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Bioremediation of four food industrial effluents

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Abstract: Some effluents ((Whey Effluent (WhE); Orange Effluent (OE); Carrot Effluent (CE) and Chocolate Effluent (ChE)) were bioremediated using some allochthonous microorganisms (*Lactobacillus delbrueckii* subsp. *bulgaricus, Saccharomyces cerevisiae* Y-1347 and *Dekkera bruxellensis*). The highest biodegradable efficiency of the Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD) and Total Organic Nitrogen (TON) of the effluents under investigation was noticed when using the allochthonous microorganisms together with the autochthonous one. *Saccharomyces cerevisiae* Y-1347 proved to be the best utilizer of Whey (WhE) organic and nitrogenous compounds with the reduction of BOD, COD and TON by 12.36, 20.09 and 68.42%, respectively. *Dekkera bruxellensis* proved to be the organism of choice on using Orange Effluent (OE) where BOD, COD and TON were reduced by 18, 20 and 53.39%, respectively. *Lactobacillus delbrueckii* subsp. *bulgaricus* proved to be the best utilizer of the Carrot Effluent (CE) constituents by reducing BOD, COD and TON by 24.27, 19.33 and 63.63%, respectively. *Dekkera bruxellensis* proved to be the best utilizer of the Chocolate Effluent (ChE) constituents by improving its quality and reducing BOD, COD and TON by 18.36 and 15.86 and 73.07%, respectively. A successful trial was made to use the treated effluents in the irrigation of *Lens culinaris* and *Phaseolus vulgaris* seeds for germination.

Keywords: Bioremediation, Allochthonous and Autochthonous Microorganisms, Industrial Effluents

1. Introduction

Water pollution is considered as a major environmental problem due to the fact that some waste materials have toxic, mutagenic and carcinogenic effects on the living organisms [1]. The chemical constituents of the effluents released by many factories are considered environmentally important because of their potential leading to the dissemination of pathogenic microorganisms, depletion of oxygen supply of the water by unstable organic matter in sewage, killing of the aquatic life, increased danger in using natural bodies of water for drinking supplies and diminished value of the water for other recreational purposes and creation of miscellaneous objectionable conditions such as offensive odors and accumulation of debris which decrease property values[2].

Several physical and physicochemical techniques have been used to clean up the effluent residues. However, bioremediation offers an effective technology for the treatment of water pollution because the majority of molecules in the effluents are biodegradable and waste-degrading microorganisms are ubiquitous [3, 4, 5]. On the other hand, bioremediation has been established as an efficient, economic, versatile and environmentally sound treatment of the water to be reused in many purposes especially [6].

Microbial degradation is considered as an important mean for the ecological recovery of polluted effluents [7, 8]. However, success depends on the bioremediation ability of the used microorganisms [9, 10] which may be native or exogenous to the contaminated water [11]. Indigenous microorganisms have been known for their potentiality to degrade the solid residues present in the effluents. Several studies have demonstrated the ability of various autochthonous together with many supplemented allochthonous microorganisms to degrade many soluble organic and inorganic compounds [12, 13].

The reuse of treated effluents for agricultural irrigation is becoming a common and rapidly increasing practice in arid and semi-arid regions around the world. This increase in the agricultural reuse of treated effluents serves goals such as promoting sustainable agriculture, preserving scarce water resources and maintaining environmental quality [14]. Also,

irrigating with effluents may reduce purification levels and fertilization costs, because soil and crops serve as bio-filters and effluents contain nutrients. Policy decisions regarding the level of purification and location of agriculture using effluents should consider multifarious aspects including costs, hazards of reuse of effluents [15].

The main objective of the present work is a trial to convert the effluents of some local factories into stable oxidized end products which can be safely and economically discharged and reused in the irrigation as one of many applications.

2. Materials and Methods

2.1. Sampling

Four different effluents were tested throughout the present work: the effluent supply from chocolate manufacturing (chocolate effluent, (ChE)), the effluent supply from whey manufacturing (whey effluent, (WhE)) and the effluent supply from carrot (carrot effluent, (CE)) and orange manufacturing (orange effluent, (OE)). These effluents were provided by three different food industries located in Beirut, Lebanon.

2.2. Chemical analysis

Effluents were collected in large sterile polyethylene bottles of 5 liters. Bottles were rinsed three times with the effluents before filled. Determination of pH and water temperature was performed in situ. Ionic chromatography and spectrophotometry were performed within 24 h after sampling. After sample collection and during transportation to the laboratory, samples were stored on ice. All the samples were filtered through a pre-acid cleaned glass filter which enables the determination of total dissolved solids (TDS) by evaporation of the filtered fraction. Samples for metal analysis were immediately acidified with nitric acid to pH<2 and stored at 4˚C for metal analysis (Zn, Cu, Pb, Cd, Mn, Co, Fe) by flame atomic absorption spectrometry (Varian, model AA50).

Working standard solutions were prepared by dilution of stock solutions (1mg metal/ml in 2% HNO_3) with milliQ water. Analytical parameters were determined with reference to official methods currently suggested [16].

Nitrate, sulphate and chloride were analyzed using an ionic chromatograph from Metrohm. The determination of nitrite and ammonium were carried out by molecular absorption spectrometry (Spectronic, Model 20 D^+). Total Organic Carbon (TOC) and Total Organic Nitrogen (TON) were analyzed by a micro analyzer N/C from Analytic Jena, Ca^{2+}, Mg^{2+} and total hardness were determined by EDTA titrimetric method. The biological Oxygen Demand (BOD) was determined according to the method described by Greenberg et al. [17] and the Chemical Oxygen demand (COD) was measured by the spectrophotometric method (Aqua Quest CECIL CE4004).

2.3. Reagents and Blank Control

All the reagents were analytical grade Merck, Panreac or Fluka. Ultrapure water was used for the preparation of solutions. All ware used for metal analysis was cleaned with detergent, thoroughly rinsed with tap water, soaked in 10% nitric acid solution overnight and finally rinsed with ultrapure water.

