Biodegradation of Food Waste by Mesophilic and Thermophilic Microorganisms in Duhok City

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Abstract

Garbage kitchen waste has been considered as one of the most difficult global issues with negative effect on the environment and health. It contains high amounts of cellulose and other organic waste which become ideal environments for the growth of pathogenic microbes and their toxic products which may reach agricultural land and water system. As a compromise, certain microbes are implemented under certain conditions to reduce and manage food waste. In the present study microorganism isolated from garbage kitchen waste collected in Duhok city. one thermophilic bacterium, two mesophilic bacteria, and fungus. All isolates were identified to the genus level and verified by morphological and biochemical characteristics. The findings show that two mesophilic bacteria were fungus from the genera Mucor and Rhizopus, one was a bacterium from the family Staphylococci, and one was a thermophilic Bacillus species. The enzyme activities of the isolates were estimated and revealed that Bacillus spp. has cellulase, protease and catalase activity while Staphylococcus spp. has only protease and catalase activity. Mucor and Rhizopus spp. have only cellulase and catalase activity. To estimate the level of garbage degradation. The level of garbage degradation was estimated by tacking the weight of the sample before and after degradation as well as detecting the content of protein and carbohydrate by using Folin Lowry and di-nitro salicyclic acid, phenol sulphuric acid method. The comparative study showed that both mesophilic (Mucor) and thermophilic microorganisms (Bacillus) showed the best degrader compared with Staphylococcus and Rhizopus.

1. Introduction:

Garbage is considered as one of the critical global issues which is produced due to the increase in industrialization and urbanization and lead to environmental pollution. Garbage is referred to as rubbish trash waste junk comprises of animal and vegetable wastes as result of the handling storage, sale and from cooking which also includes plastic bags, bottles, cloth fabrics, packing paper. Such waste is linked directly to human development both technologically and socially [1]. An increase in urbanization leads to accumulation of waste and cause environment pollution and health hazards like contamination of air, ground water, surface water and even cities water system, which as consequence results in several air and water born disease like typhoid, cholera and many others diseases [2], [3], [4], [5]. Around one-third of the food production for human consumption (1.3 billion tons) is being wasted in the world each year and private household as well as public restaurants represent the largest contributor to food wastes [6]. In Iraq the average disposal in each city around 12000 – 15000 tons of garbage every day, which is a serious problem for which massive steps need to be undertaken [7].The largest component of the municipal solid waste stream by weight is food waste, which comprises uneaten food and food preparation leftover from homes, commercial businesses like restaurants, institutional sources like school cafeterias, and industrial sources like plant lunchrooms [8],[9]. Such wastage may lead to economic losses, needless hunger, cli-

mate and environmental problems. In addition, producing methane gas which causes major effect on the greenhouse [10],[11]. An dangerous environment is created by improper waste management, which should be replaced by composting or another safer waste management method. Human effect on and adaptation to the physical environment are unavoidable as the globe moves toward a modified natural resource. As an organic fertilizer, the composting process can be very helpful in accomplishing this objective. Due to the value of composting, commercially produced chemical fertilizers will be less frequently used in favor of organic compost. It would surely be beneficial to the ecosystem and human health to reduce the amount of toxic chemicals released into the environment by reducing their consumption. [12]. Such microorganisms break down the food waste by suing certain enzymes into small particles during the composting process under aerobic and anaerobic condition [13]. Composting has the potential to enhance soil's structure, aeration, texture, and ability to store water. [14],[15]. During composting process, the volume of waste diminishes to produce high nutrient level product, which is rich with organic matter and used in agriculture sector as natural fertilizer. Through biochemical processes, microbes converting organic wastes into fiber-rich carbon containing humus rich in inorganics such as nitrogen, phosphorus [16]. Microbial activity during composting accomplished by several stages; during the first stage, microbes such as fungi and bacteria increase the temperature by oxidation of organic materials and decomposing the biodegradable wastes to stable organic products. This stage varies depend on temperature, moisture, carbon to nitrogen ratio, and the organic nature of the waste [13]. During this stage pathogens microbe and phytotoxins eliminated due to consumption of oxygen and degradation of hemicellulose, cellulose and lignin take place to produce humic material, carbon dioxide and ammonia. In the subsequently stages, the remaining organic materials is converted to stable humic substances [16],[17],[18],[19]. This Research study focuses on the handling and utilization of biodegradable food waste from restaurant, catering facility and kitchen by using conventional microbial and biochemical tests.

2. Materials and Methods:

2.1 Samples Collection:

Samples were collected from different market yards in Duhok city Which represent garbage that contain only organic food matter. The samples were collected in sterile sample bags. These samples were then used for isolation of degrading microorganism.

