring the growth of fast-growing dispersed bacteria consuming the readily biodegradable organic matter in the wastewater. After treatment at this stage, the wastewater was fed to a reactor for growth of predators consuming the bacteria. The apparent sludge yield in the process varied between 0.01 – 0.23. For a two-stage process, hydraulic retention time was equal to sludge retention time in the first stage which was very long. It was generally not feasible to apply the two-stage process in practice.

Sludge yield during the activated sludge treatment of TMP/newsprint effluent was reduced by 15%, 35% and 43%, by applying UV conditioning, anoxic conditioning and anoxic plus UV conditioning respectively. The reduction capabilities of these treatments were more pronounced when the treatment system was operated at a shorter sludge age (9 days), as opposed to a longer sludge age (19 days) (Ellott et al., 1999a). In a bench-scale trial for effluent of softwood bleached kraft mill, biomass generation was reduced upto 48% by conditioning recycled activated sludge with acidic first-stage kraft bleaching effluent in a

heated contact chamber. However, this intensive treatment of sludge reduced the BOD removal from 98% to 89%. In pilot plant trials, biomass generation was reduced by 26% in the same process without a heated contact chamber. Over a period of 23 days, sludge conditioning did not affect the removal of BOD and COD. Cost of neutralization of combined mill effluent and supplemental nutrient demand were also reduced by the sludge conditioning method (Ellott and Dorica, 1999b). Oxidative treatment using Fenton's reagent not only resulted in cell lysis but also decreased the charge density of flocs, which enhanced flocculation and settleability (Pere et al., 1993).

2.2 Different techniques applied to activated sludge (other than pulp & paper industry) for reduction of WAS

There are several processes to minimize the excess sludge generation in the biological wastewater treatment processes. The following section will present an brief overview of the significant processes:

2.2.1 Minimization of activated sludge by processes other than ozonation

Most of the researchers have worked on either biosludge of treatment of municipal wastewater or synthetic wastewater. Many of them have investigated the development of uncoupler chemicals in activated sludge process for minimization of excess sludge generation (Okey and Stensel, 1993; Low and Chase, 1998; Mayhew and Stephenson, 1998; Low et al., 2000; Chen et al., 2003; Yang et al., 2003). About 50% reduction in biomass was achieved at a pentachlorophenol (PCP) concentration of 30 mg/l as compared with a system without uncoupler (Okey and Stensel, 1993). Liu (2000) determined the observed yield by the ratio of uncoupler to biomass concentration, and reported that any dissociation of catabolism and anabolism induced by the presence of chemical uncoupler would result in a decrease of observed yield. Heavy metals and 2,4-dinitrophenol were used as chemical uncoupler using biomass from a laboratory scale oxic-settling anaerobic continuous system.

To induce uncoupled metabolism using synthetic substrate, p-nitrophenol was introduced to a bench scale activated sludge process. Presence of the protonphore caused a shift in the microbial population with protozoa being washed out of the system and proliferation of filamentous bacteria. Biomass production was reduced by 49%, but the total

substrate removal rate was also reduced by 25% (Low et al., 2000). A series of batch experiments fed with different concentration of individual metabolic uncouplers (p-chlorophenol, m-chlorophenol, m-nitrophenol and o-nitrophenol) in ASP were conducted at constant initial biomass and glucose concentration. Sludge generation was reduced with the increase of concentration of metabolic uncoupler from 0 to 20 mg/l. With application of the four chemical uncouplers, they achieved 58%, 86.8%, 65.5%, 86.1% of reduction in sludge respectively at a concentration of 20 mg/l each, while the COD removal efficiency was lowered by 8.9%, 13.5%, 13.2%, 26% respectively as compared to the control test. Among four metabolic uncouplers studied, m-chlorophenol was the most effective in reducing sludge production with less effect on the process performance. The application of chemical uncoupler for reduction of sludge increased the oxygen consumption and caused inefficient settling and dewatering. It has also been reported that after some time microorganisms became resistant to the chemical (Yang et al., 2003).

An oxic-settling anaerobic system was a simple modification of a conventional activated sludge process, in which thickened sludge from a final settling tank was returned to an aeration tank via a sludge holding tank. The working principle was to alternate anaerobic–aerobic cycling of activated sludge in order to stimulate catabolic activity, and make catabolism dissociate from anabolism, resulting in a lower sludge yield. Several authors studied the oxic and anaerobic cycling for minimizing sludge production (Ghiglizza et al., 1996; Copp and Dold, 1998; Chen et al., 2003). Liu et al., 1998 reported that observed growth yield was decreased as the substrate to biomass (S_O/X_O) ratio was increased. The biomass used for batch experiments was taken from a laboratory scale oxic-settling anaerobic continuous system**.** Such a phenomenon indicated that energy uncoupling between anabolism and catabolism occurs at higher S_O/X_O ratio.

To evaluate the significance of requirement of maintenance energy, Low and Chase, 1999b reported the effect of biomass concentration on its generation using sodium acetate as the carbon source. A laboratory chemostat containing *Pseudomonas putida* was coupled with a second stage in which the biomass in the effluent was concentrated and a portion was recycled to enhance the biomass concentration. Overall substrate removal efficiency remained constant at each of the various biomass concentrations investigated. Increasing biomass concentration from 3 to 6 g/l reduced biomass production by 12 %. Analysis of a

similar system indicated that increasing biomass concentration from 1.7 to 10.3 g/l, biomass production was reduced by 44 %. In a membrane bioreactor, solids retention time (SRT) can be controlled independently from hydraulic retention time (HRT), which resulted in higher sludge concentration (typically 15-20 g/l), and lower sludge loading rate. When the latter became low enough, little or no excess sludge was produced (Ghyooy and Verstraete 2000; Wagner and Rosenwinkel 2000; Rosenberger et al., 2002), but this option was quite expensive in terms of energy requirement and replacement of membrane due to fouling.

Saby et al., 2002 studied the effect of chlorination using two identical activated sludge membrane bioreactors that were continuously operated with synthetic wastewater. One pilot unit was used as the reference system without chlorination of excess sludge, while another served as a experimental unit. Excess sludge was taken out for conducting chlorination and the chlorinated dispersion was then returned to the aeration tank. It was observed that the sludge production could readily be reduced by 65% once chlorination was involved. Its principal disadvantages were the formation of trihalomethanes, poor sludge settlability and significant increase in soluble chemical oxygen demand in the effluent.

The reduced sludge production at high DO concentration was a consequence of DO induced metabolic changes of activated sludge. Wunderlich et al., 1985 showed that in high-purity oxygen activated sludge system, the sludge production was reduced from 0.38 to 0.28 mg VSS/mg COD removed and the SRT increased from 3.7 to 8.7 days. Abbassi et al., 1999 studied the effect of oxygen concentration on excess sludge generation using synthetic substrate and reported that the generation of sludge was reduced by 25% with the increase in concentration of DO from 2 to 6 mg/l when the organic loading was 1.7 mg BOD_5/ mg MLSS/ d. The increase of DO in bulk liquid resulted in sharp increase in the total oxygen demand and raised the aeration costs.

Springer, 1996 proposed a process for zero excess sludge by mechanically lysing the sludge in a Kady mill - a high shear device that generated heat before being recycled to the treatment reactor. The system was applied for biological sludge from a municipal wastewater treatment plant operated free of bulking. COD removal was 80%, whereas in conventional process it was 87%.

Integration of chemical and heat treatment of biosludge in biological wastewater treatment were temperature sensitive. About 60% of reduction of sludge was achieved when

the returned sludge was passed under thermal treatment at 90 ^{o}C for 3 h (Canales et al., 1994). Solubilisation of WAS obtained from brewery wastewater treatment plant was studied by self-digestion during anaerobic incubation at 60 ^{o}C after addition of NaOH at a final concentration of 0.01 N. Approximately 40% sludge became soluble within 4 days (Yuko et al., 1999). High temperature was also combined with acid or alkaline treatment to reduce or condition excess sludge. Rocher et al. (1999; 2001) showed that alkaline treatment by NaOH combined with thermal treatment (pH 10, 60 °C, 20 min) was the most efficient process to induce cell lysis. The coupling of lysis system to a bioreactor allowed a 37% reduction in the generation of excess sludge.

Chu et al., 2001 reported the effects of ultrasonic treatment on the physical, chemical, and biological characteristics of a WAS obtained from food processing unit. A critical ultrasonic power level existed above which, the floc structure was effectively disintegrated, microbial level was reduced and particulate organic compounds were sufficiently transformed into soluble state. Both ultrasonic vibration and rise in bulk temperature contributed to the treatment efficiency. Processes based on ultrasonic, thermal or thermo-chemical treatment for sludge hydrolysis suffered from high cost and poor-quality product.

2.2.2 Minimization of activated sludge by ozone application

When sludge was kept in contact with ozone in the ozonation unit, most of the activated sludge microorganisms were killed and oxidized to organic substances. There was evidence that more than 50% of the carbon obtained after ozonation was readily biodegradable (Deleris et al., 2000). This is the reason that organic substances produced during ozonation of sludge can be degraded in the post-biological treatment.

Yasui and Shibata, 1994 showed that ozonation enhanced biological degradation of activated sludge. In their experiments using synthetic substrate, MLSS concentration of 4200 mg/l was maintained in the aeration basin at 1 kg BOD /m^3/d loading without drawing excess sludge for six weeks using an ozone dose of 0.05 g O_3/g SS. TOC concentration in the effluent was slightly higher than those from the conventional ASP operated in this study.

In another study with recirculation of sludge was performed after ozonation in the activated sludge process for treatment of municipal and industrial wastewater generated from car manufacturing, in oil refinery, food, petrochemical and pharmaceutical industries. The

sludge elimination efficiency by ozonation was dependent on the nature of sludge generated during treatment of different wastewaters. In a full-scale operational experiment, no excess sludge was needed to withdraw and no significant accumulation of inorganic solids occurred in the aeration tank. Most of the inorganic compounds in the sludge were released to the soluble phase. Material balance indicated that one-third of ozonated sludge was mineralized, and thereby the requirement of sludge mass to be treated was 3.3 times as much as sludge to be eliminated. TOC of effluent was slightly higher than that of the conventional activated sludge process, indicating that refractory TOC was released from the sludge eliminated by treatment (Yasui et al., 1996). An activated sludge process which produced no excess sludge was developed at Shima sewage treatment centre. At an ozone dosing rate of 0.034 kg/kg SS, complete elimination of excess sludge was achieved when 4 times more amount of excess sludge was ozonated than that of the excess sludge expected in the treatment without ozonation. After 5 months of operation without any withdrawal of excess sludge, small amount of inorganic substances like sand and silt accumulated in the sludge (Sakai et al., 1997). In another study using synthetic sewage, activated sludge in the aeration tank was circulated via intermittent ozonation. 50% of the sludge generation was cut down with only 30% of the ozone dose required for continuous ozonation. Furthermore, the process had a remarkable effect on maintaining the sludge settling characteristics (Kamiya and Hirotsuji, 1998).

Egemen et al., 1999 carried out experiments by using excess biosludge from a continuous flow activated sludge system of municipal wastewater treatment plant by solubilising biosludge using ozone as the cell lysis agent, and then returned it to the aeration tank. The results of these preliminary studies indicated that the proposed process configuration had the potential to reduce the waste sludge production by 40% to 60%. Egemen et al., 2001 further investigated the ozone process to determine the maximum dissolution efficiency. For this purpose, a number of variables such as solid concentration in the excess sludge, ozonation time, and ozonation dosage rate were studied. Similarly a pilot-scale facility for municipal sludge was built to investigate the feasibility of the ozonation for sludge reduction and recycle. Ozonation of wastewater sludge resulted in reduction in mass and volume by mineralization, and improvement in dewatering characteristics (Ahn et al., 2002; Park et al., 2003). Sievers et al., 2004 described two full-scale sewage sludge ozonation process and subsequent aerobic as well as anaerobic stabilization as compared to

different sludge treatment processes. For both anaerobic and aerobic application, liquefaction of sludge with release of 110 and 160 mg COD per g total suspended solids (TSS) was reached at specific ozone consumption of 0.03 and 0.06 kg O_3 per kg TSS respectively. The subsequent biological treatment achieved a reduction in mass of 20–35% for the aerobic and 19% for the anaerobic stabilization. For both applications the specific ozone consumption was about 0.05 kg O_3 per kg TSS to be treated.

Partial ozonation of activated sludge decreased the production of excess sludge proportional to the amount of sludge ozonated when the ozone dosage did not surpass 0.05 g O_3 /g SS. With an ozone dosage of 0.05 g/g SS and a daily treatment of 10% of the activated sludge, sludge reduction amounted to 30% (Bohler and Siegrist, 2004; 2007). In conventional anaerobic/oxic phosphate removal system at 0.03 g O_3/g SS, 30% solubilisation of sludge was achieved; around 70% of sludge was inactivated by ozonation. At higher ozone consumption, degree of sludge solubilisation increased but the rate of solubilisation was found to decrease. An increase in concentration of soluble phosphorus was observed during ozonation (Saktaywin et al., 2005). Lee et al., 2005 carried out a study on pilot-scale activated sludge system coupled with sludge ozonation process at Jungrang wastewater treatment plant, operated for 112 days in winter season without excess sludge wasting. They observed that 2.5 –2.7 times more sludge than theoretical one was required at 0.05 kg O_3/ kg SS to get zero excess sludge generation. There was no accumulation of inorganic in biosludge; SS and COD in the effluent were identical to control.

For sequencing batch reactor (SBR) receiving partially ozonated returned activated sludge, the amount of TSS decreased linearly with ozone dose (Dytczak et al., 2006). Biomass in the alternating anoxic/aerobic reactor was easier to destroy (upto 25% reduction in comparison to the initial excess sludge) than in the aerobic (10%) reactor. Microscopic observation of sludge showed that biomass in the aerobic reactors consisted of abundant protozoa, and dense, spherical and compact flocs; while biomass in the alternating reactors consisted mostly of bacteria with fewer filamentous organisms, consisting of weak, thin and elongated flocs. Ozonation of 20% of RAS had no negative impact on the final effluent quality from the SBRs (Dytczak et al., 2007). The effects of ozonation on physical, chemical and biological properties of sludge before and after the treatment were investigated by Zhao et al., 2007. The sludge after ozonation was found to be fully compatible with the biological

system, a slight increase in effluent COD was observed but the efficiencies of COD and nitrogen removal were maintained (Paul and Debellefontaine, 2007). The combined treatment was achieved with a specific ozone dosage of 0.07 g O_3/g COD removed where the sludge yield was almost zero against that of about 0.33 g VSS/g COD removed in the control system. Similarly an evaluation of various operational parameters on the process of sludge ozonation was carried out based on semi-batch experiments by Manterola et al., 2008. The experiments were performed on sludge from urban WWTP. The solubilisation of organic matter was increased proportionally to ozone dosage at 25 and 35 mg O_3/g TSS upto the maximum COD of 430 mg/l.

The minimum threshold of ozone dose was found to be more than 0.04 g O_3/g MLSS. With the increase in ozone dose, the settlability and water content of sludge was improved but the filterability was deteriorated. There was a reduction in particle size, and increase in soluble COD, TOC, nitrogen and phosphorous with increase in ozone dose (Yan et al., 2009a). The authors studied the changes in biological nature of sludge obtained from municipal sewage treatment plant with ozone treatment. The results indicated that after the sludge was exposed to ozone at less than 0.02 g O_3/g TSS, the denaturing gradient gel electrophoresis (DGGE) fingerprint remained constant and there was still some enzyme activity, indicating that the sludge solubilization was the main process. At greater than 0.02 g O_3/g TSS, the bacteria began to break down and ozone was used to oxidize the bio-macromolecules such as proteins and DNA released from the sludge. At levels higher than 0.10 g O_3/g TSS, the disintegration of the sludge became slow and the microbes lost most of their activity; ozone was used to transform the bio-macromolecules into small molecules. However, at levels higher than 0.14 g O_3/g TSS, ozone failed to oxidize the sludge efficiently.

2.2.3 Effect of ozonation on effluent quality

Significant attention has been paid to the influence of sludge ozonation on effluent quality in the biological treatment process. During ozonation, dissolved and colloidal COD were released into the bulk solution, which led to an increase in CODs in the effluent during long-term operation. (Yasui et al., 1996; Kamiya and Hirotsuji, 1998; Deleris et al., 2002; Lee et al., 2005;). Paul and Debellefontaine, 2007 reported a slight increase in effluent COD.

The recirculation of ozonated sludge to the activated sludge process increased the nitrogen loading, and the total nitrogen concentration in effluent were found slightly higher as compared to the control run without sludge ozonation (Chiavola et al., 2007). According to Sakai et al., 1997 the slight increase in effluent SS was linked with a slightly higher effluent BOD concentration. Acid-insoluble materials, Fe and Al were more in sludge; difference in heavy metals, Mg and Ca was comparatively small.

The introduction of ozonation into activated sludge did not significantly influence effluent quality but improved the settling properties of the sludge. An operation with a suitable sludge wasting ratio was found to be necessary to prevent accumulation of inorganic and inert particles for long-term operation (Chu et al., 2009). Dignac et al., 2000 showed that sludge ozonation caused a slight increase in TOC in the effluent. The organic matter in the effluent following sludge ozonation was composed of protein and sugar moieties.

2.3 Ozonolysis of lignin and chlorophenolic compounds

Ozonolysis of lignin and its derivative compounds has widely been studied (Nkamura et al., 2004; De Los Santos Ramos et al., 2009; Mvula et al., 2009) due to low biodegradability of these compounds during biological treatment. Remediation of lignin and its derivatives by ozone oxidation resulted in increase of biodegradability of lignin decomposition products (De Los Santos Ramos et al., 2009). Chlorophenol compounds have been the subject of many studies in recent years in view of their environmental significance as persistent and potentially hazardous substances.

Compared to other oxidizing reagents, ozone oxidation was more efficient in pollutant degradation and no harmful compounds are added to treated waters. The degradation of chlorophenolics (CPs) with ozone was extensively studied by several authors (Duguet et al., 1986; Kawaguchi and Inagasaki, 1994; Kuo and Huang, 1995; Abe and Tanaka et al., 1997; Boncz et al., 1997; Trapido et al., 1997; Graham et al., 2003; Sung et al., 2008). The degradation of CPs was favored at high pH (Hautaniemi et al., 1998; Kuo, 1999; Benitez et al., 2000 & 2003; Graham et al., 2003). The faster degradation of these compounds in alkali media could be due to the fast production of $HO^{\bullet}$ radicals and the dissociation of CPs to

chlorophenolate ions that were able to react with ozone faster than non-dissociated species (Hoigne and Bader, 1983).

The reactivity of 2,4,6-trichlorophenol with ozone was studied at laboratory-scale using a simple gas bubble/liquid contacting system (Graham et al., 2003). Degradation rate constants were measured at pH 2 and 7.5 with an initial TCP concentration of 202 μM. At pH 7.5 and 15 mM ozone consumption, there was 90% degradation of TCP, which corresponded to the release of approximately 2 mol Cl^- ions per mol TCP. The presence of hydrogen peroxide in solution did not significantly increase the TCP degradation. The rate constants indicated that the reactivity of 2,4,6-TCP was much greater at neutral pH than at low pH. Manojlovic et al., 2007 reported a newly developed ozone generator consisted of coaxial dielectric-barrier-discharge reactor and operated in the air at atmospheric pressure. Ozone and ozonized water were generated in the same volume of the discharge. Five liter of water containing 6 mg/l of corresponding phenol was passed once through this ozonizer. The concentration of dissolved ozone in water was 7-40 mg/l. The ozonation lasted 1 h, but the total contact time was upto 94 h. Most of the phenol was removed during ozonation (89.5%). After 24 and 96 h the percentage removal of phenol increased to 93.3 and 98.9% respectively. With 4-chlorophenol and 2,4-dichlorophenol, 99.8 and 98.9% of corresponding phenols were removed. Sung and Huang, 2007 reported the identification of degradation product during direct ozonation of 2-chlorophenol in aqueous solution. Transient distribution of degradation products, in a semi-batch reactor under three ozone dosages were identified and determined by HPLC analysis. Results suggested that there was a dosage-dependent pathway in the direct ozonation of 2-chlorophenol. Oxalic acid was the major end product quantified.

Benitez, 2003 and Hong and Zeng, 2002 reported tetrachlorocatechol, tetrachlorohydroquinone and tetrachloro-p-benzoquinone as intermediate compounds during ozonation of PCP. These compounds were further degraded by ozone into other open-ring products like ketones and acids, and finally, into oxalic acid with quantitative release of chloride ions. Trapido et al. (1997) identified quinones during the ozonation of several CPs. Kuo and Huang (1995) detected chlorocatecol during the ozonation of p-chlorophenol.

Ozone treatment was carried out for the treatment of pulp mill effluents because of its high removal efficiencies of colour and AOX causing compounds and the increase of the wastewater biodegradability. Ozone selectively reacted with the chromophoric (colour

causing) and halogenated (AOX causing) functional groups through fast oxidation reactions as a result of the electrophilic nature of the compounds (El-Din and Smith, 2002).

From the extensive search of literature it is apparent that for the last two decades researchers across the world have studied the different techniques and aspects of sludge minimization, ozonation of municipal sludge and individual chlorophenolic compounds in aqueous phase.

Very scanty information is available on the application of ozonation method for WAS of the pulp and paper industry. Klas, 2006 worked on sewage and pulp and paper mill biosludge; response of ozone for biosludge from pulp and paper mill was poor but reasons could not be identified. The WAS from pulp and paper industry is different in chemical composition than sewage/ synthetic sludge. Many researchers have successfully worked on ozone supported excess sludge reduction from municipal / synthetic wastewater but a few works has been done on excess sludge generated form pulp and paper industry. There is considerable potential for studying the ozone assisted degradation of biosludge from pulp and paper industry; No work has been done to study the effect of ozonation on decontamination of WAS in term of organochlorine compounds. Influence of Ozone on different physical and chemical properties of WAS from pulp and paper industry has not been elucidated in detail which also necessitates further study.

Objectives of the research

Following two objectives were set for the research study:

- To study the potential of reduction of waste activated sludge at different ozone doses
- To study the potential of decontamination of the waste activated sludge from residual organochlorines

Chapter-III

3. Materials and Methods

3.1 Source of biosludge and wastewater

Sample of secondary sludge (biosludge) was collected time to time from the return line of secondary clarifier of the activated sludge process for the treatment of wastewater of pulp and paper mill in North India. This is an integrated kraft pulp and paper mill with a production capacity of 200–220 t paper/d. The mill uses mixed hard wood consisting of Eucalyptus (Eucalyptus globules) 45%, Veneer waste 25%, Poplar (*Populus alba*) 20% and Bamboo (*Bambusa ventricosa)* 10%. The bleaching sequence consists of C_D E_{OP} D_1 D_2 stages with 15-20% substitution of chlorine by chlorine dioxide in first acidic stage. The combined wastewater of different processes is clarified first in primary clarifier followed by secondary treatment in activated sludge process.

Wastewaters from chlorination (C_D) with partially chlorine dioxide substitution and alkaline extraction (E_{OP}) stages were collected which were the major sources of pollution especially of organochlorine compounds. The other source of COD and colour was spillage of black liquor from pulp mill and chemical recovery plant; weak black liquor of 16-17 % solid was collected.

3.2 Ozonation of biosludge

Ozone gas was generated in the lab with an ozone generator (INDOZ-30, ORAIPAL, India) with pure oxygen as feed gas. The capacity of ozone generator was 30 g/h at a concentration of 8-10%. The gas flow was measured by a rotameter. Another rotameter (2 lpm capacity) was used to feed the gas to the reactor; rest of the ozone gas was fed to thermal destructor (Figure 3.1).

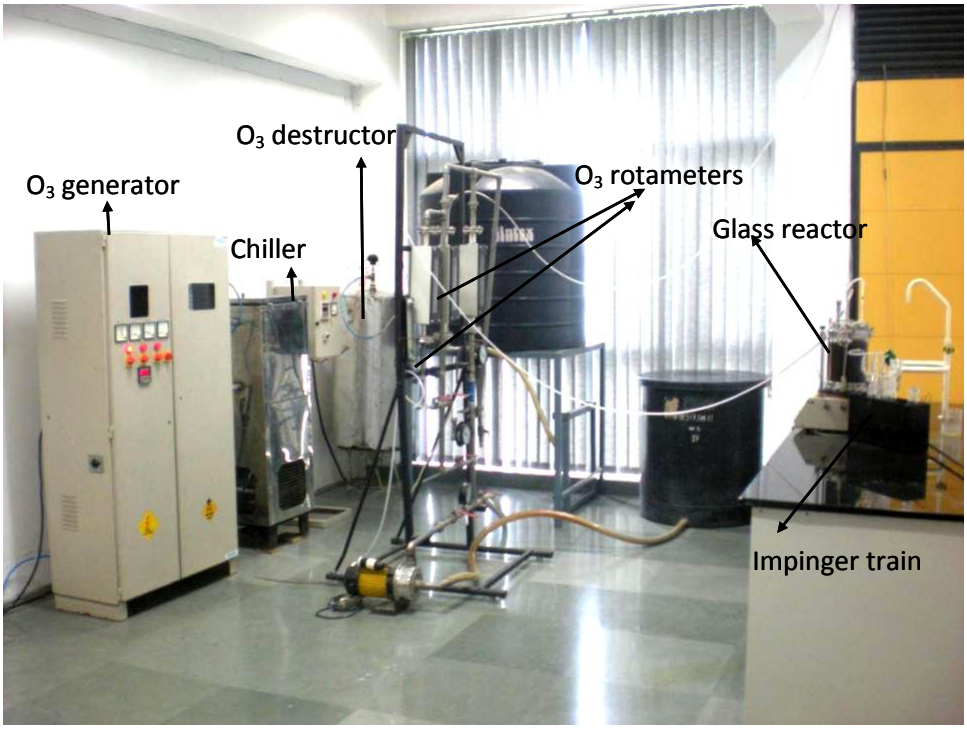


Figure 3.1: Set-up for ozone generation

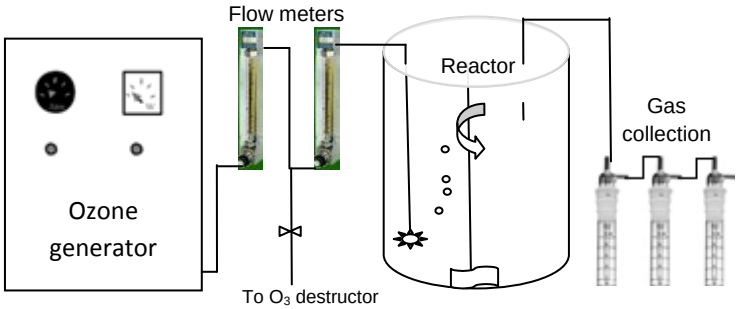


Figure 3.2: Schematic diagram of ozone treatment of biosludge

Ozonation of sludge was performed in a airtight glass reactor having 2-litre capacity (MD-250, L.E. Marubishi, Japan) with a provision of mechanical agitation at 1000 (revolution per minute) rpm. Measured volume of biosludge was taken in the glass reactor and O_3 gas was passed through G2 grade sintered diffuser dish (30 mm diameter). The gas was fed based on dry sludge (DS) at nearly neutral pH. Concentration of ozone in the feed gas was estimated as described by Masschelein et al., 1998. Unreacted ozone was estimated by connecting the outlet of reactor with impingers containing buffer KI solution (Figure 3.2), and dosage of ozone for treatment of sludge was calculated based on difference of feed and unreacted ozone.

3.3 Set-up for lab scale activated sludge process

Five laboratory scale bioreactors with capacity of 6 liter each (3 nos.) and 15 liter each (2 nos.) were used as aeration tank, followed with two settlers in series with 4.0 liter volume in each case (Figure 3.3). Activated sludge from the effluent treatment plant of the same pulp and paper mill was used as seed to start-up the bioreactors. The biosludge collected from the plant was acclimatized in 50 litre batch reactor before transferring to the continuous bioreactors.

The C_D and E_{OP} wastewaters were mixed in 2:1 ratio and WBL of 16-17% (w/v) solids was blended with it to get the requisite COD and AOX concentration in the feed. The resultant wastewater was diluted and used as the feed to bioreactors. The pH of the resultant wastewater was adjusted to 7.0 and filtered through high porosity filter paper (70 µm) to remove coarse particles. The filtrate was used as the substrate in the activated sludge process which was fed into bioreactors continuously with peristaltic pumps (Cole Parmer, USA). The wastewaters from C_D, E_{OP} streams and WBL were periodically collected from the mill and stored at 4-5 °C.

In continuous bioreactors, the temperature was maintained with glass tube immersion heater and controlled with proportional integral derivative (PID) controller. The compressed air was distributed through ring shape sand diffuser at the bottom of bioreactor and flow of air was regulated with air rotameter. The mixed liquor was kept in suspension with a mechanical agitator at 300 rpm. Urea and technical grade phosphoric acid were used as the

source of nitrogen and phosphorous respectively, and added based on soluble chemical oxygen demand (CODs) removal: N: P::100: 5: 1 with peristaltic pump.

3.4 Ozonation of biosludge and post treatment in ASP

The collected biosludge from the plant was acclimatized in the continuous bioreactors which were operated to simulate the conditions of effluent treatment plant treating pulp and paper mill effluent. The concentration of AOX compounds in wastewater from C_D and E_{OP} stages was 64.3±1.6 and 40.1±0.6 mg/l respectively. The concentration of CODs in weak black liquor (WBL), C_D and E_{OP} stage wastewaters was 149667±3512, 1141±39 and 1650±219 mg/l respectively during the study. After running the bioreactors in identical conditions, one bioreactor was kept as control and 3 bioreactors were used for ozonation of sludge at different amount of sludge and dosage of ozone. During acclimatization, the excess sludge from the bioreactors was removed on daily basis to maintain mixed liquor volatile suspended solids (MLVSS) concentration in the bioreactor. During removal of excess sludge, biomass was completely recycled to aeration tank from clarifiers and pipes. The amount of MLVSS equivalent to excess sludge was removed. The ozonated biomass was kept in a vessel under agitation at 10-12 °C by circulating chilled water in the outer chamber and fed continuously to the aeration tank with a peristaltic pump.

The recycling of disintegrated sludge in ASP was carried out in three phases, phase I (PI): first 16 days, phase II (PII): intermediate 16 days and phase III (PIII): final 20 days to evaluate the performance of biological reactors and sludge generation. The schematic diagram of ozonation of biosludge and post treatment of it is given in Figure 3.4.

Figure 3.3: Set-up for lab scale activated sludge process

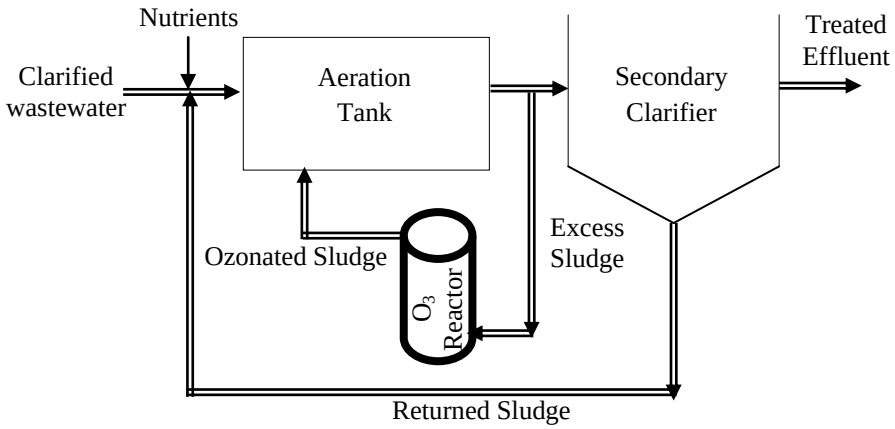


Figure 3.4: Schematic diagram of ozonation and post treatment of biosludge

3.5 Methods for characterization of biosludge and wastewater

The biosludge and wastewater samples were characterized as such as well as after pretreatment like settling/ filtration/ centrifugation etc. In case of ozonation of biosludge, the ozonated sample was allowed to settle for 8-10 h at 4 °C and the supernatant was filtered through 70 μm porosity filter paper to remove the coarse material before analysis of the sample for various parameters. Figure 3.5 summaries different pretreatments followed for analysis of various parameters.

Most of the chemicals used were of analytical or Spectroscopic grade and procured from Qualigen (Fisher Scientific), India. 4-chlorophenol (standard for AOX) was procured from Merck, Germany, whereas dichloromethane (standard for POX) and 1,2,4-trichlorobenzene (standard for EOX) were procured from Sigma Aldrich. The chlorophenol standards were procured from Helix Biotech Corporation, Canada except 2,3,4,6-tetrachlorophenol which was procured from Lancaster, England and 2,4,5-trichlorophenol and pentachlorophenol which were procured from Sigma Aldrich.

3.5.1 Characterization of physical parameters

3.5.1.1 pH (APHA- 4500 H)

pH measurement is the determination of the activity of hydrogen ion by potentiometric measurement using a pH meter (L1-127, make Elico, India).

- The pH meter was calibrated and equilibrium between electrodes and sample was established by stirring sample to ensure homogeneity.
- Electrode was rinsed with distilled water 2-3 times after reading the pH of sample and placed in the beaker containing distilled water.

3.5.1.2 Total suspended solids (APHA- 2540 D)

A well-mixed sample was filtered through a weighed glass fiber filter (Whatman GF/C, 1.2 μm pore size) and the residue retained on the filter was dried upto a constant weight at 105±2 °C. The increase in weight of the filter paper represented the total suspended solids.

- The glass microfiber filter papers were placed in the oven and the initial weight was noted after desiccating.
- A well mixed sample having at least 2.5 mg residue was filtered through glass micro fiber filter.
- It was washed 2-3 times with reagent grade water to remove any entrapped TDS.
- Filter paper from filtration apparatus was carefully removed and transferred to the oven for drying for at least 2 hour at 105±2 °C in the oven, cooled in desiccator and weighed.

Calculation:

Total suspended solids, mg/l = (A-B) x 1000/V

where,

A: final weight of filter paper and dried residue, mg

B: initial weight of filter paper, mg

V: volume of sample, ml

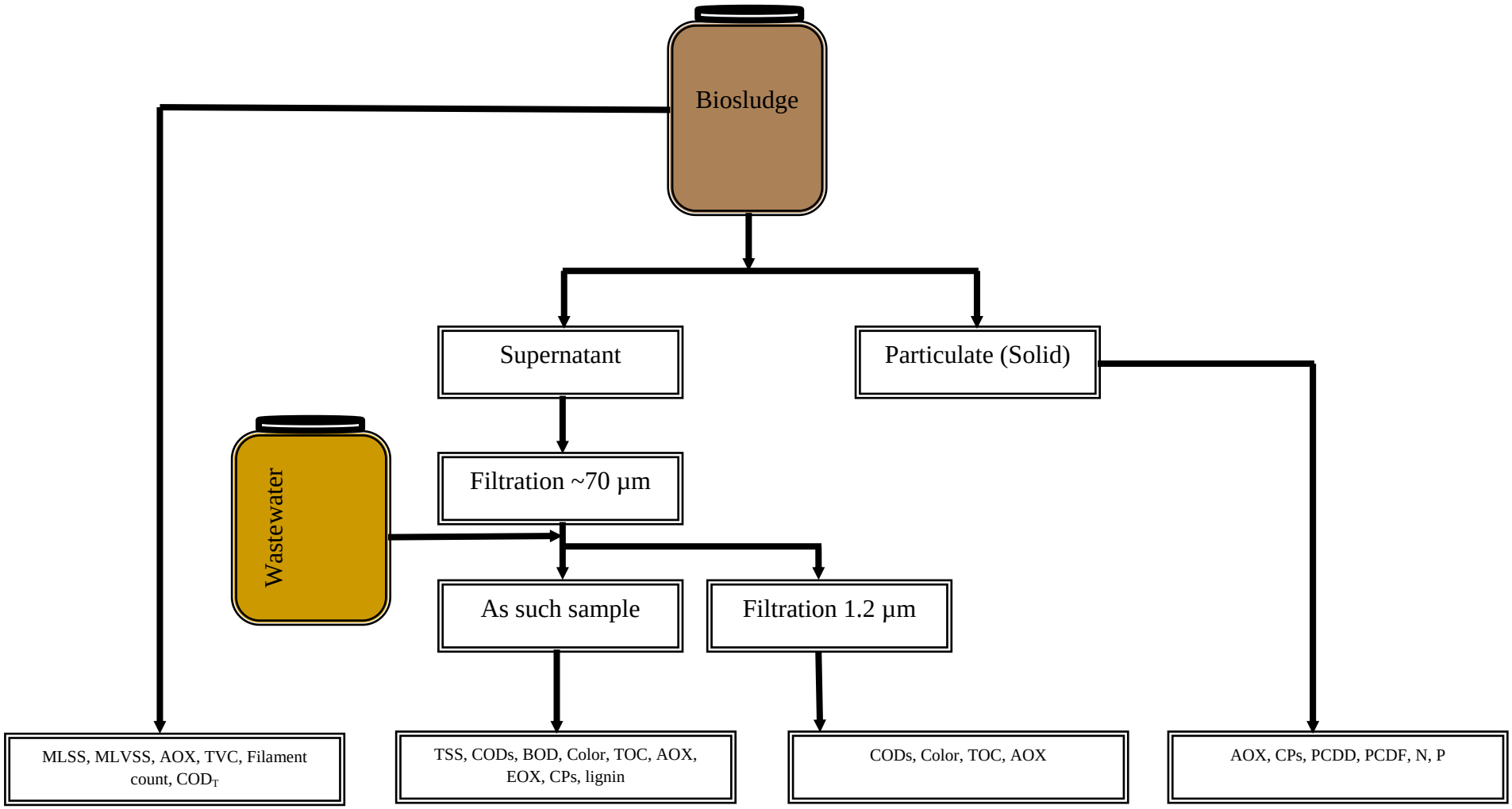


Figure 3.5: Sample preparation steps for various parameters

3.5.1.3 Total dissolved solids (APHA- 2540 C)

A well-mixed sample was filtered through a standard filter paper and the filtrate was evaporated to dryness in a weighed glass beaker and dried to the constant weight at 180±2 °C. The increase in weight of the beaker represented the total dissolved solids in the sample.

- A well-mixed sample was filtered through GFC filter paper and washed with distilled water.
- The whole filtrate was transferred in already weighed beaker (250 ml) and the latter was placed on the hot plate
- After complete evaporation of water, the beaker was transferred into oven at 180±2 °C and dried to the constant weight, cooled in desiccator and weighed.

Calculation:

Total dissolved solids, mg/l = (A-B) x 1000/V

where,

A: final weight of the dried residue and beaker, mg

B: initial weight of the beaker, mg

V: volume of sample, ml

3.5.1.4 Colour (APHA- 2120 C and manual of Hach spectrophotometer)

The platinum-cobalt method was adopted for measuring the property of wastewater in which colour is due to naturally occurring material.

- A calibration graph was prepared by using standards of 100, 200, 300, 400 and 500 Pt-Co units with UV-Vis spectrophotometer (Cary-100, make Varian, USA) .
- The samples were brought to room temperature and the pH was adjusted to 7.6 with H_2SO_4/NaOH of such a concentration that the resulting volume change did not exceed 2%.
- The suspended particles were removed by using Whatman filter paper no.1
- The absorbance was measured at 455 nm and concentration of colour was calculated by using standard graph.

Calculation:

Colour, Pt-Co unit = Colour reading x Dilution factor

3.5.1.5 Mixed liquor suspended solids and volatile suspended solids

MLVSS is the indirect measurement of the biomass present in the aeration basin. MLSS consist of micro-organisms, non–biodegradable organic and inorganic matter.

- 50-100 ml of well mixed sample was taken and centrifuged at 6000 rpm for 5 minutes.
- Supernatant was discarded and residue was washed with distilled water to remove dissolved salts
- The sample was again centrifuged and residue was transferred to pre weighed crucible and oven dried at 105±2 °C for overnight. The increase in weight of the crucible was taken as MLSS.
- The crucible was ignited at 550±25 °C in muffle furnace for 3 h and loss in weight was taken as MLVSS content in the sample.

Calculation

Mixed liquor suspended solids (g/l) = (A–B) ×1000/V

Mixed liquor volatile suspended solids (g/l) = (A–C) ×1000/V

where,

A: weight of the crucible + dried sample, g

B: weight of the empty crucible, g

C: weight of the crucible + ash, g

V: volume of sample, ml

3.5.1.6 Dissolved oxygen (APHA- 4500 O G and manual of YSI DO meter)

For activated sludge process, polarographic method was preferred to have a real time concentration of DO.

- The DO probe (YSI-58, USA) was placed in calibration chamber and calibrated by water saturated air method.
- DO probe was dipped in sample under agitation and DO values were recorded in mg/l.

3.5.1.7 Oxygen uptake rate (APHA- 2710 B and manual of YSI DO meter)

Oxygen Uptake Rate (OUR) is used to determine the rate of oxygen consumption by the microorganisms in a suspension. The sample was saturated with air and depletion of oxygen was measured with time to calculate the OUR.

- The DO probe was placed in calibration chamber and calibrated by water saturated air method.
- The temperature of the mixed liquor was noted and same was maintained during the experiment.
- Sample container was filled to overflowing with an appropriate volume (700 ml) of a representative sample of the biological suspension.
- The dissolved oxygen probe was inserted and oxygen uptake rate assembly was screwed.
- The sample was aerated at a flow rate of 1 lpm for 5 minute, initial dissolved oxygen was noted.
- The depletion in dissolved oxygen was noted with time until a constant value of DO.
- Graph was plotted against dissolved oxygen (mg/l) versus time (second) and slope of the line of best fit was determined. The slope represents the oxygen uptake rate in milligram per litre per second.

3.5.1.8 Sludge volume index (APHA- 2710 C)

Sludge Volume Index (SVI) is an overall parameter used to characterize the sludge thickening behavior. SVI is the volume in milliliter occupied by 1g of a suspension after 30 min settling. For highly bulking sludge, the sample is diluted upto an extent to have settling in the range of 200 ml.

- The MLSS content of the activated sludge was determined as per 3.5.1.5
- 1000 ml of the stirred biosludge was poured into a 1000 ml measuring cylinder and allowed to settle for 30 minutes.

- For the bulking nature of sludge, the biosludge sample was diluted upto an extent to have settling below 200 ml.

Calculation

SVI (ml/g)= V x f/ W

Where

V: volume of sludge settled, ml

f : dilution factor for DSVI

W: weight of MLSS in sample, g/l

3.5.1.9 Dissolved charge and zeta potential (manual of PCD 03 and SZP 06)

The biosludge and wastewater from pulp and paper mill carries anionic or cationic charge. The colloidal charge or ionic behavior of wastewater was examined with particle charge detector (PCD 03, make Mutek, Germany) which analyzed the colloidal dissolved charge in the form of streaming potential. The surface charge on sludge was determined in the form of zeta potential by zeta potential meter (SZP 06, make Mutek, Germany).

- For estimation of dissolved charge, 10 ml sample was taken in titration cell of PCD 03 pH meter and titrated with 0.001 N Poly diallyldimethylammonium chloride (DADMAC).
- For estimation of zeta potential, 500 ml mixed liquor was taken and analyzed with SZP 06.
- The zeta potential was directly read on the meter in mV.

Calculation

Dissolved charge, meq/l= V x N x f x 1000/10

where,

V: vol. of poly DADMAC consumed, ml

N: normality of poly DADMAC, eq/l

f : dilution factor

3.5.2 Characterization of chemical parameters

3.5.2.1 Chemical oxygen demand (APHA- 5220 B)

Organic matter is mostly oxidized by a boiling mixture of potassium dichromate and sulfuric acid, and oxidisable matter is calculated in terms of oxygen equivalent. The digestion of samples was performed in a COD digester (make Spectralab, India). For estimation of soluble COD (CODs), the suspended material was allowed to settle and supernatant was filtered through coarse filter paper (70 µm porosity) to remove any coarse particle.

