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Optimization of Physiochemical Parameters For Floc Stability and Biodegradation In Paper and Pulp Mill Effluent Using Response Surface Methodology: A Statistical Approach

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Abstract

This paper discusses the environmental factors affecting the degradation efficiency of paper mill wastewater. 2^4 full factorial central composite designs were employed for experimental plan and analyzed using response surface methodology. The physicochemical quality of pulp in paper mill effluent has been analyzed for the degradation of pulp and paper mill effluent in 72 hours incubation period using mixed culture. The temperature and pH of the effluent sample was varied between 25 to 65 ºC and 3 to 11 respectively. The effect of individual factor in the effluent treatment process indicated that the mixed culture found to degrade COD by 90% and 99% of COD at 35 ºC temperature and pH value 7. Floc stability was sustained at 35 ºC temperature and pH 7. Study on combined effect of factors showed that the optimum values of pH, temperature and days were found to be 7, 25 °C and 3 respectively.

Keywords: pulp and paper mill effluent; biodegradation; Pseudomonas alkaligenes; Bacillus subtilis; Trichoderma reesei; response surface methodology.

Introduction

In nature as well as in wastewater treatment plant, organisms are the key players in keeping water clean. The pulp and paper industry is a water intensive industry and ranks only third in the world, after the primary metals and the chemical industries, in terms of freshwater withdrawal by Kallas and Munter (1994). About 60 m^3 of water is required even with most modern and efficient operational techniques to produce a ton of paper resulting in the generation of large volumes of wastewater. Paper and pulp mill effluent is the sixth largest polluter containing a variety of gaseous, liquid and solid wastes discharged into receiving end from Ali Moharikar and Sreekrishnan (2000). The survey of various treatment processes in water and wastewater treatment shows that temperature and pH are the important factors affecting efficiency of flocculation and settling properties. In the last decades, an increased number of studies have been devoted to build up environmentally clean and non-toxic methods for industrial process (Simone de Carvalho Peixoto-Nogueira et al. 2009).

Aditi Moharikar et al., (2005) in their study reported that the microbial-based treatment systems for the degradation of organic matter have gained importance since biological treatment has several significant advantages over chemical or physical technologies. Attention has to be given to control the types of bacteria that dominate the treatment systems. Such bacteria are always present in the environment and given the right conditions of food availability, temperature and other environmental factors they grow and multiply. Besides amount, the types and functionality of bacteria is also very important in an effluent treatment plant. Shail Singh et al. (2008) argued that the mixed culture of two bacterial strains Bacillus sp. and Serratia marcescens showed potential pentachlorophenol (PCP) degradation and decolorisation of pulp and paper mill effluent.Chandra et al., (2007) reported that three potential aerobic bacterial strains ITRC S_6, ITRC S_7, ITRC S_8 were biochemically characterized and optimized for Kraft lignin degradation from pulp paper waste. Complexing agents like EDTA and DTPA are used in wide range of industrial processes such as metal finishing, photographic processes, textile industry and paper pulp industry (Kari Pirkanniemi et al., 2007). Fenton's process proved highly effective in degradation of EDTA in spiked integrated wastewater. The comparison of decolorization by different organisms show that white rot fungi P.chrysosporium and C. versicolor were suitable for efficient degradation of the recalcitrant chromophoric material in bleach plant effluents (Pratima Bajpai and Pramod K Bajpai, 1994). The use of bicarbonate to adjust p^H could be advantageous in anaerobic treatment of organochloride containing residues in paper mill sludge from Ratnieks and Gaylarde (1997).

Aerobic biological treatment process involves the use of thermophilic microorganisms has many advantages, since it includes rapid biodegradation rates, low sludge yields and excellent process stability. The thermophilic degradation of pulp and paper mill effluent does occur at temperatures of up to 60° C. Pranavenreddy, (2005). Savant et al. (2006) study on operational parameters have found that the type of the reactor used, AOX loading rate, hydraulic retention time, wastewater characteristics, wastewater dilution ratio, presence of co- substrate, pH and temperature affect the efficiency of biodegradation. Maija-Liisa Suihko and Eija Skyttä (2009) carried out a study to detect viable aerobically growing non-spore-forming bacteria from paper mill pulps containing recycled fibres with special reference to proteobacteria, including hygiene indicators and food pathogens using molecular methods. Ghanizadeh and Sarrafpour (2001) proved that temperature and pH had been the key factors that affect the efficiency of flocculation and settling properties. Hassan et al., (2009**)** have used Bacillus sp (EU 978470) for degradation of parameters in paper and pulp mill effluent. To treat pulp and paper mill effluent, biological treatment is superior to the physicochemical methods, because it has low treatment costs and possibilities of causing a secondary pollution.

An important strategy for effluent treatment is the isolation and characterization of genetically significant microorganisms together with designing and optimization of process parameter to deal with specific environment pollutants. The aim of the present study is to identify the microorganism present in pulp and paper mill effluent. The identified two bacterial (Pseudomonas alkaligenes, Bacillus sublitis) and one fungal strain (Trichoderma reesei) was prepared and the attempt was made for degradation of pulp and paper mill effluent under various pH, temperature and days to optimize the same. Mixed culture of bacterial and fungal strains Pseudomonas alkaligenes, Bacillus subtilis, Trichoderma reesei isolated from pulp and paper mill effluent showed potential degradation of pulp and paper mill effluent. Clauss et al., (1998) have approved that the density reduces and porosity increases as the floc size increases. It is important to improve the performance of the systems and to increase the yield of the processes without increasing the cost. The method used for this purpose is called optimization. Conventional and classical methods of studying a process by maintaining other factors involved at an unspecified constant level does not depict the combined effect of all the factors involved. This method is also time consuming and requires a number of experiments to determine optimum levels, which are unreliable. In any paper and pulp effluent treatment process, the main parameters to be studied for floc stability and biodegradation are pH, temperature and time (days). The existing literature focuses on biosorption treatment of paper and pulp effluent and the influence of individual parameters on degradation. Hence, it was thought of worthwhile to investigate extensively the effect of physicochemical parameters on the relationship between removal efficiency, floc size and the parameters affecting it. The objective of this present study is to optimize the physio-chemical parameters of a paper mill effluent for its efficient degradation and floc formation. The interaction between the parameters was studied and optimized using response surface methodology.

