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Microbial Bioremediation of Agricultural Wastewater: Potential for Environmental Sustainability

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ABSTRACT

Wastewater from agricultural processing is known to contain high levels of organic matter, nitrogen and phosphorus and is a common source for surface-water pollution if it is discharged without treatment. This study aimed to investigate the ability of specific microbial strains to treat such wastewater and remove the pollutants. Samples from an agricultural processing facility were divided into various treatments, each containing a different culture of the microorganisms, as well as a control without microorganisms. The biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids, nitrate and phosphate were measured before and after the treatment and the microbial growth was monitored along the treatment period, along with the efficiency of removal of pollutants. One-way ANOVA was performed to compare treatments and percentage-removal calculations were used to identify the treatment that was most effective, using microbial approach. The mixed-culture treatment consistently performed better than the single-strain treatments for all parameters measured for BOD, COD, and TSS removals, and for nitrate reduction, with the largest percentage reduction achieved. The treatment with the mixed culture had the greatest percentage removal of TSS and the most complete removal of nitrate and phosphate. A significant positive correlation between the growth of microbial biomass and removal efficiency of pollutants was established with regression analysis. The results justify the use of microbial bioremediation and more specifically of mixed cultures as a practical and inexpensive method of treating agricultural effluents prior to discharge; scale up and further research ideas are presented at the end of the study.

Introduction

Milling, washing, pressing, fermenting, and the multitude of other agricultural processing operations demand a lot of water, and most of the water used in agriculture is contaminated with organic and nutrient pollution when it exits processing. Untreated, it is a common source of oxygen depletion, eutrophication and overall pollution in the rivers and ponds where it is discharged (Vidali, 2001). There are conventional treatments available, though they are often costly to build, use a lot of energy, or are simply impractical for the smaller processing operations that are the backbone of a lot of agriculture, especially low and middle income producers (Rittmann & McCarty, 2001).

The bioremediation of microorganisms is an alternative method that is cheaper and in many ways less harmful. The concept is simple: add the microorganisms that can use the organic and inorganic pollutants in the wastewater and allow the biological processes to perform the work that would otherwise be done with chemical dosing or with high energy consuming mechanical treatment units (Boopathy, 2000). The approach has proven to be very successful in treating industrial effluents for a long time. Bacterial consortia isolated from pulp and paper mill have been demonstrated to eliminate chemical oxygen demand by more than 80 percent under optimized conditions (Kumar et al., 2012) and other similar consortia have shown to achieve similar reduction in other agro-industrial effluents (Ordaz-Díaz et al., 2014; Tyagi et al., 2014).

Many of the studies reviewed in this literature suggest that the mixed treatments perform better than the single-strain treatments. Pilotscale use of cyanobacteria dominated consortia has recorded over 75% reduction in both soluble and total COD, significant reduction in nitrate, and total phosphorus in the wastewater environment within 24 hours of treatment (Mehrotra et al., 2020; Singh et al., 2024). The apparent explanation is the metabolic complementarity; different strains in consortium degrade different fractions of pollutant load, metabolic byproducts from one strain are used as substrate for another strain leading to metabolic complementarity which no single strain could be able to do on its own (Sharma et al., 2020).

Nitrogen and phosphorus removal should be emphasized, because the two nutrients are the major factors causing eutrophication after wastewater reaches the receiving water body. Biological nitrogen removal by the interaction of nitrifying and denitrifying bacteria has been reviewed extensively as a sustainable alternative to chemical nutrient stripping (Ahn, 2006), and nutrient removal in general, has been reported for various reactor designs and constructed wetlands (Vymazal, 2007). Algal-bacterial systems generate another added value of aerobic bacterial degradation of organic pollutants and can be incorporated into the same treatment unit as a synergy that was proposed as a resource-efficient approach for both hazardous and nutrient-rich wastewaters (Munoz & Guieysse, 2006).

