

Biological Treatment of Pulp Mill Wastewater-Effect of pH and Temperature of the Influent on the Microbial Ecology and Reactor Performance

S.K. Chakrabarti*, Sanjeev Gupta*, Amandeep Kaur*, Santosh Karn*,
K.D. Sharma** and R. Varadhan**

ABSTRACT

Relatively large wastewater discharge and accompanied release of high pollution load in the environment are the sequel of high water consumption and pollution generation in the process of pulp and paper making in India. Sustained and all-round efforts are being exerted in the mills to reduce the water consumption and restrict the discharge of pollution load in the receiving aquatic body to the minimum level. Aerobic biological treatment for degradation of biodegradable organic components in the wastewater of the pulp and paper mills occupies a dominant role in the clean-up process. Continuous shifting of the boundary conditions on environmental compliance and paradigm change of process technology in the pulp and paper mills bring a new challenge in the age-old, too familiar and convenient wastewater treatment process.

The removal of biodegradable organic substances; both soluble and finely dispersed is accomplished by biological oxidation with the help of microbial consortia principally bacteria. The ecology of microbial species, in activated sludge process is having the significant role in the bio-oxidation and subsequent clarification. The microorganisms; bacteria, protozoa, fungi, and rotifers exist in the activated sludge reaction vessel and clarifier as floc. The activated sludge process works well as long as the consortia of microorganisms, typically termed as sludge, grows in a healthy way, and settles and compacts in the secondary settling tank.

A long term research project is being carried out in the Centre to address the bio-oxidation of the pulp mill wastewater in the aerobic treatment process with change of dominant and significant operating parameters and environmental conditions. The present paper is based on the research findings on the effect of two operating conditions viz., pH and temperature on the operation of activated sludge process. The study has been carried out with the laboratory scale reactors operated under specified conditions with diluted pulp mill effluent having $C_p E_{op} DD$ bleaching sequence. Aerobic biological process can be operated within the pH range of 5-9.5 without any compromise on COD and AOX reduction which are 65-71 and 37.5-43% respectively; microbial consortia is well structured and settles rapidly (SVI : 28.5 ml/g). The aerobic reactor can be operated even at pH of 9.5-11.5 with some compromise on AOX reduction (20-25%) and COD reduction (60-65%).

Activated sludge process can comfortably be operated between 30-40°C without any difficulty in operation and compromise on reactor performance, and yield COD and AOX reduction of 68 and 37% respectively though there is some pin point floc formation; SVI of the sludge is 50 ml/g.

The study indicates the importance of equalization and cooling of pulp mill influent before it is fed to the aerobic bioreactors for effective and higher pollutants removal.

Key words: Activated sludge process, pulp and paper mill wastewaters, filamentous organisms, floc forming bacteria, CODs reduction

INTRODUCTION

Pulp and paper mills around the world depends heavily on water, the most important environmental resource on the earth. Large wastewater discharge and accompanied release of high pollution load in the environment are the sequel of high water consumption and pollution generation in the process of pulp and paper making. Sustained and all-round efforts are being exerted in the mills in different parts of the globe to reduce the water consumption and restrict the discharge of pollution

load in the receiving aquatic body to the bare minimum level. Aerobic biological treatment is mainly followed for degradation of biodegradable organic components in the wastewaters of the pulp and paper mills around the world as the clean-up process (Thompson and Forster, 2003; Seka *et al.*, 2001). Continuous shifting of the boundary conditions on the environmental compliance and paradigm change of process technology in the pulp and paper mills bring a new challenge in the age-old, too familiar and convenient wastewater treatment process.

The removal of biodegradable organic substances; both soluble and finely dispersed is accomplished by biological oxidation with the help of microbial consortia principally bacteria. The ecology, or to be more precise the balance of microbial species, in activated sludge process is the significant factor in the behaviour of the biomass in the bio-oxidation and subsequent clarification (Clauss *et al.*, 1998). The microorganisms; bacteria, protozoa, fungi, and rotifers do not exist as discrete cells in the activated sludge reaction vessel and clarifier, rather they exist as floc. The activated sludge process works well as long as the

* Thapar Centre for Industrial Research & Development,
Bhadson Road, Patiala-147004

**Ballarpur Industries Limited,
Mehrauli Gurgaon Road, Gurgaon-122002

Table 1: Operating conditions and performance of ASPs prior to change to acidic pH to two reactors

Operating parameters/ Reactor performance	R1	R2	R3
pH (inlet)	6.94±0.03	6.94±0.03	6.94±0.03
pH (outlet)	8.48±0.12	8.48±0.12	8.47±0.13
Temperature (°C)	36.8±1.3	36.8±1.3	36.8±1.3
DO (mg/l)	1.7±0.5	1.6±0.5	1.7±0.6
HRT (h)	9.0±0.2	9.1±0.5	8.8±0.3
MLSS (g/l)	4.68±0.42	4.55±0.57	4.69±0.66
MLVSS (g/l)	3.66±0.39	3.57±0.47	3.65±0.53
F/M ratio (d ⁻¹)	0.23±0.02	0.25±0.03	0.25±0.05
SVI (ml/g)	60±10	61±7	53±9
Organic loading (kg CODs/m ³ /d)	0.86±0.05	0.88±0.07	0.9±0.08
CODs feed (mg/l)	474±35	478±29	478±29
CODs reduction (%)	68.1±2.2	68.9±3.1	69.3±2.6
AOX feed (mg/l)	11.9±0.26	11.9±0.26	11.9±0.26
AOX reduction (%)	42.7±1.3	42.5±1.3	40.3±0.5

Table 2a: Effect of acidic pH (6 and 5) on the ASP performance

Operating parameters/ Reactor performance	Phase I (for first 7 days)			Phase II (for next 23 days)		
	R1	R2	R3	R1	R2	R3
pH (inlet)	7.04±0.05	6.02±0.07	5.03±0.03	7.0±0.03	6.01±0.03	5.0±0.03
pH (outlet)	8.27±0.2	8.02±0.21	7.93±0.26	8.1±0.10	7.72±0.16	7.72±0.29
Temperature (°C)	36.5±0.1	36.3±0.1	36.6±0.3	36.3±0.2	36.4±0.2	36.4±0.8
DO (mg/l)	1.5±0.7	1.3±0.3	1.7±0.5	1.6±0.3	1.5±0.4	1.6±0.3
HRT (h)	9.4±0.9	9.2±0.6	9.5±1.1	8.6±0.8	8.5±0.9	8.6±1.1
MLSS (g/l)	4.50±0.40	4.33±0.64	4.41±0.73	3.85±0.42	3.68±0.65	3.81±0.52
MLVSS (g/l)	3.50±0.39	3.51±0.49	3.56±0.57	3.20±0.36	3.11±0.55	3.20±0.44
F/M ratio (d ⁻¹)	0.22±0.03	0.26±0.05	0.26±0.06	0.30±0.07	0.33±0.08	0.31±0.07
SVI (ml/g)	36±2	36±2	33±2	27±3	23±5	28±6
Organic loading (kg CODs/m ³ /d)	0.78±0.09	0.89±0.07	0.91±0.27	0.97±0.20	1.0±0.19	0.98±0.24
CODs feed (mg/l)	470±18	502±16	502±16	506±43	521±47	521±47
CODs reduction (%)	64.5±4.1	67.9±0.8	66.6±4.9	67.2±4.9	66.3±4.2	65.1±6.4
AOX feed (mg/l)	9.4±0.3	10.2±0.8	9.5±0.7	10.1±0.6	10.6±1.0	11.1±2.2
AOX reduction (%)	37.7±1.8	42.5±4.5	36.0±8.2	42.0±2.8	43.6±4.0	43.2±6.0
Colour in feed (Pt-Co unit)				1210±125	1150±87	1250±285
Colour reduction (%)				48.2±9.6	51.8±6.3	56.3±10.5

