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# Performance Optimization of Microbial Remediation of Dairy Industry Wastewater Using *Moringa Oleifera* Seeds and *Psidium Guajava* Leaves

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*Psidium guajava* Plant (Leaves and Fruits)



*Moringa oleifera* Seeds



## INTRODUCTION

- Egypt produces about 6.301 billion litres of milk annually, and uses about 5.424 billion litres in the dairy industry annually.
- Dairy wastewaters vary widely both in quantity and quality, depending on the type of the system and the methods of operation used.
- It is characterized by a high pollutants load and very rich in dissolved sugars, proteins and fat. It is primarily generated from cleaning operations including equipment cleaning and floor washing.
- Microbial treatment or biodegradation process is well established powerful technique for treating dairy effluents, where microorganisms degrade the organic matter and remove other contaminants.
- A new trend in wastewater treatment is currently being developed that uses more environmentally friendly materials, such as natural organic polymers, which reduces costs, prevents variations in the pH of the treated water, reduces sludge production, and provides greater biodegradability. They can be used on their own or integrated with microbial remediation technology.
- *Moringa oleifera* seeds are used as a primary coagulant in wastewater treatment due to the presence of a water-soluble cationic coagulant protein which is able to reduce the turbidity, chemical oxygen demand (COD), and Total dissolved solids (TDS) of the treated wastewater.
- *Psidium guajava* leaves extract was found able to be used as coagulant, fecal coliform removal and for decreasing values of some heavy metals in wastewater treatment system.

## AIM OF WORKS

The study aimed to investigate the ability of microbial treatment to decontaminate dairy industrial wastewater, in terms of removal capacity and efficiency using indigenous bacteria. Also, the study aimed to investigate, on a bench scale, the impact of natural and environmentally friendly materials, such as *Moringa oleifera* seeds powder and/or *Psidium guajava* leaves extract amendment on microbial efficiency and bioremediation performance of dairy industrial wastewater.

## CONCLUSION

- Removal efficiencies achieved by the proposed microbial consortium modified with the natural plants of MO (3.0 g/L)/ PG (1.5 g/L) achieved much higher removal efficiencies compared to unmodified bacteria or the individual MO or PG with microbial treatment.
- Results also confirmed the important role played by the bioactive compounds extracted from the agro wastes in improving the biological treatment efficiency and producing high quality effluents, complying with the environmental regulations for the safe drainage into open environment or potential reuse.

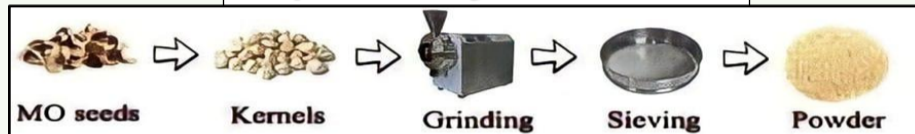
## RECOMMENDATIONS

The proposed biological treatment modified with *Moringa oleifera* powder and *Psidium guajava* leaves extract in combination is highly recommended for decontamination of dairy wastewater and similar streams due to its high capacity for removing large amounts of organic matter, the major contaminants in dairy wastewater.

## MATERIALS & METHODS

- Water samples were collected from a dairy factory in New Borg Al-Arab city, Alexandria Governorate, Egypt during the study.
- Three indigenous bacteria (R1 & R2 & R3) were investigated for the remediation of dairy wastewater (screening bioassay) as free living, to select the most promising isolate(s). The 3 strains were molecularly identified.
- According to the screening bioassay, R1 & R3 showed the best removal efficiency, therefore, they were investigated in a batch mode as individual and mixed cultures. The mixed culture (R1 & R3) that showed the highest remediation ability of dairy effluent was selected for further remediation bioassays.
- *Moringa oleifera* seeds powder and *Psidium guajava* leaves extract were prepared and their effects on bacterial remediation (using R1 & R3 mixed culture) were investigated individually at 1, 2, 3 and 0.5, 1, 1.5 g/L, respectively. Then their optimum doses of 3.0 and 1.5 g/L, respectively were investigated at room temperature in combination with the bacterial remediation to evaluate their effects in enhancing microbial treatment and compared with their control samples under the same conditions.
- Residual concentrations (RCs) or density (CFU/mL) of quality parameters {pH, BOD, COD, TSS, TDS, O&G and total viable count of bacteria (TVCB)} were determined at each exposure time in the raw and treated wastewater and their removal efficiencies (REs%) were calculated.
- The efficiency and feasibility of the proposed treatments towards the tested industrial wastewater were discussed and compared.

