Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2014.954148

56 (2015) 1908–1917
November

Taylor & Francis
Taylor & Francis Group

Biodegradation of olive mills wastewater using thermophilic bacteria

Zakaria Al-Qodah[a,b,*], Mohammad Al-Shannag[c], Khalid Bani-Melhem[d], Eman Assirey[e], Kholoud Alananbeh[f], Nahla Bouqellah[f]

[a]*Chemical Engineering Department, Taibah University, Madinah, KSA, Tel. +00966560948161; emails: z_alqodah@hotmail.com, zqudah@taibahu.edu.sa (Z. Al-Qodah)*
[b]*Chemical Engineering Department, Al-Balqa Applied University, P.O. Box 340558, Marka, Amman 11134, Jordan*
[c]*Faculty of Engineering and Technology, Chemical Engineering Department, The University of Jordan, 11942 Amman, Jordan, Tel. +00962772352778; email: mohammad_al_shannag@hotmail.com (M. Al-Shannag)*
[d]*Faculty of Natural Resources and Environment, Department of Water Management and Environment, Hashemite University, Al-Zarqa, Jordan, Tel. +00962795634630; email: k_banimelhem@yahoo.com (K. Bani-Melhem)*
[e]*Chemistry Department, Taibah University, Madinah, KSA, Tel. +00966553311211; email: eman_assirey@hotmail.com (E. Assirey)*
[f]*Biology Department, Taibah University, Madinah, KSA, Tel. +00966550040471; email: Kholoudennab@gmail.com (K. Alananbeh), Tel. +00966506563481; email: nahla.b@hotmail.co.uk (N. Bouqellah)*

Received 11 March 2014; Accepted 6 August 2014

ABSTRACT

Biodegradation of olive mill wastewater (OMW) toxic constituents was investigated using thermophilic bacteria. Three types of thermophilic bacteria were isolated from olive mills solid waste (Jift) and tested their ability to detoxify OMW. These thermophiles, namely *Bacillus polymaxa* JT6, *Bacillus Maceramns* JT7 and *Bacillus popilliae* JT8, can grow on Zebar as a main carbon source. The optimal growths of thermophiles were obtained at temperature of 65°C and pH of 6.4. The growth of the three thermophiles on Zebar was descending with increasing Zebar concentration. Complete inhibition of thermophiles was observed when the total solid concentration of Zebar was above 20 g/L. This suggests the need for a pre-treatment process for Zebar prior to the biological treatment or the use of diluted Zebar solution. Moreover, the growth rate of the thermophiles on Zebar solution of 8 mass% was described by Monod growth model.

Keywords: Thermophilic bacteria; Olive mills wastewater; Batch growth kinetics; Biodegradation

1. Introduction

Olive trees are of the most abundant fruit trees in Jordan, especially in the northern side where the climate is suitable for this tree. In addition, the produced olive oil is considered a major agricultural contributor to the national economy of Jordan [1]. However, the annual quantity of olive mills wastewater (OMW) effluents, called Zebar, exceeds 300,000 m^3, in addition to 120,000 tons of solid wastes called Jift. On the other hand, 98–99% of the olive annual world production comes from the Mediterranean countries, [2]which is accompanied by more than $3 \times 10^7 m^3$ of OMW [3]. This OMW usually contains polyphenols and sugars, volatile acids, polyalcohols, and nitrogenous compounds. The concentration of phenols could reach 10 g/L, a value that contributes to a high toxicity and antibacterial activity of OMW. Moreover, the chemical

*Corresponding author.

oxygen demand (COD) ranges from 25 to 162 kg O_2/L and the biological oxygen demand (BOD5) from 9 to 100 kg O_2/L [4].

The phenolic fraction in OMW accounts for most of the problems associated with the management of these wastes because this material is very harmful to the environment. OMW is highly colored and varies from dark red to black, depending on the age and the type of olives processed [5]. The color of this wastewater can still be detected visually after a 1/1,000 volume dilution in water. Phenolic compounds are also responsible for several biological effects, including antibiosis and phytotoxicity [6]. However, the physicochemical properties of OMW are rather variable, depending on climatic conditions, olive cultivars, degree of fruit maturation, storage time, and extraction procedure [7]. Owing to the above characteristics, the disposal of OMW in urban sewage treatment plants is impracticable and not allowable. In addition, since OMW is highly concentrated, it can, upon disposal, degrade soil and water quality, and thereby, negatively affect aquatic and terrestrial ecosystems [8].

Accordingly, the development of environmentally acceptable methods for disposal of OMW still remains a problem, whereas the degradation of the toxic compounds contained in this wastewater will certainly enhance the quality of the remediated waters and the sedimentation mud, in view of their safe utilization as fertilizers [9].

Several methods have been proposed in the last 30 years for OMW and olive pomace (Jift) management and disposal, based on evaporation ponds, thermal concentration, and physicochemical and biological treatments, as well as their application to agricultural soils as organic fertilizers either directly or after a composting process [8,10].

Among the above methods, a microbial pretreatment of OMW can positively affects its composition by reducing the toxicity of polyphenols. To achieve this goal, the selection of appropriate biological catalysts to perform OMW treatment should take into account their intrinsic capability to degrade aromatics [2]. Several investigations have been carried out using microorganisms capable of growing aerobically on diluted OMW in order to reduce the initial organic load and the phenolic content [11,12]. In particular, the pretreatment of OMW with higher fungi, which produce polyaromatic hydrocarbon-degrading enzymes, including *Aspergillus terreus* [13] and the white rot fungi *Phanerochaete chrysosporium* [14], *Pleurotus* species [15,16], *Lentinus edodes* [17], and free and immobilized mixtures of *Bacillus* sp. [18,19], have been used to detoxify and decolorize the waste and reduce considerably the COD and the total phenolic compound concentration, which is responsible for its biotoxicity. However, it is reported that their use on a large scale is difficult compared to bacteria [19].