2.4. Microorganisms and Their Maintenance

The organisms used throughout the screening experiments are three different isolates, one bacterial strain namely *Lactobacillus delbrueckii* subsp. *bulgaricus* and two yeast strains: *Dekkera bruxellensis* and *Saccharomyces cerevisiae.* These bacterial and fungal isolates were selected because of their known capacity in the bioremediation of the organic pollutants. The stock bacterial cultures were maintained on nutrient agar slants. The experimental yeast cultures were maintained on sabouraud agar slants. The slants were stored at 4˚C with transfers at monthly intervals.

2.5. Fermentation Medium

The food-industrial effluents were freshly used without any additives and dilutions (unless otherwise indicated). Cultivation with 2% of standard inocula of bacterial and yeast strains was made in 325 ml BOD bottles which were sterilized empty in oven at 180˚C for two hours.

2.6. Microbiological Analysis

Bacterial count was carried out in order to detect the number of microorganisms in the effluents. It was estimated by serial dilutions of the different effluents that were plated on nutrient agar and incubated for 48 hrs at 30˚C. The bacterial number in each of the different effluents was obtained by counting the colonies on the plates containing between 30 and 300 colonies. For fungi, the effluents were plated on sabouraud and plates were incubated for 5 days at 30˚C. At the end of the incubation, fungal isolates were identified morphologically.

2.7. Statistical Analysis

The parameters tests were expressed as means ± standard error. The values were subjected to standard one-way ANOVA with 95% confidence limits ($P \leq 0.05$) using COSTAT 2.00 statistical analysis software manufactured by Cottort Foftware Company [18].

3. Results and Discussion

3.1. Chemical Composition of Effluents (before Treatment)

The analysis of the effluents under test (Table1) indicated clearly the presence of many parameters and elements related to the pretreated water which attracted the attention to find the necessary treatment procedures to remediate

these waters to be reused as a source of public water supply in emergency cases especially with the increase demand of water. The analysis of the elements in these effluents (Table 2) indicated that they are present within the range of permissible limits.

Table 1. *Physico-chemical characteristics of the 4 tested effluents (WhE, OE, CE and ChE) before treatment*

Parameter	Whey effluent (WhE)	Orange effluent (OE)	Carrot effluent (CE)	Chocolate effluent (ChE)
Temp ˚C	19	27	25	22
pH	4.8	6.2	6.10	6.40
Total hardness mg/l	432	350	378	405
Calcium hardness mg/l	262	240	258	265
Magnesium hardness mg/l	170	110	120	140
Sulphate mg/l	65	52	50	63
TDS mg/l	1238	1210	1205	1220
Chloride mg/l	244	150	180	110
TOC mg/l	22	18	17	20
BOD mg/l	8.9	10	10.30	9.80
COD mg/l	63.7	50	60	54.20
TON mg/l	190	118	110	130
Ammonia mg/l	0.45	0.20	0.35	0.35
Nitrite mg/l	0.43	0.34	0.54	0.42
Nitrate mg/l	2.3	2.14	2.63	1.65

Table 2. *Metal contents of the 4 tested effluents(WhE, OE, CE and ChE) before treatment*

Element	Whey effluent (WhE)	Orange effluent (OE)	Carrot effluent (CE)	Chocolate effluent (ChE)
Zinc (Zn)	1.900	1.300	1.500	1.400
Manganese (Mn)	0.100	0.070	0.060	0.090
Copper (Cu)	0.345	0.175	0.200	0.100
Iron (Fe)	0.300	0.800	0.300	0.100
Cadmium (Cd)	0.006	0.004	0.0070	0.0060
Cobalt (Co)	0.027	0.003	0.0046	0.0033
Lead (Pb)	0.005	0.002	0.0040	0.0060

3.2. Microbiological Analysis

The microbiological analysis of the effluents revealed that the total bacterial count was found to be: 338, 74×103, 255×105 and 60×105 CFU/ml in WhE, OE, CE and ChE, respectively. This analysis also revealed that the effluents contained non-pathogenic bacteria especially MRSA (Methillin-Resistant Staphylococcus aureus) and E.coli; however it contains some yeast isolates. These results coincide with those reported by Chaîneau et al. [19] and Falih & Wainwright [20] who succeeded in the isolation of several bacterial strains in addition to many active non-pathogenic fungal strains from fat and oil wastewaters.

3.3. Bioremediation of Effluents

3.3.1. Screening Experiments

In these experiments, three strategies namely: (1) natural attenuation which requires the use of the autochthonous microorganisms, (2) bioaugmentation which requires the use of the autochthonous and the allochthonous microorganisms together and (3) the use of the allochthonous microorganisms alone were performed to evaluate the potency of the autochthonous and the allochthonous microorganisms to remediate the effluents used throughout the present investigation both together and one at a time and under static conditions (Table 3). The effluents (WhE, OE, CE and ChE) were dispensed in sterilized BOD bottles (325 ml), inoculated and incubated for five days; thereafter the necessary analyses were carried out (Table 3a, 3b, 3c and 3d).

Table 3a *Biomass, BOD, COD and TON of whey effluent (WhE)*

Microorganisms	Biomass mg/100ml	BOD mg/l	COD mg/l	TON mg/l	Final pH
Control		8.90^{a}	63.70^{a}	190.00^{a}	
Effluent flora (autochthonous)	32.60^{a}	8.80^{ab}	60.90^{b}	90.00^{b}	4.80^{a}
Lactobacillus delbrueckii subsp. *bulgaricus* +autochthonous	33.00^{a}	8.10^{ab}	55.20^{b}	65.00^{c}	4.60^{a}
Saccharomyces cerevisiae Y-1347+autochthonous	41.30^{a}	7.80^{ab}	50.90^{c}	60.00^{d}	4.50^{a}
Dekkera bruxellensis +autochthonous	33.10^{b}	8.10^{bc}	57.00^{d}	90.00^{e}	4.60^{a}
Lactobacillus delbrueckii subsp. *bulgaricus* (allochthonous)	38.20^{c}	8.30^{bcd}	56.30^{d}	70.00^{e}	4.60^{a}
Saccharomyces cerevisiae Y-1347 (allochthonous)	33.50^{d}	8.40^{cd}	55.40^{e}	75.00^{e}	4.50^{a}
Dekkera bruxellensis (allochthonous)	34.10^{e}	8.50^{d}	57.10^{f}	90.00^{f}	4.50^{a}

