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Bio-treatment of maize processing wastewater using indigenous microorganisms

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Abstract

Wastewater arising from maize processing industry is rich in proteins, amino acids, carbohydrates and nitrogenous compounds. Discharging of such wastewaters to the main water bodies without proper treatment creates environmental risks. The present research aimed to reduce maize processing wastewater contaminants by indigenous microorganisms. Industrial wastewater samples of maize processing factory at 10th of Ramadan city, Egypt were collected. The chemical and microbiological characteristics of samples were evaluated. An in vitro batch experiment was conducted in triplicates using 5 L Erlenmeyer flasks enriched with 2 L of the maize processing wastewater and incubated under shaking ambient conditions at 120 rpm for 30 d. Another 2 L Erlenmeyer flask was enriched with 2 L of the wastewater sample, and incubated under static ambient conditions, used as control. The biodegradation of nitrogenous and organic compounds was followed up by measuring Total Kjeldahl Nitrogen (TKN), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Dissolved Oxygen (DO) and pH. The biomass growth rate was monitored by counting the viable bacterial cells (TBC) and fungal cells. The concentration of TKN, BOD and COD was reduced from 2330, 9000 and 12,000 mg L^{-1} to 420, 220 and 430 mg L^{-1}, respectively. DO concentrations were ranged between 6 to 12 mg L^{-1} and the pH value was elevated from 6.9 to 9.3. TBC was elevated from 2×10^6 to 4×10^{10} CFU mL^{-1}, while the yeast count was reduced from 12×10^6 to 2.2×10^4 CFU mL^{-1}. Microbial identification by Analytical Profile Index (API) profiling kits indicated that *Saccharomyces cerevisiae, Bacillus subtilis, Bacillus amyloliquefaciens,* and *Bacillus licheniformis* are the strains involved in bio-treatment.

Keywords: Bacteria, Bio-treatment, Maize, Yeast, Wastewater

Introduction

The rapid growth of industries has enhanced the productivity and release of toxic substances into the environment, which creates health hazards. It has seriously affected the normal operations of ecosystems [1]. In the past, considerable attention has been paid to the industrial wastewaters, which are usually discharged on land or into different water bodies, resulting in the degradation of the aqueous environment [2]. Various physical and chemical techniques have been applied for wastewaters treatment, including; sedimentation, aeration, filtration, flotation, coagulation, degasification, chlorination, ozonation, neutralization, sorption and ion exchange [3]. But there are several limitations of physicochemical methods such as; high cost, partial treatment, and generating of secondary pollutants, so, the biological methods become an adequate alternative for the removal of pollutants [4].

Food industries always produce wastewaters rich in organic matter, fats, oil & grease, fatty acids and nitrogenous compounds [5]. The maize processing industry is one of the most common food industries world widely; concerning the production of fructose, glucose, starch, dextrose, food oil, corn flour, gluten, sorbitol, as well as, animal food production and biofuel [6]. Depending on the raw material processed, the sludge may be rich in carbohydrates, lipids or proteins. The production of canned maize produces a high volume of wastewater, with high chemical (COD) and biochemical oxygen demands (BOD). After mechanical wastewater treatment, the COD of the sludge may be more than 100 kg m^{-3},

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because of the high content of the corn starch [7]. Beszedes et al. [6] evaluated the organic content of corn processing sludge; they found that COD and BOD concentrations were 70 and 58 kg m^{-3}.

Microorganisms have been extensively used under aerobic or anaerobic conditions to remediate such industrial wastes; they have an amazing capacity to degrade a variety of organic compounds [8]. *Bacillus cereus, Bacillus subtilis, Enterobacter, Streptococcus faecalis, Escherichia coli*, and *Saccharomyces* are microorganisms frequently found in dairy wastewaters and have been found, as indigenous microflora, in bio-treatment [9, 10]. *Pseudomonas* was used for the treatment of effluent from leather industry by Selvi and co-workers [11]. *Bacillus licheniformis* was used to remove 55% of 100 mg L^{-1} of phenanthrene [12]. *Burkholderia, Pseudomonas* and Betaproteobacterium were reported by Ewida [13] as the best biodegrading bacteria for alachlor and endosulfan.