Materials and Methods

Waste water collection

The effluent obtained from a South Indian based Integrated Pulp and Paper Industry from the inlet, outlet of primary settling tank was used for study. The sample was collected in a sterile plastic container and transported to the research laboratory situated in Coimbatore within 2 hours. The effluent was stored at 4°C until required.

Physico-chemical analysis of wastewater

The wood pulping and production of the paper products generate a considerable amount of pollutants characterized by biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended solids (SS), toxicity and color when untreated or poorly treated effluents are discharged to receiving waters (Achoka, 2002; Pokhrel and Viraraghavan, 2004). The effluents from the paper industry cause slime growth, thermal impacts, scum formation, color problems and loss of aesthetic beauty in the environment. They also increase the amount of toxic substances in the water, causing death to the zooplankton and fish, as well as profoundly affecting the terrestrial ecosystem. Effluents must be treated before discharging them into the environment. The main treatment process used at pulp and paper mills plants is primary sedimentation followed by secondary treatment, generally of a biological nature. The most common biological treatment system is the activated sludge process (Pokhrel and Viraraghavan, 2004; Thompson and Forster, 2003; Thompson et al., 2001), which can operate at the loading rates as high as 1.2 kg BODm^{-3} day^{-1}. In the activated sludge process, high removals of BOD, COD, adsorbable organic halogen (AOX), and chlorinated phenolics have been achieved (Saunamäki, 1997).

The physico-chemical parameters of fresh wastewater (Table 1) were analyzed for Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Sulphate, Chlorides and pH according to the standard method for the Examination of Water and Wastewater (APHA, AWWA, WPCF, 20th ed., 1998). Chemical Oxygen Demand (COD) was analyzed by closed reflux method. Shake flask method (Pranaven Reddy et al., 2005) was used to evaluate the treatment efficiency of individual isolates and combination of isolates.

Table 1: Physiochemical parameters of waste water (paper and pulp mill effluent)

Parameter	Raw effluent
pH	7.5
Total dissolved solids (mg/l)	900
Total suspended solids (mg/l)	590
Biochemical oxygen demand (mg/l)	286
Chemical oxygen demand (mg/l)	1164
Hardness (mg/l)	56
Chlorides (mg/l)	40

Microbial analysis

Culture preparation

The fresh wastewater sample from paper and pulp mill was serially diluted using sterile pipettes from 10^{-1} to 10^{-8} dilution. For bacterial enumeration nutrient agar medium containing peptone (5 g/l), yeast extract (1.5 g/l), sodium chloride (5 g/l), agar (15 g/l) and for fungi enumeration Potato dextrose agar containing potato (200 gm), dextrose (20 gm), agar (1gm), distilled water (1000 ml) at pH 5.6 was used. The cultures were repeatedly streaked on nutrient agar medium and incubated at 37°C for 24hrs.

Isolation and identification of predominant bacteria and fungi

The incubated plates were observed for the predominant types of microorganisms. The isolated organisms were identified by colony morphology, microscopic observation and confirmation test. Colony morphology on nutrient agar plates were observed and identified based on their size, pigmentation, form (shape of the colony), margin and elevation. In microscopic observation gram staining techniques was performed to distinguish between gram positive and gram negative organisms. Bacteria are among the most diverse organisms with respect to the types of enzymes they contain. Biochemical tests like *Indole production, Methyl red test, Voges proskauer test, Citrate utilization test, Triple sugar iron test, Carbohydrate test, Catalase test, Nitrate reduction test* were performed for bacterial identification. Purified isolates from the nutrient agar media are then inoculated into a fresh, sterile nutrient broth and were incubated overnight. The isolated bacterial culture was maintained in nutrient agar slants and maintained at 4 °C for further use. The isolated fungal culture was identified as Trichoderma reesei using *Lactophenol cotton blue staining method*.

Identified bacteria and fungi

Among the identified microorganism two bacterial strain and one fungal strain was found to be more efficient during preliminary study and hence further investigations continued with the three microorganisms only. The identified organisms Pseudomonas alkaligenes, Bacillus sublitis and Trichoderma reesei were utilized to find the degradation efficiency of paper and pulp mill effluent under various temperature and pH by Pratima and Pramod, (1994). Pseudomonas is efficient slime-formers in paper mills (Desjardins and Beaulieu, 2003; Martin, 1998 and Rättö et al., 2005).

Effect of Temperature and Ph on Degradation Rate

The temperature is very important in biological wastewater treatment systems because it has effects on the microbial growth. While most microorganisms are able to exist over a broad temperature range, there is usually an optimum temperature at which each species grows best. In treatment plant, a slow adaptation occurs due to seasonal changes in temperature. However sudden change in temperature affects the microbial activity which might affect processes like flocculation due to changed surface properties of the microbial cells. pH is one of the environmental factors with the greatest relevance to the growth of microorganisms. Microbes prevail in certain ranges of pH that favor the nutrition, reproduction and survival. pH is responsible for higher degree mortality, especially when the change is sudden (Carvalho, 1978). In general fungi prefer more acidic conditions, whereas bacteria grow in a neutral to alkaline environment (Madigen et al. 2000). Degradation studies were performed in paper and pulp mill effluent in batch shake flask in orbital shaker at 100 rpm. The study was performed in two phases. The impact of temperature variation in degradation rate was analyzed in first phase at temperatures from 25°C - 55°C with intervals of 10°C. In second phase the effect of pH was assayed using 1N NAOH in pH range 3 – 9. At different time intervals aliquots were withdrawn, centrifuged at 1500 rpm and degradation efficiency were evaluated at every 24 hours interval up to 72 hours. The main principles of activated sludge is microbial growth in flocculated forms, therefore, the efficiency of the system depends on physical and biological characteristics of flocs (Clauss et al., 1998)