Table 3b *Biomass, BOD, COD and TON of orange effluent (OE)*

Microorganisms	Biomass mg/100ml	BOD mg/l	COD mg/l	TON mg/l	Final pH
Control		10.00^{a}	50.00^{a}	118.00^{a}	
Effluent flora (autochthonous)	23.40^{a}	9.50^{b}	50.00^{b}	90.00^{b}	6.20^{a}
Lactobacillus delbrueckii subsp. *bulgaricus* +autochthonous	37.50^{b}	9.30^{b}	49.00^{c}	70.00^{c}	6.00^{a}
Saccharomyces cerevisiae Y-1347+autochthonous	39.70^{c}	9.40^{b}	48.00^{d}	65.00^{d}	5.70^{a}
Dekkera bruxellensis +autochthonous	42.80^{d}	8.20^{b}	40.00^{d}	55.00^{e}	5.70^{a}
Lactobacillus delbrueckii subsp. *bulgaricus* (allochthonous)	29.50^{e}	9.30^{b}	47.00^{e}	75.00^{e}	5.70^{a}
Saccharomyces cerevisiae Y-1347 (allochthonous)	39.20^{f}	9.20^{b}	48.00^{f}	75.00^{f}	5.70^{a}
Dekkera bruxellensis (allochthonous)	41.80^{g}	9.00^{c}	45.00^{f}	60.00^{g}	5.70^{a}

Table 3c *Biomass, BOD, COD and TON of carrot effluent (CE)*

Microorganisms	Biomass mg/100 ml	BOD mg/l	COD mg/l	TON mg/l	Final pH
Control		10.30[a]	60.00[a]	110.00[a]	
Effluent flora (autochthonous)	27.40[a]	10.00[b]	59.00[b]	65.00[a]	6.10[a]
Lactobacillus delbrueckii subsp. *bulgaricus* +autochthonous	50.60[b]	7.80[b]	48.40[c]	40.00[b]	6.00[a]
Saccharomyces cerevisiae Y-1347+autochthonous	44.70[c]	9.20[bc]	57.00[d]	55.00[b]	5.90[a]
Dekkera bruxellensis +autochthonous	50.30[d]	9.80[c]	59.00[d]	45.00[b]	6.00[a]
Lactobacillus delbrueckii subsp. *bulgaricus* (allochthonous)	48.40[e]	8.50[d]	52.10[e]	40.00[c]	6.00[a]
Saccharomyces cerevisiae Y-1347 (allochthonous)	43.20[f]	8.90[de]	55.00[e]	45.00[d]	5.90[a]
Dekkera bruxellensis (allochthonous)	44.20[f]	8.70[e]	57.00[f]	45.00[e]	5.90[a]

Table 3d *Biomass, BOD, COD and TON of chocolate effluent (ChE)*

Microorganisms	Biomass mg/100 ml	BOD mg/l	COD mg/l	TON mg/l	Final pH
Control		9.80[a]	54.20[a]	130.00[a]	
Effluent flora (autochthonous)	53.20[a]	9.50[b]	54.00[b]	65.00[b]	6.40[a]
Lactobacillus delbrueckii subsp. *bulgaricus* +autochthonous	60.20[b]	9.20[c]	52.30[c]	40.00[b]	6.20[a]
Saccharomyces cerevisiae Y-1347+autochthonous	59.60[c]	9.30[cd]	53.00[d]	45.00[c]	6.00[a]
Dekkera bruxellensis +autochthonous	62.00[d]	8.00[cd]	45.60[e]	35.00[c]	6.20[a]
Lactobacillus delbrueckii subsp. *bulgaricus* (allochthonous)	53.70[e]	9.20[cd]	49.90[f]	45.00[c]	6.20[a]
Saccharomyces cerevisiae Y-1347 (allochthonous)	55.70[f]	9.00[de]	53.40[g]	45.00[d]	6.00[a]
Dekkera bruxellensis (allochthonous)	60.70[g]	8.50[e]	48.70[g]	40.00[e]	6.00[a]

Different letters in each column indicate significance at P≤0.05 as evaluated by one-way ANOVA test.

3.3.1.1. Using the Autochthonous Flora for the Bioremediation of Effluents (Natural Attenuation)

By applying natural attenuation, the autochthonous microorganisms showed a very low rate of bioremediation in the different tested effluents where in the WhE case, BOD, COD and TON were reduced by 1.12, 4.39 and 52.63%, respectively. In the OE case, BOD and TON were reduced by 5 and 23.7%, respectively. In the CE case, BOD, COD and TON were reduced by 3, 1.6 and 40.9%, respectively and in the case of ChE, BOD and TON were reduced by 3 and 50%, respectively. The findings of the present study are in agreement with those reported by Mueller et al. [21] who found that a complete assimilation of carbon compounds into biomass is not achievable under natural conditions, due to the fact that some compounds are recalcitrant or are metabolized slowly over long periods.

3.3.1.2. Using the Allochthonous Flora for the Bioremediation of Effluents

The allochthonous microorganisms namely: *Lactobacillus delbrueckii* subsp. *bulgaricus, Dekkera bruxellensis* and *Saccharomyces cerevisiae Y-1347* were incubated with effluents (after being sterilized) in order to examine their ability to degrade the waste materials.