Furthermore, it is expected that biodegradation of OMW could be achieved to a great extent if wild strains of microorganisms are used. Thermophiles, microorganisms thriving at a temperature of 50˚C or higher, could be good candidates for such processes. Such microorganisms are able to grow at relatively high temperatures where the rate of reaction could be doubled for each 10˚C rise. In addition, the stability of polyphenols at relatively high temperatures usually decreases.

Several researchers investigated the capabilities of thermophilic bacteria to degrade olive oil [20], lipids [21], fat from industry [22], and phenolic compounds [23]. Results from these studies indicate that thermophilic bacteria have superior capabilities to degrade fats and phenols over mesophiles. However, the use of thermophiles to treat OMW or Zebar has not been cited so far. For these reasons, the main objective of this investigation is to use thermophiles to treat OMW according to the following procedure. First, isolate some thermophiles from environments having OMW or its solid wastes (Jift). Second, investigate the capabilities of the isolated thermophiles to treat OMW in order to become suitable for agricultural needs. Finally, optimize the operational conditions for the biodegradation process.

2. Kinetic modeling

There is no information available in the literature about the growth kinetic of thermophilic bacteria on olive mills waste as a sole carbon source. Many researchers have applied the unstructured growth kinetic model proposed by Kono [24], for protease production by mesophilic and thermophilic *B. sphaericus* using glucose as a sole carbon source [25,26]. In this model, the cellular growth passes through six subsequent phases, including: lag phase, transition phase, exponential phase, deceleration or declining phase, stationary phase, and death phase. Since this unstructured growth has successfully been used for the kinetic analysis of a number of fermentation processes, the kinetic parameters of the cellular growth of thermophilic bacteria growing on OMW will be determined using this model. The growth rate in the exponential growth phase can be expressed by the following first-order equation:

$$\frac{dX}{dt} = \mu X \tag{1}$$

where X is the cell concentration in g/L, μ is the specific growth rate in h^{-1}, and t is the time in h. Upon integration, the above equation, within the range of the exponential growth phase, yields:

$$X = X_c e^{\mu(t-t_c)} \tag{2}$$

where X_C is the cell concentration in g/L at the boundary point between the exponential and deceleration growth phase, t_C. In this growth phase, μ is equal to the slope of the plot between ln (X/X_C) and $(t - t_C)$. On the other hand, the growth rate in the deceleration growth phase is expressed as:

$$\frac{dX}{dt} = \frac{\mu X_c (X_M - X)}{(X_M - X_C)} \tag{3}$$

where X_M is maximum or theoretical cell concentration in g/L. The integral form of Eq. (3) is:

$$X = X_M - (X_M - X_C) e^{-\frac{\mu X_C (t-t_c)}{(X_M - X_C)}} \tag{4}$$

It is clear from Eq. (4) that X is a function of time only and it can be simultaneously calculated at any time. By definition, the resultant growth rate in the stationary phase is zero, where in the death rate it can be expressed by the following first-order model:

$$\frac{dX}{dt} = -k_d X \tag{5}$$

where k_d is the death rate constant in h^{-1}. The corresponding doubling time τ_d can be calculated using the following expression:

$$\tau_d = \frac{\ln(2)}{\mu_{max}} \tag{6}$$

Monod equation [27] is the most usual empirical relationship used to describe the relation between cellular growth (μ) and substrate concentration (S). However, OMW contains high-strength organic compounds that usually cause inhibition, and this model is not entirely adequate to predict the behavior of high-strength organic compounds [28]. Consequently, substrate inhibition growth kinetics model will be assumed to explain the growth of thermophilic bacteria on OMW. The model equation is expressed as following:

$$\mu = \frac{\mu_{max} S}{k_S + S + S^2/K_{SI}} \tag{7}$$

where K_{SI} is the equilibrium constant of the substrate inhibition reaction which has the unit of g/L, k_s is the half-saturation constant of growth kinetics in g/L, and S is the substrate concentration in g/L. At low substrate concentration, the term (S^2/K_{SI}) can be neglected and Eq. (7) reduces to the following Monod equation [23]:

$$\mu = \frac{\mu_{max} S}{k_S + S} \tag{8}$$

On the other hand, at high substrate concentration, Eq. (7) is reduced to:

$$\mu = \frac{\mu_{max}}{1 + S/K_{SI}} \tag{9}$$

3. Materials and methods

3.1. OMW and Jift used

Samples of liquid OMW or Zebar, and Jift, which is a semisolid paste, were obtained from an olive oil production plant located in the Baqah Camp located 20 km to the north west of Amman city, Jordan. This plant uses a semicontinuous process for extraction of olive oil. The raw liquid samples were collected in 1 L cans from the outlet disposal pipes of the plant. These samples were transferred through ice box and then kept as stock wastewater in a refrigerator at around 4°C for further uses. Table 1 summarizes some physical, chemical, and biological characteristics of OMW used in this study. Samples of OMW were periodically withdrawn from the stock cans and centrifuged at 5,500 rpm to remove all possible suspended solids.

