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Influence of indigenous microbial isolates on acidity, salinity and ionic composition of distillery spent wash

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Abstract
Distillery spent wash (DSW) is a major industrial wastewater characterized by high organic and inorganic pollutant loads, making its treatment essential for environmental protection. An experiment was conducted during 2024-25 at the Soil and Water Management Research Unit, Navsari Agricultural University, Navsari, to investigate the effects of microbial inoculants on its physico-chemical properties and ionic composition using a Factorial Completely Randomized Design with three treatments: M_0 (uninoculated control), M_1 (*Aspergillus niger* @ 1 g/L) and M_2 (*Pseudomonas fluorescens* @ 10 mL/L), with five replications. Among the inoculants, M_1 showed greater effectiveness in reducing pH (4.10), electrical conductivity (25.02 dS/m) and bicarbonate content (12,116.73 mg/L), whereas M_2 recorded the lowest chloride concentration (9,325.00 mg/L), with an 8.68 per cent reduction over the control. Incubation period significantly affected pH, EC, ^{0}Brix and chloride, while bicarbonate remained unaffected. The interaction effect was significant only for EC, with the minimum value (24.88 dS/m) observed under M_1D_3. Both microbial isolates showed potential to alter the physico-chemical properties and ionic composition of DSW.

Keywords: Aspergillus niger, distillery spent wash, incubation period, Pseudomonas fluorescens

Introduction
Distillery spent wash (DSW) is a major liquid effluent generated during the production of alcohol from sugarcane molasses. Owing to its complex chemical composition, acidic nature and considerable concentration of dissolved constituents, its treatment and appropriate management are essential to minimize adverse environmental impacts. The characteristics of this effluent are influenced by the raw materials and processing conditions adopted during distillation (Nandy *et al.*, 2002) [3]. Its uncontrolled disposal may alter soil chemical properties, affect nutrient availability and adversely influence plant growth (Agrawal and Pandey, 1994) [1].

Microbial treatment has gained importance as an alternative approach for managing distillery wastewater because of its potential to transform complex organic constituents and modify effluent characteristics (Mohana *et al.*, 2009) [2]. *Aspergillus niger* and *Pseudomonas fluorescens* have the potentiality in microbial degradation of industrial effluents.

In view of these considerations, the present investigation was undertaken to evaluate the influence of indigenous microbial isolates (*Aspergillus niger* and *Pseudomonas fluorescens)* on pH, electrical conductivity, ^{0}Brix and ionic constituents (CO_3^{2-}, HCO_3^- and Cl^-) of distillery spent wash during different incubation periods.

Material and Methods
Distillery spent wash (DSW) was collected in October 2024 from the Distillery Plant of Gandevi Sugar Factory, Ambheta, Gandevi, Navsari district, Gujarat (20.78405° N latitude and 72.98555° E longitude). A 25% spent wash solution was prepared by diluting the raw distillery spent wash with water and was maintained to minimize its toxicity and organic load, thereby providing favorable conditions for microbial growth and metabolic activity. It was transferred into 2.5-L amber-colored glass bottles for experimental studies. The physico-chemical characteristics of the effluent were analyzed using standard analytical procedures.

The experiment was conducted in a completely randomized design with factorial concepts comprising three treatments with five replications. The treatments included uninoculated control (M_0), *Aspergillus niger* @ 1 g L^{-1} (M_1) and *Pseudomonas fluorescens* @ 10 mL L^{-1} (M_2). The samples were incubated at room temperature for 30 days with daily shaking to ensure proper mixing. Samples were analyzed periodically at 10, 20 and 30 days to determine changes in pH, electrical conductivity, ^{0}Brix, carbonate, bicarbonate and chloride content using standard analytical methods.

Table 1: Properties of raw and 25% spent wash

Sr. No.	Parameters	Method of Analysis	Reference	Raw Spent wash	25% diluted Spent wash
1.	pH	Potentiometry	Jackson (1973) [4]	4.46	4.54
2.	EC (dS/m)	Conductometry	Jackson (1973) [4]	50.38	27.36
3.	Brix	Refractometry	Spencer and Meade (1964) [6]	41.5	10
4.	Carbonate mg/L)	Titrimetric Method	Richards (1968) [5]	Ab	Ab
	Bicarbonate (mg/L)			31,873	11,590
	Chloride (mg/L)			24,532	7,937

Results and Discussion

1. Influence of Microbial Inoculation and Incubation Period on pH, EC and ^{0}Brix of Distillery Spent Wash

The significantly higher pH (4.38) was recorded in the uninoculated control (M_0), followed by *Pseudomonas fluorescens* (M_2 - pH:4.24), whereas the lowest pH (4.10) was observed with *Aspergillus niger* (M_1). Regarding incubation period, the maximum pH (4.31) was observed on the 10th day, which gradually declined to 4.20 on the 30th day and remained statistically at par with the 20th day. The reduction in pH may be associated with the production and accumulation of organic acids, such as citric, oxalic and gluconic acids, during microbial metabolism and degradation of organic constituents. The greater acidification under fungal inoculation could be attributed to the organic acid-secreting ability of *A. niger*. Similar observations were reported by Mohana *et al.* (2007) [8], who attributed the decline in distillery spent wash pH to organic acid secretion by fungi and Davidek *et al.* (2006) [7], who observed a decrease in pH during the initial stages of microbial melanoidin degradation. However, the microbial inoculation × incubation period interaction (M×D) was non-significant, indicating independent effects of both factors on pH of spent wash.

