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Kinetic modelling and process optimization of textile effluent treatment with bacterial gamma polyglutamic acid

R. Mohanraj[1], B.M. Gnanamangai[1]*, K. Ramesh[2] and P. Ponmurugan[3]

[1]Department of Biotechnology, K. S. Rangasamy College of Technology, Tiruchengode-637 215, India
[2]Tamilnadu Water Investment Company Limited, Tiruppur-641 602, India
[3]Department of Botany, Bharathiar University, Coimbatore-641 046, India

***Corresponding Author Email :** mythumithras@gmail.com ***ORCiD:** https://orcid.org/0000-0001-9681-2318

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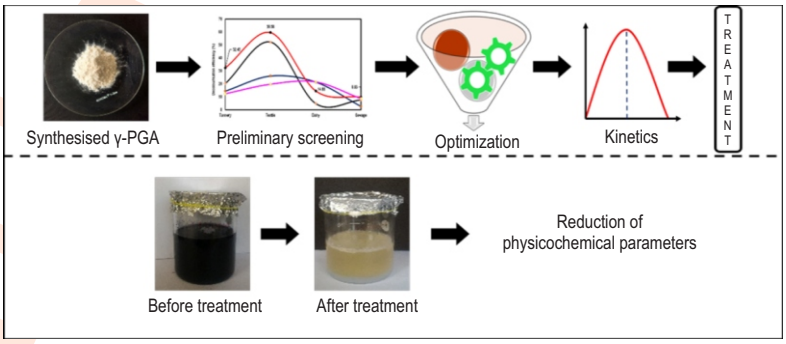
Abstract

Aim: The present study aims to synthesize gamma poly glutamic acid from the optimized starch-based glutamate-dependent medium for wastewater treatment with kinetic studies for an effective decolorization process.

Methodology: Microbial coagulant (γ-PGA) was synthesized from the optimized glutamate-dependent medium with sago as a substrate and *Bacillus subtilis* as fermenter. Synthesized γ-PGA was harvested by ethanol precipitation and lyophilization method. The presence of γ-PGA was determined by UV-visible spectrum and ninhydrin analysis and its flocculating activity was screened by ninhydrin assay. Harvested γ-PGA was treated with different types of wastewater samples (Textile, tannery, dairy and sewage) in the preliminary screening. Process optimization was performed to screen the maximal decolorization for textile effluent (light and dark color). The physical and chemical characterization of treated wastewater samples with kinetic studies was performed to improve the treatment process.



Results: The synthesized γ-PGA weighed as 41.20 g l^{-1} from the fermented medium and its presence was confirmed by characterization techniques. The flocculating activity of synthesized γ-PGA was 40.56 and 58.78 % against the kaolin suspension. The maximal decolorization percentage was 59.58% for γ- PGA treatment against textile effluent. The significance of optimized parameters such as pH, temperature, rotation speed and contact time were determined in the treatment of textile effluents (light and dark color) with maximal reduction of solids profile COD and BOD concentration. Kinetic studies revealed that the isotherm Langmuir isotherm was fit for the experimental data with R^2 as 0.9943, 0.9692 and Pseudo second order fitted with R^2 as 0.9871 and 0.9594 respective to light and dark color effluent.

Interpretation: This study confirmed the maximal yield of γ-PGA from a sago-based glutamate-dependent medium and revealed its effective flocculating and decolorization activity against textile effluent which suggest them as a better alternative coagulants.

Key words: γ- PGA, *Bacillus subtilis*, Kinetic modelling, Process optimization

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Introduction

Wastewater or effluent is a negatively impacted water from household, domestic, agriculture runoff and industrial effluents which cause the pollution into the water resources and that could not be used for human usage (Deeptha *et al*., 2015); (Crini and Lichtfouse, 2019). Wastewater is polluted by different types of pollutants like physical, chemical and biological (Ahmed *et al*., 2021). Physical and chemical pollutants originate from the runoff and different types of industries. Biological pollutants enter the water resource from domestic usage, manure runoff, food and natural products-based industries. Worldwide 80% of wastewater is directly discharged into waterways which has raised concern about the environmental and health issues (Al-Ansari *et al.,* 2019). The maximum water pollution in a highly populated country like India can lead to poor hygiene, health issues and water-borne diseases. Treatment is an essential process to recover maximal water sources from pollution as mentioned in the forthcoming section. Wastewater treatment is usually performed by chemical and biological methods. In the chemical method, different steps are followed for effective treatment such as chemical precipitation, neutralization, adsorption and disinfection.

In biological methods, microbes and plants have a major role in the treatment. Microbes are majorly involved in the aeration tanks and plants are involved in the phytoremediation process (Samer, 2015). The existing operational technology to reduce water pollution includes Effluent Treatment Plant (ETP), Sewage Treatment Plant (STP) and Common and Combined Effluent Treatment Plant (CETP) (Singh Asiwal *et al.,* 2016). Recently, Zero Liquid Discharge (ZLD) system is an effective practice to treat wastewater and effluents with a minimal concentration of solid waste. Worldwide, large-scale industries attempt to recover the maximal level of water from the effluents by this method due to heavy water scarcity. The installation cost and energy consumption of ZLD system is too high (Maiti *et al.,* 2021). In treatment plants, coagulation and flocculation play a vital role in the removal of suspended solids, color and turbidity by charge neutralization mechanism with coagulants and flocculants (Bahrodin *et al.,* 2021). Chemical coagulants are commercially used in treatment plants but it leads health issues to the humans like Alzheimer's disease. Natural coagulants and flocculants are being replaced for chemical agents due to its extensive characters and source of origin (plants, animals, microbes and biowastes) (Ahmad *et al.,* 2021; Ojewumi *et al.,* 2021; Thirugnanasambandham and Karri, 2021).

