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Evaluation of an Efficient Microorganism Product for the Treatment of Wastewater Discharged into Havana Bay, Cuba

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ABSTRACT

Nature-based solutions to mitigate the effects of wastewater discharge on the environment, including efficient microorganisms, are gaining ground among technologies for this purpose. The effectiveness of the efficient microorganism product ME Agroambiental® (MEA®) in mitigating the impact of industrial wastewater discharges into Havana Bay was evaluated. In the first half of 2022, composite samples were taken at the discharge points of three industries (with one having two wastewater discharges) dedicated to food production during peak discharge hours, with control samples taken before the application of MEA® and follow-up samples collected one month post-application. The samples were analyzed for biochemical oxygen demand (BOD_5), chemical oxygen demand, total coliforms, and thermotolerant coliforms. The results indicated a significant reduction in pollutant loads, with BOD_5 reductions ranging from 77% to 94.2% and COD reductions between 64% and 87.4% across the evaluated companies. The MEA® product demonstrated particular effectiveness in treating wastewater from kitchens, sewers, grease traps, and industrial processes, achieving up to 98.3% reduction in specific contaminants such as organic waste and fats. These findings suggest that MEA® is a viable solution for reducing pollution in Havana Bay.

Keywords: *wastewater treatment, efficient microorganisms, ME Agroambiental®, Biochemical Oxygen Demand, Chemical Oxygen Demand*

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INTRODUCTION

Wastewater treatment is essential to prevent anthropogenic pollutants, concentrated in densely populated areas or where industries generate waste containing pollutants that affect the environment, from entering ecosystems. However, many conventional treatment systems do not remove certain contaminants, such as those of emerging concern. Even treatment facilities with effective pollutant removal methods, if not properly operated and maintained, are not capable of removing pollutants from municipal or industrial wastewater (*Singh et al. 2023*). Furthermore, approximately 50% of the wastewater is discharged untreated (*Shemer et al. 2023*). On the other hand, the reuse of water for agriculture and other consumptive uses, such as supplying cities with growing populations, requires appropriate technologies, according to the specific use that will be given to the treated water, which allows for the adoption of more sustainable approaches by alleviating the pressure on available water resources (*Silva 2023*).

Thus, in the context of population growth, pollution and scarcity of water resources, and climate change, it is clear that the problem of wastewater treatment requires effective, cost-effective, and energy-efficient solutions that are attractive to the population and local authorities, ensuring the quality and environmental services of ecosystems for future generations (*Lako and Como 2024*). Nature-based solutions are an attractive options for wastewater treatment. One such solution is the use of efficient microorganisms (EMO), a feasible and economical technology for treating wastewater that was developed during the 1970s at the University of Ryukyus, Okinawa, Japan (*Ezeagu et al. 2023; Kaur et al. 2024*).

Efficient Microorganisms are defined as mixed cultures of beneficial and natural microorganisms that can be used as material for numerous biotechnological applications. An EMO is a natural fermentation product that is not chemically or genetically modified in the form of a concentrated solution. The main components of an

EMO are lactic acid bacteria, yeasts, and photosynthetic bacteria, with aerobic and anaerobic bacteria coexisting (*Safwat and Matta 2021; Celebi et al. 2024*). They are capable of removing pathogens in urban wastewater (*Begmatov et al. 2023*), combating eutrophication of reservoirs (*Tomczyk et al. 2023*), reducing contamination indicators of food industry waste to acceptable levels (*Velmurugan and Pandian 2023*); improving microbial activity in degraded agricultural soils by improving plant productivity (*Li, J 2024*), among other applications and have become part of the stock of microbiological methods available for waste treatment (*Sharma 2023*); and have improved the microbial activity in degraded soils enhancing plant productivity (*Li et al. 2024*).

Coastal ecosystems have always been attractive for human settlements. Approximately 37% of the population now lives within 100 km of the coastline, which has allowed the dumping of waste of all kinds, often untreated, affecting their valuable ecosystem services, an issue that has been pointed out for years (*UNEP 2007, Li et al. 2024*). In the case of the enclosed or pocket bays, their characteristics, with a narrow mouth that prevents the dispersion of pollutants to the open sea, make them especially sensitive to anthropogenic pollution (*Alkhalidi et al. 2023, Morell-Bayard et al. 2024*). This is precisely the case of Havana Bay, affected by hydrocarbon and industrial waste inputs, as well as municipal discharges, which led it to become one of the most polluted bays in Latin America (*Gelen et al. 2005; Degavre 2022*).