2.2 Isolation and Identification of Fungus:

Garbage samples were plated on Sabouraud dextrose agar (SDA) aseptically and then incubated at 28°C for 5 days. A pure culture was obtained and maintained by sub-culturing each of the different colonies that emerged onto the SDA plates and incubating at 28°C for 5 days. The fungal isolates were identified using cultural and morphological features such as colony growth pattern, conidial morphology, and pigmentation [20]. Oyeleke and Manga technique was used for identification of the isolated fungi using cotton blue in lactophenol stain. [21].

The identification was made by placing a drop of lactophenol stain on clean glass slide with the assistance of sterilize needle, where a small piece of the aerial mycelia from the characteristic fungi cultures was removed and placed in a drop of lactophenol and it well spread on the slide with the needle. The slide was covered with cover slip to remove air bubbles and examined under objective lenses (10X) and (40X) of light microscope.

The morphological characteristics of the fungal organisms were identified and confirmed according to Adebayo-Tayo et al. [22].

2.3 Isolation and Identification of Bacteria:

Samples were collected from garbage depot and used for isolation of degrading organism. One gram of garbage samples was suspended in 99ml of sterile distilled water and shaken vigorously for 2 minutes. Then the suspensions were serially diluted and then the selected dilution was streaked onto Nutrient Agar (Hi Media). Bacterial isolates were identified morphologically using gram stain and biochemically using catalase, methyl red, voges proskauer (MR-VP), nitrate reduction test, starch hydrolysis, gelatin liquefaction test, coagulase, indole, motility, oxidase, urease, triple sugar iron agar (TSI) and sugar fermentation as described by Bergey's manual of Determinative Bacteriology [23].

All three isolates were also studied for enzyme production. Identified organisms were checked for degradation capacity on Lab scale. For this collected sample was washed, dried and grinded in mixer. The carbohydrates and Protein content were detected by Phenol sulfuric acid and Folin Lowry method respectively. The weights of sample were taken and weighed sample was autoclaved at 121° (15lb) for 15 min. After that isolated three bacteria were inoculated in three different flasks. After 15 days of incubation the sample was filtered and solid remain on filter paper was weighed. The obtained filtrate was used for detection of total protein and carbohydrates content and also it used for enzyme assay.

2.4 Degradation of Garbage on Lab Scale:

To determine the protein and carbohydrate contents, respectively, a waste sample was collected, washed to remove surface flora, dried on filter paper, mixed in a grinder, and then subjected to the Lowry and Phenol Sulphuric Acid methods. After this 50 gm of grinded garbage sample was weighed and autoclaved then pure culture of both two fungi belonging to genus Mucor and Rhizopus isolates were inoculated in 50gm of garbage sample in two different flask and in one another flask. Thermophilic microorganism was inoculated contains

garbage sample with 200ml of autoclaved distilled water in each flask. These flasks were kept for incubation of 7 days for biodegradation process. After 7 days the garbage Sample was filtered using Whatmann filter paper no.1. the weight of the garbage sample was taken and it was subtracted from previous 50gm of Garbage Sample. The correct response displays how much waste was broken down over the course of seven days by isolated thermophilic and fungal organisms, respectively. Degradation of Garbage by taking weight of sample before inoculation and after degradation using following equation: Degradation = Initial weight of sample before Inoculation-Final weight of sample after degradation.

2.5 Enzyme Assay:

Qualitative and quantitative assay have been performed to check the presence of different enzymes in our isolated microorganisms:

2.5.1 Cellulase Enzyme:

Two assays were performed: The plate assay using sterile carboxymethyle cellulose agar medium CMC and spectrophotometric methods as shown in Table 1. The activity of cellulose was calculated by following equation

$$\text{Enzyme activity (U/ml)} = \frac{Glucose\ \ units\ formed \times dilution\ factor}{Time\ of\ incubation}$$

Table 1. Measure Cellulase activity by spectrophotometric methods.

	Test	Blank
1% 500 l	CMC	500l
Citrate phosphate 250 μl	buffer	250 μl
Enzyme (Cellulase)	250 μl	-
Incubation at 37c for 30 min		
DNSA	1 mL	1 mL
Enzyme	-	250 μl
Incubation for 10 min in boiling water bath		
Distilled water	8 mL	8 mL
	Absorbance at 540nm	

2.5.2 Protease Enzyme:

Protease testing was done to determine whether proteins were being degraded by the protease enzyme since they are the second most common component of waste. An enzyme called protease catalyzes the hydrolysis of the peptide bonds in proteins in Table 2. Two assays were performed: The plate assay of protease using milk agar media and the spectrophotometric methods for calculation the activity of protease by using the following formula:

$$\text{pecific activity } (\mu\text{g/min/ ml}) = \frac{Units\ of\ Enzyme}{Units\ of\ Protein}$$

Table 2. Measure Protease activity by spectrophotometric methods.

