

## Optimization of the activated sludge process for physicochemical improvement of pharmaceutical effluent and its application for plant growth

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### Abstract

Pharmaceutical wastewater containing high concentrations of recalcitrant organics, nutrients, and suspended solids were treated using integrated polyurethane (PU) foam adsorption and response surface methodology (RSM)-optimized activated sludge process (ASP). Initial wastewater characterization revealed elevated pH (9.2), BOD (358 mg/L), COD (469 mg/L), TDS (1054 mg/L), TSS (578 mg/L), and ammonia (358 mg/L), exceeding NEQS. PU foam adsorption effectively reduced pollutant concentrations, making it suitable as pre-biological treatment. The ASP was optimized using a Box–Behnken design with pH (6.5–8.5), dissolved oxygen (2–5 mg/L), and *Pseudomonas* concentration (0.5–2 g/kg), yielding an excellent model fit ( $R^2 = 99.81\%$ ,  $p < 0.001$ ). Under optimum conditions, residual COD decreased to 69.5 mg/L, MLSS reached 150 mg/L, and SVI was 50 mL/g. Irrigation with the treated effluent significantly enhanced plant growth, increasing root length (41.8 cm), shoot length (61.7 cm), and fruit yield (17 fruits/pot), demonstrating its potential for safe agricultural reuse.

**Keywords:** MLSS; TDS; RSM; pharmaceutical; activated sludge

### Introduction

The composition of pharmaceutical waste water includes antimicrobial agents, excipients, solvents and active pharmaceutical ingredients, which introduces serious environmental, social and health issues. Pharmaceutical contaminants continue to enter water bodies, leading to bioaccumulation, disruption of aquatic organisms' endocrine systems, antimicrobial resistance, and disturbance of ecosystems. Antibiotics, hormones, pain relievers and other drugs alter microbial communities, affect aquatic biodiversity and promote the transfer of antibiotic resistant pathogens. Exposed water reduces public trust in water safety, aggravates water scarcity problems, adds economic burden on communities, and chronic exposure via drinking water and food chains are linked to endocrine disorders, an increasing susceptibility to antibiotics, cancer risk, and developmental toxicity (Morim *et al.* 2024).

Wastewater with this composition appears light gray, which impedes microbial activity, increases biological oxygen demand (BOD), chemical oxygen demand (COD), and pH levels, while decreasing dissolved oxygen (DO) content. The lack of stringent regulations and inadequate treatment technologies exacerbate these issues, highlighting the need for more rigorous policies, advanced treatment methods, and sustainable strategies for managing pharmaceutical waste. Contaminants are eliminated through physical methods like filtration, sedimentation, and flotation. membrane filtration techniques, such as microfiltration, ultrafiltration, nanofiltration, and reverse osmosis, are frequently used to separate suspended solids and dissolved substances. These techniques often encounter challenges like membrane fouling, the necessity for regular maintenance, and high expenses (Beltran-Flores *et al.* 2023).

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Complex and resistant molecules are generated in advanced oxidation processes (AOPs), Ozonation, in Fenton's reagent and in photocatalysis. Also used for coagulation and flocculation to coagulate fine particles and dissolved organic matter for removal; chemical treatments are expensive and result in secondary pollutants that must be treated as well (Fazzino *et al.* 2024). Biological methods are those that use microorganisms to decompose organic compounds, turning them into biomass, carbon dioxide and water. This process significantly reduces the biochemical oxygen demand (BOD) and chemical oxygen demand (COD) and total suspended solids (TSS) to acceptable limits so that factory effluents can be reused and diseases associated with pharmaceutical wastewater can be prevented. Furthermore, because of their high degree of biodegradability resistance, some pharmaceutical compounds can be problematic to completely remove from the water in traditional activated sludge processes (Deshmukh *et al.* 2016).

Ashrafi *et al.* (2015) showed that under optimal ranges of environmental factors like dissolved oxygen (DO), pH, waste loading rate, heavy metal toxicity, food-to-microorganism (F/M) ratio, temperature and aeration time, microbial communities could significantly improve the efficiency of wastewater treatment. The activity of these microbes is most effective in aerobic systems and particularly in the activated sludge process (ASP) which is now widely adopted by a variety of sectors including pharmaceuticals, textiles, leather, confectionery and dairy processing. In this study, Morim *et al.* (2024) performed bench scale tests on combination of activated sludge process (ASP) with advanced oxidation process (AOP) for the treatment of chemical oxygen demand (COD) and color removal of 74.8% and 58.3% respectively.

The optimization of the ASP for the treatment of the fish-canning wastewater was also conducted by using Response Surface Methodology (RSM), and optimal conditions were obtained at 6.4 hour of residence time and 406.2 mg/L of initial dissolved organic carbon (DOC) in the wastewater (Cristovao *et al.* (2015). Several other works have also investigated integration of ASP with activated carbon for the removal of high-COD organic pollutants and dye residue from industrial wastewater (Yeh *et al.* 2021). Combination of effluent characteristics, biological dynamics and reactor chemistry determine the overall efficiency of the activated sludge process. Optimization of operational variables is essential to transform the complicated organic pollutant to simpler and more stable compounds. Optimizing each parameter individually, however, can be time consuming and may be misleading because of interactions

between the parameters. This has given rise to the use of factorial design in research and development to evaluate the combined effect of more than one factor on the treatment performance (Kumar *et al.* 2023). Abdulgader *et al.* (2020) Response Surface Methodology (RSM) was used to successfully optimize coagulation and flocculation parameters, resulting in substantial reduction in biochemical oxygen demand (BOD) and COD in palm oil mill effluent in order to minimize the number of experiments required. In a similar study, Darvishmotevalli *et al.* (2019) optimized the performance of electrocoagulation process with potassium ferrate to treat textile effluents by using RSM. RSM has also been used to optimize enzyme activity, Fenton oxidation and nanoporous membrane processes in wastewater treatment. Alattabi *et al.* (2019) applied RSM for optimization parameters of activated sludge process in pharmaceutical wastewater including pH, reaction time and Mixed Liquor Suspended Solids (MLSS). Their results validated the efficiency of RSM in improving the efficiency of the removal of organic pollutants. However, a thorough literature review indicates that much is yet to be done, as there is a lack of evidence of optimization studies on only the treatment of pharmaceutical industrial wastewater using the activated sludge process.