The objective of this work is to show the effectiveness of using EMO, specifically the ME Agroambiental® product (MEA®), to mitigate the impact of food processing industrial waste in Havana Bay.

MATERIALS AND METHODS

Characteristics of the Study Area and Industries

Havana Bay, Cuba is classified as a deep-water pocket bay with a protected port. It has a surface area of 5.2 km^2 and an average depth of 9.20 m. It receives an average freshwater flow of 4.00 m s^{-1}, coming from a catchment area of approximately 68 km^2 (G*elen et al. 2005; Degavre 2022*). Several industries are located on its shores, which discharge their waste with little or no treatment, three of which were selected, all dedicated to food production: EA1– for the production of edible oils and fats, EA2– for the production of sausages, selecting two processing centers of the same EA2A and EA2B, which have independent waste discharge points, and finally, EA3– for the production of various foods (**Figure 1**).

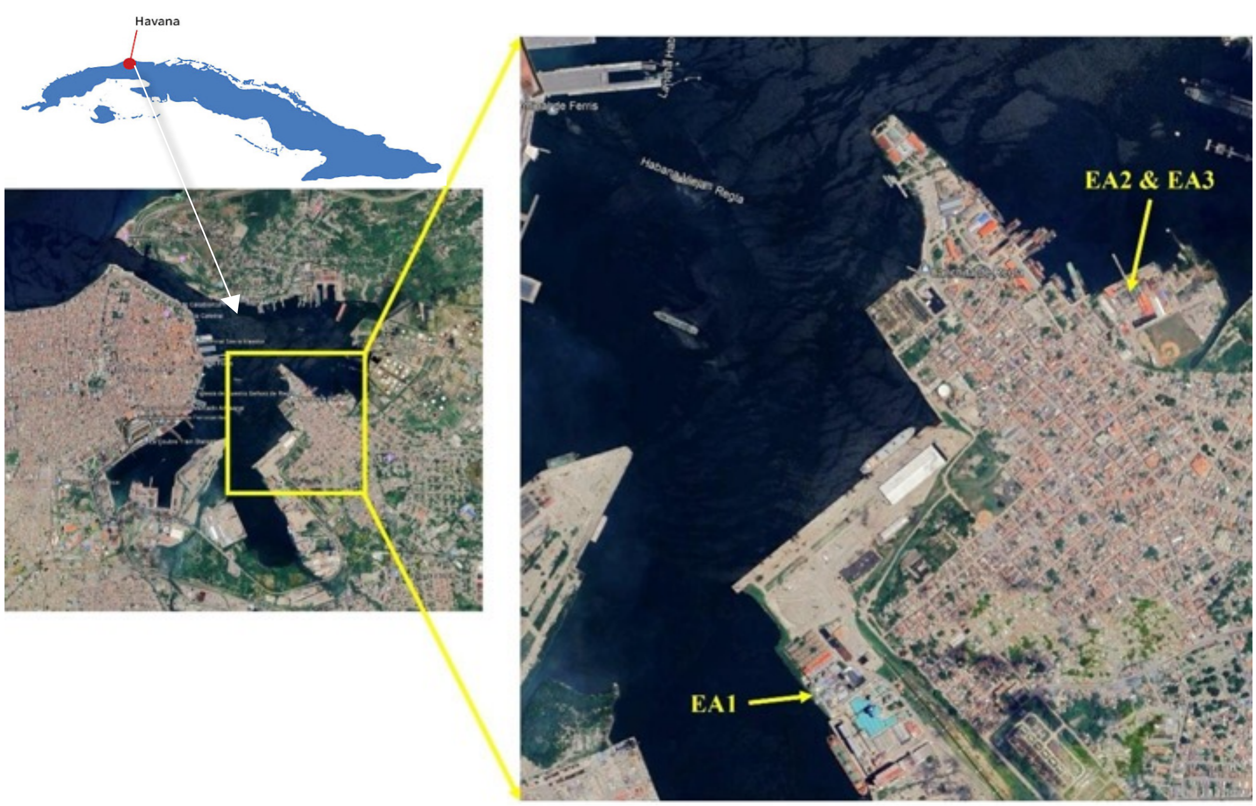

Figure 1. Havana Bay, Cuba and location of the studied industries.