The bioremediation rate on using these allochthonous microorganisms was higher than that achieved by the autochthonous ones where in the WhE sample, *Lactobacillus delbrueckii* subsp. *bulgaricus* was found to be the most promising microorganism showing a BOD , COD and TON reduction by 6.74 ,11.6 and 63.15%,respectively. In the OE sample, *Dekkera bruxellensis* was found to be the most promising organism with a reduction in BOD, COD and TON by 10, 10 and 49.15%, respectively. In the CE sample, *Lactobacillus delbrueckii* subsp. *bulgaricus* was found to be the organism of choice showing a reduction in BOD, COD and TON by 17.4, 13.1 and 63.6%, respectively. In the case of ChE, *Dekkera bruxellensis* was found to be the most promising organism showing a reduction in BOD, COD and TON by 13.26, 10.14 and 69.2%, respectively.

3.3.1.3. Using the Autochthonous Flora with the Allochthonous Ones for the Bioremediation of Effluents (Bioaugmentation Experiments)

The bioaugmentation experiments were carried out to evaluate the effect of the added strains with the autochthonous microorganisms on the effluent bioremediation. Bioaugmentation of the native microorganisms with the allochthonous ones exhibited the highest bioremediation rate where in WhE sample, *Saccharomyces cerevisiae* Y-1347 proved to be the organism of choice upon growing on WhE and reducing BOD, COD and TON by 12.35, 20 and 68.42%, respectively. *Dekkera bruxellensis* proved to be the best utilizer of OE contents of organic and nitrogenous compounds with the reduction of BOD, COD and TON by 18, 20 and 53.38%, respectively. *Lactobacillus delbrueckii* subsp. *bulgaricus* proved to be the organism of choice upon growing on CE and reducing BOD, COD and TON by 24.27, 19.3 and 63.63%, respectively. *Dekkera bruxellensis* also proved to be the organism of choice upon growing on ChE with the reduction of BOD, COD and TON by 18.36, 15.86 and 73%, respectively. The findings of the present study are in agreement with those obtained by Supaphol et al. [22] who found that the addition of commercial microbial cultures (bioaugmentation) did significantly enhance the rates of waste bioremediation. Lee et al. [23] demonstrated that inoculation with commercial strains of waste-degrading bacteria was effective.

Therefore, the bioaugmentation of the different effluents was of higher efficiency than both natural attenuation and

the use of the allochthonous microorganisms alone in addition that the capacity of the different allochthonous microorganisms to utilize the tested effluents was also varied from organism to organism according to the type of effluent. So according to the bioaugmentation results, the most promising allochthonous microorganisms were selected to grow on the most suitable effluents (with their native microbial community and with the optimization of certain factors in further steps to be improved for reuse and irrigation.

3.3.2. Physiological and Environmental Factors Affecting the Bioremediation of the Effluents

The aim of the this part in the study was to evaluate the physiological and environmental requirements of the allochthonous and the autochthonous microorganisms under investigation to increase the bioremediation rate of BOD and COD of the effluents under test to be improved and reused in various applications.

3.3.2.1. Effect of Dilution Rate

From the previous experiments, the most promising organisms were chosen to grow on the most suitable effluents together with the autochthonous microorganisms for further investigations; *Saccharomyces cerevisiae* Y-1347 on whey effluent (WhE); *Lactobacillus delbrueckii* subsp. *bulgaricus* on carrot effluent (CE) and *Dekkera bruxellensis* grown on both orange effluent (OE) and chocolate effluent (ChE).

Serial dilutions 1:10, 1:20, 1:30, 1:40 and 1:50 (v/v) were prepared from the effluents under test, inoculated with the suitable allochthonous microorganisms and incubated at 20˚C for 5 days in order to test the effect of the dilution rate on the biodegradability of BOD and COD in the different trials. The results obtained (Fig. 1a,1b,1c and 1d) revealed that the tested dilutions showed a variable influence on the metabolic activities of the experimental organisms. These results showed that the increase of the dilution ratio to 1:50 (v/v) was significant for *Saccharomyces cerevisiae* Y-1347 grown on WhE with COD and TON reduction by 26.9 and 41.6%, respectively. The same dilution ratio (1:50, (v/v)) was also significant for *Dekkera bruxellensis* grown on OE with BOD, COD and TON reduction by 32.9, 38 and 47.27%, respectively. On contrary, lower dilution (1:30, (v/v)) was significant for *Dekkera bruxellensis* when grown on ChE with BOD, COD and TON reduction by 33.75, 43.85 and 28.57%, respectively. The lowest dilution 1:10 was favorable for *Lactobacillus delbrueckii* subsp. *bulgaricus* grown on CE with BOD, COD and TON reduction by 41, 37.8 and 30%, respectively. The lowest dilution 1:10 was favorable for *Lactobacillus delbrueckii* subsp. *bulgaricus* grown on CE with BOD, COD and TON reduction by 41, 37.8 and 30%, respectively. The results obtained by the use of the different dilution ratios revealed that the reduction in pollutants is not only due to the microbial activity but also due to the high level of O_2 provided by the increase in the volume of the diluted water which causes an increase in the remediation process. The findings of the present study are in agreement with those reported by LIoyd & Macaskie [24] who reported that as a result of great dilution, the load of contaminating organic materials would be relatively small and normal biological processes occurring in the water could transform these materials to stable end products.