	Test	Blank
Citrate phosphate buffer	415 μl	415 μl
Enzyme	100 μl	100 μl
	Incubation at RT for 40 min	
5% TCA		1 mL
Substrate	100 μl	100 μl
	Incubation at RT for 40 min	
5% TCA	1 mL	
	Absorbance at 280nm	

3. Results And Discussion:

All of the microorganisms that were isolated from the waste sample that was collected from the Duhok market yard were subsequently morphologically identified and kept at 4 °C. Before and after the breakdown process, the protein and carbohydrate content had been determined on a lab scale using the folin Lowry and phenol Sulphuric acid technique.

3.1 Isolation, Screening, and Identification of Enzyme Producing Bacteria:

The biochemical characterizations of the isolated strains are presented in Table 3. A microscopic examination revealed that all isolated strains were gram positive bacteria; one strain was rod shaped and two strain were cocci arranged in cluster shape. According to Table 3, one isolated bacterium (III) required a temperature of 55 °C, whereas two isolated bacteria (I, II) preferred 37 °C. The genera Staphylococcus spp. and one Bacillus spp. were represented among the isolates, which were all identified using Bergey's handbook of determinative bacteriology. The thermophilic Bacillus species that was isolated was present.

3.2 Quantitative Analysis of Enzymes Activity by Bacteria:

In order to measure the enzyme activity quantitatively, two methods were used: plate assay and spectrophotometer methods.

3.2.1 Plate assay for enzyme activity:

The cellulase activity was assayed by using 3, 5-dinitrosalicy -lic acid (DNS) assay. Carboxymethyl cellulose (CMC) solution was used as a carbon source to assay the cellulose activity formed during hydrolysis as shown in table 4. In addition, skimmed milk agar plate assays were used for qualitative determinations of protease activity and the hydrolysis zone on the milk agar measure to the amount of protease produced as shown in Table 4. Bacillus species have shown the high zone of cellulose hydrolysis (1 mm) and protein hydrolysis (15 mm) compared to other two strains of Staphylococcus.

Table 3. Biochemical tests for Identifying isolated Bacteria.

Identification Bcriteria	Isolates		
	I	II	III
Aerobic growth	+	+	+
Anaerobic growth	+	+ -	
Oxides test	-	+	-
Catalase test	+	+	+
Sugar utilization			
1. D-glucose	AG	A	A
2 .mannitol	-	A	-
3. D-lactose	-	A	-
Nitrate utilization	A	-	A

(+) = Organism shows positive test
(-) = Organism shows negative test
(A) = Acid production (G) =gas production

Table 4. Cellulase and protease activity in the culture filtrate of isolated bacteria.

	Zone in diameter (mm)	
Microorganisms	Cellulase	Protease
Staphylococcus spp. I	0	0.8
Staphylococcus spp. II	0.5	12
Bacillus spp.	1	15

3.2.2 Spectrophotometric Methods for Enzyme Activity:

Spectrophotometric method was used for determining cellulase and protease activity based on the quantity of the generated reducing substrate at 530 and 280 nm, respectively as shown in Table 5. Among all isolated species, Bacillus spp. has shown the highest level of the cellulase activity (0.83U/mL) and protease activity (3 U/mL).

Table 5. Enzyme activity produced by isolated bacteria using spectrophotometric methods.

	Absorbance in nm		Enzyme activity	
Microorganisms	Cellulase A530 nm	Protease A280 nm	Cellulase (U/mL)	Protease (U/mL)
Staphylococcus spp. I	0	0.04	0	0.66
Staphylococcus spp. II	0.04	0.04	2	0.66
Bacillus spp.	0.06	0.06	3	0.83

3.2.3 Garbage Degradation:

Isolated organism was added to garbage and degradation was observed after 7 days. Among all biodegradation bacteria estimated in the current study, Bacillus spp. II shows the highest level of carbohydrate degradation (0.1 mM/mL) and protein degradation (12 mM/mL), followed by Staphylococcus spp I and Staphylococcus spp II as shown in Table 6.

The highest degradation (1.078 gm) was observed by Bacillus spp. followed by Staphylococcus spp II (0.634 gm) and Staphylococcus spp I (0.457 gm). Thermophilic Bacillus spp. was a best garbage degrader. The Bacillus spp. was a maximum cellulase and protease Table 7.