Table 2b: Effect of acidic pH (4.5, 4) on the ASP performance

Operating parameters/ Reactor performance	Phase I (for first 7 days)			Phase II (for next 24 days)		
	R1	R2	R3	R1	R2	R3
pH (inlet)	7.01±0.01	4.5±0.02	4.01±0.01	7.01±0.01	4.49±0.10	4.01±0.01
pH (outlet)	8.09±0.11	7.32±0.19	6.94±0.24	8.01±0.23	6.75±0.45	6.23±0.59
Temperature (°C)	35.7±1.6	36.4±0.4	35.5±0.2	36.0±0.7	36.1±0.5	35.4±0.7
DO (mg/l)	1.5±0.6	1.6±0.6	1.5±0.5	1.9±0.5	1.4±0.5	1.4±0.5
HRT (h)	8.1±0.6	8.6±0.7	8.3±0.6	8.9±1.2	8.9±0.7	9.5±2.8
MLSS (g/l)	4.02±0.58	3.93±0.56	3.94±0.56	4.04±0.27	3.68±0.29	3.34±0.44
MLVSS (g/l)	3.94±0.53	3.47±0.53	3.39±0.52	3.41±0.25	3.24±0.28	2.95±0.38
F/M ratio (d ⁻¹)	0.37±0.13	0.34±0.07	0.36±0.12	0.28±0.05	0.27±0.04	0.24±0.05
SVI (ml/g)	20±2	19±4	33±5	19±2	15±5	22±13
Organic loading (kg CODs/m ³ /d)	1.12±0.15	1.09±0.19	1.03±0.21	0.95±0.15	0.88±0.12	0.69±0.16
CODs feed (mg/l)	541±34	558±24	558±24	523±26	510±30	510±30
CODs reduction (%)	70.3±3.9	70.0±2.0	68.3±4.7	65.9±4.3	63.5±4.1	53.4±6.8
AOX feed (mg/l)				14.94±1.06	14.90±1.32	15.75±0.73
AOX reduction (%)				37.9±2.9	31.7±5.4	32.5±5.0
Colour in feed (Pt-Co unit)	1010±63.7	863±145	840±67	1011±38	851±78	821±35
Colour reduction (%)	60.8±4.9	52.1±3.2	47.1±1.3	51.5±0.5	41.5±6.6	28.1±10.9

consortia of microorganisms, typically termed as sludge, grows in a healthy way, and settles and compacts in the secondary settling tank to the extent that the return sludge concentration is high enough to maintain the mixed liquor suspended solids concentration

(MLSS) at a desired level (Novak *et al.*, 1993).

Solids separation problem in activated sludge process arises due to various factors viz., dispersed growth of microorganisms, non-filamentous

bulking, filamentous bulking, fungal bulking, pin or pinpoint floc, blanket rising, foaming and scum formation. Although any of the stated factors can adversely affect the functioning of the secondary clarifier, filamentous bulking is the predominant cause of sludge bulking (Lou and de Ios Reyes, 2005).

Poor settling in the activated sludge process is a world wide phenomenon, and pulp and paper mill wastewater seem to be particularly prone to the problem. A long term project is being carried out in the Centre in the laboratory scale to address the bio-oxidation behaviour of the pulp mill wastewater in the aerobic treatment process with the change of dominant and significant operating parameters and environmental conditions. The objective of the long term study is to critically examine the causes of bulking in activated sludge plants treating the wastewaters from pulp and paper mills. The present paper is based on the research findings on the effect of two operating conditions viz., pH and temperature.

MATERIALS & METHODS

Materials

Effluent of a pulp mill having C_DE₀₁DD bleaching sequence along with dilute black liquor for COD make-up was used as the substrate for feeding into the activated sludge process. Effluent samples were periodically collected from the mill and stored in the refrigerated condition.

Activated sludge samples from the effluent treatment plant in the same pulp and paper mill along with cow dung were used for the seeding in the biological reactors. The proportion of the two was equal; cow dung was screened first with coarse screen.

Three laboratory scale reactors (Fig. 1) having volume capacity of 6 litres were used for biological reaction. Aeration tank was followed with two settlers in series having 2.5 litres volume of each. Feeding in the reactors was done with Peristaltic pumps of Cole Parmer, USA. Inlet feed solution was partially refrigerated at a temperature of 10-12 °C to arrest biodegradation. Temperature controller was used in each reactor. Air (after passing through rotameter) was supplied in the reactors; reactor biomass was circulated with

Table 2c: Effect of acidic pH (3.5, 4) on the ASP performance

Operating parameters/ Reactor performance	Phase I (for first 6 days)			Phase II (for next 6 days)		
	R1	R2	R3	R1	R2	R3
pH (inlet)	7.01±0.01	3.52±0.01	4.01±0.01	7.01±0.01	3.51±0.01	4.01±0.01
pH (outlet)	7.98±0.19	5.77±0.38	6.23±0.21	7.94±0.14	5.79±0.15	5.9±0.30
Temperature (°C)	36.0±0.3	36.1±0.4	34.9±0.6	36.1±0.7	36.2±0.2	35.5±0.7
DO (mg/l)	2.1±0.5	1.4±0.2	1.3±0.2	1.5±0.3	1.1±0.1	1.2±0.2
HRT (h)	9.7±0.9	9.4±1.3	8.7±0.3	9.0±0.1	8.8±0.4	8.3±0.3
MLSS (g/l)	4.18±0.30	3.47±0.41	3.12±0.22	3.52±0.1	3.06±0.12	2.89±0.06
MLVSS (g/l)	3.46±0.24	3.11±0.36	2.82±0.20	3.52±0.1	3.06±0.12	2.89±0.06
F/M ratio (d ⁻¹)	0.28±0.04	0.25±0.03	0.30±0.06	0.26±0.05	0.24±0.04	0.26±0.04
SVI (ml/g)	20±1	13±4	10±3	20±1	16±2	10±3
Organic loading (kg CODs/m ³ /d)	0.98±0.15	0.77±0.12	0.83±0.14	0.99±0.04	0.72±0.11	0.75±0.11
CODs feed (mg/l)	562±28	547±35	547±35	535±20	521±44	521±44
CODs reduction (%)	70.0±3.8	54.4±4.9	54.6±6.1	69.0±1.7	50.9±4.1	52.0±5.6
AOX feed (mg/l)	13.01	14.05	14.49	14.45±0.41	14.64±0.27	14.97±0.42
AOX reduction (%)	44.4	39.6	40.9	37.1±1.6	29.1±1.5	31.0±1.6