- A pinch of $HgSO_4$ was added to COD vessel and 5 ml sulphuric acid reagent was added very slowly with stirring to dissolve $HgSO_4$. 10.0 ml sample (as such or after dilution) containing COD less than 900 mg/l was placed in COD vessel.
- 5 ml of 0.25 N $K_2Cr_2O_7$ solution was added followed by 10 ml of sulfuric acid reagent. The air condenser was attached; mixture was mixed for homogenization and refluxed for 2 hour at 150 °C.
- After digestion, the condenser was washed with distilled water. The reflux condenser was disconnected and mixture was diluted to about twice its volume (60 ml) with distilled water.
- The samples were cooled to room temperature and excess $K_2Cr_2O_7$ was titrated with ferrous ammonium sulphate solution (FAS) using 2-3 drops of ferroin indicator. The end point was the sharp colour change from blue-green to reddish-brown.
- In the same manner, blank containing the reagents and distilled water were treated.
- Normality of FAS solution was estimated by taking 5 ml of 0.25N $K_2Cr_2O_7$ and 15 ml of H_2SO_4.

Calculation

COD as mg O_2/l = (A–B) x N x 8000/ V

where,

A: vol. of FAS used for blank, ml

B: vol. of FAS used for sample/standard, ml

N: normality of FAS

V: vol. of sample, ml

3.5.2.2 Biochemical oxygen demand (IS 3025: part 44)

Determination of the biochemical oxygen demand (BOD_3) is an empirical test in which standardized laboratory procedures are used to determine the relative oxygen requirement of effluent and polluted waters.

- Dilution water was prepared by bubbling compressed air in distilled water for overnight at 27 °C.
- One ml of each phosphate buffer, magnesium sulphate, calcium chloride and ferric chloride solution were added for each liter of dilution water and mix thoroughly. 1 ml of treated wastewater (after aerobic biological treatment) was also added as seed for each liter of dilution water.
- pH of dilution water was adjusted to 7.0 by using NaOH or HCl.
- Suitable portion of the sample was taken in one-liter volumetric flask, diluted to one liter, and mixed thoroughly. Three BOD bottles (300 ml each) were filled with the above solution.
- Entrapping of air bubble was avoided during mixing and transfer of sample.
- Properly marked two bottles were kept for 3 days in incubator at 27 °C. The DO of these bottles was determined after third day. Similarly 2 bottles were prepared for blank.
- The DO concentration in the first bottle was determined immediately. Similarly 2 bottles were prepared for blank.

Calculation

BOD_3, mg/l = $[(D_1-D_2)]-[(B_1-B_2)]$ x f

where,

D_1: initial DO of diluted sample/standard, mg/l

D_2: final DO of diluted sample/standard after 3 days incubation at 27 °C, mg/l

B_1: initial DO of blank sample, mg/l

B_2: final DO of blank sample after 3 days incubation at 27 °C, mg/l

f : dilution factor

3.5.2.3 Adsorbable organic halogen (AOX) in wastewater (ISO 9562: 1989)

The water sample was acidified with nitric acid and the AOX compounds in the water sample were adsorbed on activated carbon. The inorganic halogen was removed with sodium nitrate solution and loaded carbon was ignited at 950 °C in a stream of oxygen, the combustion converted the organically bound halogens to hydrogen halides. The halogen content in the flue gases was determined by microcoulometric titration with total chlorine analyzer (ECS-1200, make Thermo Scientific, USA).

- The instrument was turned on and oxygen gas was allowed to flow. After stabilization of temperature and the background current, the microcoulometric titration system for Cl^- detection was validated by injecting various amounts of the sodium chloride calibration standards directly into the titration cell.
- 100 ml as such or diluted sample having AOX content near to 0.1 mg/l was taken and 50 mg of activated carbon, 5 ml of nitrate stock solution were added.
- The sample was shaked for at least 2 hour on a mechanical shaker at 200 rpm and filtered through a quartz frit under nitrogen gas pressure.
- The filter cake was washed with about 25 ml of nitrate wash solution in several portions and finally with distilled water.
- After filtration and washings, the moist filter cake together with the quartz frit was placed into quartz combustion boat.
- The quartz boat was introduced into the heated zone of the quartz tube under oxygen stream at 950 °C and AOX content of sample was calculated after coulometric titration.

Calculation

AOX, mg/l = (S-B) x f/1000

where

S: AOX content of sample, µg/l

B: AOX content of blank, µg/l

f : dilution factor

3.5.2.4 Adsorbable organic halogen in sludge

The dewatered sludge sample was dispersed in acidified water and the AOX compounds in the suspension were adsorbed on activated carbon and estimated as per section 3.5.2.3.

3.5.2.5 Extractable organic halogen (EOX) in wastewater (DIN 38409-P 8)

The organically bound halogens were extracted from the water in two stages using hexane. The extract was burnt in an oxygen-argon atmosphere and mineralized products were determined by microcoulometric titration with total chlorine analyzer (ECS-1200, make Thermo Scientific, USA).

- 1000 ml of sample was taken in a beaker and pH of the sample was adjusted to 6-8 with sulphuric acid or sodium hydroxide solution. 20 g of sodium sulphate and sample were added to a 1000 ml volumetric flask having a low-level measuring mark.
- A magnetic bead was added and flask was placed on magnetic stirrer.
- 15 ml of hexane was added and sample was stirred for 10 minutes at 1000 rpm. The extract was pipetted off carefully after phase separation and transferred to a 50 ml volumetric flask.
- The sample was extracted 2 times more with another 15 ml aliquot of hexane and separated as earlier. 5 ml of hexadecane was added and volume made upto 50 ml with hexane (hexane: hexadecane = 9:1).
- The instrument was stabilized and validated as per 3.5.2.3
- 50 µl blank solution (hexane + hexadecane) was taken in syringe and injected in EOX cassette. Similarly, 50 µl aliquot of sample and standard (trichlorobenzene, 10 mg/l) were run in the same way as blank.

Calculation

EOX, mg/l = (S-B) x f x 50 / (1000 x 1000)

where,

S: EOX content of sample, µg/l

B: EOX content of blank, µg/l

f : dilution factor

3.5.2.6 Extractable organic halogen in sludge (EPA Method 9023)

The sludge sample was extracted with ethyl acetate with the help of probe sonicator to isolate organic halides and extracted sample was injected into a pyrolysis furnace using a stream of oxygen and argon gas; hydrogen halide, the pyrolysis product was determined by microcoulometric titration.

- An aliquot of dewatered sludge containing approximate 1g of sludge on OD basis was taken in 25 ml glass vial. 1 ml of reagent water was added followed by 5 ml of ethyl acetate to the sample and cap was placed tightly.
- The sample was shaked vigorously for 30-60 seconds and the suspension was agitated directly with a sonic probe. The sample was sonicated 3-4 times for one minute duration under ice bath.
- The suspension was kept at 4 °C and centrifuged at 10,000 rpm under cooled condition for five minutes.
- The ethyl acetate layer was transferred to a clean 10 ml vial.
- The instrument was stabilized and validated as per 3.5.2.3
- Two blank samples containing 50 µl of ethyl acetate were injected followed by 50 µl aliquots of the trichlorobenzene working standard solution and samples into the furnace.

Calculation

EOX, mg/ kg = (S-B) x 5 x 1000/ (1000 x W)

where,

S : EOX content of sample, mg/l

B : EOX content of blank, mg/l

W: OD weight of sample, g

3.5.2.7 Purgeable organic halogen (POX) in sludge (DIN 38414 - part 18 and EPA method 9021)

- An aliquot of dewatered sludge containing approximate 1g of sludge (on OD basis) was taken in two POX sample bottles containing 80 ml reagent water and dispersed.
- The instrument (ECS-1200, make Thermo Scientific, USA) was stabilized and validated as per 3.5.2.3
- The purge and furnace temperature were set at 60 and 950 °C respectively.
- Two sample bottles containing reagent water were placed in POX cassette for 15 minutes and the temperature was raised to 60 °C.
- After attaining the temperature the gas needles were inserted in the bottle for purging of volatile organochlorine compounds.
- The purged gases were passed through the heated zone of the quartz tube under oxygen stream at 950 °C and POX content of sample was calculated after coulometric titration.
- In the similar way, sample (dispersed sludge) and standard solution (dichloromethane) were run to estimate the POX compounds.

Calculation

POX, mg/ kg = (S-B) x 1000/(1000 x W)

where,

S : POX content of sample, µg/l

B : POX content of blank, µg/l

W: OD weight of sample, g

3.5.2.8 Metals in sludge (APHA- 3111 B)

- Five to ten g of sludge was taken in beaker, dispersed in 50 ml water and acidified with conc. HNO_3.
- Additional 5 ml conc. HNO_3 was added and evaporated on a hot plate to 15-20 ml.

- 10 ml of conc. HNO_3 and 5 ml of $HClO_4$ was added, and evaporated gently on a hot plate until dense white fumes of $HClO_4$ just appeared.
- If necessary, last step was repeated to complete the digestion of sludge.
- The sample was dried completely on sand bath, ground and baked to convert silicic acid to silica.
- The dried material was dissolved in 1:1 HCl by heating and filtered through Whatman no. 42 ashless filter paper and volume made upto 100 ml.
- The various metals were analyzed using atomic absorption spectrophotometer (NovAA 300 (flame) and NovAA 400 (furnace), make Analytik jena, Germany).

3.5.2.9 Chlorophenolic compounds in wastewater (NCASI method CP-85.01)

The method comprised of in-situ derivatization, extraction and determination of the chlorophenolic compounds by gas chromatography (GC-450, make Varian, Netherland).

- 50 ml (450 ml for low concentration) of sample was neutralized to pH 7.0-7.1 with sulphuric acid/ NaOH, 1.3 ml of potassium carbonate (60 % w/v) was added and pH was adjusted to 11.6±0.1
- The sample was transferred to a 125 ml separating funnel and spiked with 25 µl of internal standard (dibromophenol).
- 1 ml of acetic anhydride was added and sample was shaked with frequent venting and allowed to stand for 5 minutes.
- 5 ml of hexane was added and shaked vigorously for 2 minutes with frequent venting and allowed to stand for phase separation.
- In case of formation of emulsion, the extracted phase was centrifuged and clear hexane layer was stored in 5 ml chromatographic vials with teflon-lined screw cap.
- The standard solution of each of the chlorophenolic compound was prepared by dilution of the primary standard stock solution (Table 3.1).
- Known quantity of secondary stock standard (2.5 to 200 µl) was spiked in 50 ml of reagent grade water and processed in a manner analogous to sample.

- The GC with ECD detector was used for the determination with fused silica VF-1 column (15 m, 0.25 mm I.D) and nitrogen as carrier gas.
- Injector temperature was 210 °C and gas flow through the column was 0.8 ml/min. The column oven was programmed from initial 45 to 100 °C at 15 °C/min and then at 2 °C/min to 120 °C. The temperature was increased at 5 °C/min to 150 °C and then at 20 °C /min to 250 °C. The holding time at 45, 120 and 150 °C was 1, 20 and 0.7 minute respectively. The temperature and make-up gas flow in detector was 300 °C and 25 ml/min respectively.
- 1 µl sample/ standard was injected for split less injection technique. The area of individual compound based on its retention time (Table 3.2) was calculated after getting the chromatogram of the mixed standard solution (Figure 3.6).
- The calibration curve was plotted for each compound taking concentration at X axis and area at Y axis, and equation for linear regression was calculated.

Calculation

Concentration (µg/l) = ((As/ Ais) x Aisa x 1000)/ (Ms x V)

where,

As : area of the analyte ion

Ais : area of the internal standard in sample

Aisa : average area of the internal standard

Ms : slop from calibration curve for analyte ion

V : volume of sample taken for extraction

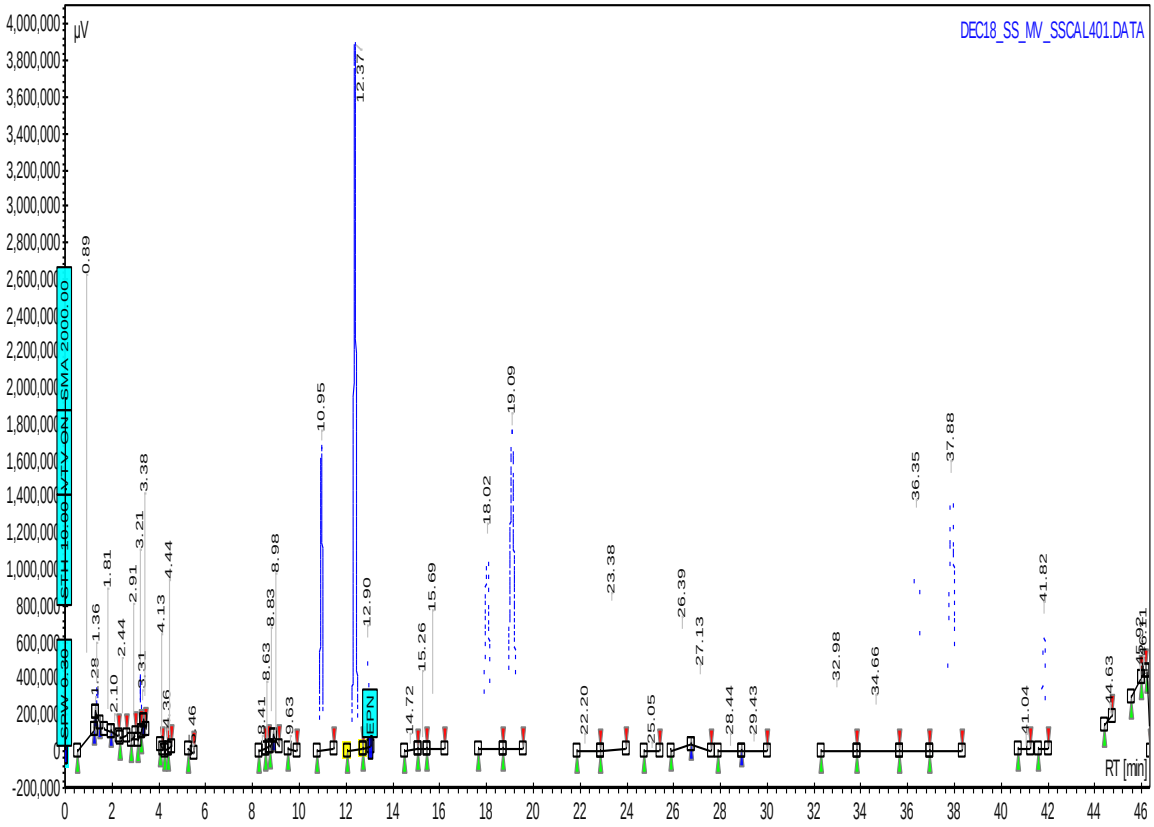


Figure 3.6: Chromatogram of mix standards of 12 chlorophenolic compounds

Table 3.1: Concentration of 12 chlorophenolic compounds in standard solution

Compound	Primary stock solution (mg/50 ml)	Standard solution (mg/25 ml)
2,4,6-trichlorophenol	40.20	0.4020
2,4,5-trichlorophenol	42.00	0.4200
2,3,4,5-tetrachlorophenol	41.54	0.4154
3,4,6-trichloroguaiacol	36.12	0.7224
3,4,5-trichloroguaiacol	40.40	0.8080
4,5,6-trichloroguaiacol	40.28	0.8056
3,4,6-trichlorocatecol	40.32	1.6128
Pentachlorophenol	10.50	0.2100
3,4,5-trichlorocatecol	42.96	1.2888
Tetrachloroguaiacol	42.28	0.8456
Trichlorosyringol	40.62	0.8124
Tetrachlorocatecol	39.76	0.7952
Dibromophenol (IS)	40.20	0.4020

Table 3.2: Retention time and detection limit of 12 chlorophenolic standards

Compound	RT (min.)	Detection limit (µg/l)[#]	Detection limit (µg/kg)*
2,4,6-trichlorophenol	10.95	0.05	21.1
2,4,5-trichlorophenol	12.90	0.16	71.2
2,3,4,5-tetrachlorophenol	18.02	0.06	25.9
3,4,6-trichloroguaiacol	19.09	0.10	44.3
3,4,5-trichloroguaiacol	23.38	0.11	50.3
4,5,6-trichloroguaiacol	26.39	0.11	49.0
3,4,6-trichlorocatecol	27.13	0.19	85.1
Pentachlorophenol	32.92	0.02	10.7
3,4,5-trichlorocatecol	34.66	0.12	51.6
Tetrachloroguaiacol	36.35	0.13	58.7
Trichlorosyringol	37.88	0.12	55.1
Tetrachlorocatecol	41.82	0.07	30.8
Dibromophenol (IS)	12.37	-	-

for 450 ml of wastewater sample
* for 1 g of biosludge sample

3.5.2.10 Chlorophenolic compounds in sludge (NCASI method CP-85.01)

The dewatered biosludge samples were dispersed with the help of probe sonicator. The method involved in situ derivatization, extraction and determination of the chlorophenolic compounds by gas chromatography.

- An aliquot of dewatered sludge containing approximate 1g of sludge on OD basis was taken in 250 ml of flat bottom glass bottle and 25 ml reagent water was added.
- The sample was sonicated 2 times for one minute duration under ice bath.
- The sample was spiked with 25 µl of internal standard. 2 ml of potassium carbonate was added (pH: 11.6±0.1) under agitation (magnetic stirring) followed by 4 times sonication (for 1 minute duration each time) under ice bath.
- 2 ml of acetone was added under agitation to the suspension and sonicated 2 times (for 1 minute duration each time) under ice bath.
- 1.5 ml of acetic anhydride was added under agitation followed by 2 minutes of sonication. The addition of acetic anhydride produced significant foaming.
- 0.5 ml of acetic anhydride was added at last to kill the foam and 5 ml hexane was added under agitation.
- Finally the sample was sonicated 2 times (for 1 minute duration each time) under ice bath and agitated for 10 minutes under ice bath with air tight teflon lined cap.
- The sample was centrifuged at 4 °C and 6000 rpm and water layer was removed by syringe.
- After removal of complete water, the sample was centrifuged at 4 °C and 10000 rpm to separate the hexane from sludge.
- The clear solvent was collected in chromatographic vial and processed as 3.5.2.9.

Calculation

Concentration, (µg/kg) = (As/ Ais) x Aisa x 1000/(Ms x W)

where,

As : area of the analyte ion

Ais : area of the internal standard in sample

Aisa : average area of the internal standard

Ms : slop from calibration curve for analyte ion

W : OD weight of sludge

3.5.2.11 Total organic carbon (APHA- 5310 C)

The wastewater samples were oxidized with persulphate-ultraviolet light and evolved CO_2 was estimated by NDIR detector (HyperTOC, make Thermo Scientific, USA).

- For organic carbon, calibration curve was prepared with potassium hydrogen phthalate from 0-1000 mg/l range.
- For inorganic carbon, calibration curve was prepared with a mixture of Na_2CO_3 and $NaHCO_3$ from 0-1000 mg/l range.
- The filtered sample was transferred in TOC vial and total carbon content were measured in triplicates.
- The inorganic carbon content was measured using nitric acid to convert carbonates and bicarbonates into carbon dioxide.

Calculation

TOC, mg/l = ((Cso-Bso) – (Csi- Bsi)) x f

where,

Cso: total carbon content of sample, mg/l

Bso: total carbon content of blank, mg/l

Csi : inorganic carbon content of sample, mg/l

Bsi : inorganic carbon content of blank, mg/l

f : dilution factor

3.5.2.13 Nitrogen in sludge (IS 10158- 1982)

The sample was digested with concentrated sulphuric acid in the presence of catalyst to convert the organic nitrogen into ammonium sulphate and nitrogen content was estimated as per micro-Kjeldahl method.

- An aliquot of dewatered sludge containing approximate 1g of sludge on OD basis was taken into the 100 ml Kjeldahl flask.
- 5 g of anhydrous sodium sulphate, 0.68 g of copper sulphate, 0.068 g of selenium powder and 15 ml of conc. sulphuric acid were added to the flask.
- Flask was placed in an inclined position on heater and closed loosely with hollow glass stopper to prevent the loss of sulphuric acid and entry of dust.
- Mixture was heated gently in a fume cupboard until the initial frothing was ceased, and further heated until the solution became clear and then boiled for 2 h more.
- The sample was cooled and transferred into the 100 ml volumetric flask using water.
- Distillation assembly was fitted and 10 ml of the digested sample was added into distillation flask followed by 10 ml of NaOH solution.
- 50 ml of distillate was collected in the indicator boric acid solution and ammonia present in the distillate was titrated with sulphuric acid.

Calculation

Nitrogen, % = 14 x V x N x 10 x 100/ W

where,

V: volume of standard H_2SO_4 used in titration, ml

N: normality of H_2SO_4

W: weight of sample taken on oven dried (OD) basis, g

3.5.2.15 Phosphorous in sludge (APHA- 4500 P E)

The sludge was acid digested to convert organically bound phosphates to soluble orthophosphate which was measured spectrophotometrically.

- An aliquot of dewatered sludge containing approximate 5 g of sludge on OD basis was taken in the glass beaker and digested with mixture of perchloric - nitric acid until solution became colorless.
- The sample was cooled, diluted with distilled water and filtered through whatman no. 42 into 100 ml volumetric flask.
- A part of the sample was taken, pH was adjusted to neutral and excessive colour (if present) was removed by shaking the sample with 200 mg of activated carbon in an Erlenmeyer flask and filtered.
- 50 ml or less of sample, containing 0.5 to 2.0 mg of phosphorus (as P) was taken and 8 ml of combined reagent (H_2SO_4 + ammonium molybdate + potassium antimonyl tartrate) was added and mixed.
- In the same way reagent blank and standard solution (0.05-2.0 mg/l) were processed
- Absorbance was measured against reagent blank at 880 nm with Hach-2500 D spectrophotometer within 10-30 minutes.

Calculation

Phosphorus (as P), % = C x 100 x 100/ (1000 x 1000x W)

where,

C: concentration of phosphorus from calibration curve, mg/l

W: weight of sample taken on OD basis, g

3.5.2.16 Lignin in wastewater (CPPRI TMI-A8)

This method involves the determination of concentration of soluble lignin by measuring the absorbance at 280 nm wavelength (Cary-100, make Varian, USA).

- Lignin standard were prepared with indulin (softwood pine lignin) and mixed hard wood extracted lignin in the range of 10 to 100 ppm concentration by dissolving in 0.05 N NaOH solution.
- The samples were filtered and the absorbance of the samples was measured at 280 nm wavelength with ultraviolet-visible spectrophotometer.

Calculation

Lignin, mg/l = A x 0.0239

where,

A : absorbance at 280 nm

0.0239: multiplication factor calculated from the equation of linear line

3.5.2.17 Chloride in wastewater (APHA- 4500 Cl^- D, F and manual of Orion 960 and IC 882)

The chloride concentration in higher range (<100 mg/l) were estimated with potentiometric method using Orion 960 titrator (make Orion, USA), whereas the same at lower concentration (<20 mg/l) were estimated with ion chromatograph (IC-882, make Metrohm, Switzerland).

- For potentiometric analysis, 10 ml of sample was treated with 2 ml of concentrated nitric acid and titrated with 0.0141 N $AgNO_3$ solution.

Calculation

Chloride, mg/l = V x 0.0141 x 35.5 x 1000/10

where,

V: volume of standard silver nitrate consumed, ml

- The ion chromatograph was calibrated with requisite chloride standard solutions and concentration of the same in sample was calculated directly.

3.5.2.18 Ozone gas concentration (Masschelein et al., 1998)

This method involves the determination of ozone concentration in outlet of ozone generator as well as residual ozone concentration in gas coming out from reactor by iodometric method.

- 2% buffer KI solution was taken in 3 impingers placed in train (150, 50 and 50 ml).
- The ozone gas was supplied at 1 lpm flow rate from gas rotameters for 1 minute to first impinger inlet (for estimation of gas concentration) or from outlet of sludge reactor (for estimation of residual ozone).
- After reaction, the KI solutions from all the impingers were mixed in a flask and 10 ml 2N H_2SO_4 was added.
- The liberated iodine was titrated with thiosulphate solution.

Calculation

Concentration of ozone, mg/l of gas = $24 \times V_T \times N / V_G$

where,

V_T: volume of thiosulphate consumed, ml

N : normality of thiosulphate solution, eq/l

V_G: volume of gas passed, l

3.5.3 Characterization of biological parameters

3.5.3.1 Total viable count (APHA- 9215 C)

- 18 g nutrient agar media (NAM) was dispersed in 1000 ml distilled water and sterilized at 15 psi pressure and 121°C for 15 minutes.
- NAM plates were prepared by pour-plate technique.
- The samples were diluted by serial-dilution method by taking 9 ml distilled water (autoclaved) and one ml of sample.
- 100 µl of as such or diluted sample was poured on NAM plate and spreaded with aseptic spreader in duplicate.

- Plates were incubated at 37 ºC temperature for 24 h and bacterial colonies grown on NAM were counted in duplicate plates.

Calculation

Number of cells/ 100 µl = Cell count x dilution factor

3.5.3.2 Filamentous organisms (Jenkins et al., 1993)

The filament organisms were examined with Optical Microscope (Axio Scope-A1, make Zeiss, USA)

- The sludge samples were mixed properly to have homogenous sample. 2 ml of mixed liquor was taken in 100 ml volumetric flask and volume made upto the mark with NaOH solution having pH 10. The deflocculation of sludge was better in NaOH solution.
- Different dilutions of sludge sample were made for counting of filamentous organisms. 1000 time dilution was found suitable for counting; 20 µl of this dilution contained 200-250 count of filamentous organisms.
- 20 µl of diluted sample was placed on a clean glass slide in the form of drop and dried in oven at 45 °C temperature.
- One glass slide was marked with fine marker into 0.5 to 0.8 mm squares and the sample slide was placed on marked slide.
- The whole drop was observed under image analyzer at 10x magnification with the help of marked slide and image of each portion of sample was carefully captured and saved.
- The number and length of filamentous organisms were measured using the image analysis software.

Calculation

Total extended filament length (volume basis), µm/ml MLSS = N x L x f

Total extended filament length (weight basis), µm/g MLSS= N x L x f/ W

where,

N: total no. of filament

L: average length of filaments, µm

f : dilution factor

W: conc. of MLSS, g/ml

3.5.3.3 Morphology of organisms (Jenkins et al., 1993)

- The sludge samples were mixed properly to have homogenous sample
- Two separate drops of the sample were poured on glass slide and one drop was covered with glass cover slip.
- The morphology was studied at 5 and 10x resolution with Optical Microscope.

3.6 Parameters for evaluation of process performance

3.6.1 Hydraulic retention time (HRT) in bioreactor

The treated wastewater coming out of the clarifier was collected in buckets during night period to know the flow rate of wastewater.

HRT, h = V/ Q

where,

V: volume of bioreactor, l

Q: flow rate of wastewater, l/h

3.6.2 Food to microorganism (F/M) ratio

F/M ratio = CODsr /(HRT x A_{MLVSS})

where,

CODsr : reduction in CODs, mg/l

A_{MLVSS}: concentration of MLVSS in the bioreactor, mg/l

HRT : hydraulic retention time, d

3.6.3 Organic load

Organic load, $kg/m^3/d$ = CODsr/V

where,

CODsr: reduction in CODs, kg/d

V : volume of bioreactor, m^3

3.6.4 Sludge retention time (SRT)

SRT, d = A_{MLSS} x V/(B+C+D)

where,

A_{MLSS}: concentration of MLSS in the bioreactor, g/l

V: vol. of bioreactor, l

B: amount of biomass removed for excess sludge, g/d

C: amount of biomass removed for MLSS estimation, g/d

D: amount of TSS in the treated wastewater, g/d

3.6.5 Specific dissolution of biosludge

Specific dissolution = A/B

where,

A: amount of solubilised MLSS or MLVSS, mg

B: dosage of ozone, mg

3.6.6 Specific oxygen consumption rate

Specific oxygen consumption rate, (mg/g/h)= OUR x 60 x 60/ W

where,

OUR: Oxygen uptake rate, mg/l/s

W: MLVSS concentration, g/l

3.6.7 Sludge yield

The sludge yield was presented as biomass generation/g of CODs removal during biological treatment. The various parameters were evaluated on daily basis to estimate the sludge yield.

Sludge yield =(A+B+C+D)–(E+F)/CODsr

where,

A : amount of MLVSS in the bioreactor, g

B : amount of MLVSS taken for analysis per day, g

C : VSS amount in effluent per day, g

D : amount of MLVSS removed for excess sludge per day, g

E : VSS amount in influent per day, g

F : amount of MLVSS in bioreactor on previous day, g

CODsr: CODs removal per day, g

Chapter-IV

4. Characterization of biosludge

The biosludge collected from the pulp and paper mill was characterized for the physio-chemical properties and biological nature. The same sludge was used as seed for continuous bioreactor i.e. activated sludge process.

4.1 Physio-chemical characteristics of biosludge

The nature of biosludge from effluent treatment plant of the integrated pulp and paper mill had resemblance with the characteristics of the influent wastewater. The pH of biosludge was near neutral; 6.8-7.8. The colour of biosludge was brown due to presence of various lignin compounds and its derivatives in the wastewater; part of the same was adsorbed on the biosludge during biological treatment of the wastewater. The concentration of MLSS in returned sludge was 4.0 to 8.5 g/l; the MLVSS content was 70.8±5.0%, which was perceived as the concentration of microbial biomass in the sample (the value represent mean±standard deviation). The carbon content of the dewatered sludge was 32.1±1.1%, whereas the Kjeldahl nitrogen content was 5.4±0.1%. Based on MLVSS, carbon and nitrogen contents were 45.3% and 7.6% respectively. The amount of these two elements was quite similar to that in the bacterial cell (Tchobanoglous et al., 2003). The sulpher content in the biosludge was relatively higher (0.5±0.2%) due to contamination of sulpher bearing compounds in black liquor accompanied with the pulp in the integrated pulp and paper mill. Phosphorous content in the sludge was comparatively lower than that present in bacterial biomass; the concentration was 412±19 mg/kg.

The biosludge carried negative surface charge and the Zeta potential was -16.5±0.8 mV. Flocs in activated sludge have been reported to carry negative charge at neutral pH, usually between -10 to -20 mV (Jia et al., 1996). This is due to ionization of the anionic functional groups, such as carboxyl and phosphate. As the electro-negativity of the floc surface is quite high (less than -20 mV), repulsion might occur and the particles might move apart; thus, the settling properties of the sludge deteriorate (Morgan et al., 1990). The dissolved charge in the aqueous layer was -0.06 to -0.08 meq/l.

The biosludge was rich in calcium which was upto 4% of the dry weight of biosludge (Table 4.1). Other metals like sodium, potassium, magnesium, iron and aluminum were also present in significant amount. Traces of heavy metals like Cr, Zn, Cu, Co were also observed. Heavy metals like Hg, As and Se were not detectable. The possible sources of the metals were the raw materials (wood and others), process chemicals (NaOH, lime, PAC, fillers, make-up salt, dyes and pigments etc.) and chemicals (alum, lime etc.) used in wastewater treatment.

4.1.1 Characterization of organochlorine compounds

Application of chlorine or chlorine derivatives e.g. ClO_2 during bleaching of pulp generates organochlorine compounds. AOX is a measure of the total halogens, which are present either as dissolved or adsorbed on suspended organic matter in the wastewater sample.

The AOX content of the sludge is determined by adding observed AOX content and POX content. The POX content is added to compensate the amount of AOX compounds that may be lost during drying and grinding of biosludge for dispersion of the same (DIN, 1989). To evaluate the volatilization of AOX compounds of biosludge samples due to heating, sludge samples were dried at 45 and 100 °C for overnight and in the presence of sun light (with an average temperature of 45-47 °C) for 12 h duration. Highest loss of AOX compounds was observed at 100 °C (49.0%). The extent of volatilization of AOX compounds at 45 °C and under sun light was 20.1 and 29.6% respectively (Table 4.2). Volatilization of AOX compounds from biosludge was dependent on extent of temperature. Shomar (2007) reported a reduction in AOX compounds upto 66% of its original concentration in the sludge due to exposure in the sunlight for three months. The purgeable organic halogen content of the biosludge was negligible. As an advanced method for determination of AOX in biosludge, the latter was dispersed in water with a sonicator and proceeded as per the method adopted for the samples in soluble or colloidal condition.

Table 4.1: Metallic contamination in biosludge

Parameter	Value (mg/kg)
Calcium	41467±3129
Magnesium	4685±2326
Aluminum	4190±1937
Sodium	1917±956
Iron	1854±546
Potassium	1727±480
Manganese	106±25
Zinc	74±2
Copper	24±6
Chromium	22±15
Nickel	15±4
Cobalt	2.0±1.1

Table 4.2: Effect of drying on reduction in AOX compounds in biosludge

Drying temperature (°C)	AOX (mg/kg)	Reduction (%)
45 (overnight)	1782 ± 14	20.1
45-47 (12 h sunlight)	1571 ± 36	29.6
100 (overnight)	1138 ± 47	49.0

AOX of original sample: 2231±137 mg/kg

The volatile organochlorine compounds were adsorbed on biosludge during biological treatment; van der Waals forces, chemical or hydrogen bonding might also be involved (Juhasz et al., 2002). These compounds were stripped off by application of higher energy i.e. heat during drying but were not purgeable in aqueous medium at 60 °C. The increase in temperature enhanced penetration of organochlorine compounds through cell wall (Wandan et al., 2006). The release of water vapors during the drying process facilitated the release of volatile compounds, whereas desorption and release of these compounds was not possible with application of lesser energy during estimation of POX in aqueous phase. Negligible release of volatile compounds during estimation of POX resulted in lower AOX content than actual concentration of the same. There was no loss of POX compounds during disintegration of sludge with sonication. The method gave higher AOX value than estimated as per DIN (1989).

The concentration of AOX in dewatered biosludge collected from the mill at different intervals was 2119±200 mg/kg, whereas the same was 7.3±1.4 mg/l in the aqueous phase; AOX in the influent to secondary treatment was 11.7±0.9 mg/l. The concentration of AOX compounds in biosludge was dependent on its concentration in the influent. There was 36-38% removal of organochlorine compounds during biological treatment.

The EOX content of biosludge was 641±84 mg/kg in the dewatered sludge and the same was 0.23±0.02 and 0.09±0.02 mg/l in the influent and aqueous phase respectively. The ratio of EOX/AOX compounds in dewatered sludge and aqueous phase was 30 and 1.2% respectively. Due to lipophilic nature of EOX compounds, their accumulation in biosludge was higher as the cell membrane is composed of lipids.

The biosludge samples were also characterized for twelve chlorophenolic compounds identified by USEPA for regulation (Figure 4.1). Chlorophenolic compounds include phenols, guaiacols, syringols and catechols substituted with one to five chlorine atoms. 11 compounds were detected in the wastewater before biological treatment except 4,5,6-trichloroguaiacol. The concentration of the chlorophenolic compounds in feed wastewater varied from 0.42±0.13 for PCP to 8.45±0.72 µg/l for 3,4,5-TCC. The total concentration of all the chlorophenolic compounds in the feed wastewater was 37.9±2.1 µg/l. During biological treatment a part of the organochlorine compounds was retained on biosludge. The latter was contaminated with 10 chlorophenolic compounds. The concentration of 3,4,5-TCG and 4,5,6-TCG was below detection limit, whereas other compounds were present in significant amount. The

concentration of 3,4,5-TCC was highest i.e. 0.319±0.032 mg/kg (Table 4.3). The toxicity of these compounds increase with increase in number of chlorine atom. Pentachlorophenol is the most toxic compound in the 12 members of chlorophenolic compounds.

Table 4.3: Chlorophenolic compounds in feed, biosludge and treated wastewater

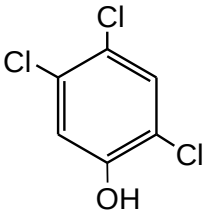
Compound	RT (min.)	Feed (µg/l)	Treated wastewater (µg/l)	Biosludge (mg/kg)
2,4,6-trichlorophenol	10.95	7.16±0.90	1.92±0.13	0.311±0.023
2,4,5-trichlorophenol	12.90	0.56±0.15	<0.16	0.085±0.014
2,3,4,5-tetrachlorophenol	18.02	1.30±0.29	<0.06	0.132±0.005
3,4,6-trichloroguaiacol	19.09	1.25±0.28	0.45±0.08	<0.044
3,4,5-trichloroguaiacol	23.38	1.91±0.16	0.82±0.13	0.089±0.038
4,5,6-trichloroguaiacol	26.39	<0.11	<0.11	<0.049
3,4,6-trichlorocatecol	27.13	5.96±0.50	3.94±0.38	0.240±0.025
Pentachlorophenol	32.92	0.42±0.13	0.22±0.04	0.259±0.032
3,4,5-trichlorocatecol	34.66	8.45±0.72	4.22±0.87	0.319±0.032
Tetrachloroguaiacol	36.35	3.21±0.31	1.04±0.20	0.064±0.012
Trichlorosyringol	37.88	4.48±0.85	2.7±0.47	0.233±0.037
Tetrachlorocatecol	41.82	3.20±0.32	2.11±0.41	0.311±0.054

The toxicity of chlorophenolic compounds was calculated in a similar fashion to that of chlorinated dioxins and furans by use of a relative toxicity scale (Berry et al., 1991; Kovacs et al., 1993)**.** This scale provides a single value from the attribute of chlorophenolic compounds present in the wastewater on chronic toxicity to fish, expressed in terms of its pentachlorophenol toxicity equivalency (TEQ). Based on the degree of chlorination, the toxicity equivalent factor (TEF) has been assigned (Table 4.4). The respective concentration of the compound is calculated by multiplying the absolute value with the TEF value; the TEQ due to 12 chlorophenolics from feed, treated wastewater and biosludge was 9.46, 4.29 µg/l and 717 µg/kg respectively.

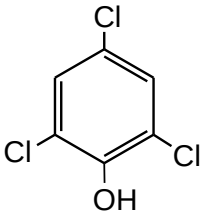
Table 4.4: Chronic toxicity equivalency factor (TEF) of chlorinated phenolic compounds

Degree of chlorination of phenolic compound	TEF value
Monochloro	0.03
Dichloro	0.07
Trichloro	0.20
Tetrachloro	0.40
Pentachloro	1.00

Source: Berry et al., 1991; Kovacs et al., 1993



2, 4, 5- Trichlorophenol



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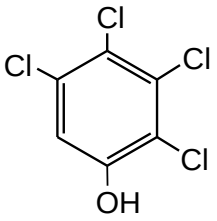
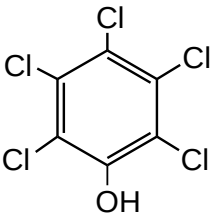
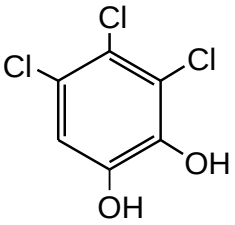


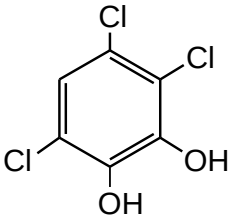
2, 3, 4, 5- Tetrachlorophenol



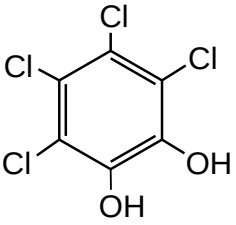
Pentachlorophenol



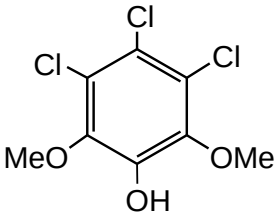
3, 4, 5- Trichlorocatecol



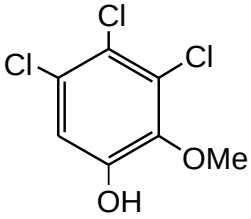
3, 4, 6- Trichlorocatecol



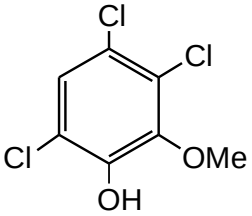
Tetrachlorocatecol



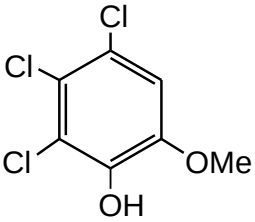
Trichlorosyringol



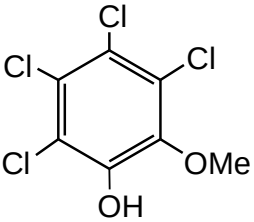
3, 4, 5- Trichloroguaiacol



3, 4, 6- Trichloroguaiacol



4, 5, 6- Trichloroguaiacol



Tetrachloroguaiacol

Figure 4.1: Structure of 12 chlorophenolic compounds

Dewatered biosludge was characterized for PCDD and PCDF compounds as per EPA 1613 by isotope dilution method using HRGC-HRMS (ALS Laboratory Group, Czech Republic). The concentration of, most toxic compounds of PCDD/Fs family, 2,3,7,8-TCDD and 2,3,7,8-TCDF was 16 and 210 pg/g of dry sludge (DS) respectively (Table 4.5). The upper bound I-TEQ from PCDD/Fs was 54 pg/g DS. The dioxin congener consists of two benzene rings connected by two oxygen bridges. There are eight positions where substitution of hydrogen atoms by other atoms or by organic or inorganic radicals can occur (Figure 4.2). 2,3,7,8-TCDD is one of 75 dioxin congeners and is the most toxic. The chlorinated dibenzofurans have similar structure, but have only one oxygen bridge rather than two. 2,3,7,8-TCDF is the most toxic of 135 chlorinated dibenzofurans.

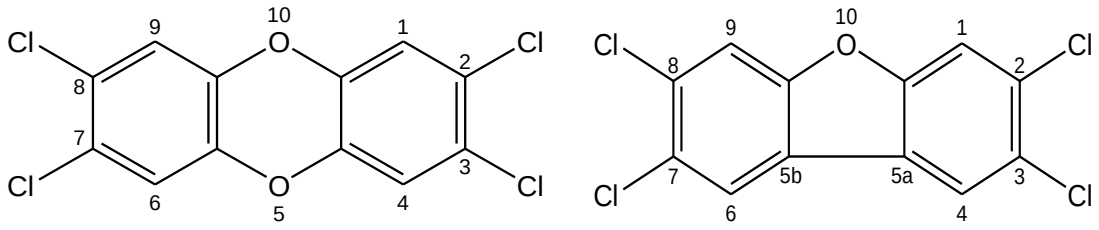


Figure 4.2: Structure of 2,3,7,8-TCDD (a) and 2,3,7,8-TCDF (b)

Typically, bleaching process with chlorine results in the formation of 2,3,7,8-TCDD and 2,3,7,8-TCDF and also higher substituted tri-, tetra-, and penta-chlorinated compounds (USEPA, 2000). An enzyme mediated formation of PCDD and PCDF from 2,4,5- and 3,4,5-trichlorophenol has been reported to be responsible for the biogenic formation (Fiedler, 2003). Dioxin and furan are not effectively degraded during wastewater treatment; they pass into receiving waters untreated or adsorbed in the biosludge (USEPA 2000). The present finding corroborates with the earlier observations (Leuenberger et al., 1985). Elementary chlorine free (ECF) bleaching minimizes the formation of chlorophenols and PCDD/Fs. Still measurable quantities of PCDD/Fs are formed.

Table 4.5: Concentration of PCDD and PCDF compounds in biosludge

PCDD/PCDF	TEF	Biosludge (pg/g DS)
2,3,7,8-TCDD	1	16
1,2,3,7,8-Pe CDD	0.5	<2.0
1,2,3,4,7,8-Hx CDD	0.1	ND
1,2,3,6,7,8- Hx CDD	0.1	2.2
1,2,3,7,8,9-HxCDD	0.1	<2.4
1,2,3,4,6,7,8-HpCDD	0.01	250
OCDD	0.001	8300
2,3,7,8-TCDF	0.1	210
1,2,3,7,8-PeCDF	0.05	7.4
2,3,4,7,8-PeCDF	0.5	6.1
1,2,3,4,7,8-HxCDF	0.1	<1.4
1,2,3,6,7,8- HxCDF	0.1	<1.4
1,2,3,7,8,9- Hx CDF	0.1	ND
2,3,4,6,7,8-HxCDF	0.1	3.0
1,2,3,4,6,7,8- HpCDF	0.01	23
1,2,3,4,7,8,9-HpCDF	0.01	ND
OCDF	0.001	87
Lower bound I-TEQ from PCDD/Fs	-	52
Upper bound I-TEQ from PCDD/Fs	-	54

Estimation of uncertainty of each PCDD/F congener: 30%

4.2 Characterization of biosludge for biological parameters

The biosludge samples were rich in pin point flocs and filamentous organisms. The average diameter and length of the latter was 1-1.2 and 50-54 μm respectively. The flocs

were diffused in nature; higher organisms like protozoa and nematode were not observed (Figure 4.3). The poor morphological nature of biosludge was due to low dissolved oxygen (DO) concentration in the aeration tank. The DO values (at the surface to 2 feet depth) were 0.4-0.6 and the same was 0.2-0.3 mg/l at the bottom of the aeration tank.