Standardization of temperature and pH

Temperature not only affects the metabolic activities of the microbial population but also influences the gas-transfer rates and the settling characteristics of activated sludge. In general, the rates of biochemical reactions and of substrate transfer processes increase with higher temperature. However, the solubility of oxygen decreases in the mixed liquor as temperature increases, resulting in poor biodegradation conditions for aerobic microbes. Thus, an increase in temperature generates two reciprocal effects on biochemical reactions. In first phase the treatment was carried out at different temperatures (25ºC, 35ºC, 45ºC and 55ºC) and the optimum temperature was determined from the obtained value. Table 2 gives the comparative analysis of treatment process at various temperatures and the results represent the superiority of effluent remediation at the temperature of 35ºC parameter re*du*ction. In second phase investigation the effect of pH on treatment was determined by studying the remediation process at various pH ranges 3-9. The percentage reduction of parameters was analyzed and calculated for three consecutive days. Table 3 proves that pH variation have greater impact on treatment process. Maintaining pH at 7 maximizes the percentage reduction thereby treatment efficiency.

Table 2: Effect of temperature on treatment process

Parameters Studied	**Percentage reduction**											
	Temp 25 °C			**Temp 35** °C			**Temp 45** °C			**Temp 55** °C		
	1st day	**2nd day**	**3rd day**	**1st day**	**2nd day**	**3rd day**	**1st day**	**2nd day**	**3rd day**	**1st day**	**2nd day**	**3rd day**
TDS	0	0	0	0	22	22	0	11	0	0	0	0
TSS	32	43	43	36	62	60	35	34	28	18	17	14
BOD	51	48	48	79	98	85	55	62	60	41	40	38
COD	49	49	47	72	90	74	42	41	41	33	32	31
Hardness	21	25	25	32	63	59	12	0	0	12	8	6
Chlorides	0	0	0	12	40	34	12	0	0	0	0	0

Table 3: Effect of pH on treatment process

Parameters studied	**Percentage reduction**											
	pH 3			**pH 5**			**pH 7**			**pH 9**		
	1st day	**2nd day**	**3rd day**	**1st day**	**2nd day**	**3rd day**	**1st day**	**2nd day**	**3rd day**	**1st day**	**2nd day**	**3rd day**
TDS	0	0	0	0	7	8	0	22	0	0	0	0
TSS	25	23	23	36	39	25	35	65	61	18	28	25
BOD	51	48	48	59	72	69	68	99	85	41	57	61
COD	49	49	47	46	56	53	72	90	90	33	38	37
Hardness	21	25	25	12	17	18	39	62	53	12	15	14
Chlorides	0	0	0	0	12	0	12	43	34	0	0	0

Activated sludge Floc

Microorganisms in the sludge suspension of biological wastewater treatment reactors are in the form of aggregated flocs that are known to be highly fractal (Jiang and Logan, 1991; Li and Ganczarczyk, 1989; Logan and Kilps, 1995). The fractal nature of activated sludge flocs suggests that the flocs may be filled by gaps of all sizes (Li and Ganczarczyk, 1989). The overall floc structure is negatively charged and is the result of physico-chemical interactions between microorganisms (mainly bacteria), inorganic particles (silicates, calcium phosphate and iron oxides), EPS and multivalent cations (Neyens and Baeyens, 2003). The activated sludge must be settled and separated from the treated water before it is discharged into any receiving water body. The effectiveness of settling activated sludge from the treated mixed liquor is dependent on the ability of the activated sludge to form floc. When the activated sludge flocculates poorly, the level of suspended solids in the effluent increases, and it is often associated with weak floc structures. Deflocculation and poor biodegradation can be the result of transient operating conditions and environmental stresses such as shift in temperature, toxic compounds, metal, dissolved oxygen concentration, pH, ionic strength, shift in substrate loading and nutrient characteristics or concentration.

Effect of pH on floc stability

The role of pH in floc stability was studied by varying pH value from 5 to 9 and the floc size was measured by microscope for every 24 hour till 10th day of incubation. The results presented in Table 4 highlights that the floc stability at pH 7 was maximum up to ten days of incubation. At pH 5 and 6 and 8 the flocs were smaller in size and showed delayed formation. No floc formed at the alkali pH. During the course of the study when the pH was raised from 7 to 9, the floc disintegrates from its consistency signifying the floc instability at alkali pH. The studies on activated sludge systems show that most of the biological systems and bacteria are activate in pH 4-9 (Sedlac, 1991)

Table 4: Influence of pH on floc stability

pH	Floc size (mm)									
	1st day	**2nd day**	**3rd day**	**4th day**	**5th day**	**6th day**	**7th day**	**8th day**	**9th day**	**10th day**
5	0*	0*	1.2	1.2	1.2	1.1	1.1	1.1	1.1	1.1
6	0*	1.3	1.3	1.3	1.3	1.5	1.5	1.4	1.4	1.4
7	0*	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
8	0*	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
9	0*	0*	0*	0*	0*	0*	0*	0*	0*	0*

0* No floc formation

Effect of temperature on floc stability

The importance of temperature maintenance for floc steadiness was studied by considering the treatment process at different temperatures. The degradation process was carried out 25ºC, 35ºC, 45ºC and 55ºC and the optimum temperature for floc permanence was established. The data projected in Table 5 represents the influence of temperature on floc stability. Dense floc was formed at the temperature of 35ºC whereas no floc was formed at 55ºC temperature and loose flocs formed at 25ºC temperature. Degradation of pulp and paper mill effluent occurs at temperatures of up to 60ºC; once final degradation is obtained, it decreases significantly as temperature increases (Pranaven Reddy et al. 2005).