The present study aimed to develop and optimize an integrated treatment methodology for pharmaceutical wastewater by combining polyurethane foam adsorption as a pre-treatment step with a Response Surface Methodology (RSM)-optimized activated sludge process (ASP). The effects of pH, dissolved oxygen (DO), and *Pseudomonas* concentration were optimized to maximize the reduction of COD and BOD in the effluent. The novelty of this work lies in the integration of adsorption and statistically optimized biological treatment, followed by the evaluation of the treated effluent for potato cultivation, thereby assessing both treatment efficiency and the potential for agricultural reuse. In addition, the physicochemical characteristics of the influent and treated effluent were determined to evaluate improvements in wastewater quality.

## Materials and methods

Pharmedic pharma Lahore wastewater samples were collected using a combination of grab and composite sampling at key discharge points, ensuring representative analysis of effluent quality. Samples were stored in pre-cleaned, chemically resistant containers, preserved at 4°C, and transported promptly to GCWB Lahore. Waste water characteristics were analyzed by conducting

parameters like pH (9.2), BOD (358 mg/l), COD (469 mg/l), TDS (1054 mg/l), TSS(578 mg/l), Ammonia Nitrogen (358 mg/l), and total phosphate (198 mg/l) (Table I).

**Table. I Characteristics of effluent**

| Parameters                | Effluent | PU     | Activated sludge process |
|---------------------------|----------|--------|--------------------------|
| pH                        | 9.2      | 7.58   | 6.95                     |
| BOD (mg/l)                | 358      | 204.06 | 53.05                    |
| COD (mg/l)                | 469      | 267.33 | 69.50                    |
| TDS (mg/l)                | 1054     | 643.78 | 241.75                   |
| TSS (mg/l)                | 578      | 398.46 | 150.83                   |
| NH <sub>3</sub> -N (mg/l) | 358      | 204.06 | 53.05                    |
| TP (mg/l)                 | 198      | 112.86 | 29.71                    |

#### *Treatment of effluent by polyurethane (PU) foam*

##### *Polyurethane foam packed-bed adsorption column*

A laboratory-scale fixed-bed adsorption column made of transparent acrylic was used for the pre-treatment of pharmaceutical wastewater. The column had a total height of 60 cm, an internal diameter of 5 cm, and a 30 cm packed bed containing washed polyurethane (PU) foam cubes (1–2 cm). A 5 cm gravel layer was placed at the bottom to support the packing and ensure uniform flow distribution, while the remaining 25 cm served as freeboard. The bed volume was 589 cm<sup>3</sup> (0.589 L). Wastewater was introduced in down-flow mode at a constant flow rate of 0.5 mL min<sup>-1</sup>, and all experiments were conducted at 25 ± 2°C. The treated effluent from the adsorption column was subsequently used as the influent for the optimized activated sludge process.

##### *Production of Pseudomonas bacteria*

For preparing the culture of Pseudomonas bacteria, the laboratory was used a growth medium, like Nutrient Agar. The medium was dissolved in double distilled water, sterilized by autoclaving at 121°C for 15 minutes and poured into sterile Petri dishes and allowed to solidify. Soil was used to collect the bacterium sample, and was streaked on the surface of the agar with a sterile inoculation loop. The plates are then allowed to grow for 24–48 hours at 30–37°C with sufficient oxygen levels and a pH range of 6.5–8.5, Pseudomonas being an aerobic bacterium. Following incubation, bacterial colonies were checked for typical greenish or bluish colouration, especially in Pseudomonas aeruginosa which produces pyocyanin. The bacteria was identified by using Gram staining (Gram-negative rods) and biochemical tests such as the oxidase and catalase tests. The isolated colonies were subculture onto fresh media for storage to use in activated sludge process.

#### *The activated sludge process is used to treat the effluent*

When all the suspended solids settled, the supernatant was disposed of from the activated sludge collected from the effluent treatment plant. The sludge was well mixed and aerated using an air pump before it was used for further research. In order to cultivate the activated sludge, it was inoculated in a locally constructed aeration tank for 20 days at lab temperature (30°C) and aerated every day for 4 hours at a rate of 3 lit/mint at pH 6.5–8.5. A biological treatment method, activated sludge is made up of various compartments that help to treat wastewater. A storage tank, flow controller, aeration tank, aeration pump, clarifier, and effluent outlet were all part of a laboratory-scale reactor. All batches were treated with aerated sludge, per RSM. The activated sludge process is determined by the amount of solids in the aeration tank. So mixed Liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), Sludge Volume Index (SVI) settled sludge volume (SSV) and sludge density index (SDI) were measured following Standard Methods for Examination of Waste and Wastewater.

$$MLSS \left( \frac{mg}{l} \right) = \frac{A_2 - A_1 \times 1000}{V} \quad (1)$$

$$MLVSS \left( \frac{mg}{l} \right) = A_2 - A_3 \times 1000/v \quad (2)$$

$$SVI \left( \frac{ml}{g} \right) = SSV \times \frac{1000}{MLSS \left( \frac{mg}{l} \right)} \quad (3)$$

$$SDI \left( \frac{g}{ml} \right) = \frac{MLSS \left( \frac{g}{l} \right)}{SSV \left( \frac{ml}{g} \right)} \quad (4)$$

$$SSSV = SVI \times MLSS \left( \frac{g}{l} \right) \quad (5)$$

A<sub>1</sub> = weight of clean, dry filter paper (mg)

A<sub>2</sub> = weight of filter paper + dried residue after filtering mixed liquor (mg)

A<sub>3</sub> = weight after ignition at 550°C (ash removed)

V = volume of mixed liquor sample filtered (mL).