Anju *et al.* (2017) and Kumar *et al.* (2018) reported that polyglutamic acid (PGA) is a water-soluble and biodegradable anionic homopolyamide made up of L and D glutamic acid units with peptide bonds from peptide transformation, chemical synthesis, microbial fermentation and biotransformation. PGA is made up of non-essential aminoacids, especially glutamic acid (Dahiya *et al.,* 2021). PGA is considered as a structural compound of few bacteria (*Bacillus megaterium* and *Bacillus anthracis*) and maximum of reports clearly describe that *Bacillus* species is an effective strain for PGA production. PGA has various industrial and medicinal applications such as heavy metals adsorption, surgical adhesive, cryoprotectant, flocculant, food, water treatment, plastic and agriculture (Mohanraj *et al.,* 2019 and Dahiya *et al.,* 2021). Biopolymers are reported as an effective compound to remove heavy metals, dyes, organic and inorganic pollutants from effluents with maximal pollutant reduction and it can also fit into part of water treatment process (Udayakumar *et al.,* 2021). The present study aims to investigate the role of process parameters and decolorization efficacy in effluent treatment with microbial biopolymer (γ-PGA) which has been harvested from the optimized sago-based glutamate-dependent medium with *Bacillus subtilis* 2756. Commercially availed practiced techniques require high initial investment and complex process methods so this present study focus on a simple technique to remove the maximal of pollutants and color from the effluent at primary stage. In the previous publications, maximum of decolorization studies were done with crude dyes but in this study real effluent was used. Especially, process parameters optimization was performed for light and dark color textile effluents and their physicochemical parameters were investigated in the optimized condition. The relationship between adsorbed and adsorbent was screened by kinetic studies.

Materials and Methods

Synthesis and purification of γ-PGA: Based on previous study, six hour seed culture was prepared using *Bacillus subtilis* 2756 as a subjective strain. Based on the previous studies, seed culture was inoculated into a sterile optimized glutamate-dependent medium in 1:10 ratio. The inoculated media were incubated at 37°C for 96 hrs in a shaker with 80 rpm. Synthesized γ-PGA was purified by ethanol precipitation method, where the cells were separated by centrifugation at 20,000 rpm for 30 min at 4°C. The supernatant containing γ-PGA was poured into four volumes of ethanol and incubated at 4°C for 12 hrs, followed by centrifugation at 12,000 rpm for 30 min at 4°C. Obtained pellets were lyophilized and used for further testing Mohanraj *et al.* (2019).

Characterization of γ-PGA: UV-Visible spectrometry method was used to confirm the presence of γ-PGA in the fermented medium by measuring the optical density at 216 nm at room temperature (Zeng *et al*., 2012). The qualitative ninhydrin test was performed to detect the presence of amino acids in the purified sample reported by Tiwari (2015).

Flocculating activity of γ-PGA: The flocculating activity of γ-PGA was screened with kaolin suspension as per the protocol developed by Bhunia *et al.* (2012) and Taniguchi *et al.* (2005). Synthesized γ-PGA powder (0.1 g) was added with 10 ml of kaolin suspension and vigorously mixed and kept for sedimentation. The optical density of upper phase of the sample was read on a UV-Vis spec at 550 and 660 nm, likewise for the control sample that isdevoid of γ-PGA. The flocculating activity was calculated in percentage.

Collection and characterization of wastewater: Four different types of wastewater samples were screened for this study.

Wastewater samples were collected in clean borosilicate bottles. Tannery effluent was collected from the Leather Processing Industry, Agraharam, Erode, Tamil Nadu. Textile effluents were collected from the Textile industry, Kumarapalayam, Namakkal, Tamil Nadu. Dairy processed effluent was collected from the Dairy Industry, Kollampalayam, Namakkal, Tamil Nadu and Sewage water was collected from Varapallayam, Namakkal, Tamil Nadu. The collected samples were subjected to physico-chemical parameters by standard protocols. The color of the wastewater samples was identified by the visual monitoring method. Odor of the collected samples was observed by direct smelling method with required precautions. The pH of the collected wastewater samples was measured by a standardized pH meter. The solids profile and oxygen demand ranges were estimated following the standard protocols of APHA (2017). Total Solids (TS) (2540-B),Total Dissolved Solids (TDS) (2540-C), Total Suspended Solids (TSS) (2540-D), Chemical Oxygen Demand (COD) (5220-C)and Biological Oxygen Demand (BOD) was estimated by sensor method with respirometric and electronic pressure sensors (BOD system BD 600, Lovibond).

Screening of optimal wavelength by UV-visible spectral analysis: The optimal wavelength of collected wastewater samples was studied by monitoring the changes in its absorption spectrum (200-600 nm) (UBP 2900, Hitachi) spectrometer with their respective control. The peak value was considered as the optimal wavelength as demonstrated by Ayed *et al.* (2011) for analyzing the decolorization percentage of wastewater samples.