Application Method of MEA®

The product is applied directly to the liquid waste to be treated. A shock or initial dose is applied only once, at a rate of 2 L of MEA® m^{-3} of reservoir capacity and a contaminant load ≤ 1000 mg L^{-1} of BOD_5 or 4 L of MEA® m^{-3} of reservoir capacity and a contaminant load ≥ 1000 mg L^{-1} of BOD_5. After that, a maintenance dose is directly applied, every three days at a rate of 1 L of MEA® m^{-3} of reservoir capacity and a contaminant load equal to or less than 1000 mg L^{-1} of BOD_5, or 2 L of MEA® m^{-3} of reservoir capacity and a contaminant load equal to or greater than 1000 mg L^{-1} of BOD_5.

Sampling

In each industry, on production days, composite samples were taken as described in Cuban Standard NC 27:2012 (from different sites and depths of the wastewater retention reservoirs during peak production hours). In each company evaluated, two groups of five composite samples were taken: one over the course of four months before the application of MEA®, considered as control samples, and another of five samples taken on five consecutive days one month after the application of the product, a practically useful time that allows the effects of EMO to be evaluated.

Analysis

The samples collected were sent to the laboratories of the Hydraulic Resource Analysis and Technical Services Company (ENAST), accredited in: ISO 9001: 2021 and ISO/IEC 17025, complying with the Operational Work Procedures, Good Laboratory Practices, and the provisions of the Quality Management System of these laboratories. Chemical oxygen demand (COD) was determined by the Potassium Dichromate method, biological oxygen demand (BOD_5) by the titrimetric method and total coliforms (CT) and thermotolerant coliforms (TTC) according to NC 1095:2015: Water Microbiology- Detection and enumeration of Coliform, most probable number (MPN) technique, multiple tube procedure. Temperature and pH were also monitored, considering that these variables can influence the activity of microorganisms and the interpretation of the results. Given the intermittent nature of the production processes, flow measurements were not taken. The obtained results were compared with the Average Maximum Permissible Limits (LMPP) of the current standard for waste discharge according to the classification of the receiving body in the Cuban Mandatory Standard NC 27:2012 (2012). Discharge of Wastewater into Terrestrial Lagoons and Sewers and *NC 521: 2007* (2007). Discharge of Wastewater into the Coastal Zone and Marine Waters.

RESULTS AND DISCUSSION

Sample Characteristics

To determine whether treatment with efficient microorganisms (EMO) was suitable in the control samples of each industry, biodegradability (BioD) was determined and calculated from the BOD_5/COD ratio. If BioD ≥ 0.4, the water is considered to be highly biodegradable, if BioD ≤ 0.2, it is considered to be moderately biodegradable, and if BioD < 0.2, the wastewater is slightly biodegradable or not biodegradable (**Figure 2**).

It can be concluded that the treatment with EMO is viable and that, a broad spectrum of BOD5 and COD values was studied, which allows to affirm the validity of this treatment for similar industries (**Figure 2**). In all control samples, the presence of TC and TTC was detected in average quantities of >1600, 430; >1600, >1600; >1600, 850, and >1600, >1600 for samples EA1, EA2A, EA2B, and EA3, respectively. These values correspond to the fact that in the final wastewater from the industries studied, process water is mixed with sewage water.

Temperature and pH values remained stable and at levels conducive to EMO growth: between 29.5°C and 31.2°C and 6.9 to 7.2°C, respectively.

Removal Results

It should be noted that the NC 27:2012 establishes the following regulations: Average Maximum Permissible Limits for BOD_5 (mgL^{-1}) < 300, for COD (mg L^{-1}) < 700; TC and TTC not regulated. Biochemical Oxygen Demand (BOD_5) was reduced by an average of 77% compared to the control in the period analyzed. In the case of COD, the reduction achieved is 64% (**Table 1**). The TC and TTC are not regulated by the NC 27:2012 standard, but they have decreased significantly.

In EA2A and EA2B, the reduction of the pollutant load reached values between 84 % and 98%, even managing to reduce the values of these parameters to levels accepted by NC 27:2012 (2012), the regulation applicable to this company. In the case of EA2A, a reduction of 91% BOD5 and 85% for COD was achieved, and in EA2B, 88.34% BOD5 and 84.92% for COD.