Table 3: Effect of reversal to normal pH from acidic pH on the ASP performance

Operating parameters/ Reactor performance	Phase I (for first 7 days)			Phase II (for next 7 days)			Phase III (for next 7 days)		
	R1	R2	R3	R1	R2	R3	R1	R2	R3
pH (inlet)	7.01±0.01	7.01±0.01	7.01±0.01	7.01±0.01	7.01±0.01	7.01±0.01	7.01±0.01	7.01±0.01	7.01±0.01
pH (outlet)	7.83±0.21	7.89±0.29	8.10±0.16	7.97±0.14	8.11±0.10	8.23±0.12	7.95±0.14	8.08±0.13	8.16±0.13
Temperature (°C)	35.9±1.1	36.1±0.5	35.1±0.9	36.0±1.6	35.4±1.4	35.2±1.7	33.7±1.9	34.4±1.7	33.8±0.8
DO (mg/l)	1.7±0.5	1.3±0.2	1.4±0.2	1.6±0.8	1.5±0.3	1.7±0.2	2.0±0.5	2.1±0.6	2.3±0.5
HRT (h)	9.1±0.1	8.8±0.4	8.0±0.6	8.1±0.2	8.9±0.2	8.5±0.5	8.4±0.2	8.9±0.2	8.6±0.2
MLSS (g/l)	4.15±0.16	3.6±0.12	3.47±0.15	4.05±0.46	3.62±0.31	3.46±0.37	4.05±0.16	3.85±0.84	3.94±0.15
MLVSS (g/l)	3.49±0.13	3.14±0.09	3.06±0.12	3.43±0.42	3.12±0.30	3.02±0.36	3.46±0.13	3.21±0.70	3.42±0.12
F/M ratio (d ⁻¹)	0.30±0.02	0.26±0.02	0.32±0.05	0.35±0.08	0.30±0.07	0.33±0.11	0.30±0.02	0.31±0.09	0.28±0.03
SVI (ml/g)	22±1	17±3	20±1	24±3	25±2	25±2	19±3	28±4	31±1
Organic loading (kg CODs/m ³ /d)	1.03±0.04	0.82±0.07	0.99±0.15	1.16±0.15	0.92±0.09	0.97±0.14	1.05±0.07	0.95±0.07	0.96±0.09
CODs feed (mg/l)	571±15	571±15	571±15	559±31	559±31	559±31	538±22	538±22	538±22
CODs reduction (%)	68.3±1.3	52.5±3.4	57.5±4.4	69.3±2.7	61.0±3.3	61.1±4.0	68.3±2.5	65.5±3.2	63.6±4.7
AOX feed (mg/l)	14.82	14.82	14.82	11.54±3.45	11.54±3.45	11.54±3.45	14.08±0.42	14.08±0.42	14.08±0.42
AOX reduction (%)	36.4	36.9	36.6	34.5±0.8	35.2±0.7	34.7±0.7	31.9	30.4	30.8
Alkalinity (Inlet) (mg/l)	287±27	287±27	284±27	278±26	278±26	278±26	397±35	297±35	297±35
Alkalinity (Outlet) (mg/l)	272±7	276±24	292±32	281±8	257±17	244±45	254±8	255±9	260±13

Table 4: Operating conditions and performance of ASPs prior to change to alkaline pH to two reactors

Operating parameters/ Reactor performance	R1	R2	R3
pH (inlet)	7.01±0.01	7.01±0.01	7.01±0.01
pH (outlet)	7.9±0.1	7.9±0.1	7.9±0.1
Temperature (°C)	35.9±0.1	36.4±0.1	35.2±0.5
DO (mg/l)	1.4±0.7	0.9±0.5	1.2±0.6
HRT (h)	8.5±0.1	8.3±0.1	8.4±0.8
MLSS (g/l)	4.08±0.14	4.06±0.10	4.0±0.14
MLVSS (g/l)	3.4±0.1	3.4±0.09	3.4±0.09
F/M ratio (d ⁻¹)	0.28±0.02	0.29±0.04	0.3±0.05
SVI (ml/g)	23±5	23±4	23±4
Organic loading (kg CODs/m ³ /d)	0.96 ±0.09	1.0±0.15	1.02±0.16
CODs feed (mg/l)	499±31	499±31	499±31
CODs reduction (%)	68.2±4.8	66.7±5.2	69.1±4.4
AOX feed (mg/l)	11.3±0	11.3±0	11.3±0
AOX reduction (%)	32.2±0	36.8±0	38.2±0
Alkalinity (Inlet) (mg/l)	255±13	252±12	247±10
Alkalinity (Outlet) (mg/l)	201±23	201±12	204±19
TSS (mg/l)	19.1±5.3	19.1±5.3	19.1±5.3

mechanical stirrers. Urea and technical grade phosphoric acid were used as the source of nutrient; CODs:N:P ratio of 100:5:1 was maintained throughout the study.

Methods

Analytical Methods

Adsorbable Organic Halides (AOX) was determined as per ISO Method No. 9562:1989 using Euroglas make AOX analyzer (Model No. ECS 2000). p-chlorophenol (SD Fine Chemicals, India) was used as the standard.

Chemical Oxygen Demand (COD)

was determined by open reflux method as per APHA method No. 5220 D using Spectra Lab make COD digestion system.

Colour was determined by spectrophotometric technique as per Hach method No. 8025. The pH of the wastewater was first adjusted to 7.6 by using H₂SO₄ or NaOH solution and filtered through Whatman filter no. 1.

Alkalinity was determined by titrametric method using Indian standard IS 3025 (Part 23) 1986.

Suspended solids was determined by filtration and gravimetric method using Indian standard IS 3025 (Part 17) 1984.

MLVSS was determined by incinerating the suspended solids at 500± 50 °C using Indian standard IS 3025 (Part 18) 1984.

DO was determined by Winkler's titration method using Indian standard IS 3025 (Part 38) 1989.

Morphological characterization of organisms was done with Image analyzer (Buehler, USA; Ominimet model)

RESULTS AND DISCUSSION

Nature of seed sludge

The secondary sludge samples was collected from an industrial plant. Microscopic analysis of the sludge in different times either as such or with different stains like Gram and Neisser stains indicates that the sludge is rich in filamentous organisms (Figure 2 and 3). Filaments are mostly irregular in shape and located partly within the floc and majority of the same are free.

Table 5a: Effect of alkaline pH (8.5 and 9.5) on the ASP performance

Operating parameters/ Reactor performance	Phase I (for first 8 days)			Phase II (for next 13 days)		
	R1	R2	R3	R1	R2	R3
pH (inlet)	7.2±0.3	8.44±0.12	9.3±0.55	7.3±0.2	8.4±0.1	9.42±0.1
pH (outlet)	7.8±0.1	8.08±0.07	8.2±0.1	7.9±0.10	8.2±0.1	8.4±0.1
Temperature (°C)	35.7±0.2	36.5±0.1	36.2±0.6	35.8±0.3	36.4±0.1	36.3±0.7
DO (mg/l)	1.1±0.4	1.1±0.2	1.2±0.3	1.1±0.4	1.3±0.3	1.7±0.4
HRT (h)	8.6±0.1	8.3±0.1	8.6±0.1	8.4±0.3	8.2±0.3	8.4±0.2
MLSS (g/l)	4.08±0.14	4.06±0.1	4.0±0.14	4.0±0.14	4.14±0.14	4.2±0.17
MLVSS (g/l)	3.38±0.1	3.38±0.1	3.36±0.1	3.4±0.1	3.5±0.1	3.4±0.13
F/M ratio (d ⁻¹)	0.31±0.02	0.31±0.03	0.3±0.04	0.29±0.03	0.29±0.02	0.30±0.03
SVI (ml/g)	23±5	23±4	23±4	21±1	20±1	24±2
Organic loading (kg CODs/m ³ /d)	1.04±0.05	1.04±0.1	1.0±0.1	1.01±0.12	1.02±0.09	1.01±0.06
CODs feed (mg/l)	531±25	525±37	522±29	513±28	504±19	496±25
CODs reduction (%)	70.0±1.9	68.4±3.0	66.7±3.5	68.9±4.6	69.3±3.4	71±2.0
AOX feed (mg/l)	9.9±0.8	8.8±0.3	7.8±0.51	10.5±0.03	9.1±0.2	8.4±0.04
AOX reduction (%)	44.5±2.2	37.1±5.1	34.8±8.4	42.9±3.5	36.9±0.3	37.5±4.4
Colour in feed (Pt-Co unit)	1200±52	1257±15	1281±81	1291±183	1239±51	1094±17
Colour reduction (%)	42±2.1	39.5±2.5	34.4±1.6	48.7±4.4	42±3.3	39.3±3.4
Alkalinity (Inlet) (mg/l)	267±0	295±21	329±37	230±29	309±69	381±55
Alkalinity (Outlet) (mg/l)	180±25	252±23	256±42	214±62	296±60	354±63