##### *Storage tank*

Storage tank is consisted of plastic drum having volume of 20 litter. It contained pharmaceutical waste water already treated by polyurethane foam; was transferred to aeration tank. A silicon tube connected with tank and placed above 4mm from bottom to avoid the settled solids flow with waste water.

### Flow controller

It was like pinch cock that installed on silicon tube to control or regulate the flow of solid free water to aeration tank.

### Aeration tank

It is main part of activated sludge reactor. It is locally fabricated of plastic container with dimensions of height: 40 cm and internal diameter 15 cm working volume of aeration tank was 5 lit. Air diffuser were placed 40 mm above the bottom of the tank to mix the activated sludge and maintained the oxygen supply to microorganisms. The microorganisms consume the organic matter in the wastewater as food, breaking it down into carbon dioxide, water, and biomass.

### Settling tank/clarifier

The waste water treated in aeration tank flows in to clarifier and activated sludge settles out from the treated water due to gravity. It is separated from aeration tank by a glass plate, which act as weir to discharge flows to final clarifier. The size of clarifier is height 35 cm internal diameter 15 cm.

### Effluent outlet

Treated water exit from reactor through this outlet to compare with NEQS.

### Optimization of activated sludge process by RSM

Response Surface Methodology (RSM) is an optimization technique that involves more than two parameters, including one independent and one dependent variable. This approach was utilized to evaluate how pH, DO and Pseudomonas (Psd) affect the reduction of Chemical Oxygen Demand (COD) in pharmaceutical industrial effluent using the activated sludge process. The parameters' operating ranges were chosen based on those typically used in the activated sludge process: pH (6.5-8.5), Dissolved Oxygen (DO) (2-5 mg/l), and Pseudomonas (0.5-2 g/kg) (Table-II). The Box Behnken design version 15, which has three levels -1 (low), 0 (medium), and +1 (high) is effective for managing and interpreting comparisons. In this study, a two by three factorial design was applied to fit a second-order polynomial model, representing experimental run 15, to optimize the conditions for the activated sludge process and enhance COD reduction efficiency. The independent variables and their mathematical relationship with the response Y were estimated using a quadratic equation.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \quad (6)$$

To calculate the reduction in effluent COD, the conditions of pH (6.5-8.5), DO (2-5 mg/l), and Pseudomonas bacterium (0.5-2 g/kg) were optimized for the activated sludge process

**Table. II Box–Behnken design matrix along with experimental, predicted values and activated sludge optimization parameters for COD reduction**

| Sr.NO | pH  | DO  | Psb  | Experimental values | Predicted values | MLSS (mg/l) | MLVSS (mg/l) | MLSS (g/l) | SVI (l/g) | SSV (ml/l) | SDI (g/ml) |
|-------|-----|-----|------|---------------------|------------------|-------------|--------------|------------|-----------|------------|------------|
| 1     | 7.5 | 5   | 0.5  | 121.2               | 119.05           | 400         | 315          | 0.4        | 59        | 23.6       | 0.017      |
| 2     | 7   | 3.5 | 1.25 | 69.5                | 70.2             | 150         | 95           | 0.15       | 50        | 7.5        | 0.02       |
| 3     | 6.5 | 5   | 1.25 | 105.6               | 106.925          | 387         | 240          | 0.387      | 70        | 27.09      | 0.014      |
| 4     | 7   | 3.5 | 1.25 | 69.5                | 70.2             | 150         | 95           | 0.15       | 50        | 7.5        | 0.02       |
| 5     | 7.5 | 2   | 2    | 90                  | 92.15            | 198         | 106          | 0.198      | 95        | 18.81      | 0.01       |
| 6     | 7.5 | 5   | 2    | 96.8                | 97.6             | 195         | 112          | 0.195      | 106       | 20.67      | 0.009      |
| 7     | 8.5 | 5   | 1.25 | 134.1               | 135.625          | 407         | 298          | 0.407      | 173       | 70.411     | 0.006      |
| 8     | 8.5 | 3.5 | 0.5  | 144.3               | 143.525          | 401         | 304          | 0.401      | 165       | 66.165     | 0.006      |
| 9     | 6.5 | 3.5 | 0.5  | 111.2               | 110.425          | 396         | 254          | 0.396      | 125       | 49.5       | 0.008      |
| 10    | 8.5 | 3.5 | 2    | 120.2               | 121.775          | 389         | 249          | 0.389      | 169       | 65.741     | 0.006      |
| 11    | 7.5 | 2   | 0.5  | 101.7               | 103.4            | 205         | 98           | 0.205      | 101       | 20.705     | 0.010      |
| 12    | 6.5 | 2   | 1.25 | 98.54               | 97.375           | 190         | 108          | 0.19       | 98        | 18.62      | 0.010      |
| 13    | 6.5 | 3.5 | 2    | 98                  | 99.475           | 201         | 97           | 0.201      | 100       | 20.1       | 0.01       |
| 14    | 8.5 | 2   | 1.25 | 125.36              | 124.075          | 410         | 295          | 0.41       | 156       | 63.96      | 0.006      |
| 15    | 7   | 3.5 | 1.25 | 69.5                | 70.2             | 150         | 95           | 0.15       | 50        | 7.5        | 0.02       |

following the RSM protocol. A total of 15 trials were executed in a batch format, with each batch's conditions meticulously controlled in a laboratory setting. An automatic control system was used to regulate the process's DO and pH. Each batch's sludge bed was prepared according to the RSM, using 5 liters of effluent for treatment. After the ten-day treatment period, samples were collected and analyzed for COD and other relevant parameters to distinguish between the different RSM trials.