Table 5: Influence of temperature on floc stability

Temperature	Floc size (mm)									
	1st day	**2nd day**	**3rd day**	**4th day**	**5th day**	**6th day**	**7th day**	**8th day**	**9th day**	**10th day**
25ºC	0*	0*	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
35ºC	0*	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
45ºC	0*	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
55ºC	0*	0*	0*	0*	0*	0*	0*	0*	0*	0*

0* No compact floc formation

Statistical Analysis

Factorial experimental design

The physical parameters viz. temperature, pH and days were optimized using central composite design (Box and Wilson, 1951; Box and Hunter, 1951). These parameters were chosen as independent variables and the removal efficiency (percentage reduction) of TDS, TSS, BOD, COD, Hardness and Chloride in paper and pulp mill effluent as output response. According the Central Composite Design (CCD), the total number of treatment combinations is $2^k + 2k + n_0$ where k is the number of independent variables and n_0 the number of repetitions of the experiments at the center point. For statistical calculations, the variables X_i have been coded as x_i according to the following transformation equation:

$$x_i = \frac{(X_i - X_0)}{\delta X} \qquad \text{(Eqn.1)}$$

Where x_i is dimensionless coded value of the variable X_i, X_0 the value of the X_i at the center point and δX the step change. A 2^4 factorial design with six axial points and six replicates at the center point and thus a total of 20 experiments were employed for optimizing parameters in this study. The center point replicates were chosen to verify any change in the estimation procedure, as a measure of precision property. The analysis was focused on how the degradation (removal efficiency) is influenced by independent variables, i.e., pH (X_1), temperature (X_2) and days (X_3) The dependent output variable is maximum removal efficiency (percentage reduction).

The behavior of the system was explained by the following quadratic equation

$$Y = \beta_0 + \Sigma\beta_i x_i + \Sigma\beta_{ii} x_i^2 + \Sigma\beta_{ij} x_i x_j \qquad \text{(Eqn.2)}$$

where Y is the predicted response, β_0 is the intercept term, β_i is the linear effect, β_{ii} is the squared effect and β_{ij} is the interaction effect. The regression equation (2) was optimized for maximum value to obtain the optimum conditions and the results of the experimental design were studied and interpreted by statistical software, MINITAB 14 (PA, USA) to estimate the response of the dependent variable.

Results and Discussion

Experimental analysis

Sludge deflocculation increases and the flocculation physicochemical properties deteriorate under temperature shift from 30 °C – 45 °C (Morgan-Sagastume, Allen, 2005). Decreased microbial activity caused by a temperature reduction leads to increased deflocculation of activated sludge (Wilén et al., 2000). The experimental study reveal that the up-shifts in temperature from 25 °C – 45 °C had major effects on removal efficiency in effluent chemical oxygen demand, suspended solid concentration and chlorides. It has also been found that microbial activity is sustained by temperature rise which leads increased flocculation of activated sludge in effluent. It is understood that there was no degradation of TDS, chlorides at 25 ºC and 55 ºC temperature (Table 2) at pH 3, 9 (Table 3). Maximum floc formation was noticed at

35 ºC temperature (Table 5) and pH of 7 (Table 4) on the second day and was stable throughout the treatment process, however no floc formation neither at 55 ºC (Table 5) nor at pH 9 (Table 4). The study reveals that the degradation rate occurs in the down trend for BOD, COD, TSS, chlorides and TDS for temperature at 35 ºC (Table 2) and pH 5 (Table 3). This study infer that the mixed culture was found very potential towards COD reduction up to 90 % at temperature of 35 ºC and pH value of 7 (Figure 1). Further floc stability was maintained on the second day of incubation at temperature of 35 ºC and pH 7. No floc formed at the alkali pH.

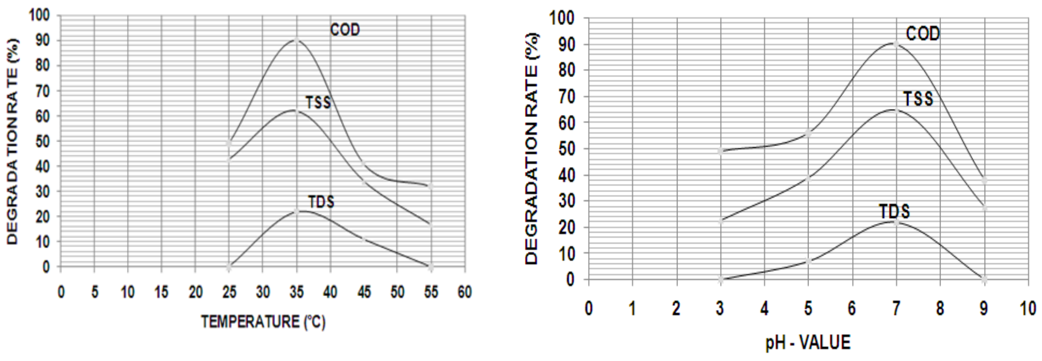

Figure 1: Temperature and pH influence in treatment process

Statistical Analysis

RSM consists of a group of mathematical and statistical techniques that can be used to characterize the relationships between the response and the independent variables. RSM defines the effect of the independent variables, alone or in combination, in the processes. In addition to analyzing the effects of the independent variables, this experimental methodology could also generate a mathematical model. In this research paper statistical analysis was used to examine the interaction between the physical parameters viz, pH, temperature and days in relation to the degradation of TDS, TSS, BOD, COD, Hardness and Chloride in paper and pulp mill effluent using RSM. A Central Composite Design was used for studying the interaction of these variables within a range of -1.68 to +1.68 in relation to the microbial degradation. Experiments were performed with various combinations of independent variables as given in Table 6.