On the other hand, samples of the Jift were taken from the Jift heap at a depth of about 30 cm below the free surface. These Jift samples were kept in a refrigerator at a temperature of 4°C in order to be used for isolating thermophiles. Fresh Jift is made mainly with water, seed, and pulp [29]. The Jift exhibits similar damages of OMW to the environment. It has phytotoxic and antimicrobial properties, low pH, relatively high salinity and organic load, and the phenolic and lipid constituents [30].

3.2. Thermophilic bacteria isolation

In this part of the investigation, attempts were made to isolate lipid- or oil-degrading microorganisms from Jift. Jift samples were inoculated by thermophiles

Table 1
Characteristics of OMW used in this study

Parameter	Value
pH	5.2
Total COD (g/L)	114
Density (g/L)	1.08
Dissolved COD (g/L)	62
BOD_5 (g/L)	55
Total solids (TS) (g/L)	91.2
Ammonia (g/L)	0.16
Total sugars (g/L)	19
Total phenolic compounds (g tannic acid/L)	5.5

in three flasks. Each flask contained 100 mL MA broth. The mass of Jift was 1.0, 1.5, and 2.0 g in flasks 1, 2, and 3; respectively. The flasks were incubated at 45˚C for 24 h. After this period, a sample of liquid was withdrawn from each flask and analyzed for the presence of viable cells by subculturing on solid media. Inoculums of 100 μL of each sample were spread on the surface of MA plates. After 24 h, the plates were visualized under the microscope.

After the detection of cellular growth in the plates, several experiments were conducted to test the tolerance of these microorganisms toward higher temperatures, different pH values, and quantity of Zebar in the growth media. The effect of temperature was conducted by consequent procedures of culturing and subculturing at a higher temperature. The first subculturing was made at 50˚C, then 55, 60, 65, 70, 75, and 80˚C. The effect of pH was investigated by varying its value from 4 to 11 on the cultures isolated at 65˚C. On the other hand, the effect of Zebar quantity was tested on cultures isolated at 65˚C and pH of 6.4. The procedure was conducted as follows: a mixture of 1 g Zebar and 14 g peptone was dissolved in 100 mL distilled water and incubated by a culture isolated at 65˚C and pH of 6.4. The culture was incubated for 24 h in a shaker incubator. After that, a sample of 1 mL of the broth was transferred to another mixture containing 2 g Zebar and 13 g peptone and incubated for 24 h. This procedure of culturing, incubation, and transferring continues until a mixture of 14 g Zebar and 1 g peptone was reached. This process was conducted to acclimate the thermophiles to the highest possible concentrations of Zebar.

3.3. Thermophilic bacteria characterization

Samples of the isolated thermophiles were transferred to three agar media, including nutrient agar, EMB agar, and MacConkey agar, using calibrated loop. The plates of cultures and subcultures were incubated at different temperatures from 45 to 80˚C for 48 h. After incubation, a mixed culture of different types of bacteria was obtained. Number of colonies in each sample was calculated using a dark field colony counter (Leica, USA). Isolation of each species was carried out using the streaking plate method. Bacterial colonies were identified using morphological characteristics, microscopic appearances, and a set of biochemical tests (API 50 CHB, bioMerieux sa, Inc, France), in addition to gram staining.

3.4. Batch growth of the thermophilic isolates

After the determination of the optimal conditions for culturing the three isolates C_1, C_2, and C_3, a series of batch growth experiments were conducted in a laboratory-scale one-liter-size fermenter with a working volume of 700 mL. The fermenter was equipped with suitable controllers including temperature, pH, oxygen concentration, and stirring speed. The fermentation was conducted at 65˚C and pH 6.4. The pH was maintained by the controlled addition of 1 M NaOH. The agitation speed was set to 250 rpm and an aeration rate was adjusted to 80 L/h. The dissolved oxygen concentration was monitored using a standard amperometric pO_2 probe (Mettler-Toledo Prozeβanalytic GmbH, Stein-batch, Germany). The aeration flow rate was enough to maintain pO_2 above 10% saturation to avoid oxygen limitation. Each of the batch processes continued until the optical density reached a maximum and started to decrease after the stationary phase. The thermophiles cell dry weight was determined by centrifuging a certain volume of the fermentation broth and then washing the pellets twice with deionized water, followed by drying of the washed pellets at 105˚C for 2 h. During the fermentation process the turbidity of the broth was monitored by measuring the optical density at 600 nm which is a direct measure of cellular growth. The fermentation process continued for 14 h in each case. Fig. 1 shows a calibration curve between cell dry density, ρ_d, and both optical density, ρ_o, and cell count (C_c).

4. Results and discussion

4.1. Characterization and identification of the microorganism

In this investigation, thermophilic bacterial strains were isolated from olive solid wastes or Jift. These strains were cultured on different types of agar as mentioned above. After identification and characterization, three strains were isolated and purified from the Jift samples. These strains are *Bacillus polymaxa*, *Bacillus Maceramns*, and *Bacillus popilliae*. Actually,