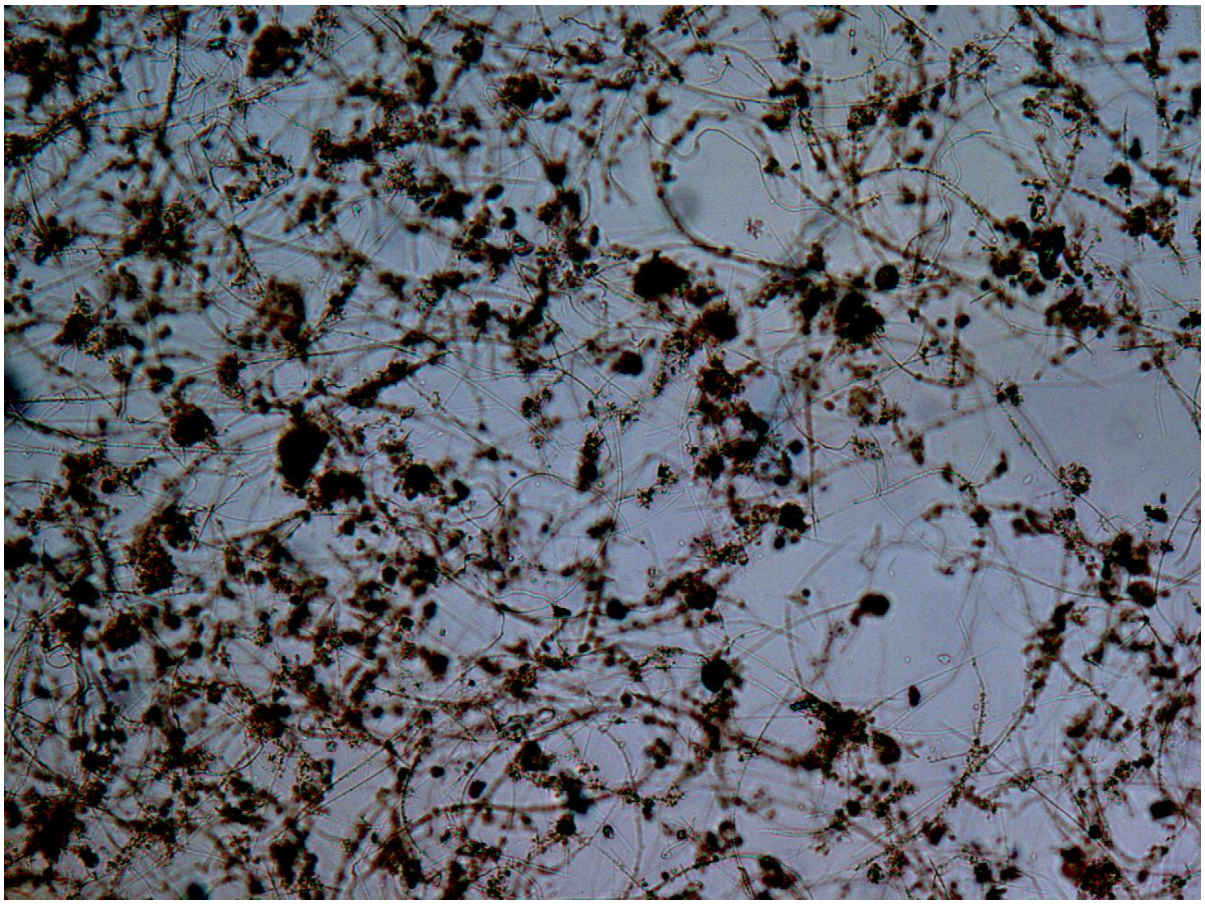


Figure 4.3: Morphology of biosludge from ASP of pulp and paper mill (5 x)

The collected samples were diluted to 10^4 to 10^5 times with autoclaved water for determination of total viable count which was 1.01 x 10^7 to 1.30 x 10^7/ 100 µl sample. The average filament count for the biosludge samples was 5.28 x 10^7/ml when the MLSS content of the same sample was 7.68±0.12 g/l. The average extended filament length was 2.81 x 10^9 µm/ml of MLSS or 3.66 x 10^{11} µm/g of MLSS. About 64% of the filamentous organisms were 1-50 µm in length and the maximum length of the filamentous organisms was as high as 400 µm.

Summary

pH of the biosludge samples was in the neutral range and colour was dark brown due to sorption of lignin and its derivative compounds. The surface charge of biomass was negative in nature. Biosludge was rich in calcium, sodium, potassium, magnesium, iron and aluminum. Traces of some other heavy metals like Cr, Zn, Cu, Co were also observed. It contained excessive amount of filamentous organisms and pin point flocs. The flocs were diffused in nature; higher organisms were absent.

The estimation of AOX content after drying and grinding resulted in lower concentration of AOX compounds. The method using dispersion of biosludge with sonicator overcame the limitation of the DIN 38414 method and provided a more precise and accurate concentration of AOX compounds in biosludge. The concentration of AOX and EOX compounds in dewatered biosludge was 2119 and 641 mg/kg respectively. The ratio of EOX/AOX compounds in dewatered sludge and influent was 0.3 and 0.02 respectively. Due to lipophilic nature of EOX compounds, accumulation of the same was higher in biosludge. Eleven out of 12 chlorophenolic compounds were detected in the wastewater before biological treatment except 4,5,6-trichloroguaiacol. The total concentration of all the chlorophenolic compounds in the feed wastewater was 37.9 µg/l. During biological treatment the part of the chlorophenolic compounds was retained on biosludge which was contaminated with 10 chlorophenolic compounds. Toxicity equivalence due to chlorophenolics in feed, treated wastewater and biosludge was 9.46, 4.29 µg/l and 717 µg/kg respectively. The biosludge was contaminated with significant concentration of polychlorinated dibenzodioxin and dibenzofurans. The concentration of 2,3,7,8-TCDD and 2,3,7,8-TCDF was 16 and 210 pg/g DS respectively. The upper bound I-TEQ from PCDD/Fs was 54 pg/g DS.

Chapter-V

5. Evaluation of sludge yield in activated sludge process

During biological treatment of wastewater, the organic substrate is decomposed to simple end product and energy is liberated. A part of this generated energy is utilized by microorganisms for new cell growth. The production of new biomass depends on nature of the substrate, operating parameters and type of process. To evaluate the sludge yield in activated sludge process treating pulp and paper mill wastewater, one lab scale bioreactor having ASP configuration was run for more than 6 months. The characteristics of wastewater fed to the bioreactor during the study are given in Table 5.1.

Table 5.1: Characteristics of the wastewater used as feed in the experimental bioreactor

Sample	CODs (mg/l)	BOD:CODs ratio	AOX (mg/l)	Colour (Pt-Co)
C_D wastewater	1150±225	1:1.9	90±20	600±30
E_{OP} wastewater	1225±235	1:3.8	55±15	1500±90
Weak black liquor	149667±3512	1:5.7	-	471400±10824

The bioreactor was run under ideal operating and environmental conditions as given in Table 5.2. The pH of the feed was neutral and temperature during the study was 36.0±0.3 °C. The dissolved oxygen was maintained near to 1.5±0.3 mg/l and HRT was 8.6±0.3 h. The average sludge retention time was 18 days during the study. The concentration of CODs in the feed was 536±11 mg/l and reduction of CODs was 68±3%. Based on CODs removal, the organic load and F/M ratio were 0.98±0.06 kg/m^3/d and 0.28±0.02 d^{-1} respectively. The biomass was flocculating in nature and SVI was 39±14 ml/g throughout the study. The free filamentous organisms were not observed and higher organisms like protozoa and rotifer were present in good number. The average sludge yield during the study was 0.31±0.02 g/g of CODs removal, the sludge yield varied from 0.27 to 0.33 g/g of CODs removal (Figure 5.1).

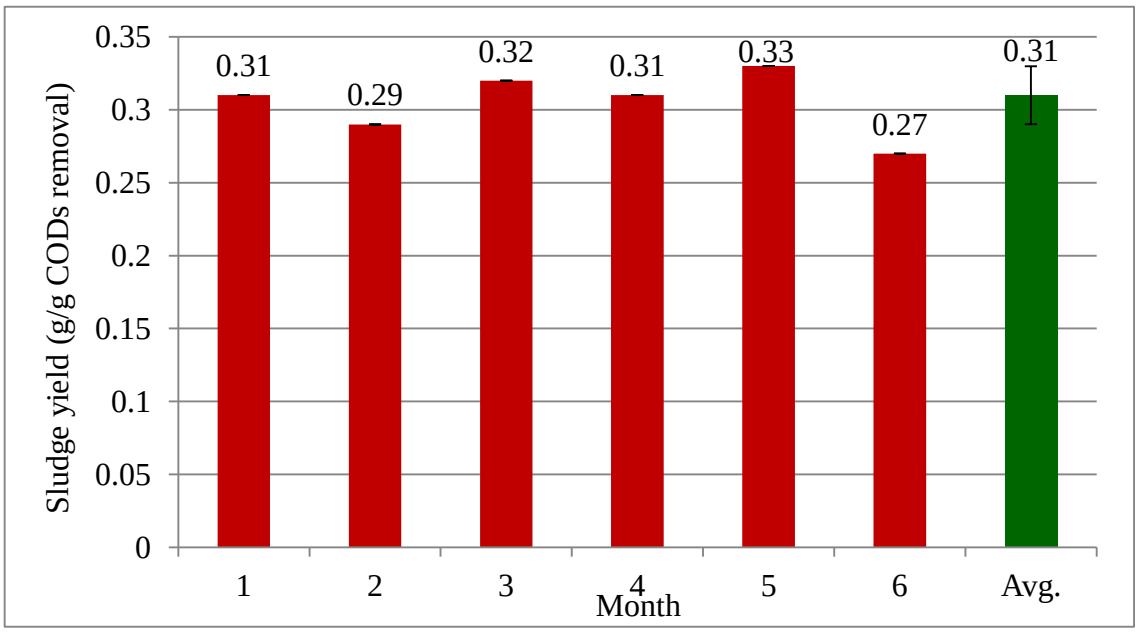


Figure 5.1: Sludge yield in lab scale ASP treating pulp and paper mill effluent

5.1 Effect of varying load of organochlorine compounds on operation of ASP

The various chlorophenolic compounds present in pulp and paper mill wastewater have been reported as metabolic uncoupler. Bacterial anabolism is coupled with catabolism of substrate through rate limiting respiration. In the presence of these compounds excess free energy would be directed away from anabolism so that the production of biomass can be reduced (Liu et al., 1998; Low et al., 2000). The current study is aimed at evaluating the sludge yield and performance of the process at varying load of organochlorine compounds.

Table: 5.2: Operating and environmental conditions, and performance of bioreactor during the study

Parameter	Month-I	Month-II	Month-III	Month-IV	Month-V	Month-VI
pH	7.41±0.12	7.36±0.09	7.71±0.14	7.28±0.21	7.47±0.12	7.39±0.19
Temperature, ($^{\circ}$C)	36.0±0.7	35.9±1.8	35.6±1.1	35.9±1.4	36.4±0.2	36.2±0.2
DO, (mg/l)	1.91±0.48	1.86±0.57	1.71±0.58	1.44±0.69	1.16±0.51	1.26±0.50
HRT, (h)	9.21±0.98	8.27±0.53	8.64±0.55	8.40±0.47	8.41±0.39	8.59±0.17
Organic load, (kg/m^3/d)	0.96±0.12	1.0±0.11	0.95±0.12	0.94±0.10	1.09±0.12	0.96±0.09
F/M ratio,(d^{-1})	0.27±0.04	0.30±0.05	0.27±0.04	0.27±0.03	0.31±0.04	0.28±0.03
SVI, (ml/g)	39±5	29±8	42±10	26±4	23±7	24±3
CODs reduction, (%)	67.3±3.7	65.9±4.0	67.5±5.0	69.7±2.8	72.7±3.3	66.4±3.4
AOX reduction, (%)	38.7±3.3	33.0±1.2	33.8±2.6	39.7±6.4	37.4±12.4	41.2±9.2

Influent CODs: 536±11 mg/l, AOX: 13.1±0.3 mg/l

5.1.1 At normal AOX load

Under normal AOX load, the three bioreactors (6 liter capacity) were run for 20 days under the specified environmental and operating conditions (Table 5.3). The conditions were used to grow flocculating biomass. Though the pH of feed was neutral, it was higher (7.7 to 8.1) in treated wastewater in all the three bioreactors due to increase in alkalinity. Temperature and DO were maintained at 34-37 °C and 0.9-1.5 mg/l respectively. SVI of the sludge in three bioreactors was in the range of 13-28 ml/g. Reduction in CODs and AOX was 68-72 and 39-43% respectively in the bioreactors. Dense flocs were developed with a few filamentous organisms coming-out of flocs in all the three bioreactors. Abundance of higher organisms like protozoa, rotifer and nematode was quite significant in all the bioreactors.

Table 5.3: Operating conditions and performance of bioreactors under normal AOX load

Parameter	R1	R2	R3
pH	7.91±0.20	7.92±0.22	7.90±0.12
Temperature (°C)	35.8±1.2	35.6±1.1	36.0±0.9
DO (mg/l)	1.2±0.3	1.1±0.2	1.3±0.3
HRT (h)	8.7±0.2	8.5±0.3	8.9±0.2
MLSS (g/l)	4.12±0.25	4.09±0.35	4.00±0.27
MLVSS (g/l)	3.44±0.21	3.42±0.27	3.34±0.22
F/M ratio (d^{-1})	0.23±0.02	0.25±0.02	0.22±0.05
Organic load ($kg/m^3/d$)	0.80±0.06	0.82±0.02	0.84±0.03
SVI (ml/g)	20±3	16±3	24±4
CODs reduction (%)	69.8±1.3	70.1±1.2	69.9±1.4
AOX reduction (%)	41.3±1.2	40.1±1.3	41.7±0.9

Influent pH: 7.01±0.01, CODs: 482±10 mg/l, AOX: 12.2±0.4 mg/l

5.1.2 At moderate, low and high AOX load

The first bioreactor (R_1) in the previous run was maintained as control (R_c) throughout the study (moderate AOX load), whereas the second (R_2) and third (R_3) bioreactors were run at reduced (R_{rl}) and higher (R_{hl}) AOX load respectively (Table 5.4). The other operating and environmental conditions were similar in all the three bioreactors (Table 5.5). The study on varying load of AOX compounds was conducted in three phases to observe the impact of its concentration with time.

Table 5.4: AOX load in bioreactors at moderate, low and high concentrations

Bioreactor		R_c	R_{rl}	R_{hl}
Phase I (First 10 days)	AOX (mg/l)	11.64	4.51	29.30
	AOX load ($g/m^3/d$)	34.9	13.5	87.9
Phase II (Next 20 days)	AOX (mg/l)	11.36 ± 0.18	4.59 ± 0.10	28.69 ± 0.40
	AOX load ($g/m^3/d$)	34.1 ± 0.5	13.8 ± 0.4	86.1 ± 1.2
Phase III (Next 30 days)	AOX (mg/l)	10.94 ±0.42	4.70 ± 0.57	27.61 ± 0.95
	AOX load ($g/m^3/d$)	32.8 ± 1.3	14.1 ± 1.7	82.8 ± 2.9

During phase I (PI), MLVSS to MLSS ratio in R_{hl} decreased to 81.8 from 87.0% (Table 5.5) due to higher proportion of C_D and E_{OP} wastewaters in the feed and accumulation of salts present in wastewater from bleaching streams. During phase II (PII) and phase III (PIII), MLVSS content in bioreactor R_{hl} further decreased to 76.9 and 71.9 % respectively, whereas the same increased to some extent in R_{rl} (Table 5.5).

The average removal of CODs in control bioreactor remained in the range of 67.6 to 71.5% throughout the study (Table 5.6). In R_{rl} reduction of CODs also was decreased to 64-65 from 70.1±1.2%. The lower removal in CODs in R_{rl} was due to (i) relatively higher amount of low biodegradable weak black liquor in the feed to make-up the required CODs concentration and (ii) relatively lower amount of biodegradable substrate (C_D and E_{OP}) to have low AOX content.

Table 5.5: Operating conditions of bioreactors during moderate, low and high AOX load

Operating parameter		R_c	R_{rl}	R_{hl}
MLSS (g/l)	PI	4.06 ± 0.11	3.96 ± 0.16	4.23 ± 0.10
	PII	3.97 ± 0.16	3.86 ± 0.16	4.42 ± 0.19
	PIII	3.89 ± 0.13	3.85 ± 0.19	4.62 ± 0.19
MLVSS (g/l)	PI	3.52 ± 0.09	3.48 ± 0.14	3.46 ± 0.11
	PII	3.49 ± 0.13	3.46 ± 0.15	3.39 ± 0.14
	PIII	3.44 ± 0.12	3.50 ± 0.18	3.32 ± 0.13
MLVSS/MLSS (%)	PI	86.7 ± 0.5	87.9 ± 0.7	81.8 ± 1.2
	PII	87.9 ± 0.9	89.6 ± 1.5	76.7 ± 2.1
	PIII	88.4 ± 1.0	90.9 ± 1.4	71.9 ± 1.9
F/M ratio (d^{-1})	PI	0.27 ± 0.02	0.26 ± 0.02	0.30 ± 0.05
	PII	0.30 ± 0.04	0.28 ± 0.04	0.29 ± 0.03
	PIII	0.27 ± 0.04	0.27 ± 0.03	0.26 ± 0.04
HRT (h)	PI	8.7 ± 0.3	8.9 ± 0.4	8.5± 0.1
	PII	8.4 ± 0.5	8.5 ± 0.8	8.6 ± 0.3
	PIII	8.4 ± 0.5	8.5 ± 0.4	8.7 ± 0.8
Organic load (kg CODs removal/m^3/d)	PI	0.94 ± 0.07	0.91 ± 0.07	1.04 ± 0.19
	PII	1.03 ± 0.14	0.98 ± 0.14	0.97 ± 0.09
	PIII	0.94 ± 0.13	0.94 ± 0.11	0.85 ± 0.13

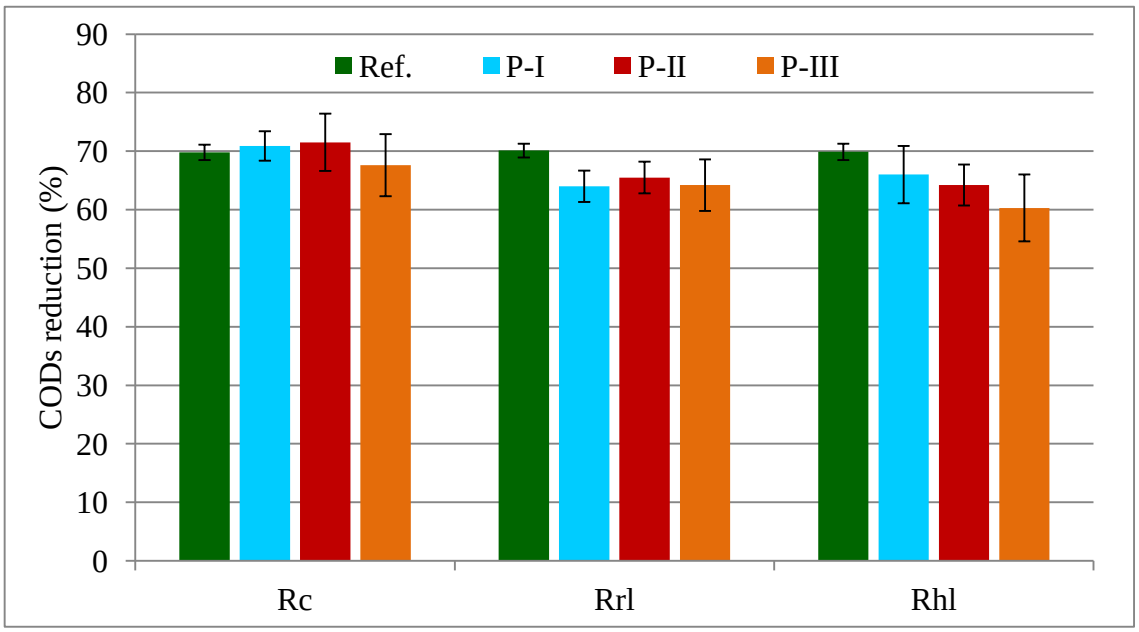


Figure 5.2: Effect of AOX load on reduction of CODs

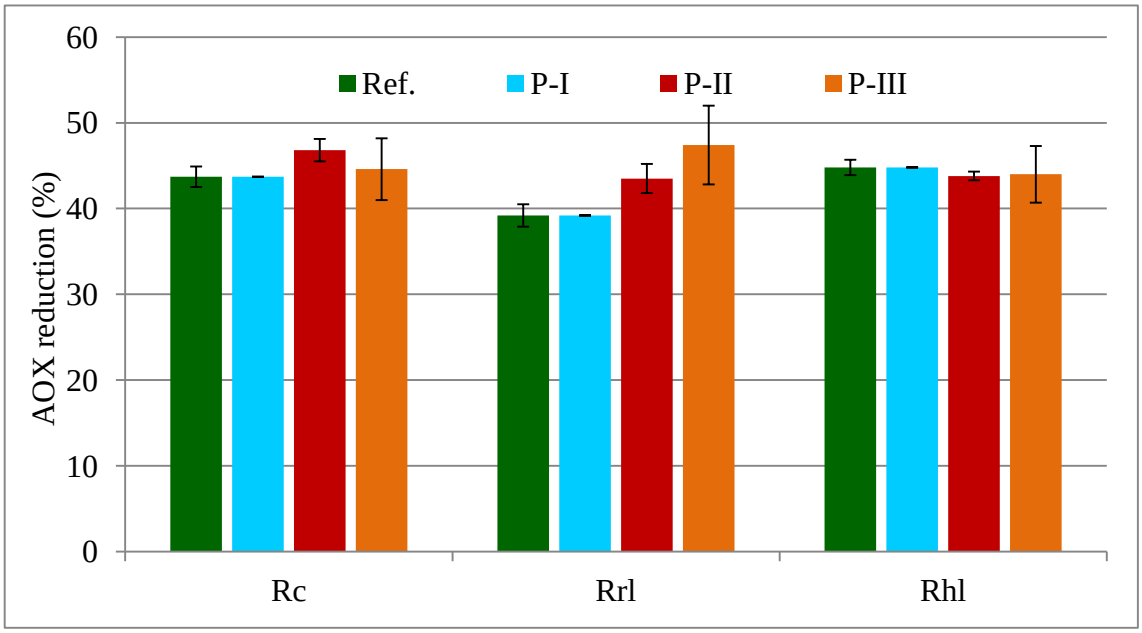


Figure 5.3: Effect of AOX load on reduction of AOX compounds

The lowering in CODs removal was observed in P-I for both R_{rl} and R_{hl} and it remained almost same during the study in R_{rl} (Figure 5.2). In R_{hl}, the removal of CODs was decreased to 66.0±4.9 from 69.9±1.4% in P-I and further to 64.2±3.5 and 60.3±5.7 % during P-II and P-III respectively.

The performance of bioreactors for reduction of AOX was comparable (Figure 5.3); it varied between 39-47% irrespective of influent AOX concentration. The final discharge concentration of AOX was highest in R_{hl} followed by R_c and R_{rl} due to higher concentration of the same in the feed in R_{hl}. Lower reduction in CODs and higher SVI value indicated that increase in AOX compounds (i) resulted in diffused flocs and, (ii) hampered the metabolism. However, The degradation of AOX was not affected at higher AOX load during the study period.

Table 5.6: Performance of ASP during moderate, low and high AOX load

Parameter		R_c	R_{rl}	R_{hl}
CODs (mg/l)	PI	480±14	522±19	523±12
	PII	505±34	525±29	540±22
	PIII	485±25	516±28	515±34
CODs removal (%)	PI	70.9±2.5	64.0±2.7	66.0±4.9
	PII	71.5± 4.9	65.5 ±2.7	64.2±3.5
	PIII	67.6±5.3	64.2±4.4	60.3±5.7
AOX removal (%)	PI	43.7	39.2	44.8
	PII	46.8±1.3	43.5±1.7	43.8±0.5
	PIII	44.6±3.6	47.4±4.6	44.0±3.3
SVI (ml/g)	PI	35±5	19±2	29±1
	PII	44±11	28±12	46±14
	PIII	34±9	37±12	114±25

During P-I, the morphology of organisms in all the bioreactors remained similar. The motility of higher organisms in all the bioreactors was also comparable. The morphology of microorganisms started to change during the second phase (P-II) with a change in AOX load. In R_{rl} there were good dense flocs with a few diffused one. Though a few filamentous organisms were observed, the motility of the higher organisms was good. In R_{hl} dense flocs started to disintegrate with the development of thin and pinpoint flocs (Figure 5.4). The motility of higher organisms was stressed to some extent.

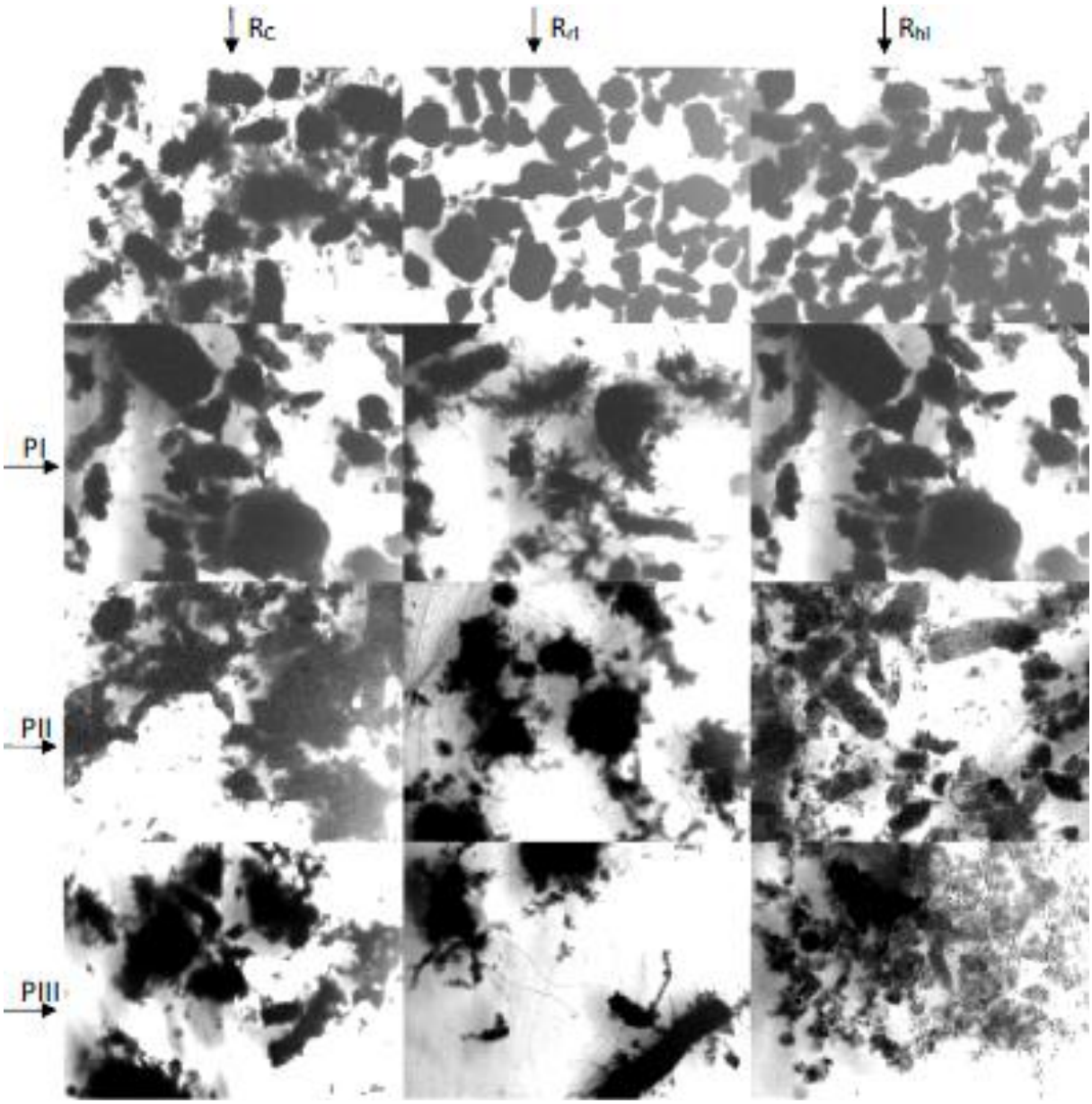


Figure 5.4: Morphological characteristics during moderate, low and high AOX load

In Phase III, there was a drastic change in structure of sludge in R_{hl}; most of the same was in the form of diffused flocs though free forms of filamentous organisms were not detected. Motility of higher organisms was quite comparable to R_c. A few new flocs started to grow at the end due to gradual adaptation in the changed conditions. Due to stable morphology of biosludge in R_c and R_{rl}, the SVI value remained similar to that of reference (Figure 5.5), whereas in R_{hl} the SVI values were more than 100 ml/g in the last phase (P-III) of the study due to change in morphology.

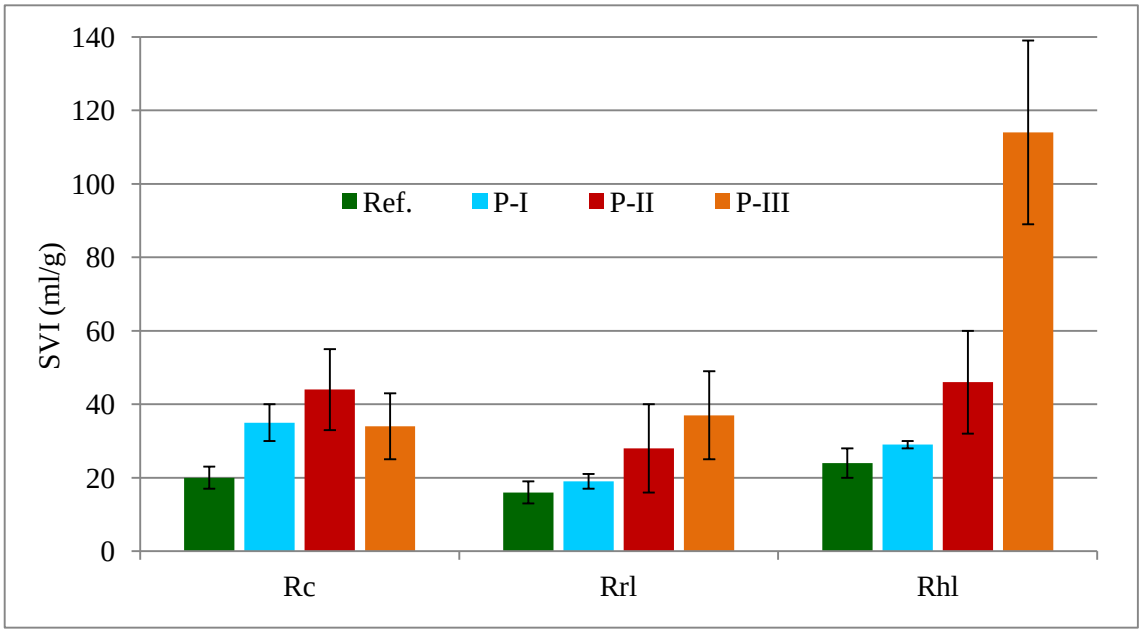


Figure 5.5: Effect of AOX load on SVI

5.1.3 Effect of AOX load on sludge yield

In biological treatment processes, growth of cell is well linked with the oxidation of substrate. Sludge generation at both moderate and low AOX load was 0.29±0.05 and 0.30±0.02 g/g of CODs removal respectively, whereas at high AOX load the same was reduced to 0.18±0.06 g/g of CODs removal (Table 5.7). The increased concentration of organochlorine compounds affected both the growth of microorganisms and the oxidation efficiency. The organochlorine compounds worked as metabolic uncouplers and resulted in low sludge generation.

Table 5.7: Sludge generation at moderate and high AOX load

Parameter	Moderate load	High load
AOX conc. in influent (mg/l)	9.35±1.87	23.67±5.41
AOX load ($g/m^3/d$)	28.1±5.6	71.0±16.2
AOX reduction (%)	40.8±6.0	41.3±3.7
Sludge yield (g/g CODs removal)	0.29±0.05	0.18±0.06

5.2 Mode of removal of AOX compounds

Three set of samples were analyzed as a prelude to find the mechanism of removal of AOX compounds. At moderate load, 45.0±4.1% AOX compounds were dechlorinated during biological degradation, whereas 2.3±0.6% of the same accompanied with waste activated sludge in adsorbed form (Table 5.8). The overall AOX removal was 47.3±3.7%. Similarly, at high load, 44.7±3.3% AOX compounds were dechlorinated, whereas 1.6±0.2% of the same accompanied with waste activated sludge. The overall AOX reduction was 46.3±3.3%. The concentration of AOX compounds in sludge was dependent on concentration of the same in influent; it was 2690±969 and 7659±750 mg/kg at moderate and high AOX load respectively. The major mode of AOX removal was dechlorination (~95 % of the AOX removal) in both the bioreactors. During biological treatment, 43-51% AOX compounds was removed and rest (49-57%) compounds were released with treated effluent; only 1.6-2.3% AOX compounds were adsorbed on waste activated sludge.

Table 5.8: Removal of AOX compounds at different AOX loads

Parameter	Moderate load	High load
AOX in sludge (mg/kg)	2690±969	7659±750
HRT (h)	8.6±0.2	8.9±0.5
CODs in influent (mg/l)	450±22	445±31
CODs in effluent (mg/l)	152±3	171±34
Biomass (g/g CODs removal)	0.29±0.05	0.18±0.06
AOX conc. in influent (mg/l)	9.95±1.77	23.43±4.57
AOX conc. in effluent (mg/l)	5.29±1.30	12.63±2.93
AOX removal (%)	47.3±3.7	46.3±3.1
AOX dechlorination (%)	45.0±4.1	44.7±3.3
AOX adsorbed on sludge (%)	2.3±0.6	1.6±0.2

Though van der Waals forces, chemical and hydrogen bonding were considered to be ways of adherence, biosorption was the most important factor in the removal of organochlorines in secondary treatment system of pulp and paper mill effluent (Gloria et al., 1994).

To confirm the sorption of organochlorine compounds in biosludge, dichloromethane (DCM) was used as spiking agent. DCM is volatile organochlorine compound and used as standard for estimation of POX compounds. 0.1g sludge was dispersed in 80 ml water and spiked with dichloromethane (DCM) solution containing 50 µg/l as POX compounds; the recovered concentration of DCM was 49.96 µg/l. Similarly 0.2, 0.35, 0.5 and 1.0 g biosludge was dispersed and spiked with the same amount of dichloromethane solution (Table 5.9). As the concentration of biosludge was increased, there was higher sorption of dichloromethane. The stripping of the same was decreased with increasing concentration of biosludge (Figure 5.6). The increase in sorption of DCM with increase in biosludge concentration was due to its equilibrium distribution between the solid sludge and aqueous phase. Recovery of the standard from water containing the same amount of DCM was 50.28±0.09 µg/l.

Table 5.9: Effect of biosludge concentration on desorption of dichloromethane (DCM) as POX compounds

Sludge amount (g/80 ml)	Recovered POX concentration (µg/l)	Recovery of POX (%)
0.1	49.96	99.9
0.2	49.41	98.8
0.35	38.67	77.3
0.5	25.62	51.2
1.0	24.01	48.0

Further to check the sorption behavior of biosludge, 0.35 and 1.0 g sludge were dispersed in 80 ml of water, spiked with DCM as described above and shaked for 1.0 h at 200 rpm. In case of 0.35 g sludge sample, recovery of DCM was 29.81 µg/l and sorption of DCM increased from 22.7 to 40.4%. For the second case, the sorption of DCM increased from 52.0 to 62.2 % and recovery was 18.9 µg/l. Higher sorption of DCM is due to increased concentration of biosludge, agitation and time of exposure (Leuenberger et al., 1985).

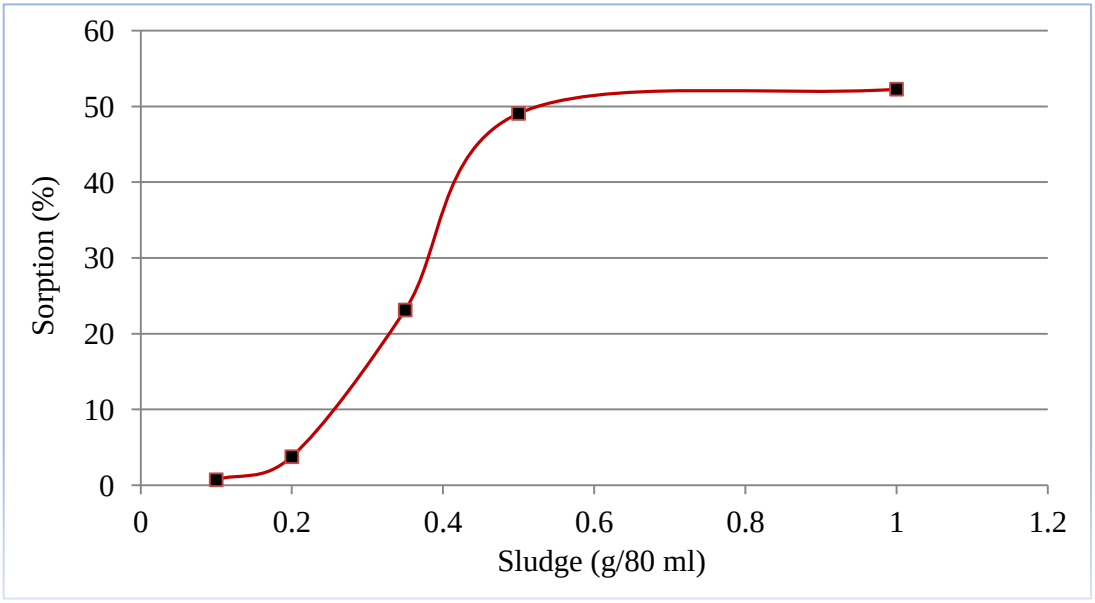


Figure 5.6: Effect of biosludge concentration on sorption of dichloromethane (DCM)

Summary

The growth of biomass in the biological treatment of wastewater depends on the nature of substrate, operating parameters and type of process. As the wastewater of pulp and paper mills is rich in organochlorine compounds, it affects the growth of organisms in the process. The higher load of AOX compounds had a bearing on the performance as well as morphology of biomass. Well dense structure of microbial flocs started breaking and resulted in diffused and pin point flocs when operated at higher AOX load of 84 g/m^3/day. Though the AOX reduction remained more or less same in the range of 39-46 % at moderate and higher AOX load, there was 5-7 % lower CODs removal at higher AOX load. The sludge volume index (SVI) was above 100 ml/g at higher AOX load. During biodegradation, the major mode of AOX removal was dechlorination and only 1.6-2.3% AOX compounds were adsorbed on waste activated sludge. The concentration of AOX compounds in sludge was dependent on the AOX concentration in influent. The sludge yield was 0.29 and 0.18 g/g of CODs removal during low to moderate and higher AOX load respectively. The sorption with dichloromethane revealed that biosludge from pulp and paper mill was a good adsorbent of volatile organochlorine compounds.

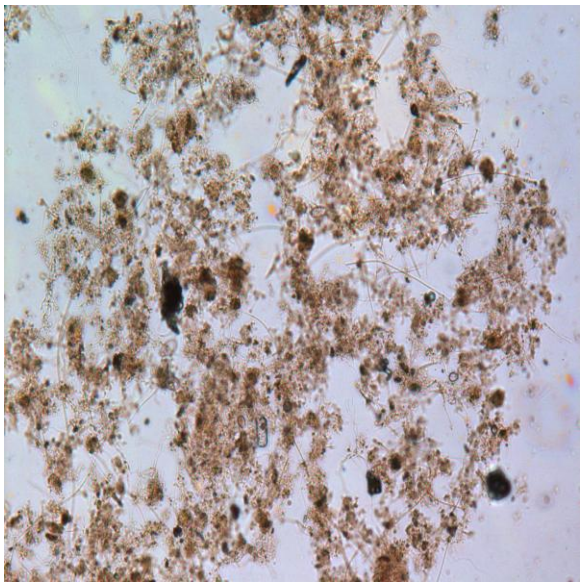
Chapter-VI

6. Ozonation of biosludge of laboratory reactors

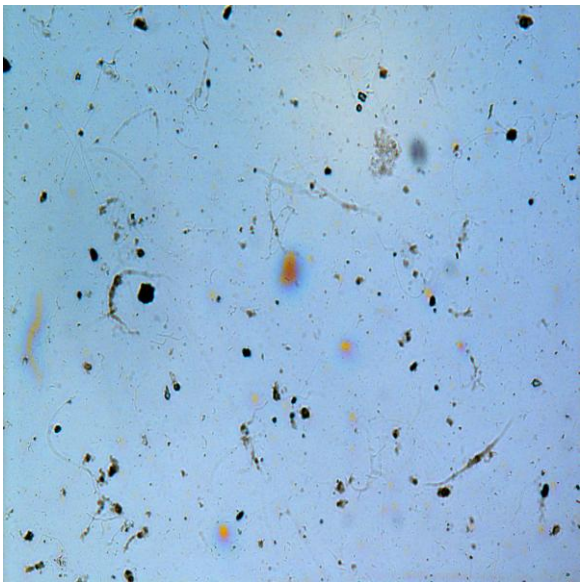
It is well studied and reported that ozonation of the biosludge depends on its nature which is directly linked with the substrate, operating and environmental conditions. As the wastewater from pulp and paper mill is very complex in nature; morphological changes of biosludge has been well reported by different researchers throughout the world. The present chapter depicts a concise picture of the response of bulking and flocculating nature of biosludge from pulp and paper mill effluent treatment plant towards ozonation. The biosludge sample collected from the effluent treatment plant (sludge A) was of bulking in nature (Figure 6.1a). The biosludge was kept in active condition in batch reactor in laboratory by providing air, nutrients and the same substrate from the mill. The flocculating sludge (sludge B) was developed by mixing sludge A with cow dung in 1:1 proportion and acclimatized in batch reactor under controlled condition in the laboratory. Acclimatization of biosludge for 15-20 days under controlled condition converted the biomass to good settling one which contained very dense flocs (Figure 6.2a).

The ozonation of biosludge (sludge A & B) was performed at 500 rpm of agitation. Pin point flocs and filamentous organisms were more susceptible to ozonation due to higher exposed surface area and started to get ruptured with increase in ozone dosage. In case of sludge A, most of the diffused flocs and filamentous organisms were ruptured after ozonation at 50 mg O_3/g DS dosage (Figure 6.1a & b). In case of sludge B, diffused and pinpoint flocs were ruptured but there was no appreciable change in structure of dense flocs at the same ozone dosage (Figure 6.2a & b). Mobility and survival of higher organisms was found to be linked with dosage of ozone. At low dosage of the oxidant, the organisms had low mobility but at an ozone dosage near to 30 mg/g DS, organisms were totally inactive.

Number of microbial survivors too decreased sharply with increase in ozone dosage. There was an average reduction of 70% in colony forming units (CFU) at an ozone dosage near to 40 mg O_3/g DS in both the sludge samples (Figure 6.3).

