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Reduction of turbidity and Escherichia coli ATCC 25922 in wastewater of dairy industry after treatment with Moringa oleifera LAM

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Abstract*— Several liquid wastes are generated daily in the agribusiness sector. In small dairy industry for example, the main concern is the volume of treated water used for less noble purposes within the company and the difficulty in wastewater treatment before disposal in receiving water bodies. Thus, this study aimed to evaluate the reduction of turbidity and Escherichia coli in synthetic agribusiness wastewater after treatment with Moringa seed extracts for reuse purposes and/or disposal. The extracts at a concentration of 20 mg L^{-1}, 40 mg L^{-1}, 60 mg L^{-1} and 80 mg L^{-1} were applied in wastewater made with reconstituted whole milk. Turbidity analysis and counts of contamination bioindicators were performed in the three repetitions. The extract of lower concentration proved to be undesirable for this treatment because it has increased the turbidity and not showed effectiveness against E. Coli to the treated samples. The results showed a turbidity reduction of 87.9% for effluents treated with 80 mg L-1 extract. The concentrations of 60 mg L^{-1} and 80 mg L^{-1}, in a real situation, would be appropriate for the treatment of effluents with turbidity < 240 NTU, for later release on Class II and III water bodies. Similar to the turbidity test, after T_{IV}-MO80mg L-1 treatment decreased the population density of E. coli to, approximately, 2.2 x 10^2 UFC 100 mL^{-1}. The routine work using cleaner production, combined with the treatment of wastewater from natural coagulant, tend to minimize the negative impacts of such waste on the environment.

***Keywords*— dairy, moringa oleifera, plant extract, wastewater.**

I. INTRODUCTION

A great part of Brazilian dairy companies are small and have difficulties in managing and treating their wastewater. Thus, while the activities of the dairy industry represent an important economic source [1], they have become a significant source of pollution of water resources from effluents generated in their production processes, equipment cleaning and environments [2].

The effluents generated by agribusiness, due to its characteristics, when released untreated into receiving water bodies, tend to change certain parameters such as Biochemical Oxygen Demand - BOD, dissolved oxygen, hydrogen potential, temperature and turbidity.

NEC Resolution 430 of 2011 defines a number of parameters that must be observed for the discharge of effluents into receiving water bodies, without changing their quality class [3]. Whereas NEC Resolution 357 of 2005 define quality classes in the water bodies [4]. According to this resolution most of water bodies in Brazil are within 2 and 3 quality classes that allows turbidity value to have 100 nephelometric turbidity units – NTU, and the presence of coliforms depending on the purpose of the water, varying 1000 - 4000 coliform per100 milliliters.

Efficient wastewater treatment can become possible its reuse in less noble purposes that do not require drinking water, monitoring of turbidity [5] and fecal coliform [6]. To this purpose, chemical coagulants such as aluminum and iron salts and following by chlorine are commonly used. Although the efficiency gains trigger a number of harmful side-effects such as Alzheimer's disease and other neuropathologies [7]. Due to these significant toxic effects, the treatment of wastewater constitutes a public health problem [8].

In contrast, natural coagulants such as Moringa Oleifera are presented as a promising alternative to different

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effluent treatment in order to remove turbidity [9], [10], [11], [12], [13], [14], [15], [16], [17].

She has a protein with cationic property with high molecular mass [18], [19] that destabilizes negatively charged particles dispersed in the liquid medium, flocculating them, resulting in improving turbidity.

Regarding the inhibiting pathogens properties of Moringa Oleifera many studies have been done on different types of pathogens [20], [21], [22], [23], [24]. In this studies were evaluated the antibacterial properties in vitro tests with different ways of extraction, such as: aqueous, ethanol, methanol and chloroform extracts.

Natural coagulants have low biological risk because they are biodegradable and present a decrease in the volume of residual sludge [25]. In addition the reduction of the raw material in the environment reduces the availability of carbohydrates and protein to microorganisms that are likely to develop the dairy effluent [24] and consequently microbial growth.

In this context, this study aimed to evaluate the clotting action of Moringa oleifera Lam. extract in reducing Escherichia coli and turbidity in effluent from the dairy industry (SDE) to later disposal in water bodies and / or reuse.

