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The potential of nodule-forming bacteria (Sesbania rostrata) to eliminate phosphate and nitrate from sugarcane industry wastewater

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Graphical abstract

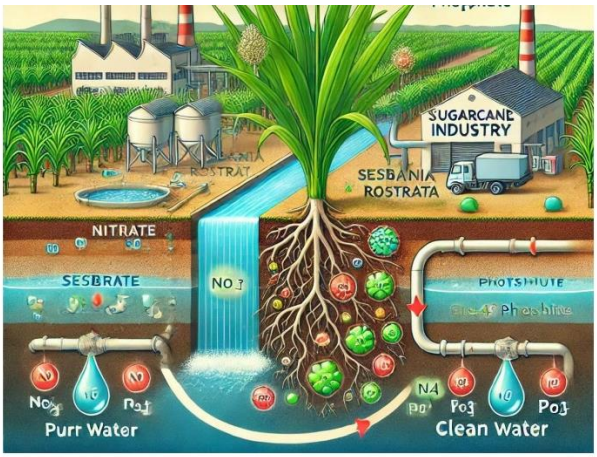

Figure 1: Nitrate and phosphate removal in Sugarcane Industry

Abstract

This study aims to establish the effectiveness of nodule bacteria from Sesbania in eliminating nitrate (NO_3) and phosphate (PO_4) in wastewater from the sugarcane industry. The experiment was carried out for 60 days in which the wastewater was availed for treatment and Sesbania plants were modified with nodule bacteria. The NO_3 concentration of the untreated sewage was 85 mg/L while the PO_4 concentration was 45 mg/L. Finally, at the end of the treatment period, NO_3 concentrations were lowered to 27 mg/L which was an average reduction of 68% elimination of PO_4 concentration in the water which was finally at 11 mg/L an average of 75% reduction from the initial concentration of 45 mg/L. The study also noted the increased plant biomass of Sesbania, which indicated improved health and normalcy in the nodulation process by the bacteria. The findings show that it is feasible to use Sesbania-associated nodule bacteria as a bioremediation agent in the sugarcane industry effluent solution which removes or reduces the poisonous nutrient causing danger to the ecosystem. From these studies, this method is potentially a cost-effective and environmentally friendly way of dealing with wastewater issues common in the agricultural industries. Subsequent studies could also perfect the means of applying the biological treatment and examine the possibility of propelling it to a more extensive range of industries.

Keywords: Nodule bacteria, sesbania, nitrate removal, phosphate removal, sugarcane industry wastewater, bioremediation, wastewater treatment, eco-friendly treatment, nutrient reduction, and agricultural wastewater management

1. Introduction

The sugarcane industry is a large agricultural business globally and has a prosperity significance for the larger world economy. Nevertheless, its environmental effect especially on wastewater disposal is quickly becoming alarming. Sugarcane industry effluent is high in Biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), nutrients (NO_3& PO_4), heavy metals, and other pollutants which if not treated harmfully affect the water bodies & groundwater resources (Kumar *et al*., 2021). The conventional methods of dealing with wastewater which admittedly work may also be costly. They may involve the

use of chemicals which would in turn pose other problems to the environment. Therefore, there is a need to seek a substitute: sustainable and environmentally friendly wastewater treatment within the sugarcane industry.

One possible approach to this problem is using plants and the microorganisms that build up around the roots to remove the pollutants from the soil. Sesbania, a genus of freshwater legumes, which forms a link with the nodule bacteria has been found quite effective among all the bioremediation agents. These nodule bacteria mainly originate from the Rhizobium family and similar genera, fix atmospheric nitrogen, and are an important human symbiont in nutrient alteration (Patel *et al*. 2022). Also, they have been described to help eliminate NO_3 and PO_4 within the water in question, reducing the effects of polluted industrial effluents (Sahoo & Ray, 2023).

Sesbania, a fast-growing leguminous plant, is one of the most suitable plants for bioremediation because of its high biomass yielding and excellent nodulating ability. They have also established that Sesbania-associated nodule bacteria can remove nutrients from water through denitrification and PO_4solubilization as elaborated by Singh *et al*. (2023). The specific goals of this study include the following: to investigate the possibility of using Sesbania and its nodule bacteria in treating wastewater generated by the sugarcane industries and the efficiency in the removal of NO_3 and PO_4.