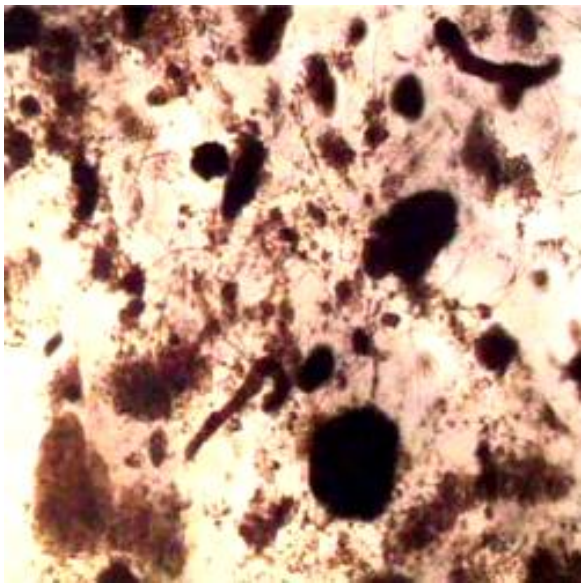


6.1a: Before ozonation

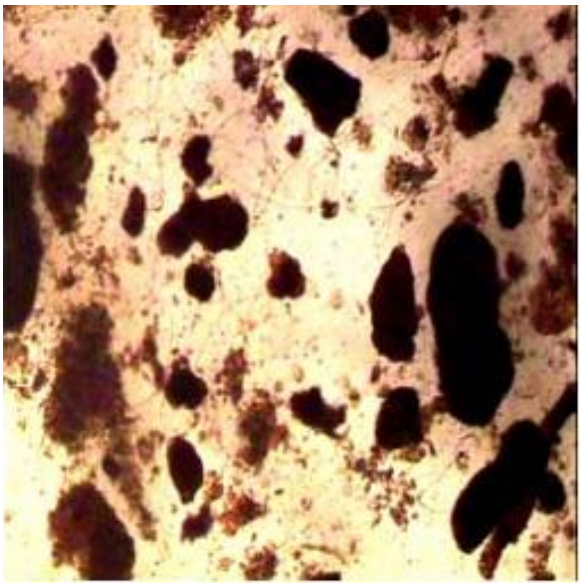


6.1b: After ozonation

Figure 6.1: Morphology of bulking sludge (sludge A) before and after ozonation



6.2a: Before ozonation



6.2b: After ozonation

Figure 6.2: Morphology of flocculating sludge (sludge B) before and after ozonation

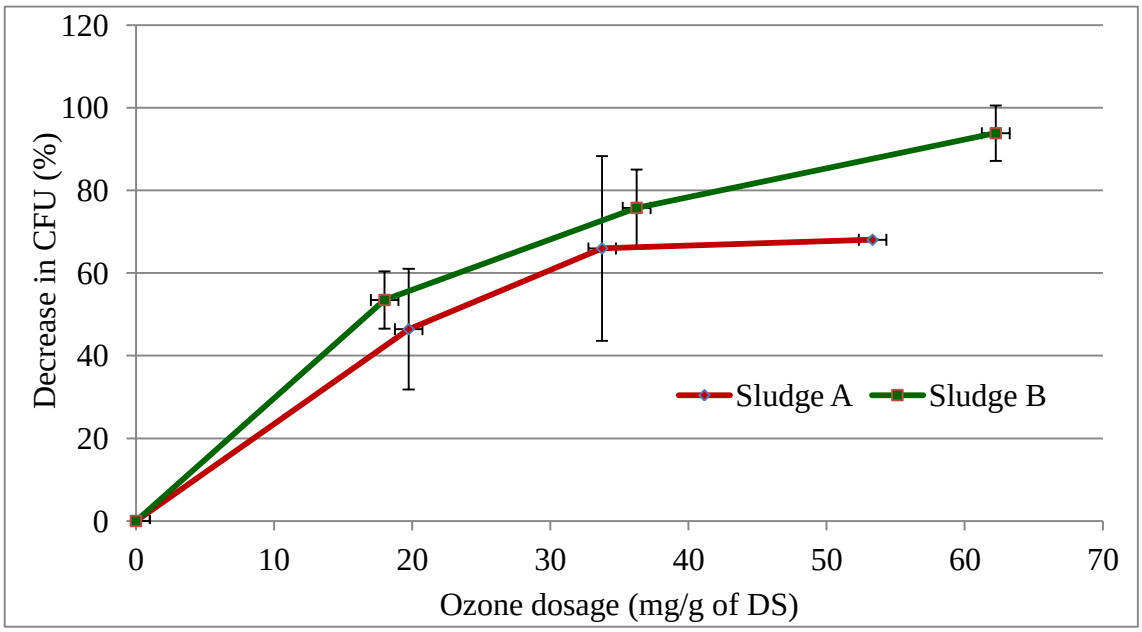


Figure 6.3: Effect of ozone on viable count

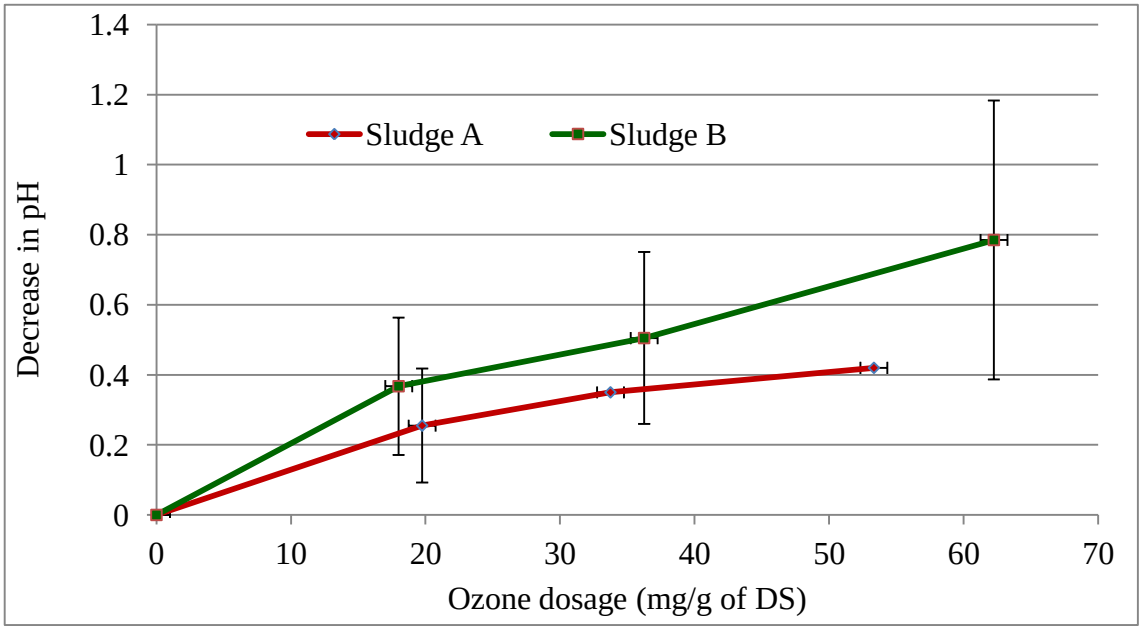


Figure 6.4: Effect of ozonation on pH

During ozonation higher molecular weight compounds might be oxidized to lower molecular weight ones and pH decreased due to formation of fatty acids. The same observation has been reported elsewhere (Fontanier et al. 2005; Poznyak and Vivero 2005). The change in pH was 0.3–0.4 and 0.5–0.7 unit respectively in sludge A and sludge B at ozone dosage from 30–55 mg/g DS (Figure 6.4).

With the increase of ozone dosage, dissolution of biomass was increased. Sludge A, which had diffused flocs and excessive filamentous organisms, was more susceptible to ozonation than sludge B; Wijnbladh, 2007 has also reported the same observation. MLSS and MLVSS concentration in the case of bulking sludge (sludge A) ranged from 4.0–7.5 and 2.5–4.9 g/l respectively, it had the organic content of 62-74%. It was concentrated to 5 to 11 g/l MLSS for ozone application. There was 9–18% dissolution of MLSS at ozone dosage from 30 to 55 mg O_3/g DS (Figure 6.5). The MLSS concentration in the case of flocculating sludge (sludge B) varied from 3–4 g/l and was concentrated to 7.5–8 g/l for ozone application. The organic content of the biosludge was 73-78%. Although it contained higher organic content, there was lesser dissolution of biomass than sludge A due to the dominance of compact flocs and presence of a few pin point flocs, filamentous organisms in sludge B. Biomass dissolution was only 4–6% at the same ozone dosage.

The concentration of colour in aqueous phase was 325±35 Pt-Co unit. Initially ozone reacted less with the sludge and more with soluble and easily oxidisable organic compounds. This resulted in increase in color very slowly. During the disintegration process, presumably due to dissolution of adsorbed lignin compounds and sludge material, color was increased at a faster pace (Figure 6.6). At ozone dosage of 55 mg /g DS, there was about 2.4 fold increase in color in the aqueous phase of sludge A. The release of lignin was lower due to low dissolution of biomass in case of sludge B and resulted in only 1.7 fold increase in colour in the aqueous phase at the same ozone dosage.

During disintegration of biomass, refractory organic compounds were converted into easily biodegradable compounds and dissolution of biomass resulted in an increase of BOD and CODs in aqueous phase. Initially due to complete oxidation of easily oxidisable compounds, increase in CODs and BOD was less and after 10–20 mg O_3/g DS dosage, there was sharp increase in CODs (Figures 6.7) and BOD (Figures 6.8) in the aqueous phase.

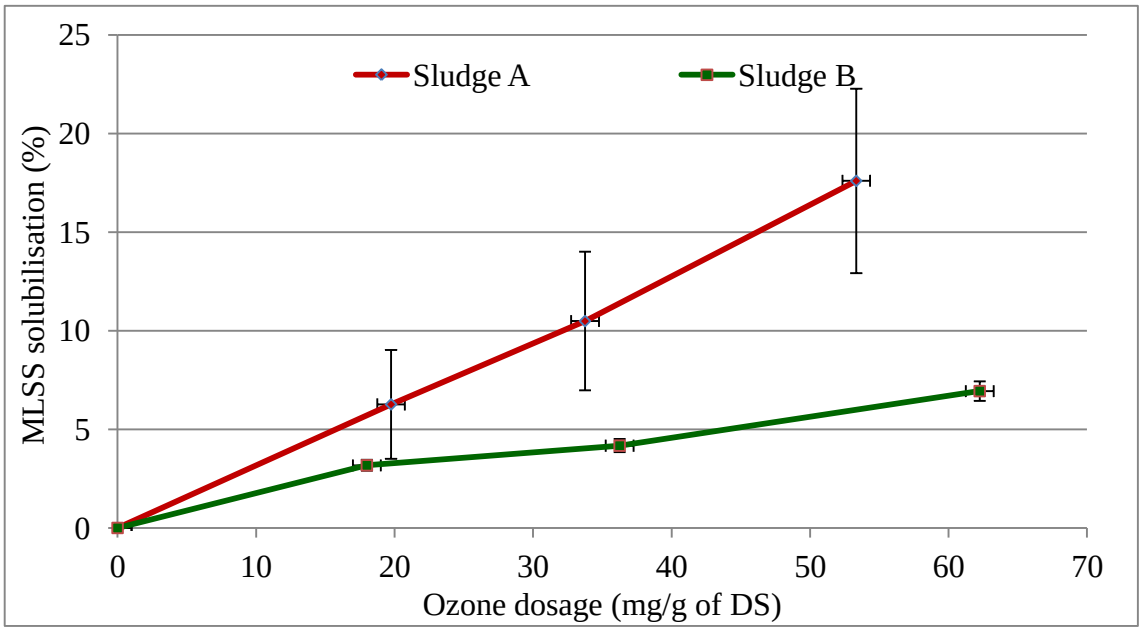


Figure 6.5: Effect of ozonation on dissolution of biomass

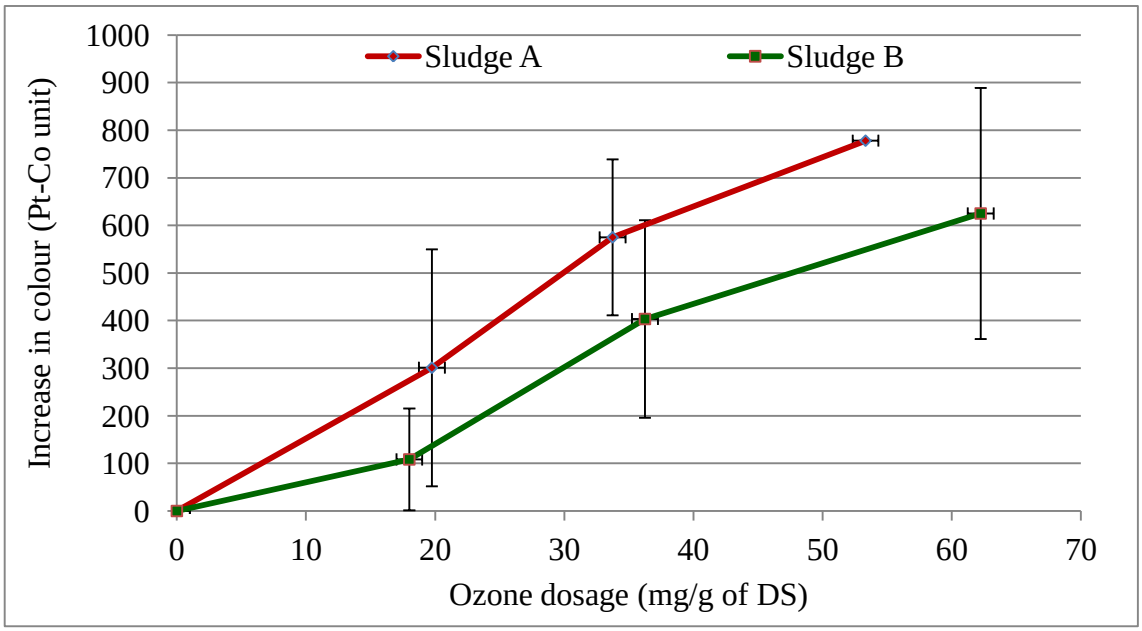


Figure 6.6: Effect of ozonation on release of colour

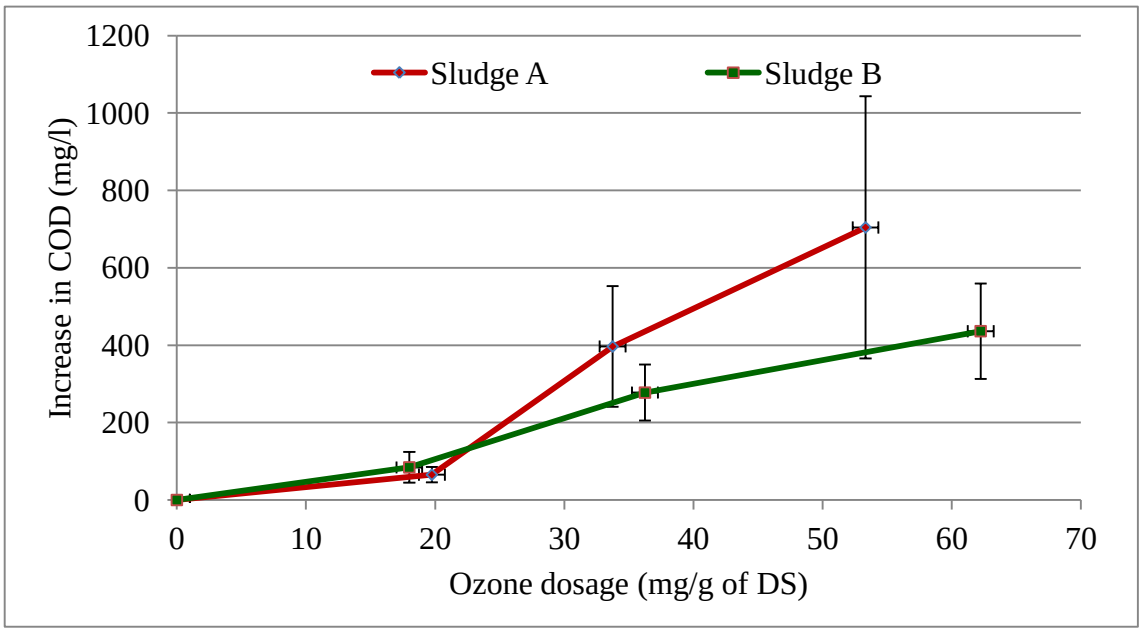


Figure 6.7: Effect of ozonation on increase in CODs in aqueous phase

Initial BOD and CODs concentration in the aqueous phase of sludge A were 18.5±1.5 and 280±112 mg/l respectively, whereas those for sludge B were 2.4±1.4 and 125±34 mg/l respectively. In case of sludge A, there was 75–175 mg/l increase in BOD and 300–710 mg/l increase in CODs with 30 to 55 mg O_3/g DS. Higher deviation of solubilised CODs concentration through ozonation might be due to variation in organic content of biosludge. For sludge B, there was 50–85 mg/l increase in BOD and 200–390 mg/l increase in CODs at the same ozone dosage.

In case of sludge A, the concentration of AOX compounds in sludge and aqueous phase was 2568±207 mg/kg and 5.51±1.19 mg/l respectively, whereas influent AOX concentration was 7.0–9.0 mg/l. Similarly, in case of sludge B, the concentration of AOX compounds in sludge and aqueous phase was 3282±449 and 5.32±0.31 mg/l respectively and influent AOX concentration was 10–12 mg/l. Initially ozone mineralized the AOX compounds thus reducing the AOX level in liquid phase. In the latter stage, AOX compounds from the sludge were released in the aqueous phase and concentration of the same was increased but remained lower than AOX concentration of initial sample. The dechlorination of AOX compounds was 18–26% and 16– 20% in sludge A and sludge B respectively at ozone dosage from 30–55 mg/g DS (Figure 6.9).

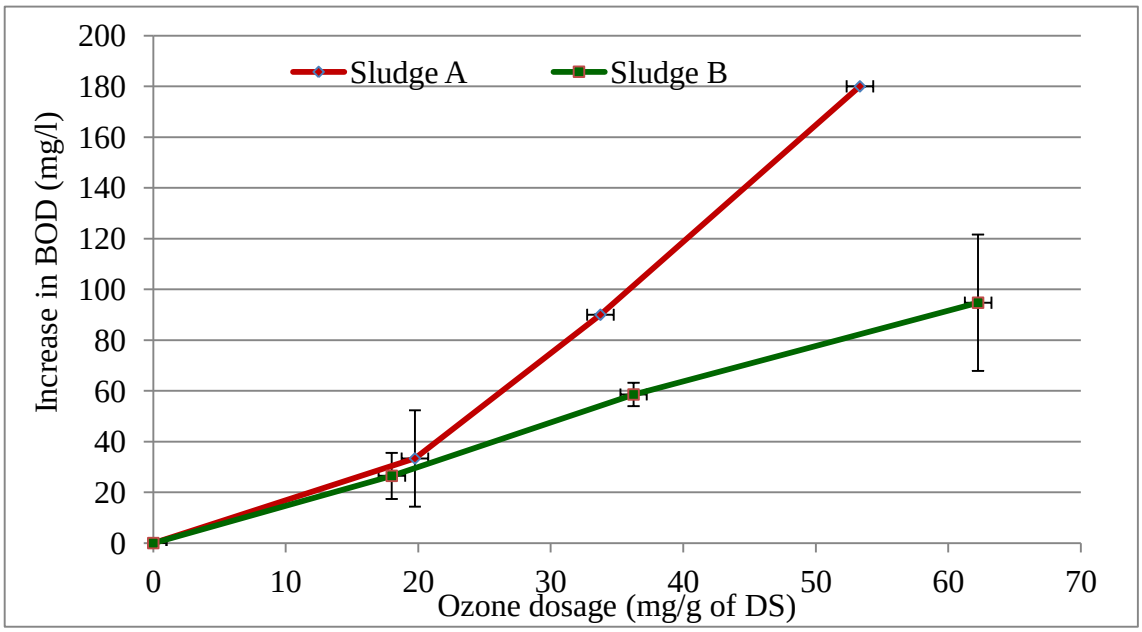


Figure 6.8: Effect of ozonation on increase in BOD content of aqueous phase

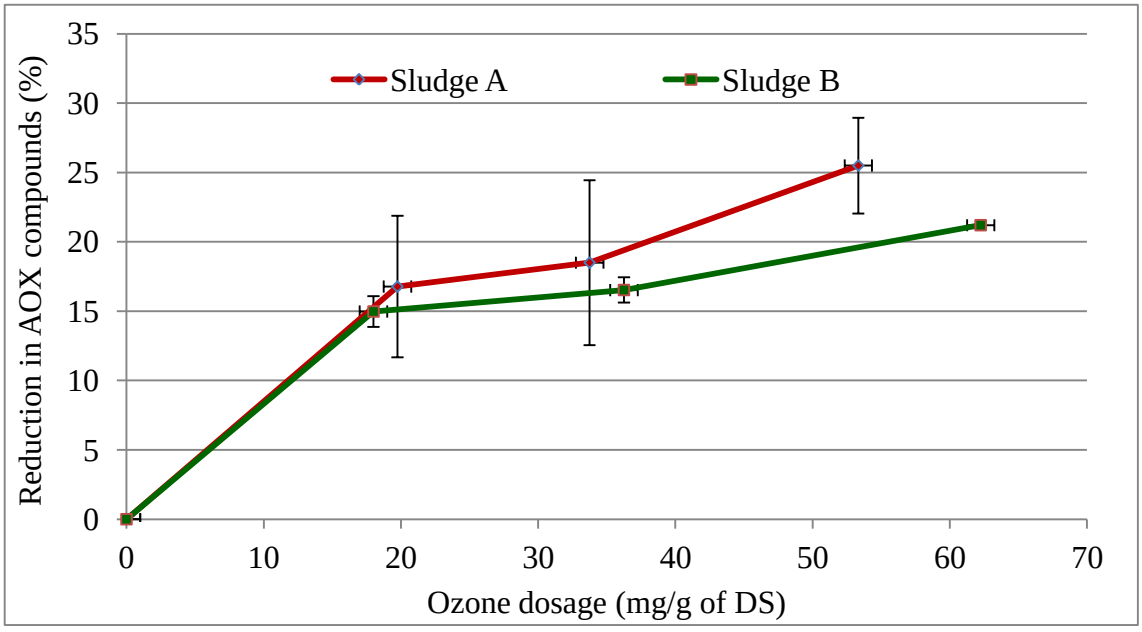


Figure 6.9: Effect of ozonation on removal of AOX content

Summary

Number of microbial survivors decreased sharply with increase in ozone dosage in both the bulking (sludge A) and flocculating (sludge B) sludges. The pH of biosludge was reduced by 0.3–0.4 and 0.5–0.7 unit respectively in sludge A and sludge B at ozone dosage from 30–55 mg/g DS. The dissolution of MLSS with ozone dosage of 30 to 55 mg O_3/g DS was 9–18 and 4–6% in bulking and flocculating sludge respectively. Dissolution of biosludge and subsequent desorption of lignin compounds resulted in increase in colour in the aqueous phase. At ozone dosage of 55 mg /g DS, there was ~2.4 and 1.7 fold increase in color in the aqueous phase of sludge A and B respectively. The release of lignin was lower due to low dissolution of biomass in case of sludge B and resulted in low colour in the aqueous phase.

In case of sludge A, there was 75–175 mg/l increase in BOD and 300–710 mg/l increase in CODs at ozone dosage of 30 to 55 mg O_3/g DS. For sludge B, there was 50–85 mg/l increase in BOD and 200–390 mg/l increase in CODs at the same ozone dosage. Low dissolution of the biomass in case of sludge B resulted in less CODs and BOD. The dechlorination of AOX compounds in sludge A and B was 18–26% and 16–20% respectively at ozone dosage of 30–55 mg/g DS. The pin point and filamentous organisms were disintegrated with ozone, whereas the compact flocs were hard to be disintegrated with ozone.

Chapter-VII

7. Ozonation of biosludge of pulp and paper mill

The activated sludge process in pulp and paper mill suffers from problem of bulking of sludge due to proliferation of filamentous organisms. Present chapter deals with the ozonation of industrial sludge in greater details while considering the variables of the ozonation process, constituents of the biosludge and the potential contaminants.

7.1 Evaluation of impact of operating conditions on ozonation of biosludge

The impact of various operating conditions like agitation speed, ozone dosage, sludge concentration, presence of catalyst, pH and temperature have been studied for dissolution of solids and removal/ modification of contaminants.

7.1.1 Effect of agitation on ozonation

The ozonation of biosludge was performed at two agitation speed of 500 and 1000 rpm (Table 7.1). The experiments were performed at ambient temperature and neutral pH. The residual ozone was inconsistent at 500 rpm and the reacted ozone was low. The residual ozone concentration was low at higher agitation speed due to intimate contact of the ozone gas with biosludge particles and higher mass transfer. The low values of standard deviation at 1000 rpm revealed that the reaction of ozone with biomass was more consistent. Formation of foam was also observed at lower agitation speed due to escape of more unreacted gas. Therefore it was not possible to treat the sludge with more than 75-80 mg O_3/g DS dosage. At higher agitation there was no foaming at lower dosage due to high mixing and reaction of ozone with biosludge.

Table 7.1: Effect of agitation on mass transfer of ozone

Agitation: 500 rpm		Agitation: 1000 rpm	
Ozone dosage (g/g DS)	Reacted ozone (%)	Ozone dosage (g/g DS)	Reacted ozone (%)
22.3±2.5	77.7±3.5	22.7±1.5	88.6±0.6
41.7±3.5	65.3±6.5	45.3±2.1	83.9±0.4

MLSS content: 7.529 g/l.

7.1.2 Effect of ozone dosage on ozonation of biosludge

The sludge was ozonated at different ozone dosage from 20 to 100 mg O_3/g DS. The reaction of ozone was higher at the lower concentration and as the dosage of ozone was increased, the amount of unreacted ozone was increased (Table 7.2).

Table 7.2: Dissolution of biomass and unreacted ozone at varying ozone dosage

Ozone dosage (mg/g DS)	Reacted ozone (%)	Dissolution of MLSS (%)	Specific dissolution of MLSS (mg/mg of O_3)
21.6	89.1	9.0	4.2
47.4	82.3	13.7	2.9
71.2	79.8	19.9	2.8
95.3	75.2	26.9	2.8

MLSS content: 8.241 g/l

The specific dissolution of biosludge was calculated by dividing the amount of solubilized biosludge with the amount of ozone. Initial high specific dissolution of MLSS indicated the dissolution of easily oxidisable material at lower ozone dosage (Figure 7.1).

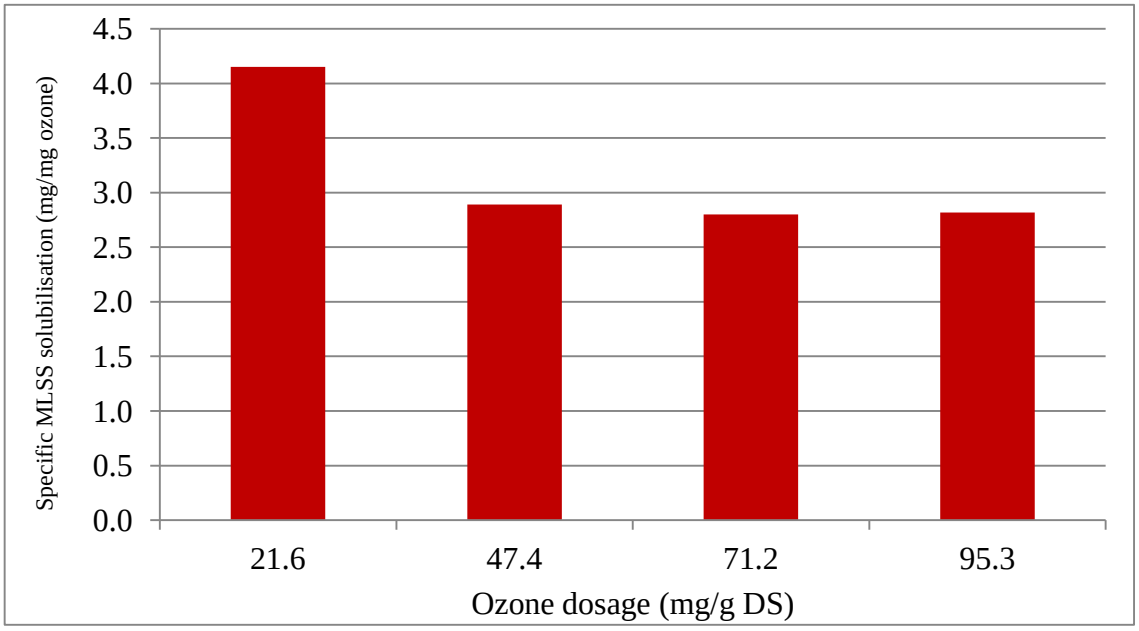


Figure 7.1: Specific dissolution of MLSS at varying ozone dosage

In biological process, bacterial cells form macroflocs (Jorand et al., 1995) and these macroflocs have a typical size ranging from 100 to 150 µm. The microflocs comprise mainly active cells, organized in micro-colonies, covered with exopolymers and having a size of approximately 10 or 15 µm (Paul and Debellefontaine, 2007). It is postulated that most of the inert particulate material like colloids, mineral particles, and ionic component such as divalent cations are located outside the microflocs (Nielsen et al., 2004). Ozone is able to break-up the macroflocs and small particles (Figure 7.2). However, the microflocs are quite stable due to the presence of extracellular polymeric substances (EPS) which are composed of carbohydrates, proteins, nucleic acids, lipids, uronic acid and deoxyribonucleic acids. These compounds are principally responsible for floc formation (Tian, 2008). The initial higher specific dissolution of biomass is due to disintegration of diffused macroflocs and releasing the adsorbed inert material in the aqueous phase. Further increase in dosage of ozone resulted in average specific dissolution in the range of 2.8-2.9 mg/mg of ozone dosage.

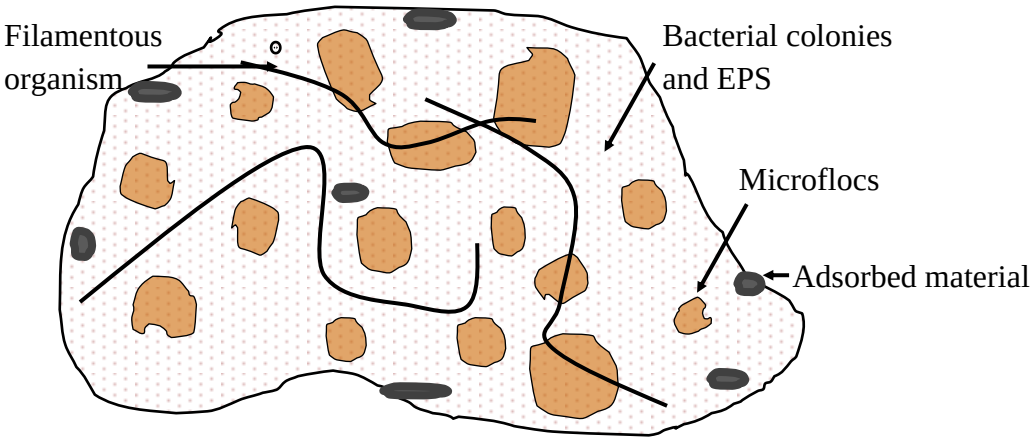


Figure 7.2: Schematic representation of activated sludge flocs

7.1.3 Ozonation of biosludge with change in sludge concentration

Based on the effect of agitation, ozone dosage and residual ozone concentration, ~50 mg O_3/g of DS at 1000 rpm was selected for further study. In these conditions more that 80% of the ozone reacted with the sludge.

The MLSS concentration of the returned sludge from the plant during the study varied from 4.0 to 8.5 g/l. This was concentrated to 5.5-11.0 g/l by settling and decantation of the aqueous phase. Ozonation was performed with the partially concentrated sludge.

Table 7.3: Impact of biosludge concentration on dissolution during ozonation

Set	MLSS (g/l)	Ozone dosage (mg/g DS)	Dissolution of MLSS (g/l)
I	5.50	48.7	0.788
II	7.83	49.1	1.113
III	11.02	48.2	1.534

The dissolution of biomass in the medium was increased with increase in biomass concentration while ozone dosages were 48.2 to 49.1 mg/g DS (Table 7.3). The absolute amount of ozone (mg) fed was proportional to the biomass concentration. It was almost double the amount that was fed to biosludge in set-III in comparison to Set-I (Figure 7.3). During ozonation formation of foam was observed in the reactor; the amount of foam was increased with the increase in concentration of sludge.

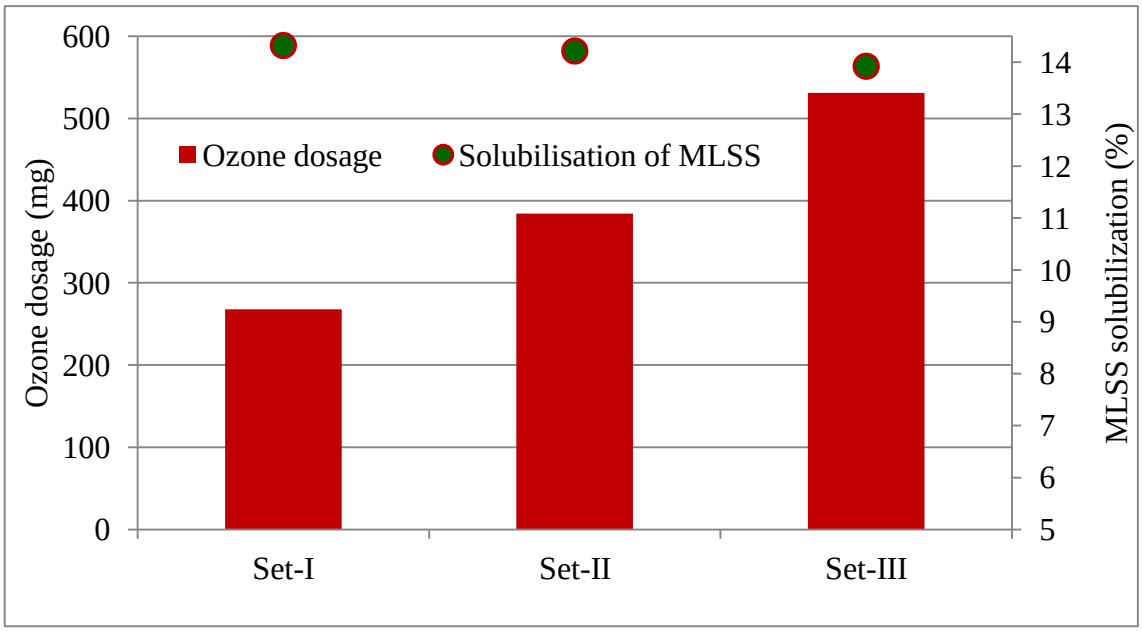


Figure 7.3: Impact of sludge concentration on dissolution

The dissolution of biosludge in all the experimental sets was comparable and dissolution varied from 13.9 (Set-III) to 14.3% (Set-I).

7.1.4 Effect of pH and temperature on ozonation of biosludge

The pH of the sludge of the mill was near to neutral and it was adjusted to 4.5 (with 1 N HCl) and 9.0 (with 1 N NaOH) during the study. The dissolution of biomass was observed during addition of HCl (Table 7.4). $CaCO_3$ is used as filler during paper making; lime is used for causticization and treatment of wastewater during primary treatment. Part of finely dispersed $CaCO_3$ was carried over after primary clarification. During biological treatment, this dispersed filler is adsorbed on the surface of biomass and contribute to inorganics; the same was dissolved in the acidic conditions. The dissolution of biomass was comparable at 30 and 45 °C temperature. There was no significant impact of pH and temperature on dissolution of biomass although slightly higher dissolution was observed at acidic pH due to high organic content of the biomass.

Table 7.4: Impact of pH and temperature on dissolution of biosludge during ozone treatment

Set		pH	Temperature (°C)	Initial MLSS (g/l)	Ozone dosage (mg/g DS)	Dissolution of MLSS (%)
Control	Set-I	7.1	30.0	8.504	49.7	14.5
Change of pH	Set-II	4.5	30.0	6.219	50.1	15.2
	Set-III	9.0	30.0	8.548	50.4	14.7
Change of temperature	Set-IV	7.1	45.0	8.476	49.6	14.7

Catalytic behavior of hydrogen peroxide was also assessed. The biosludge was spiked with 10 mg H_2O_2/l and ozonated at a 49.9 mg O_3/g DS dosage. 14.7% dissolution of MLSS was observed. The efficiency of H_2O_2 might be lost by decomposition with metal ions present in the sludge (Table 4.1). Deactivation of H_2O_2, due to presence of metal ion, has also been reported by Khan, 2006.

7.2 Effect of ozone treatment on disintegration of biomass

Based on the previous experimental findings, the sludge was ozonated under agitation of 1000 rpm while maintaining neutral pH and ambient temperature. Ozone dosage was

varied from 22.7±1.5 to 92.3±1.5 mg ozone /g DS. The ozonation of biosludge resulted in disintegration of biomass and subsequent reduction in the concentration of MLSS and MLVSS content (Table 7.5). Through the ozonation of sludge, refractory organic molecules were oxidized and converted into biodegradable low-molecular weight compounds. The disintegration or lysis process might break down cell walls and release intracellular compounds into the medium. Thus ozonation of sludge acted in two ways; it destructed the cell wall thereby releasing the biodegradable compounds, and converting the refractory molecules to low molecular weight biodegradable components.

Table 7.5: Disintegration of biomass with change in dosage of ozone

Ozone dosage (mg/g DS)	MLSS (g/l)	MLVSS (g/l)	Dissolution (%)	
			MLSS	MLVSS
0.0	7.687±0.116	5.657±0.128	-	-
22.7±1.5	6.912±0.209	5.058±0.209	10.1±1.4	10.6±1.7
45.3±2.1	6.613±0.108	4.752±0.126	13.9±0.4	16.0±0.5
67.0±2.6	6.155±0.252	4.353±0.197	19.9±2.3	23.1±1.8
92.3±1.5	5.577±0.035	3.872±0.031	27.4±0.9	31.5±1.6

Ozonation of biosludge changed the MLSS and MLVSS profile with increase in ozone dosage. MLVSS/MLSS ratio, which was 0.736±0.007 in the untreated sludge, was dropped to 0.694±0.001 at 92.3±1.5 mg O_3/ g DS (Figure 7.4).

Figure 7.5 depicts that specific dissolution of MLSS and MLVSS was highest at lower dosage of ozone (i.e. 22.7±1.5). At higher ozone dosage (45.3 mg/g DS and above), the specific dissolution of MLSS and MLVSS was 3.0-3.1 and 2.5-2.6 mg/mg of ozone respectively. The initial higher specific dissolution of biomass was due to disruption of diffused macro-flocs and release of adsorbed inorganic and organic material into the aqueous phase.

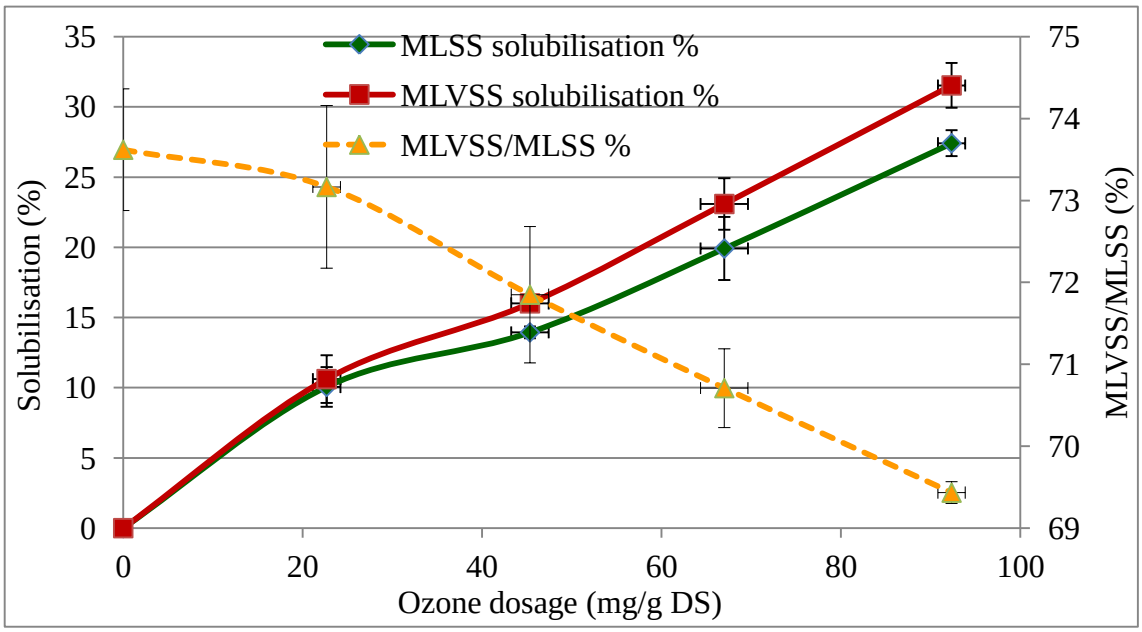


Figure 7.4: Effect of ozonation on disintegration and dissolution of biomass

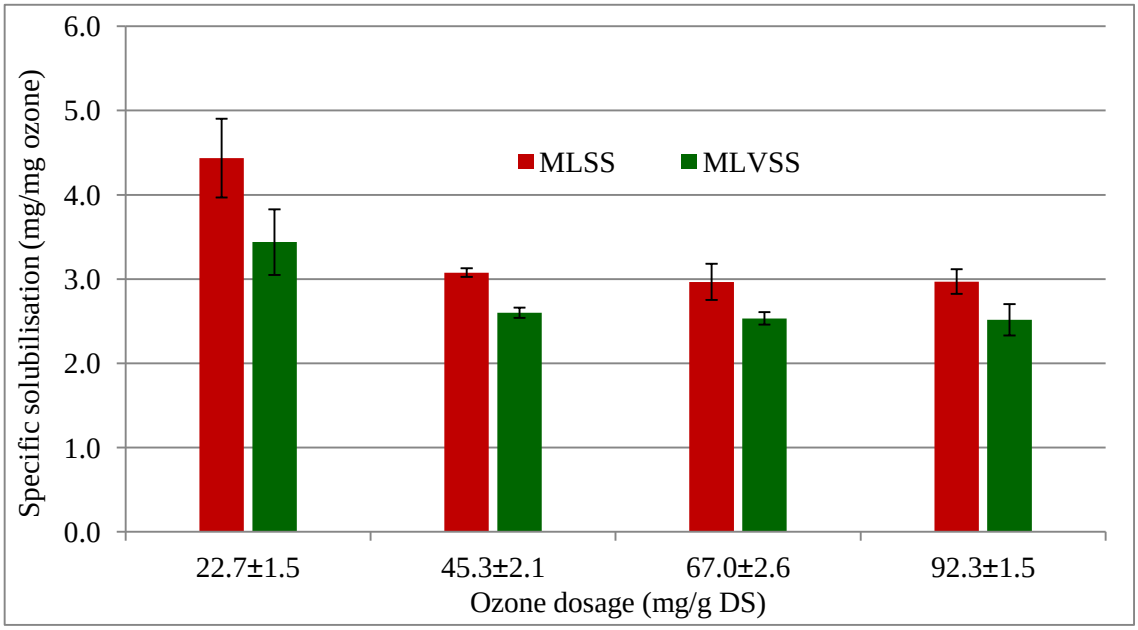


Figure 7.5: Specific dissolution of MLSS and MLVSS at varying ozone dosage

The absolute dissolution of MLSS and MLVSS per liter of the sludge as well as ratio of solubilised MLVSS to MLSS is given in Figure 7.6.