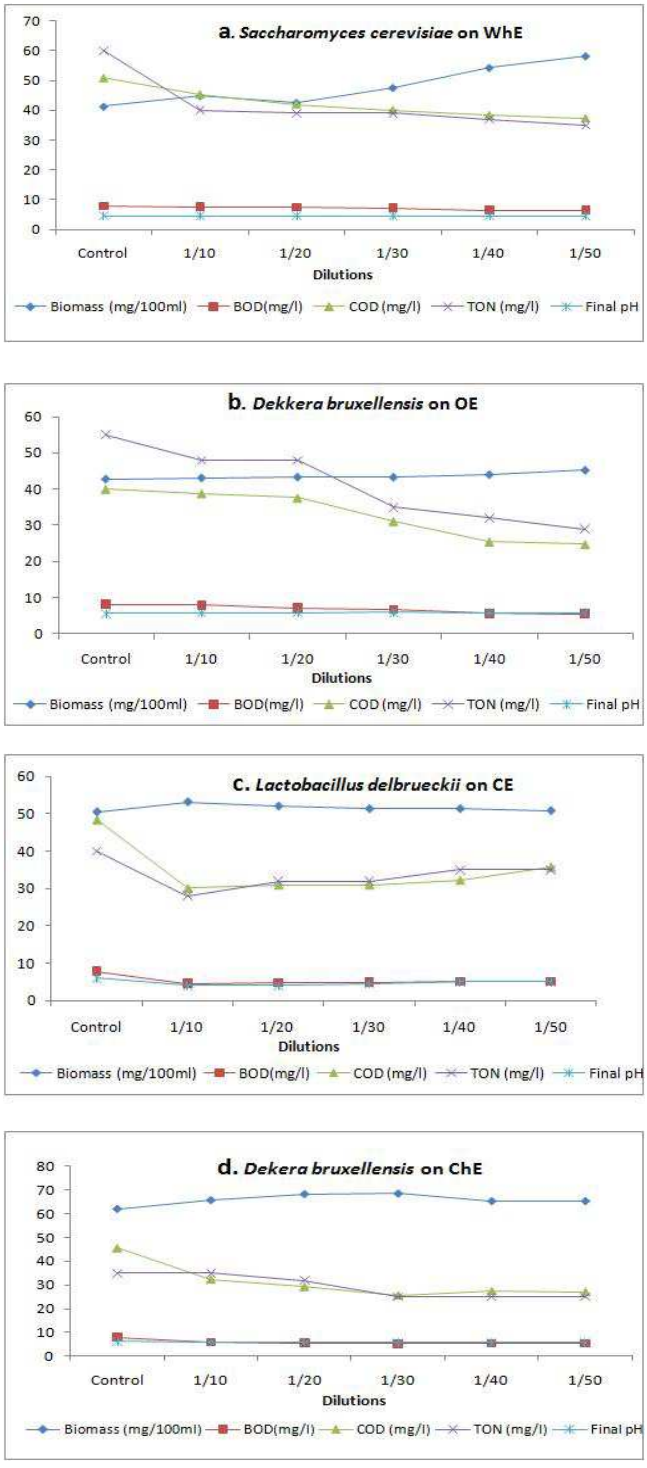


Figure 1. *Biomass, BOD, COD and TON of the tested effluents as affected by the dilution rate*

3.3.2.2. Effect of Incubation Period

In the present study, the bioremediation process was monitored during the different phases of growth and the analysis of the fermentation process was carried out daily using the suitable dilution. This study revealed that the values of BOD, COD and TON were high at the beginning of the experiment and reduced gradually till the 5th day of the incubation period referring to the beginning of the stationary phase of the tested yeast strains [25, 26, 27].