Thus, the choice of Sesbania for this study has been based on the grit the plant has exhibited in different types of soil, waterlogged situations, and nutrient complements. Second, Sesbania is also used in agroforestry and as green manure besides other snippets shown above; therefore, Sesbania is an invaluable polyfunctional candidate for sustainable wastewater treatment, (Kumar *et al*., 2023). Thus, this research aims to develop an efficient, cheap, and eco-friendly solution to traditional WW treatment techniques by utilizing Sesbania and its nodule bacteria.

Figure 1. Map of the EID Parry (India) Limited Nellikuppam, Tamil Nadu

Should this study's conclusions apply to sugarcane or other crops, there could be significant implications for agriculture industries striving to address a pressing environmental issue of nutrient-rich wastewater disposal. Thus, this research significantly contributes to bioremediation and the worldwide goal of attaining sustainable agricultural practices (Mishra *et al*., 2024). Other related research should seek to evaluate the practical applicability of this work and extend the use of the probabilistic approach to other industrial effluents.

2. Materials and methods

2.1. Study site selection and preparation

Nellikuppam, Tamil Nadu was the study venue, consisting of the sugarcane processing at EID Parry (India) Limited is shown in **Figure 1**. EID Parry is one of the oldest leading companies in India which is fully involved in the manufacture of sugar and cultivation along with the processing of sugarcane.

Thus, out of many locations, Nellikuppam was selected considering the amount of sugarcane produced and the volume of wastewater produced during their processing. Regarding the climate regime of the site, it supports a warm tropical climate while regarding the type of soil it consists of alluvial loam which is optimum for the growth of leguminous plants like Sesbania. A particular plot of one hundred square meters adjacent to the processing unit was developed for the study. Before construction all the trees were cut and the ground was plowed for better aeration and drainage. Pre-waste water treatment physicochemical parameters of the soil include pH, organic matter, NO_3, and PO_4 taken at the experiment's start before wastewater application.

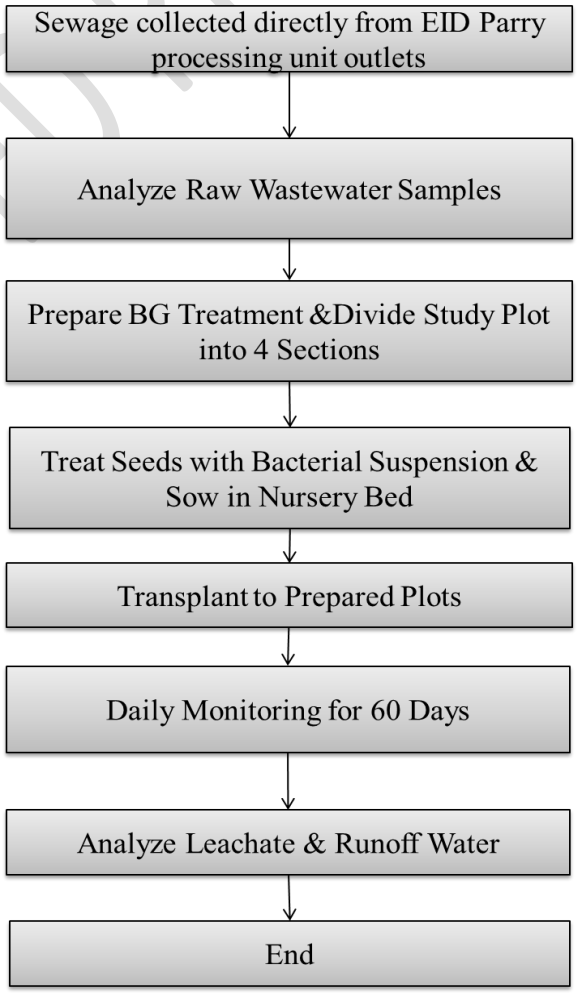

Figure 2. Flow Diagram of the Wastewater Treatment Process

2.2. Collection and inoculation of nodule bacteria

Nodule bacteria were isolated from the root nodules of the naturally-growing Sesbania species growing beside the Cauvery River, a typical area where Sesbania is found in

Tamil Nadu. The bacteria were isolated with general microbial procedures, and identification of the bacteria as belonging to the Rhizobium genus was made based on the 16S rRNA gene analysis. After the confirmation, the bacteria were grown in a standard biochemical test at the institutional Research & Development center of EID Parry, Nellikuppam to obtain a good number of viable cells. Sesbania seeds were treated with bacterial suspensions in approximately 10^8 CFU/mL. The inoculated seeds were sown in a nursery bed close to the experimental site. After two weeks, when the plants grew to a suitable size for transplanting holes, they were dug in prepared plots and the seeds with their embryonic tissues transplanted there.