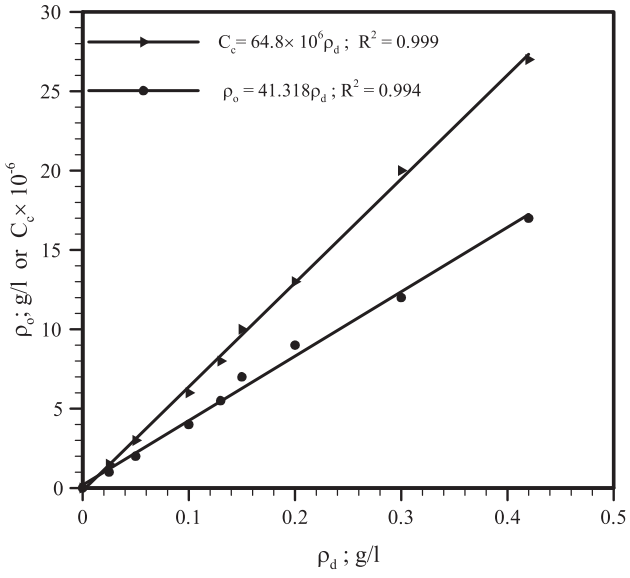

Fig. 1. Calibration curves between cell dry density, ρ_d, vs. both optical density, ρ_o, and cell count (C_c).

these thermophiles are considered as the first thermophiles isolated from olive mills wastes. In previous studies, most researchers acclimate known bacteria to utilize olive mills wastes rather than following the procedure illustrated in this study [17–19]. For this reason, the isolated strains are new and prefixes of JT6, JT7, and JT8 will be added to denote *B. polymaxa*, *B. Maceramns*, and *B. popilliae* thermophiles; respectively. Other thermophiles of prefixes JT1 to JT5 were previously isolated from Jordanian hot springs [26,31,32]. As shown in Table 2, Gram staining indicated that the three thermophiles are Gram positive. The microscopic photo of *B. polymaxa* (JT6) shows that this Gram-positive strain as the others is strictly aerobic, rod shaped organism and was motile. The cells possessed endospore at the center. On solid media the colonies were round.

4.2. Optimization of the growth conditions

After identifying the three isolates, a series of experiments was conducted to examine the effects of temperature, pH, and mass quantity of OMW (Zebar) added to the medium in order to optimize their growth conditions.

4.2.1. Effect of temperature

Several plates containing the three isolates were incubated at different temperatures. The procedure of culturing and subculturing mentioned above was applied. It is evident that all the three thermophiles showed a very good growth at temperature of 65˚C. On the other hand, plates incubated at 80˚C showed a relatively low growth.

Samples of the three thermophiles grown at 65˚C were inoculated in the bioreactor. In each run, the bioreactor temperature was adjusted to a certain temperature. The pH was 7, and the medium contained 12 g Zebar and 3 g Peptone/L. These batch experiments were continued for 12 h in order to measure the maximum medium turbidity at each temperature. Fig. 2 shows the results of thermophiles growth at different temperatures.

It is evident from Fig. 2 that the optimum temperature of growth of the three thermophiles is about 65˚C. The turbidity and consequently the cell dry density increase as the temperature increases until it reaches a maximum. Then, the turbidity starts to decrease until a minimum value of 80˚C. However, Fig. 2 shows that JT8 thermophile had a higher growth values than JT6 and JT7 at all temperatures. At 64˚C, the thermophiles JT6, JT7 and JT8 had a cell dry density of 2.7, 2.7, and 3 g/L, respectively. At 80˚C, these values were 1.1, 1.2, and 1.7 g/L, respectively.

4.2.2. Effect of pH

The previous procedure was repeated at different values of pH for the three thermophiles at 65˚C to investigate the optimum pH for the cellular growth. The results showed that the optimum pH was between 6 and 7. The best growth for JT6, JT7, and JT8 thermophiles was at a pH of about 6.4. Subsequently, samples of the three thermophiles grown at

Table 2
Some properties of the *Bacillus* strains isolated from Jift

Strain	Type	Gram staining	MacConkey agar	EMB agar
C_1	*Bacillus polymaxa* (JT6)	+ve	+ve	+ve
C_2	*Bacillus Maceramns* (JT7)	+ve	+ve	+ve
C_3	*Bacillus popilliae* (JT8)	+ve	+ve	+ve

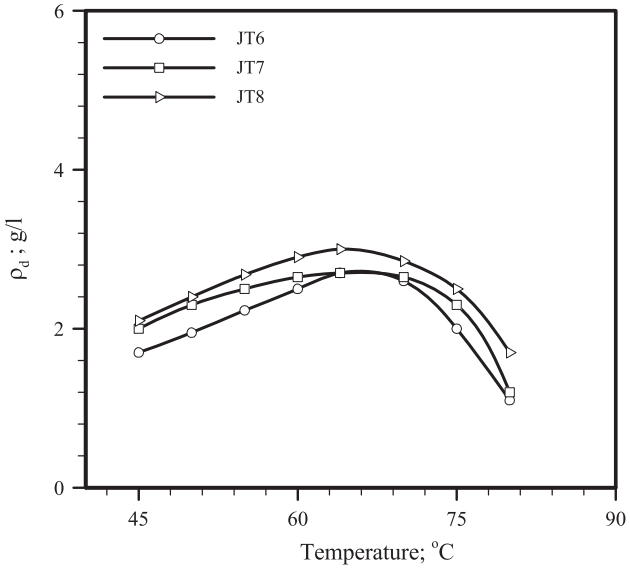

Fig. 2. Effect of temperature on the growth, in terms of ρ_d, of the three thermophiles cultured originally at 65°C.