Table 5b: Effect of alkaline pH (10.5) on the ASP performance

Operating parameters/ Reactor performance	(for 9 days)		
	R1	R2	R3
pH (inlet)	7.01±0.01	7.01±0.01	10.5±0.04
pH (outlet)	8.08±0.07	8.14±0.11	8.7±0.06
Temperature (°C)	35.0±0.3	36.0±0.1	35.0±1.0
DO (mg/l)	1.0±0.26	2.0±0.27	1.0±0.19
HRT (h)	8.4±0.16	9.0±0.36	9.0±0.54
MLSS (g/l)	4.03±0.42	4.17±0.51	4.25±0.58
MLVSS (g/l)	3.37±0.39	3.47±0.42	3.38±0.48
F/M ratio (d ⁻¹)	0.23±0.03	0.23±0.03	0.22±0.06
SVI (ml/g)	18±2	20±2	25±2
Organic loading (kg CODs/m ³ /d)	0.76±0.05	0.79±0.04	0.79±0.13
CODs feed (mg/l)	426±49	426±49	430±61
CODs reduction (%)	67±8	70±8	65±9
AOX feed (mg/l)	8.8	8.8	6.9
AOX reduction (%)	42.5	46	29
Colour in feed (Pt-Co unit)	1210	1210	1082
Colour reduction (%)	37.4	45.5	25.1
Alkalinity (Inlet) (mg/l)	302±3	306±6	535±34
Alkalinity (Outlet) (mg/l)	256±25	259±10	498±34

Filaments are having diameter of 1-1.4 . And average length varies from 113 to 181.Gram, Neisser and Sulphur staining of the sludge indicate the dominance of *N. limicola* 2, *N. limicola* 1 and *M. parvicola* species. Abundance of either floc forming or filamentous bacteria in cow dung is limited.

Laboratory reactor operation with controlled condition

Organisms are acclimatized in laboratory environment in batch reactor supplied with aeration and continuous

mixing with mechanical stirrer. Diluted feed was added each day along with proportional amount of nutrients. Supernatant is decanted and fresh feed was added. This operation is continued for acclimatizing and growth of organisms. Requisite amount of organisms are transferred to each laboratory scale reactor which was then operated continuously. 15-20 days of continuous operation transformed the nature of organisms from extremely filamentous to sufficiently floc forming biomass. Severe oxygen deficiency in the industrial scale plant might be the

major cause of proliferation of filamentous bacteria. 1.0 to 1.7 mg/l of DO in the controlled reactor was found to be adequate to control the proliferation of filamentous organisms and producing sufficiently abundant floc forming consortia. Similar observation has been reported elsewhere (Gaval and Pernelle, 2003).

Operation of reactors with change of pH

Acidic pH

Before the change of environmental condition i.e. pH all the three reactors were run continuously for 15 days maintaining almost near environmental and operating conditions (Table 1). The near ideal conditions were selected so that biological aeration system run with flocculating bacteria. SVI of the sludge in three reactors were in the range of 53- 61 ml/g. CODs reduction of 68-69% and AOX reduction of 40-43% were observed in the reactors.

pH of the second and third reactors were changed to 6 and 5 respectively and that of the first one was maintained at 7.0. In these changed conditions the three reactors were run for 30 days. The reactors R2 and R3 were adapted to the new conditions very quickly. These resulted in good flocculating sludge with very less filamentous organisms outside of flocs (Figure 4). Protozoa and rotifers which were very less in the seed sludge were in large abundance in all three reactors. The performance of the reactors at pH 6 and 5 were comparable with that of the control reactor operated at pH 7. CODs, AOX and colour reduction in these three reactors were in the range of 64.5-67, 36- 43 and 48-56% respectively (Table 2a). For a developed flocculating sludge with less filamentous proliferation sustained operation with high level of biodegradation was possible even for pH of 5. For an industrial plant it is imperative that the incoming wastewater should first be made homogenous in pH with neutralization and equalization.

The pH of the two experimental reactors were then lowered to pH of 4.5 (R2 reactor) and 4 (R3 reactor) and run for 31 days in two phases and subsequently pH was further lowered to 3.5 (R2 reactor) whereas the R3 reactor was run at pH 4.0. The reactors were run in this condition for 12 days. Even at

Table 5c: Effect of alkaline pH (10.5 and 11.5) on the ASP performance

Operating parameters/ Reactor performance	(for 11 days)		
	R1	R2	R3
pH (inlet)	7.01±0.03	11.5±0.01	10.5±0.04
pH (outlet)	8.13±0.08	9.2±0.07	8.73±0.11
Temperature (°C)	34.8±0.2	36.4±0.1	35.0±0.2
DO (mg/l)	1.2±0.3	1.1±0.3	1.4±0.3
HRT (h)	8.5±0.2	8.5±1.0	8.5±0.2
MLSS (g/l)	4.02±0.12	4.05±0.15	4.24±0.09
MLVSS (g/l)	3.45±0.11	3.29±0.13	3.37±0.06
F/M ratio (d ⁻¹)	0.25±0.03	0.25±0.07	0.25±0.04
SVI (ml/g)	17±2	23±1	30±2
Organic loading (kg CODs/m ³ /d)	0.86±0.09	0.82±0.24	0.85±0.12
CODs feed (mg/l)	472±22	438±50	457±33
CODs reduction (%)	64.6±4.0	64.3±6.7	65.8±4.5
AOX feed (mg/l)	8.08±0.08	5.77±0.24	6.2±0.21
AOX reduction (%)	36.8±3.3	23.5±1.9	25.3±0.5
Colour in feed (Pt-Co unit)	1271±126	553±16	914±18
Colour reduction (%)	39.2±3.2	-3.6±4.0	31.1±12.1
Alkalinity (Inlet) (mg/l)	309±8	591±85	500±135
Alkalinity (Outlet) (mg/l)	275±10	655±48	499±26

Table 6: Effect of reversal to normal pH from alkaline pH on the ASP performance

Operating parameters/ Reactor performance	(for 8 days)		
	R1	R2	R3
pH (inlet)	7.01±0.01	7.01±0.01	7.01±0.01
pH (outlet)	8.10±0.05	8.2±0.06	8.21±0.03
Temperature (°C)	34.9±0.1	36.4±0.3	35.2±0.3
DO (mg/l)	0.5±0.3	0.6±0.3	1.0±0.3
HRT (h)	8.5±0.2	8.5±1.0	8.5±0.2
MLSS (g/l)	3.92±0.26	4.18±0.23	4.23±0.22
MLVSS (g/l)	3.4±0.23	3.44±0.18	3.44±0.19
F/M ratio (d ⁻¹)	0.26±0.03	0.28±0.02	0.29±0.02
SVI (ml/g)	16.6±1.7	27.6±3.7	28.3±3.1
Organic loading (kg CODs/m ³ /d)	0.89±0.05	0.97±0.02	0.97±0.04
CODs feed (mg/l)	508±16	508±16	508±16
CODs reduction (%)	69.7±2.6	69±2.4	70.9±2.1
AOX feed (mg/l)	7.7±0.2	7.7±0.2	7.7±0.2
AOX reduction (%)	45.2±5.4	44.9±6.8	44.9±9.0
Colour in feed (Pt-Co unit)	1302	1302	1302
Colour reduction (%)	35.3	59.1	55.1
Alkalinity (Inlet) (mg/l)	341±33	329±36.7	337.3±55.2
Alkalinity (Outlet) (mg/l)	329±29	329±36.7	311.5±25.5

lower pH very few free filamentous organisms were observed; flocs were more or less dense only few were broken. Rotifer, protozoa and nematodes were abundantly present in very active form (Figure 5). Foaming in the reactor is pronounced when the reactors were run below pH 5.0. Initially COD reduction was quite high

(more than 68% even at pH 4.0). CODs reduction dropped marginally in the long run. At pH 4.0 the CODs reduction was 53.4%. AOX reduction is more than 31% at these lower pH too. Colour reduction is appreciably low at lower pH (Table 2b & 2c).