On the other hand, the results of the company EA3,

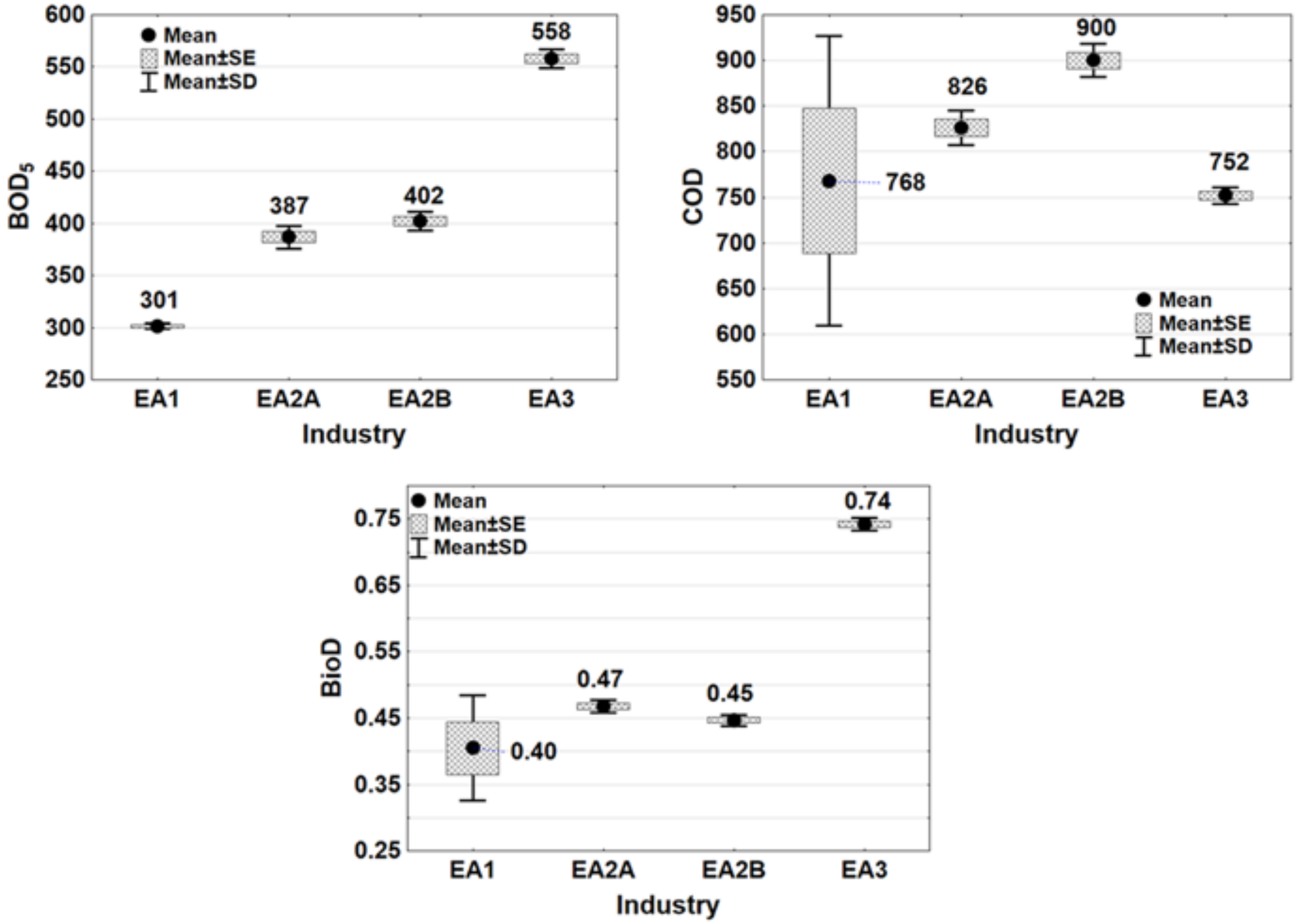

Figure 2. Box plots of BOD5, COD, and BioD values for the studied industries.

Table 1. Wastewater characteristics of the food processing industries under study after applying ME Agroambiental®.