#### *Impact of effluent and influent on plant growth*

Potato tubers of the Asterix were planted in air-dried, 2 mm-sieved sandy clay loam soil (pH 7.6; N 0.05%; P 6.4 mg/kg; K 1.21 mg/kg; CEC 8.9 c mol/kg; Organic Matter 1.03%) contained in pots measuring 40 cm in diameter and 40 cm in height, each filled with 10 kg of soil. The experiment was conducted in triplicate, with three replicate pots for each treatment. Irrigation was carried out weekly using untreated pharmaceutical wastewater (influent) and activated sludge-treated effluent, with each pot receiving 2.5 L of water through a sprinkler irrigation system. No pesticides or herbicides were applied during the experiment, and weeds were removed manually as required. The potato plants were grown for four months before harvesting. After harvest, the physical growth characteristics of the plants were evaluated to assess the effects of irrigation with untreated influent and activated sludge-treated effluent on potato growth and development.

#### *Analytical methods*

The influent and effluent samples were analyzed for pH, BOD, COD, TDS, TSS and ammonia nitrogen, according to the Standard Method for water and wastewater. The soil's carbon, hydrogen, nitrogen, sulfur, and oxygen content were measured using a CHNS analyzer (Elementar-Germany). A 5% aqueous soil slurry was filtered to determine pH (HANNA-1122). Soil concentrations of magnesium (Mg), calcium (Ca), and lead (Pb) were measured using atomic absorption spectroscopy, while sodium (Na<sup>+</sup>) and potassium (K<sup>+</sup>) were detected with a flame photometer (Jenway).

#### *Statistical analysis*

Each pharmaceutical waste water, soil parameters were reported averaged taking into consideration three analyses, whereas triplicate samples were each analyzed twice to measure the analytical error. Microsoft office 2017 was used for standard deviation/standard error along with the average value.

## **Results and discussion**

Pharmaceutical wastewater contains high concentrations of COD and BOD because of the presence of complex organic compounds, solvents and active pharmaceutical ingredients which are hard to degrade. The presence of nitrogenous compounds from drug formulations and chemical synthesis process causes the ammonia level to be high. These pollutants cause high oxygen demand and toxicity which makes the pollutants difficult to remove effectively unless treated with advanced technology.

#### *Characteristics of effluent*

The pH of wastewater is one of the major variables that suggests the chemical properties, efficiency of the processes and conditions in the pharmaceutical manufacturing systems. It is a representation of the ratio of acidic to basic substances produced during formulation, synthesis and cleaning operations. The measured pH in the present study was 9.2 and was found to be outside the NEQS limits (6 to 9), indicating alkaline effluents. The reason for this higher pH value could be the utilization of alkaline chemicals (reagents, buffers, alum etc.) in different chemical and biological processes including the production of drugs, pH adjustment and cleaning of equipment. The pH profile of the effluent is also affected by the acid or base neutralisation steps that occur during its production.

Biochemical oxygen demand (BOD: 358 mg/L) and chemical oxygen demand (COD: 469 mg/L) exceeded permissible limits, which meant that there is a high organic load. The contamination is primarily due to the presence of active pharmaceutical ingredients (APIs), solvents, intermediates and non-biodegradable organic residues. A number of pharmaceuticals (antibiotics, hormones, surfactants) are not degraded by biological processes and therefore add to the excessive oxygen demand. In addition, the use of oxidising agents and chlorinated compounds at the disinfection and synthesis steps aggravates the production of recalcitrant organic matter, which increase the COD level. The high levels of BOD and COD indicate that there is a critical need for effective pre-treatment and tertiary treatment facilities to mitigate environmental harm.

The total dissolved solids (TDS) (1054 mg/L) and total suspended solid (TSS) (578 mg/L) were also found to be high. These are high values that are related to salts, undissolved particles, precipitates and fine organic particles created during mixing, crystallization and purification processes. High TDS and TSS may lead to high conductivity and turbidity of the effluent which will

reduce light penetration and impact photosynthetic activity in receiving waters. This leaves a residue of such solids that makes the settling and filtration process of conventional wastewater treatment plants even more complicated.

The ammonia concentration (358 mg/L) in the effluent was higher than the NEQS limits, probably as a result of the use of nitrogen-based intermediates, amines and ammonium compounds in the formulation of pharmaceuticals and for pH control. Biological activity can also liberate ammonia as a result of the breakdown of nitrogen containing compounds such as protein and organic nitrogen during the process. Excessive nitrogen can lead to eutrophication, and to the imbalance of oxygen in aquatic systems.

The coloured nature of the pharmaceutical effluents is mainly due to the presence of complex aromatic structures, dyes, and by-products of synthetic and fermentation operations. Chemically stable and non-biodegradable, causing persistent colour after primary treatment. The colouration impacts on the look of the water body, and also has an impact on the aquatic ecosystems, through the impact on light penetration.

The high concentrations of pH, BOD, COD, TDS, TSS and ammonia compounds underscore the complicated and long-term nature of pharmaceutical wastewater. The results highlight the importance of combined treatment methods such as adsorption and activated sludge process to meet NEQS.

#### *Treatment of effluent by polyurethane foam*

The use of polyurethane (PU) foam based adsorber was very effective in the reduction of the pollution load of the pharmaceutical wastewater, which can be observed as a reduction in the major physicochemical and nutrient parameters. A decrease in pH from 9.2 to 7.58 is the result of buffering and surface interaction processes which increase effluent stability. The high reduction in Biochemical Oxygen Demand (358-204.06 mg/L) and Chemical Oxygen Demand (469-267.33 mg/L) indicates an efficient removal of biodegradable and refractory organic components by the processes of physicochemical adsorption, pore diffusion and hydrophobic interaction in the polyurethane matrix, which has been reported for polymeric sorbents applied to complex industrial effluents (Babel and Kurniawan 2003). The reduction of the total dissolved solids (TDS) (1054-643.78 mg/L) indicates that

the pharmaceutical residues and ionic species have been sequestered by surface binding and entrapment processes, which corresponds to the adsorption isotherm type behaviour and mass transfer controlled process as reported by Foo and Hameed (2010). Similarly, the decrease in Total Suspended Solids (TSS) (578-398.46 mg/L) suggests that particulates get filtered by the interconnected pore structure of PU foam and simultaneously get adhered to its pores, which is due to their porous nature, and further corroborates the use of porous adsorbents in simultaneous filtration-adsorption systems, as reported in the literature (Tran *et al.* 2017). The removal of nutrients was also significant, ammonia-nitrogen (358-204.06 mg/L) and total phosphate (198-112.86 mg/L), which was attributed to electrostatic attraction, surface complexation and diffusion-controlled uptake, thus reducing nutrient eutrophication (Crini and Lichtfouse 2019). All the results indicate that polyurethane foam is an effective adsorptive media which can be used to reduce the amount of organic content, particulate content and nutrient content, thereby proving the suitability of using polyurethane foam as a polishing or pre-biological treatment stage for the remediation of pharmaceutical wastewater.