Screening of γ-PGA efficiency: γ-PGA was added to collected wastewater samples in different ratios (0.05:10, 0.1:10 and 0.15:10) in individual vessels and gently mixed to screen the flocculating activity of γ-PGA. The mixture was incubated at room temperature and its absorbance was read at 540 nm by a UV-Visible spectrometer to estimate the decolorization percentage.

Optimization of process parameter for textile effluent: From the preliminary screening optimal ratio of γ-PGA and volume of wastewater was determined as 0.1:10 with efficient flocculant activity on textile effluent compared to other wastewater samples. Textile effluent was selected for further studies. Two different color effluents (light and dark) were extensively studied for the optimization process. The optical density of light and dark color effluents were detected by continuous wavelength from 200 -600 nm on a UV-Visible spectrometer. The influence of pH was studied at 5, 6, 7, 8 and 9; Temperatures studied include 30, 35, 40 and 45°C; Rotation speed was followed at 50, 100, 150 and 200 resolution per minute (rpm); The complete adsorption efficiency was studied under the influence of contact time at 12, 24 and 36 hrs as per the methodology derived by Rajamohan *et al.* (2014).

Treatment: Optimized parameters and dosage were maintained for the collected textile effluents (light and dark) with γ-PGA. After the incubation period, the optical density was read at 540 and 416nm to find the decolorization efficiency respective to light and dark effluents.

Physico-chemical characteristics of treated textile effluents: γ-PGA was treated with one liter of textile effluents (0.1:10 ratio) at optimized conditions. Treated effluents were poured into one liter of measuring cylinder and maintained under stirring condition at 4 rpm for 30 min. After the incubation period, the settled sludge volume in ml l^{-1} was measured by direct observation and suspended solids (2710-D; APHA, 2017).The color of the wastewater samples was determined by the DurchsichtsFarbZahl (DFZ) method (Europa Norm, 1994).

Adsorption isotherm and kinetics: The adsorption isotherms were studied from the optimized parameter such as pH, temperature, shaking speed and contact time with γ- PGA as an adsorbent. The absorbance value was measured at optimal wavelength for light effluent as 540 nm and dark effluent as 416 nm. The most widely accepted adsorption models for single-solute systems are Langmuir and Freundlich models that follow equilibrium studies. Kinetic studies on adsorption are used to find the isotherm relationship between the mass of the solute adsorbed per unit, the mass of the adsorbent and the equilibrium concentration of solute. A pseudo-second-order model was used to determine the rate constant and percentage of decolorization. The linearity of plot showed the applicability of pseudo-second order in the kinetic model with regression coefficient values.

Pseudo second order rate equation is

$$t/q_t=(1/k^2q_1^2+1/q_1)t$$

where, q_1 - maximum adsorption; q_t - amount of color adsorbed at time, t; k^2 –second order rate

Graph was plotted between t/q_t vs t. Straight lines of the models represent the best fitness of experimental data and R^2 square value also noted as well.

Results and Discussion

Synthesized γ- PGA was harvested from a fermented medium by ethanol precipitation method. The γ-PGA 41.20 g yield of was obtained from one liter of fermented optimized sago-based glutamate-dependent medium due to the presence of required nitrogen and carbon sources. The lyophilized γ-PGA was stored at 4°C for further process. So far previous studies have reported varying yields by different strains that include, *Bacillus subtilis* (natto) strain MR 141 (Ogawa *et al*., 1997), *Bacillus amyloliquefaciens* (Anju *et al.,* 2017), *Bacillus subtilis* ZJU-7 (Moraes *et al*., 2013), *Bacillus subtilis* D7 (Lee *et al*., 2014), *Bacillus subtilis* and *Bacillus licheniformis* (Gomaa, 2016).

The γ-PGA yield varied based on the microbial species, production medium and process parameters.In our previous study, γ-PGA was synthesized from the glutamate-dependent and independent medium with sago and soybean as substrate and *Bacillus subtilis* 2756 as an effective strain. The maximum γ-PGA yield was as 39.8 g l^{-1} obtained from a sago-based glutamate-dependent medium (Mohanraj *et al.*, 2019). The carbon and

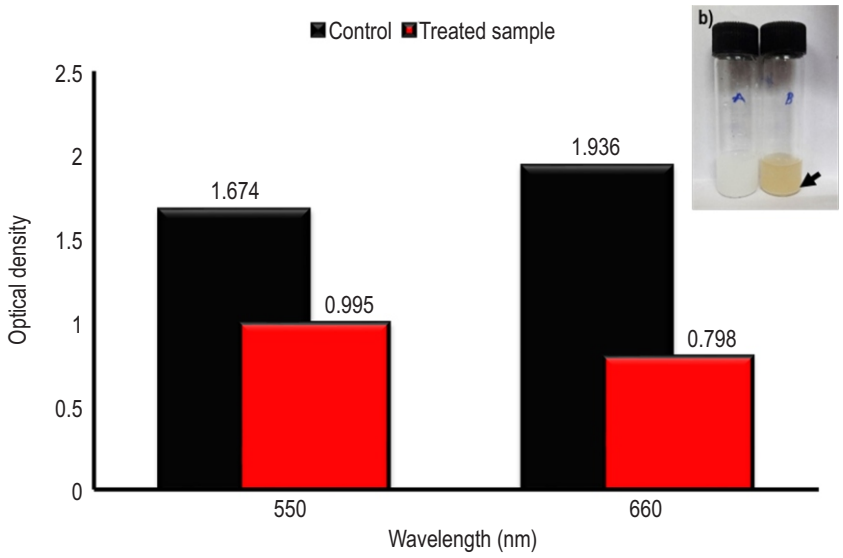

Fig. 1: Flocculation activity of synthesized γ-PGA; b) Settled flocs from flocculation process.