The operating conditions of the reactors

were then changed and all were run at pH 7 for 21 days. After the pH stress was withdrawn the bacterial biomass became more active gradually which is evidenced by the CODs reduction. In the three weeks run the pH stressed reactors came to near normal condition of biodegradation (Table 3).

Alkaline pH

Before changing of pH of two reactors to alkaline condition all the three reactors were run for 15 days with almost identical environmental and operating conditions with fresh sludge from the buffer batch reactor. Well developed sludge with flocs having abundance of protozoa and rotifers and very less proliferation of filamentous organisms resulted in high biodegradation of organic substances (Table 4).

pH of the second and third reactors were changed to 8.5 and 9.5 respectively and that of the first one was maintained at 7.0. In these changed conditions the three reactors were run for three weeks. The reactors R2 and R3 were adapted to the new conditions. These resulted in good flocculating sludge with very less filamentous organisms outside of flocs (Figure 6); though in the third reactor flocs were little dispersed and large flocs were disintegrated. MLSS and MLVSS concentration in all three reactors were also in the same level which shows that there is no loss of organisms at higher pHs. Protozoa and rotifers were also in large abundance in all three reactors. The performance of the reactors at pH 8.5 and 9.5 were comparable with that of the control reactor operated at pH 7 with respect to CODs reduction. Marginally lower reduction of both AOX and colour was observed in these two reactors; lowest reduction were in the reactor operated in pH 9.5 (Table 5a). Alkaline pH showed more stabilization of AOX and colour containing substances.

All the three reactors were then brought back to normal operating condition; pH were 7 in each case. After running for seven days the pH in the third reactor (R3) were changed to 10.5 whereas other two reactors were run at pH 7. In this sequence the reactors were run for 8 days. Subsequently pH of the second reactor (R2) was changed to 11.5 whereas the third reactor was run at the same condition of 10.5. Even in these two extreme alkaline conditions

Table 7: Operating conditions and performance of ASPs prior to change of temperature of two reactors

Operating parameters/ Reactor performance	R1	R2	R3
pH (inlet)	7.01±0.01	7.01±0.01	7.01±0.01
pH (outlet)	7.95±0.12	7.97±0.15	7.97±0.14
Temperature (°C)	36.2±1.3	36.3±0.7	36.2±1.1
DO (mg/l)	1.3±0.5	1.5±0.4	1.5±0.5
HRT (h)	8.6±0.6	8.5±0.3	8.6±0.4
MLSS (g/l)	4.0±0.1	4.0±0.1	4.0±0.1
MLVSS (g/l)	3.5±0.1	3.5±0.1	3.5±0.1
F/M ratio (d ⁻¹)	0.26±0.02	0.27±0.02	0.27±0.02
SVI (ml/g)	32±5	27±4	35±4
Organic loading (kg CODs/m ³ /d)	0.9 ±0.1	0.9±0.1	0.9±0.1
CODs feed (mg/l)	477±30	477±30	477±30
CODs reduction (%)	68.5±2.7	69.3±2.6	69.1±2.7
AOX feed (mg/l)	9.8±0.4	9.8±0.4	9.8±0.4
AOX reduction (%)	33.1±0	32.7±0	30.4±0
Alkalinity (Inlet) (mg/l)	274±13	274±13	274±13
Alkalinity (Outlet) (mg/l)	279±25	279±25	279±25
TSS (mg/l)	5.0±4.3	9.0±5.3	6.0±4.7

Table 8a: Effect of temperature (36, 30 and 40°C) on the ASP performance

Operating parameters/ Reactor performance	Phase I (for first 7 days)			Phase II (for next 12 days)		
	R1	R2	R3	R1	R2	R3
pH (inlet)	7.01±0.01	7.01±0.01	7.01±0.01	7.01±0.01	7.01±0.01	7.01±0.01
pH (outlet)	8.21±0.38	8.07±0.18	8.13±0.12	8.08±0.13	8.01±0.13	8.27±0.13
Temperature (°C)	36.6±0.2	30±0.1	39.7±1.0	36.8±0.4	30.3±0.2	40.5±0.1
DO (mg/l)	1.5±0.6	1.8±0.6	1.1±0.5	1.2±0.5	1.2±0.5	1.4±0.3
HRT (h)	8.2±0.5	8.5±0.1	7.8±0.9	8.5±0.2	8.5±0.1	8.5±0.1
MLSS (g/l)	4.0±0.3	3.9±0.4	4.0±0.2	4.1±0.1	3.9±0.1	4.2±0.2
MLVSS (g/l)	3.5±0.2	3.4±0.3	3.5±0.1	3.5±0.1	3.4±0.1	3.5±0.2
F/M ratio (d ⁻¹)	0.29±0.03	0.27±0.02	0.3±0.03	0.28±0.03	0.27±0.04	0.27±0.03
SVI (ml/g)	36.3±1.7	26.3±5.0	45±0.6	37.5±1.2	21±2.0	50.1±5.1
Organic loading (kg CODs/m ³ /d)	1.0±0.1	0.9±0.15	1.0±0.1	1.0±0.1	0.9±0.1	0.9±0.1
CODs feed (mg/l)	471±13	471±13	471±13	485±45	485±45	485±45
CODs reduction (%)	70.8±2.6	68.2±1.8	70.1±2.9	69.4±2.9	67.4±3.1	68.1±3.0
AOX feed (mg/l)	13.0±1.7	13.0±1.7	13.0±1.7	13.6±0.7	13.6±0.7	13.6±0.7
AOX reduction (%)	48.9±5.4	48.8±4.7	47.3±5.5	40.6±5.9	40.1±5.4	34.1±9.7
Colour in feed (Pt-Co unit)	1032±49	1032±49	1032±49	1152±129	1152±129	1152±129
Colour reduction (%)	47.9±2.6	41.6±3.0	50.6±0.6	53.5±3.0	44.8±0.8	51.4±0.6

Table 8b: Effect of temperature (36, 45 and 50 °C) on the ASP performance

Operating parameters/ Reactor performance	Phase I (for first 7 days)			Phase II (for next 12 days)		
	R1	R2	R3	R1	R2	R3
pH (inlet)	7.01±0.01	7.01±0.01	7.01±0.01	7.01±0.01	7.01±0.01	7.01±0.01
pH (outlet)	8.17±0.15	8.37±0.1	8.42±0.14	8.06±0.14	8.29±0.15	8.40±0.16
Temperature (°C)	36.2±0.1	45.1±0.9	50.2±1.3	36.3±0.1	45.2±0.1	50.2±1.6
DO (mg/l)	1.1±0.7	0.5±0.4	0.1±0.2	1.0±0.3	0.5±0.2	0.3±0.2
HRT (h)	8.4±0.5	9.0±0.6	9.4±0.5	8.4±0.2	8.4±0.3	8.6±0.2
MLSS (g/l)	4.17±0.3	3.78±0.41	3.16±0.45	4.10±0.15	3.75±0.24	2.9±0.1
MLVSS (g/l)	3.57±0.27	3.24±0.38	2.59±0.39	3.51±0.12	3.15±0.19	2.25±0.09
F/M ratio (d ⁻¹)	0.33±0.03	0.27±0.03	0.29±0.03	0.29±0.02	0.22±0.02	0.26±0.03
SVI (ml/g)	36±2	24±3	24±3	27±2	41±20	27±6
Organic loading (kg CODs/m ³ /d)	1.19±0.7	0.88±0.11	0.74±0.08	1.0±0.07	0.7±0.06	0.59±0.06
CODs feed (mg/l)	554±20	554±20	554±20	497±22	497±22	497±22
CODs reduction (%)	74.9±2.7	58.9±6.9	52.9±7.2	70.2±4.0	49±2.6	42.7±3.4
AOX feed (mg/l)	12.8±0.8	12.8±0.8	12.8±0.8	13.47±0.9	13.47±0.9	13.47±0.9
AOX reduction (%)	46±0	30±0	28.8±0	48±6.7	35.6±7.9	33.6±4.8
Colour in feed (Pt-Co unit)	1371±75	1371±75	1371±75	1303±57	1303±57	1303±57
Colour reduction (%)	59.5±2.9	32.0±7.6	27.7±12.6	54.1±0.9	31.7±5.9	17.4±7.7
Alkalinity (Inlet) (mg/l)	333±20	333±20	333±20	304±0	304±0	304±0
Alkalinity (Outlet) (mg/l)	318±16	382±20	383±21	328±0	352±0	352±0
TSS (mg/l)	11.3±4.0	76±51	201±46	37.3±20.7	136.3±16	167±23