Table 6: coded and uncoded values of physical parameters used for central composite design

Variables	Coded values				
	-1.68	-1	0	+1	+1.68
pH (X_1)	3	5	7	9	11
Temperature (X_2)	25	35	45	55	65
Days (X_3)	1	2	3	4	5

Experimental plan showing the coded value of the variables together with parameter (TDS, TSS, BOD, COD, Hardness and Chloride) degradation are given in Table 7 (*Annexure I*).

The following presumptions were arrived from the statistical analysis:

- The degradation rate i.e. removal efficiency was highest when pH, temperature and days were at 0, -1.68 and 0 level respectively (Run order # 18).
- The degradation rate of physiochemical parameters and floc formation was poor when the pH and day were at the centre point (0 level) and the temperature is at 1.68 level (Run order # 5). This shows that the microorganisms can survive at a temperature higher but the enzymes responsible for bringing out degradation and floc were not optimally active at those temperatures.
- Floc stability and the rate of degradation (removal efficiency) were not much different in cases of where pH, temperature and days were at 0 levels indicating that the effect of one of the variable was compensated by the other (Run order # 2, 3, 11, 14, 15, 17).

The results obtained from the CCD experiments were fitted to a second order polynomial equation to explain the dependence of microbial degradation on the medium components. Following second order regression equations shows the dependence of pH, temperature and days on TDS, TSS, BOD, COD, Hardness and Chloride degradation. Multiple regression analysis of the experimental data generated the parametric equation for the determination of respective output responses (Y):

$$Y_{TDS} = (22.5403) + (-0.8824X_1) + (-0.3607X_2) + (-0.8838X_3) + (0.0284X_1^2) + (0.0008X_2^2) + (0.0819X_3^2) + (0.0005X_1X_2) + (-0.0046X_1X_3) + (0.0092X_2X_3) \quad \text{(Eqn.3a)}$$

$$Y_{TSS} = (77.0736) + (-1.2221X_1) + (-0.5774X_2) + (-2.1963X_3) + (-0.0144X_1^2) + (-0.0012X_2^2) + (0.06551X_3^2) + (-0.0001X_1X_2) + (0.3747X_1X_3) + (-0.0264X_2X_3) \quad \text{(Eqn.3b)}$$

$$Y_{BOD} = 100.241 + (0.813X_1) + (-0.758X_2) + (-1.851X_3) + (-0.085X_1^2) + (-0.001X_2^2) + (0.395X_3^2) + (0.003X_1X_2) + (-0.021X_1X_3) + (0.022X_2X_3) \quad \text{(Eqn.3c)}$$

$$Y_{COD} = (107.688) + (-2.297X_1) + (-0.806X_2) + (-2.860X_3) + (0.014X_1^2) + (0.001X_2^2) + (0.129X_3^2) + (0.002X_1X_2) + (0.212X_1X_3) + (0.006X_2X_3) \quad \text{(Eqn.3d)}$$

$$Y_{Hard} = (63.6463) + (-0.9170X_1) + (-0.7620X_2) + (0.7185X_3) + (0.0161X_1^2) + (-0.0010X_2^2) + (-0.1694X_3^2) + (0.0067X_1X_2) + (-0.0469X_1X_3) + (0.0104X_2X_3) \quad \text{(Eqn.3e)}$$

$$Y_{Chl} = (28.4993) + (0.7405X_1) + (-0.3245X_2) + (0.8261X_3) + (-0.0009X_1^2) + (-0.0013X_2^2) + (-0.2198X_3^2) + (-0.0125X_1X_2) + (-0.1725X_1X_3) + (0.0230X_2X_3) \quad \text{(Eqn.3f)}$$

$$Y_{Floc} = (0.27048) + (0.2043X_1) + (0.0082X_2) + (0.4013X_3) + (-0.0116X_1^2) + (-0.00034X_2^2) + (-0.0484X_3^2) + (-0.002013X_1X_2) + (-0.0143X_1X_3) + (0.003525X_2X_3) \quad \text{(Eqn.3g)}$$

Statistical testing of the model was performed by the fisher's statistical test. The ANOVA result (*Table 8 in Annexure II*) shows that the F- value (the ratio of mean square due to regression to the mean square due to error) was highest for regression followed by the linear and square for TDS, BOD and Hardness, but was insignificant for the interaction. Whereas the F- value was highest for the regression followed by the linear and interaction for TSS and COD and was insignificant for the square. Further the analysis shows that the F- value was highest for the regression followed by square and was insignificant for the linear and interaction in respect of floc stability. According to the ANOVA (Table 8), the $F_{statistics}$ values for all the regression were higher. This large value of F indicates that most of the variation in the response can be explained by the regression model equation. In general, larger the magnitude of T and smaller the value of P, the more significant is the corresponding coefficient term (Montgomery, 1991). The regression coefficient, T and P values for all the linear, quadratic and interaction effects of the parameter are given in Tables 8. It was observed that the coefficients for the linear effect of temperature and pH ($P = 0.000$ for TDS, COD, Hardness and $P = 0.099$ for BOD and $P = 0.006$ for TDS respectively) was highly significant and coefficient for the linear effect of time was least significant expect for COD ($P = 0.059$). The coefficient of the quadratic effect of pH, temperature and time was highly insignificant. The coefficients of the interactive effects among the variables did not appear to be very significant in comparison to the linear effects. However, the interaction effect between pH and temperature, pH and days (P 0.9) for TDS, BOD; temperature and days (P 0.7) for BOD, COD and Chlorides were found to be significant. The significance of these interaction effects between the variables would have been lost if the experiments were carried out by conventional methods. The associated P-value is used to judge whether $F_{statistics}$ is large enough to indicate statistical significance, P-value lower than 0.05 indicates that the model is considered to be statistically significant by Bas D and Boyaci IH (2007). The P-values for all of the regression lower than 0.01 means that at least one of the terms in the regression equation has a significant correlation with the response variables. The ANOVA Table 8 (*Annexure II*) also shows a term for residual error, which measures the amount of variations in the response data left unexplained by the model. The form of the model chosen to explain the relationship between the factors and the response is correct. The analysis of variance of the quadratic regression model also suggests that the model was very significant as indicated by a low probability value $[(P_{model}>F) =0]$. Also the values of R^2 indicated that the model predicted values are in perfect agreement with the experimental values as shown in Table 7 (*Annexure I*). Regression equations also predict that the interaction of variables pH and days affect the microbial degradation to some extent. Coefficients of interaction terms were significant as compared to linear terms indicating that the interaction between the variables cannot be neglected.