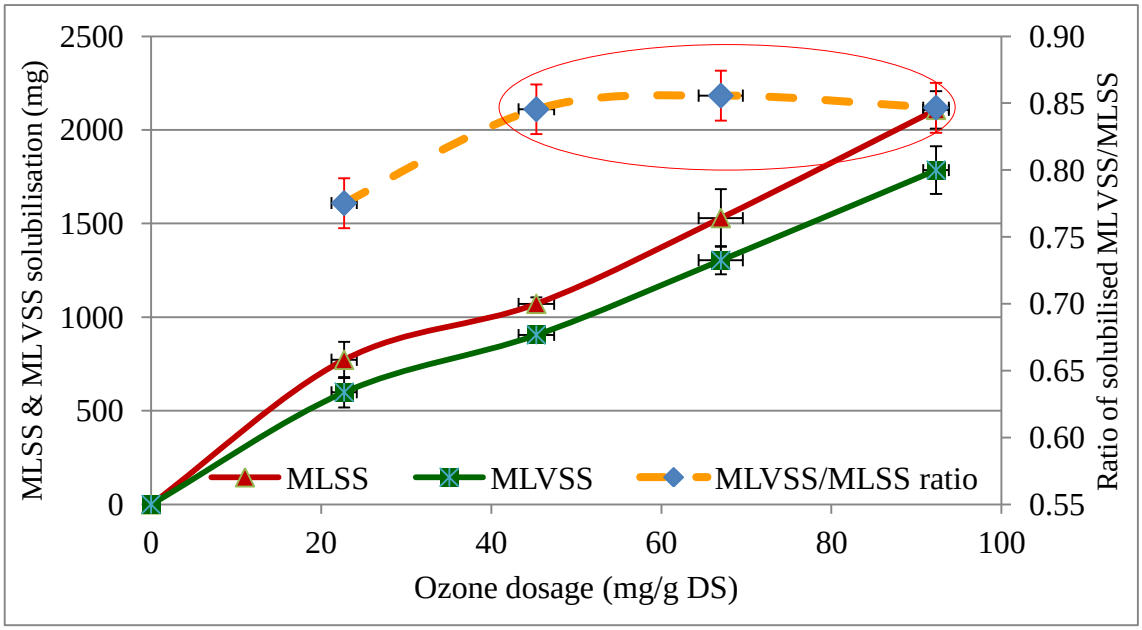


Figure 7.6: Absolute dissolution of MLSS and MLVSS at varying ozone dosage

At low dosage of ozone, the dissolution of MLVSS content was less in comparison to total dissolution due to release of adsorbed inorganic material; with increase in ozone dosage the dissolution ratio of MLVSS/MLSS increased and remained constant at higher dosage. There was no impact of increasing dosage of ozone on specific dissolution of MLSS and MLVSS.

The decomposition of biomass resulted in increase in TSS and TDS content in the aqueous phase (Table 7.6). The initial TSS and TDS content in the aqueous phase were 17±5 and 1721±135 mg/l respectively. Major portion of the solubilised biomass contributed on increasing the TDS; only 10.9-14.6 % of the increase was in the form of TSS. These particles were in finely dispersed form. The material balance revealed that solubilised MLSS was converted into TSS and TDS.

Table 7.6: Increase in TSS and TDS content with varying ozone dosage

Ozone dosage (mg/g DS)	Dissolution of MLSS (mg/l)	Dissolution of MLVSS (mg/l)	TSS (mg/l)	TDS (mg/l)	Increased TSS+TDS (TS) (mg/l)
0.0	-	-	17±5	1721±135	-
22.7±1.5	772±97	599±82	91±10	2341±222	694±87
45.3±2.1	1071±35	905±25	160±16	2558±144	980±53
67.0±2.6	1529±154	1304±76	213±17	2928±281	1403±155
92.3±1.5	2107±101	1785±128	272±27	3387±60	1921±112

7.3 Effect of disintegration of biosludge on COD and BOD

The ozonation of biomass disrupted the cell walls of microorganisms in waste activated sludge and cytoplasm was eluted into the solution. The mixed sludge sample as well as filtered aqueous phase were analysed for COD and BOD content to evaluate the effect of ozonation on biosludge. The total COD (COD_T) of mixed biosludge was 7873±249 mg/l or 1392±16 mg/g of MLVSS. With ozonation a decrease in COD content was observed which indicated the oxidation of organic material to CO_2 (Table 7.7). The complete mineralization of biomass to CO_2 was insignificant and a decreasing trend was observed with the increase in ozone dosage. Several researchers have reported different results; Kamiya and Hirotsugi, 1998 reported that ozonation not only solubilized the sludge, but also mineralized it. A little mineralization was observed by Yasui and shibata, 1994 and Salsabil, 2008. Whereas Bougrier, 2006 reported that ozonation did not mineralize sludge. Based on the reported findings and present research it can be inferred that the extent of mineralization depends on nature of sludge and dosage of ozone.

The dissolution of biomass during ozone treatment resulted in increase in CODs in aqueous phase while reducing the MLSS and MLVSS. The COD_T was 1392±16 mg/g and 1939±51 mg/g of MLVSS at 0 and 92.3±1.5 mg O_3/g DS dosage (Figure 7.7). Based on the empirical formula of biomass; $C_5H_7NO_2$, the oxygen equivalent (COD) of it (typically measured as MLVSS) is approximately 1.42 g COD/g of biomass (Tchobanoglous et al., 2003).

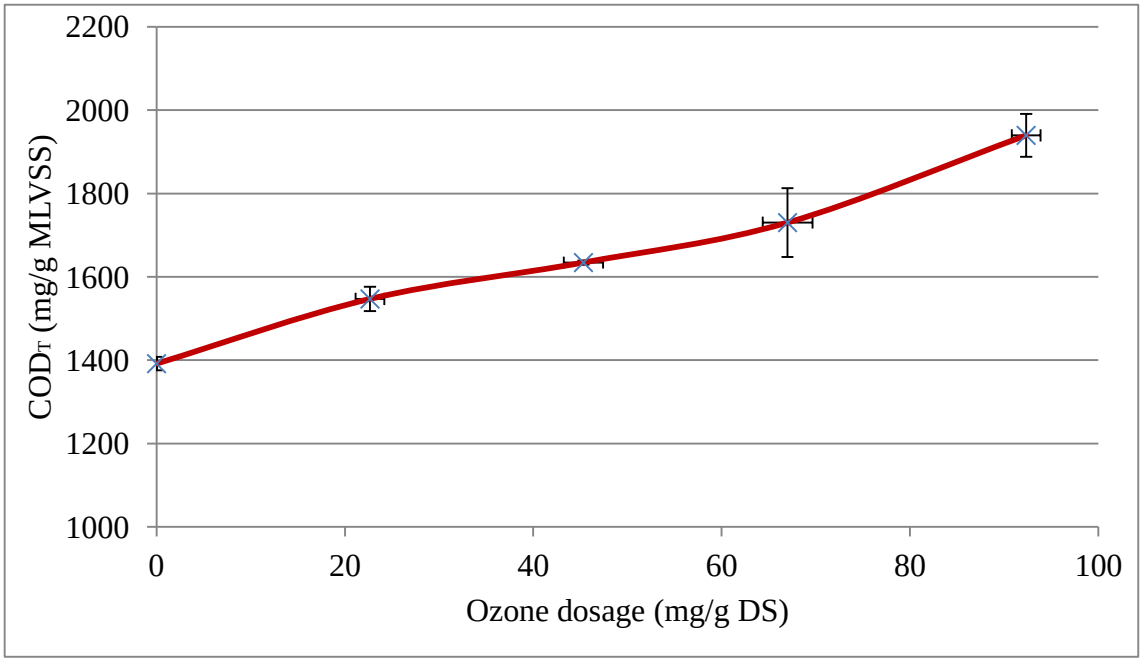


Figure 7.7: Contribution in COD_T due to destruction of MLVSS content

The change in COD and BOD with ozone treatment is given in Table 7.7. After settling of biomass, the supernatant aqueous phase was quite clear before ozone treatment, whereas it was quite turbid even after 24 h due to presence of disrupted cell components by ozonation.

Table 7.7: Effect of disintegration of biosludge with varying dosage of ozone on COD and BOD

Ozone dosage (mg/g DS)	COD_T (mg/l)	CODs (mg/l)	BOD (mg/l)
0.0	7873±249	268±10	16±8
22.7±1.5	7746±274	617±22	111±13
45.3±2.1	7700±269	1276±61	363±23
67.0±2.6	7522±253	1783±74	860±98
92.3±1.5	7511±253	2365±293	1629±113

The initial BOD to CODs ratio was 6.2±3.2% in the aqueous phase. With increase in ozonation, the ratio was changed to 69.6±10.1% (Figure 7.8) which indicated that the disintegrated material was biodegradable in nature. The increase in CODs and BOD at 92.3±1.5 mg O_3/g DS dosage was 2098±293 and 1614±110 mg/l respectively in the aqueous phase.

Bijan and Mohseni, 2004 reported the behavior of pulp and paper mill effluent during ozone treatment. Ozonation enhanced the biodegradability of the wastewater by 30-40%. The ozonation degraded the refractory high molecular weight (HMW) compounds into low molecular weight (LMW) one and increased the biodegradability. The biodegradability of color causing organics was increased by 60%, whereas that of HMW fraction was increased by about 50%. Corollary to it, ozonation of biosludge releases the low molecular weight biodegradable compounds.

At ozone dosage of 45.3±2.1 and 92.3±1.5 mg/g DS, there was an increase of CODs content of 178±8 and 371±48 mg/g of MLVSS dissolution respectively. Increase in CODs in aqueous phase is directly proportional to the ozone dosage; 2.08 times increase in ozone dosage resulted in 2.03 times increase in CODs content (Figure 7.9). The CODs increase per gram of disintegrated MLVSS was lowest at 22.7±1.5 mg O_3/g DS dosage and it was only 0.6±0.1 g/g of MLVSS dissolution. At higher dosage from 45.3±2.1 to 92.3±1.5 mg O_3/g DS, the CODs build-up was constant and it was 1.1±0.1 g/g of MLVSS dissolution. The initial disintegration of biomass resulted in highest dissolution of MLSS but it contributed less in CODs build-up. The constant ratio in the range of 1.1±0.1 at higher dosage is indicative of the fact that with increase in ozone dosage conversion of biomass to CODs was stable.

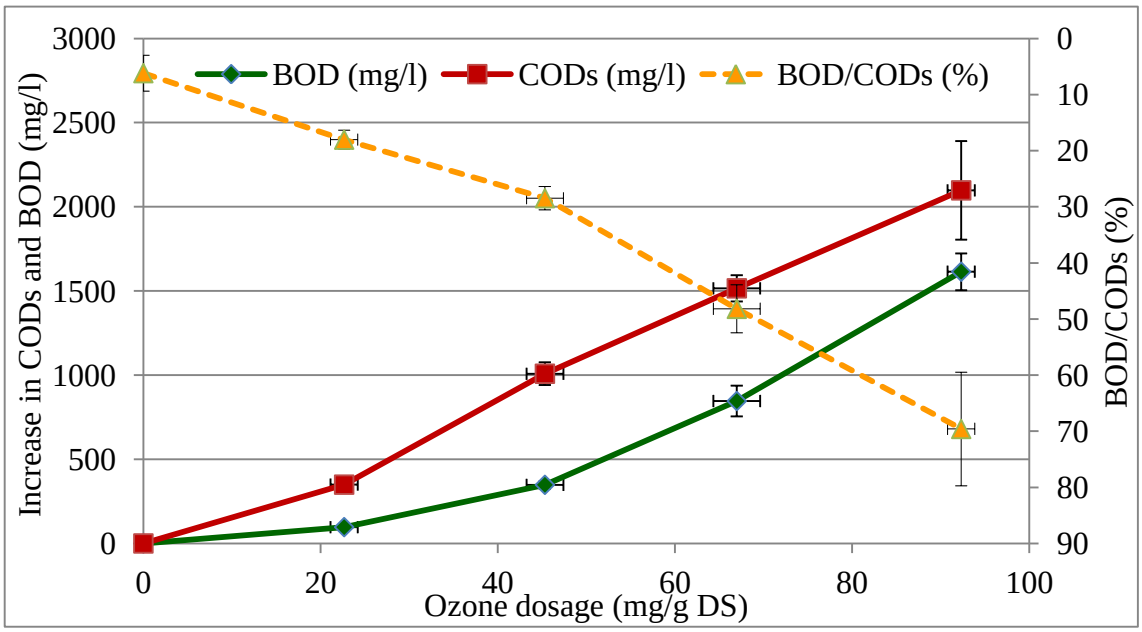


Figure 7.8: Build-up of organic material with ozonation

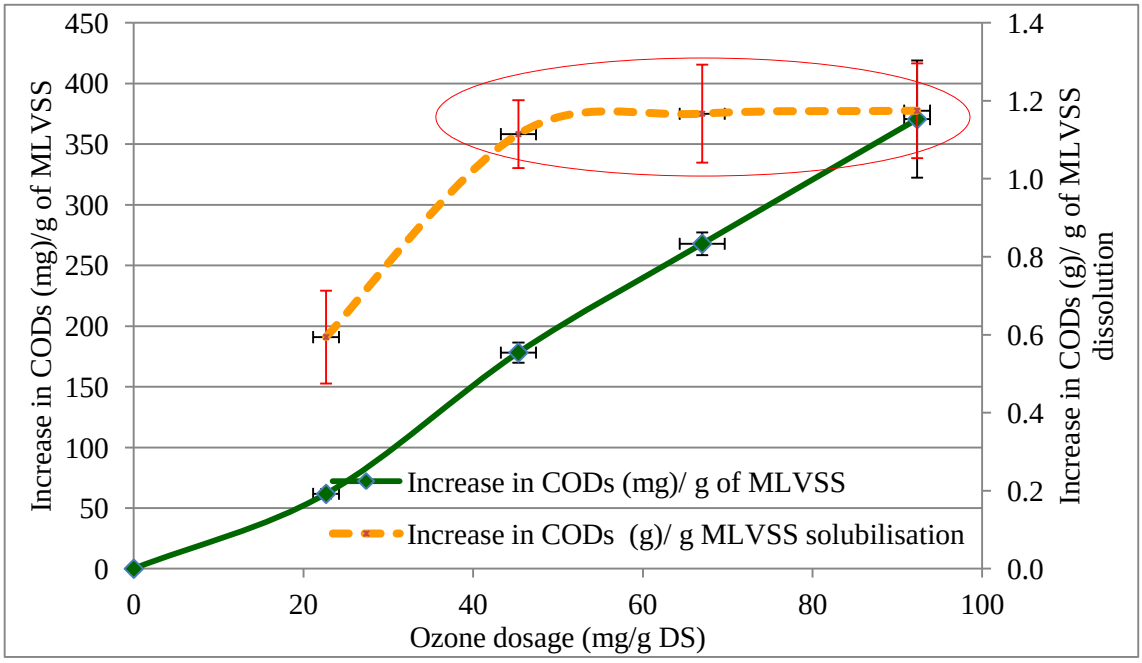


Figure 7.9: Increase in CODs with dissolution of MLVSS content

To estimate the relative contribution of dispersed material to COD in the aqueous phase, the sample was filtered through GFC filter paper (porosity 1.2 µm) and analyzed for COD (Table 7.8). The initial ratio of COD in 1.2 µm to 70 µm filtrate samples was 0.929, whereas biodegradability of the wastewater was very poor. The ratio was reduced to 0.85, 0.86, 0.85 and 0.79 at 22.7±1.5, 45.3±2.1, 67.0±2.6, 92.3±1.5 mg O_3/g DS dosage respectively. Although the ratio of COD in 70 and 1.2 µm filtrate sample was decreased, biodegradability of the dispersed material was high.

Table 7.8: Effect of disintegration of biosludge on TOC and COD

Ozone dosage (mg/g DS)	TOC (mg/l)	COD (mg/l)	
		70 µm filtrate	1.2 µm filtrate
0.0	95±4	268±10	249±12
22.7±1.5	230±12	617±22	522±18
45.3±2.1	483±32	1276±61	1102±69
67.0±2.6	694±43	1783±74	1512±93
92.3±1.5	935±90	2365±293	1877±315

The dissolution of biomass resulted in increase in TOC content in the aqueous phase. The initial ratio of CODs to TOC was 2.83±0.03 and with dissolution of biomass, the ratio decreased to 2.53±0.16 at 92.3±1.5 mg O_3/g DS dosage (Figure 7.10). Increase of TOC in aqueous phase conclusively proves the dissolution of organic material.

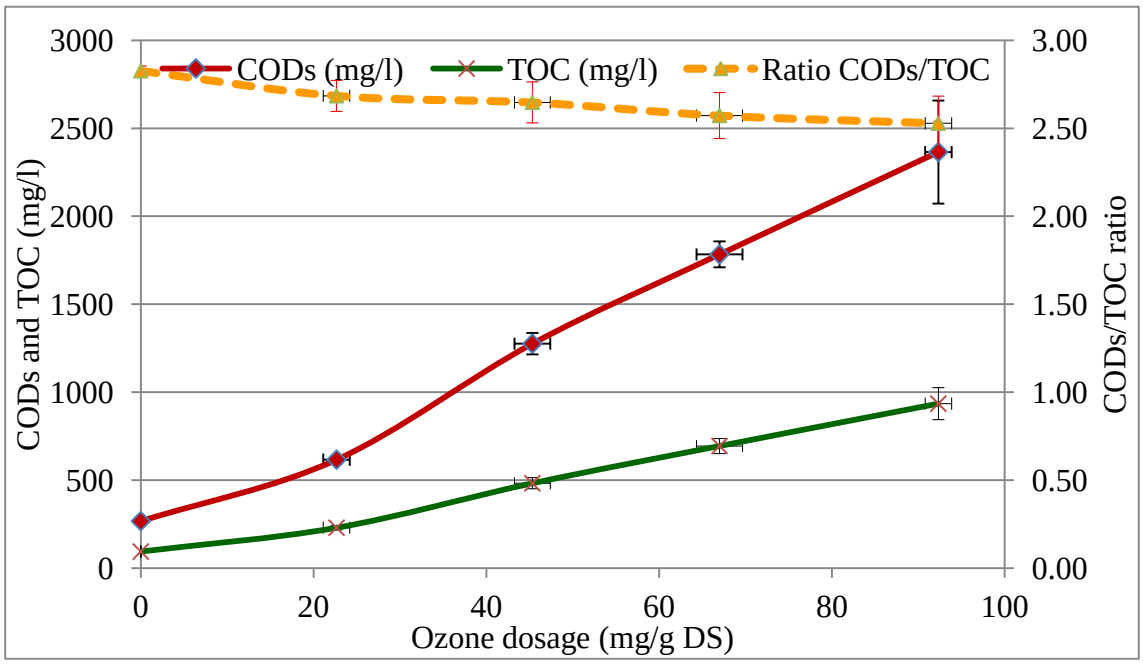


Figure 7.10: Relationship of CODs and TOC content in the aqueous phase

7.4 Effect of ozonation of biosludge on physio-chemical properties in the aqueous phase

Ozonation of biosludge resulted in decrease in pH of the aqueous phase. There was a drop of pH by 0.4 to 0.8 from the initial pH of 7.1±0.1 at ozone dosage of 22.7±1.5 to 92.3±1.5 mg/g DS (Table 7.9). During ozonation, compounds of higher molecular weight and fatty matter were oxidized to lower molecular weight compounds and fatty acids which resulted in drop in pH (Fontanier et al. 2005; Poznyak & Vivero 2005, Chu et al., 2008).

Table 7.9: Effect of ozonation of biosludge on change of pH, release of colour and lignin in aqueous phase

Ozone dosage (mg/g DS)	pH	Colour (Pt-Co unit)	Lignin (mg/l)
0.0	7.1±0.1	312±15	62±3
22.7±1.5	6.7±0.1	691±28	115±7
45.3±2.1	6.5±0.1	1019±38	163±8
67.0±2.6	6.3±0.1	1545±50	241±13
92.3±1.5	6.3±0.1	1883±34	281±10

The wastewater from pulp and paper mill contains dark brown colour due to presence of lignin and its derivatives (Etiegni et al., 2007; Singh, 2007). The initial colour in the aqueous phase was 312±15 Pt-Co unit and corresponding lignin content was 62±3 mg/l (Table 7.9). Lignin and its derivatives are adsorbed on the surface of the biosludge during treatment of wastewater. With the progress of ozonation, the colour causing substances and lignin were eluted in the aqueous phase. Ozone has been found to be an effective oxidizing agent for degradation of lignin compounds and well reported for removal of colour from pulp and paper mill wastewater (Joss et al., 2007; Morais et al., 2008; Assalin, 2009; Rosner, 2009; Tran, 2009). The ratio of lignin to colour showed a marginally decreasing trend from 0.17 to 0.15 at ozone dosage from 22.7±1.5 to 92.3±1.5 mg/g DS respectively (Figure 7.11). The decrease in ratio is indicative of partial oxidation of lignin molecules to low molecular weight compounds having chromophoric groups which have contributed to colour.

In the present case ozone preferentially reacted with the macro and micro flocs of the biosludge. The lignin and other colour causing substances, which were adsorbed on the surface of the biosludge, were driven out. Reaction with lignin was a secondary phenomenon.

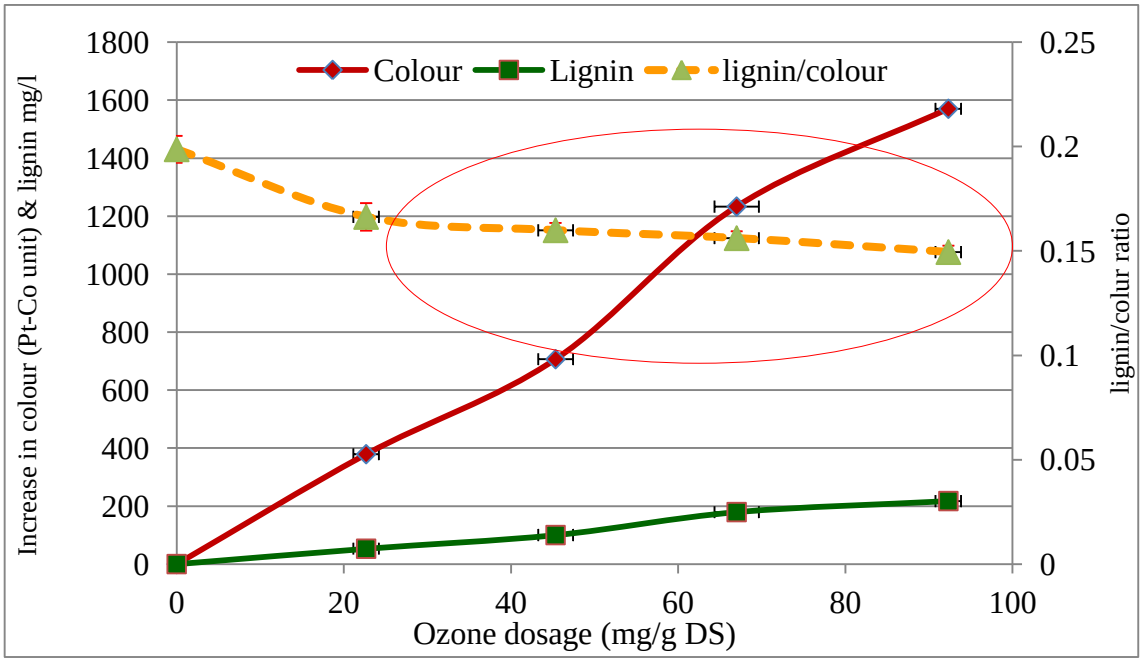


Figure 7.11: Effect of ozone dosage on release of colour and lignin compounds

7.5 Effect of ozonation on organochlorine compounds

As described earlier (section 4.1.1) the biosludge under study contained different forms of organochlorine like AOX, EOX, chlorophenols, PCDD and PCDF. The following section will describe the effect of ozonation on these compounds.

7.5.1 Degradation of AOX and EOX compounds at different ozone dosage

Effect of ozonation on the concentration of AOX compounds in various physical state of biosludge and aqueous phase is given in Table 7.10. The initial concentration of AOX compounds in mixed sludge, dewatered sludge and aqueous phase was 23.5±0.3 mg/l, 1980±42 mg/kg and 8.2±0.4 mg/l respectively.

Table 7.10: Effect of ozonation on AOX compounds in the biosludge and aqueous phase

Ozone dosage (mg/g DS)	AOX compounds			
	Mixed sludge (mg/l)	Dewatered sludge (mg/kg)	Aqueous phase (mg/l)	
			70 µm filtrate	1.2 µm filtrate
0.0	23.5±0.3	1980±42	8.2±0.4	7.4±0.4
22.7±1.5	18.8±0.3	1769±89	6.7±0.8	6.2±0.5
45.3±2.1	18.2±0.8	1693±44	6.8±0.1	6.3±0.2
67.0±2.6	15.7±0.1	1376±107	7.2±0.3	6.4±0.1
92.3±1.5	13.9±0.3	1080±85	7.9±0.5	6.8±0.3

With increase in ozone dosage considerable removal of organochlorine compounds was observed. Initially there was 20±1% removal of AOX compounds at 22.7±1.5 mg O_3/g DS dosage. With increase in ozone dosage, the removal of the same was increased (Figure 7.12). Initially the concentration of AOX compounds was decreased in aqueous phase due to oxidation of easily oxidisable organic compounds with ozone. The adsorbed organochlorine compounds were released in aqueous phase during disintegration of biomass and concentration was increased. The increase in concentration of AOX compounds in aqueous phase was not same as that was released due to dissolution of biomass. The release of organochlorine compounds from biosludge and degradation of the same in aqueous phase

took place simultaneously and the concentration of AOX compounds in the aqueous phase remained same. Similar observation on dechlorination of AOX compounds in the effluent of pulp and paper mill was reported by Balcioglu et al., 2007. When the samples of aqueous phase were filtered through GFC filter paper (1.2 µm) and analyzed for AOX content, it was observed that the average value of AOX in 1.2 µm filtrate was 90% of that in 70 µm filtrate. This indicated that finely dispersed particles have some contribution of the AOX compounds.

The dechlorination of organochlorine compounds resulted in release of chloride ions in the solution. Chloride content in the aqueous phase increased from 683±8 to 694±7 mg/l with ozone treatment. The increase in concentration of chloride was corroborated by dechlorination of AOX compounds. Oxidative degradation of organochlorine compounds resulted in dehalogenation of the same. Dechlorination of AOX compounds during ozonation of alkali extraction stage effluent was also reported by Wang et al., 2004.

As reported earlier, the concentration of EOX compounds in the dewatered biosludge and in aqueous phase was 599±19 mg/kg and 0.09±0.02 mg/l respectively. With ozone treatment, reduction in EOX content of the sludge was also observed, whereas the same was not detectable in the aqueous phase. During ozonation of biomass, the significant portion of adsorbed EOX compounds were oxidized and resulted in 67±1% removal of the same at 92.3±1.5 mg O_3/g DS dosage (Figure 7.13).

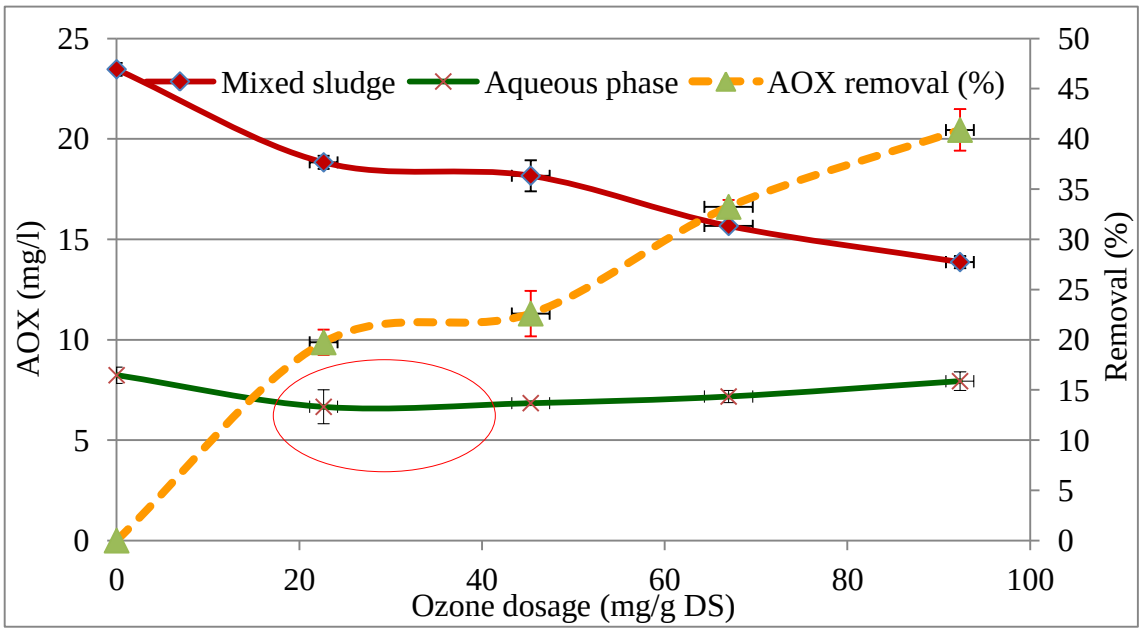


Figure 7.12: Removal of AOX compounds with ozonation

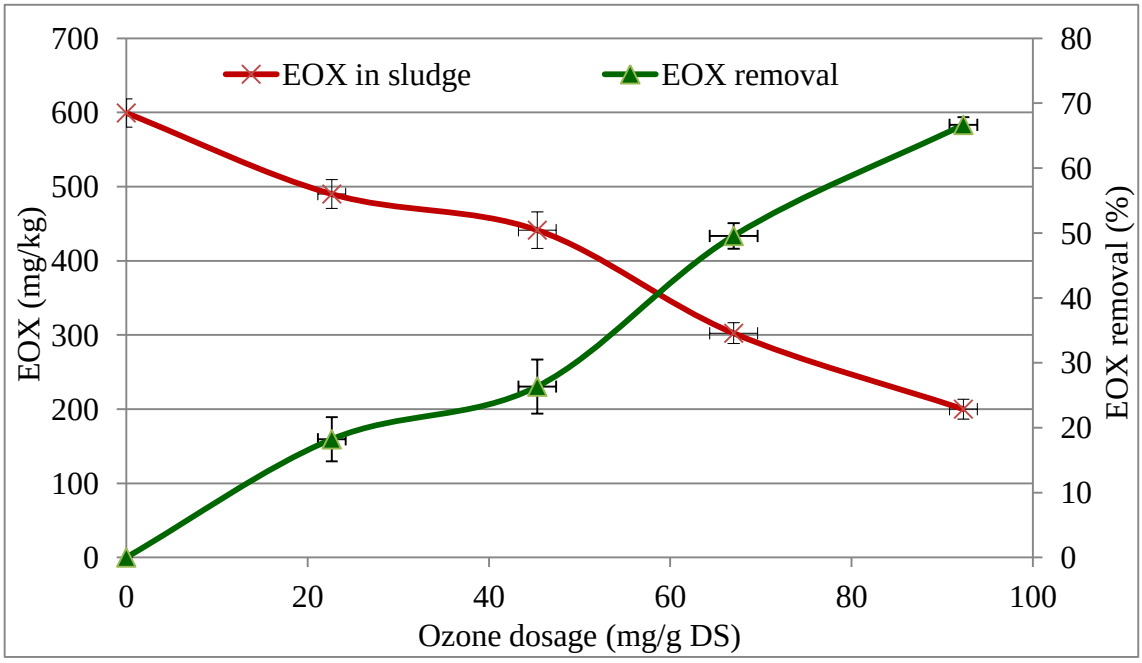


Figure 7.13: Removal of EOX compounds with ozonation

7.5.2 Degradation of chlorophenolic compounds at varying ozone dosage

As described earlier (section 4.1.1) 11 higher molecular weight chlorophenolic compounds were detected in the wastewater before biological treatment. 10 such compounds were present in the biosludge (Table 7.11). The total concentration of chlorophenolic compounds in the biosludge was 2087 µg/kg which was equivalent to 717 µg/kg with respect to toxicity equivalency. The ozonation made significant changes in degradation of all the chlorophenolic compounds in the biosludge.

Table 7.11: Chlorophenolic compounds in biosludge before and after ozonation

Compound	Feed (µg/l)	Ozone dosage (mg/g DS)				
		0.0	22.7±1.5	45.3±2.1	67.0±2.6	92.3±1.5
		Chlorophenolic compounds (µg/kg)				
2,4,6-TCP	7.16±0.90	311±23	247±11	129±47	104±10	74±10
2,4,5-TCP	0.56±0.15	85±14	56±5	44±4	41±1	40±2
2,3,4,5-TeCP	1.30±0.29	132±5	67±4	42±11	37±7	35±4
3,4,6-TCG	1.25±0.28	<43	ND	ND	ND	ND
3,4,5-TCG	1.91±0.16	89±38	82±4	63±8	58±6	52±4
4,5,6-TCG	< 0.11	<49	ND	ND	ND	ND
3,4,6-TCC	5.96±0.50	240±25	183±9	140±14	135±11	107±11
PCP	0.42±0.13	259±32	181±8	124±38	110±27	106±15
3,4,5-TCC	8.45±0.72	319±32	257±13	221±31	196±16	159±17
TeCG	3.12±0.31	64±12	55±3	51±1	49±1	45±4
TCS	4.48±0.85	233±37	209±10	190±10	166±16	166±34
TeCC	3.20±0.32	311±54	273±7	248±19	214±7	204±9
TEQ	9.46	717	546	418	370	339

In case of biosludge, the degradation of chlorophenolic compounds was increased with increase in ozone dosage; the same was varied from 7.3±4.5 (3,4,5-TCG) to 49.3±3.4% (2,3,4,5-TeCP) at 22.7±1.5 mg O_3/g DS dosage (Figure 7.14). The maximum degradation

was observed for 2,3,4,5-TeCP at initial ozone concentration of 22.7±1.5 (49.3±3.4%). Subsequently it was 68.1±8.6 and 72.0±5.1% at ozone dosage of 45.3±2.1 and 67.0±2.6 mg O_3/ DS respectively. The same was highest for 2,4,6-TCP (76.3±3.2%) at ozone dosage of 92.3±1.5 mg/g DS. 3,4,6-TCG and 4,5,6-TCG were below detection limit in the virgin biosludge and after ozone treatment both the compounds were not detected. The concentration of total chlorophenolic compounds was 1611, 1254, 1111 and 986 µg/kg after ozonation at 22.7±1.5, 45.3±2.1, 67.0±2.6 and 92.3±1.5 mg O_3/g DS dosage respectively. The corresponding removal was 22.8, 40.0, 46.8 and 52.7% respectively. None of the compounds was detectable in the aqueous phase after ozone treatment. Similar results were reported by Wang et al., 2004 during ozone treatment of alkali extraction stage wastewater where most of the major chlorophenols identified in the E-1 effluent were not detected after treatment with ozone. The TEQ in the biosludge was decreased by 23.9, 41.7, 48.4 and 52.7% at ozone dosage of 22.7±1.5 45.3±2.1, 67.0±2.6 and 92.3±1.5 mg O_3/g DS respectively. Almost complete removal of TEQ was observed in aqueous phase due to the absence of chlorophenolic compounds.

To confirm the degradation of organochlorine compounds in aqueous phase, 1.2 liter of water was spiked with known quantity of all the chlorophenols in the range of 60-500 µg/l and ozonated. There was 92-100 % degradation of all the compounds at very low concentration of ozone varying from 10-30 mg O_3/l. To understand the mode of degradation of chlorophenolic compounds during ozonation, 1.5 litre of water samples were spiked separately with 2,4,5-TCP, 2,4,6-TCP, 2,3,4,5-TeCP and PCP at a concentration of 9.71, 7.23, 8.60 and 7.57 mg/l respectively. The samples were characterized for individual chlorophenolic compound, AOX content and chloride content before and after ozonation. The initial AOX content of these samples was 5.03, 3.69, 5.43 and 4.84 mg/l respectively. The samples were treated at three different low ozone dosages of 1.0, 2.1 and 4.8 mg O_3/l respectively. The ozone treatment of the sample was performed at low dosage to avoid the complete degradation of organochlorine compounds and to know the mode of degradation. During the ozonation of all the four compounds, with increase in ozone dosage, the concentration of AOX and chlorophenolic compounds was decreased and concentration of inorganic chloride ions was increased.

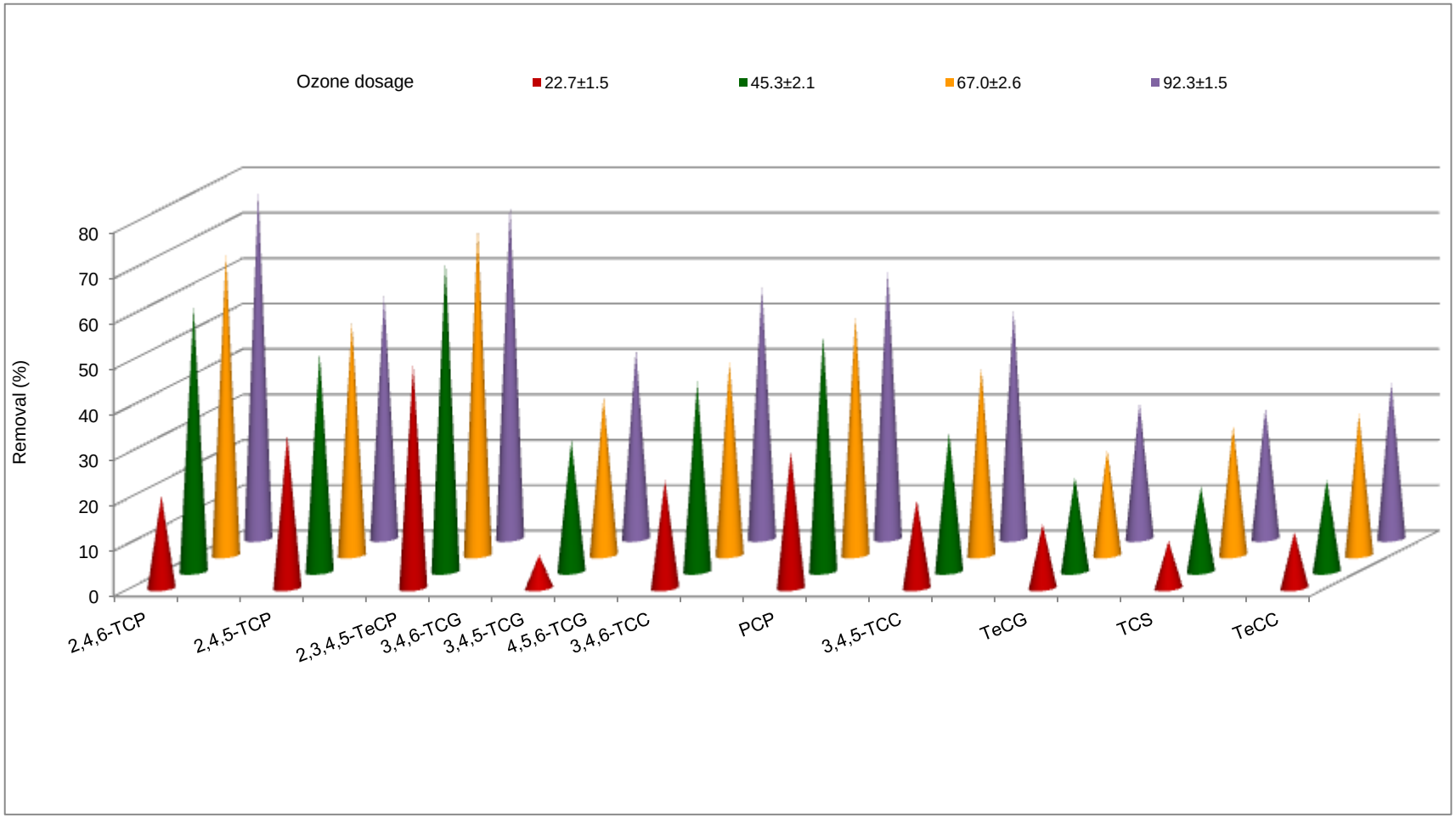


Figure 7.14: Removal of chlorophenolic compounds with ozone dosage

In case of 2,4,5-TCP, 20.5, 29.6 and 50.3% removal of AOX and 37.7, 69.4 and 96.0% removal of chlorophenolic compound was observed at ozone dosage of 1.0, 2.1 and 4.8 mg O_3/l respectively (Figure 7.15). Similarly the degradation of AOX and 2,4,6-TCP was 21.4, 35.0, 45.8% and 27.9, 66.7 and 97.4% respectively (Figure 7.16). There was 26.3, 40.5, 61.3% reduction in AOX and 31.1, 74.3, 96.5% reduction in chlorophenolic compound respectively for 2,3,4,5-TeCP (Figure 7.17). During ozonation of pentachlorophenol, other tri and tetra chlorophenols were not detected in the degradation product. It supported that the removal of pentachlorophenol was accomplished by degradation of the same into low molecular weight compounds and subsequent dehalogenation instead of only dehalogenation without ring opening. The degradation of the AOX and PCP was 26.9, 40.7, 64.3 % and 46.0, 80.8 and 97.7% at ozone dosage of 1.0, 2.1 and 4.8 mg O_3/l respectively (Figure 7.18). In all the cases, the decrease in concentration of AOX resulted in equivalent increase in concentration of inorganic chloride. It revealed that the ozonation of chlorophenolic compounds was accomplished by partial dechlorination as well as decomposition into low molecular weight organochlorine compounds.

The degradation of chlorophenols in aqueous phase with ozone has been extensively studied by Sung and Huang, 2007 and suggested that there was a dosage-dependent pathway in the direct ozonation of 2-chlorophenol; oxalic acid was the major end product. The ozone reaction with substituted phenolic compounds formed a Criegee intermediate that undergoes further rearrangement resulting in ring cleavage to generate substituted muconic, maleic, and other linear olefinic carboxylic acids. Continued ozonation of these intermediate products leads to formation of oxalic acid (Lesko et al., 2006).

7.5.3 Degradation of PCDD/Fs at varying ozone dosage

The dewatered biosludge was analyzed for PCDD and PCDF compounds before and after ozonation (Table 7.12). The ozonation was found to be effective for degradation of all PCDD and PCDF compounds. 92-100% degradation of the compounds was observed at an ozone dosage of 46 mg/g DS. The upper bound I-TEQ from PCDD/Fs was reduced to 3.7 pg/g DS with a reduction of 93%. A few reports are available on ozone assisted degradation of PCDD/Fs compounds; Wang et al., 2008 reported removal of gaseous PCDD/Fs with catalytic ozonation and 94% destruction efficiency was achieved.