activated sludge process could be operated. Floc were initially in good form but started breaking over time due to high pH stress condition. More pin point and diffused flocs were seen in the reactor operated at pH 11.5 (Figure 7). Higher organisms like protozoa, rotifers and nematodes were in high abundance. Bio-oxidation in the two reactors operated at high pH conditions were reasonably high (COD reduction was more than 65%). AOX reduction was low (23.5-25%); this might be due to higher solubilization of AOX compounds in high pH conditions (Table 5b & 5c).

The reactors were brought back to normal operating condition of pH 7.0 after running for 20 days. Within 8 days of operation in this condition the reactors came to same level of performance (Table 6). Organisms are highly floc forming with a few free filamentous ones (Figure 8).

Operation of reactors with change of temperature

Before changing of temperature of two reactors all the three reactors were run for 15 days in almost identical environmental and operating conditions with fresh sludge from the buffer batch reactor like in the previous occasions. Well developed sludge with flocs having abundance of protozoa and rotifers, and marginal proliferation of filamentous organisms outside of the flocs resulted in high biodegradation of organic substances (Table 7).

Temperature of the second and third reactors were changed to 30 and 40 °C respectively and that of the first one was maintained at 36°C. In these changed conditions the three reactors were run for 19 days. The reactors R2 and R3 were adapted to the new conditions within 7 days. These resulted in good flocculating sludge with some filamentous organisms outside of flocs (Figure 9). Protozoa and rotifers were also in large abundance in all three reactors. The performance of the reactors R2 and R3 were comparable with that of the control reactor operated at pH 7 with respect to CODs, AOX and colour reduction in the initial phase (Table 8a). Marginally lower reduction of AOX was observed in the second phase which was primarily due to longer period of storage of feed solutions of C_D and EOP stages. In spite of marginally lower AOX reduction it was

Table 9: Effect of reversal of temperature on the ASP performance

Operating parameters/ Reactor performance	Phase I (for first 7 days)			Phase II (for next 12 days)			Phase III (for next 7 days)		
	R1	R2	R3	R1	R2	R3	R1	R2	R3
pH (inlet)	7.01±0.01	7.01±0.01	7.01±0.01	7.01±0.01	7.01±0.01	7.01±0.01	7.01±0.01	7.01±0.01	7.01±0.01
pH (outlet)	8.04±0.17	8.04±0.24	8.10±0.22	8.06±0.15	8.10±0.15	8.09±0.15	8.04±0.10	8.09±0.12	8.07±0.11
Temperature (°C)	36.3±0.2	36.5±0.1	37.2±0.2	36.3±0.1	36.6±0.1	37.0±0.1	36.1±0.2	36.4±0.3	36.7±0.2
DO (mg/l)	0.9±0.2	0.5±0.3	0.7±0.4	1.5±0.5	1.5±0.3	1.5±0.3	1.6±0.4	1.7±0.2	1.7±0.2
HRT (h)	8.5±0.1	8.1±0.4	8.6±0.3	8.8±0.2	8.7±0.5	8.6±0.1	8.5±0.1	8.5±0.2	8.4±0.3
MLSS (g/l)	4.09±0.38	4.11±0.32	3.84±0.48	3.96±0.14	4.04±0.08	4.13±0.08	3.95±0.15	3.96±0.14	4.11±0.16
MLVSS (g/l)	3.54±0.28	3.45±0.23	3.03±0.47	3.42±0.13	3.45±0.08	3.39±0.07	3.42±0.14	3.41±0.11	3.46±0.12
F/M ratio (d ⁻¹)	0.27±0.03	0.25±0.02	0.25±0.03	0.27±0.02	0.27±0.04	0.26±0.03	0.29±0.03	0.31±0.02	0.32±0.05
SVI (ml/g)	23±2	68±38	63±31	19±3	51±2	46±8	16±1	45±6	70±9
Organic loading (kg CODs/m ³ /d)	0.95±0.4	0.87±0.05	0.76±0.07	0.91±0.09	0.92±0.14	0.89±0.11	0.99±0.09	1.07±0.09	1.13±0.2
CODs feed (mg/l)	515±18	515±18	515±18	506±29	506±29	506±29	527±27	527±27	527±27
CODs reduction (%)	65.2±1.4	56.9±2.3	52.8±6.9	65.9±2.9	65.5±7.0	62.6±6.1	66.9±4.4	72.1±2.4	74.7±10.8
AOX feed (mg/l)	8.7±0.25	8.7±0.25	8.7±0.25	11.54±3.45	11.54±3.45	11.54±3.45	14.08±0.42	14.08±0.42	14.08±0.42
AOX reduction (%)	42.5±0	32.7±2.0	30.0±4.5	52.1±10.3	47.5±12.7	46.5±10.6	55.2±4.0	51.5±4.9	50.7±3.5
Colour in feed (Pt-Co unit)	1269±28	1269±28	1391±82	1350±30	1350±30	1350±30	1399±17	1399±17	1399±17
Colour reduction (%)	50.9±0.6	43.1±2.4	28.6±11.3	47.9±3.3	49.5±5.2	50.4±3.3	41.8±2.3	54.0±0.4	51.1±1.5
Alkalinity (Inlet) (mg/l)	326±18	326±18	326±18	348±0	348±0	345±0	333±0	333±0	323±0
Alkalinity (Outlet) (mg/l)	307±18	339±18	342±5	342±0	373±0	336±0	296±0	280±0	311±0
TSS (mg/l)	28±0	35±0	17±0	20±0	24±0	49±0	39±0	52±0	42±0

Figure 2: Abundance of filamentous organisms in secondary sludge

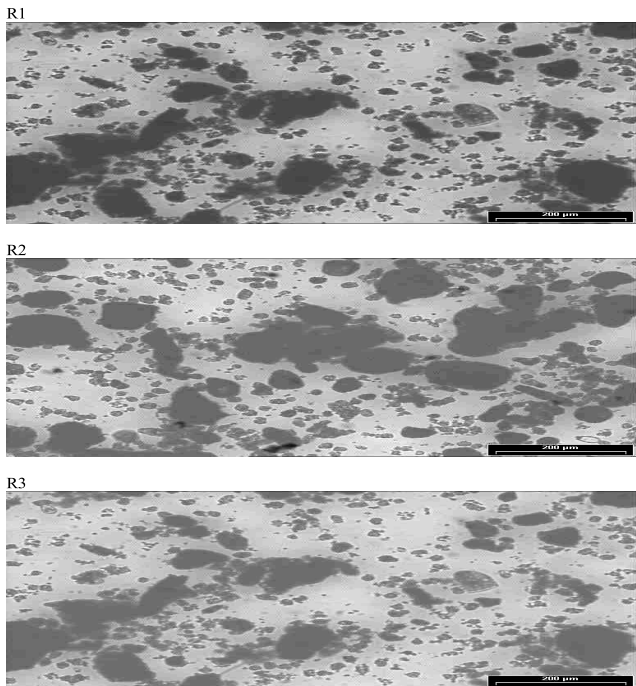
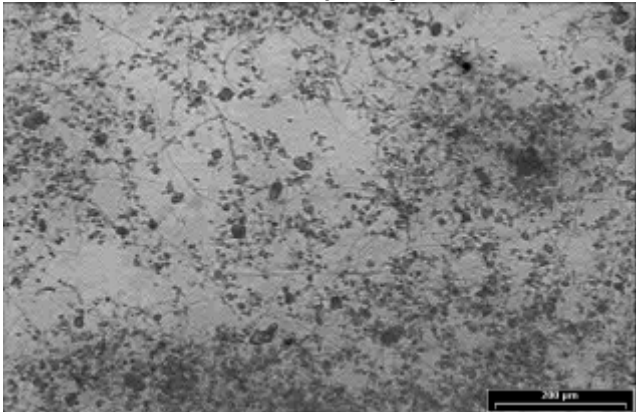