II. METHODS

2.1 Preparation of synthetic wastewater

The preparation of the effluent consisted of a basic mix of reconstituted whole milk (LIR) of Itambé® (main components are shown in TABLE 1) and distilled water.

Table 1: Chemical composition[1] of whole milk powder instant Itambé®.

Constituents	Amount (grams) [2]
Carbohydrates	9.6
Protein	6.7
Total Fat	7.1
Saturated Fat	4.4

[1]Constituents with the highest concentration; [2]Amount corresponding to 26 grams of product.

The mix aimed to obtain a synthetic effluent turbidity of 240 NTU (nephelometric turbidity units), similar to dairy effluent. The synthetic effluent was homogenized for 30 minutes at room temperature using magnetic stirring. The turbidity of the effluent was measured by a turbidimeter Del Lab®; each sample was sterilized by moist heat for 15 minutes at 121 ºC and after reaching ambient temperature the reading was taken.

2.2 Obtaining aqueous extract of Moringa oleifera Lam.

The pods of Moringa oleifera Lam. used to prepare the aqueous extract were obtained from a species cultivated in the city limits of Catalão - GO. These were placed in plastic bags and sent to the laboratory in Federal University of Goias - Regional Catalão.

The bark and pods were removed, the seeds were ground at room temperature in a household mixer for 5 minutes. Then 30 grams of the powder was weighed and 150 mL of distilled water was added to give a stock solution of 200 mg mL-1. Subsequently, the mixture was filtered on Melitta® paper (No. 103) and then filtered on membrane units consisting of cellulose ester (MEC) with 0.45 mm in porosity, and wrapped in sterile Boeco® bottle.

2.3 Standardization of inoculum

The overnight culture of Escherichia coli ATCC 25922 was standardized in similar turbidity to McFarland nephelometric scale tube 0.5 [27] and subsequently serially diluted to the standardized inoculum of 103 cells in final volume of 500 mL synthetic effluent. This cell concentration was used to simulate an effluent to be disposed of in Class 3 freshwater [3].

2.4 Coagulant action of Moringa oleifera Lam.(MO) extract

The synthetic effluent was treated with 4 different concentrations of Moringa Oleifera Lam extract. Thus, aliquots equivalent to 50 µL, 100 µL, 150 µL and 200 µL were pipettes in final volume of 500 mL of effluent, resulting in treatments with equal concentrations. at 20 mg L^{-1}, 40 mg L^{-1}, 60 mg L^{-1} and 80 mg L^{-1}, respectively. The control group consisted of coagulant-free synthetic effluent.

Control groups underwent the same operation conditions for comparison to the treated samples (TABLE 2).

Table 2: Tests with the aqueous extract of Moringa oleifera Lam.seeds.

Group	Description
Control I - C_I	Becker presenting RWM
Control II - C_{II}	Becker presenting RWM + 1 mL *E. coli* ATCC 25922
Treatment I - T_I	Becker presenting RWM + 1 mL *E. coli* ATCC 25922 + $MO_{20mg\,L^{-1}}$
Treatment II -	Becker presenting RWM + 1 mL *E. coli*

T_{II}	ATCC 25922 + $MO_{40mg\,L^{-1}}$
Treatment III - T_{III}	Becker presenting RWM + 1 mL *E. coli* ATCC 25922 + $MO_{60mg\,L^{-1}}$
Treatment IV - T_{IV}	Becker presenting RWM + 1 mL *E. coli* ATCC 25922 + $MO_{80mg\,L^{-1}}$

RWM: Reconstituted whole milk; MO: Aqueous extract of *Moringa Oleifera* Lam.

The containers were previously marked on their external faces, to define two sampling levels (LI: lower referring to markings between 200 mL and 300 mL, LII: upper referring to markings between 300 mL and 400 mL). Each level was taken 100 ml of the sample which were duly packed in sterile bottles for further analysis.

2.4.1 Turbidity determination

The method used was the nephelometric. Thus, 20 mL of each SDE were transferred to specific containers of equipment that had been previously calibrated with standards (< 0.10 NTU, 10 NTU 100 NTU to 1000 NTU) before reading the samples that occurred in triplicate.

At the end of each reading, the container was washed with a brush, followed by decontamination with 70% alcohol, rinsed with distilled water and kept at rest until complete drying.

Comparisons between treatment and control groups were performed at the relative concentration.