### Preparation of *Moringa oleifera* Seeds Powder



Dairy Wastewater Cultures during the Batch Microbial Treatment (Mixed Culture of R1 & R3) Integrated with *Moringa oleifera* Seeds



Dairy Wastewater Cultures during the Batch Microbial Treatment (Mixed Culture of R1 & R3) Integrated with *Psidium guajava* Leaves Extract



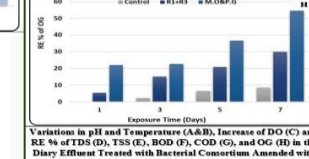
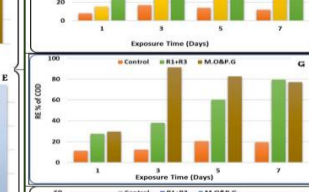
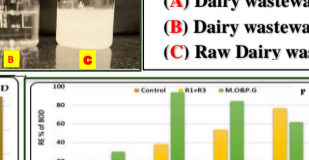
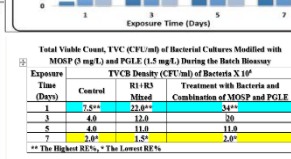
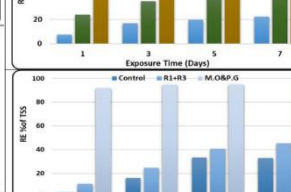
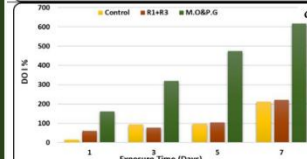
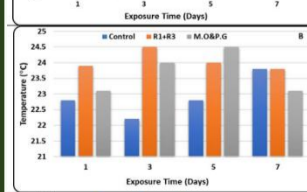
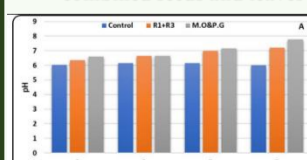
Dairy Wastewater Cultures during the Batch Microbial Treatment (Mixed Culture of R1 & R3) Integrated with *Psidium guajava* Leaves Extract and *Moringa oleifera* Seeds (In Combination)



Preparation *Psidium guajava* Leaves Extract

## RESULTS

1. **Screening Bioassay**
  - Raw dairy wastewater contained very high levels of COD (7360 mg/L) and BOD (5152 mg/L), and a high level of TSS (1523 mg/L) confirming high organic and suspended matter load. It also contained high levels of TDS (4153 mg/L) and Oil & Grease, OG (1260 mg/L) and low DO levels (1.29 mg/L) due to high consumption during microbial oxidation demand for organic content.
  - Isolates R1 and R3 showed the highest activity for selective removal of the tested pollutants during the treatment screening test of the dairy effluent. Therefore, they were selected to precede together with the following bioremediation assays of the contaminated dairy effluents.
2. **Batch Mode Treatment**
  - Raw dairy wastewater contains very high levels of COD (5165 mg/L), BOD (3408 mg/L) and high levels of TDS (2959 mg/L), intermediate levels of TSS (1033 mg/L) and OG (984 mg/L) and low DO level (0.99 mg/L).
  - The mixed culture (R1 and R3) together achieved the highest removal efficiency of BOD (71.03%), COD (70.95%), OG (19.20%), TSS (38.52%) and TDS (50.18%) as well as DO increase (389.89%).
  - R1 and R3 mixed culture proved to be the most promising for either minimization or decontamination of pollution load (mostly organic) from dairy wastewater. Therefore, they were molecularly identified and selected and modified with *Moringa oleifera* seeds and *Psidium guajava* leaves extract and examined to remediate the contaminated dairy wastewater.
3. **Molecular Characterization of the Tested Bacteria**
  - R1, R2 and R3 were molecularly identified as *Bacillus cereus* ATCC 14579, *Bacillus aureus* strain 24K and *Bacillus cereus* ATCC 14579 with accession numbers of NR-074540.1, NR-118439.1 and NR-074540.1 and similarity of 99.64, 99.36 and 98.96% to the closest neighbours.
4. **Integrated Microbial Treatment with *M. oleifera* Seeds Powder (MO) and *Psidium guajava* Leaves Extract (PG) on Individual Basis**
  - Raw dairy wastewater contains very high levels of COD (8010 mg/L), BOD (2243 mg/L) and a high level of TSS (1903 mg/L), high levels of TDS (2510 mg/L), OG (743 mg/L) and low DO level (0.59 mg/L).
  - MO seeds powder-modified culture at 3.0 g/L achieved the highest removal efficiency of BOD (85.20%), COD (84.88%), OG (35.40%), TSS (71.44%) and TDS (84.30%), as well as DO increase of 330.51% compared to their controls.
  - PG leaves extract-modified culture at 1.5 g/L achieved the highest removal efficiency of BOD (87.87%), COD (80.09%), OG (42.93%), TSS (84.81%) and TDS (67.65%) as well as DO increase (533.90%) compared to their controls.
5. **Integrated Microbial Treatment with *M. oleifera* Seeds Powder (MO) and *Psidium guajava* Leaves Extract (PG) in Combination**
  - Raw dairy wastewater contains very high levels of COD (12550 mg/L), BOD (5211 mg/L), high levels of TSS (912 mg/L), OG (317 mg/L), TDS (1621 mg/L) as well as low DO level (0.68 mg/L).
  - The MO/PG-modified bacterial mixture (R1+R3) achieved the highest RE of BOD (93.84%), COD (91.31%), OG (54.57%), TSS (94.85%) and TDS (87.79%) as well as DO increase (616.18%).
  - The control (unseeded wastewater) showed the lowest activity towards the dairy effluent's contaminants confirming the superiority of the combined seeds and leaves extract with bacteria.