#### *Treatment of effluent by activated sludge process under RSM*

The Box-Behnken design was used in Response Surface Methodology (RSM) to optimize the removal of Chemical Oxygen Demand (COD) in pharmaceutical effluents by the activated sludge process. RSM is well known to be a statistical and mathematical technique that can be used for modeling and optimizing complex environmental and biotechnological processes that involve several interacting variables affecting the performance of the process (Montgomery, 2017; Myers *et al.* 2016). The combined effects of pH (6.5 – 8.5), dissolved oxygen (2 – 5 mg/L) and *Pseudomonas* (0.5 – 2 g/kg) were systematically investigated with a total of 15 experimental runs. The second order polynomial model that was developed showed good agreement between experimental and predicted COD reduction values, thus the goodness of the quadratic model in describing the biodegradation process was considered satisfactory. The regression model was statistically highly significant ( $p < 0.001$ ) as the selected independent variables were able to explain the variation in COD removal efficiency. The coefficient of determination ( $R^2 = 99.81\%$ ) indicated that the model explained 99.81% of the variance in the COD reduction; the adjusted  $R^2$  (99.48%) was very close to the predicted

$R^2$  (97.02%) indicating that no over fitting had occurred and that the model was robust and predictive. Wastewater treatment optimization has been reported to have similar high predictive efficiency for RSM as seen in the other environmental studies (Bezerra, 2008; Yetilmezsoy, 2009).

$$\begin{aligned} \text{COD}_{\text{exp}} = & 1848.4 - 458.2 \text{ pH} - 43.93 \text{ DO} - 56.13 \text{ Psb} + \\ & 31.71 \text{ pH} \times \text{pH} + 6.981 \text{ DO} \times \text{DO} + 32.41 \text{ Psb} \times \text{Psb} + \\ & 0.280 \text{ pH} \times \text{DO} - 3.63 \text{ pH} \times \text{Psb} - 2.822 \text{ DO} \times \text{Psb} \end{aligned} \quad (7)$$

Those parameters that were investigated, pH was found to have the greatest impact on the removal of COD. The linear and quadratic terms of pH are highly significant, suggesting that the efficiency of microbial degradation is strongly dependent on acidity or alkalinity of the environment. The pH sensitivity of microbial enzymatic activity, transport of substrates and metabolic pathways is known to be very sensitive to changes in pH, and is generally found to be more favorable for aerobic biodegradation at slightly alkaline conditions (Metcalf and Eddy, 2014).

Any pH outside the optimum range will negatively affect the growth of microorganisms and enzyme activity, and thus treatment efficiency. The effect of dissolved oxygen was also statistically significant on COD reduction, and the results indicated that the reduction of COD is closely related to the oxidation of organic pollutants. In activated sludge systems, oxygen is the final electron acceptor for the microbial respiration, and therefore its availability affects the rate of organic matter degradation

(Tchobanoglous). However, the presence of a significant quadratic term indicates that there is not a proportional improvement in removal efficiency with increased aeration, which may be attributed to limitations in oxygen transfer during aeration and increased aeration energy requirement (Table III).

The presence of *Pseudomonas* species resulted in a significant contribution to COD reduction, showing the significance of microbial biomass in the treatment of pharmaceutical wastewater. *Pseudomonas* spp. are well known for their metabolic versatility and capacity to degrade a myriad of complex organic compounds, pharmaceutical residues (Madukasi *et al.* 2010). The quadratic effect is large, suggesting that there is an optimum biomass level for enhanced biodegradation capacity, beyond which, there is a possibility of a limitation in substrate or mass transfer effect. Interaction analysis also showed that COD removal was not only dependent on the individual variables, but also on the interaction between these variables. The interaction between pH and *Pseudomonas* concentration is significant, which indicates that environmental conditions of pH are significant factors influencing microbial activity, and the interaction between dissolved oxygen and microbial concentration is significant, suggesting that the availability of oxygen directly influences the metabolic performance of the microbes. The results are in agreement with earlier reports, which focused on the synergistic effects between aeration, microorganisms, and environmental factors in activated sludge systems (Zhang *et al.* 2012).

The optimum COD reduction conditions were optimized numerically for the regression model and were found within an operating window of slightly alkaline pH, medium to high

**Table. III Analysis of Variance for COD reduction of pharmaceutical effluent**

| Source      | DF | Adj SS  | Adj MS  | F-Value | P-Value |
|-------------|----|---------|---------|---------|---------|
| Regression  | 9  | 7473.41 | 830.38  | 297.39  | 0.000   |
| Linear      | 3  | 2424.59 | 808.20  | 289.44  | 0.000   |
| pH          | 1  | 1529.60 | 1529.60 | 547.80  | 0.000   |
| DO          | 1  | 221.55  | 221.55  | 79.34   | 0.000   |
| Psb         | 1  | 673.45  | 673.45  | 241.18  | 0.000   |
| Square      | 3  | 3459.07 | 1153.02 | 412.94  | 0.000   |
| pH*pH       | 1  | 2762.22 | 2762.22 | 989.24  | 0.000   |
| DO*DO       | 1  | 843.    | 843.34  | 302.03  | 0.000   |
| Psb*Psb     | 1  | 1136.29 | 1136.29 | 406.94  | 0.000   |
| Error       | 5  | 13.96   | 2.79    |         |         |
| Lack-of-Fit | 3  | 13.96   | 4.65**  |         |         |
| Pure Error  | 2  | 0.00    | 0.00    |         |         |
| Total       | 14 | 7487.37 |         |         |         |