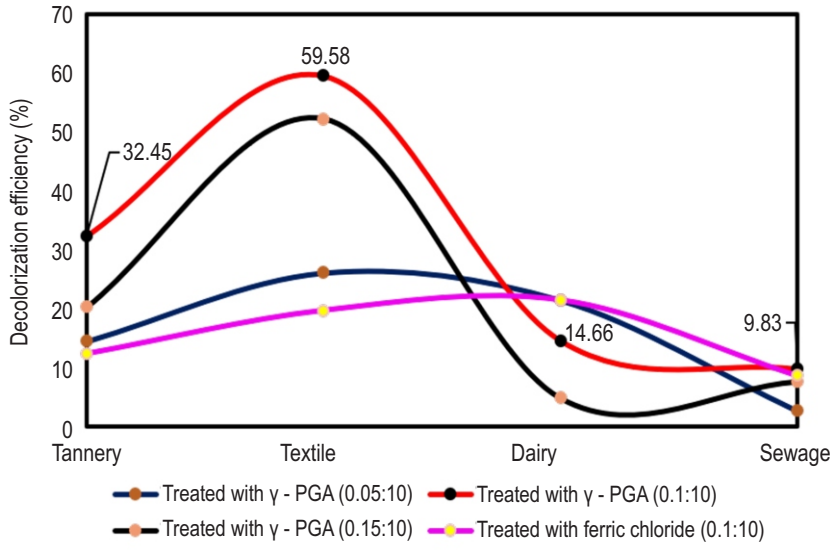

Fig. 2: Screened decolorization efficiency of γ-PGA with different ratios for preliminary screening.

nitrogen sources play a vital role in the microbial production of γ-PGA. The maximal yield of γ-PGA was observed in the optimized sago-based glutamate-dependent medium when compared with the unoptimized sago-based glutamate medium which may be confirmed by the presence of required limit of carbon and nitrogen sources in the medium. The presence of γ-PGA was determined by the UV-Visible spectrophotometer at 216 nm.

The optical density of fermented medium was found to be 0.891from spectrum analysis which indicated the presence of a high concentration of γ-PGA.The maximal absorption peaks of γ-PGA were reported as 216 nm from scanning at different continuous wavelengths of 190-340 and 190-800 nm, respectively (Zeng *et al.,* 2012; Chatterjee *et al.,* 2019). The peptide chains that are not attributed for the polymers were confirmed by the absence of peaks at 260-280 nm (Wang *et al.,* 2017).The presence of alpha amino acids namely glutamic acid was confirmed through deep blue color formation which was due to the interaction of ninhydrin molecules that act as an oxidizing agent and react with alpha amino acids to form a deep blue color