(A) Dairy wastewater treated by bacterial strain only  
(B) Dairy wastewater treated by bacterial strain with MO&PG  
(C) Raw Dairy wastewater

Name	Molecular Formula	Mol. wt.	Area	Int.	R. Time	EDH	
1-Butanol, 2-methyl-, (p)	C <sub>8</sub> H <sub>18</sub> O	126	234688	41.00	3.8214	1	
Caproic acid	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>	114	46646	119.00	11.157	2	
Bicyclo[2.2.0]hexane-4-one, 4,1,1-trimethyl-, 5-methylene-, (E)	C <sub>12</sub> H <sub>20</sub>	152	204	166.00	41.00	11.618	3
1H-Cyclopropylidene, acetyleno-1,1,2-trimethyl-, 4-methyl-, (E)	C <sub>12</sub> H <sub>20</sub>	152	304	832.00	41.00	11.793	4
Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylbutyl)-	C <sub>20</sub> H <sub>30</sub>	274	174	133.00	139.00	12.465	5
1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	C <sub>22</sub> H <sub>40</sub> O	324	222	587.00	49.00	12.643	6
Caryophyllene oxide	C <sub>15</sub> H <sub>24</sub> O	204	230	843.00	43.00	13.077	7
1H-Cyclopropylidene, 4-oxo-, dimethyl-, 1,1,4,7-tetraethyl-, (E)	C <sub>18</sub> H <sub>30</sub>	238	204	535.00	136.00	13.491	8
Hexadecanoic acid, ethyl ester	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	266	284	401.00	89.00	15.729	9
9-Hexadecenoic acid, 9-oxo-, ethyl ester, (E)	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	264	304	465.00	55.00	16.030	10
9-Octadecenoic acid (Z)-, 9-oxo-, ethyl ester, (Z)	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	274	332	1892.00	55.00	16.723	11
ethyl Octanoate	C <sub>10</sub> H <sub>20</sub> O <sub>2</sub>	158	310	354.00	58.00	17.286	12
Octyl alcohol, heptafluorooxybutyl ether	C <sub>18</sub> H <sub>33</sub> F <sub>7</sub> O	444	444	—	—	—	13
Octyl alcohol, tetrafluoroethyl ether	C <sub>18</sub> H <sub>33</sub> F <sub>4</sub> O	384	213.00	69.00	49.444	14	
Cyclohexanemethanol	C <sub>6</sub> H <sub>12</sub> O	98	118	657.00	55.00	49.707	15

Total Viable Count, TVC (CFU/ml) of Bacterial Cultures Modified with MOSP (3 mg/L) and PGLE (1.5 mg/L) During the Batch Bioassay

Exposure Time (Days)	Control	Mixed Culture of R1 & R3	Combination of MOSP and PGLE
1	2.0e+08	2.0e+08	2.0e+08
3	4.0e+08	1.0e+08	1.0e+08
5	6.0e+08	1.0e+08	1.0e+08
7	8.0e+08	1.0e+08	1.0e+08

• The Highest RE%, • The Lowest RE%

Variations in pH and Temperature (A&B), Increase of DO (C) and RE % of TDS (D), TSS (E), BOD (F), COD (G), and OG (H) in the Dairy Effluent Treated with Bacterial Consortium Amended with MOSP (3 mg/L) and PGLE (1.5 mg/L)