The mathematical model was formulated using MINITAB 14 software to generate the optimal values for the above three variables. The optimum values of pH, temperature and days were found to be 7, 25 °C and 3 respectively. However the experimental investigation reveals that the optimal values of pH, temperature and days were as 7, 35 °C and 2 respectively for the individual treatment. The model predicted value of the rate of degradation of physiochemical parameters from the experimental study were found to be 5.37 % TDS, 45.37 % TSS, 73.87 % BOD, 63.98 % COD, 32.59 % Hardness, 17.59 % Chlorides and Floc size of 1.16 mm which is nearly ¾ of degradation under optimized conditions. In all cases the optimum rate of degradation and floc size predicted by the plots matched with the experimentally predicted value. To verify the optimal conditions obtained using CCD, experiments were performed under the optimal conditions and compared with centre points.

Concluding Remarks

Temperature, pH and time are the most important factors that affect the flocculation efficiency and settling processes in various treatment method of water and wastewater treatment. Control measures are necessary to counter the colonization of microorganisms in paper mills, especially when white-water systems are being closed to reduce water consumption. By monitoring different environmental parameters such as temperature, pH, COD, BOD, TSS, TDS, an indication of the microbial activity can be maintained. The mixed culture of these bacterial and fungal strains was found more potential over pure strains and showed maximum degradation efficiency of the parameters in short duration. Experiments were carried out covering a wide range of operating conditions. The influence of pH, temperature and treatment period was critically examined. It was observed from this investigation that the removal efficiency is significantly influenced by pH, temperature and treatment period (day). A 2^4 full factorial central composite design was successfully employed for experimental design and analysis of results. The experimental data were analyzed using response surface methodology to get hold of the individual and combined parameter effects on degradation rate (removal efficiency) and floc stability. Regression equations were developed using experimental data and solved using the statistical software MINITAB 14. It was observed that model predictions are in good agreement with experimental observations. Under optimal values of process parameters around 83.11% BOD, 70.35% COD and 52.81% TSS degradation was achieved. RSM was successfully used to optimize the physical parameters and study the interaction effect among the same for treating the paper and pulp mill effluent. Optimization results show that higher degradation of physiochemical parameters could be achieved with some modification in physical parameters viz pH, temperature and day as well as the degradation could be enhanced.

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Annexure - I

Table 7: Experimental Plan (Central Composite Design) for Optimizing Physiochemical parameters by mixed culture

Run Order	Ph (X_1)	Temp (X_2) °C	Days X_3	Removal efficiency (degradation) in percentage													
				TDS		TSS		BOD		COD		Hardness		Chlorides		Floc	
				RE expt	RE pred	RE expt	RE pred	RE expt	RE pred	RE expt	RE pred	RE expt	RE pred	RE expt	RE pred	RE expt	RE pred
1	1.68	0	0	1.01	0.80	36.78	36.28	65	65.49	50.33	50.45	22.86	22.50	11.26	10.69	0.6	0.65
2	0	0	0	2.66	2.71	37.21	37.49	66.97	66.71	54.33	55.93	25.97	24.63	11.74	12.10	1.4	1.01
3	0	0	0	2.66	2.71	37.79	37.49	65.38	66.71	55.57	55.93	24.75	24.63	10.98	12.10	1.4	1.01
4	1	1	-1	-0.74	-0.80	29.26	29.59	57.34	56.96	45.77	46.32	15.97	15.95	6.49	7.43	0.3	0.39
5	0	1.68	0	-2.33	-2.44	22	22.17	51	51.17	41.83	41.51	8.75	9.09	3.11	2.84	0.45	0.48
6	-1	-1	-1	6.43	6.26	47.84	46.13	74	73.72	66.90	66.22	33.53	33.62	17.47	18.10	1.23	1.28
7	1	-1	1	4.26	4.33	44.94	44.17	72.99	75.24	60.73	60.06	30.57	31.18	14.36	15.36	1.23	1.26
8	1	1	1	-0.63	-0.82	28.36	28.85	58.37	59.7	45.98	45.64	15.94	15.64	5.56	6.10	0.65	0.73
9	-1	1	-1	1.12	1.10	32.32	30.80	58.26	58.18	51.77	51.80	17.95	18.08	8.75	8.84	0.67	0.76
10	0	0	-1.68	2.78	2.73	37.76	38.23	64.43	63.97	56.99	56.62	24.33	24.95	12.72	13.43	0.63	0.67
11	0	0	0	2.67	2.71	37.74	37.49	66.74	66.71	55.76	55.93	24.94	24.63	10.86	12.10	0.98	1.01
12	1	-1	-1	4.61	4.35	44	44.91	72.56	72.5	60.37	60.74	31.73	31.49	17.98	16.69	0.92	0.92
13	-1	1	1	1.17	1.08	29.21	30.06	59.75	60.92	50.67	51.12	17.58	17.76	7.43	7.51	1.23	1.10
14	0	0	0	2.41	2.7	37.76	37.49	67.34	66.71	56.33	55.93	24.47	24.63	11.95	12.10	0.97	1.01
15	0	0	0	2.73	2.71	37	37.49	67.34	66.71	56.28	55.93	23.95	24.63	11.75	12.10	0.975	1.01
16	-1	-1	1	6.2	6.24	45	45.38	74.31	76.46	65.19	65.54	33.46	33.30	17	16.77	1.55	1.62
17	0	0	0	1.98	2.71	37.9	37.49	68.36	66.71	55.74	55.93	24.63	24.63	17.29	12.10	0.964	1.01
18	**0**	**-1.68**	**0**	**8.00**	**7.87**	**52**	**52.81**	**83.11**	**82.25**	**70**	**70.35**	**39.98**	**40.17**	**20.87**	**21.36**	**1.518**	**1.54**
19	-1.68	0	0	4.93	4.62	37.73	38.70	67.35	67.93	61.47	61.41	27.21	26.76	13.76	13.51	1.273	1.37
20	0	0	1.68	2.90	2.69	37.73	36.74	73.79	69.45	55.37	55.25	23.87	24.31	10.57	10.77	1.223	1.35