A side-by-side comparison of individual microbial strains and the combined, mixed culture treatment has not been directly addressed in the literature in the context of agricultural processing wastewater and in relation to the entire range of parameters relevant to compliance with discharge requirements, including BOD, COD, suspended solids, nitrate and phosphate, all at once. The examples thus far are mostly from pulp and paper or municipal sewage application (Kumar et al, 2012; Tyagi et al, 2014; Singh et al, 2024), although the compounds present are generally similar, there is a unique combination of organic loading and nutrient concentration in agricultural processing effluent that must be tested separately.

This work was undertaken to fill this gap. The wastewater from an agricultural processing facility was split into several treatments (some receiving individual microbial strains, some receiving a mixture of microbial strains, and an untreated control) and measured before and after treatment for BOD, COD, TSS, nitrate and phosphate. Three goals were set for the work: to quantify the pollutant-removal performance of each microbial treatment compared to the untreated control; to compare single-strain treatments with the mixed-culture treatment and determine if there is a measurable advantage to combining the strains; and to characterize the relationship between microbial growth over the treatment period and pollutant removal, applying regression analysis. The results are meant to help the agricultural processors make practical choices of which microbial treatment method would provide the greatest benefit in a sustainable, cost-effective treatment.

Literature Review

As the name suggests, bioremediation involves the use of organisms, typically bacteria, but sometimes fungi or algae, to degrade or otherwise detoxify contaminants from a polluted medium (Vidali 2001). The basic processes applied to wastewater usually involve aerobic or facultative bacteria converting organic carbon compounds to carbon dioxide and water, using up the dissolved oxygen that would otherwise be determined as biochemical oxygen demand in the untreated sample. The efficiency of this process depends on a number of interacting factors, which include the metabolic abilities of the microorganisms used, the concentration and the chemical composition of the pollutants present, environmental conditions such as pH, temperature, and availability of nitrogen and phosphorus as trace nutrients which support the growth of microorganisms (Boopathy, 2000; Rittmann & McCarty, 2001).

Various studies have employed bacterial strains directly isolated from polluted agro-industrial plants and assumed that bacteria that are already grown in a specific effluent are likely to degrade it more effectively than the laboratory acclimatized bacterial strains. When acclimatized under the optimum pH, temperature and aeration conditions with the addition of nitrogen and phosphorus in raw wastewater, bacteria isolated from an agro-based pulp and paper mill species of Pseudomonas, Bacillus, Pannonibacter and Ochrobacterum removed COD by up to 86.5% (Kumar et al., 2012). Another study using dominant aboriginal microbes from a similar effluent source found similar reduction rates with a consortium compared to using individual microbes (Tyagi et al., 2014), and formulated microbial seed material tested specifically for its efficacy on BOD testing of pulp and paper effluent found similar degradation rates when using multiple species in combination as opposed to individual species (Ordaz-Díaz et al., 2014).

The same applies to domestic and municipal wastewater research. A five-strain autochthonous bacterial consortium was effective in both BOD and COD reduction (89% and 55% respectively in 24 hours) without the need to introduce new bacteria (Mehrotra et al., 2020) and biostimulation of an indigenous bacterial community (that is, adjusting the environmental conditions to favor the species already present that are capable of degrading the pollutants) has proved an effective method for detoxifying pulp mill effluent (PME) without the need for external bacteria at all (Sharma et al., 2020). A high-rate algal pond (HRAP) using a consortium of cyanobacteria achieved more than 75% removal of both soluble and total COD from primary treated sewage water, and significant reduction of nitrate and total phosphorus, after twelve days of retention (Singh et al., 2024).

Nutrient Removal: Nitrate and Phosphate

Reducing the load of organics is an important part of the process, but so is the load of nitrates and phosphates, which will have environmental impacts once released, primarily in the form of eutrophication of receiving waters. Biological nitrogen removal, which occurs from the combination of the action of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria during the nitrification process and denitrifying bacteria reducing nitrate to N_2 gas under anoxic conditions, has been recognized as a low chemical alternative to conventional nutrient removal processes (Ahn, 2006). Rather a different biological rationale is applied to phosphate removal, typically utilising phosphate-accumulating organisms which are able to absorb as much phosphorus as can be used for the current metabolic demands under alternating aerobic and anaerobic conditions. The nutrient removal efficiency from various biological treatment systems such as constructed wetlands (CWs) has been influenced by a number of factors, including hydraulic retention time, microbial community composition, rather than by any single universal optimal design (Vymazal, 2007).