The aerobic biodegradation of food processing liquid wastes with high content of COD was previously suggested by McIntosh and McGeorge [14] and applied by Eckenfelder [15] who have been use aerated lagoons to remove high contents of COD and BOD from liquid wastes of canning of fruits and vegetables. When the waste effluent content of COD and BOD were ranged between 700 and 900 mg L^{-1} the removal percentage was up to 90%. As their values increased between 3000 to 4000 mg L^{-1} the removal percentage was 70%. The percentage of removal was decreased to 55% when the values of COD and BOD reached 7000 to 8000 mg L^{-1}. The author mentioned that the reason for such effect was attributed to the depletion in dissolved oxygen (DO) concentration during the experiment [15]. Where DO concentration was 1.5 mg L^{-1} before starting aeration, and reached 6 mg L^{-1} during the test, and finally come down to 0.3 mg L^{-1} at the end, which created unsuitable conditions for biodegradation.

In the present work, an in-vitro experiment was conducted under ambient conditions, with extensive aeration (to recover the problem of DO depletion) for maize processing wastewater. The following objectives were considered; (i) evaluation of the chemical and microbiological characteristics of maize processing wastewater, (ii) performing a bio-treatment experiment to reduce the high content of organic and nitrogenous compounds found in such wastewater, (iii) isolation, as well as, identification of fungal and bacterial isolates capable of removing the high organic content, to be used as bioremediation tools.

Materials and methods

Samples collection

Three maize processing wastewater samples were collected (in triplicates) from an industrial factory for maize products, located in 10th of Ramadan city, Egypt. The main activity of that factory is to produce starch, dextrose, gluten, fructose and corn flour from maize. The collected samples were black in their color, and characterized by their high content of solids.

Quality assessment studies of maize processing wastewater samples

As soon as samples are received, they quickly subjected to physical, chemical and microbiological investigations. All the analyses were carried out according to the Standard Methods for the Examination of Water and Wastewater [16].

Physico-chemical assessment

The pH was measured at 25 °C using pH meter (InoLab WTW level 1, electrode with ATC probe WTW Sentix 4), electric conductivity (EC) was measured at 25 °C using conductivity meter (InoLab Cond level 1), and total suspended solids (TSS) was also measured by keeping a known volume of sample overnight at 105 °C. Phosphate and sulfate were measured using Ion Chromatography, Dionex product, model DX5000. Trace metals; aluminum, barium, cobalt, chromium, copper, iron, manganese, nickel, lead and zinc were measured using the inductively coupled plasma-mass spectrometry, Perkin-Elmer product model SCIEX Elan 9000.

Organic content; Total Kjeldahl Nitrogen (TKN), which is the sum of ammonia-nitrogen plus organically bound nitrogen, was measured using Kjeldahl apparatus (VELP, SCIENTIFICA). BOD; using (OxiTop® system WTW) at 20 °C incubation in a thermostatic incubator chamber for 5 d. COD; using dichromate reflux method, the intensity of the formed complex was measured by the visible Spectrophotometer (HACH 2000).

Microbiological assessment

Samples were highly turbid, so, serial dilution of samples was carried out until dilution of 10^5. Total count of bacteria (TBC) at 22 and 35 °C, was nominated by spread plate method using plate count agar medium (DIFCO, USA). The total count of coliform bacteria and fecal coliforms was nominated by membrane filter technique using m-endo agar LES and m-FC agar, respectively (MERCK, Germany). Furthermore, the total fungal count (TFC) (including molds and yeasts) was measured by spread plate method using sabauroud agar medium (DIFCO, USA) with direct identification of fungi based on colonial morphology (diameter, color, texture, mode of growth, pigmentation and exudates production).

Salmonella was detected by adding 10 mL of each wastewater sample to 50 mL of sterile tetrathionate broth base, and incubated at 37 °C for 5 d. One milliliter was inoculated on bismuth sulfite agar plate in each day and incubated at 37 °C for further 24 h, the growing of black colonies was considered as presumptive *Salmonella*.

Bio-treatment of maize processing wastewater using indigenous microorganisms

Conducting an in-vitro experiment

It was designed according to McIntosh and McGeorge [14] who recommended the incubation of food processing wastewaters under aeration to reduce their high COD content. A modification has been performed by creating excessive oxygen during the days of the experiment. So, a mixture of the three maize processing wastewater samples was made using 3 L of each. The experiment was initiated using a set of triplicate batches of 5 L Erlenmeyer flasks, enriched with 2 L of the mixture sample. The three 5 L batches were incubated under ambient conditions (25 °C) with shaking at 120 rpm for 30 d (to create excessive aeration; a large volume of air was left in each flask, and all were incubated with shaking). Another 2 L Erlenmeyer flask enriched with 2 L of the mixture sample was kept static at room temperature as a control.