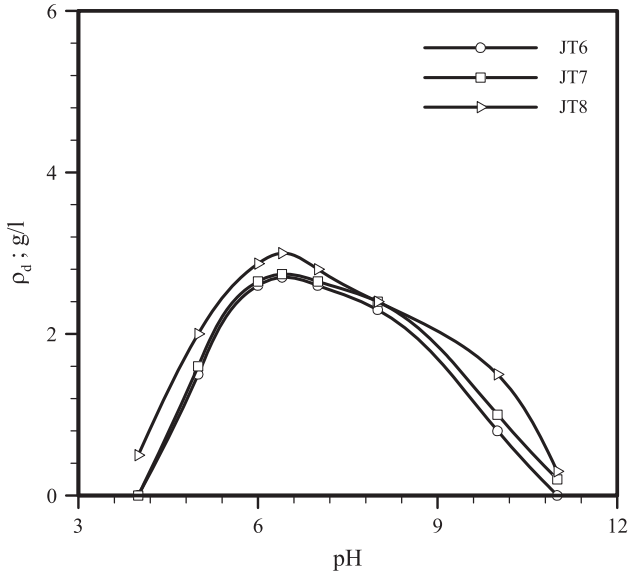

Fig. 3. Effect of pH on the growth, in terms of ρ_d, of the three thermophiles cultured originally at a pH of 6.4.

pH 6.4 were inoculated in the bioreactor. In each run, the acidity level of the broth inside the bioreactor was adjusted to the desired pH value using 0.1 M NaOH. The temperature was 65°C, and the medium contained 12 g Zebar and 3 g Peptone/L. Again, these batch experiments were continued for 12 h in order to measure the maximum medium turbidity at each pH value. Fig. 3 shows the results of thermophiles growth at different pH values.

Fig. 3 depicts that the optimum pH for growth of the three thermophiles is about 6.4. The turbidity and consequently the cell dry density increases as the pH values increase until it reaches a maximum cell dry density of 2.7, 2.74, and 3 g/L for JT6, JT7 and JT8; respectively. Then, the turbidity started to decrease until a minimum value at pH of 11 where the cell dry density values fall to 0, 0.2, and 0.3 for JT6, JT7, and JT8, respectively. Furthermore, JT7 showed no growth at a pH of 4.

4.2.3. Effect of Zebar ratio in the broth

Several plates were prepared with different proportions of Zebar to peptone as carbon sources in order to examine the effect of Zebar concentration in the medium and its inhibition to the cellular growth. In all experiments, the temperature was 65°C, the pH was 6.4, and the agar percentage was 2 mass%. The results indicate that all strains show good growth until the concentration of Zebar reaches 9 g/L or 60% of the total carbon sources. After that, the growth started to decrease as a result of Zebar inhibition.

Batch experiments using liquid media were performed using different proportions of Zebar. In all experiments, the temperature was 65°C and the pH was 6.4. The results are shown in Fig. 4. It is clear from Fig. 4 that low Zebar ratio in the carbon sources has no adverse effect on the cellular growth. However, when this ratio exceeds 60 mass%, the turbidity and consequently the cell dry density sharply decrease. When the Zebar mass ratio was 60%, the cell dry density for JT6, JT7, and JT8 were 4.8, 4.9, and 5 g/L,

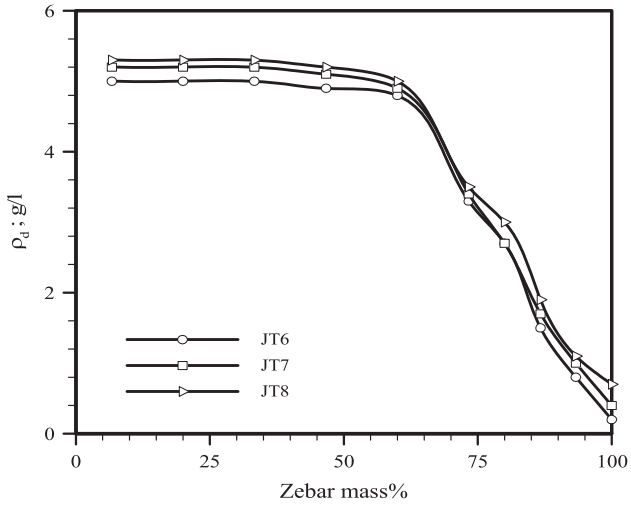

Fig. 4. Effect of Zebar mass percent as a carbon source on the growth, in terms of ρ_d, of the three thermophiles at temperature of 65°C and pH of 6.4.

respectively. When the Zebar mass ratio reaches 100%, the corresponding values were 0.2, 0.4 and 0.7 g/L; respectively. This behavior could be attributed to the negative effects of the polyphenols found in the Zebar on the cellular growth. This also could be attributed to another important factor related to the low nitrogen and phosphorus content in the Zebar. These nutrients are necessary for an adequate aerobic fermentation process [11]. Accordingly, the Zebar should be diluted before biodegradation if no pretreatment process is to be used. In addition, sufficient amounts of N and P should be added to the fermentation broth.

4.3. Batch growth of thermophiles on OMW

The results of traditional batch growth experiments of the three thermophiles, *B. polymaxa* (JT6), *B. Macera-mns* (JT7), and *B. popilliae* (JT8) are shown in Fig. 4. The medium contains 12 g OMW and 3 g peptone per 100 mL.

It is evident from Fig. 5 that the batch growth curves of the three isolates resemble the traditional growth curve in batch cultures of other mesophiles. This curve usually passes in six phases: lag, exponential, deceleration, constant, decay, and death phases. However, there were some differences between the three thermophiles in the extent of each phase. For example, JT8 shows a very short lag phase of about half an hour and a relatively long exponential one of about 5 h and a short constant growth phase. On the other hand, JT6 shows a relatively long lag phase of about two hours and a short exponential phase of three hours. Moreover, the growth of these thermophiles depends on Zebar and peptone concentrations as main carbon sources. The Zebar usually has its natural content of polyphenols, but in diluted concentrations of about 10 mass% of the crude Zebar, this content of polyphenols is low to cause inhibition. Accordingly, these strains show appreciable capabilities for degrading natural or normal diluted Zebar.