Annexure – II

Table 8: ANOVA results for physiochemical parameter degradation and floc size : effect of pH, temperature and day

Response Surface Regression: TDS versus pH, TEMP, DAYS

Estimated Regression Coefficients for TDS

Term	**Coef**	**SE Coef**	**T**	**P**
Constant	22.5403	2.13400	10.562	0.000
pH	-0.8824	0.25243	-3.495	0.006
TEMP	-0.3607	0.05169	-6.979	0.000
DAYS	-0.8838	0.49990	-1.768	0.108
pH*pH	0.0284	0.01057	2.683	0.023
TEMP*TEMP	0.0008	0.00042	1.891	0.088
DAYS*DAYS	0.0819	0.04229	1.935	0.082
pH*TEMP	0.0005	0.00375	0.128	0.900
pH*DAYS	-0.0046	0.03749	-0.122	0.906
TEMP*DAYS	0.0092	0.00750	1.225	0.249

S = 0.2121 R-Sq = 99.6% R-Sq(adj) = 99.3%

Analysis of Variance for TDS

Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**
Regression	9	121.466	121.46630	13.496255	300.11	0.000
Linear	3	120.930	2.24898	0.749660	16.67	0.000
Square	3	0.467	0.46733	0.155778	3.46	0.059
Interaction	3	0.069	0.06894	0.022978	0.51	0.684
Residual Error	10	0.450	0.44971	0.044971		
Lack-of-Fit	5	0.041	0.04130	0.008261	0.10	0.987
Pure Error	5	0.408	0.40841	0.081682		
Total	19	121.916				

Response Surface Regression: TSS versus pH, TEMP, DAYS

Estimated Regression Coefficients for TSS

Term	**Coef**	**SE Coef**	**T**	**P**
Constant	77.0376	7.76655	9.919	0.000
pH	-1.2221	0.91869	-1.330	0.213
TEMP	-0.5774	0.18811	-3.070	0.012
DAYS	-2.1963	1.81934	-1.207	0.255

pH*pH	-0.0144	0.03848	-0.373	0.717
TEMP*TEMP	-0.0012	0.00154	-0.787	0.449
DAYS*DAYS	0.0651	0.15392	0.423	0.681
pH*TEMP	-0.0001	0.01364	-0.007	0.995
pH*DAYS	0.3747	0.13644	2.746	0.021
TEMP*DAYS	-0.0264	0.02729	-0.969	0.355

S = 0.7718 R-Sq = 99.4% R-Sq(adj) = 98.8%

Analysis of Variance for TSS

Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**
Regression	9	953.770	953.7703	105.9745	177.91	0.000
Linear	3	948.069	5.6153	1.8718	3.14	0.074
Square	3	0.649	0.6491	0.2164	0.36	0.781
Interaction	3	5.052	5.0517	1.6839	2.83	0.093
Residual Error	10	5.957	5.9566	0.5957		
Lack-of-Fit	5	5.280	5.2799	1.0560	7.80	0.021
Pure Error	5	0.677	0.6767	0.1353		
Total	19	959.727				

Response Surface Regression: BOD versus pH, TEMP, DAYS

Estimated Regression Coefficients for BOD

Term	**Coef**	**SE Coef**	**T**	**P**
Constant	100.241	17.2151	5.823	0.000
pH	0.813	2.0363	0.399	0.698
TEMP	-0.758	0.4170	-1.817	0.099
DAYS	-1.851	4.0327	-0.459	0.656
pH*pH	-0.085	0.0853	-0.992	0.345
TEMP*TEMP	-0.001	0.0034	-0.347	0.736
DAYS*DAYS	0.395	0.3412	1.159	0.273
pH*TEMP	0.003	0.0302	0.095	0.926
pH*DAYS	-0.021	0.3024	-0.070	0.945
TEMP*DAYS	0.022	0.0605	0.368	0.721

S = 1.711 R-Sq = 97.2% R-Sq(adj) = 94.7%

Analysis of Variance for BOD

Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**
Regression	9	1012.27	1012.2700	112.4744	38.43	0.000
Linear	3	1002.74	14.7096	4.9032	1.68	0.235
Square	3	9.10	9.0964	3.0321	1.04	0.418

Interaction	3	0.44	0.4370	0.1457	0.05	0.984
Residual Error	10	29.27	29.2661	2.9266		
Lack-of-Fit	5	24.50	24.4952	4.8990	5.13	0.048
Pure Error	5	4.77	4.7709	0.9542		
Total	19	1041.54				