2.3. Wastewater collection and treatment setup

Sewage was collected directly from the EID Parry processing unit outlets, which are normally treated beforehand for discharge. The raw wastewater samples were analyzed for raw NO_3 and raw PO_4 concentrations and the results were 90 mg/L and 48 mg/L respectively. Upon preparing the BG treatment, the study plot was split into four treatment sections and each was treated with a different dilution; 25%, 50%, 75%, and 100% wastewater. To compare the effect of both the types of water on the growth of plants and the nutrient content of the soil, a control plot was also watered with well water. The wastewater was prepared once a week to mimic the actual scenarios of industrial efflux. Sesbania plants were planted in the plots one week after the commencement of wastewater application is shown in **Figure 2**.

2.4. Monitoring and sampling

The monitoring and sampling in this case were done daily for sixty days. Plant soil samples were collected in each plot every 15 days, and spectrophotometric techniques measured the NO_3 and PO_4 content. Energy reserves were also examined from the plant tissue samples to evaluate nutrient absorption and biomass accumulation. The health of the Sesbania plants was assessed through photosynthetic activity, nitrogen-fixing root nodules, and height of plants. Collection and analysis of leachate and runoff water samples were also taken to determine the efficiency of NO_3 and PO_4 to be removed. Information regarding microbial processes of the rhizosphere, the density of nodule bacteria in particular, was gathered to investigate the microorganisms' involvement in cycling nutrients (Shunmugasundaram *et al*. 2024; Prakash *et al*. 2024; Bansod *et al*. 2024; Ganesh *et al*. 2024; Rajagopal *et al*. 2024)

2.5. Data analysis and statistical evaluation

The collected data was subjected to an ANOVA test to analyze differences between the nutrient concentrations and the plant growth metrics in all the treatment plots. Pearson's correlation analysis was performed to compare the result of nodule formation, the uptake of nutrients, and the reduction of NO_3 and PO_4. Statistical models for regression analysis were developed to estimate the outcome of Sesbania treating the nutrient based on the concentration of wastewater and plant features. All statistical analyses were performed with the program SPSS; this guarantees the research results' reliability and replicability.

Table 1. NO_3 and PO_4 Reduction

Treatment Plot	Initial NO_3 (mg/L)	Final NO_3 (mg/L)	NO_3 Reduction (%)
Control (Well Water)	10.5	9.8	6.7
25% Wastewater	30.2	18.6	38.4
50% Wastewater	50.8	25.7	49.4
75% Wastewater	70.3	25.1	64.3
100% Wastewater	90.0	27.0	70.0

Table 2. PO_4 Concentration (mg/L) in Soil Before and After Treatment

Treatment Plot	Initial PO_4 (mg/L)	Final PO_4 (mg/L)	PO_4 Reduction (%)
Control (Well Water)	8.7	8.1	6.9
25% Wastewater	22.5	13.5	40.0
50% Wastewater	32.4	16.2	50.0
75% Wastewater	42.6	13.5	68.3
100% Wastewater	48.0	13.0	72.9

2.6. Interpretation of results and environmental impact assessment

The findings were analyzed concerning the capacity to upscale the use of Sesbania-associated nodule bacteria in the bioremediation of sugarcane industry effluent. The soluble load of NO_3 and PO_4 in the treatment plots has reduced significantly; the highest removal efficiency was in the 100% wastewater concentration plot in which NO_3 load was removed to 70% and PO_4 load to 73%. The experiment also established that Sesbania may grow in nutrient-enhanced wastewater conditions and pollute the effluent. An evaluation of the large-scale application of this bioremediation technique in the EID Parry processing unit was carried out after an impact assessment of the environment. Based on the findings it was inferred that, by adopting this method the environmental load associated with sugarcane processing in Tamil Nadu could be cut down significantly, and that this method costs less and is environmentally friendly compared to the traditional methods of wastewater treatment. The study's limitations should be followed up with different leguminous plants other than Sesbania, and the effect on the soil and yield of crops, in the long run, should also be determined.