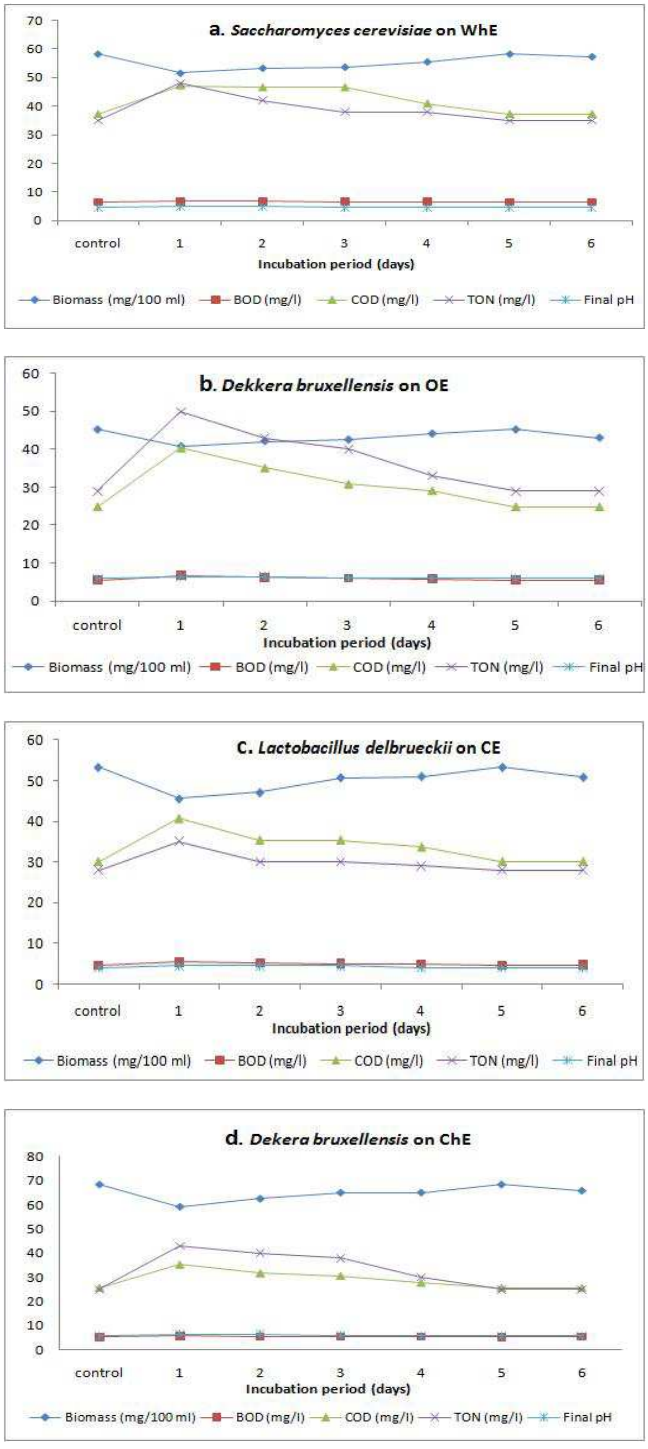


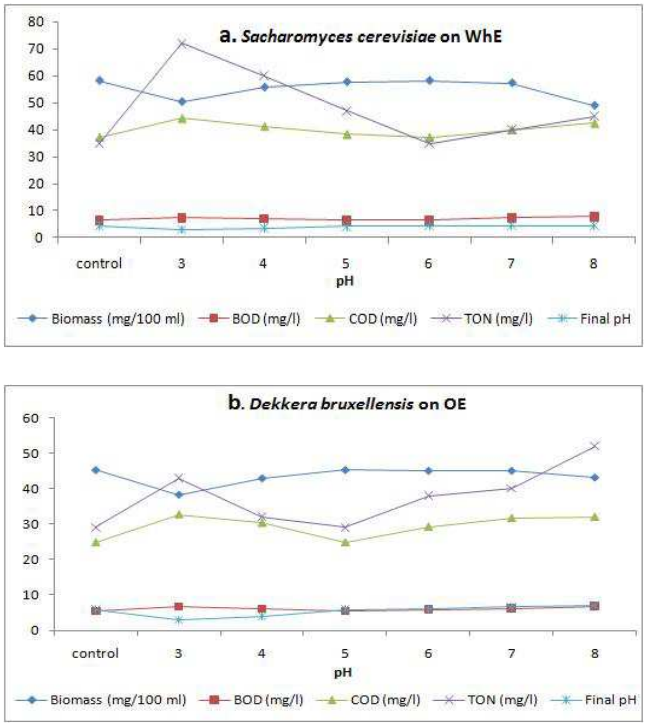
Figure 2. *Biomass, BOD, COD and TON of the tested effluents as affected by the incubation period*

The results of the present study coincide with those reported by Baker & Herson [28] where the incubation period of the cultivated microorganisms used was 5 days.

As for *Lactobacillus delbrueckii*, it was noticed that the stationary phase started from the 3rd day till the 6th day of growth (Fig. 2a, 2b, 2c and 2d). These findings are in accordance with that obtained by St-Arnaud et al. [29] who reported that the incubation time of *Nitrosomonas europaea* for maximum reduction of BOD and COD was five days. Therefore, the fermentation processes were allowed to grow for five days in all the experiments [30, 31].

3.3.2.3. Effect of pH

In the present work, the effect of different starting pH values on the activity and the biodegradability of the tested organisms grown on the different effluents were investigated. The biodegradability of the tested organisms responded differently to the reaction of the medium. Maximum growth and bioremediation activities (Biomass, 58.2 mg/100ml; BOD, 6.4 mg/l; COD, 37.2 mg/l and TON, 35 mg/l) were achieved at pH 6 by Saccharomyces cerevisiae Y-1347 grown on WhE. This was followed by Dekkera bruxellensis grown on OE where maximum bioremediation activities (Biomass, 45.2 mg/100ml; BOD, 5.5 mg/l; COD, 24.8 mg/l and TON, 29 mg/l) were achieved at pH 5. Furthermore, Lactobacillus delbrueckii subsp. bulgaricus grown on CE showed maximum growth and bioremediation activities (Biomass, 53.2 mg/100ml; BOD, 4.6mg/l; COD, 30.1mg/l and TON, 28 mg/l) at pH 4. Finally, Dekkera bruxellensis grown on ChE showed maximum growth and bioremediation activities (Biomass, 68.5 mg/100ml; BOD, 5.3 mg/l; COD, 25.6 mg/l and TON, 25 mg/l) at pH 5 (Fig.3a, 3b, 3c and 3d).



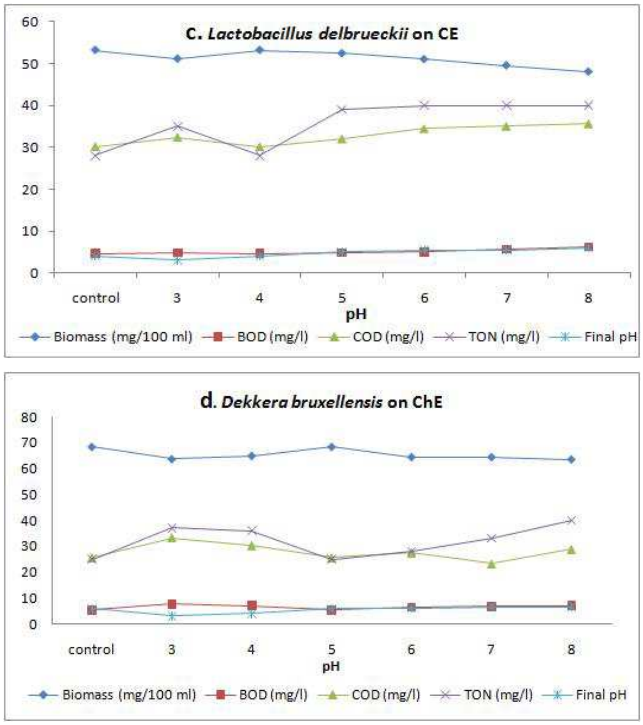


Figure 3. *Effect of hydrogen ion concentration of the tested effluents on the biomass, BOD, COD and TON on using different microorganisms*

Data in the present investigation revealed that pH beyond these values was inhibitory to the growth of all the tested organisms and the bioremediation of the effluents under test was best achieved at slightly acidic pH (6, 5, 4 and 5 for WhE, OE, CE and ChE, respectively). These results are in agreement with those obtained by Norris [6] who reported that bioremediation precedes well in aquifers at a slightly acidic pH values (4.5-5). Furthermore, Blais et al. [32] reported that Thiobacillus thiooxidans and strain VA-4 were cultivated at pH 4 while T. thioparus; T. intermedius; T. neapolitanus; T. denitrificans and strain VA-7 were cultivated at pH 7 for metal removal from the municipal sludge and hence the reduction of BOD and COD. Similarly, Borja and Banks [33] reported that pH 4.8 was the best for the anaerobic digestion of soft drink wastewater.

3.3.2.3. Effect Age of Seed Culture

The following experiment was carried out to determine the optimal seed culture age of the bacterial and yeast strains under test which leads to maximum reduction in BOD and COD in the different trials.