Parameter	Mean before MEA® n=5	Mean after MEA® n=5	Max	Min	Std.Dev	% Removal
EA1						
BOD_5 (mgL^{-1})	301.34	70.2	76	62	5.2	77
COD (mg L^{-1})	768.0	274.1	296	251	21.1	64
TC	> 1600	285.8	800	154	287.4	82
TTC	430	19.1	34	14	8.6	96
EA2A						
BOD_5 (mgL^{-1})	386.8	33.6	37.5	30.2	3	91.3
COD (mg L^{-1})	826.3	127.8	130.2	125.6	1.9	84.5
TC	>1600	206.3	356.3	150.2	100	87.1
TTC	>1600	35.6	39.7	31.2	4.3	97.7
EA2B						
BOD_5 (mgL^{-1})	402	46.8	48.7	45.2	1.4	88.3
COD (mg L^{-1})	900	135.7	137.9	133.2	2	84.9
TC	>1600	182.8	188.5	179.6	4	88.5
TTC	850	61.7	63.2	60.6	1.1	92.7
EA3						
BOD_5 (mgL^{-1})	558.03	32	33.2	30.5	1.1	94.2
COD (mg L^{-1})	752.8	94.7	97.6	91.5	2.5	87.4
TC	>1600	156.8	162.3	154.3	3.6	90.2
TTC	>1600	27.2	30	23.5	3.2	98.3

where the ME Agroambiental® showed greater effectiveness on this type of waste, achieving reduction levels in the order of 87.4 to 98.3%, for organic waste, fats and oils, detergents, sewage, hot water (60 to 80 °C) from the industrial process.

The obtained results were similar to those reported by other authors in the treatment of different types of wastewater using EMO (**Table 2**). Although the results reported by *Aguila-Michelena et al.* (*2024*) reached 95% in one of the sugar mills studied, in the comparison, it must be considered that these results correspond to 60 days after treatment.

From the analysis of wastewater characteristics of the food processing industries under study after applying MEA® (**Table 1**), it can be concluded that the highest removal values were obtained in EA3, which can be attributed to its greater biodegradability (**Figure 2**).

It should be noted that the differences in BOD_5 and COD values before and after treatment were statistically significant ($p<0.05$).

The Advantages of Using MEA®

The values of the percentage reduction of the parameters studied from the use of MEA® are similar to results obtained by the different authors treating wastewater, which used processes that combine different methods of contamination removal, including advanced processes (**Table 3**); although variability is appreciated according to the type of residual to be treated, due to the complexity of food processing wastewaters (*Pan et al. 2022*). Some results were superior to those obtained with MEA®, but the technological simplicity of the process using this product implies considerable economic advantages, especially due to the minimum use of energy and the facility requirements for the execution of the treatment.

Removal and BioD

To evaluate the correspondence between BioD and the removal results, a nonlinear fixed regression was performed ,which resulted in the following equations for the removal of BOD_5 (BOD_R) (Equation 1) and COD (COD_R) (Equation 2):

$$BOD_R = -581.59-1122.81BioD+1751.53\sqrt{BioD} \quad (1)$$

$$COD_R = -993.00-1828.52BioD+2828.85\sqrt{BioD} \quad (2)$$

The correspondence between the observed and

Table 2. Removal of BOD_5 and COD using efficient microorganisms (EMO).

Wastewater Type	BOD_5 Removal (%)	COD Removal (%)	Reference
From a carbonated[1] beverage factory	77	- 66	(*Carmo 2023*)
Domestic	68		(*Calderon-Huamaní et al. 2019*)
Domestic[2]	50-67		(*Centeno et al. 2019*)
Urban[3]	68-69	55-63	(*Ortiz et al. 2021*)
Sugar mill factories[4]		60-95	(*Aguila-Michelena et al. 2024*)
Domestic	57-81	10	(*Kaur et al. 2024*)

Notes: 1 – After 20 days; 2 – a consortium of microorganisms composed of *Lactobacillus* ssp., *Schizosaccharomyces pombe*, and non-sulfurous red bacteria isolated from *Brassica oleracea* and water from Huanchaco swamps; 3 – Microorganisms captured from areas rich in diversity and suitable climatic conditions; 4 – LEBAME® after 60 days.

Table 3. BOD_5 and COD removal using different processes.