Figure 4: Morphological characteristics of control reactor (R1) (pH 7) and other two reactors; R2 (at pH 6) and R3 (at pH 5)

Figure 1: Laboratory scale biological reactors for activated sludge process



Figure 3: Gram stain of the secondary sludge

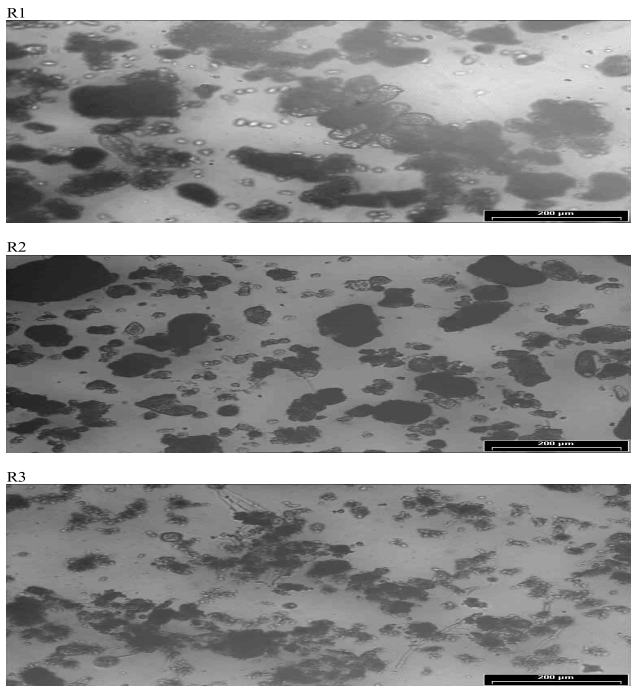
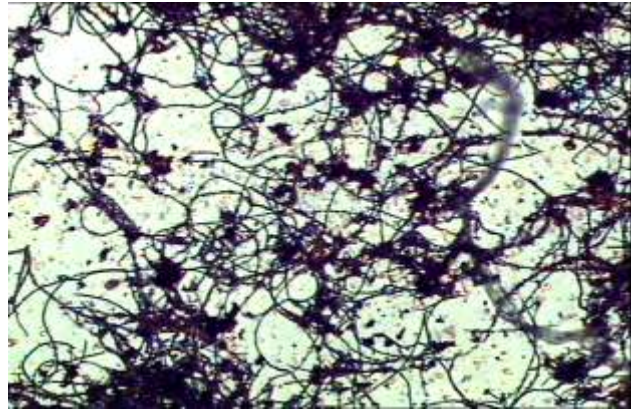


Figure 5: Morphological characteristics of control reactor (R1) (pH 7) and other two reactors; R2 (at pH 4.5) and R3 (at pH 4)

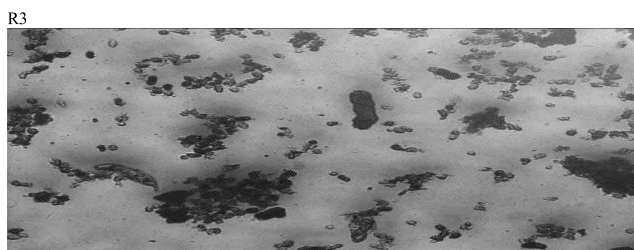
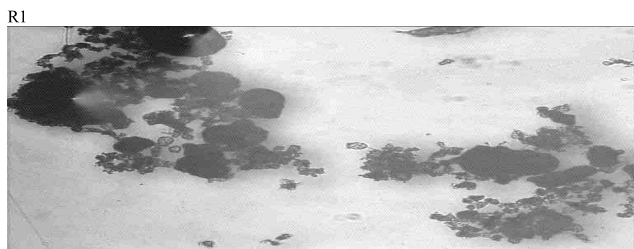


Figure 6: Morphological characteristics of control reactor (R1) (pH 7) and other two reactors; R2 (at pH 8.5) and R3 (at pH 9.5)

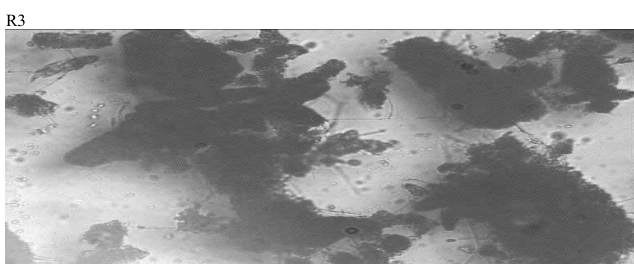


Figure 8: Morphological characteristics of two reactors; R2 (at pH 11.5) and R3 (at pH 10.5) after reversal to normal operating condition of pH of 7

experienced that ASP can be operated upto the temperature of 40°C.

Temperature of the second and third reactors were subsequently changed to 45 and 50 °C respectively and that of the first one was maintained at 36 °C. In these changed conditions the three reactors were run for 19 days. Performance of the reactors R2 and R3 started deteriorating gradually; DO came down to less than 0.5 mg/l; MLSS and MLVSS were reduced indicating lower biomass growth and wash-out thus resulting in higher TSS in the effluent. OLR came down in proportion to biomass degeneration. Performance of the reactors showed continual fall with respect to CODs, AOX and colour reduction (Table 8b). Microbial flocs disintegrated as

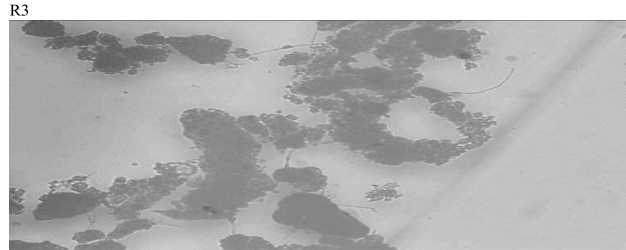
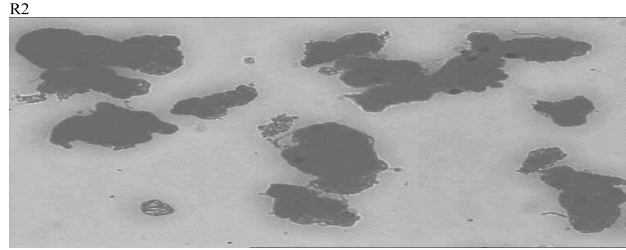
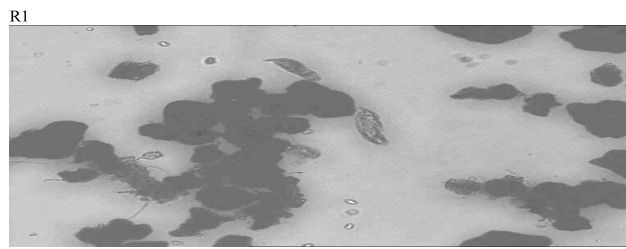