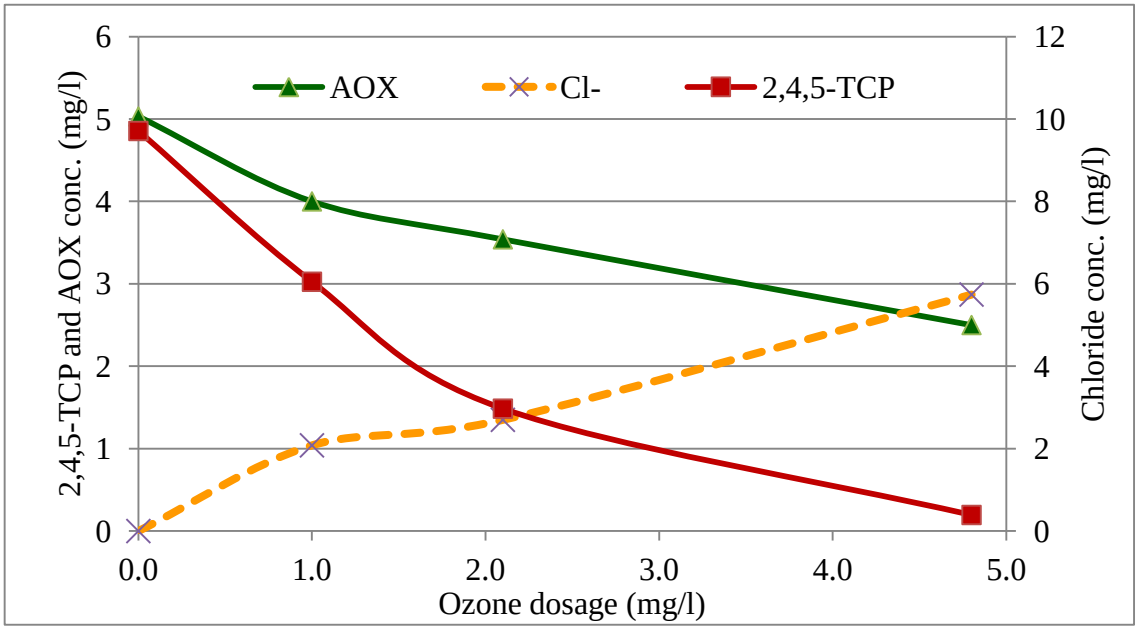


Figure 7.15: Degradation of 2,4,5-TCP with ozone dosage

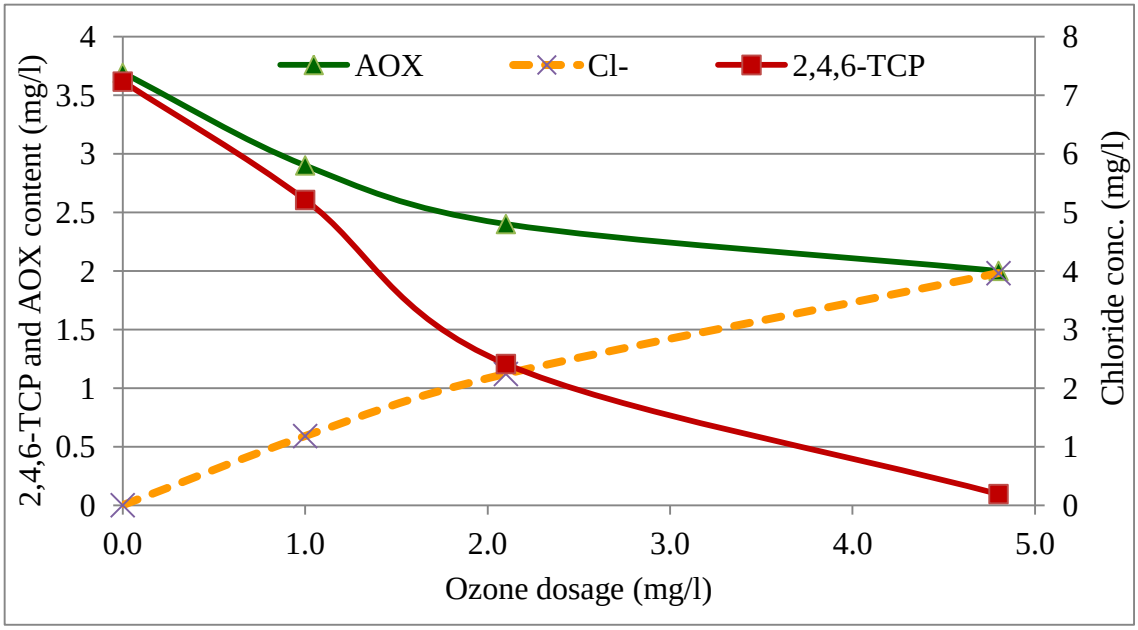


Figure 7.16: Degradation of 2,4,6-TCP with ozone dosage

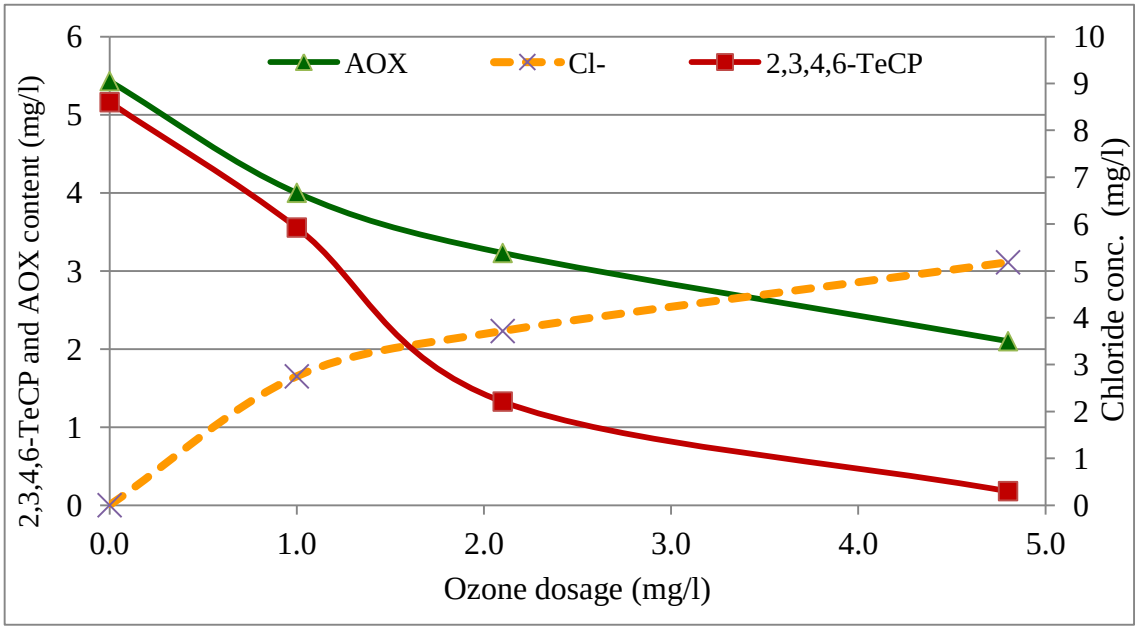


Figure 7.17: Degradation of 2,3,4,6-TeCP with ozone dosage

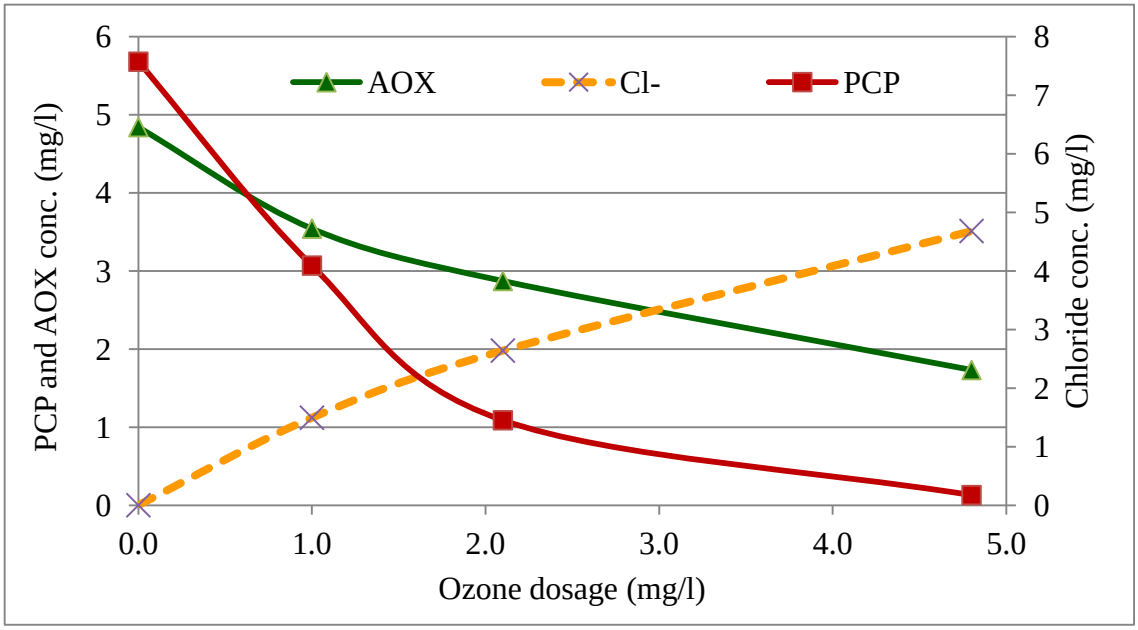


Figure 7.18: Degradation of pentachlorophenol with ozone dosage

Table 7.12: Concentration of PCDD and PCDF compounds in biosludge

PCDD/PCDF (pg/g DS)	TEF	Biosludge*	Ozonated biosludge	Removal (%)
2,3,7,8-TCDD	1	16	ND	~100
1,2,3,7,8-Pe CDD	0.5	<2.0	ND	~100
1,2,3,4,7,8-Hx CDD	0.1	ND	ND	-
1,2,3,6,7,8- Hx CDD	0.1	2.2	ND	~100
1,2,3,7,8,9-HxCDD	0.1	<2.4	ND	~100
1,2,3,4,6,7,8-HpCDD	0.01	250	19	92
OCDD	0.001	8300	120	99
2,3,7,8-TCDF	0.1	210	8.1	96
1,2,3,7,8-PeCDF	0.05	7.4	ND	~100
2,3,4,7,8-PeCDF	0.5	6.1	ND	~100
1,2,3,4,7,8-HxCDF	0.1	<1.4	ND	~100
1,2,3,6,7,8- HxCDF	0.1	<1.4	ND	~100
1,2,3,7,8,9- Hx CDD	0.1	ND	ND	-
2,3,4,6,7,8-HxCDF	0.1	3.0	ND	~100
1,2,3,4,6,7,8- HpCDF	0.01	23	<3.2	~100
1,2,3,4,7,8,9-HpCDF	0.01	ND	ND	-
OCDF	0.001	87	<4.7	~100
Lower bound I-TEQ from PCDD/Fs		52	1.1	98
Upper bound I-TEQ from PCDD/Fs		54	3.7	93

*Refer table 4.5

7.6 Effect of ozonation on morphological and biological properties of biosludge

The biosludge was rich in filamentous organisms and diluted sludge volume index (DSVI) was 509±19 ml/g.

7.6.1 Impact of ozonation on viable count

The viable count of the biomass represented the amount of active colony forming bacteria. The colony-forming unit (CFU) of mixed sludge collected from the plant was $116x10^5 \pm 6x10^5$ count/100 µl. The same was reduced to $14x10^5 \pm 4x10^5$ and $52x10^4 \pm 15x10^4$ at ozone dosage of 45.3±2.1and 92.3±1.5 mg/g DS dosage respectively (Figure 7.19).

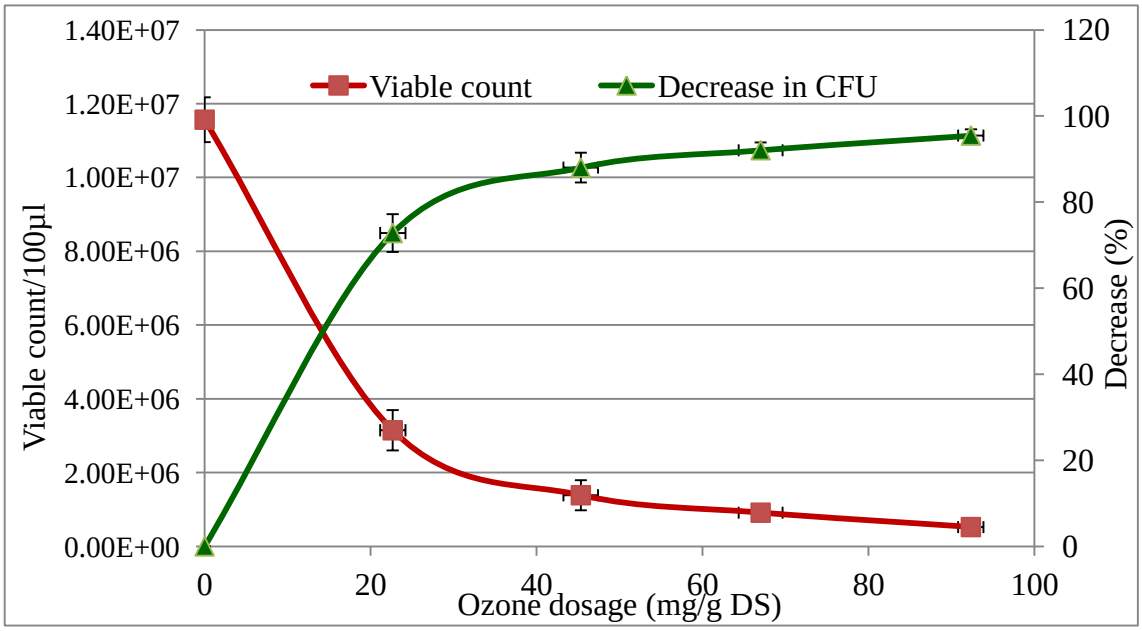


Figure 7.19: Reduction in viable count with ozone dosage

Earlier researchers have reported on disinfection of water with ozonation. Near to 100% deactivation of microorganisms has been observed with ozone treatment (Cho et al., 2003).

7.6.2 Impact of ozonation on settling properties

The bulking nature of sludge was due to the presence of excessive filamentous organisms (Figure 4.3). With increase in ozone dosage, significant decrease in DSVI values was observed (Table 7.13). The DSVI values were 255±31 and 166±25 ml/g at ozone dosage of 45.3±2.1and 92.3±1.5 mg /g DS respectively. At low ozone dosage, reduction in SVI value was only 14±6% and by two fold increase in ozone dosage, reduction was about 3.6 times. Figure 7.20a-e showed that at lower ozone dosage, filamentous organisms were in dominant conditions.

Table 7.13: Settling characteristics of biosludge at varying ozone dosage

Ozone dosage (mg/g DS)	DSVI (ml/g)	Reduction (%)
0.0	509±19	-
22.7±1.5	439±24	14±6
45.3±2.1	255±31	50±6
67.0±2.6	205±29	60±5
92.3±1.5	166±25	67±5

The morphology of biosludge at different dosage of ozone indicated that the abundance of filamentous organisms was decreased with increase in ozone dosage. The length of filamentous organisms varied from 1 to 576 µm and average length varied from 50-54 µm. The extended filament length of the control sample was $28.1x10^{8}$±$2.9x10^{8}$ (µm/ml) and a sharp reduction in both average and extended length was observed with increase in ozone dosage (Table 7.14). The extended filament length of filamentous organisms was decreased by 64±2% at ozone dosage of 91-94 mg O_3/g DS. The filamentous bacteria were vulnerable to ozone treatment since they had a high surface area to volume ratio, and could maintain a higher rate of mass transfer across cell boundaries than floc forming microorganisms. Similar observation on destruction of filamentous organisms and improvement in settling properties was reported by Wijnbladh, 2007.

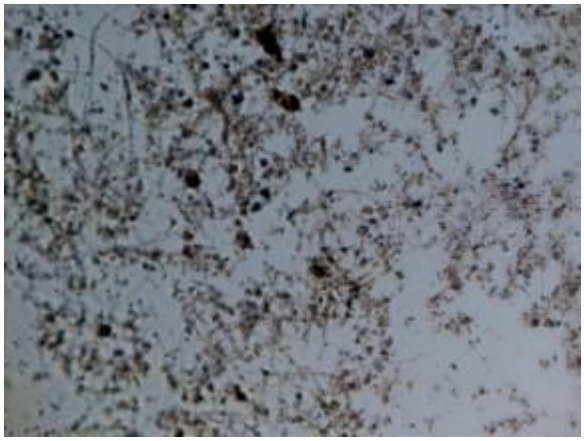


Figure 7.20a

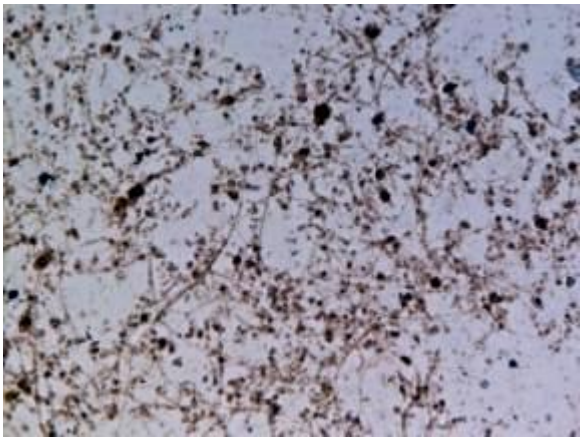


Figure 7.20b

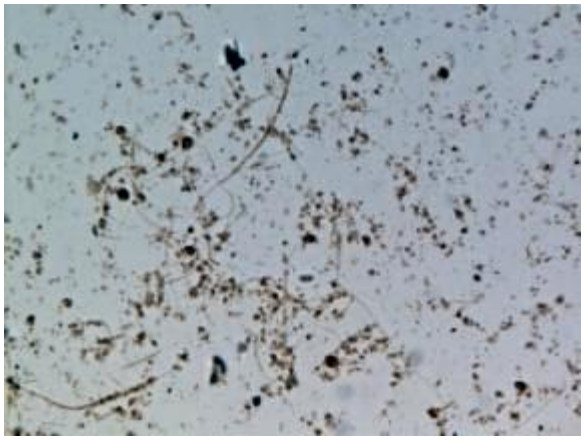


Figure 7.20c

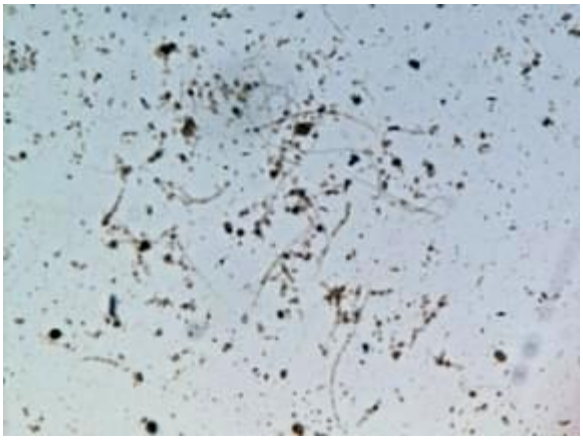


Figure 7.20d

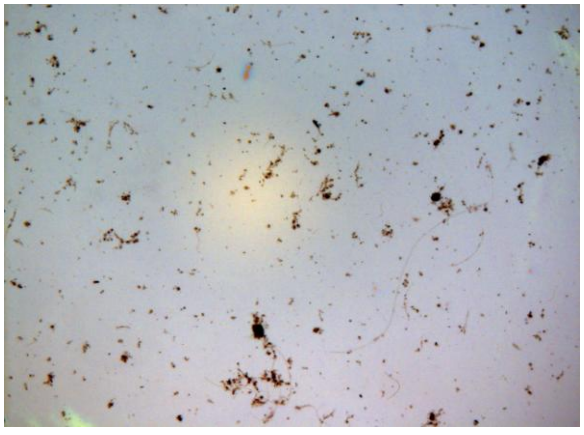


Figure 7.20e

Figure 7.20: Impact of ozone on morphology of biosludge;

a) control sludge
b) Ozone dosage 24 mg/g DS
c) Ozone dosage 47 mg/g DS
d) Ozone dosage 70 mg/g DS
e) Ozone dosage 94 mg/g DS

Table 7.14: Impact on number and length profile of filamentous organisms with ozone dosage

Ozone dosage (mg/g DS)	Average length (μm)	No. of filament/ml	Extended filament length (μm/ml)	Extended filament length (μm/g initial MLSS)
0.0	53±2	$52.8x10^6±4.3x10^6$	$28.1x10^8±2.9x10^8$	$366x10^9±39x10^9$
22.7±1.5	53±4	$29.6x10^6±0.9x10^6$	$15.8x10^8±0.9x10^8$	$205x10^9±12x10^9$
45.3±2.1	46±1	$26.7x10^6±2.2x10^6$	$12.2x10^8±0.8x10^8$	$159x10^9±8x10^9$
67.0±2.6	46±3	$24.2x10^6±1.9x10^6$	$11.0x10^8±0.7x10^8$	$143x10^9±8x10^9$
92.3±1.5	38±3	$26.3x10^6±2.8x10^6$	$10.0x10^8±0.8x10^8$	$130x10^9±10x10^9$

The zeta potential of the untreated biosludge was –17.1±0.2 mV. Flocs in activated sludge carry negative charge at neutral pH, usually between -10 to -20 mV (Jia et al., 1996). This was due to the ionization of the functional groups, such as carboxylic and phosphate, on the sludge surface. If the electronegativity of the floc surface was sufficiently large, repulsion might occur, hold the particles far apart and hinder the settling of biosludge (Morgan et al., 1990). During ozonation, the zeta potential of biomass was –13.0±0.1 mV at ozone dosage of 92.3±1.5 mg /g DS. On the contrary, the electronegativity of the aqueous phase was –69±3 μeq/l and increased to -1378±21 μeq/l due to dissolution of negatively charged biomass (Table 7.15).

Table 7.15: Effect of ozonation on electrochemical properties of biosludge

Ozone dosage (mg/g DS)	Dissolved charge (μeq/l)	Zeta Potential (mV)
0.0	-69±3	-17.1±0.2
22.7±1.5	-372±11	-15.9±0.2
45.3±2.1	-948±17	-14.6±0.1
67.0±2.6	-1204±18	-13.7±0.2
92.3±1.5	-1378±21	-13.0±0.1

The change in zeta potential resulted in lowering of intermolecular repulsion. The lysis of filamentous organisms and reduction in zeta potential combinedly resulted in low SVI values (Figure 7.21). Though the decrease in filament length was significant at 22.7±1.5 mg O_3/g DS dosage, corresponding decrease in SVI was insignificant.

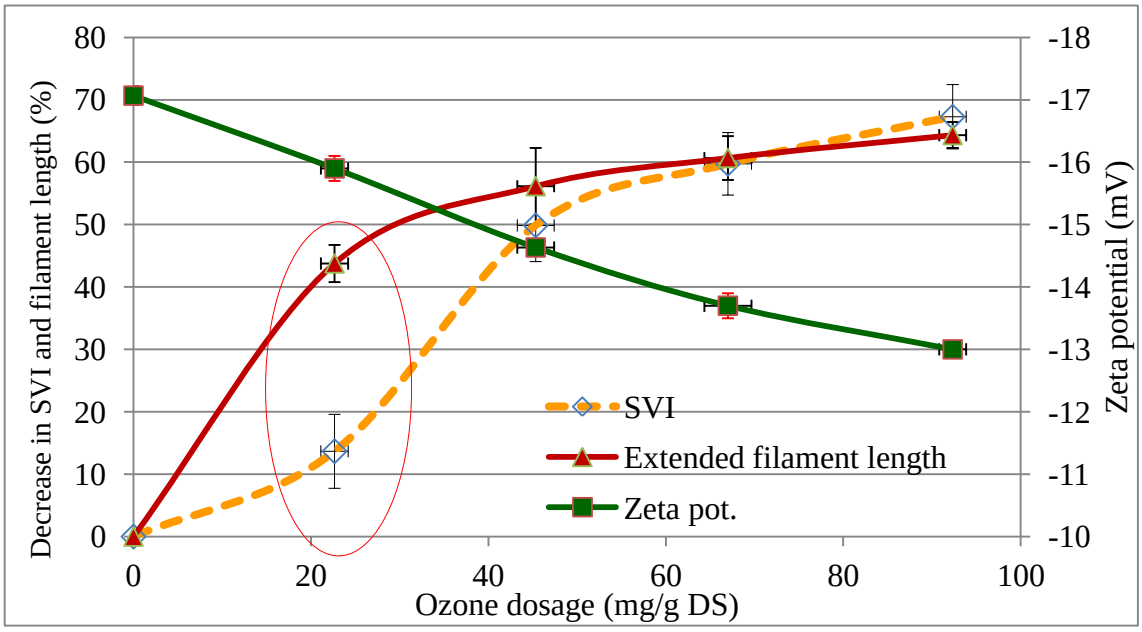


Figure 7.21: Change in sludge volume index, zeta potential and filamentous length with ozone dosage

Summary

Ozonation of biosludge altered the physical, chemical and biological characteristics. Elution of cytoplasm into bulk solution by the lysis of cell wall of organisms and conversion of refractory molecules into low molecular weight biodegradable compound are the result of the ozonation. Due to disintegration of biomass, concentration of MLSS and MLVSS was decreased with increased dosage of ozone. The reduction in MLSS concentration was accomplished with its partial dissolution. The specific dissolution of MLSS and MLVSS was highest at lower dosage of ozone (i.e. 22.7 mg/g DS) due to initial disruption of diffused macro-flocs and subsequent release of adsorbed inorganic material into the aqueous phase. The dissolution of MLVSS content was lesser at lower dosage of ozone but degree of dissolution was high due to release of adsorbed material. The extent of solubilization of MLSS and MLVSS depended on the ozone dosage. Solubilization of MLSS and MLVSS was 10.1 to 27.4% and 10.6 to 31.5% respectively at ozone dosage of 22.7 and 92.3 mg/g DS. The disintegration of biomass resulted in increase in total solids in the aqueous phase; 10.9-14.6 % of the total solids were in the form of TSS.

The ozonation of biosludge resulted in a little mineralization of COD_T content . With increase in ozone dosage and subsequent dissolution of MLVSS, the CODs in aqueous phase was increased from 268 to 2365 mg/l at 92.3 mg O_3/g DS dosage. The dispersed material was highly biodegradable in nature; the initial BOD to CODs ratio was 6.2% which was increased to 69.6% with increase in ozonation. The dissolution of biosludge also resulted in higher TOC concentration in the aqueous phase.

During ozonation of biosludge, a decrease in pH of the aqueous phase was observed. There was 0.4 to 0.8 unit drop in pH values at ozone dosage of 22.7 to 92.3 mg/g DS. The treatment increased the colour content in aqueous phase due to release of adsorbed lignin on biosludge.

With increase in ozone dosage organochlorine compounds were reduced considerably. The oxidative degradation of organochlorine compounds resulted in partial dehalogenation of the same. The adsorbed AOX and EOX compounds were oxidized with ozone resulting in 41 and 67% removal respectively at 92.3 mg O_3/g DS dosage. The total concentration of the identified twelve chlorophenolic compounds in the feed wastewater was 37.9 µg/l and the

same was 2087 µg/kg in the biosludge. The ozonation considerably degraded all of these chlorophenolic compounds in the biosludge. The maximum degradation (49.3%) was observed for 2,3,4,5-TeCP at ozone dosage of 22.7 mg/DS dosage. The concentration of chlorophenolic compounds was decreased by 22.8, 40.0, 46.8 and 52.7% at ozone dosage of 22.7, 45.3, 67.0 and 92.3 mg/g DS dosage respectively. None of the compounds was detectable in the aqueous phase after ozonation. Similarly, the TEQ in the biosludge was decreased by 23.9, 41.7, 48.4 and 52.7% respectively. The ozonation of chlorophenolic compounds resulted in partial dechlorination as well as decomposition into low molecular weight organochlorine compounds. 92-100% removal of PCDD and PCDF compounds was noticed at an ozone dosage of 46 mg/g DS. The upper bound I-TEQ from PCDD/Fs was reduced to 3.7 pg/g DS with a reduction of 93%.

The ozonation was very effective in destroying the organisms; 95.5% colony forming organisms were decreased at 92.3 mg O_3/g DS dosage. Significant decrease in SVI values was observed with ozonation; at low ozone dosage, SVI value was reduced by 14% and two fold increase of ozone dosage resulted in 3.6 times reduction in SVI. The filamentous organisms in the wastewater of integrated pulp and paper mill were highly vulnerable to ozonation due to the high surface area to volume ratio. The lysis of filamentous organisms and change in zeta potential were combinedly responsible for lower SVI values at higher ozone dosage.

Chapter-VIII

8. Treatment of ozonated sludge in activated sludge process

Ozonation of biosludge caused destruction of diffused flocs and cell wall by the oxidative power, and released organic substances into the aqueous phase. The released material was biodegradable. Hence the resultant wastewater was treated in the aerobic biological process to minimize the net sludge generation. From the study (reference chapter VII) ~50 mg/g DS ozone dosage was found to be suitable for relatively higher dissolution of biosludge with minimum amount of residual ozone. The biosludge from all the three bioreactors was removed and ozonated at a targeted dosage of 50 mg O_3/ g DS. The first two bioreactors (RI and RII) were made of higher capacity (15 l) to get higher biomass for ozonation i.e. one and two times of excess sludge respectively. The average mixed sludge removal from bioreactor RI, RII and RIII was 659±109, 1519±340 and 1190±135 ml per day based on one, two and three times of excess sludge respectively. Various operating and performance conditions of all the bioreactors prior to ozone treatment are given in table 8.1.

Table 8.1: Operating conditions and performance of bioreactors prior to ozone treatment

Parameter	Rc	RI	RII	RIII
Bioreactor capacity (l)	6.0	15.0	15.0	6.0
pH	7.40 ± 0.13	7.27 ± 0.06	7.37 ± 0.09	7.57 ± 0.08
Temperature (°C)	35.1 ± 0.2	35.3 ± 0.2	35.5 ± 0.2	35.2 ± 0.2
MLSS (g/l)	4.076 ± 0.240	4.228 ± 0.253	4.127 ± 0.198	4.098 ± 0.208
MLVSS (g/l)	3.305 ± 0.193	3.283 ± 0.187	3.219 ± 0.217	3.237 ± 0.154
CODs reduction (%)	46.6±6.6	46.4 ±2.6	47.1 ±5.1	46.9 ±3.2
AOX reduction (%)	44.9 ± 1.6	44.5±1.3	44.8±1.1	45.1±2.1
HRT (h)	8.3 ± 0.5	8.1±0.5	8.2±0.5	8.2±0.5
F/M ratio (d^{-1})	0.21 ± 0.05	0.22 ± 0.02	0.22 ± 0.04	0.22 ± 0.07
Organic load (kg CODs removal/m^3/d)	0.69 ± 0.13	0.74 ± 0.05	0.75 ± 0.09	0.75 ± 0.11
SVI (ml/g)	477±32	459±30	482±17	469±35

Inlet feed pH: 7.0±0.1, CODs: 550±22 mg/l, AOX: 14.69±0.27 mg/l, DO in bioreactor: 0.5± 0.1 mg/l

8.1 Effect of aerobic treatment on sludge yield

In case of control bioreactor (Rc) and RI, biomass was withdrawn on daily basis to maintain the MLVSS concentration near to 3.2 g/l, whereas for RII, the biomass was withdrawn after two or three days during phase I. Due to higher amount of biosludge withdrawal for ozone treatment in RIII, biomass was removed only one time to maintain MLVSS concentration during phase I. The sludge yield during phase I in Rc, RI, RII and RIII bioreactors was 0.29, 0.25, 0.15 and 0.06 g/g of CODs removal respectively.

While withdrawing the excess sludge from RI and RII bioreactors in the aforesaid amount, ozonated and recycled into the bioreactors, maintenance of the requisite amount of MLVSS in the bioreactors (3.2 g/l) was difficult; as concentration of MLVSS was continuously increasing and hence drainage of some more sludge was necessary. The removed biomass, to maintain the concentration of MLVSS, contained some part of ozonated biomass. In earlier studies 2.5 to 4 times of excess sludge was ozonated to obtain near to zero biomass for disposal (Yasui and Shibata, 1994 & 1996; Sakai et al., 1997; Lee et al., 2005). Based on the findings during phase I in RIII where the biomass generation was 0.06 g/g of CODs removal, while withdrawing 3 times of excess sludge, the rest of the study was performed with ozonation of the same amount from the RIII bioreactor which was renamed as ozone bioreactor (Ro) only.

The MLVSS concentration in Rc was maintained near to 3.2 g/l, whereas due to limited withdrawal of biomass from Ro and recycling of the ozonated biomass to aeration tank, the MLVSS concentration was increased to 4.3 g/l during phase I and some biomass was removed (Figure 8.1). After gradual adaptation of organism in ASP on dual feed (wastewater and ozonated biomass), there was no need to drain biosludge from the system. MLSS and MLVSS concentration in Ro was comparable to Rc during rest of the study. The ozonation of three times of excess sludge followed by biological treatment was capable to biodegrade about 1/3 of the substrate. The MLVSS to MLSS ratio in Rc and Ro bioreactors was 80.8±1.5 and 80.9±1.4 % during the study. Similar MLVSS/ MLSS ratio in Ro pointed out that there was no accumulation of inorganic salts in the biosludge due to recycling of the ozonated biosludge. Though, part of the inorganic material was solubilised, during ozonation it was not redeposited on the biomass. In earlier studies (Yasui et al., 1996; Sakai et al., 1997) on the ozonation of sewage sludge, a little accumulation of inorganic material was reported

on the biosludge. As there was no primary sedimentation in the sewage treatment plant, sand and silt particles in the raw sewage might accumulate in the sludge, if excess sludge was not withdrawn (Sakai et al., 1997). The continuous removal of excess sludge in conventional process facilitated the removal of sand and silt particles, whereas the same remained in the system during ozonation and subsequent recycling of the biosludge. In case of pulp and paper mill, the clarified wastewater after primary treatment contained 20-100 mg/l of suspended solids. During lab experiments, the concentration of carryover suspended solids was only 27±5 mg/l with a very high ratio of organic to inorganic material (81-83%). The low concentration of TSS and its high ratio of organic to inorganic indicated that the feed wastewater did not affect the organic content of the biomass during the time of recycling of the ozonated sludge.

The sludge yield in Rc corroborated the earlier lab experiments (Section 5) and the same was 0.31±0.02 g/g of CODs removal during the study. Similar results have been observed by Ammary, 2004. The sludge yield in ozone bioreactor varied from 0.057 to 0.062 during the three phases of study and average sludge yield was 0.059±0.003 g/g of CODs removal (Figure 8.2). In Ro, growth of higher organisms like protozoa, rotifers etc. was observed; the development of higher organisms was also contributory factor in low sludge generation as these organisms were able to graze on fragmented debris of bacterial biomass (Yan et al., 2009b).

Reduction of net sludge generation by 81% was achieved with ozonation of biosludge at an average ozone dosage of 46.4±3.2 mg/g DS. The ozone dosage was 9.8 ±2.0 mg/g of DS in term of total MLSS concentration in the bioreactor. Kamiya and Hirotsuji, 1998 reported 50% reduction in excess sludge at intermittent ozone dosage of 11 mg/g of aeration tank MLSS with synthetic sewage with meat extract and peptone.

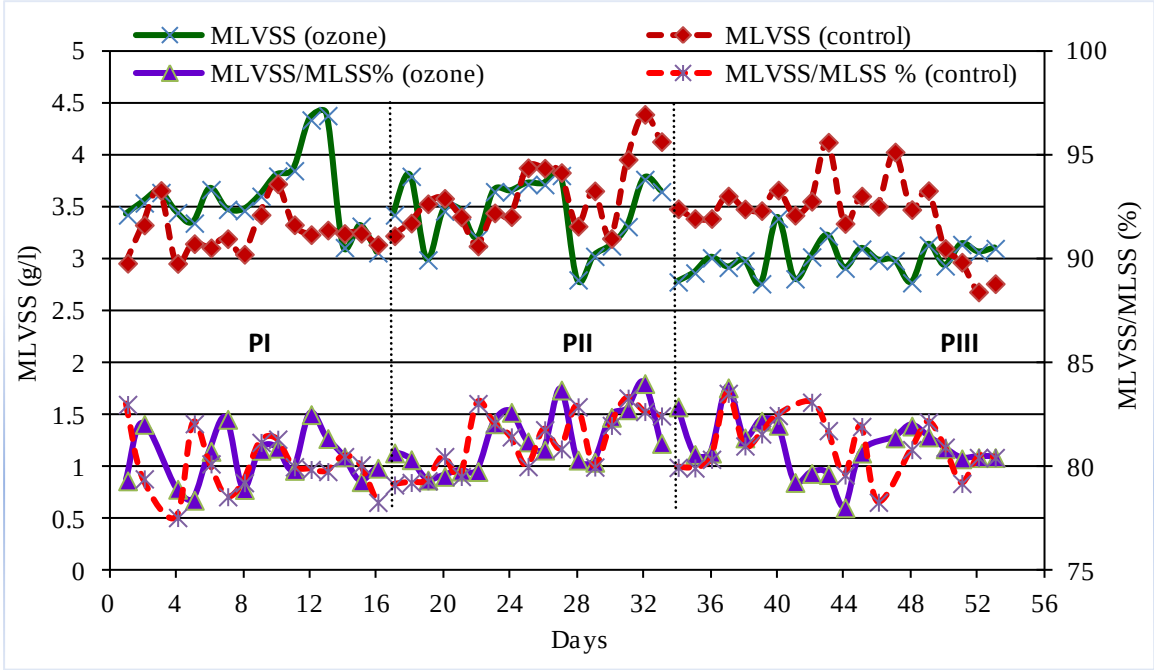


Figure 8.1: MLSS and MLVSS concentration in control and ozone bioreactor

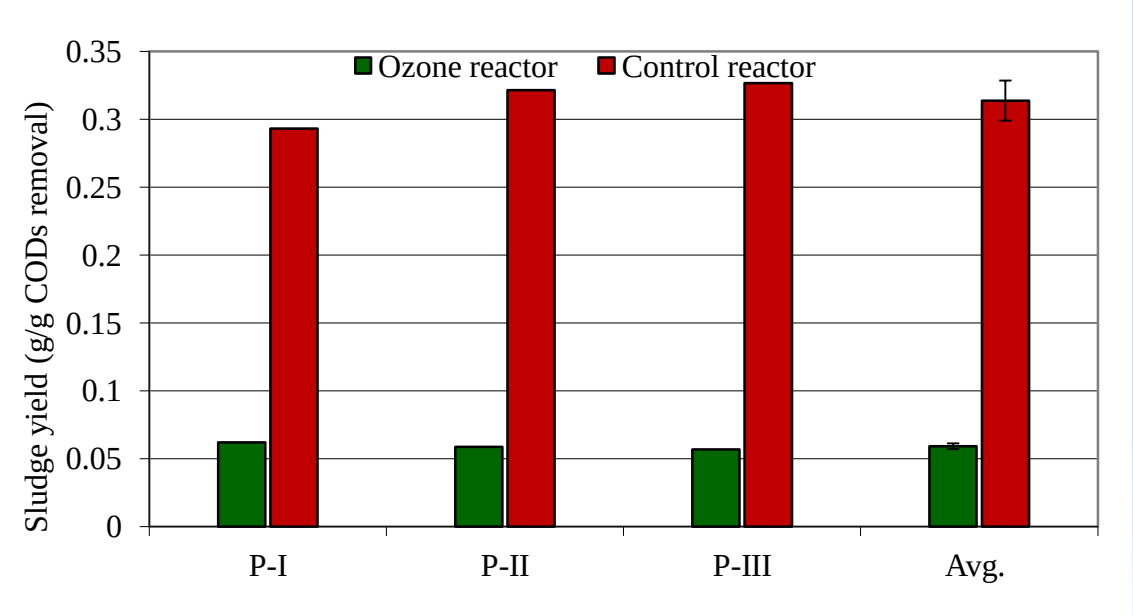


Figure 8.2: Sludge yield in control and ozone bioreactor

8.2 Effect of recycling of ozonated biosludge on performance of ASP

The performance of activated sludge process after recycling of ozonated sludge was compared with control bioreactor with respect to quality of treated wastewater for CODs, colour, AOX, TOC, TSS and TDS concentration. The concentration of first three parameters in feed wastewater was taken for performance evaluation, and effect of extra load due to recycling of ozonated sludge was evaluated in the treated wastewater. The operating as well as performance parameters for both the bioreactors are given in table 8.2.

The average concentration of CODs in influent to both the bioreactors was 543±47 mg/l which corresponded to organic loading of 1.7±0.3 and 1.8±0.2 kg/m^3/d in Rc and Ro respectively in terms of concentration of CODs in feed. The average removal of CODs was 53±9 and 55±7% respectively in Rc and Ro bioreactors and organic loading based on CODs removal was 0.89±0.23 and 0.97±0.17 kg of CODs removal/m^3/d.

During the study, an average amount of MLVSS of 4.2±0.8 g was ozonated daily at an average ozone dosage of 46.4±3.2 mg/g DS. There was 178±8 mg increase of CODs per gram of MLVSS dissolution at ozone dosage of 45.3±2.1 mg/g DS (section 7.3). This generated 748±138 mg extra CODs load (7%) to Ro. The total increase in COD load due to feeding of ozonated sludge might be higher because biodegradable portion of partially dispersed biomass also contributed to the parameter in the aerobic bioreactor. The concentration of CODs in treated wastewater from Rc and Ro was 255±49 and 246±41 mg/l respectively and the reduction in CODs is given in figure 8.3. The extra load due to recycling of ozonated biosludge did not affect the performance of ASP. Similar results have been reported by Lee et al., 2005 during ozone treatment of sewage sludge. The performance of both control and ozone bioreactors was a little low during phase II which might be due to relatively low biodegradability of the feed.

Table 8.2: Operating conditions and performance in control and ozone bioreactors

Parameter	Control bioreactor			Ozone bioreactor		
	PI	PII	PIII	PI	PII	PIII
Operating condition						
pH	7.6±0.3	7.4±0.3	7.5±0.2	7.5±0.2	7.3±0.3	7.5±0.2
Temperature (°C)	36.1±0.2	35.8±0.5	35.2±0.4	35.8±0.3	36.1±0.3	35.0±0.6
MLSS (g/l)	4.05±0.23	4.45±0.42	4.27±0.43	4.45±0.43	4.25±0.36	3.72±0.22
MLVSS (g/l)	3.26±0.22	3.62±0.36	3.45±0.35	3.60±0.37	3.45±0.32	3.00±0.16
MLVSS/MLSS (%)	80.5±2.1	81.2±1.5	81.3±1.6	80.8±2.0	81.2±1.5	81.0±1.3
DO (mg/l)	0.62±0.07	0.58±0.14	0.51±0.12	0.53±0.17	0.61±0.11	0.58±0.15
Organic load (kg CODs removal/ m^3/d)	0.80±0.21	0.76±0.18	1.07±0.17	0.96±0.13	0.96±0.22	0.98±0.17
Performance						
$CODs_{inlet}$ (mg/l)	552±34	512±61	562±27	552±34	512±61	562±27
$CODs_{outlet}$ (mg/l)	277±51	271±51	224±25	266±32	231±37	242±46
AOX_{inlet} (mg/l)	15.1±0.8	16.0±1.8	17.8±1.0	15.1±0.8	16.0±1.8	17.8±1.0
AOX_{outlet} (mg/l)	9.0±0.7	10.3±0.6	11.9±0.3	8.6±0.7	9.9±0.7	11.1±0.6
$Colour_{inlet}$ (Pt-Co unit)	1090±211	917±44	918±24	1090±211	917±44	918±24
$Colour_{outlet}$ (Pt-Co unit)	798±218	682±33	577±71	800±207	638±57	607±48
SVI (ml/g)	420±108	349±131	340±33	282±84	157±30	67±34
Sludge yield (g/g CODs removal)	0.293	0.321	0.327	0.062	0.059	0.057

The concentration of colour in feed wastewater was 982±152 Pt-Co unit and reduction of the same in Rc and Ro was 30.4±11.7 and 30.5±8.0% respectively. The release of adsorbed lignin and its derivatives from the biomass increased the colour in aqueous phase during ozone treatment of biomass. Desorption of lignin and its derivatives contributed 3% extra load of colour to the biological reactor (Ro). The concentration of colour in treated wastewater was 686±166 and 687±155 Pt-Co unit in Rc and Ro bioreactors respectively

(Figure 8.4). Combined ozone-biological treatment was effective on removal of lignin and its derivatives over biological treatment alone. The released compounds were converted to low molecular weight fractions in the aqueous phase with ozonation. Similar observation has been reported elsewhere (Ruas et al., 2007).

The concentration of TOC in the feed wastewater was 194.1±17.3 mg/l and the ratio of TOC to CODs was 0.353±0.032. During biological treatment of the wastewater, there was about 50% reduction in TOC and its concentration was 101.4±12.7 and 94.7±8.2 mg/l in the treated wastewaters of Rc and Ro respectively. The ratio of TOC to CODs was slightly higher in treated wastewaters; 0.426±0.069 and 0.406±0.102 in Rc and Ro respectively. The extra TOC load through recycling of ozonated sludge had no impact on the treated wastewater quality.