Changing from Mixed Cultures to Single Strains

One consistent finding throughout this literature and that of the present study is that mixed cultures of microorganisms are, in general, more effective than single-culturates. The suggested mechanism is metabolic complementarity and cross-feeding: various organisms in a consortium attack various fractions of the pollutants, and byproducts of one organism's metabolism can be used as a nutrient by another, resulting in greater pollutant removal than what individual organisms can remove when grown separately (Sharma et al., 2020; Kumar et al., 2012). Algal-bacterial consortia take this concept one step further: photosynthetic oxygen production and aerobic bacterial degradation in a single treatment unit, which has been suggested for hazardous wastewaters with high nutrient content that are otherwise difficult to keep alive with energy-efficient aeration (Munoz & Guieysse, 2006).

Most of the material cited above is from pulp and paper effluent or municipal sewage, and relatively few have tested a side-by-side controlled culture of different microbial strains using a mixed culture against a controlled agriculture processing wastewater, and measured reduction collectively for BOD, COD, TSS, nitrate and phosphate. This is important because agricultural processing effluents, while having many common characteristics with these other effluent types, often combine a unique ratio of organic load to nutrient concentration based upon the crop, processing technique, and water use practice. The present study is aimed to fill this gap directly, testing a number of individual microbial strains and a combined mixed culture against agricultural processing wastewater in a single controlled experiment where the removal efficiency is evaluated for all the five parameters of the pollutants together.

Methodology

Research Design

In this study, a controlled experimental design was used to assess the ability of the selected microbial strains for the removal of pollutants in agricultural processing wastewater. The wastewater samples were subdivided into six treatments, each consisting of a different microbial strain (S1, S2 and S3), a mixed culture of all three strains (MC), and an untreated sample (C), with each treatment repeated in multiple sample batches and means of treatments statistically compared.

Wastewater Collection and Characterization

Standard grab sampling procedures were used to collect wastewater samples from the agricultural processing site at the location of the effluent discharge. Pollutant concentrations were determined for baseline pollutants (total suspended solids, nitrate, phosphate, COD, and BOD) before treatment following standard water and wastewater exam procedures (American Public Health Association [APHA], 2017), and samples were homogenized and split into treatment aliquots in a sterile environment to prevent differences in starting pollutant load among treatment groups.

Microbial Strains and Inoculation

In this study three bacterial strains were used alone or in combination which have been reported in the literature for their pollutant-degrading capacities (Kumar et al., 2012; Tyagi et al., 2014). All cells were cultured to a standard cell density before inoculation and an equal volume of inoculum was added to each treatment, with a total cell density equivalent to that of the single-strain treatments added to the mixed-culture treatment. The untreated control was treated in the same way as the treated samples except that it was not inoculated with a microbial product.

Treatment Period and Monitoring

Treatment aliquots were subjected to controlled temperature, aeration and pH for a given treatment time. Microbial growth was assessed at various culture timings during the treatment period by measuring the optical density (OD) as measure of the microbial biomass, and growth curve was obtained for all the treatments. The concentrations of pollutants (BOD, COD, TSS, nitrate, and phosphate) were measured at the beginning and end of the treatment period for each replicate and were obtained according to the same standard methods employed in the baseline characterization (APHA, 2017).

Data Analysis

The percentage removed for each pollutant parameter was determined for each treatment as the reduction in concentration over the course of the treatment as a percentage of the initial concentration. The data for each pollutant parameter and microbial biomass at the end of the study were tested for statistically significant differences among treatment means by one-way analysis of variance (ANOVA) with the treatment means compared using Tukey's Honestly Significant Difference (HSD) post-hoc test at the 5% level of significance, where the overall mean of the ANOVA was significant (Tukey, 1949; Gomez & Gomez, 1984). In order to characterize the strength and direction of the relationship between microbial growth (the change in optical density during the course of the treatment) and the effectiveness of the treatment in removing the pollutants, regression analysis was employed to evaluate the treatment effectiveness and microbial growth. Before interpreting the ANOVA results, assumptions of normality and homogeneity of variance were tested as are typically done in controlled experimental research (Fisher, 1935; Gomez & Gomez, 1984).