Results obtained (Fig. 4a, 4b, 4c and 4d) revealed that a good bioremediation of whey effluent (COD, 19.20 mg/l; BOD, 4.2 mg/l and TON, 20 mg/l) was exhibited by *Saccharomyces cerevisiae* Y-1347 of 3 days old seed culture. A good bioremediation of the carrot effluent (CE) (COD, 15.00 mg/l; BOD, 3.8 mg/l and TON, 15 mg/l) was exhibited by *Lactobacillus delbrueckii* subsp. *bulgaricus* of 36 hours old seed culture. As the seed culture grew older (3 days old), a maximum reduction of BOD, COD and TON were achieved by *Dekkera bruxellensis* grown on both orange effluent (OE) (BOD, 4 mg/l; COD, 17 mg/l and TON, 18 mg/l) and chocolate effluent (ChE) (BOD, 4.2 mg/l; COD, 17.10 mg/l and TON, 19 mg/l). However, seed cultures of younger or older ages showed lower efficiencies in the bioremediation rate with higher biomass yield. This might be due to the high metabolic activity of the cells at this stage of growth [34] beside that using an inoculum of actively growing cells might result in a fast increase in sugar consumption and consequently accelerate the reduction rate of BOD, COD and TON of all the tested wastes and enhanced the biomass production [35, 36].

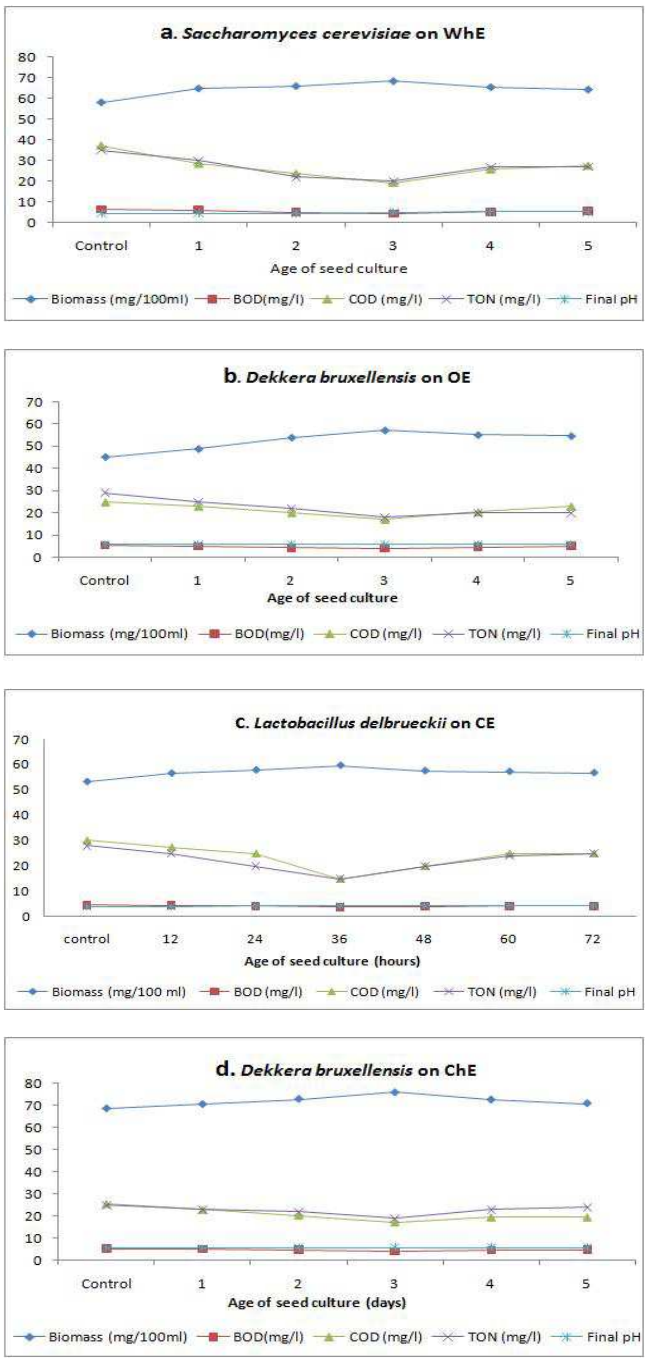


Figure 4. *Effect of age of the seed cultures of the tested microorganisms on the biomass, BOD, COD and TON of the tested effluents*

3.4. Chemical Composition of the Treated Effluents

In the previous experiments, emphasis has been imposed to outline the important aspects which lead to the highest biodegradable efficiency of the measured parameters (BOD, COD and TON) and the highest biomass output. The results of these investigations paved the way to determine the ability of the autochthonous microorganisms together with the tested allochthonous ones to reduce BOD, COD and TON contents of the effluents. Data given in Table 4 and 5 revealed that all the measured parameters and the element contents of the treated effluents were reduced with the improvement of their chemical and physiological characteristics. These results were in accordance with those obtained by Old & Primrose who reported that microorganisms can be used not only in the extraction of metals from low grade ores but also to reduce the pollution load in the environment. Recovery of metals using microorganisms would only be a fraction of the cost of physical and chemical recovery processes, therefore the active utilization of suitable microorganisms to remove metals from wastewaters appears very attractive both environmentally and economically[38, 39].