The TSS concentration in feed wastewater was 27±5 mg/l with an organic content of 81-83%. A little higher concentration of TSS in the wastewater of ozone bioreactor (Ro) was observed; the concentration of the same in treated wastewater from Rc and Ro was 35.9±5.1 and 39.1±4.8 mg/l respectively. The concentration of total dissolved solids in the feed was 1624±47 mg/l and the same was 1547±32 and 1560±36 mg/l in Rc and Ro respectively. The decrease in TDS in both the bioreactors was due to mineralization of organic material during biological treatment. From the dissolution of MLSS, it appeared that released inorganic substances resulted in slightly higher concentration of TDS in the wastewater from the bioreactor. The mineralization of organic material during biological treatment evolved CO_2 which reacts with released metal ions viz. Na, K, Ca and Mg, and resulted in increase in carbonate-bicarbonate alkalinity. This phenomenon also resulted in increase in conductivity of the treated wastewater (Figure 8.5). Slight increase in effluent conductivity was also observed after biological treatment of the textile mill wastewater probably due to increased inorganic content in the wastewater during the biological treatment (Sahinkaya et al. 2008).

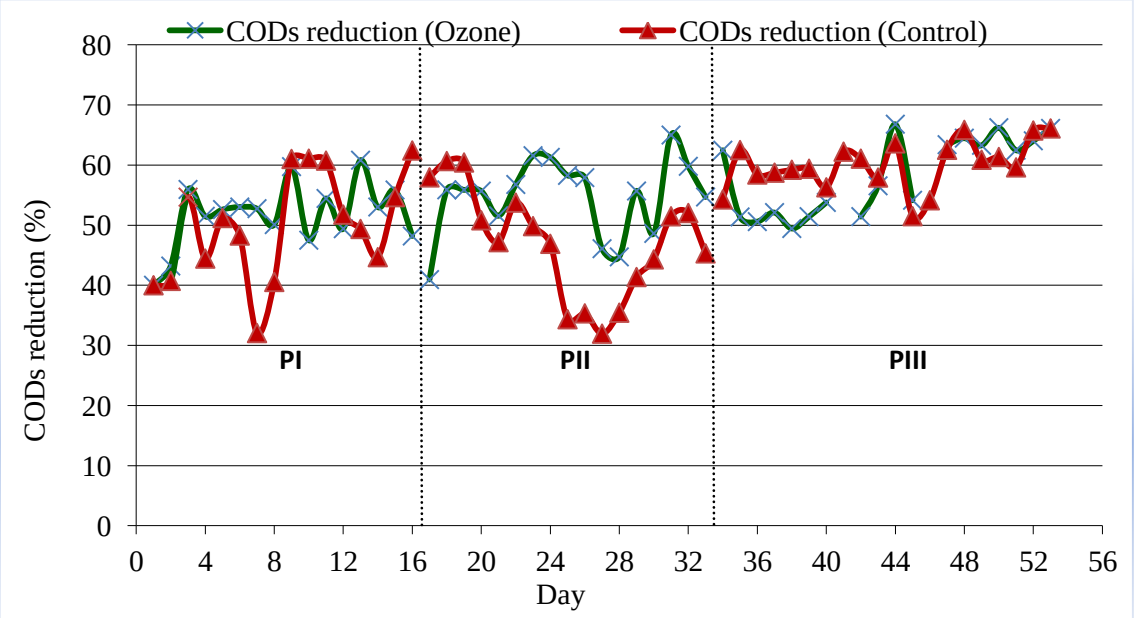


Figure 8.3: Reduction in CODs in control and ozone bioreactors

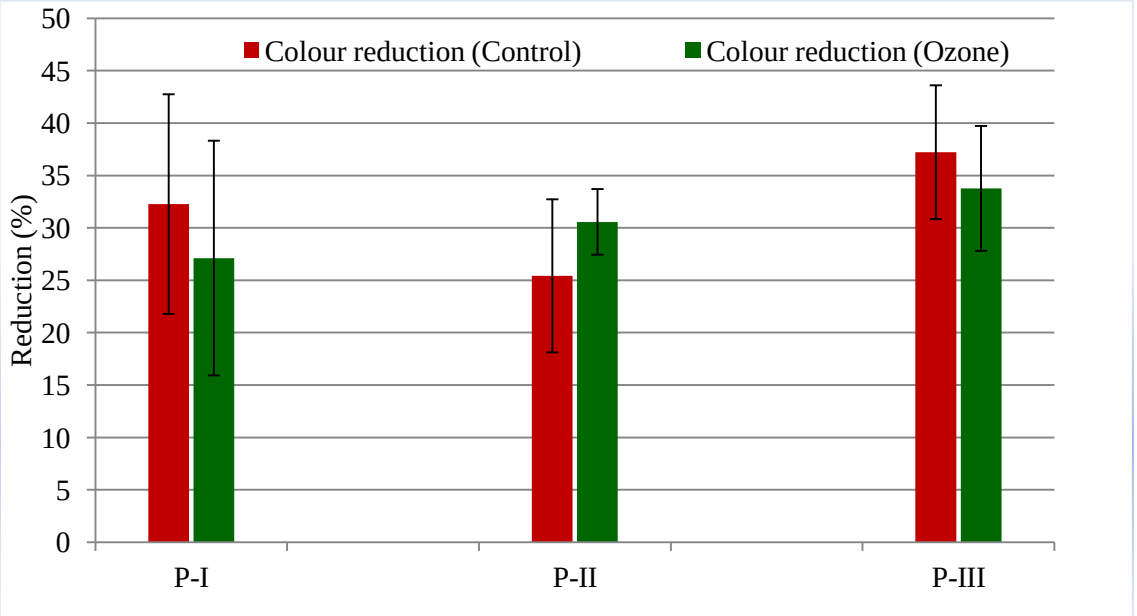


Figure 8.4: Removal of colour in control and ozone bioreactor

8.3 Effect of recycling of ozonated biosludge on operating parameters

Due to recycling of ozonated biomass to ozone bioreactor, the values of various process parameters were changed. The food to microorganism ratio in Rc and Ro was 0.26±0.08 and 0.30±0.06 d^{-1} respectively based on MLVSS content present in the bioreactors. The relatively higher value in the latter indicated the lower concentration of MLVSS in the bioreactor. Based on the remained biosludge after the withdrawal for ozonation, the F/M ratio appeared to be 0.37±0.09 d^{-1}. Similarly based on extra loading of CODs content from the biomass contributing towards higher food, the F/M ratio appeared to be 0.42±0.10 d^{-1}. The contribution of CODs in the aqueous phase was accounted for calculation of the above F/M ratio.

During the study, the average sludge retention time (SRT) in Rc was 21 days though the SRT for conventional ASP has been reported from 3-15 days (Tchobanoglous et al., 2003) with MLSS concentration of 1000-3000 mg/l in the aeration tank. The higher value of SRT in control bioreactor was due to higher MLSS concentration (~4 g/l) in the aeration tank. SRT was only 4 days in Ro due to removal of 3 times of excess sludge for ozone treatment. After ozone treatment the sludge is recycled to the system. If biosludge removal for ozonation is not considered for withdrawal of biomass, the SRT in ozone bioreactor was 24 days. Yan et al., 2009b have reported solid retention time in both the bioreactors approximately 15 days during their study.

8.4 Fate of organochlorine compounds during biological treatment

The concentration of AOX compounds in feed wastewater was 16.35±1.64 mg/l during the study period. During biological treatment, the degradation of AOX in Ro was higher than that in Rc. The removal of AOX compounds in Rc and Ro was 36.0±5.1 and 39.4±4.8% respectively. Accordingly the concentration of the same in treated wastewater was 10.58±1.87 and 9.90±1.18 mg/l respectively (Figure 8.6). The recycling of ozonated biomass did not affect the treated wastewater characteristics in term of AOX concentration. During ozonation of biosludge, the desorbed AOX compounds from the biosludge into the aqueous phase were oxidized and subsequently mineralized during biological treatment. Thus the concentration of the AOX compounds remained same in both the samples.

To find the probable mode of removal of the AOX compounds, a few samples of feed, treated wastewater and biosludge at different time interval were evaluated for the parameter (Table 8.3). The concentration of AOX compounds in the analyzed feed wastewater was 16.52±2.12 mg/l. In control bioreactor, 36.5±4.8% of AOX compounds were removed during biological treatment; 34.1% of the same were mineralized to inorganic halides and rest 2.4% were adsorbed with waste activated sludge. Whereas the total removal of AOX compounds was 39.5±4.4% in Ro; 39.1% of the same were mineralized to inorganic chloride and only 0.4% of compounds were adsorbed in the waste activated sludge.

There was no additional accumulation of AOX compounds in the biosludge due to recycling of ozonated sludge; the accumulation of the same was dependent on the concentration of the AOX compounds in the feed wastewater (Figure 8.7). The average concentration of AOX compounds in biosludge from control and ozone bioreactors was 4146±953 and 3872±784 mg/kg respectively.

Table 8.3: Removal of AOX compounds during biological treatment

A. Bioreactor operating condition		
	Control bioreactor (Rc)	Ozone bioreactor (Ro)
HRT (h)	8.0±1.3	7.3±0.9
CODs removal (g/d)	5.61±1.6	5.94±1.8
Sludge yield (g/d)	1.74	0.36
B. Removal of AOX compounds		
Outlet AOX (mg/l)	10.45±1.29	9.97±1.17
Sludge AOX (mg/kg)	4146±953	3872±784
AOX removal (%)	36.5±4.8	39.5±4.4
Sorption on sludge (%)	2.4	0.4
AOX dechlorination (%)	34.1	39.1

Inlet AOX concentration: 16.52±2.12 mg/l

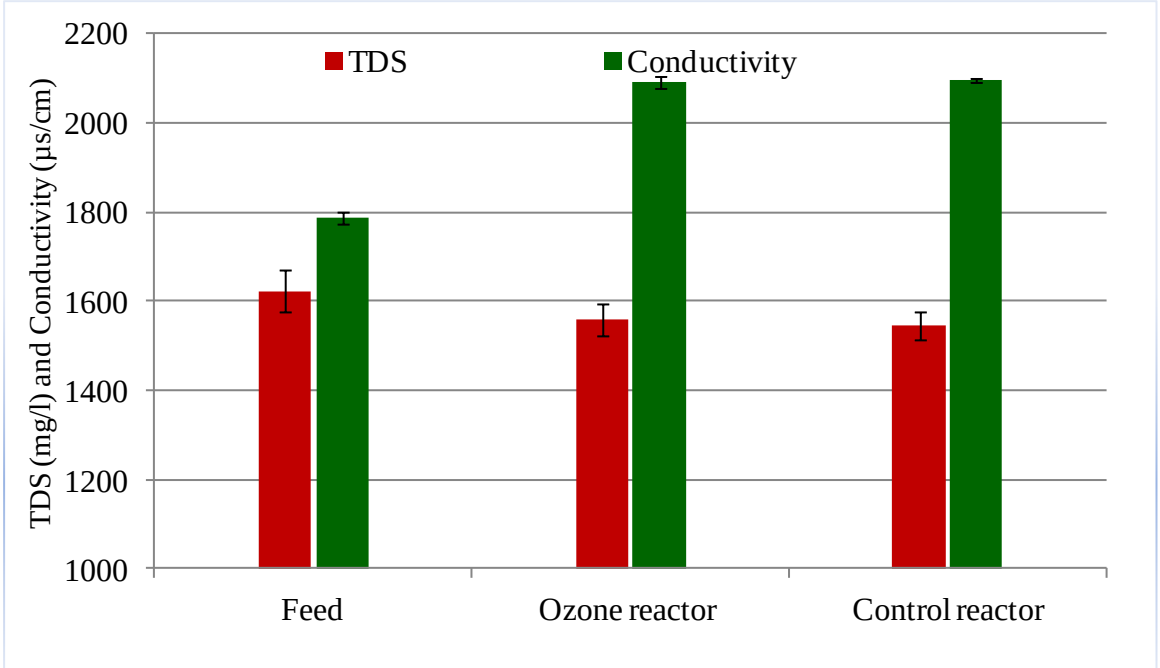


Figure 8.5: Total dissolved solids and conductivity before and after biological treatment

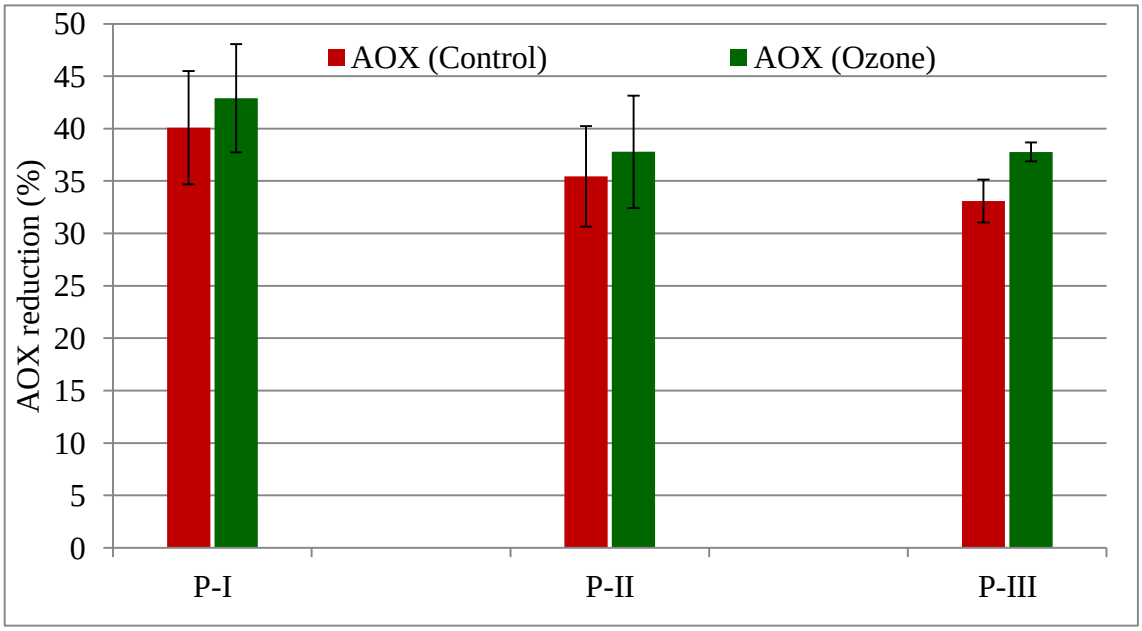
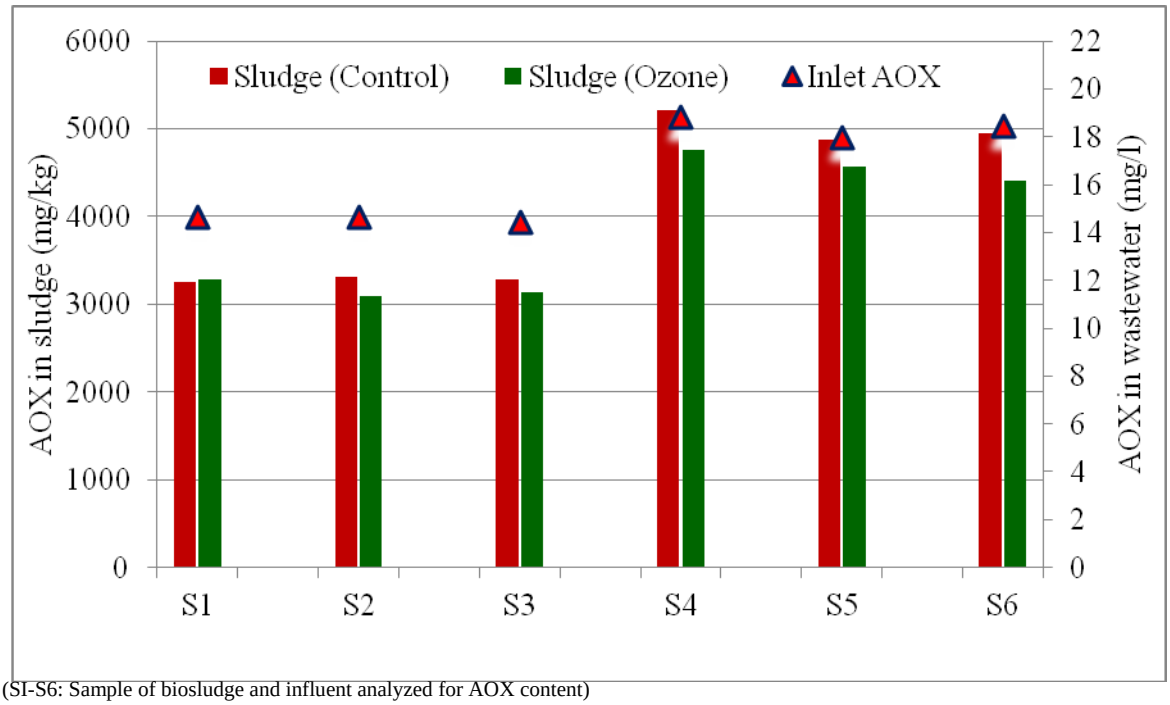


Figure 8.6: Reduction of AOX compounds during biological treatment

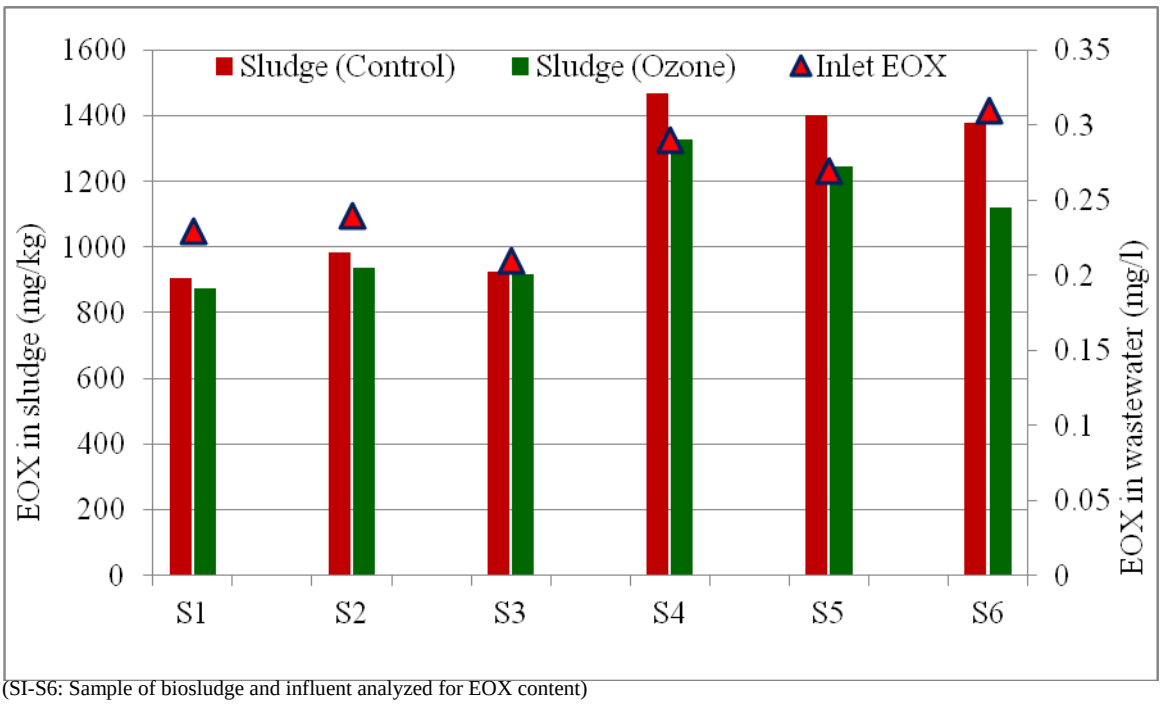
It has been reported that generally 1-3% of AOX compounds, measured as extractable organic halogen (EOX) compounds, are relatively lipophilic, potentially toxic and bioaccumulable (Berry et. al., 1991; Kovacs et al., 1993). In the wastewater under study, feed contained about 1.6% of EOX compounds, and the EOX content in the biosludge was 27-28% of AOX compounds due to its lipophilic nature. The concentration of EOX compounds in the feed wastewater was 0.26±0.04 mg/l. During biological treatment, EOX compounds were mostly adsorbed on the biosludge (43.7%) in the control bioreactor and only 4.9% of the compounds were dechlorinated (Table 8.4). The lipophilic EOX compounds were difficult to remove by conventional biological treatment. Ozonation of biosludge oxidized part of these compounds and formed smaller molecules, which were amenable to biological treatment. 53.5% removal of EOX compounds was observed during biological treatment. Higher dechlorination (46.1%) and less sorption (7.4%) of the same on sludge during ozonation were the result of ozone assisted degradation. In the case of control bioreactor 89.9% of the removed EOX compounds were adsorbed on WAS and only 13.8% of the compounds were dechlorinated, whereas in ozone bioreactor, the major mode of removal was dechlorination; only 10.1% of the removed EOX compounds remained with WAS and 86.2% of the compounds were dechlorinated. Due to low sludge yield and degradation of organochlorine compounds during ozone treatment, dechlorination of EOX compounds was much higher in comparison to the control bioreactor.

Similar to AOX compounds, no accumulation of EOX compounds was observed in the biosludge due to recycling of ozonated sludge. The concentration of the same in control and ozone bioreactors was 1177±264 and 1070±190 mg/kg respectively. The accumulation of EOX compounds in the biosludge was dependent on the concentration of the same in the feed wastewater (Figure 8.8).



(SI-S6: Sample of biosludge and influent analyzed for AOX content)

Figure 8.7: Concentration of AOX compounds in biosludge in control and ozone bioreactors



(SI-S6: Sample of biosludge and influent analyzed for EOX content)

Figure 8.8: Concentration of EOX compounds in biosludge in control and ozone bioreactors

Table 8.4: Removal of EOX compounds during biological treatment

A. Bioreactor operating condition		
	Control bioreactor (Rc)	Ozone bioreactor (Ro)
HRT (h)	8.0±1.3	7.3±0.9
CODs removal (g/d)	5.61±1.6	5.94±1.8
Sludge yield (g/d)	1.74	0.36
B. Removal of EOX compounds		
Outlet EOX (mg/l)	0.13±0.01	0.12±0.02
Sludge EOX (mg/kg)	1177±264	1070±190
EOX removal (%)	48.6±5.7	53.5±4.7
Sorption on sludge (%)	43.7	7.4
EOX dechlorination (%)	4.9	46.1

Inlet EOX concentration: 0.26±0.04 mg/l

Out of 12 identified chlorophenolic compounds, 11 compounds were present in the feed wastewater except 4,5,6-trichloroguaiacol. The concentration of compounds was in the range from 0.15±0.02 (pentachlorophenol) to 13.08±2.19 µg/l (trichlorosyringol). Among the 12 compounds, 9 compounds were detected in the biosludge in both the bioreactors in significant concentration varying from 98±6 (pentachlorophenol) to 393±22 (2,4,6-trichlorophenol) µg/kg (Table 8.5). The presence of appreciable amount of chlorophenolic compounds in the feed wastewater and sorption of the same during biological treatment were responsible for the accumulation of the same in biosludge. The TEQ of the feed wastewater was 9.61 µg/l and after biological treatment the same was 3.55 and 3.77 µg/l respectively in the treated wastewater from control and ozone bioreactors. The TEQ of biosludge from Rc and Ro was 557 and 533 µg/kg respectively.

Table 8.5: Concentration of chlorophenolic compounds in biosludge before and after biological treatment

Chlorophenolic compound	Feed (µg/l)	Control bioreactor (Rc)		Ozone bioreactor (Ro)	
		Treated effluent (µg/l)	Biosludge (µg/kg)	Treated effluent (µg/l)	Biosludge (µg/kg)
2,4,6- Trichlorophenol	6.16±0.27	1.33±0.19	369±20	1.41±0.07	393±22
2,4,5- Trichlorophenol	0.21±0.04	<0.16	<71	<0.16	<71
2,3,4,5- Tetrachlorophenol	1.05±0.14	0.44±0.08	152±9	0.49±0.09	149±16
3,4,6- Trichloroguaiacol	1.10±0.14	0.31±0.04	<44	0.24±0.03	<44
3,4,5- Trichloroguaiacol	3.51±0.45	1.60±0.21	108±8	1.31±0.22	106±6
4,5,6- Trichloroguaiacol	<0.11	<0.11	<49	<0.11	<49
3,4,6- Ttrichlorocatecol	4.49±0.11	1.57±0.38	196±11	1.94±0.06	218±18
Pentachlorophenol	0.15±0.02	0.05±0.005	104±6	0.05±0.01	98±6
3,4,5- Trichlorocatecol	6.58±0.91	4.33±0.19	340±11	3.99±0.52	328±22
Tetrachloroguaiacol	4.50±0.60	1.70±0.29	153±15	1.47±0.19	143±9
Trichlorosyringol	13.08±2.19	4.71±0.32	381±30	4.20±0.98	334±33
Tetrachlorocatecol	0.56±0.05	0.25±0.03	130±13	0.26±0.02	107±6
TEQ	9.61	3.55	557	3.77	533

Unlike the earlier reports on degradation of chlorophenolic compounds during biological treatment (Leuenberger et al., 1985; Yan and Allen, 1994), it was established that the degraded chlorophenolic compounds were mostly decomposed during aerobic biological treatment instead of sorption on biosludge (Table 8.6).

Table 8.6: Removal of chlorophenolic compounds during biological treatment

A. Bioreactor operating condition		
	Control bioreactor (Rc)	Ozone bioreactor (Ro)
HRT (h)	8.0±1.3	7.3±0.9
CODs removal (g/d)	5.61±1.6	5.94±1.8
Sludge yield (g/d)	1.74	0.36
B. Removal of chlorophenolic compounds		
Outlet CPs (µg/l)	15.4	16.3
Sludge CPs (µg/kg)	1934	1876
CPs removal (%)	62.80	60.62
Sorption on sludge (%)	0.45	0.08
CPs dechlorination (%)	62.35	60.54

Inlet CPs concentration: 41.4 µg/l

The cumulative concentration of the chlorophenolics was 41.4 µg/l in the feed wastewater and TEQ was 9.61 µg/l. During biological treatment the degradation of CPs in control bioreactor was 62.8% and the same was 60.6% in ozone bioreactor. Correspondingly the reduction in TEQ in the treated wastewater of control and ozone bioreactors was 63.1 and 60.8% respectively. 34-78% degradation of chlorophenolic compounds took place during biological treatment in both the bioreactors. The sorption of these compounds on waste activated sludge was only 0.45 and 0.08% in case of control (Rc) and ozone bioreactor (Ro) respectively. Concentration of individual compound in the biosludge was comparable in both the bioreactors and recycling of ozonated biosludge did not affect the removal efficiency of chlorophenolic compounds in ozone bioreactor (Ro). The two stage process comprising of ozonation of biosludge and subsequent downstream biological treatment in activated sludge process resulted in 80.1, 81.2 and 79.9% less discharge of AOX, EOX and chlorophenolic compounds respectively with sludge in comparison to conventional biological treatment. The TEQ due to chlorophenolic compounds was 80.2% lower in the ozonated biosludge. The lower discharge of organochlorine compounds with biosludge was due to partial

dechlorination to inorganic chloride and conversion to low molecular weight compounds which were amenable to biodegradation in the biological oxidation stage.

8.5 Effect of ozonation and downstream treatment in ASP on biological properties of biosludge

The ozonation of biosludge resulted in improved settling properties of the biosludge in ozone bioreactor. Initially, biomass in both the bioreactors was bulking in nature and SVI values were 478±32 and 459±30 ml/g respectively for control and ozone bioreactor. Ozonation of biosludge and subsequent treatment in activated sludge process resulted in gradual decrease in SVI values (Figure 8.9). The average SVI values for control bioreactor during PI, PII and PIII were 420±108, 349±134 and 340±33 ml/g respectively, whereas the same were 284±84, 157±30 and 67±34 respectively for ozone bioreactor.

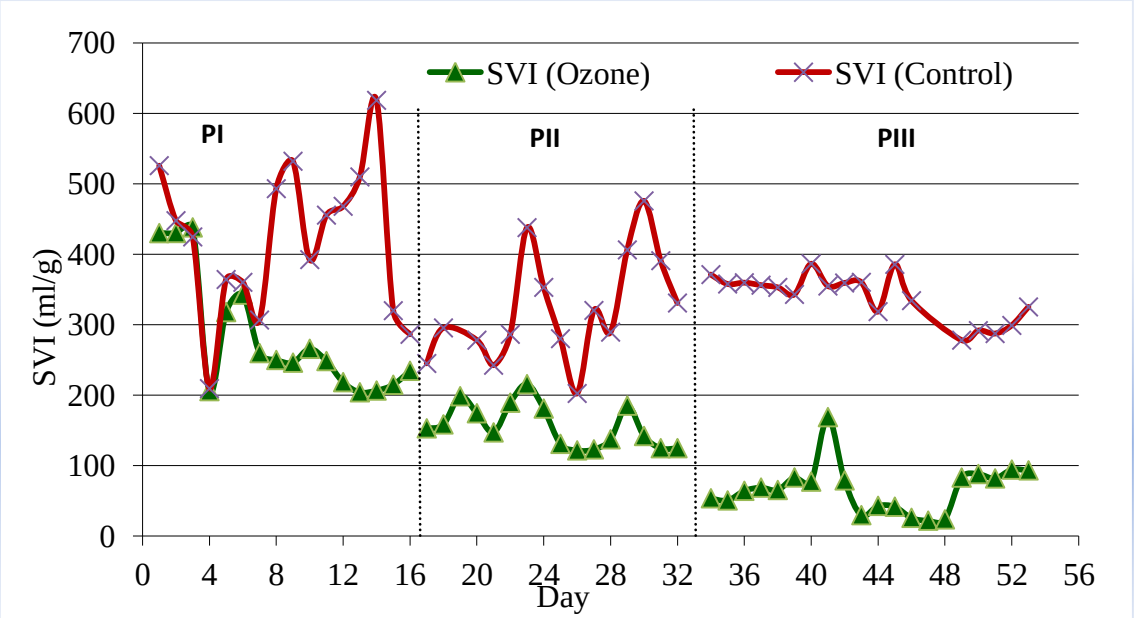


Figure 8.9: Settling characteristics of biosludge in control and ozone bioreactors

As indicated in section 6, filamentous organisms were more susceptible to ozonation due to their higher surface area. Reduction in filamentous organisms was observed during ozonation and subsequent recycling of the biosludge to activated sludge process (Table 8.7).

Table 8.7: Profile of filamentous organisms in control and ozone bioreactors during phase III

Parameter	Control (Rc)	Ozone (Ro)
MLSS (g/l)	4.57±0.17	4.01±0.13
Average length (μm)	47.2±4.0	37.2±4.2
No. of filaments/ml	$23.3x10^6 \pm 1.5x10^6$	$12.6x10^6 \pm 1.0x10^6$
Extended filament length (μm/ml)	$11.0x10^8 \pm 1.0x10^8$	$4.7x10^8 \pm 0.9x10^8$
Extended filament length (μm/g of MLSS)	$240x10^9 \pm 16x10^9$	$117x10^9 \pm 21x10^9$

The average length of filamentous organisms in Ro was 21.2% lesser than that in control bioreactor. The total filament count as well as extended filament length was 45.9 and 57.3% lower in the ozone bioreactor. Drastic reduction of filamentous organisms was responsible for better settling properties of the biomass in ozone bioreactor.

In the beginning of the study, the microbial flocs were in diffused form and presence of filamentous organisms was abundant, whereas higher organisms like protozoa, rotifer and nematode were absent in both the bioreactors. (Figure 8.10 and 8.11). The higher SVI values corroborated the characteristics of biomass. Recycling of ozonated biomass in Ro resulted in development of flocs of bacteria on filamentous organisms; although dense compact flocs were not developed like the previously described in section 5.1 (Figure 8.12 and 8.13).

The zeta potential of biosludge from Rc and Ro was -16.2 and -15.6 mV respectively during PIII. Ozone treatment of biosludge was responsible for changing the potential and intermolecular repulsion. The cumulative effect on control of proliferation of filamentous organisms and lowering of intermolecular repulsion in Ro were responsible for better settling properties of the biosludge.

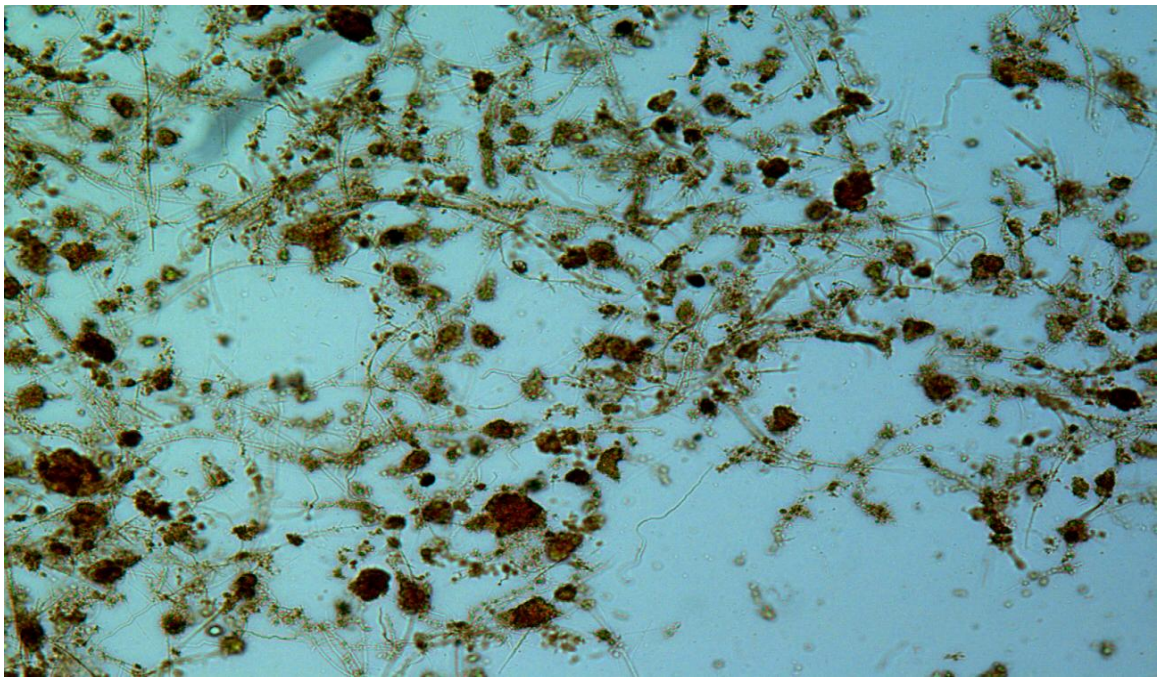


Figure 8.10: Morphology of biomass in control bioreactor in phase I

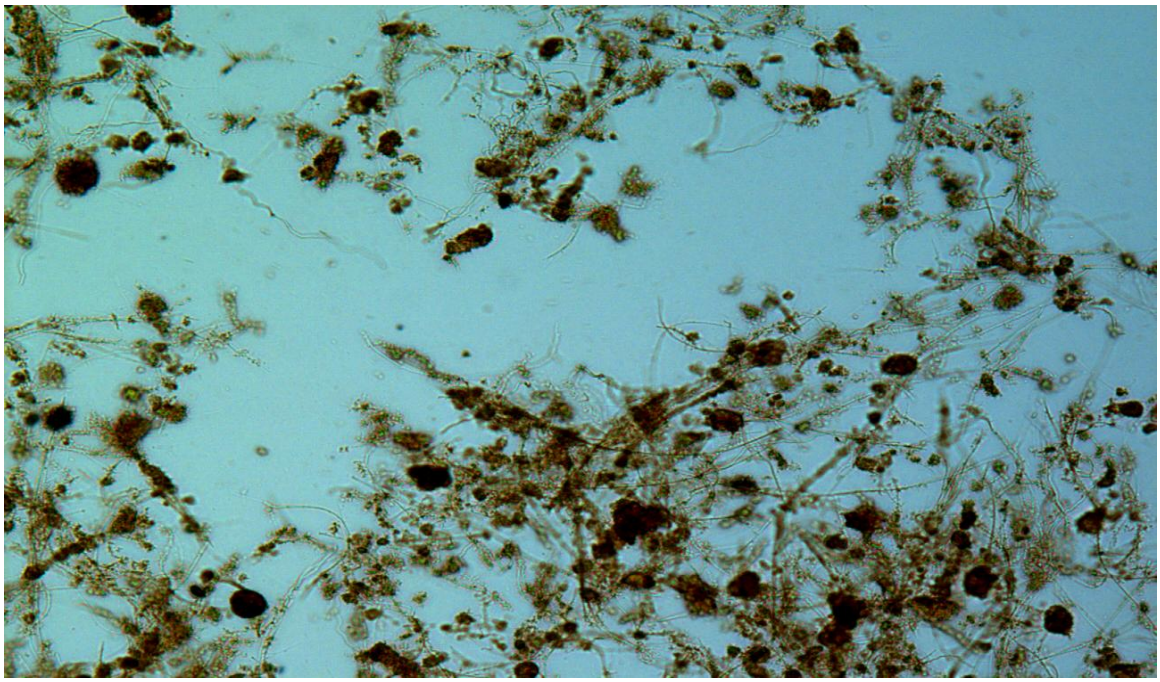


Figure 8.11: Morphology of biomass in ozone bioreactor in phase I

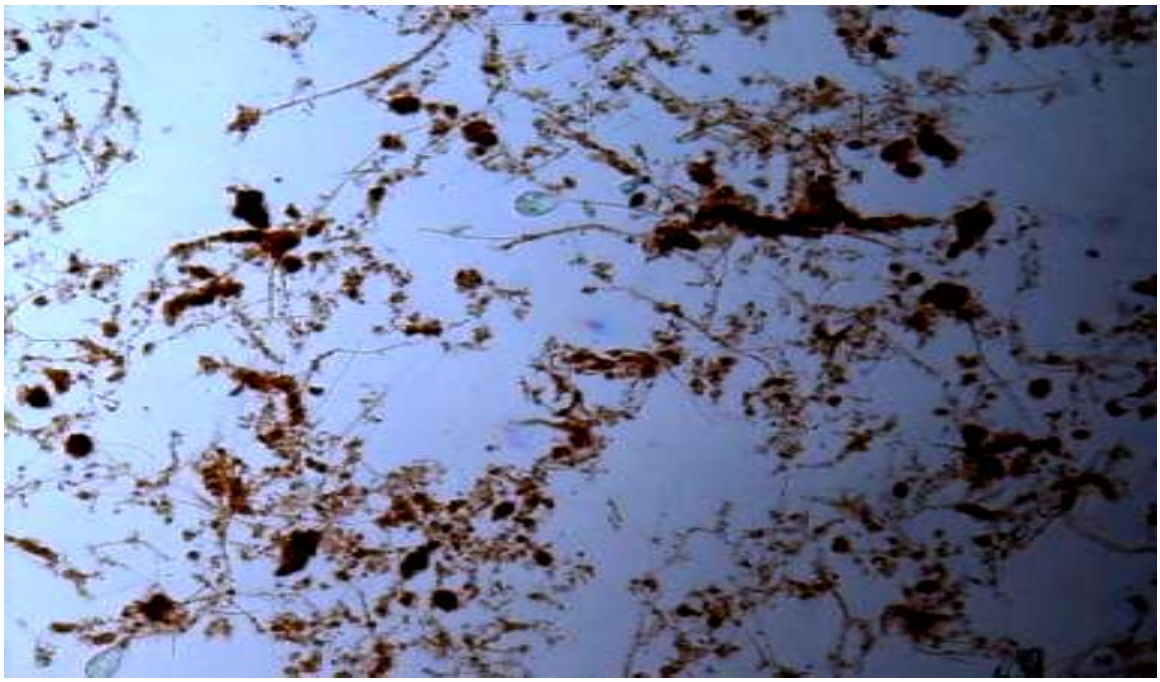


Figure 8.12: Morphology of biomass in control bioreactor in phase III

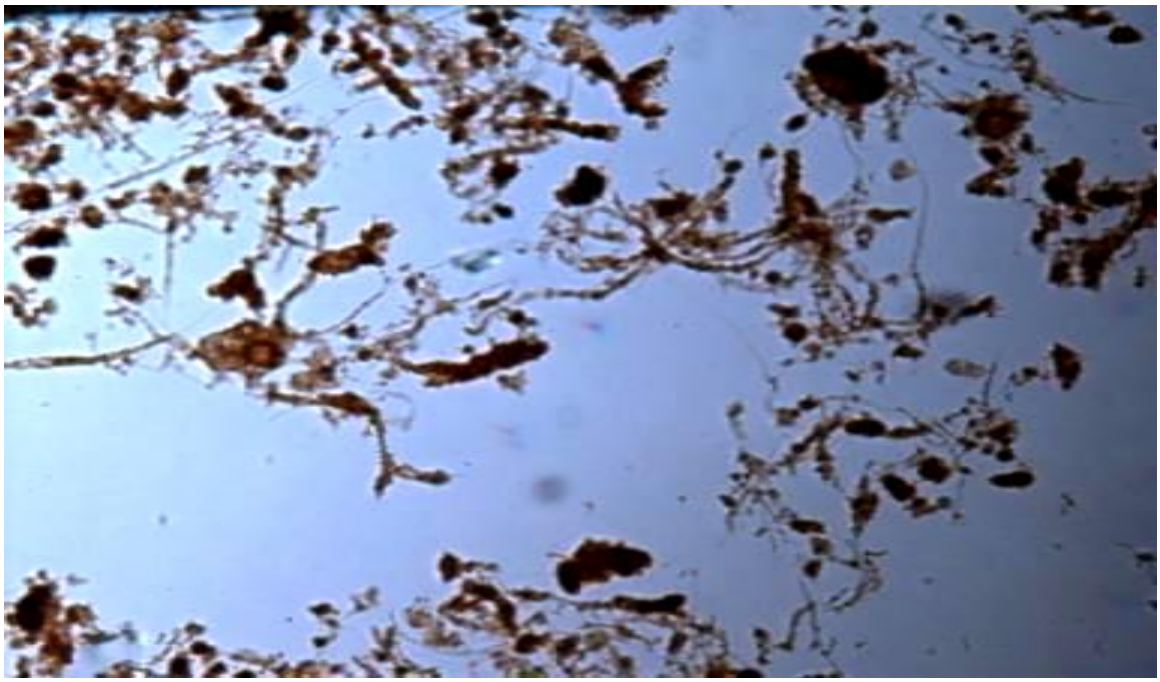


Figure 8.13: Morphology of biomass in ozone bioreactor in phase III

The biosludge samples were also evaluated for viable count as well as oxygen uptake rate (OUR). The average viable count in the control and ozone bioreactor was $76.7x10^5 \pm 6.1x10^5$ and $85.7x10^5 \pm 12.1x10^5$ count/100 µl respectively during phase III. The higher viable count in ozone bioreactor indicated that ozonation of excess sludge did not affect the active organisms in the continuous process. The easily biodegrdable material after ozone treatment was responsible for better growth of bacteria.

Oxygen uptake rate (OUR) of ozone bioreactor was a little higher than that in control bioreactor. The average OUR in Rc and Ro was 13.7±0.4 and 15.7±0.8 mg/l/h. The specific oxygen uptake rate (SOUR) was calculted by dividing the OUR with MLVSS content of the sample. SOUR of the bioreactors was 4.3±0.3 and 4.7±0.4 mg/g/h respectively. The increased OUR was an indication of higher activity of organisms which was made possible due to the prevalence of large amount of easily biodegradable material in the ozone bioreactor (Erden et al., 2010). The probable reasons for better performance of the ozone bioreactor are:

i. Availability of low molecular weight biodegradable substrates after ozonation
ii. There are relatively young organisms in the bioreactor due to lower SRT
iii. Higher DO concentration in the feed from the ozonation of sludge
iv. Comparatively higher growth of flocculating organisms

The ozone treatment resulted in decreased abundance of filamentous bacteria. The recycling of ozonated sludge improved the settling properties of bulking sludge without interfering with other important microbiological activities. Ruas et al., 2007 also reported that ozonation led to higher biomass activity during biological treatment. Ozonation of organic compounds usually produces oxygenated products and low molecular weight acids that are less toxic and more biodegradable (Adams et al. 1997; Sandra et al. 2005). Combination of ozone with biological treatment for pulp and paper mill effluent has resulted in higher degradation of COD, colour and AOX (Helble et al. 1999).