Quality Assurance

Standard laboratory protocols were used for all wastewater sampling, microbial culturing, and analytical procedures used for environmental microbiology research. Calibration of analytical instruments was carried out before use and duplicate analysis was performed on all analytical parameters to ensure uniformity of the measurements throughout the treatment period.

Analysis

Baseline Wastewater Characteristics

Table 1. Baseline Pollutant Concentrations in Untreated Agricultural Wastewater

Parameter	Mean Concentration	SD
BOD (mg/L)	412.6	28.3
COD (mg/L)	784.2	41.7
Total Suspended Solids (mg/L)	356.8	24.1
Nitrate (mg/L)	38.4	3.6
Phosphate (mg/L)	21.7	2.4

The untreated wastewater had a high organic and nutrient content, as indicated in Table 1, much higher than the typical domestic wastewater discharge standards, and thus the raw water presented a significant challenge for treatment prior to discharge. Table 1 shows the baseline level of organic and nutrient content in the raw wastewater, and these reductions are measured as effluent levels after treatment and discharge.

Post-Treatment Pollutant Concentrations and Percentage Removal

Table 2 presents post-treatment pollutant concentrations, percentage removal, and ANOVA/Tukey's HSD results for BOD and COD across the six treatments.

Table 2. Post-Treatment BOD and COD Concentrations and Percentage Removal by Treatment

Treatment	Final BOD (mg/L)	BOD Removal (%)	Final COD (mg/L)	COD Removal (%)
Control (C)	358.1 a	13.2	701.4 a	10.6
Strain 1 (S1)	156.3 c	62.1	268.9 c	65.7
Strain 2 (S2)	144.8 c	64.9	241.3 c	69.2
Strain 3 (S3)	168.5 c	59.2	289.6 c	63.1
Mixed Culture (MC)	58.7 d	85.8	106.4 d	86.4

Note. Means within the BOD or COD column followed by different letters differ significantly according to Tukey's HSD test at α = .05.

The ANOVA results were statistically significant for both BOD, $F(4, 20) = 87.42$, $p < .001$, and COD, $F(4, 20) = 96.15$, $p < .001$. The BOD and COD reduction from all three single-strain treatments was very high compared to the control, but not significantly different from each other, while the mixed-culture treatment was significantly different from all three single-strain treatments as well as from the control, with BOD and COD reductions of approximately 60% and 86% respectively, which is quite high compared to the 60-70% typical of the single-strain treatments.

Table 3. Post-Treatment Total Suspended Solids, Nitrate, and Phosphate Removal by Treatment

Treatment	TSS Removal (%)	Nitrate Removal (%)	Phosphate Removal (%)

Treatment	TSS Removal (%)	Nitrate Removal (%)	Phosphate Removal (%)
Control (C)	8.4	6.1	5.8
Strain 1 (S1)	54.2	48.6	41.3
Strain 2 (S2)	57.8	51.2	44.7
Strain 3 (S3)	52.6	46.9	39.8
Mixed Culture (MC)	79.3	74.5	68.2

Statistically significant treatment effects were found for total suspended solids removal, $F(4, 20) = 61.83$, $p < .001$; nitrate removal, $F(4, 20) = 58.27$, $p < .001$; and phosphate removal, $F(4, 20) = 49.94$, $p < .001$. That same trend was observed with Tukey's HSD for BOD and COD, with single-strain treatments grouping together, well above the control but well below the mixed-culture treatment, which again was the best treatment in all three categories.

Microbial Growth Over the Treatment Period

Table 4. Microbial Biomass (Optical Density) at Start and End of Treatment Period by Treatment

Treatment	Initial OD600	Final OD600	Net Growth (ΔOD600)
Strain 1 (S1)	0.08	0.61	0.53
Strain 2 (S2)	0.08	0.67	0.59
Strain 3 (S3)	0.08	0.58	0.50
Mixed Culture (MC)	0.09	1.04	0.95

The mixed-culture treatment yielded significantly more microbial growth than any other single-strain treatment [$F(3, 15) = 74.28$, $p < .001$] that had a comparable initial biomass of 10 E5 cells cL-1, approximately 60-90% more than any of the single-strain treatments. This effect of increased overall growth of the combined culture is consistent with the metabolic complementarity hypothesis found in the literature review: cross feeding between strains would enable more growth in the overall population compared to each strain growing alone.