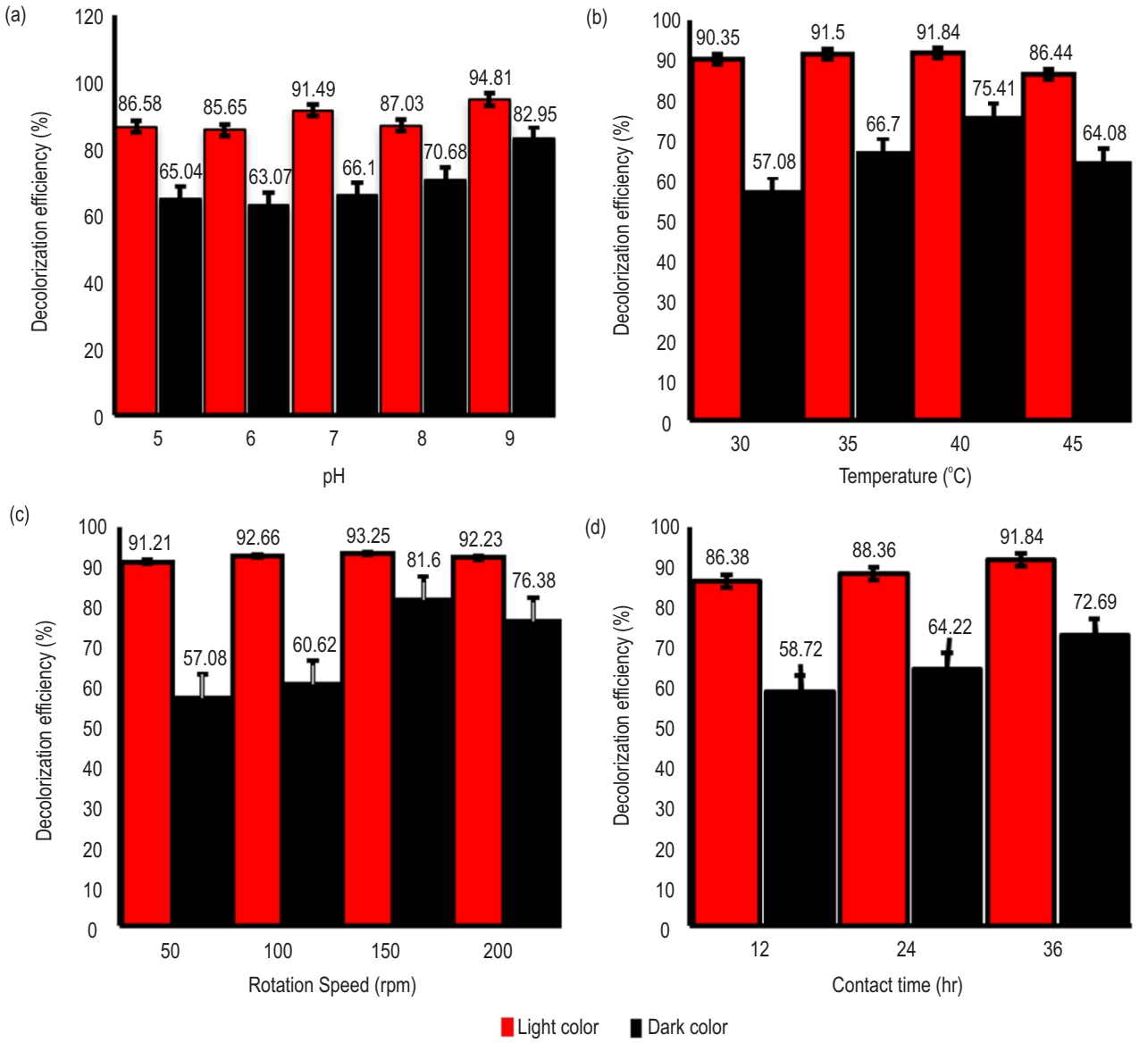

Fig. 3: Screened decolorization efficiency for process parameters for optimization process (a) pH; (b) Temperature; (c) Rotation speed and (d) Contact time.

(Tiwari, 2015). The flocculating activity of γ-PGA was screened from the UV-Vis spec analysis at 550 and 660 nm.

The settled flocs were visually observed within 10 min from the γ-PGA and kaolin suspension (Fig. 1b). Flocculating activity of γ-PGA was 40.56% and 58.78% at 550 and 660 nm respectively (Fig. 1a). The biodegradable and non-toxic properties of γ-PGA were impacted as an environmental friendly flocculant in the wastewater treatment plant Luo *et al.* (2016). The flocculant percentage of palm oil mill effluent biomass based crude bioflocculant (2.9 g l^{-1}) was reported as 80 % at 550 nm spectrum analysis by Nurul-Adela *et al*. (2016). Similarly, the flocculant activity of γ-PGA (10 mg l^{-1}) was reported as 14.53±1.2 1/OD against the kaolin suspension at 550 nm due to the effect of cations and pH in the flocculation process. Cations like calcium, aluminum, ferrous ions, magnesium ions and potassium ions were majorly involved in the ion's neutralization process with flocs formation and the previous report also briefs that maximal flocculant activity (21.4±1.2 1/OD) was found for calcium ions at 10 mM ratio. In the flocculation process, pH has a vital role and pH 7 has been reported as an efficient point for the highest flocculant activity (Bajaj and Singhal, 2011; Bhunia *et al.*, 2012). Taniguchi *et al.* (2005) reported flocculant activity of γ-PGA and crosslinked polyaluminium calcium-γ-PGA as 2.74 and 5.48 % with 1 µg ml^{-1}