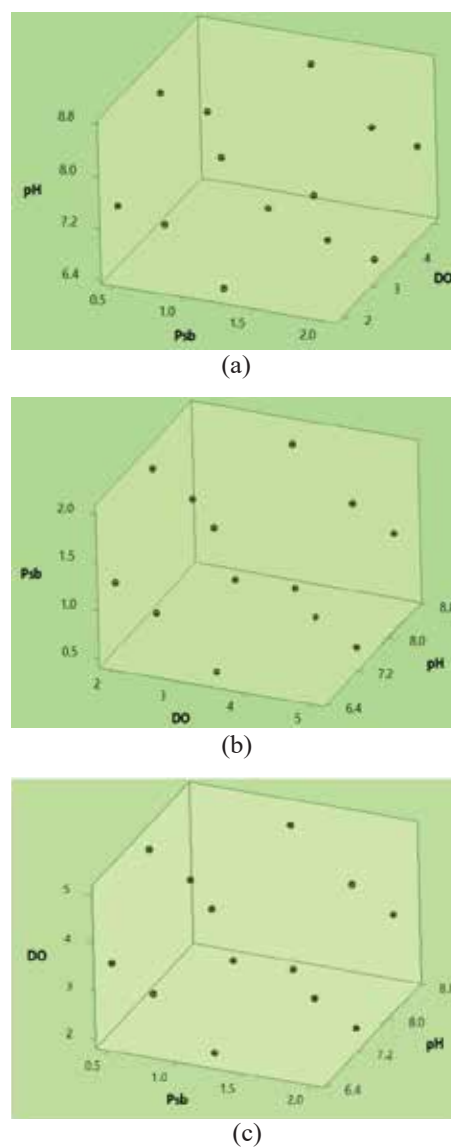
**Table. IV Estimated regression coefficients for COD reduction of pharmaceutical effluent**

| Term            | Effect  | Coef SE | Coef  | T-Value                     | P-Value                      |
|-----------------|---------|---------|-------|-----------------------------|------------------------------|
| Constant        |         | 68.49   | 1.17  | 58.46                       | 0.000                        |
| pH              | 27.655  | 13.828  | 0.591 | 23.41                       | 0.000                        |
| DO              | 10.525  | 5.262   | 0.591 | 8.91                        | 0.000                        |
| Psb             | -18.350 | 9.175   | 0.591 | 15.53                       | 0.000                        |
| pH*DO           | 0.840   | 0.420   | 0.836 | 0.50                        | 0.637                        |
| pH*Psb          | 5.450   | -2.725  | -3.26 | 0.022                       | 0.022                        |
| DO*Psb          | -6.350  | -3.175  | 0.836 | -3.80                       | 0.013                        |
| $R^2 = 99.81\%$ |         |         |       | $R^2 = (\text{adj})99.48\%$ | $R^2 = (\text{pred})97.02\%$ |

DO and optimized microbial concentration. The response surface showed curvature, thus second-order polynomial model was necessary to accurately predict and optimize. (Myers, 2016) The validation of the model revealed that the values calculated by the model were in good agreement with the experimental results in all experimental runs, which further supported the reliability and predictive ability of the developed equation. In conclusion, the results illustrate the potential of RSM as a valuable and effective technique for optimizing pharmaceutical wastewater treatment processes. The optimized conditions obtained in this study can help to improve the efficiency of COD removal, operational stability and manage the pharmaceutical industrial effluent in an environmentally friendly way (Table IV).

The normal probability plot shows that the points are close to the straight line, which means that the residuals are normally distributed, a necessary condition for the validity of ANOVA and regression analysis. The histogram also has a bell-shaped distribution around zero, which further supports normality. Random scatter of residuals about fitted values indicates that there is no funnel shape, or constant variance (homoscedasticity). The residual plot is shown with the order on the x-axis, and there is no obvious pattern or trend, suggesting independence of experimental runs. The results obtained demonstrate the validity of the quadratic RSM model, which is suitable for the experimental data with no systematic errors.

The 3-D scatter plots show good distribution of the experimental points over the ranges of pH, DO, and Pseudomonas concentration. This uniform distribution is important to allow accurate estimation of linear, interaction (pH×DO, pH×Psb, DO×Psb), and quadratic effects and is important for coverage of the design space. Center and extreme points make the prediction capability stronger and enhance the stability of the model. The RSM model meets the regression assumptions (Figure 1) and the design space is well explored (Figure 2), making the model statistically valid