Response Surface Regression: COD versus pH, TEMP, DAYS

Estimated Regression Coefficients for COD

Term	**Coef**	**SE Coef**	**T**	**P**
Constant	107.688	5.72298	18.817	0.000
pH	-2.297	0.67696	-3.392	0.007
TEMP	-0.806	0.13861	-5.817	0.000
DAYS	-2.860	1.34063	-2.134	0.059
pH*pH	0.014	0.02835	0.511	0.620
TEMP*TEMP	0.001	0.00113	0.541	0.600
DAYS*DAYS	0.129	0.11342	1.138	0.282
pH*TEMP	0.002	0.01005	0.185	0.857
pH*DAYS	0.212	0.10054	2.109	0.061
TEMP*DAYS	0.006	0.02011	0.285	0.781

S = 0.5687 R-Sq = 99.7% R-Sq(adj) = 99.4%

Analysis of Variance for COD

Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**
Regression	9	956.104	956.1039	106.2338	328.45	0.000
Linear	3	954.162	11.2665	3.7555	11.61	0.001
Square	3	0.465	0.4652	0.1551	0.48	0.704
Interaction	3	1.476	1.4764	0.4921	1.52	0.269
Residual Error	10	3.234	3.2344	0.3234		
Lack-of-Fit	5	0.598	0.5982	0.1196	0.23	0.935
Pure Error	5	2.636	2.6361	0.5272		
Total	19	959.338				

Response Surface Regression: HARDNESS versus pH, TEMP, DAYS

Estimated Regression Coefficients for HARDNESS

Term	**Coef**	**SE Coef**	**T**	**P**
Constant	63.6463	5.10302	12.472	0.000
pH	-0.9170	0.60363	-1.519	0.160
TEMP	-0.7620	0.12360	-6.165	0.000

DAYS	0.7185	1.19540	0.601	0.561
pH*pH	0.0161	0.02528	0.636	0.539
TEMP*TEMP	-0.0010	0.00101	-1.020	0.332
DAYS*DAYS	-0.1694	0.10113	-1.675	0.125
pH*TEMP	0.0067	0.00896	0.746	0.473
pH*DAYS	-0.0469	0.08964	-0.523	0.612
TEMP*DAYS	0.0104	0.01793	0.579	0.576

S = 0.5071 R-Sq = 99.7% R-Sq(adj) = 99.5%

Analysis of Variance for HARDNESS

Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**
Regression	9	985.784	985.7841	109.53157	425.93	0.000
Linear	3	984.301	12.4504	4.15013	16.14	0.000
Square	3	1.183	1.1834	0.39448	1.53	0.266
Interaction	3	0.300	0.2995	0.09985	0.39	0.764
Residual Error	10	2.572	2.5716	0.25716		
Lack-of-Fit	5	0.322	0.3216	0.06433	0.14 0.974	
Pure Error	5	2.250	2.2500	0.44999		
Total	19	988.356				

Response Surface Regression: CHLORIDES versus pH, TEMP, DAYS

Estimated Regression Coefficients for CHLORIDES

Term	**Coef**	**SE Coef**	**T**	**P**
Constant	28.4993	18.0488	1.579	0.145
pH	0.7405	2.1350	0.347	0.736
TEMP	-0.3245	0.4371	-0.742	0.475
DAYS	0.8261	4.2280	0.195	0.849
pH*pH	-0.0009	0.0894	-0.010	0.992
TEMP*TEMP	-0.0013	0.0036	-0.373	0.717
DAYS*DAYS	-0.2198	0.3577	-0.614	0.553
pH*TEMP	-0.0125	0.0317	-0.394	0.702
pH*DAYS	-0.1725	0.3171	-0.544	0.598
TEMP*DAYS	0.0230	0.0634	0.363	0.724

S = 1.794 R-Sq = 91.8% R-Sq(adj) = 84.5%

Analysis of Variance for CHLORIDES

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	9	361.556	361.556	40.1729	12.49	0.000
Linear	3	358.175	4.034	1.3447	0.42	0.744

Square	3	1.505	1.505	0.5018	0.16	0.923
Interaction	3	1.875	1.875	0.6251	0.19	0.898
Residual Error	10	32.169	32.169	3.2169		
Lack-of-Fit	5	2.814	2.814	0.5627	0.10	0.989
Pure Error	5	29.356	29.356	5.8712		
Total	19	393.726				

Response Surface Regression: FLOC SIZE versus TEMP, pH, DAYS

Estimated Regression Coefficients for FLOC SIZE

Term	**Coef**	**SE Coef**	**T**	**P**
Constant	0.270480	1.62370	0.167	0.871
TEMP	0.008183	0.03933	0.208	0.839
pH	0.204330	0.19207	1.064	0.312
DAYS	0.401261	0.38036	1.055	0.316
TEMP*TEMP	-0.000345	0.00032	-1.073	0.309
pH*pH	-0.011599	0.00804	-1.442	0.180
DAYS*DAYS	-0.048398	0.03218	-1.504	0.163
TEMP*pH	-0.002013	0.00285	-0.706	0.497
TEMP*DAYS	0.003525	0.00570	0.618	0.550
pH*DAYS	-0.014250	0.02852	-0.500	0.628

S = 0.1614 R-Sq = 89.6% R-Sq(adj) = 80.2%

Analysis of Variance for FLOC SIZE

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	9	2.24165	2.24165	0.249072	9.57	0.001
Linear	3	2.11054	0.05209	0.017363	0.67	0.591
Square	3	0.10171	0.10171	0.033903	1.30	0.327
Interaction	3	0.02940	0.02940	0.009800	0.38	0.772
Residual Error	10	0.26035	0.26035	0.026035		
Lack-of-Fit	5	0.01758	0.01758	0.003516	0.07	0.994
Pure Error	5	0.24277	0.24277	0.048555		
Total	19	2.50200				