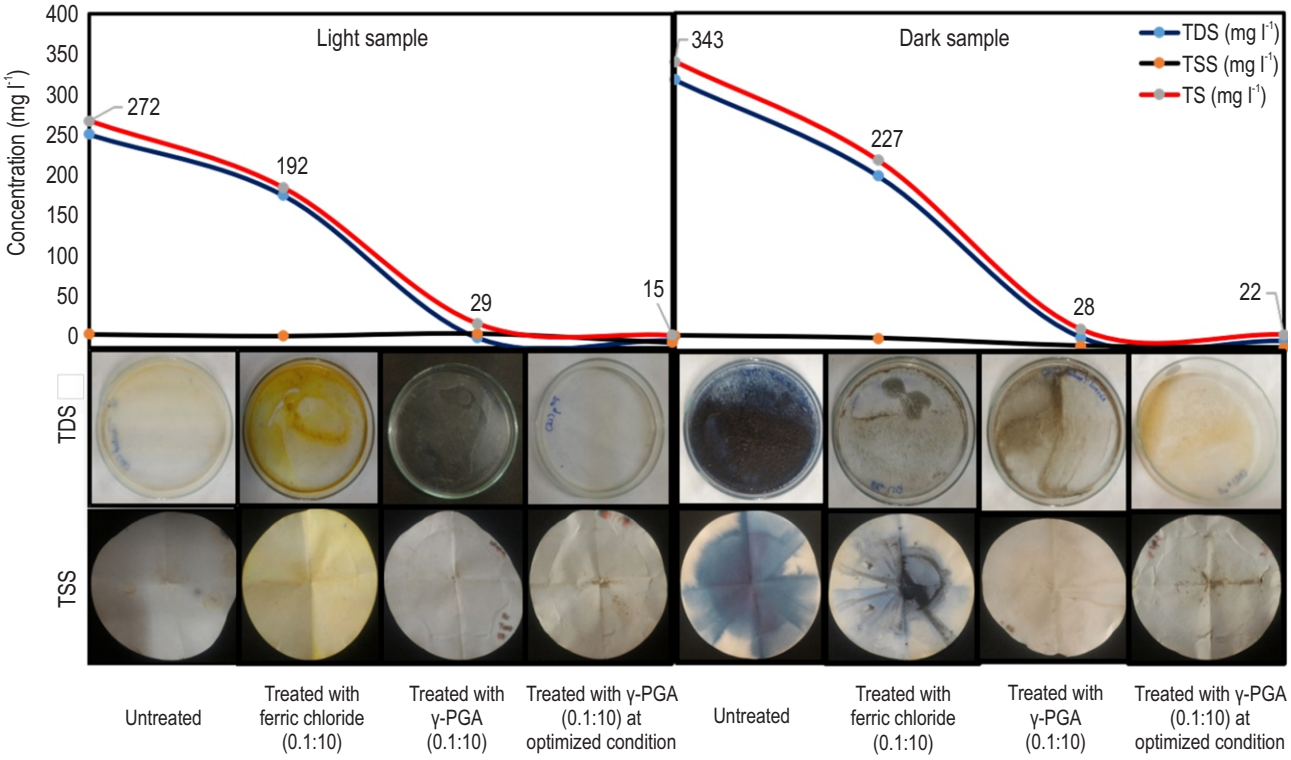

Fig. 4: Estimated solids profile for light and dark sample.

concentration at 660 nm against the kaolin suspension. The results for color and odor of the samples differed from each sample which varied from greyish, lime yellow, pale white and slightly blackish color. The pH of wastewater samples were alkaline in nature. Tannery effluents had high solid profiles, maximal BOD and COD concentrations followed by textile, dairy and sewage samples. The perusal of data showed that the samples were highly polluted with different organic and inorganic compounds.

In the current study, the physico-chemical parameters wastewater samples varied because of their nature, chemical compositions and sources of substrates (Table 1). The tannery industry were discharged 80 % of liquid and solid wastes from the raw material process Verma and Sharma (2022). The physicochemical parameters of tannery effluents were reported by Saied *et al*. (2021). Color of the tannery effluent was reported as greenish blue due to the presence of chrome ions and pH as 10.5, TSS as 877.6 mg l^{-1}, TDS as 15720 mg l^{-1}, BOD as 2345.7 mg $l^{-1}$and COD as 641.7 mg l^{-1}. The physiochemical parameters of textile effluents from Monastir, Tunisia were studied and reported as concentration of the effluent's parameters were beyond the regulation. The pH of the effluents was alkaline in nature and the TSS as 425 to 592 mg l^{-1}, COD as 1505 to 1951 mg $l^{-1}$and BOD as 495 to 760 mg l^{-1}. The adverse effect of the textile influents and effluents were reported from the ecotoxicology investigation by Methneni *et al.* (2021). Sewage water samples from Chennai, Malaysia and Indonesia were analysed and reported by Ishak *et al.* (2021). The physiochemical parameters are as follows pH (7.06, 7.14 and 7.58); TSS (110, 99 and 105 mg l^{-1}); TDS (580, 512 and 320 mg l^{-1}); BOD (320, 355 and 298 mg l^{-1}) and COD (830, 910 and 750 mg l^{-1}). Gaur *et al.* (2021) analysed and reported the parameters of dairy industrial wastewater such as, BOD, COD, TDS and TSS was 1445 mg l^{-1}, 441 mg l^{-1}, 2180 ppm and 1260 mg l^{-1}respectively. The extensive reports indicated and confirmed with the present study that, maximal concentration of solids and oxygen demands were found in the tannery effluents due to their process nature and ingredients. In the tannery process industry heavy metals, acids and salts were used for leather processing. These components may increase the oxygen demands and solids profile which may leads the pollution (Tadesse and Guya, 2017). Next to the tannery effluents were textile effluents, dairy and sewage effluents. In the textile industries, strong dyes and various chemical agents were used from the sizing to finishing process for efficient cloth. These types of dyes and chemicals may free in the effluent as an excess form or react within the chemicals and convert into a complex pollutant (Yaseen and Scholz, 2019).

Preliminary screening was performed with different concentrations of γ-PGA (0.05:10; 0.1:10 and 0.15:10) over the collected wastewater samples at room temperature. The maximal efficacy of γ-PGA was found at 0.1:10 ratio in all the treated samples, except in dairy sample (0.05:10). The preliminary screening showed decolorization percentage as 32.45, 59.58,