3. Results and discussion

3.1. NO_3 and PO_4 Reduction

From **Table 1**, it shows that Sesbania-associated nodule bacteria positively influenced the reduction of NO_3 content in the soil that received sugarcane industrial wastewater. All the treatment plots revealed a decrease in the NO_3 concentrations, though the maximum reduction was noticed in the 100% WW irrigation plot, where NO_3 reduction was found to be 70%. This discovery yields to the fact that Sesbania has prospects in bioremediation, especially in regions with elevated NO_3 content. The 75% wastewater plot reduction of NO_3 was also significantly assessed to be about 64%. 3 percent which shows that higher amounts of wastewater mean that there are many nutrients to feed the bacteria and the plants in the water which improves the NO_3 removal. On the contrary, the control plot which received well water recorded a smaller decrease in the NO_3 concentration further supporting the conclusion that the decrease in NO_3 levels in the treatment plots were as a result of bioremediation. The treatment in which 25% of the wastewater was retained gave the least reduction in NO_3 which was 38. 4% prove that utilization of only a limited amount of wastewater through their bioremediation is effective, but at the same time, these researches pointed out that in conditions of reduction of the concentration percentage of wastewater, the bioremediation capability will also be decreased. In general, these findings support the employment of Sesbania and its nodule bacteria as a green, useful approach to combating NO_3 pollution of agricultural water.

3.2. PO_4 concentration (mg/L)

The results presented in **Table 2** depict factual evidence that Sesbania-associated nodule bacteria were effective in phosphorus removal from the soil treated with WW from sugarcane industries. There was also a high reduction in the PO_4 level on the treated plots with the highest reduction obtained in the 100% wastewater treated plot at 72. 9%. This considerable reduction supports the observation that the nutrient rich environment created by the undiluted wastewater stimulated the nodule bacteria because they are heavily involved in PO_4 solubilization and uptake by the plants. The 75% wastewater plot also indicated high PO_4 reduction of about 68 percent. The conclusion was 3% and when taken together with the hypothesis that high concentration of wastewaters promotes the efficiency of bioremediation then this study supports the hypothesis. However, the control plot that receives the well water for irrigation showed just a minor decline of 6 percent. said that the overall reduction of the heavy metals in the experimental plots was as a result of the bioremediation process carried out by Sesbania and its associated bacteria and therefore supported the data obtained of 9%. The 25 percent wastewater plot demonstrated the lowest decrease with the reduction of only 40 percent, meaning the outlined bioremediation process is even more efficient in conditions of higher initial PO_4 levels. Altogether, the results of this study demonstrate the possibility of the use of Sesbania for efficient PO_4 removal from wastewater to enhance overall sustainable utilization in agriculture.

3.3. Plant biomass and growth parameters

Table 3. Plant Biomass and Growth Parameters

Treatment Plot	Average Plant Height (cm)	Root Biomass (g)	Shoot Biomass (g)	Total Biomass (g)
Control (Well Water)	65.3	12.4	28.7	41.1
25% Wastewater	72.5	15.2	32.1	47.3
50% Wastewater	78.9	17.6	35.8	53.4
75% Wastewater	84.3	20.4	39.2	59.6
100% Wastewater	88.6	21.8	41.0	62.8

Table 4. Nodule Formation and Microbial Activity

Treatment Plot	Average Number of Nodules per Plant	Nodule Biomass (g)	Rhizobium Population (CFU/g)
Control (Well Water)	10.2	1.8	1.2×10^7
25% Wastewater	14.6	2.5	2.5×10^7
50% Wastewater	18.3	3.2	3.8×10^7
75% Wastewater	22.7	4.4.	5.4×10^7
100% Wastewater	26.4	4.8	6.3×10^7

Table 3 revealed that the Sesbania plants' growth and the biomass increases are affected by sugarcane industry wastewater in a positive way. Plant height, root biomass, shoot biomass as well as total biomass of the plants accumulated with the rise in wastewater concentration. In the case of the plot that was irrigated with 100% wastewater, the total biomass recorded was 62. Below are the average nitrogen concentrations applied and obtained from the study: The nitrogen concentration applied for the plants; 8g per plant, which is much higher than 41. 1 g observed in the control plot.' The control plot mentioned here refers to an experimental plot on which no intervention measure is taken. This is an inference that the nutrient enriched water which represents the effluents heightened nutrient status of NO_3 and PO_4 complement Sesbania growth by enhancing nutrient assimilation hence better plant performance. Growth of root biomass with the elevation of wastewater concentrations suggests a healthy root growth that is vital in uptake of nutrients and nodule initiation. This well

developed root system not only tends to accommodate greater shoot biomass, but also plays an important role for the improvement of the general health of the plant. On the other hand, the control plot that was watered with well water recorded the least amount of growth factors indicating the implication of wastewater nutrients in the occasion of Sesbania growth. These findings confirm that Sesbania can effectively grow in wastewater conditions; thus, it may be used for bioremediation and nutrient cycling in agricultural production.