Figure 7: Morphological characteristics of control reactor (R1) (pH 7) and other two reactors; R2 (at pH 11.5) and R3 (at pH 10.5)

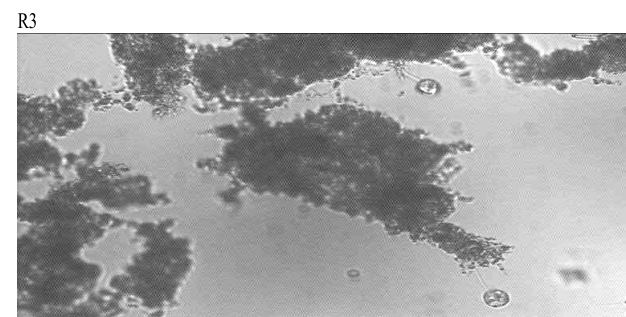
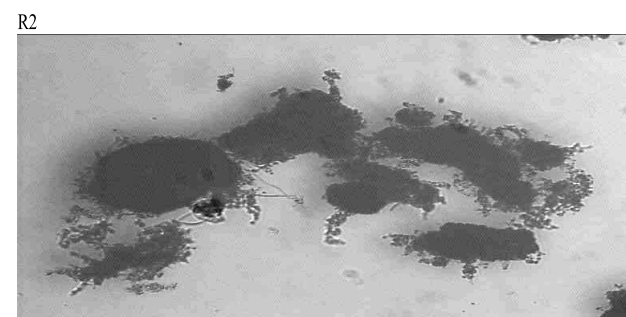
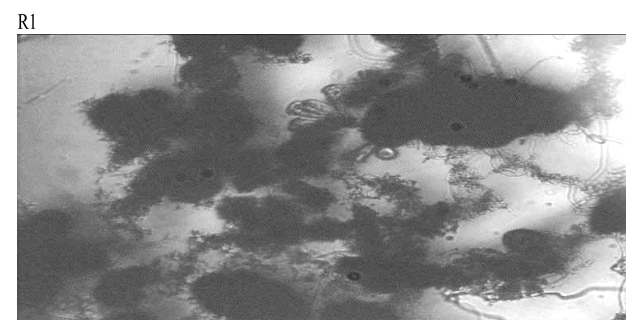


Figure 9: Morphological characteristics of control reactor (R1) (at temperature 37 °C) and other two reactors; R2 (at temperature 30 °C) and R3 (at temperature 40 °C)

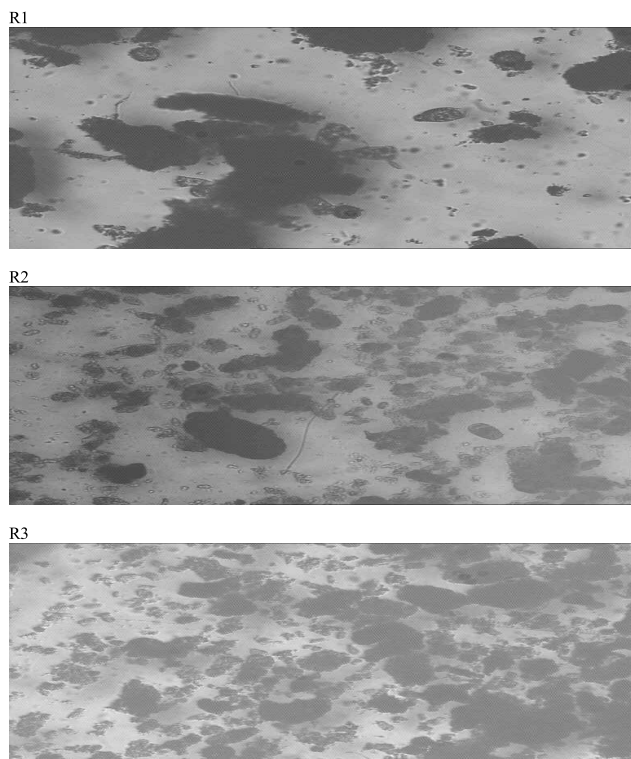


Figure 10: Morphological characteristics of control reactor (R1) (at temperature 37 °C) and other two reactors; R2 (at temperature 45 °C) and R3 (at temperature 50 °C)

the temperature was increased. Diffused or pin point flocs was more pronounced in reactor R3 (Figure 10). Similar findings have been reported by Morgan-Sagastume and Allen, 2005. Filamentous organisms in both the two cases were less proliferated. Low DO and higher temperature are not conducive to growth of filamentous organisms.

Reversal of temperature to 37°C of two reactors

The goal of the study is to see if it is, at all, possible to return to the original state of microbial structure once the temperature of the two reactors was brought down to 37 °C (i.e., mesophilic condition). The study was conducted in three phases; phase I for 7, second phase for 12 and last phase for 7 days. In the initial 7 days temperature shock was relieved, DO and hence MLSS and MLVSS started showing improvement. Organic loading automatically increased. Performance of the reactors with respect to biodegradation, colour and AOX reduction got stabilized after two weeks (Table 9). Morphological characteristics of the reactor (R2) was totally regained after three weeks (Figure 11) but that of the reactor the temperature of which was 50 °C required longer time to come to the original condition (not shown in the text). The study clearly demonstrates

the requirement of cooling of the wastewater below 40°C for obtaining floc forming bacteria and good reactor performance.

CONCLUSIONS

The study of microbial behaviour with change of pH and temperature reveals that the microorganisms once developed properly having less dominance of filamentous organisms can withstand both lower and higher pH of the incoming wastewater (from 5 to 9.5). 68-71% CODs, 37-43% AOX reductions are possible in the activated sludge process. Microorganisms get less stressed in alkaline condition as compared to acidic condition. Excessive foam is generated at pH lower than 5.0. The study indicates the importance of homogenization of influent through equalization basin. Microbial ecology is not disturbed upto the temperature of 40 °C. Sharp deterioration of reactor performance was observed once the temperature was raised above 45°C. Cooling of the wastewater is required once the temperature exceeds 40°C.

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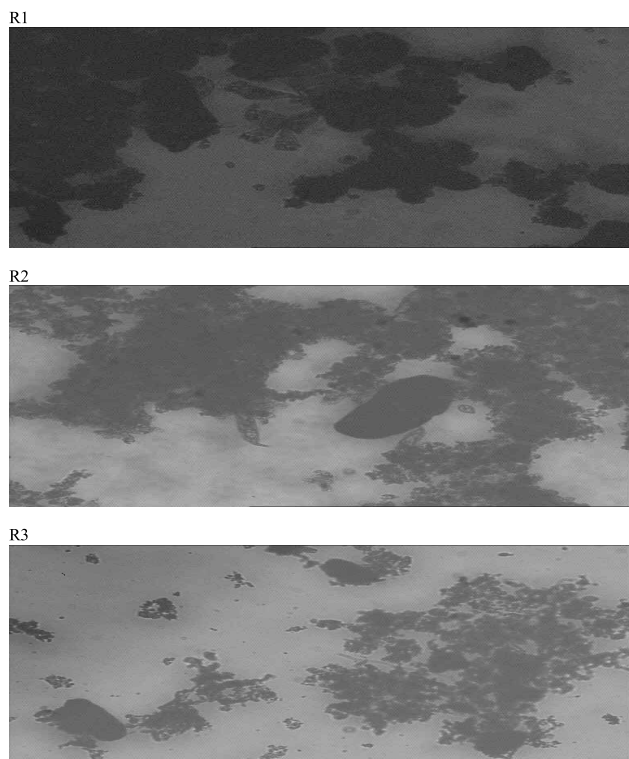


Figure 11: Morphological characteristics of control and other two reactors after revert back to temperature of 37 °C

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