Wastewater Type	Treatment Process	BOD5 Removal (%)	COD Removal (%)	Reference
Sago industry	HUASB-EMO	-	88	(*Priya et al. 2015*)
Dairy effluent	EC	82	93	(*López and Harnisth 2016*)
Meat processing	MBR	71-76	68-77	(*Joudah and Racoviteanu 2019*)
Food processing	EF-MA	-	85	(*Arias et al. 2024*)
Dairy effluent	MC/AF	83-85	85-86	(*Porwal et al. 2015*)
Dairy effluent	EMO/AF	96	96	(*Ali et al. 2021*)
Seafood processing	AnMBR/AO	-	98	(*Hang et al. 2024*)
Fruit processing	CP	-	72	(*Güven and Ateş 2024*)
Dairy/palm oil effluents	CMT	-	96/92	(*Rani et al. 2024*)

Notes: EMO – Efficient microorganisms; HUASB – Hybrid Upflow Anaerobic Blanket reactor; EF – Electro-Fenton; MA – microalgae; MC – microbial cultures; A/F – aeration-filtration tanks or reactors; MBR – Membrane bioreactor; AnMBR – Anaerobic membrane bioreactor; AO – anoxic-oxic process; CMT – ceramic membrane technology with pressure; CP- Chemical precipitation a

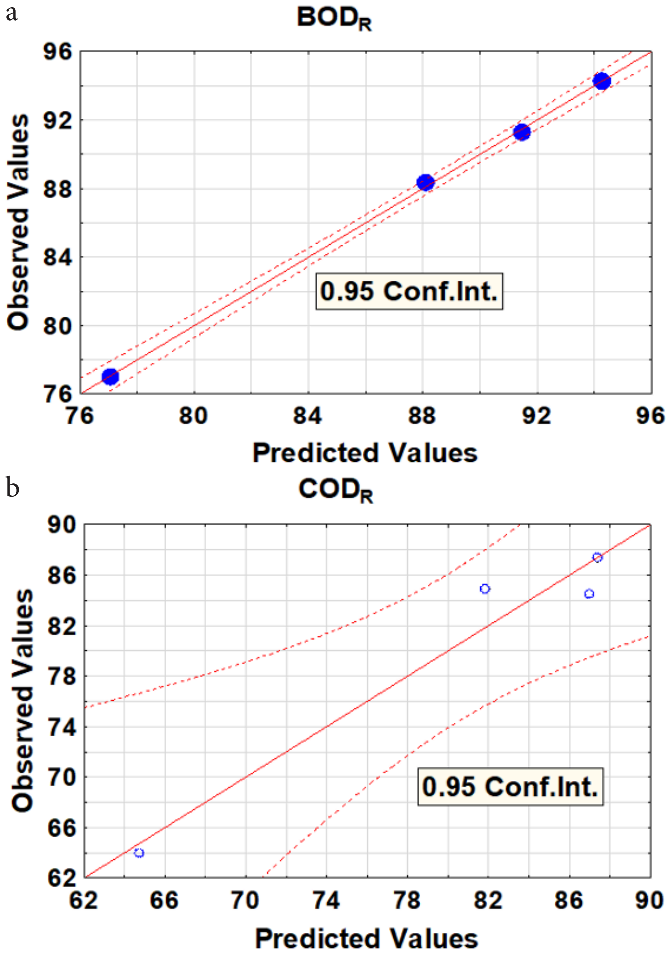

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Figure 3. Observed vs. predicted values for BOD_R (a) and COD_R (b).

predicted values is as expected, a better prediction for the BODR values is observed (**Figure 3**).

CONCLUSIONS AND RECOMMENDATIONS

The effectiveness of the applied doses of the efficient microorganisms, ME Agroambiental® (MEA®) product was evaluated in the Companies EA1, EA2A, EA2B, and EA3, under the specific conditions of their waste, through composite monitoring, after one month of treatment with efficient microorganisms. The MEA® demonstrated effectiveness in the kitchen-dining room, sewer, grease trap, and industrial waste, belonging to the companies EA2A, EA2B, and EA3, from companies with different industrial productions. With the use of the MEA®, in the evaluated processing centers, the reduction of the contaminant load of BOD5, and COD was achieved. The percentage reduction in the BOD5 and COD pollutant load in EA1 was 77% for BOD5 and 64% for COD; in the case of EA2A, 91% for BOD5 and 85% for COD; for EA2B, a reduction of 88% for BOD5 and 85% for COD was achieved; and for company EA3, 94.2% for BOD5 and 87.4% for COD. It is recommended to continue applying the MEA® at the sampled points of wastewater from companies that discharge into Havana Bay, thus reducing the pollution load until reaching values below the LMPP regulated by NC27:2012 (2012) for this type of waste.

It is important to highlight that the product's technological simplicity and low energy demand allow it to be considered for the development of economically feasible public policies for the cleanup of contaminated water bodies.

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