**Fig. 1. 3D Scatterplot for COD reduction (a) DO vs Psb vs pH (b) pH vs Do vs Psb (c) pH vs Psb vs DO**

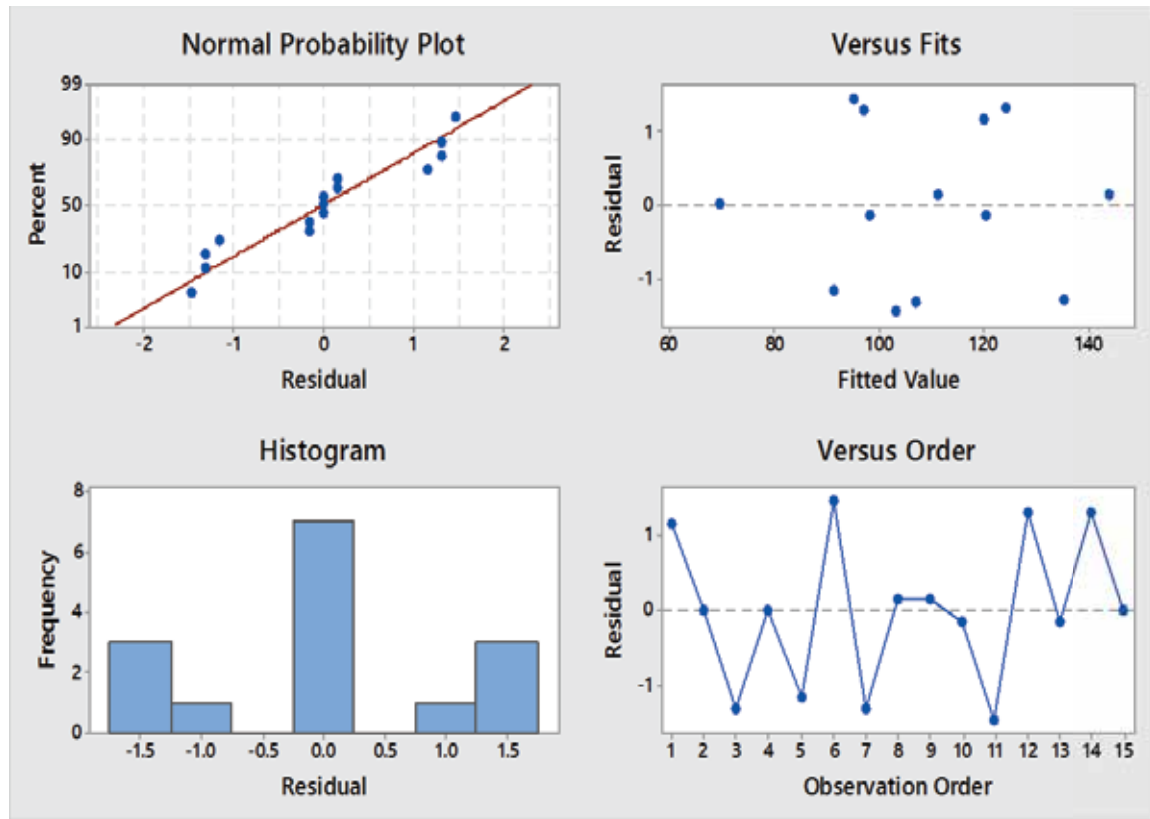


Fig. 2. Residual diagnostic plots for the COD removal experimental model

and reliable for prediction and optimization of COD reduction in the activated sludge process.

The present study revealed a consistent correlation between the development of biological solids and the residual COD concentration in the activated sludge treatment process of pharmaceutical wastewater through the use of the response surface experimental matrix. As may be observed in the experimental data, the higher the COD concentration of the trial, the higher the biomass production and the worse the settleability of the sludge. For instance, the Sr.8 recorded the highest COD value (144.3 mg/L) when MLSS 401 mg/L, MLVSS 304 mg/L, SVI 165 mL/g, and SSV 66.17 mL/L, which is the sign of the high amount of sludge volume and poor floc compaction. Likewise, Sr. 7 (COD 134.1 mg/L; MLSS 407 mg/L; SVI 173 mL/g) and Sr. 10 (COD 120.2 mg/L; MLSS 389 mg/L; SVI 169 mL/g) showed that an increase in the organic loads caused the growth of microbes which led to scoured floc structure and poor treatment efficiency. In Sr. 3 and Sr. 14, COD above 105 mg/L was correlated with MLSS above 380 mg/L and with SVI above 70–156 mL/g, indicating that increased sludge biomass results is not beneficial

for the removal of organic matter, but it increases sludge volume and decreases the efficiency of sludge clarification.

On the other hand, tests performed to prove treatment effectiveness were with moderate biomass concentration and compact sludge structure. The lowest COD residual (69.5 mg/l) was observed for Sr. 2, Sr. 4 and Sr. 15 under the following parameter values of MLSS (150 mg/l), MLVSS (95 mg/l), SVI (50 mL/g) and SSV (7.5 mL/l), which shows a good microbial activity and phase separation. In Sr. 9, COD was relatively low (111.2 mg/L) and sludge characteristics were fairly balanced (MLSS 396 mg/L; SVI 125 mL/g), which further suggests the importance of sludge settleability in the treatment performance. The relatively low SVI in Sr. 2, 5, 9 and 15 indicate that compact floc morphology is beneficial to biomass retention and organic oxidation. The results of the study carried out by Parivallal *et al.* (2022) on different sewage treatment plants for reduction of effluent load showed that the trend in the values of MLSS and MLVSS were coherent with the present study. The sewage waste water in activated sludge process has been also analyzed by Fazzino *et al.* (2024) and the results got are significant advancement of the knowledge on the effects of such C-based

CMs to realistic and scalable AD process conditions with above present study.

The results showed that the higher the COD after treatment, the greater was the microbial proliferation, which resulted in higher value of MLVSS and MLSS as well as the higher value of SSV and SVI. However, high biomass concentration leads to the volume of sludge increasing and treatment efficiency decreasing because of limited oxygen transfer and floc stability. On the other hand, if COD removal is increased, biomass concentration is kept under control with sludge structure becoming denser as seen from the reduction of SSV accompanied by an increase in SDI which signifies better settling behavior. The results indicate that the removal efficiency of COD is not just the function of biomass, but also a function of microbial growth and sludge compaction.

The observed behavior during the present study is in accordance with the current wastewater engineering concept of operating under moderate biomass conditions and stable floc architecture for optimal biological treatment. International studies on industrial and pharmaceutical effluent treatment also have documented that having too much MLSS leads to more sludge, but not corresponding to the improvement in biodegradation that might be achieved; that is because excess MLSS makes it more difficult to transfer oxygen and because diffusion limitations in the floc increase in relation to the increase in MLSS. The other aspect of the interaction between the organic loading, biomass growth and settling characteristics in activated sludge systems is also confirmed by the modern process modeling studies, which proved that the response surface methodology is adequate to represent this interaction. The results confirm the need to control organic loading to achieve stable microbial activity and effective treatment, as outlined in Wastewater Engineering: Treatment and Resource Recovery and corroborated by recent international studies on the optimization of biological treatment and the dynamics of the biodegradation of pharmaceutical wastewater.