Table 2: Influence of microbial inoculation and incubation period on pH of DSW

Microbial Inoculation	pH			
	Incubation period (Day)			Mean (M)
	10th (D_1)	20th (D_2)	30th (D_3)	
M_0	4.43	4.37	4.33	4.38
M_1	4.17	4.05	4.08	4.10
M_2	4.32	4.22	4.19	4.24
Mean (D)	4.31	4.22	4.20	4.24
Source	S.Em. (±)		CD at 5%	CV (%)
M	0.02		0.05	1.49
D	0.02		0.05	
M×D	0.03		NS	

The significantly lowest EC (25.02 dS/m) was recorded under *Aspergillus niger* inoculation (M_1), whereas the highest value (26.56 dS/m) was observed in the uninoculated control (M_0), which was statistically at par with M_2 (26.25 dS/m). Across incubation periods, EC gradually declined from 26.44 dS/m on the 10th day to 25.60 dS/m on the 30th day, which was statistically at par with the 20th day (25.79 dS/m). The reduction in EC may be attributed to microbial assimilation and utilization of dissolved ions, including K, Na and sulphates, along with their transformation into biomass-bound or insoluble forms during microbial growth and metabolism. The interaction effect (M×D) was significant, with the minimum EC (24.88 dS/m) recorded under M_1D_3 and the maximum (27.40 dS/m) under M_0D_1, indicating that the influence of microbial inoculation varied with incubation duration. Similar reductions in EC were reported by Kumar *et al.* (2022) [9] using *Bacillus albus* (3.59 to 2.18 µS/cm) and Venkatachalam and Gounder (2017) [10] during microbial treatment of distillery spent wash (31.1 to 10.4 mS/cm).

Table 3: Influence of microbial inoculation and incubation period on EC(dS/m) of DSW

Microbial Inoculation	EC (dS/m)			
	Incubation period (Day)			Mean (M)
	10th (D_1)	20th (D_2)	30th (D_3)	
M_0	27.40	26.06	26.21	26.56
M_1	25.16	25.03	24.88	25.02
M_2	26.75	26.29	25.72	26.25
Mean (D)	26.44	25.79	25.60	25.94
Source	S.Em. (±)		CD at 5%	CV (%)
M	0.10		0.29	1.49
D	0.10		0.29	
M×D	0.17		0.50	

The ^{0}Brix content was significantly influenced by the incubation period, whereas microbial inoculation and its interaction with incubation duration (M×D) had non-significant effects. The minimum ^{0}Brix (10.21%) was recorded on the 10th day, which was statistically at par with the 20th day (10.32%), while the maximum value (10.63%) was observed on the 30th day. The initial decline followed by an increase in ^{0}Brix may be associated with microbial degradation of complex organic compounds, resulting in the release of soluble substances and sugars into the medium. However, the absence of significant differences among M_0, M_1 and M_2 suggests that the microbial treatments had a limited influence on ^{0}Brix, possibly due to variations in sugar consumption and release during metabolism. In contrast, Misale *et al.* (2019) [11] reported a reduction in ^{0}Brix during microbial treatment of distillery effluent, attributed to microbial utilization of sugars as an energy source for growth and metabolic activities.

Table 4: Influence of microbial inoculation and incubation period on ^{0}Brix content of DSW

Microbial Inoculation	^{0}Brix			
	Incubation period (Day)			Mean (M)
	10th (D1)	20th (D2)	30th (D3)	
M0	10.30	10.43	10.64	10.46
M1	10.16	10.32	10.54	10.34
M2	10.16	10.22	10.70	10.36
Mean (D)	10.21	10.32	10.63	10.39
Source	S.Em. (±)		CD at 5%	CV (%)
M	0.07		NS	2.54
D	0.07		0.20	
M×D	0.12		NS	

2. Influence of Microbial Inoculation and Incubation Period on CO_3^{-2}, HCO_3^- and Cl^- Content of Distillery Spent Wash

Carbonate ions were not detected in distillery spent wash, which may be attributed to its highly acidic nature. Under such acidic conditions, carbonate ions are converted into bicarbonate and dissolved CO_2, preventing their presence in appreciable amounts**.**