On the other hand, and to evaluate the kinetic parameters of the thermophiles growth in pure Zebar as a whole carbon source, a set of batch experiments were conducted using Zebar at different concentrations; see Fig. 6. It is clear in Fig. 5 that the growth and consequently the cell dry density increase as the Zebar substrate concentration increases until a maximum value. It is evident from Fig. 6 that the three thermophiles were able to grow on Zebar as a whole carbon source. The cellular growth increases as Zebar concentration increases from 1 to 8 g/L where it reaches a maximum. The maximum cell dry density of JT6, JT7, and JT8 were 3, 3.2, and 3.5 g/L, respectively. At low substrate concentration, the thermophiles grow with no inhibition according to Monod model described by Eq. (8). The value of growth rate at each Zebar concentration was calculated using the batch growth curve in the exponential growth phase and Eq. (2). Then, the Monod model described by Eq. (8) was liberalized by the double reciprocal (Lineweaver-

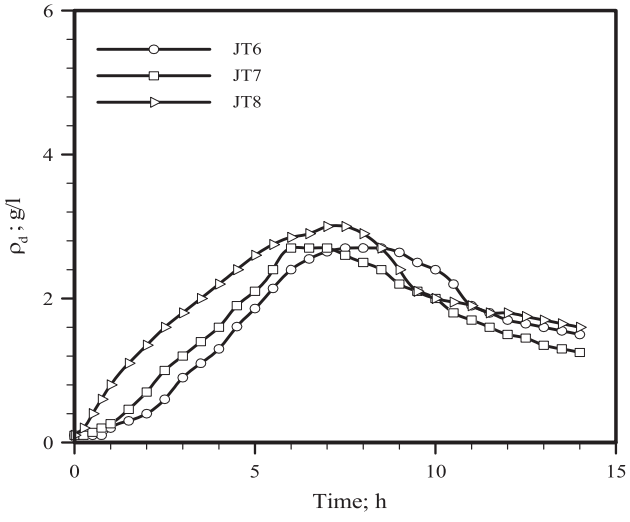

Fig. 5. Batch growth curves in terms of ρ_d for the three thermophiles on OMW at temperature of 65°C, pH of 6.4, and agitation speed of 250 rpm. The culture medium contains 3 g peptone and 12 g Zebar /L.

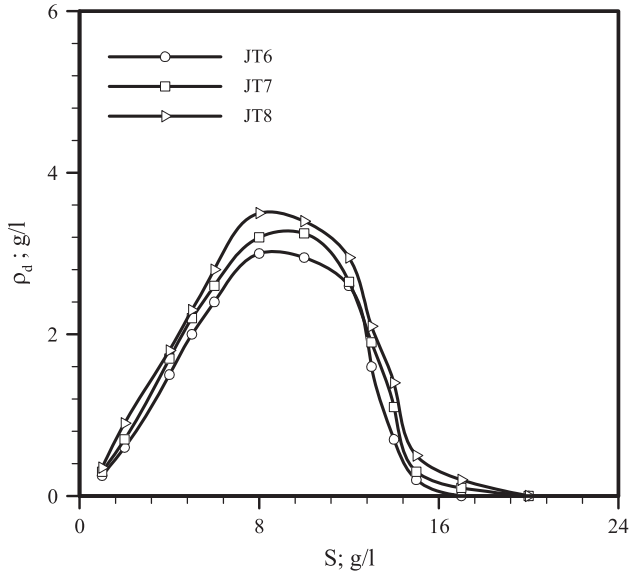

Fig. 6. Effect of substrate (Zebar) concentration, S, as a carbon source on the cell dry density, ρ_d, of the three thermophiles at temperature of 65°C, pH of 6.4, and agitation speed of 250 rpm.

Burk) plot method to obtain the model constants. These constants are the maximum growth rate, μ_{max}, and Monod saturation constant, K_s, and they were evaluated for the three strains. Fig. 7 shows the double-reciprocal plot for the three thermophiles.

The values of the Monod model constants for the three thermophiles obtained from Fig. 7 in addition to other constants corresponds to Eqs. (4) and (6) are summarized in Table 3.

In this part of research, Zebar is considered as one compound rather than a solution containing several different compounds of different chemical properties including inhibition. For this reason, any adverse effect of increasing Zebar concentration will be considered as a substrate inhibition. Actually, polyphenols present in Zebar are the most effective inhibitors for cellular growth. However, the relative polyphenol concentration to Zebar is constant throughout all experiments.

Referring to Fig. 6, it could be noted that as the Zebar concentration increases beyond 8 g/L, the cell dry density sharply decreases due to inhibition. The cell dry density of the three thermophiles reaches zero when the Zebar concentration exceeds 18 g/L. This substrate inhibition is a result of Zebar constituents of polyphenolic compounds. The value of the substrate inhibition constant K_I was determined from a plot of $1/\mu$ vs. S which will produce a straight line of slope $1/\mu_{max}$ and intercept of $1/(K_I\ \mu_{max})$. The values of K_I obtained from the corresponding slopes were 150, 142, and 133 mg/L for JT6, JT7, and JT8 thermophiles, respectively.