Overall, comparison of all the experimental trials shows that Sr. 2 is the most effective operational condition, resulting in the lowest residual COD and better sludge settle ability. The findings have shown that the treatment of pharmaceutical wastewater in activated sludge systems is highly dependent on the balance between organic loading and microbial growth and compaction, in order to achieve an efficient COD removal and stability in the system.

#### *Impact of effluent and influent on plant growth*

Under controlled pot conditions, the vegetative and reproductive responses of all were consistently better in plants grown with activated sludge treated pharmaceutical effluent (ET) than with the raw pharmaceutical effluent (IT).

Soil type and irrigation volume (2.5 L week<sup>-1</sup>) and cultivation practices were standardized. Treatment status significantly affected root development. The mean root length of ET was 58.01 cm which was greater than both IT (41.3 cm) and the control (53.9 cm). The improvement in rooting following the treatment with effluent indicates better aeration in the rhizosphere zone and a decrease in phytotoxic stress due to elimination of a significant concentration of organic load and inhibitory pharmaceuticals by the Activated Sludge Process (ASP). Root growth structure was also enhanced with greater efficiency in acquisition of nutrients and water, similar to the concurrent improvement of the stem girth and yield-related parameters. In agricultural reuse studies, reductions in COD and toxicity also have been reported to improve root physiology and biomass in plants. Treatment also resulted in improved structural growth. The stem girth is also increased from 1.15 cm (IT) to 1.44 cm (ET) and leaf number from 9 (IT) to 13 (ET) which shows improved cell expansion and photosynthetic capacity (Table V). These effects are probably due to the decreased osmotic and toxic effects of the treated effluent, and to the presence of incompletely removed nutrients, such as nitrate and phosphate, at no inhibitory levels in the treated effluent. The reproductive performance was also observed to be similar, with fruit number increasing from 9 (IT) to 14 (ET) and fresh fruit weight from 298.2 g (IT) to 351.08 g (ET) respectively, showing that ASP treatment alleviates growth inhibition and can support productivity when effluent quality is required to meet irrigation standards. These trends were statistically verified. One-way ANOVA revealed that irrigation treatment significantly influenced root length ( $F_{2,6} = 72.781$ ,  $p < 0.001$ ), fresh fruit weight ( $F_{2,6} = 17.921$ ,  $p = 0.0029$ ), and stem girth ( $F_{2,6} = 5.184$ ,  $p = 0.049$ ). Tukey's HSD test showed that plants irrigated with treated effluent (ET) exhibited significantly greater root length, fruit weight, and stem girth than those irrigated with influent wastewater (IT), while the control generally showed intermediate values. These findings indicate that the activated sludge treatment effectively improved the quality of pharmaceutical effluent, making it more suitable for plant growth. In contrast, the number of fruits per pot ( $F_{2,6} = 3.316$ ,  $p = 0.107$ ) and the number of leaves per plant ( $F_{2,6} = 1.147$ ,  $p = 0.379$ ) did not differ significantly among treatments, although ET produced slightly higher mean values (Table VI). Overall, the statistical analysis demonstrates that treated effluent significantly enhanced vegetative growth and fruit biomass, supporting its potential for sustainable agricultural reuse.

**Table. V Impact of influent and effluent on plant growth**

| Parameters                  | ( IT) | (ET)   | (C)    |
|-----------------------------|-------|--------|--------|
| Root length (cm)            | 41.3  | 58.01  | 53.9   |
| No of fruit/pot             | 9     | 14     | 13     |
| Fresh fruit weight/pot (gm) | 298.2 | 351.08 | 314.10 |
| Stem girth (cm)             | 1.15  | 1.44   | 1.32   |
| No of leaves/plant          | 9     | 13     | 12     |

**Table. VI One-way ANOVA results for the effects of influent wastewater (IT), treated effluent (ET), and control (C) on plant growth parameters**

| Parameters         | df (between) | df (within) | F-value | P-value |
|--------------------|--------------|-------------|---------|---------|
| Root length        | 2            | 6           | 72.781  | <0.001  |
| No. of Fruits      | 2            | 6           | 3.316   | 0.107   |
| Fresh fruit Weight | 2            | 6           | 17.921  | 0.0029  |
| Stem girth         | 2            | 6           | 5.184   | 0.049   |
| No. of leaves      | 2            | 6           | 1.147   | 0.379   |

## Conclusion

This study demonstrated the effectiveness of an integrated pharmaceutical wastewater treatment system combining polyurethane (PU) foam adsorption with a Response Surface Methodology (RSM)-optimized activated sludge process (ASP). PU foam adsorption effectively reduced the organic load, suspended solids, and nutrients, providing a more stable influent for biological treatment. Optimization of the ASP showed excellent model reliability ( $R^2 = 99.81\%$ ), with pH identified as the most influential operating parameter, followed by dissolved oxygen and *Pseudomonas* concentration. Under the optimized conditions, balanced biomass (MLSS 150 mg/L) and good sludge settleability (SVI 50 mL/g) achieved a residual COD of 69.5 mg/L, demonstrating that treatment performance depends on maintaining an optimal balance between microbial activity and organic loading rather than excessive biomass accumulation. Furthermore, irrigation experiments confirmed that the treated effluent significantly improved potato growth and yield compared with untreated wastewater, highlighting its potential for agricultural reuse.

Although the study demonstrates the technical feasibility of the integrated PU–ASP process, it was limited to laboratory-scale experiments using a single pharmaceutical wastewater source and potato as the test crop. Future research should focus on pilot- and industrial-scale validation, long-term operational stability, evaluation of pharmaceutical micropollutant removal, and assessment of treated effluent for a wider range of crops under field conditions. These investigations will further establish the practical applicability and sustainability of the proposed treatment system for pharmaceutical wastewater management.

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