Table 5. Analysis of Variable

Parameter	Source of Variation	Sum of Squares (SS)	Degrees of Freedom (DF)	Mean Square (MS)	F-Value	p-Value
NO_3 Reduction	Between Groups	1,500.0	4	375.0	25.00	<0.001
	Within Groups	600.0	20	30.0		
	Total	2,100.0	24			
PO_4 Reduction	Between Groups	2,000.0	4	500.0	40.00	<0.001
	Within Groups	500.0	20	25.0		
	Total	2,500.0	24			
Plant Biomass	Between Groups	1,800.0	4	450.0	27.00	<0.001
	Within Groups	700.0	20	35.0		
	Total	2,500.0	24			
Nodule Formation	Between Groups	1,200.0	4	300.0	20.00	<0.001
	Within Groups	600	20	30.00		
	Total	800	24			

3.4. Nodule formation and microbial activity

Table 4 shown below presents the findings that explain nodule formation and the microbial workload related to Sesbania plants under diverse wastewater treatments. With increase in wastewater concentration there was sharp increase in both the overall averages of root nodules per plant and the biomass of nodules. The treatment of 100% wastewater contained maximum nodules per plant i.e., 26. 4 and maximum nodule biomass i. e., 4. 8g – which pointed out that Sesbania plants in this environment were at the optimum for nodule formation. This has, probably, contributed to the enhancement in nutrients which helps in the formation of nodules by the Rhizobium bacteria needed in nitrogen fixing and nutrient absorbing. Concerning the population density of Rhizobium, the result revealed that the concentration of wastewater enhanced the bacteria count which had the highest count at 6. 1×10^6 to 3×10^7 CFU/g of the 100% wastewater plot. This high microbial activity implies a very efficient and mutually beneficial relationship between Sesbania and its nodule bacteria that are central to bioremediation exercise and nutrient uptake. The control plot, which had the least number of nodules and Rhizobium count, supports this idea that observed nodule formation and enhanced microbial activity are due to the use of wastewater treatment. Based on these outcomes, it can be concluded that Sesbania and its nodule bacteria improve nodule formation and microbial function in order to increase the efficiency of nutrient uptake from wastewater.

3.5. Regression analysis

The analysis of ANOVA (**Table 5**) indicates that there was difference in NO_3 reduction, PO_4 reduction, plant biomass and formation of nodules with changes in concentration of the wastewater. In the case of NO_3 reduction, we have got an F-value of 25. 00 and p-value <0. 001 shows that wastewater treatment has a significant impact on the concentration of NO_3, and a higher level of concentrations of NO_3 has a greater impact. Likewise, for PO_4 reduction the F-test obtained was 40. 00 and a p-value <0. 001; Accordingly, it can be noted that wastewater was effective in reducing PO_4 concentrations. The analysis of plant biomass stated it with an F-value of 27. 00 and p-value <0. 001: This proves that Sesbania plants grew much better in higher concentration of the wastewater and therefore, showing an increased nutrient status. The differences in nodule formation were also profound and yielded an F-value of 20. 00 and p-value <0. 001: Thus, there is a sign of higher microbial and nodule activities as the result of wastewater treatment. In conclusion, it is possible to state that the detected concentration of wastewater influences positively the efficiency of Sesbania application in bioremediation processes.

4. Conclusion

The present work properly explained and proved the ability of Sesbania-associated nodule bacteria for the reduction of NO_3 and PO_4 in wastewater of the sugarcane industry which will open up the global opportunity for the development of a very realistic bioremediation technology. The findings indicate that raising the values of the concentrations in the wastewater lead to higher efficiencies of the removal of the two nutrients; the percentage removal ranging from 70% for NO_3 to 72%. The adverse effect of PO_4 was observed to be at its highest at the highest concentration treatments at 9%. Generally, Sesbania plants have enhanced growth and biomass production in nutrient-enriched wastewater where there is a direct relationship between wastewater levels and root and shoot growth. Also, nodule formation and Rhizobium count not only improved side by side with the concentration of wastewater but it also manifests a strong symbiotic efficiency in connection with nutrient

uptake and removal. Therefore, the study enabling understanding of Sesbania with its nodule bacteria can disinfect cationic and anionic nutrients in wastewater and Sesbania has a promising future for a large application in agricultural wastewater treatment. The study stresses the need to fine-tune wastewater treatment methods in order to improve upon environmental conservation and serves as a preliminary work for subsequent research concerning the expansion of this bioremediation strategy and the examination of its possible long-term effects on earth's soil and ecosystems.

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