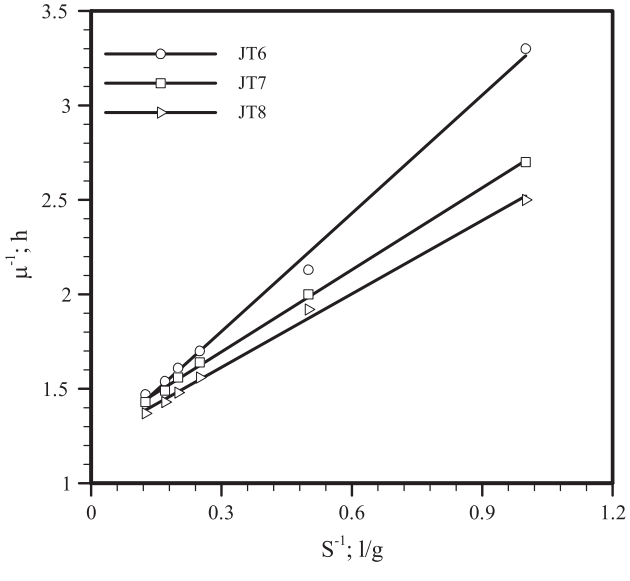

Fig. 7. Linearized plots used to obtain the model constants μ_{max} and K_s of Monod equation for the three thermophiles at temperature of 65℃, pH of 6.4, and agitation speed of 250 rpm.

Table 3
Kinetic parameters of batch Zebar biodegradation by three thermophiles

Variable	JT6	JT7	JT8
μ_{max}; h^{-1}	0.80	0.82	0.85
K_s; g/L	1.40	1.36	1.30
τ_d; h	0.87	0.84	0.82
X_C; g/L	2.65	2.70	2.90
X_M; g/L	5.20	5.50	6.00

5. Conclusions

Three thermophilic bacteria were isolated from olive mills solid wastes (Jift). These are *B. polymaxa* (JT6), *B. Maceramns* (JT7), and *B. popilliae* (JT8). The optimum growth temperature was about 65℃ and the optimum pH was around 6.4. The thermophiles grew on diluted Zebar as a whole carbon source. Inhibition was recognized when Zebar concentration increased beyond 8 g/L and fell to zero at 20 g/L. Therefore, a pretreatment stage is recommended for Zebar prior to a biological treatment stage in order not to impede microbial activity. The growth kinetic parameters of the thermophiles were investigated.

Acknowledgments

The authors are grateful for the Higher Council of Science and Technology (HCST), Jordan, Al-Balqa University, Jordan, and Deutsche Forschungsgemeinschaft, DFG (The German Research Foundation), for funding the project. The technical support of Hashemite University, Jordan and Hamburg Technical University, Germany are greatly appreciated for their help in conducting parts of the experiments.

Nomenclature

k_d — death rate constant (h^{-1})
k_s — half-saturation constant of growth kinetics (g/L)
K_{SI} — equilibrium constant of the substrate inhibition reaction (g/L)
S — substrate concentration (g/L)
X — cell concentration (g/L)
X_C — cell concentration at the at the boundary point between the exponential and deceleration growth phase (g/L)
X_M — maximum or theoretical cell concentration (g/L)

t — time (h)
t_C — time at the boundary between the exponential and deceleration growth phase (h)

Greek symbols

μ — specific growth rate (h^{-1})
μ_{max} — maximum growth rate (h^{-1})
τ_d — doubling time (h)