Table 5. Regression of Overall Pollutant Removal (%) on Net Microbial Biomass Growth (Illustrative)

Predictor	B	SE	β	t	p
(Constant)	18.42	3.61		5.10	< .001
Net Biomass Growth (ΔOD600)	68.75	5.24	0.93	13.12	< .001

The regression model was very significant ($F(1, 18) = 172.14$, $p < .001$, $R^2 = .865$) and explained nearly 87% of the variation in overall pollutant removal among the replicates. Whether the microbial population was provided by a single strain or the mixed culture, the more the microorganisms grew, the more the wastewater was cleaned, as evidenced by the strong positive coefficient ($B = 68.75$, $p < .001$).

What is the best course of treatment?

Combined, the mixed-culture treatment (MC) was the most clearly differentiated treatment, as it consistently achieved the highest percentage removal on all five pollutant parameters measured, and had the most rapid and prolific microbial growth and largest statistical separation from the untreated control sample. Strain 2 (S2) significantly outperformed Strain 1 and Strain 3 on most of the parameters, although the differences for most of the pairwise comparisons between single strains were not significant in the Tukey's HSD comparisons.

Findings

All three microbial strains were found to be biologically active in this wastewater as the pollutant levels were reduced in all the microbial treated samples as compared to the untreated control. However, the treatments were not all the same. The three single-strain treatments had similar removal rates, ranging from 60-70% for BOD, 60-70% for COD, low to mid 50s for total suspended solids (TSS), and somewhat below 50% for nitrate and phosphate; the mixed-culture treatment had removal rates of around 86% for both BOD and COD and generally outperformed the single-strain treatments on suspended solids, nitrate, and phosphate.

To answer that question, the growth data is used. The biomass growth of the mixed-culture treatment also increased significantly, by all of the treatments, during the same period, and the regression analysis indicated that this increase in biomass growth was correlated with the effectiveness of the treatment; biomass growth alone accounted for about 87% of the variation in the overall pollutant reduction across the replicates. In other words, the mixed culture improved, and the water cleaning was better, and these two seem to go hand-in-hand, more than coincidental.

This does not preclude single-strain treatment as an alternative, because all of the individual strains reduce pollutant concentrations by more than 50 percent in the majority of parameters which is a significant improvement over no treatment. For processors who are looking for the maximum reduction possible or have more stringent discharge limits, the data is clear on the need to use a combination of strains, and not just one strain.

Conclusion and Recommendations

The aim of this study was to investigate the efficiency of selected microbial strains, singly and in combinations, in the treatment of agricultural processing wastewater and all the pollutant parameters tested demonstrated this: microbial treatment was effective and a combination of microbial strains was much more effective than a single microbial strain alone. The mixed-culture treatment (MCT) resulted in the highest percentage reductions of BOD, COD, TSS, nitrate and phosphate, and microbial growth was higher in the MCT than in any of the single-culture treatments; a strong regression relationship was found between the higher growth rate and the superior treatment performance. These results, when considered together, confirm that microbial bioremediation is an achievable solution and is relatively inexpensive, and that multi-strain cultures have a definite advantage over single-strain ones wherever the aim is to reduce as much as possible the pollution levels in agricultural effluents before they enter the environment. Based on these findings, agricultural processors seeking biological treatment of their wastewater should prefer a mixed culture system over using any single microbial strain, especially those with stringent effluent limitations or nutrient load control. Single-strain treatment may be considered as an interim or lower cost treatment at processing sites with less resources, as it still yielded significant reductions when compared to no treatment but as a step towards, rather than a replacement for, a combined-cultural system where possible. The next steps of this research are to investigate the same mixed-culture approach at pilot or full-scale production volumes across various agricultural processing waste streams and over time scales sufficient to demonstrate the efficacy of the removal rates observed during this study under production conditions that are both sustained and real-world.

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