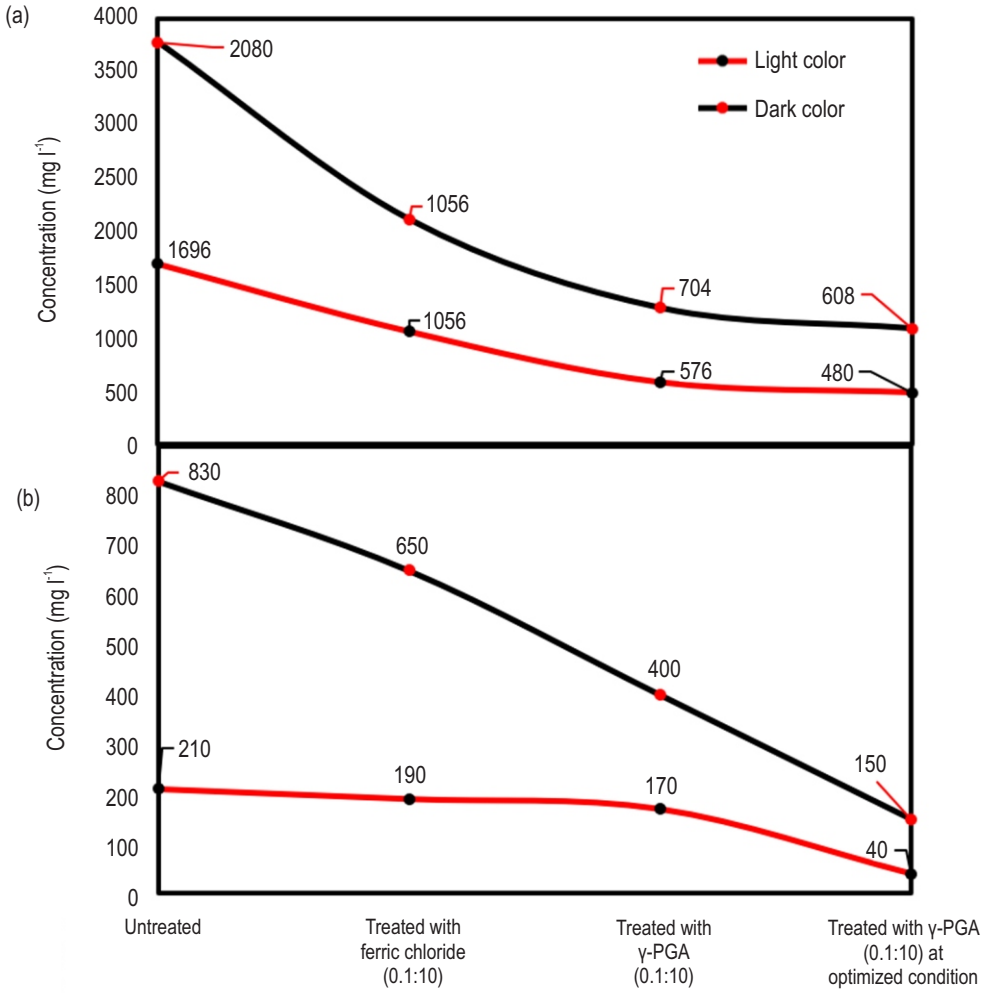

Fig. 5: Concentration reduction of oxygen demands (a) COD ranges and (b) BOD ranges.

14.66 and 9.83 % for 0.1:10 ratio of γ-PGA for tannery, textile, dairy and sewage samples respectively. Similarly, ferric chloride was used as a chemical coagulant in 0.1:10 ratio for all collected samples that attained the decolorization percentage of 12.47, 19.75, 21.5 and 8.74% (Fig. 2). Based on the preliminary screening, the efficacy of γ-PGA was determined at 0.1:10 ratio. The maximal decolorization percentage of 59.58 was noted in 0.1:10 ratio for textile effluent sample. Based on the preliminary screening, textile samples were used for further treatment and optimization.

Two different colors of wastewater samples (light and dark) were collected from the textile industry for optimization process. The optimal wavelength of light and dark color samples were read at 540 and 416 nm from the continuous wavelength scan of 200-600 nm in the UV-Vis Spectroscopy. In the optimization process, four essential parameters (pH, temperature, rotation speed and contact time) were studied in the decolorization screening. The maximal decolorization percentage was 94.1 and 82.95 at pH 9 (Fig. 3a), 91.84 and 75.41 at 40° C (Fig 3b), 93.25 and 81.6 at 150 rpm (Fig. 3c) and 91.84 and 72.69 at 36 hrs (Fig. 3d) for light and dark color samples, respectively. Rajamohan et al. (2014), reported earlier about the absorbance effect of activated *Eucalyptus camaldulensis* barks powder and role of process parameters in metal removal against aluminium solution. From the process, 27.1 mg g^{-1} of metal removed at pH 5, 82% of metal reduction showed at 6 g l^{-1} treatment; 29.6 mg g^{-1} of metal removed at 30 min as contact time with 600 rpm as shaking speed. The results described the maximal level of metal removal was achieved at specific and optimized points for each parameter. The optimized parameters were maintained for γ-PGA with light and dark color textile effluents (01:10 ratio). The decolorization percentage of light and dark color samples were determined as 96.8 and 84.81 % from the spectral analysis at 540 and 416 nm with respective to light and dark textile effluent. Hence it can be predicted that there is an influence of the optimized parameters in increasing the decolorization percentage.

Table 1: Physicochemical parameters of collected wastewater samples

Parameters	Tannery		Textile		Dairy		Sewage	
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
Colour	Greyish	-	Lime yellow	-	Pale white	-	Blackish	-
Odour	Rotten egg	-	Odourless	-	Pungent	-	Rotten smell	-
pH	8.3	0	11.7	0.04	7.6	0.05	7.4	0.04
TDS (mg l^{-1})	2130	0.47	1360	0.47	881	0.47	655	0.47
TSS (mg l^{-1})	1250	0.47	650	0.47	427	0.47	27	0.47
TS (mg l^{-1})	3380	0.47	2010	0.82	1308	0.82	682	0.47
COD (mg l^{-1})	4464	0.82	2000	0.47	3200	0.82	1126	0.82
BOD (mg l^{-1})	3400	1.63	1200	0.47	2472	0.94	628	0.47

coefficient of variation ranges were found as ,1 which indicates the low variance