2.4.2 Enumeration of Escherichia Coli ATTCC 25922

For the analysis, 1 ml of each sample was transferred into sterile plates followed by the addition of Eosin Methylene Blue Agar - EMB previously melted and cooled to 45 ºC. After homogenization and solidification, the plates were incubated at 37 °C for 48 hours. Then the Colony Forming Units (CFU) and the results expressed CFU per 100 mL^{-1} [28].

2.5 Data analysis

The averages for the parameters of fecal coliform and turbidity in triplicate were calculated. The results obtained for the contamination bioindicators were compared to the parameters applicable to non-potable water for reuse purposes [6] and receiving water body classes for effluents to be discarded [4]. For the turbidity analysis the mean scores were evaluated and analyzed for reuse purposes [5] and disposal [4].

III. RESULTS AND DISCUSSION

The results of the turbidity values and population density of E. coli ATCC 25922 in the synthetic effluent were relatively promising after treatment with the aqueous extract of Moringa oleifera Lam. - MO.

In general the average scores for turbidity in "LI" were higher than those recorded in "LII" (TABLE 3).

Table 3: Turbidity results (NTU) obtained after stirring in jar test.

Group	Level I	Level II
C_I	312.22	274.66
C_{II}	265.89	263.78
T_I	502.00	491.78
T_{II}	358.44	364.59
T_{III}	94.28	68.16
T_{IV}	49.59	33.23

In TABLE 3, we can see that the average values of turbidity in the control group "CI - RWM" were 312.22 NTU (LI) and 274.66 NTU (LII). After treatment "TI - MO $_{20mg\ L-1}$", there was an increase in turbidity for "LI" (502 NTU) and "LII" (492.77 NTU). Considering that the coagulant protein of Moringa Oleifera seeds neutralize the suspended particles coagulating the negatively charged colloids through subsequent flocculation and it is believed that the observed values "LI" are due to the deposition and / or presence of organic matter from the seeds of Moringa Oleifera in the sedimentation process during collection.

In TABLE 4 the variation of turbidity for the different levels and treatments is shown.

Table 4: Concentration relative (%) of change in turbidity after stirring in jar test and Moringa Oleifera Lam treatment at different concentrations.

Group	Level I	Level II
C_{II}	14.84	3.96
T_I	-60.78	-79.05
T_{II}	-14.80	-32.74
T_{III}	69.80	75.18
T_{IV}	84.12	87.90

As can be observed in TABLE 4, the increase in turbidity was equal to 60.8% for "NI" and 79% for "NII". Similar to the "TI" the "TII" had the opposite of expected. There was an increase in "NI" (14.8%) and "NII" (32.7%).

Better reduction of turbidity from the "TIII" and "TIV", especially the latter ("NI": 84.10%; "NII": 87.9%) was observed. Both treatments were effective to remove organic matter in the synthetic effluent, leaving it appropriate to discharge into a water body, since the turbidity was less than 100 NTU [4]. The lowest average

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score for turbidity was detected in level II after "TIV" (33.23 NTU). However, this concentration is not enough after the treatment of wastewater for reuse purposes, as parameters presented by [5]. In order to increase the reduction efficiency of turbidity can be used Moringa Oleifera seed extract associated with others natural or chemical coagulants as tested [15], [16] for concrete wastewater.

In general, the largest reduction of population density of E. coli ATCC 25922 was observed in "NII" (Fig. 1).