Table 4. *Physico-chemical characteristics of the 4 treated effluents (WhE, OE, CE and ChE) (%reduced)*

Parameter	Whey effluent (WhE)	Orange effluent (OE)	Carrot effluent (CE)	Chocolate effluent (ChE)
pH	8.3	3.23	29	7.82
Total hardness mg/l	82	83	87	86
Calcium hardness mg/l	81	87	90	89
Magnesium hardness mg/l	84	76	80	80
Sulphate mg/l	70	65	70	68
TDS mg/l	69	71	71	71
Chloride mg/l	61	60	57	24
TOC mg/l	54	76	60	65
Ammonia mg/L	66	50	71	71
Nitrite mg/l	76	41	81	28
Nitrate mg/l	26	34	54	33

Table 5. *Metal contents of the4 treated effluents (WhE, OE, CE and ChE) (%reduced)*

Element	Whey effluent (WhE)	Orange effluent (OE)	Carrot effluent (CE)	Chocolate effluent (ChE)
Zinc (Zn)	75.27	42.31	63.34	69.29
Manganese (Mn)	76.00	72.86	66.67	61.12
Copper (Cu)	91.00	80.00	83.50	57.00
Iron (Fe)	89.00	96.75	96.67	77.00
Cadmium (Cd)	52.24	71.74	84.29	41.67
Cobalt (Co)	83.71	46.88	43.48	60.61
Lead (Pb)	68.00	33.34	70.00	61.67

3.5. Irrigation Quality of the Treated Effluents

The treated effluents were used as irrigation water in the following experiment: Two kinds of seeds, *Lens culinaris* and *Phaseolus vulgaris* were subjected to germination by irrigation (daily for 1 month) with the four treated and untreated effluents (WhE; OE; CE and ChE) and with ordinary water as control. The fresh and dry weight of the seedlings was estimated.

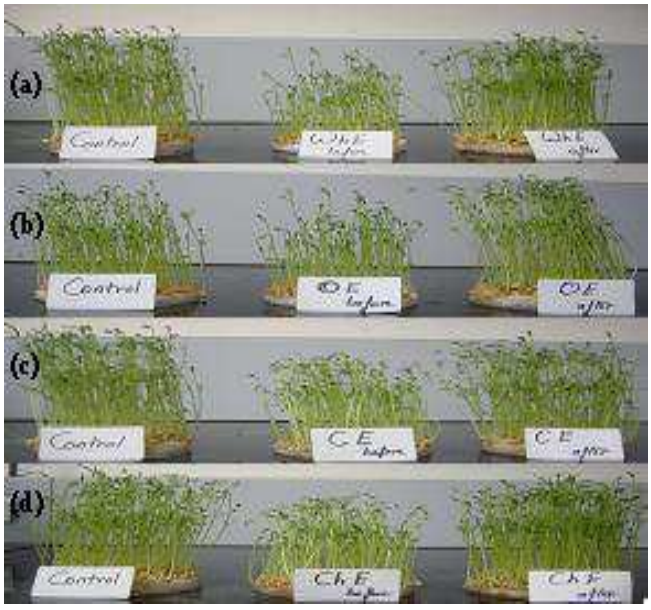


Figure 5. *Germinating Lens culinaris seeds as affected by the biologically treated effluents (a. WhE, b. OE, c. CE and ChE)*

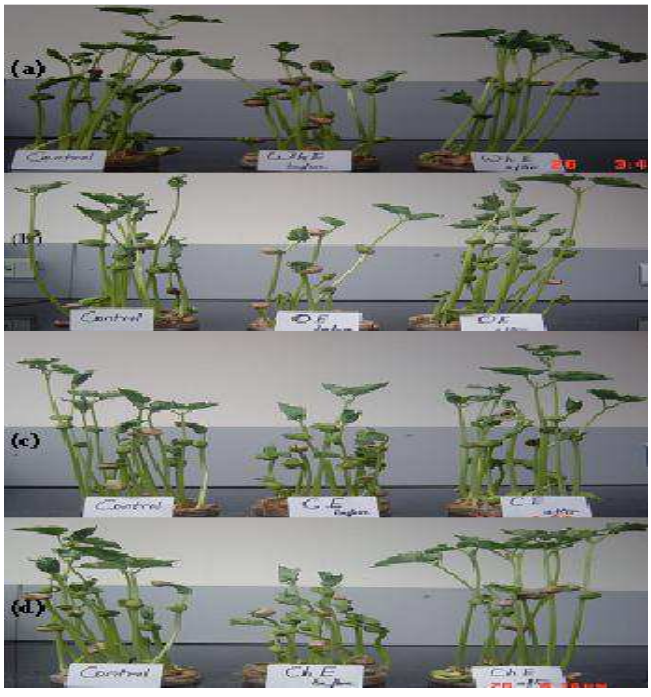


Figure 6. *Germinating Phaseolus vulgaris seeds as affected by the biologically treated effluents (a. WhE, b. OE, c. CE and ChE)*

Fig. 5 & 6 revealed that the tested seeds irrigated with the untreated effluents were able to grow, but their growth were not as much as those irrigated with the treated effluents which proved to be suitable for irrigating the tested seeds and was confirmed by the fresh and the dry weights of the

germinating seeds (seedlings) (Table 4). This might be due to the presence of high metal concentrations in the untreated effluents which exerted an inhibitory effect on the growth of the embryo hence retardation of the growth of the seeds irrigated with the untreated effluents and it might be also due to the improvement of the quality of the effluents under test as compared to the irrigation water quality according to the recommended *values* (EPA).

In addition, the tested seeds irrigated with ordinary water as control showed that their growth was better than the seeds irrigated with the untreated effluents but also their growth was not better than the seeds irrigated with the treated effluents and this is may be due to the presence of low concentrations of organic materials in the ordinary water and which were consumed rapidly by the seeds and which explain why their growth was not as efficient as those irrigated with the treated effluents.

Table 6. *Fresh and dry weights of the germinating seeds as affected by treated effluents*

Type of seedling	Treated irrigating effluent	Seedling fresh weight (g)	Seedling dry weight (g)
Phaseolus vulgaris	Control	2.25	0.70
	Whey (WhE)	2.10	0.60
	Orange (OE)	2.10	0.70
	Carrot (CE)	2.10	0.60
	Chocolate (ChE)	2.10	0.60
Lens culinaris	Control	0.32	0.05
	Whey (WhE)	0.32	0.05
	Orange (OE)	0.35	0.06
	Carrot CE)	0.32	0.05
	Chocolate (ChE)	0.30	0.04

4. Conclusion

The bioaugmentation strategy (using autochthonous and allochthonous microorganisms) in all the tested wastewater effluents showed to have the highest potential to convert the waste materials into stable oxidized end products which can be safely discharged and successfully used in irrigation.

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