Bio-treatment assay

The biodegradation of the nitrogenous and organic compounds in the mixture of corn processing wastewater was monitored by measuring; TKN, BOD, COD, DO and pH. The biomass growth rate was followed up by measuring TBC and TFC by pour plate method at 30 °C, using plate count agar and sabouraud agar media, respectively. All the tests were measured twice a week through the period of the experiment. At the end of the experiment, nitrite and nitrate concentrations were also evaluated to check for the extent of nitrification.

Microbial identification

The identification of microorganisms involved in bio-treatment was carried out by comparing the community profile of the mixture of corn wastewater samples, at the beginning of the bio-treatment and after it finished. The predominated microbial strains were identified by means of Analytical Profile Index (API) 20 C kit profiling (for yeasts), API 20 E profiling (for Enterobacteriaceae), API 50 CHB (for bacilli) and API staph (for staph bacteria).

Results and discussion

Quality assessment studies for maize processing wastewater samples

The results of the physico-chemical analyses of maize processing wastewater samples are described in Table 1. They were characterized by a high content of phosphate and sulfate; where their values were ranged from 3650 to 3900 mg L^{-1} and 3900 to 4700 mg L^{-1}, respectively. Heavy metals were also detected in high concentrations, especially copper, iron, manganese and zinc where their values were ranged from 217 to 280, from 226 to 273, from 390 to 430 and from 228 to 240 mg L^{-1}, respectively.

The maize processing wastewater samples were also very rich in their content of nitrogenous and organic compounds where the TKN was ranged from 2190 to 2330 mg L^{-1}, the BOD was ranged from 8800 to 9000 mg L^{-1} and the COD was ranged from 11,200 to 12,100 mg L^{-1}. Concerning the high values obtained from the physico-chemical analyses of maize processing wastewater samples, there are many authors who have been evaluated the chemical composition of corn processing sludge all over the world [17–20]. Cronje [21] found that phosphate and sulfate constituted about 0.37 and 0.43% of the dried sludge produced from wastewater treatment of corn processing for the production of glucose, dextrose and corn flour in South Africa. van Lune [22] reported the high metal concentration in soil fertilized by sludge produced from maize by-products in Netherland.

The high content of nitrogenous and organic compounds obtained in the present study was expected, as it is well known that corn kernel is consists mainly from starch and gluten (70%) [6], so, the wastewaters come up from such highly enriched constituents will be saturated with carbohydrates, proteins, amino acids and nitrogenous substances. In accordance with the present results, Ross [18] reported that the mean value of BOD, COD and TKN of corn processing sludge collected from a factory manufacturing corn flour from maize in South Africa was 7194, 9210 and 1900 mg kg^{-1}, respectively.

The microbiological assessment of maize processing wastewater samples indicated their contamination with elevated counts of bacteria, as stated in Table 1. The presence of coliform bacteria in such high counts (more than 10 million CFU 100 mL^{-1} of total coliforms and more than 1 million CFU 100 mL^{-1} of fecal coliforms) might be due to the mixing of sewage effluent with industrial wastewater, by the factory. The test for *Salmonella* as pathogenic bacteria was negative. On the other hand, the community of fungi was yeast only, where the grown colonies on each plate were the same in cultural characteristics (homogenate community).

Bio-treatment of maize processing wastewater using indigenous microorganisms

The aerobic biodegradation was the tool used in the present study; the batch experiment was conducted by diffusing excessive oxygen through the wastewater contents, taking into account, the high carbon, nitrogen and other elements content of such wastewater, as well as, the elevated counts of bacteria and yeasts. The values of TKN, BOD, COD, DO, pH, TBC and TFC were followed up in triplicates and the mean was recorded. The