The significantly highest HCO_3^- concentration (14,943.20 mg/L) was recorded in the uninoculated control (M_0), followed by *Pseudomonas fluorescens* (M_2; 12,193.07 mg/L), whereas the lowest value (12,116.73 mg/L) was observed under *Aspergillus niger* (M_1), which was statistically at par with M_2. The incubation period and its interaction with microbial inoculation (M×D) had non-significant effects on HCO_3^- concentration, although a gradual increase was observed from 12,249.47 mg/L on the 10th day to 13,666.47 mg/L on the 30th day. This variation may be associated with CO_2 released during microbial decomposition of organic matter, which dissolves in the medium and contributes to bicarbonate formation. The minimum HCO_3^- concentration (10,906 mg/L) was observed under M_1 on the 10th day. The reduction in bicarbonate under microbial treatments may be attributed to microbial-induced acidification and the conversion or neutralization of bicarbonate ions during organic matter degradation. Chandra *et al.* (2008) [12], highlighted the role of microbial activity in bicarbonate reduction through acidification and changes in carbonate-bicarbonate equilibrium.

Table 5: Influence of microbial inoculation and incubation period on HCO_3^- content (mg/L) of DSW

Microbial Inoculation	HCO_3^- (mg/L)			
	Incubation period (Day)			Mean (M)
	10th (D1)	20th (D2)	30th (D3)	
M0	14,062.80	15,712.80	15,054.00	14,943.20
M1	10,906.00	12,557.40	12,886.80	12,116.73
M2	11,779.60	11,741.00	13,058.60	12,193.07
Mean (D)	12,249.47	13,337.07	13,666.47	13,084.33
Source	S.Em. (±)		CD at 5%	CV (%)
M	437.13		1,258.86	12.93
D	437.13		NS	
M×D	757.13		NS	

The significantly lowest Cl^- concentration (9,325.00 mg/L) was recorded under *Pseudomonas fluorescens* (M_2), followed by *Aspergillus niger* (M_1; 9,504.00 mg/L), whereas the highest value (10,210.93 mg/L) was observed in the uninoculated control (M_0), indicating reductions of 8.68 and 6.92 per cent under M_2 and M_1, respectively, over M_0. Regarding incubation period, the minimum Cl^- concentration (9,088.20 mg/L) was recorded on the 10th day, which was statistically at par with the 20th day, while the maximum (10,403.20 mg/L) was observed on the 30th day. The reduction under microbial treatments may be associated with microbial-mediated transformation and bioaccumulation of chloride-containing compounds, whereas its subsequent increase with incubation time could indicate the release or accumulation of chloride ions in the medium. However, the interaction effect (M×D) was non-significant, suggesting independent effects of microbial inoculation and incubation duration. Similar findings were reported by Chandra *et al.* (2018) [13], who observed a 70.68 per cent reduction in chloride (2,746 to 805 mg/L) following bacterial consortium treatment of distillery effluent, and Kumar *et al.* (2022) [9], who reported a decline from 347 to 137 mg/L due to microbial bioaccumulation and enzymatic transformations.

Table 6: Effect of microbial inoculation and incubation period on Cl^- content (mg/L) of DSW

Microbial Inoculation	Cl^- (mg/L)			
	Incubation period (Day)			Mean (M)
	10th (D1)	20th (D2)	30th (D3)	
M0	9,340.80	10,291.00	11,001.00	10,210.93
M1	9,020.00	9,076.00	10,416.00	9,504.00

M_2	8,903.80	9,278.60	9,792.60	9,325.00
Mean (D)	9,088.20	9,548.53	10,403.20	9,679.98
Source	S.Em. (±)		CD at 5%	CV (%)
M	238.53		686.92	9.54
D	238.53		686.92	
M×D	413.14		NS	

Conclusion

This study revealed that microbial inoculation significantly influenced the physico-chemical properties and ionic composition of distillery spent wash during incubation. Among the microbial treatments, *Aspergillus niger* (M_1) was more effective in reducing pH (4.10), EC (25.02 dS/m) and HCO_3^- concentration (12,116.73 mg/L), whereas *Pseudomonas fluorescens* (M_2) showed greater reduction in Cl^- content (9,325.00 mg/L), representing an 8.68 per cent decrease over the uninoculated control. Incubation period significantly influenced pH, EC, ^{0}Brix and Cl^-, with the 10th day recording the lowest ^{0}Brix (10.21%) and Cl^- (9,088.20 mg/L), while the 30th day showed the lowest pH (4.20) and EC (25.60 dS/m). However, HCO_3^- concentration was not significantly affected by incubation duration. The interaction between microbial inoculation and incubation period was significant only for EC, indicating treatment-dependent changes in electrical conductivity over time. Overall, both microbial isolates showed potential to modify the physico-chemical properties and ionic composition of distillery spent wash, indicating their usefulness in microbial treatment for improving its quality before further utilization or disposal.

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