The physicochemical analysis of treated samples showed a maximal reduction of solid profile (TS, TDS and TSS), COD and BOD concentration. The TS concentration was reduced upto 94.49 and 93.59%; similarly, TDS to 96.48 and 98.45%; TSS to 62.5 and 66.67% (Fig 4) with respective to light and dark color effluents. The COD concentration was reduced to 71.70 and 70.77%; BOD reduced to 80.95 and 81.93 % concentration was determined from the optimized treated light and dark effluents (Fig 5a and b). Sludge volume index of untreated light and dark color textile effluents were determined as 30; 20 ml l^{-1} and similarly for treated light and dark color textile effluents as 18.76; 8.5 ml l^{-1}. The DFZ values were determined for untreated light and dark textile effluents as 89.1 and 61.7 m^{-1} at 436 nm; 76 and 144.5 m^{-1} at 525 nm and 68.2 and 259 m^{-1} at 620 nm. The DFZ values of treated light and dark effluents were found as 16.4 and 18.8 m^{-1} at 436 nm; 12.4 and 38.4 m^{-1} at 525 nm; 10.4 and 63.9 m^{-1} at 620 nm. Uysal et al. (2014) previously reported that DFZ values of textile industrial effluents from Bursa city as 21 m^{-1} at 436 nm; 15.2 m^{-1} at 525 nm; 8.8 m^{-1} at 620 nm. Similarly, Acarbabacan et al. (2002) mentioned the DFZ values of synthetic wastewater as 8 9.5 m^{-1} at 436 nm; 93.2 m^{-1} at 525 nm; 22.2 m^{-1} at 620 nm and efficient color removal percentage was reported as 91 % at 425 nm; 98 % at 525 nm and 96 % at 620.

Textile effluent treatment was studied with different process parameters and natural coagulants like *Strychnos potatorum* and *Eirchornia crassipes* mixtures. The effective coagulant was reported as *Eirchornia crassipes* with optimal dosage as 8 g l^{-1} and reduction percentage of TSS, TDS, BOD and COD as 36.49, 92.90, 65.24 and 50.80 % respectively Prabhakaran *et al.* (2020). The current era in the tannery effluent research is utilised it as a source of energy for electricity production by microbial fuel cell (MFC) mechanism. Chauhan *et al.* (2022) reported that maximum voltage (940 mV) was found from the tannery effluent and synthetic electrolytic solution based MFC with reduction percentage of COD, BOD and TDS as 88, 74 and 94 % respectively. Hybrid technologies showed the vital role in the dairy wastewater treatment with coagulant and electro-fenton (EF) process. In this study poly aluminium chloride (PAC) used as a coagulant and electrodes and current used as EF components for the effective and affordable effluent treatment. Coagulants play a vital role in the flocculation and coagulation process with floc formation and the EF process performed by hydroxy radicals which considered as highly reactive free radicals involved in the decompose of organic compounds in the wastewater. The optimised process with 100 mg l^{-1} of PAC, 60 min of contact time, 20 V of voltage, 2 m of electrode distance, 1.5 g l^{-1} of H_2O_2 and pH3 was showed effective reduction of TSS, BOD and COD as 87, 87.25 and 90.3 % respectively (Zakeri *et al.*, 2021). The present study confirmed that, γ-PGA is an effective coagulant with reduction of physico-chemical parameters which is observed to have a varying range of process parameters according to its coagulating nature.

The optimized parameters were used for isotherm analysis. The parameters obtained from the models were used of adsorption mechanisms, surface properties and affinities of the adsorbent. Langmuir isotherm and Freundlich isotherm were performed to analyze the adsorption of both light and dark color effluent by γ-PGA with Ce/q versus Ce. From the Langmuir isotherm model, the R^2 value of light and dark color effluents were found as 0.9943 and 0.9692. In the Freundlich isotherm model, the R^2 value was calculated as 0.9444 and 0.912 for light and dark color effluents. From the isotherm studies, obtained R^2 values of γ-PGA treated light and dark color effluent were found as near to one in Langmuir isotherm model than the Freundlich isotherm. Based on the isotherm analysis, the Langmuir isotherm model was screened as the best-fitted model. The studies on the rate of removal are essential for the proper design of wastewater treatment facilities. A pseudo second-order model was followed for the experimental data to analyze the adsorption kinetics by γ-PGA. Kinetic study of γ-PGA treated light and dark color effluent were evaluated with the Pseudo second-order model which showed the R^2 value of 0.9871 and 0.9594. Earlier in a study conducted by Santhi *et al.* (2010), the Langmuir and Freundlich isotherm models were followed to fit the experimental data. Best-fitting isotherms were identified based on the linear regression. Their study explored for adsorption effect of activated carbon with Langmuir, Tempkin, Dubinin-radushkevich and Freundlich isotherm and the fitted isotherm was found as a Langmuir isotherm. Similar to our study, the adsorption isotherm and kinetic studies were reported for reactive Red M5B dye and activated

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carbon from citrus fruit peels. From the isotherm studies, Langmuir isotherm was found as fitted model with maximal R^2 value (0.98) similar to the pseudo-second order in the kinetics studies with maximal R^2 as more than 0.99 compared to other models. This adsorption studies revealed that monolayer coverage between the dye and surface of the adsorbent and also its confirmed the distribution of active sites as homogeneous on the adsorbent Babu *et al.* (2011).

The present study concludes that, γ-PGA is aneffective natural coagulant and decolorizing compound against different effluents with solids reduction. γ-PGA can be used as a primary coagulant in the effluent treatment plant to simplify the secondary and tertiary treatment processes. This technique will act as an eco-friendly effluent treatment process for small-scale dyeing and effluent treatment units.

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