Table 1 Quality assessment of maize processing wastewater samples

Analysis	Unit	Maize processing wastewater		
		Sample 1	Sample 2	Sample 3
Physico-chemical characteristics				
pH		7.3	7.4	7.2
EC	μS cm^{-1}	1475	1520	1290
TSS	mg L^{-1}	160,000	190,000	130,000
Phosphate	mg L^{-1}	3700	3900	3650
Sulfate	mg L^{-1}	4300	4700	3900
Aluminum	mg L^{-1}	26	24	19
Barium	mg L^{-1}	19	12	7
Cobalt	mg L^{-1}	9	10	6
Chromium	mg L^{-1}	18	16	9
Copper	mg L^{-1}	217	280	240
Iron	mg L^{-1}	226	273	230
Manganese	mg L^{-1}	427	430	390
Nickel	mg L^{-1}	24	19	12
Lead	mg L^{-1}	3	1.2	2.1
Zinc	mg L^{-1}	228	240	236
Organic content				
TKN	mg L^{-1}	2330	2190	2210
BOD	mg L^{-1}	8800	9000	9220
COD	mg L^{-1}	11,200	12,100	12,000
Microbiological assessment (Count × 10^4)				
TBC 22 °C	CFU mL^{-1}	220	135	190
TBC 35 °C	CFU mL^{-1}	300	240	290
Total coliforms count	CFU 100 mL^{-1}	1660	1420	1760
Fecal coliforms count	CFU 100 mL^{-1}	1250	1120	1190
Salmonella	Presence/Absence	absent	absent	absent
TFC	CFU mL^{-1}	1000	1200	700

influence of microorganisms on the wastewater content was obvious comparing the characters of the mixture of wastewater before and after the test is done, as ensured in Fig. 1.

The concentration of TKN in the mixture sample of maize processing wastewater was 2330 mg L^{-1}. The application of bio-treatment under excess oxygen gives the chance to indigenous microorganisms to reduce the concentration to 420 mg L^{-1} with removing percentage of 82%, as explained by Fig. 2a. The effect of biodegradation was observed from the first day of the experiment, but it starts to distend from the day 6 to day 27. The concentrations of nitrite and nitrate were also measured at the end of the experiment; the first was less than 0.2 mg L^{-1}, while the last was 44 mg L^{-1}, ensuring that there is no extent for nitrification and the by-products of biodegradation of nitrogenous compounds are nontoxic.

Concerning the reduction of carbon content during the bio-treatment, the results were illustrated in Fig. 2b and c. The BOD and COD values were come down extensively as the test is going on. BOD values were reduced from 9000 to 220 mg L^{-1} with removing percentage of 98%, COD content was reduced from 12, 000 to 430 mg L^{-1} with removing percentage up to 97%.

It is clear that BOD and COD biodegradation set of from day 3 to 24 of the expirement. These results are in accordance with those recorded by Tricolici et al. [23] who studied the bio-treatment of dairy industry wastewater rich in organic and nitrogenous compounds in Romania. They found that bacteria with help of microalgae could remove 68 and 91% of TN and COD, respectively. The reduction in COD was also reported by Ross [18] who used indigenous bacteria to treat corn processing wastewater sludge; it was ranged from 91 to 95%.

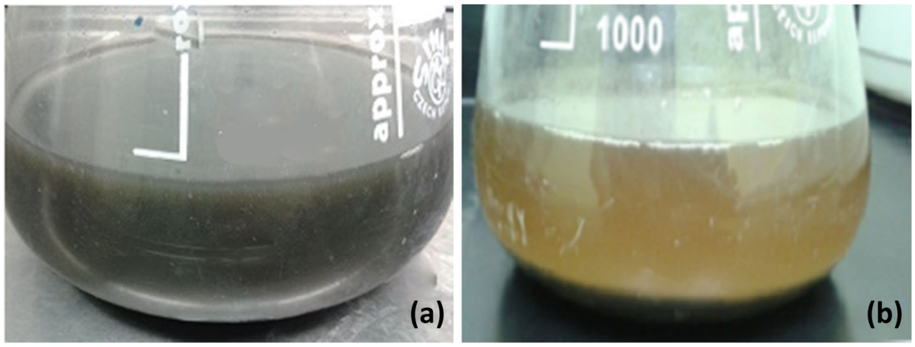

Fig. 1 Influence of indigenous microorganisms on maize processing wastewater (**a**) wastewater at the beginning of the test (**b**) at the end