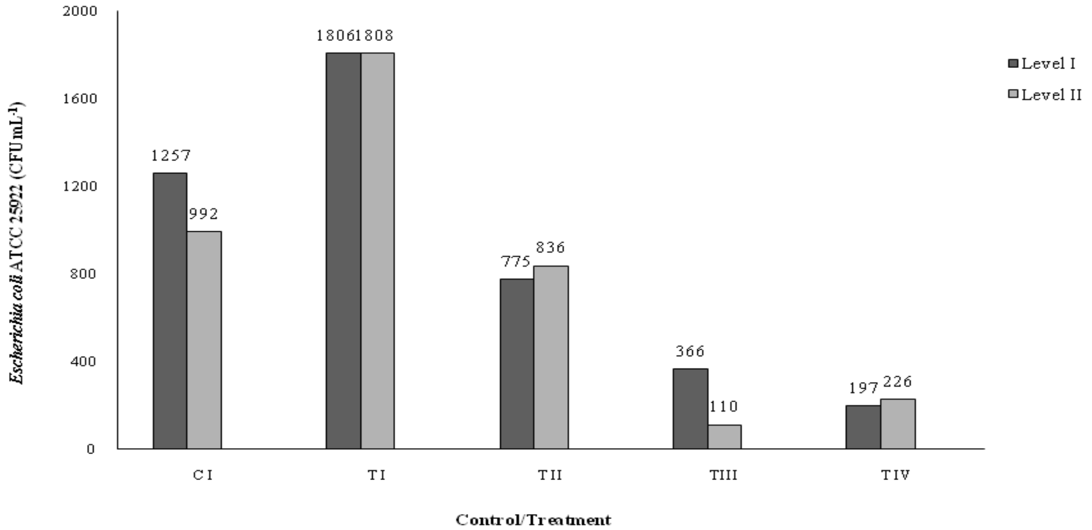

Fig.1: Escherichia coli ATCC 25922 (CFU 100 mL^{-1}) count in the samples after stirring jar test in synthetic wastewater and treatment with Moringa oleifera Lam at different concentrations.

Similar to turbidity tests after "TI " and "TII " reduction of Escherichia coli ATCC 25922 was not observed (Figure 1). In this study it likely the suspended organic matter arising from Moringa Oleifera seeds of 20 mg L^{-1} extract was assimilated by the microorganism test, resulting in an increase in population [29] and [22] not observed significant bactericide effect in vitro test for doses around 20 mg L^{-1}. In this extract, an increase in average scores was observed on two levels ("NI" and "NII": 1.8 x 10^{3} UFC 100 mL^{-1}) compared to the "CII".

Several studies highlight the importance of Moringa Oleifera in inhibiting pathogens [30], [20], [31], [32], [33], [22], [23], [24], [34]. [23] and [34] observed that aquaous extract of Moringa oleifera exhibited a zone of inhibition against E. coli Whereas [33] observed that E. Coli tested against seed ethanol extract and found to be sensitive at 50 g L^{-1}. Although in this study the results did not differentiate from those obtained by [21] that verified the resistance in vitro of Escherichia coli ATCC 25922 to the action of Moringa Oleifera Lam. This situation can be explained since different strains have different resistances and although [33] have tested the same species they used different extract in a dosage more than six times our highest dosage. In addition it is important to highlight that the efficiency of any bactericide in a real situation of a wastewater treatment it is much smaller than in vitro situation. This happens because the particles presents in wastewater protect the microorganism from bactericide action.

The largest decreases were found for "TIII " and "TIV", especially the latter ("NI": 1.7 x 10^{2} CFU 100 mL^{-1}; "NII": 2.2 x 10^{2} CFU 100 mL^{-1}). Even with this reduction, in a real situation the highest concentration of extract was not adequate to ensure the absence of Escherichia coli ATCC 25922 in a hundred milliliters of effluent; therefore it would be inappropriate for reuse purposes [6].

Based on the results obtained relating to turbidity and the microbial bioindicator count, those treated effluent concentrations of 60 mg L^{-1} and 80 mg L^{-1}, in a real situation could be disposed of Class II and III receptor in the water bodies [4].

IV. CONCLUSIONS

With the results it was possible to conclude that:

- The concentration of aqueous extract of Moringa Oleifera lam. 20 mg L^{-1} contributed to increase of the turbidity due to the accumulation of organic matter in the trials.

- The lowest average values of turbidity and microbial cell count occurred after application of aqueous extract of Moringa Oleifera lam. of 80 mg L^{-1} .

- none of the treatments was effective for adequacy of effluent for reuse purposes within the dairy industry, however it can be used in less noble activities in other sectors as water gardens and wash external areas.

- Concentrations of 60 mg L^{-1} and 80 mg L^{-1} demonstrated effectiveness in the adequacy of the effluent for disposal purposes in water bodies class 2 and 3.

- The aqueous extract of Moringa Oleifera lam. posses inhibitory effect against many types microorganism as the studies have shown, but against E. Coli specifically, even in vitro, the dosage is much higher than those tested in this work. So is imperative that new other tests with higher dosages be carry out in wastewater treatment.

- Adoption of strategies for cleaner production, combined with the treatment of wastewater from natural coagulant, can minimize the negative impacts of such waste in the environment.

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