References

[1] A. Khdair, E. Qasim, Integrated Waste Management for Olive Oil Pressing Industries in Lebanon, Syria and Jordan, Phase 1-Report, Ministry of Environment, Amman, Jordan, 2006.
[2] A. D'Annibale, D. Quaratino, F. Federici, M. Menice, Effect of agitation and aeration on the reduction of pollutant load of olive mill wastewater by the white-rot fungus *Panus tigrinus*, Biochem. Eng. J. 29 (2006) 243–249.
[3] O. Yesilada, S. Sik, M. Sam, Biodegradation of olive oil mill wastewater by *Coriolus versicolor* and *Funalia trogii*: Effects of agitation, initial COD concentration, inoculum size and immobilization, World J. Microbiol. Biotechnol. 14 (1998) 37–42.
[4] M.S. Fountoulakis, S.N. Dokianakis, M.E. Kornaros, G.G. Aggelis, G. Lyberatos, Removal of phenolics in olive mill wastewaters using the white-rot fungus *Pleurotus ostreatus*, Water. Res. 36 (2002) 4735–4744.
[5] D.M. Gonzalez, N.E. Moreno, J.Q. Sarmiento, A. Ramos-Cormenzana, Studies on antibacterial activity of waste waters from olive oil mills (Alpechin): Inhibitory activity of phenolic and fatty acids, Chemosphere 20 (1990) 423–432.
[6] R. Robles, R. Lucas, G.A. Cienfuegos, A.G. Alvez, Biomass production and detoxifcation of wastewaters from the olive oil industry by strains of *Penicillium* isolated from wastewater disposal ponds, Bioresour. Technol. 74 (2000) 217–221.
[7] A. Fiorentino, A. Gentili, M. Isidori, P. Monaco, A. Nardelli, A. Parrella, F. Temussi, Environmental effects caused by olive mill wastewaters: Toxicity comparison of low-molecular-weight phenol components, J. Agric. Food Chem. 51 (2003) 1005–1009.
[8] S. Ntougias, K. Bourtzis, G. Tsiamis, The microbiology of olive mill wastes, Biomed. Res. Int. (2013) 1–16.
[9] M. Bressan, L. Liberatore, N. Alessandro, L. Tonucci, C. Belli, G. Ranalli, Improved combined chemical and biological treatments of olive oil mill wastewaters, J. Agric. Food Chem. 52 (2004) 1228–1233.
[10] C. Paredes, M.P. Bernal, J. Cegarra, A. Roig, Bio-degradation of olive mill wastewater sludge by its co-composting with agricultural wastes, Bioresour. Technol. 85 (2002) 1–8.
[11] K. Fadil, A. Chahlaouia, A. Ouahbib, A. Zaid, R. Borjac, Aerobic biodegradation and detoxification of wastewaters from the olive oil industry, Int. Biodeterior. Biodegrad. 51 (2003) 37–41.
[12] H. El Hajjouji, G. Ait Baddi, A. Yaacoubi, H. Hamdi, P. Winterton, J.C. Revel, M. Hafidi, Optimisation of bio-degradation conditions for the treatment of olive mill wastewater, Bioresour. Technol. 99 (2008) 5505–5510.
[13] L. Martinez Nieto, S.F. Garrido Hoyos, F. Camacho Rubio, M.P. Garsia Pareja, A. Ramos Cormenzana, The biological puri¢cation of waste products from olive oil extraction, Bioresour. Technol. 43 (1993) 215–219.
[14] S. Sayadi, R. Ellouz, Roles of lignin peroxidase and manganese peroxidase from *Phanerochaete chrysosporium* in the decolorization of olive mill wastewaters, Appl. Environ. Microbiol. 61 (1995) 1098–1103.
[15] E. Sanjust, R. Pompei, R. Rescingo, A. Rinald, M. Ballero, Olive milling wastewaters as a medium for growth of four *Pleurotus* species, Appl. Biochem. Biotechnol. 31 (1991) 223–235.
[16] G. Zervakis, P. Yiatras, C. Balis, Edible mushrooms from olive oil mill wastes, Int. Biodeterior. Biodegrad. 38 (1996) 237–243.
[17] C. Ehaliotis, K. Papadopoulou, M. Kotsou, I. Mari, C. Balis, Adaptation and population dynamics of *Azotobacter vinelandii* during aerobic biological treatment of olive-mill wastewater, FEMS Microbiol. Ecol. 30 (1999) 301–311.
[18] M.P. Prasad, K. Manjunath, Comparative study on Biodegradation of lipid-rich wastewater using lipase producing bacteria species, Indian J. Biotechnol. 10 (2011) 121–124.
[19] A.L. Galaction1, D. Caşcaval, R. Rotaru, A.M. Lupasteanu, M. Turnea, Kinetic studies on biodegradation of lipids from olive oil mill wastewater with free and immobilized *Bacillus* sp. cells, Sci. Study Res., Chem. Chem. Eng., Biotechnol., Food Ind. 13 (2012) 049–062.
[20] P. Becker, D. Köster, M.N. Popov, S. Markossian, G. Antranikian, H. Märkl, The biodegradation of olive oil and the treatment of lipid-rich wool scouring wastewater under aerobic and thermophilic conditions, Water. Res. 33 (1998) 653–660.
[21] S. Markossian, P. Becker, H. Märkl, G. Antranikian, Isolation and characterization of lipid-degrading *Bacillus thermoleovorans* IHI-91 from an Icelandic hot spring, Extremophiles 4 (2000) 365–371.
[22] I. Reimann, A. Klatt, H. Märkl, Treatment of wastewater containing fat from food industry with a thermophilic microorganism, Eng. Life Sci. 2 (2002) 398–401.
[23] H. Feitkenhauer, S. Schnicke, R. Müller, H. Märkl, Kinetic parameters of continuous cultures of *Bacillus thermoleovorans* sp. A2 degrading phenol at 65°C, J. Biotechnol. 103 (2003) 129–135.
[24] T. Kono, Kinetics of microbial cell growth, Biotechnol. Bioeng. 10 (1968) 105–131.
[25] J. Singh, R.M. Vohra, D.K. Sahoo, Enhanced production of alkaline protease by *Bacillus sphaericus using* fed-batch culture, Process Biochem. 39 (2004) 1093–1101.
[26] Z. Al-Qodah, H. Daghstani, Ph. Geopel, W. Lafi, Determination of kinetic parameters of α-amylase producing thermophile *Bacillus sphaericus*, African J. Biotechnol. 6 (2007) 699–706.
[27] J. Monod, The growth of bacterial cultures, Annu. Rev. Microbiol. 3 (1949) 371–376.
[28] A. Vlyssides, E.M. Barampouti, A.S. Mai, H. Loukakis, E. Tsimas, Kinetic of thermophilic aerobic degradation of olive mill wastewater (2005). Available from: http://www.srcosmos.gr/srcosmos/showpub.aspx?aa=11080.

[29] B. Gómez-Muñoz, D.J. Hatch, R. Bol, R. García-Ruiz, The compost of olive mill pomace: From a waste to a resource-environmental benefits of its application in olive oil groves (2012). Available from: http://dx.doi.org/10.5772/48244.
[30] J. Sierra, E. Marti, M.A. Garau, R. Cruanas, Effects of agronomic use of olive oil mill wastewater–field experiment, Sci. Total Environ. 378 (2007) 90–94.
[31] Z. Al-Qodah, Production and characterization of thermostable α-amylase by thermophilic *Geobacillus stearothermophillus*, Biotechnol. J. 1 (2006) 850–857.
[32] Z. Al-Qodah, H. Daghistani, K. Alananbeh, Isolation and characterization of thermostable protease producing *Bacillus pumilus* from thermal spring in Jordan, African J. Microbiol. Res. 7 (2013) 3711–3719.