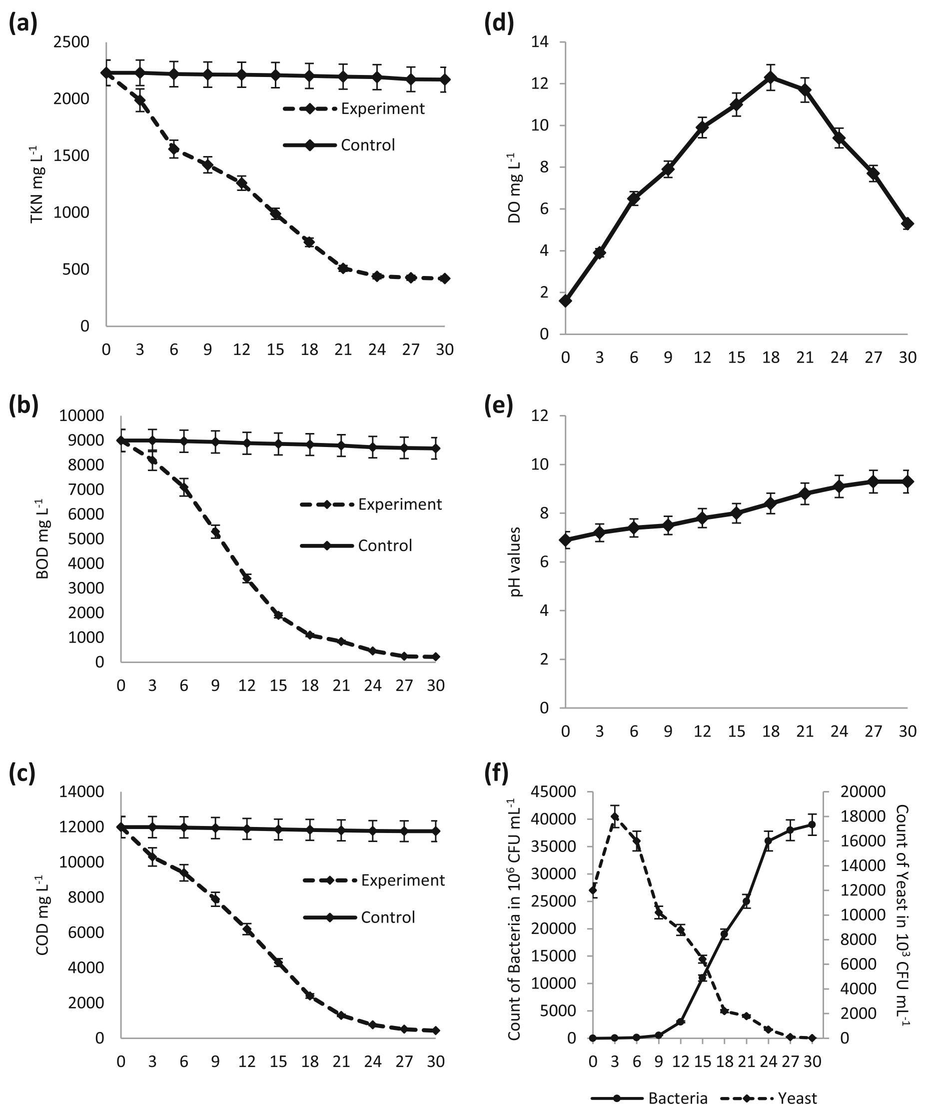

Fig. 2 Illustrations concerning bio-treatment of maize processing wastewater by indigenous bacteria (**a**) reduction in TKN (**b**) reduction in COD (**c**) reduction in BOD (**d**) DO concentrations during the experiment (**e**) elevation in pH values (**f**) growth rates of bacteria and yeast during the experiment

Moreover, Abdel-Fatah and co-workers [24] applied the same idea of excessive aeration on corn processing wastewater in Egypt. They recorded that COD and BOD were reduced from 8000 to 700 mg L^{-1}, and from 4500 to 400 mg L^{-1}, respectively.

The initial DO concentration of maize processing wastewater was very low before starting aeration by shaking (1.6 mg L^{-1}) and increased to 7 mg L^{-1} at day 6, by the effect of shaking. The metabolic activities of indigenous microorganisms gradually increased by the effect of excess oxygen diffused in wastewater, even so, the expected depletion of DO was recovered during the period of day 6 to 27, due to continuous shaking where DO ranged from 6 to 12 mg L^{-1} (the period of maximum activity for biodegradation obtained by indigenous microorganisms as ensured by the previous results). Then the DO was reduced to 5.3 at the end of the test (Fig. 2d). Such findings are in accordance with those reported by Abdel-Fatah et al. [24] who mentioned that increasing the oxygen content in the reactor and elevating the biomass concentration lead to high biodegradation capacity. Concerning the elevated DO concentrations during day 12 to 24 (ranged between 9 to 12 mg L^{-1}) which beyond the known saturation level (8–9 mg L^{-1}). That might be arise from the fact that equilibration of the oxygen content of water with the air above it is happen in slow-flowing streams but in fast-flowing streams, the DO readings were elevated over 100% air saturation. DO concentration reached 15–18 mg L^{-1} in natural water falls [25]. So, the saturation level is considered when the surface of water is in static phase, but in case of shaking conditions, the air's oxygen permeates through the water, leading to over saturation conditions. McDaniel and Bailey [26] have been used shaking and agitation to produce high DO concentration in culture media.