Summary

Ozonation of three times of excess sludge followed by biological oxidation resulted in significant reduction in discharge of waste activated sludge during biological treatment of pulp and paper mill wastewater. The biosludge was ozonated at an average ozone dosage 46.4 mg/g of dry sludge. The dissolution of biomass during ozonation was only 13-14%; a significant amount of biomass in disintegrated form was amenable to biodegradation. The MLVSS to MLSS ratio in both the bioreactors remained comparable during the study and it was 80.8 and 80.9% in control and ozone bioreactors respectively. The very close MLVSS concentration in the Ro revealed that there was no accumulation of inorganic content in the biosludge due to recycling of the ozonated biosludge. The solubilised inorganic material marginally increased the TDS in the treated wastewater. The sludge yield in Rc and Ro was 0.31 and 0.059 g/g of CODs removal during the study. The growth of higher organisms like protozoa, rotifers etc. additionally assisted in low sludge generation in Ro.

The recycling of ozonated biosludge to activated sludge process increased the load of pollutants but did not affect the performance of the process. Removal efficiency of pollutants was comparable or a little better than that in control bioreactor with respect to CODs, colour, AOX and other parameters. There was no accumulation of AOX and EOX compounds in the biosludge due to recycling of ozonated sludge and the same was dependent on the concentration of the same in the feed wastewater. Significant removal of identified chlorophenolic compounds was observed during biological treatment; 34-78% degradation of chlorophenolic compounds took place during biological treatment in both the bioreactors. The sorption of these compounds on waste activated sludge was negligible in comparison to biological degradation. The two stage process comprising of ozonation of biosludge in combination with biological treatment through activated sludge process resulted in 80.1, 81.2 and 79.9% lower discharge of AOX, EOX and chlorophenolic compounds with sludge respectively in comparison to conventional biological treatment without affecting the concentration of these parameters in the treated wastewater. The lower discharge of organochlorine compounds with biosludge was due to partial dechlorination to inorganic chloride and conversion to low molecular weight compounds in the biosludge which were amenable to biodegradation in the biological oxidation stage.

The ozonation and subsequent recycling of biosludge in activated sludge process was effective in controlling the proliferation of filamentous organisms which resulted in good settling characteristics of biosludge; the average SVI values in the last phase of the experimentation were below 100 ml/g. The good viable count and higher oxygen uptake rate in ozone bioreactor indicated that the activity of organisms was better in the bioreactor. The higher performance of the ozone bioreactor was due to relatively young organisms, lower SRT, higher DO level in the feed after ozonation, control of proliferation of filamentous organisms, availability of low molecular weight highly biodegradable substrate and growth of higher organisms like protozoa and rotifers.

Conclusion and further scope

The biological processes specially activated sludge process are efficiently capable of treating pulp and paper mill wastewater with generation of excess biomass as byproduct. Biosludge of the integrated pulp and paper mill contained excessive filamentous organisms and pin point flocs. Contamination of heavy metals in the biosludge was not significant. The sludge was highly contaminated with organochlorine compounds. The developed method determined the AOX compounds accurately and precisely in the biosludge. The concentration of AOX and EOX compounds in the biosludge was 2119 and 641 mg/kg respectively. Ratio of EOX and AOX in the biosludge was 0.3, whereas the same was 0.02 in the influent. Ten out of 12 classified chlorophenolic compounds were present in the biosludge. Toxicity equivalency (TEQ) due to chlorophenolics in the biosludge was 717 μg/kg. The concentration of 2,3,7,8-TCDD and 2,3,7,8-TCDF was 16 and 210 pg/g of dry sludge respectively. The upper bound I-TEQ from PCDD/Fs was 54 pg/g of dry sludge.

Organochlorines present in the wastewater of the integrated pulp and paper mill influenced the growth of biomass and nature of organisms, and affected the removal of CODs and colour. In the normal AOX concentration of 10-16 mg/l, the sludge yield was 0.29-0.31 which was reduced to 0.18 g/g of CODs removal in the higher concentration (28-33 mg/l). Pinpoint and diffused flocs were dominant in the case of wastewater having higher AOX concentration. The major mode of AOX removal in the aerobic process was dechlorination; only 1.6-2.3% of the AOX compounds were removed in adsorbed form with waste activated sludge.

Ozonation of the filamentous and flocculating sludge nurtured in the laboratory caused a drop of pH of 0.3-0.4 and 0.5-0.7 respectively at ozone dosage of 30-55 mg/g DS. It increased 75-175 mg/l BOD and 300-710 mg/l COD in the case of filamentous biosludge. For the flocculating biosludge, the corresponding increase of BOD and COD was 50-85 and 200-390 mg/l respectively. The filamentous biosludge was disintegrated with ozone while the compacts flocs were resistant towards ozonation.

Ozonation of mill sludge altered its physical, chemical and biological characteristics. At ozone dosage of 22.7 to 92.3 mg/g DS, 0.4-0.8 drop in pH was observed due to formation of low molecular weight and acidic end products. In these ozone dosage, the solubilisation of MLSS and MLVSS was 10.1-27.4 and 10.6-31.5% respectively. Disintegration of biomass

increased the total solids in the aqueous phase; 10.9-14.6% of the total solid was in the form of TSS. CODs in the aqueous phase was 268-2365 mg/l. Biodegradability increased with ozonation.

There was drastic decontamination from the organohalogen compounds due to ozonation. The adsorbed AOX and EOX compounds were oxidized resulting in 41 and 67 % removal respectively at an ozone dosage of 92.3 mg/g DS. TEQ in the biosludge due to chlorophenolic compounds was decreased by 23.9, 41.7, 48.4 and 52.7% at ozone dosage of 22.7, 45.3, 67.0 and 92.3 mg/g DS. The ozonation of chlorophenolic compounds resulted in partial dechlorination as well as decomposition into low molecular weight organochlorine compounds. 92-100% removal of PCDD and PCDF compounds occurred at an ozone dosage of 46 mg/g DS. The upper bound I-TEQ from PCDD/Fs was reduced by 93% to 3.7 pg/g DS.

Ozonation destroyed the active organisms; 95.5% colony forming organisms were decreased at an ozone dosage of 92.3 mg/g DS. The filamentous organisms in the biosludge of pulp and paper mill wastewater were highly vulnerable to ozonation due to high surface to volume ratio. The lysis of the organisms and change in zeta potential were combinedly responsible for lower SVI values at higher ozone dosage.

Ozonation of three times of excess biosludge at 46.4 mg O_3/g DS dosage followed by biological oxidation reduced the excess sludge generation from 0.31 to 0.059 g/g of CODs removal. Inorganic constituents of the biosludge were solubilised during ozonation resulting in marginal increase in TDS in the treated wastewater. The two stage process comprising of ozonation of the biosludge followed by biological oxidation in the activated sludge process resulted in 80.1, 81.2 and 79.9 % lower discharge of AOX, EOX and chlorophenolic compounds with sludge without affecting the concentration of these parameters in the treated wastewater. The lower discharge of the compounds was due to dechlorination during ozonation and biodegradation in the aerobic reactor of the degraded molecules. The combined process was effective in controlling the proliferation of filamentous organisms, and promoting the growth of young, active and flocculating organisms.

Cost of ozonation varies based on size of the treatment plant. The cost of ozone treatment is becoming favorable due to advancement in ozone generation technology. Caffaz et al., 2005 reported a saving of 54% of the total sludge treatment cost in the WWTP, using the combined treatment of ozone-aerobic digestion. The cost of disposal of the hazardous biosludge from integrated pulp and paper mill will be higher due to its hazardous nature

which involves transportation to the designated hazardous material handling site and downstream treatment for detoxification. Considering the higher cost of sludge disposal, the operational cost and the investment needed for sludge ozonation can be compensated.

Based on the outcome of the current research following future work is recommended:

- Development of appropriate method for treating flocculating sludge
- Treatment of the biosludge in continuous ozonation reactor and pilot plant study for generation of design data
- Appropriate protocol for net zero sludge discharge
- Increasing oxidative efficiency of ozonation for complete elimination of organochlorine compounds from the biosludge

References

1. Abbassi B., Dullstein S. and Rabiger N., (1999). Minimisation of excess sludge production by increase of oxygen concentration in activated sludge flocs; Experimental and theoretical approach. Water Res., 34 (1), 139-146.
2. Abe K.I., Tanaka K., (1997). Fe3+ and UV-enhanced ozonation of chlorophenolic compounds in aqueous medium. Chemosphere, 35, 2837-2847.
3. Adams C.D., Cozzens R.A. and Kim B. J. (1997). Effects of ozonation on the biodegradability of substituted phenols. Water Res., 31(10), 2655-2663.
4. Ahn K.H., Park K.Y., Maeng S.K., Hwang J.H., Lee J.W., Song K-G and Choi S., (2002). Ozonation of wastewater sludge for reduction and recycling. Water Sci. Technol., 46 (10), 71-77.
5. Ali M. and Sreekrishnan T.R., (2000). Anaerobic treatment of agricultural residue based pulp and paper mill effluents for AOX and COD reduction. Process Biochem., 36, 25-29.
6. Ammary B.Y., (2004). Nutrients requirements in biological industrial wastewater treatment. Afr. J. Biotechnol., 3 (4), 236-238.
7. APHA (1992). Standard Methods for the Examination of Water and Wastewater, 18th Edition, American Public Health Association/ American Water Work Association/Water Environment Federation, Washington, DC, USA.
8. Assalin M.R., Almeida E.S., Duran N., (2009). Combined system of activated sludge and ozonation for the treatment of kraft E1 effluent. Int. J. Environ. Res. Public Health., 6, 1145-1154.
9. Bajpai P. and Bajpai P.K., (1997). Reduction of organochlorine compounds in bleach plant effluents. Adv. Biochem. Eng./Biotechnol., 57, 213-259.
10. Balcioglu, I.A., Tarlan E., Kivilcimdan C. and Sacan M.T. (2007). Merits of ozonation and catalytic ozonation pre-treatment in the algal treatment of pulp and paper mill effluents. J. Environ. Manage., 85, 918-926.

11. Barroca M.J.M.C., Seco I.M., Fernandes P.M.M., Ferreira L.M.G.A. and Castro J.A.A.M., (2001). Reduction of AOX in the bleach plant of a pulp mill. Environ. Sci. Technol., 35, 4390-4393.

12. Benitez F.J., Beltran-Heredia J., Acero J.L. and Rubio F.J., (2000). Contribution of free radicals to chlorophenols decomposition by several advanced oxidation techniques. Chemosphere, 41, 1271-1277.

13. Benitez F.J., Acero J.L., Real F.J. and Garcia J., (2003). Kinetics of photodegradation and ozonation of pentachlorophenol. Chemosphere, 51, 651-662.

14. Berry R.M., Luthe C.E., Voss R.H., Wrist P.E., Axegard P., Gellerstedt G., Lindblad P-O and Popke I., (1991). The effect of recent changes in bleached softwood kraft mill technology on organochlorine emission: An international perspective. Pulp Pap-Canada, 92 (6), 43-54.

15. Bijan L. and Mohseni M., (2004). Using ozone to reduce recalcitrant compounds and to enhance biodegradability of pulp and paper effluents. Water Sci. Technol., 50 (3), 173-182.

16. Biester, H., Keppler F., Putschew A., Cortizas A.M. and Petri M. (2004). Halogen retention, organohalogens, and the role of organic matter decomposition on halogen enrichment in two Chilean peat bogs. Environ. Sci. Technol. 38, 1984-1991.

17. Bohler M. and Siegrist H., (2004). Partial ozonation of activated sludge to reduce excess sludge, improve denitrification and control scumming and bulking. Water Sci. Technol., 49 (10), 41-49.

18. Boehler M. and Siegrist H., (2007). Potential of activated sludge ozonation. Water Sci. Technol., 55 (12), 181-187.

19. Boncz M.A., Bruning H., Rukens W.H., Sudholter E.J.R., Harmsen G.H. and Bijsterbosch J.W., (1997). Kinetic and mechanistic aspects of the oxidation of chlorophenols by ozone. Water Sci. Technol., 35, 65-72.

20. Bougrier C., Albasi C., Delgenes J.P. and Carrere H., (2006). Effect of ultrasonic thermal and ozone pre-treatment on waste activated sludge solubilisation and anaerobic biodegradability. Chem. Eng. Process., 45, 711-718.

21. Canales A., Pareilleux A., Rolls J.L., Goma G. and Huyard A., (1994). Decreased sludge production strategy for domestic wastewater treatment. Water Sci. Technol., 30, 96-106.

22. Cesbron D., Deleris S., Debellefontaine H., Roustan M. and Paul E., (2003). Study of competition for ozone between soluble and particulate matter during activated sludge ozonation. Chem. Eng. Res. Des., 81 (9), 1165-1170.

23. Chakrabarti S. K., (2005). Emerging challenges in handling, disposal and utilization of biosludge: Indian context- an overview. Journal IAEM, 32 (2), 74-82.

24. Chen G.H., An K.J., Saby S., Brois E. and Djafer M., (2003). Possible cause of excess reduction in an oxic-settling-anaerobic activated sludge process (OSA process). Water Res., 37(16), 3855-3866.

25. Chiavola A., Naso M., Rolle E. and Trombetta D., (2007). Effect of ozonation on sludge reduction in a SBR plant. Water Sci. Technol., 56 (9), 157-165.

26. Chinnaraj S., Sankaralingam P., Ravi K. and Subrahmanyam S.V., (2011). Integrated approach for water conversion and pollution reduction: A step towards minimum impact mill. IPPTA J., 23 (4), 115-120

27. Cho M., Chung H. and Yoon J., (2003). Disinfection of water containing natural organic matter by using ozone-initiated radical reactions. Appl. Environ. Microbiol., 69(4), 2284-2291.

28. Chu L., Yan S., Xing X-H, Sun X. and Jurcik B., (2009). Progress and perspectives of sludge ozonation as a powerful pretreatment method for minimization of excess sludge production. Water Res., 43, 1811-1822.

29. Chu L., Yan S., Xing X.H., Yu A., Sun X. and Jurcik B., (2008). Enhanced sludge solubilization by microbubble ozonation, Chemosphere, 72, PP. 205–212.

30. Chu C.P., Chang B.V., Liao G.S., Jean D.S. and Lee D.J., (2001). Observations on changes in ultrasonically treated waste-activated sludge. Water Res., 35(4), 1038-1046.

31. Copp J.B. and Dold P.L., (1998). Comparing sludge production under aerobic and anoxic conditions. Water Sci. Technol., 38, 285-294.

32. CPCB (2011). Proposed discharged standards: central pollution control board, New Delhi. Inpaper India, 14 (6), 54-55.

33. CPPRI (2001). Laboratory manual of testing procedure, Central Pulp and Paper research Institute, Saharanpur, India.

34. De Los Santos Ramos W., Poznyak T., Chairez I. and Cordova R., (2009). Remediation of lignin and its derivatives from pulp and paper industry wastewater by the combination of chemical precipitation and ozonation. J. Hazard. Mater., 169, 428-434.

35. Deleris S., Geaugey V., Camacho P., Debellefontaine H. and Paul, E., (2002). Minimization of sludge production in biological processes: an alternative solution for the problem of sludge disposal. Water Sci. Technol., 46(10), 63-70.

36. Deleris S., Paul E., Audic J.M., Roustan M. and Debellefontaine H., (2000). Effect of ozonation on activated sludge solubilization and mineralization. Ozone Sci. Eng., 22(5), 473-487.

37. Diez M.C., Castillo G., Aguilar L., Vidal G. and Mora M.L., (2002). Operational factors and nutrient effects on activated sludge treatment of Pinus radiata kraft mill wastewater. Biores. Tech., 83, 131–138.

38. Dignac M.F., Derenne S., Ginestet P., Bruchet A., Knicker H. and Largeau C., (2000). Determination of structure and origin of refractory organic matter in bio-epurated wastewater via spectroscopic methods. Comparison of conventional and ozonation treatments. Environ. Sci. Technol., 34 (16), 3389-3394.

39. DIN 38409-part 8 (1984). Determination of extractable organically bonded halogens (EOX). Detshes Instituy fur Normung, Berlin.

40. DIN 38414-part 18 (1989). Sludge and sediments (Group S)- Determination of adsorbable organically bound halogens (AOX). Detshes Instituy fur Normung, Berlin.

41. Dugal H.S., (2009). The paper industry – Where is it going? Past, Present, Future. 9th International Technical Conference on Pulp, Paper and Allied Industry, 4-6 December, New Delhi, India, 1-11.

42. Duguet J.P., Dussert B., Bruchet A. and Mallevialle J., (1986). The potential use of ozone and peroxidase for removal of aromatic compounds from water by polymerization. Ozone Sci. Eng., 8, 247-260.

43. Dytczak M.A., Londry K., Siegrist H. and Oleszkiewicz J.A., (2006). Extracellular polymers in partly ozonated return activated sludge: impact on flocculation and dewaterability. Water Sci. Technol., 54 (9), 155-164.

44. Dytczak M.A., Londry K.L., Siegrist H. and Oleszkiewicz J.A., (2007). Ozonation reduces sludge production and improves denitrification. Water Res., 41, 543-550.

45. Dziurla M.A., Salhi M., Leroy P., Paul E., Ginestet P. and Block J.C., (2005). Variations of respiratory activity and glutathione in activated sludges exposed to low ozone doses. Wat. Res., 39, 2591-2598.

46. Endlay N., Mishra S., Gupta M.K., Farid M., Jain K., Panwar S. AND Mathur R.M., (2011). Reduction of water footprints in agro and RCF based pulp and paper mills scope, possibilities and available options. IPPTA J., 23 (4), 115-120.

47. Egemen E., Corpening J., Padilla J., Brennan R. and Nirmalakhandan N., (1999). Evaluation of ozonation and cryptic growth for biosolids management in wastewater treatment. Water Sci. Technol., 39 (10-11), 155-158.

48. Egemen E., Corpening J. and Nirmalakhandan N., (2001). Evaluation of an ozonation system for reduced waste sludge generation. Water Sci. Technol., 44 (2-3), 445-452.

49. El-Din M.G. and Smith D.W., (2002). Ozonation of kraft pulp mill effluents: process dyanamics. J. Environ. Eng. Sci., 1, 45-57.

50. Ellott A., Dorica J. and Bbrousseau Y., (1999a). Pilot scale evaluation of sludge reduction techniques at a TMP/newsprint mill. Pulp Pap-Canada, 100 (1), 59-63.

51. Ellott A. and Dorica J. (1999b). Reduced biomass production using acid effluent from a kraft bleach plant. Pulp Pap-Canada, 100 (5), 138-142.

52. EPA method 9021 (1992): Purgeable organic halides (POX). United State Environmental Protection Agency, USA.

53. EPA method 9023 (1996): Extractable organic halides (EOX). United State Environmental Protection Agency, USA.

54. Erden G., Demir O. and Filibeli A., (2010). Disintegration of biological sludge: Effect of ozone oxidation and ultrasonic treatment on aerobic digestibility. Bioresour. Technol., 101, 8093-8098.

55. Etiegni L., Oricho O.D., Ofosu-Asiedu K., Senelwa K.A., Surtan K.G. and Omutange E.S., (2007). Removal of colour from a kraft pulp and paper mill effluent in Kenya using a combination of electrochemical method and phosphate rock. Water Sci. Technol., 55 (6), 15–22.

56. Fiedler H., (2003). Persistent organic pollutants. The Handbook of Environmental Chemistry Vol. 3, Springer, Germany, 164-173.

57. Freire C.S.R., Silvestre A.J.D. and Neto C.P., (2003). Carbohydrate derived chlorinated compounds in ECF bleaching of hardwood pulps: Formation, degradation and contribution to AOX in a bleached kraft pulp mill. Environ. Sci. Technol., 37, 811-814.

58. Fontanier V., Farines V., Albet J., Baig S. and Molinier J., (2005). Oxidation of organic pollutants of water to mineralization by catalytic ozonation. Ozone Sci. Eng., 27(2), 115-128.

59. Gaudy A.F. and Gaudy E.T., (1981). Microbiology for environmental scientists and engineers. McGraw-Hill, International student edition, Tokyo, Japan, 175-205.

60. Ghiglizza R., Lodi A., Converti A., Nicolella C. and Rovarti M., (1996). Influence of the ratio of the initial substrate concentration to biomass concentration on the performance of a sequencing batch reactor. Bioprocess Eng., 14, 131-137.

61. Ghyooy W. and Verstraete W., (2000). Reduced sludge production in a two-stage membrane assisted bioreactor. Water Res., 34, 205-215.

62. Gloria Y. and Allen D.G., (1994). Biosorption of high molecular weight organochlorines in pulp mill effluent. Water Res., 28 (9), 1933-1941.

63. Graham N., Chu W. and Lau C., (2003). Observations of 2,4,6-trichlorophenol degradation by ozone. Chemosphere, 51, 237-243.

64. Gunten, U.V., (2003a). Ozonation of drinking water: Part I. Oxidation kinetics and product formation. Water Res., 37 1443-1467.

65. Gunten, U.V., (2003b). Ozonation of drinking water: Part II. Oxidation kinetics and product formation in presence of bromide, iodide or chlorine. Water Res., 37, 1469-1487.

66. Hautaniemi M., Kallas J., Munter R. and Trapido M., (1998). Modeling of chlorophenol treatment in aqueous solutions. 2. Ozonation under basic conditions. Ozone Sci. Eng., 20 (4), 283-302.

67. Helble A., Schlayer W., Liechti P.A., Jenn, R. and Mobius C.H. (1999). Advanced effluent treatment in the pulp and paper industry with a combined process of ozonation and fixed bed biofilm reactor. Water Sci. Technol., 40(11-12), 343-350.

68. Hoigne J. and Bader H., (1983). Rate constants of reactions of ozone with organic and inorganic compounds in water-I. Water Res., 17, 173-183.

69. Hong P.K.A. and Zeng Y., (2002). Degradation of pentachlorophenol by ozonation and biodegradability of intermediates. Water Res., 36, 4243-4254.

70. Industry guide, http://www.skcinc.com/instructions/1448.pdf, accessed on April 25, 2011.

71. IS 10158 (1982): Methods of Analysis of Solid Wastes (Excluding Industrial Solid Wastes). Bureau of Indian Standard, India.

72. ISO method 9562 (1989): Water quality- Determination of adsorbable organic halogens (AOX). International organization for standardization, Switzerland.

73. Jenkins D, Richard M.G. and Daigger G.T., (1993), Manual on the causes and control of activated sludge bulking and foaming. 2nd Edition, Lewis publishers, Michigan, USA, 3-75.

74. Jia X.S., Fang H.H.P. and Furumai H., (1996). Surface charge and extracellular polymer of sludge in the anaerobic degradation process. Water Sci. Techno., 34(5-6), 309-316.

75. Jorand, F., Zartarian F., Thomas F., Block J.C., Bottero J.Y., Villemin G., Urbain V. and Manem J., (1995). Chemical and structural (2d) linkage between bacteria within activated sludge flocs. Water Res., 29(7), 1639-1647.

76. Joss E.N., McGrouther K.G. and Slade A.H., (2007). Comparison of the efficacy of oxidative processes and flocculation for the removal of colour from E_{OP} effluent. Water Sci. Techno., 55(6), 57-64.

77. Juhasz A.L., Smith E., Smith J. and Naidu R., (2002). Biosorption of organochlorine pesticides using fungal biomass. J. Ind. Microbiol. Biotechnol., 29 (4), 163-169.

78. Kamiya T. and Hirotsuji J., (1998). New combined system of biological process and intermittent ozonation for advanced wastewater treatment. Water Sci. Technol., 38(8-9), 145-153.

79. Kansal S.K., Singh M. and Sud D. (2008). Effluent quality at kraft/soda agro-based paper mills and its treatment using a heterogeneous photocatalytic system. Desalination, 228, 183-190.

80. Kawaguchi H. and Inagasaki A., (1994). A kinetics of ferric ion promoted photodecomposition of 2-chlorophenol. Chemosphere, 28, 57-62.

81. Khan A.F., (2006). Study on effect of heavy metals and the stabilization of hydrogen peroxide bleach bath by polycarboxylic acid, amino polycarnoxylic acid, poly aminophosphonic acid, and their salts. Ph.D Thesis, Department of Applied Chemistry and Chemical Technology, University of Karachi, Pakistan, 12-13.

82. Klas S., (2006). Reduction of excess sludge production by ozonation. Master Thesis, Department of Water and Environmental Engineering, Lunds Institute of Technology, Lund University, Sweden, 49-51.

83. Knudsen L., Pedersen J.A. and Munck J., (1994). Advanced treatment of paper mill effluents by two stage activated sludge process. Water Sci. Technol., 30 (3), 173-181.

84. Kovacs T.G., Martel P.H., Voss R.H., Wrist P.E. and Willes R.F., (1993). Aquatic toxicity equivalency factors for chlorinated phenolic compounds present in pulp mill effluents. Environ. Toxicol. Chem., 12 (2), 281-289.

85. Kuo C.H. and Huang C.H., (1995). Aqueous phase ozonation of chlorophenols. J. Hazard. Mater., 41, 31-45.

86. Kuo W. S., (1999). Synergistic effects of combination of photolysis and ozonation on destruction of chlorophenols in water, Chemosphere, 39 (11), 1853-1860.

87. Lee J.W., Cha H-Y, Park K-Y, Song K-G and Ahn K-H, (2005). Operational strategies for an activated sludge process in conjunction with ozone oxidation for zero excess sludge production during winter season. Water Res., 39, 1199-1204.

88. Lee N.M. and Welander T., (1996). Reducing sludge production in aerobic wastewater treatment through manipulation of the ecosystem. Water Res., 30 (8), 1781-1790.

89. Lesko T., Colussi A.J. and Hoffmann M.R. (2006). Sonochemical decomposition of phenol: evidence for a synergistic effect of ozone and ultrasound for the elimination of total organic carbon from water. Environ. Sci. Technol., 40, 6818-6823.

90. Leuenberger C., Giger W., Coney R., Graydon J.W. and Kubica E.M., (1985). Persistent chemicals in pulp mill effluents- Occurrence and behavior in an activated sludge treatment plant. Water Res., 19 (7), 885-894.

91. Liu Y., Chenand G-H and Etinne P., (1998). Effect of the S_O/X_O ratio on energy uncoupling in substrate sufficient batch culture of activated sludge. Water Res., 32 (10), 2883-2887.

92. Liu Y., (2000). Effect of chemical uncoupler on the observed growth yield in batch culture of activated sludge. Water Res., 34 (7), 2025-2030.

93. Low E.W. and Chase H.A., (1998). The use of chemical uncouplers for reducing biomass production during biodegradation. Water Sci. Technol., 37 (4-5), 399-402.

94. Low E.W. and Chase H.A., (1999a). The effect of maintenance energy requirements on biomass production during wastewater treatment. Water Res., 33 (3), 847-853.

95. Low E.W. and Chase H. A., (1999b). Reducing production of excess biomass during wastewater treatment. Water Res., 33 (5), 1119-1132.

96. Low E.W., Chase A.C., Milner M.G. and Curtis T.P., (2000). Uncoupling of metabolism to reduce biomass production in the activated sludge process. Water Res., 34(12), 3204-3212.

97. Mahmood T. and Elliott A., (2006). A review of secondary sludge reduction technologies for the pulp and paper industry. Water Res., 40, 2093-2112.

98. Manojlovic D., Ostojic D.R., Obradovic B.M., Kuraica M.M., Krsmanovic V.D. and Puric J., (2007). Removal of phenol and chlorophenols from water by new ozone generator. Desalination, 213, 116-122.

99. Manterola G., Uriarte I. and Sancho L., (2008). The effect of operational parameters of the process of sludge ozonation on the solubilisation of organic and nitrogenous compounds. Water Res., 42, 3191-3197.

100. Masschelein W.J., Blaich L., Langlais B., Thieben E., Bell J. and Reading A., (1998). Ozone science & engineering special issue on quality assurance in ozone practice. Ozone Sci. Eng., 20, 433–487.

101. Mayhew M. and Stephenson T., (1998). Biomass yield reduction: Is biochemical manipulation possible without affecting activated sludge process efficiency? Water Sci. Technol., 38 (8-9), 137-144.

102. MoEF, (2010). Technical EIA guidance manual for pulp and paper industry. IL&FS Ecosmart Limited, Hyderabad, India.

103. MoEF (Ministry of Environment and Forest) notification, 2008. www.moef.nic.in/legis/hsm/HAZMAT_2265_eng.pdf, last accessed on April 09, 2011.

104. Monte M.C., Fuente E., Blanco A. and Negro C., (2009). Waste management from pulp and paper production in the European Union. Waste Manage., 29, 293-308.

105. Morais A.D.A., Mounteer A.H. and Silveira D.S.A. (2008). Improvement of eucalyptus bleached kraft effluent treatment through combined ozone–biological treatment. Tappi J., 7(2), 211–218.

106. Morgan J.W, Forster C.F. and Evison L. (1990). A comparative study of the nature of biopolymers extracted from anaerobic and activated sludges. Water Res., 24(6), 743-750.

107. Muller J.A., (2000). Pretreatment processes for the recycling and reuse of sewage sludge. Water Sci. Technol., 42 (9), 167-174.

108. Mvula E., Naumov S. and Sonntag C.V., (2009). Ozonolysis of lignin models in aqueous solution: Anisole, 1,2-Dimethoxybenzene, 1,4-Dimethoxybenzenr, and 1,3,5-Trimethoxybenzene. Environ. Sci. Technol., 43, 6275-6282.

109. Nakamura Y., Daidai M. and Kobayashi F., (2004). Ozonolysis mechanism of lignin model compounds and microbial treatment of organic acids produced. Water Sci. Technol., 50 (3), 167-172.

110. NCASI method CP-85.01 (1997). Chlorinated phenolics in water by in-situ acetylation/GC-ECD determination. NCASI, West Coast Regional Centre, North Carolina.

111. Nielsen P.H., Thomsen T.R. and Nielsen J.L., (2004). Bacterial composition of activated sludge – importance for floc and sludge properties. Water Sci. Technol., 49 (10), 51-58.

112. NPC (National productivity council), (2006). Development of guidelines for water conservation in pulp and paper sector. Final report, India, 52-54.

113. Okey R.W. and Stensel D.H., (1993). Uncouplers and activated sludge- the impact on synthesis and respiration. Toxicol. Environ. Chem., 40, 235-254.

114. Park K.Y., Ahn K.H., Maeng S.K., Hwang J.H. and Kwon J.H., (2003). Feasibility of sludge ozonation for stabilization and conditioning. Ozone Sci. & Engg., 25 (1), 73-80.

115. Paul E. and Debellefontaine H., (2007). Reduction of excess sludge produced by biological treatment processes: effect of ozonation on biomass and on sludge. Ozone Sci. & Engg., 29 (6), 415-427.

116. Pere J., Alen R., Viikari L. and Eriksson L., (1993). Characterisation and dewatering of activated sludge from pulp and paper industry. Water Sci. Technol., 28 (1), 193-201.

117. Perez-Elvira S.I., Nieto Diez P. and Fdz-Polanco F., (2006). Sludge minimisation technologies. Rev. Environ. Sci. Biotechnol., 5, 375-398.

118. Poznyak T. and Vivero J., (2005). Degradation of aqueous phenol and chlorinated phenols by ozone. Ozone Sci. Eng., 27(6), 447-458.

119. Ruas D.B., Mounteer A.H., Lopes A.C., Gomes B.L., Brandao F.D. and Girondoli L.M., (2007). Combined chemical biological treatment of bleached eucalypt kraft pulp mill effluent. Water Sci. Technol., 55 (6), 143–150.

120. Ray A.K., (2006). Paper mill pollutants, their monitoring and measurement techniques. IPPTA J., 18(4), 45-53.

121. Reeve D.W., (1991). Organochlorine in bleached kraft pulp. Tappi J., 74 (2), 123-126.

122. Rocher M., Goma G., Pilas Gegue A., Louvel L. and Rolls J. L., (1999). Towards a reduction in excess sludge production in activated sludge processes: biomass physicochemical treatment and biodegradation. Appl. Microbiol. Biotecnol., 51, 883-890.

123. Rocher M., Roux G., Goma G., Begue A.P., Louvel L. and Rols J.L., (2001). Excess sludge reduction in activated sludge processes by integrating biomass alkaline heat treatment. Water Sci. Technol., 44 (2-3), 437-444.

124. Rosenberger S., Kruger U., Witxig R., Manz W., Szewzyk U. and Kraume M., (2002). Performance of a bioreactor with submerged membranes for aerobic treatment of municipal wastewater. Water Res., 36(2), 413-420.

125. Rosner M., (2009). Ozone treatment of process and wastewater in the pulp and paper industry. 9th International technical conference on pulp, paper and allied industry, December 4–6, 2009, Delhi, India, 150–163.

126. Roy, M., Chakrabarti S.K., Bharadwaj N.K., Chandra S., Kumar S., Singh S., Bajpai P.K. and Jauhari M.B., (2004). Characterization of chlorinated organic material in eucalyptus pulp bleaching effluent. J. Sci. Ind. Res., 63, 527-536.

127. Ruas D.B., Mounteer A.H., Lopes A.C., Gomes B.L., Brandao F.D. and Girondoli L.M. (2007). Combined chemical biological treatment of bleached eucalypt kraft pulp mill effluent. Water Sci. Technol., 55 (6), 143-150.

128. Saby S., Djafer M. and Chen G.H., (2002). Feasibility of using a chlorination step to reduce excess sludge in activated sludge process. Water Res., 36(3), 656-666.

129. Sahinkaya E., Uzal N., Yetis U. and Dilek F.B., (2008). Biological treatment and nanofiltration of denim textile wastewater for reuse. J. Hazard. Mater., 153(3), 1142-1148.

130. Sakai Y., Fukase T., Yasui H. and Shibata M., (1997). An activated sludge process without excess sludge production. Water Sci. Technol., 36(11), 163-170.

131. Saktaywin W., Tsuno H., Nagare H., Soyama T. and Weerapakkaroon J., (2005). Advanced sewage treatment process with excess sludge reduction and phosphorus recovery. Water Res., 39, 902-910.

132. Salsabil M. R., (2008). Comparative study of the digestion of urban biological sludge pretreated by physical, chemical, and thermal processes: applied to reduce sludge production. Ph.D Thesis, University of Limoges, France, 46-48 & 147-150.

133. Sandra C., Piatkowska J., Rodriguez M., Sans C. and Esplugas S. (2005). Biodegradability improvement of aqueous 2,4-dichlorophenol and nitrobenzene solutions by means of single ozonation. Ozone Sci. Eng., 27(5), 381-387.

134. Savant D.V., Abdul-Rahman R. and Ranade D.R., (2006). Anaerobic degradation of adsorbable organic halides (AOX) from pulp and paper industry wastewater. Bioresour. Technol., 97(9), 1092-1104.

135. Seviour R. and Nelsen P.H., (2010). Microbial ecology of activated sludge, IWA Publishing, London, UK, 57-66.

136. Shomar B., (2007). Sources of adsorbable organic halogens (AOX) in sludge of Gaza. Chemosphere, 69, 1130-1135.

137. Sievers M., Ried A. and Koll R., (2004). Sludge treatment by ozonation- evaluation of full-scale results. Water Sci. Technol., 49 (4), 247-253.

138. Singh P., (2007). Sequential anaerobic and aerobic treatment of pulp and paper mill effluent in pilot scale bioreactor. J. Environ. Biol., 28(1), 77-82.

139. Smook, G.A., (1982). Handbook for pulp and paper technologists, Joint Textbook Committee of the Paper Industry. TAPPI, USA, 384-388.

140. Springer A. M., Dietrich-Velazquez G. Higby C.M. and Digiacomo D., (1996). Feasibility study of sludge lysis and recycle in the activated-sludge process. Tappi J., 79 (5), 162-170.

141. Sung M., Lee S.Z. and Huang C.P., (2008). Ozonation of pentachlorophenol in unsaturated soils. J. Contam. Hydrol., 98, 75-84.

142. Sung M. and Huang C.P., (2007). Kinetics of the degradation of 2-chlorophenol by ozonation at pH 3. J. Hazard. Mater., 141, 140-147.

143. Taghipour F. and Evans G.J., (1996). Radiolytic elimination of organochlorine in pulp mill effluent. Environ. Sci. Technol., 30 (5), 1558-1564.

144. Tchobanoglous, G., Burton, F.L. and Stensel, H.D., (2003). Wastewater engineering: Treatment and reuse, 4th ed., Metcalf & Eddy Inc., Tata McGraw-Hill Publishing, New Delhi, India, 555-588, 661-747.

145. Tian Y., (2008). Behaviour of bacterial extracellular polymeric substances from activated sludge: a review. Int. J. Environ. Pollut., 32 (1), 78-89.

146. Tran A.I., (2009). Removal of COD and color loads in bleaching kraft pulp effluent using ozone. Tappi J. 8(6), 211–218.

147. Trapido M., Hivronen A., Veressinina Y., Hentunen J. and Munter R., (1997). Ozonation, ozone/UV and UV/H_2O_2 degradation of chlorophenols. Ozone Sci. Eng., 19 (1), 75-96.

148. USEPA, (2002). EPA office of compliance sector notebook project: profile of pulp and paper industry. 2nd ed, Washington, DC 20460, USA: EPA/310-R-02-002.

149. USEPA, (2000). Permit guidance document: pulp, paper and paperboard manufacturing- point source category. Washington, DC 20460, USA, EPA-821-B-00-003.

150. USEPA, (1999). Office of enforcement and compliance assurance, office of compliance, manufacturing, energy and transportation division: kraft pulp mill compliance assessment guide (CAA, CWA, RCRA and EPCRA). EPA/310-B-99-001.

151. Wandan E.N., Adouby K. and Chantal A., (2006). The effect of environmental parameters on contaminant uptake by a passive sampler device. J. Appl. Sci. Environ. Mgt., 10(1), 15-23.

152. Wang H.C. , Chang S.H., Hung P.C., Hwang J.F. and Chang M.B., (2008). Catalytic oxidation of gaseous PCDD/Fs with ozone over iron oxide catalysts. Chemosphere, 71 (2), 388-397.

153. Wang L.K., Shammas N.K. and Hung Y.T., (2007). Biosolids treatment processes, Hand book of environmental engineering, vol. 6, Humana Press Inc., USA, 269-273.

154. Wang R., Chen C.L. and Gratzl J.S., (2004). Dechlorination and decolorization of chloro-organics in pulp bleach plant E-1 effluents by advanced oxidation processes. Bioresour. Technol., 94, 267-274.

155. Wagner J. and Rosenwinkel K.H., (2000). Sludge production in membrane bioreactors under different conditions. Water Sci. Technol., 41(10-11), 251-258.

156. Wei Y., Houten R.T.V., Borger A.R., Eikelboom D.H. and Fan Y., (2003). Minimization of excess sludge production for biological wastewater treatment. Water Res., 37, 4453- 4467.

157. Wijnbladh E., (2007). Ozone technology for sludge bulking control. Master thesis, Department of Microbiology, Swedish University of Agricultural Sciences, Uppsala, Sweden, 19-27.

158. Wood N., Tran H. and Master E. (2009). Pretreatment of pulp mill secondary sludge for high-rate anaerobic conversion to biogas. Bioresour. Technol., 100, 5729-5735.

159. Wunderlich R., Barry J., Greenwood D. and Carry C., (1985). Startup of a high-purity, oxygen-activated sludge system at the Los Angeles County Sanitation Districts' Joint Water Pollution Control Plant. J. Water Pollut. Control Fed., 57, 1012-1018.

160. Yan S.T., Chu L.B., Xing X.H., Yu A.F., Sun X.L. and Jurcik B., (2009 a). Analysis of the mechanism of sludge ozonation by a combination of biological and chemical approaches. Water Res., 43, 195–203.

161. Yan, S.T., Zheng H., Li A., Zhang X., Xing X.H., Chu L.B., Sun X.L., Jurcik B. and Ding G., (2009 b). Systematic analysis of biochemical performance and the microbial community of an activated sludge process using ozone-treated sludge for sludge reduction. Bioresour. Technol., 100, 5002–5009.

162. Yan G. and Allen D.G., (1994). Biosorption of high molecular weight organochlorines in pulp mill effluent. Water Res., 28(9), 1933-1941.

163. Yang X.F., Xie M.L. and Liu Y., (2003). Metabolic uncouplers reduce excess sludge production in an activated sludge process. Process Biochem. 38, 1373-1377.

164. Yasui H. and Shibata M., (1994). An innovative aroach to reduce excess sludge production in the activated sludge process. Water Sci. Technol., 30(9), 11-20.

165. Yasui H., Nakamura K., Sakuma S., Iwasaki M. and Sakai Y., (1996). A full-scale operation of a novel activated sludge process without excess sludge production. Water Sci. Technol., 34(3-4), 395-404.

166. Yoon S.H. and Lee S., (2005). Critical operational parameters for zero sludge production in biological wastewater treatment processes combined with sludge disintegration. Water Res., 39, 3738–3754.

167. Yuko S., Seiji I., Chikako I., Yasushi K., Yasushi O. and Hikaru K., (1999). Solubilisation of activated sludge by self-digestion. Water Res., 33 (8), 1864-1870.

168. Zhao Y.X., Yin J., Yu H.L., Han N. and Tian F.J., (2007). Observations on ozone treatment of excess sludge. Water Sci. Technol., 56 (9), 167-175.

169. Zheng Y. and Allen D.G., (1996). Biological dechlorination of model organochlorine compounds in bleached kraft mill effluents. Environ. Sci. Technol., 30, 1890-1895.

List of publications

Publication in journals:

- Gupta S., Chakrabarti S.K. and Singh S. (2010). Effect of ozonation on activated sludge from pulp and paper industry, Water Sci. Technol., 62(7), 1676-1681.
- Gupta S., Chakrabarti S.K. and Singh S. (2011). Effect of varying load of organochlorine compounds on activated sludge process, IPPTA J., 23 (1), 175-179.
- Gupta S., Purwar M., Chakrabarti S.K. and Singh S., (2012). Influence of drying of biosludge from pulp and paper industry on organochlorine compounds, J. Environ. Biol., 33, 85-88.
- Gupta S., Chakrabarti S.K. and Singh S., Oxi-bioremediation of hazardous biosludge from pulp and paper industry, Ozone Sci. Eng., (In press).
- Gupta S., Chakrabarti S.K. and Singh S., Effect of ozonation on the degradation of organochlorine compounds in biosludge of pulp and paper industry, Ozone Sci. Eng., (communicated).

Conference Presentations:

- Chakrabarti S. K., Gupta S., Karn S., Sharma K. D. and Varadhan R., (2009). Effect of AOX compounds on the microbial community structure and reactor performance in activated sludge process, 9th IWA Symposium on Forest Industry Wastewater, Fredricton, New Brunswick, Canada, June 14–17, 2009, 92.
- Gupta S., Singh S. and Chakrabarti S. K., (2009). Effect of ozonation on waste activated sludge of pulp & paper industry, 9th IWA Symposium on Forest Industry Wastewater, Fredricton, New Brunswick, Canada, June 14–17, 2009, 93.
- Gupta S., Chakrabarti S. K. and Singh S., (2011). Reduction in quantity and contamination of hazardous biosludge from pulp and paper industry by ozonation, 14th Punjab Science Congress on Role of Scientific Innovations & Knowledge in Economic Development, Longowal, Sangrur (Punjab), India, February 7-9, 2011, 231-232 **(Mecaster Young Scientist Award Winner).**

- Gupta S., Purwar M., Chakrabarti S.K. and Singh S., (2011). Influence of drying of biosludge from pulp and paper industry on organochlorine compounds, 14th Punjab Science Congress on Role of Scientific Innovations & Knowledge in Economic Development, Longowal, Sangrur (Punjab), India, February 7-9, 2011, 135-136.
- Gupta S., Chakrabarti S. K. and Singh S., (2012). Minimisation of hazardous biosludge from pulp and paper industry by ozone oxidation, 10th IWA Symposium on Forest Industry Wastewater, Concepcion, Chile, January 8-11, 2012, 52.