The pH was monitored during the batch experiment period; the results were clarified in Fig. 2e. It was neutral through the first week and starts to be slightly alkaline, ranging from 7.5 to 8 through the second week. As the biodegradation products increased with time, the pH of the mixture increased, and the solution became moderately alkaline at the end, where the pH reached 9.3.

The biomass growth rate was carried out by counting of the viable bacterial and yeast cells, the results obtained were illustrated in Fig. 2f. The count of bacteria exploded during bio-treatment, where it was 2×10^6 CFU mL^{-1} at the beginning of the test, and become 4×10^{10} CFU mL^{-1} at the end. On the other hand, yeasts were predominated in the fungal community, the mycelial fungi were not present. The count of yeast was 12×10^6 CFU mL^{-1} at the start time and decreased during bio-treatment to become 2.2×10^4 CFU mL^{-1} at the end. The identification of the microbial community before and after bio-treatment indicated that yeast group constituted 26% and bacterial group 74%, of the microbial community of the tested maize processing wastewater. Yeast was identified by means of API 20 C kits profiling as *Saccharomyces cerevisiae*, while bacteria, were identified by means of API 20 E, API 50 CHB and API staph kits profiling as *E. coli, Enterobacter, Citrobacter, Proteus, Bacillus lichiniformis, Bacillus amyloliquefaciens, Bacillus subtilis, Staphylococcus* and non-identified Gram-negative bacteria. They were counting for 8, 6, 4, 5, 10, 12, 12, 5 and 12% of the community, respectively. At the end of bio-treatment the survived microorganisms were *S. cerevisiae, B. lichiniformis, B. amyloliquefaciens and B. subtilis* constituting 1, 29, 37 and 33% of the community, respectively.

Biodegradation of organic compounds of maize processing wastewater by the indigenous microorganisms was noticed from the first day of the in vitro conducted experiment. That could be initiated by yeast (*S. cerevisiae*) which constituted the largest percentage in the microbial community. *S. cervisiea* has been reported in the literature as a strong bio-degrader especially for wastes rich in protein and carbohydrates [27–29]. As the pH of the solution tends to be alkaline; the growth of yeast and consequently, their influence on biodegradation was slowed down. It was reported by Yalcin and Ozbas [30] that *S. cerevisiae* show better activity at pH 5 to 7, and strongly suppressed at pH 10. As the bio-treatment experiment going on, in the third week, bacteria start to explode in numbers and their influence in biodegradation become obvious.

Actually not all the bacterial genera identified at the beginning of the experiment have been shared in biodegradation, because Gram-negative bacteria as *E. coli, Enterobacter, Citrobacter and Proteus,* as well as, Gram-positive cocci as *Staphylococcus* were found to be sensitive to alkaline pH. On the other hand, *B. lichiniformis, B. amyloliquefaciens and B. subtilis* had shown strong ability to survive alkaline pH, they grown well and continuing the bio-treatment process successfully. Padan et al. [31] found that *E. coli* when grown in Luria-Bertani media at pH 7, all genes encoding for adenosine triphosphate (ATP) cycle were working well, but at pH 8.5 those genes did not express, so, the ATP cycle corrupted, consequently, bacterial cells destroyed. While the encoding genes for ATP cycle in *B. subtilis* are still expressed even at pH 9.5 and give the same bacterial density as at pH 7.

Conclusions

Maize processing wastewater was enriched with a high content of nitrogenous and organic compounds. The indigenous yeast and bacteria, under ambient conditions with diffusion of excessive oxygen could be removed up to 85% of nitrogenous compounds and up to 95% of organic matters. The microorganisms shared in biodegradation are *S. cerevisiae, B. lichiniformis, B. amyloliquefaciens and B. subtilis.* The use of such technique for industrial wastewater treatment before discharge to the sewerage system is recommended. Further studies should be carried out on the produced sludge to be used as fertilizer.

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Availability of data and materials
All data generated or analyzed during this study are of my own work and it is my pleasure to be available publically.

Competing interests
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