3.3 Isolation, Screening, and Identification of Enzyme Producing Fungus:

The results show full growth of white mycelia which was Rhizopus where as another plate after 24 hours shows black growth all over the plate of PDA at 37C which was Mucor. Both of which had been identified by slide culture and Wet mount technique.

3.3.1 Quantitative Aalysis of Ezymes Activity by Fungus:

In order to measure the enzyme activity quantitatively, spectrophotometer methods were used. Enzyme assay for cellulase and protease was performed quantitatively and the results showed that cellulase enzymes gave positive results, while protease gave negative result among all isolated strains as can been seen in Table 8. Isolated fungus was added to garbage and degradation was observed after one week. Among all biodegradation fungus detected in the current study, Rhizopus shows the highest level of carbohydrate degradation (0.1 mM/mL) and protein degradation (12 mM/mL), followed by Mucor as shown in Table 9. The degradation of garbage by taking weight of sample before inoculation and after degradation was illustrated in Table 10. The highest degradation amount (2.943 gm) was observed by Mucor spp. Followed by Rhizopus (1.933 gm) as shown in the Table 10.

4. Disussion:

The results of all trials carried out in earlier research showed that the temperature fluctuated between 24.6 °C and 66.1 °C during the composting process, peaking after 5 days of composting and promoting the development of thermophilic bacteria such Bacillus cereus [24]. Although, mesophilic organism also has degrading capacity, but due to excess metabolism heat is released which support enhancement of thermophiles and at same time mesophilic are inhibited. Cellulose degradation is considered as important steps of composition process. Bacillus cereus showed the high cellulolytic potential compared to other bacteria species [25],[26]. Among protease producers microorganisms, Bacteria are the most dominant group and the genus Bacillus being the most prominent source due to their highly ability for producing proteins [27],[28]. Several studies conducted for developing microbial consortiums for purpose of accelerating composting and biodegradation of garbage [29], [30]. Among bacteria, Bacillus and Actinobacteria considered as the best biodegrade microorganisms for compositing the vegetable products and increase the humification of compost and as consequence enhanced the quality of agriculture products [31]. When compared to bacteria, fungi are the best degraders. In addition to benefiting our gardens,

Table 6. Biodegradation of Carbohydrate and protein contents by isolated bacteria.

	Before Degradation	After Degradation		
Contents	level of degradation	Staphylococcus spp. I	Staphylococcus	spp II Bacillus spp.
Carbohydrate Content (mM/mL)	0.9 mM/mL	0.4 mM/mL	0.18 mM/mL	0.1 mM/mL
Protein Content (mg/mL)	16 mM/mL	14 mM/mL	14 mM/mL	12 mM/mL

Table 7. Biodegradation of garbage by isolated bacteria.

Microorganisms	Weight of sample Before Degradation (gm)	Weight of sample a After Degradation (gm)	Observed Degradation (gm)
Staphylococcus spp. I	50	49.543	0.457
Staphylococcus spp. II	50	49.366	0.634
Bacillus spp.	50	48.922	1.078

Table 8. Enzyme activity produced by isolated fungus using spectrophotometric methods.

	Absorbance in nm		Enzyme activity	
Microorganisms.	Cellulase A530 nm	Protease A280 nm	Cellulase (U/mL)	Protease (U/mL)
Mucor	0.09	0	4	0
Rhizopus	0.06	0	3	0

Table 9. Biodegradation of Carbohydrate and protein contents by isolated fungus.

Contents	Before degradation	After degradation	
		Mucor	Rhizopus
Carbohydrate (mM/50)	0.9	0.2	0.1
Protein (mg/50)	16	13	12

food, and ecosystem, utilizing fungal strains in the composting process is a fantastic way to combat the issue of rubbish pollution. Reconnecting our garbage with the earth can boost soil nutrition and renew soil fertility. Fungal strains produce a wide variety of hydrolytic enzymes that break down complicated sugar and cellulose polymers [32]. Some research studied reported the ability of some fungal strains such as Penicillium purpurogenum, Neurospora crassa and Mucor sp for degradation of coffee silverskin and release large number of phenolic compounds [33]. Some studied reported that after rainfall, more biodegradation fungi and bacteria were collected from garbage waste compared to those isolated on hot sunny days and that may be due to that the spores are re-germinated in the presence of water, while remain dormant during hot days [34]. Regarding the biodegradation process, fungal species showed a unique capacity of the degradation more than bacteria due to their saprophytic properties and larger surface area, besides their ability to produce larger quantity of extracellular enzymes than bacteria for their survival among wastes.

5. Conclusion:

Result shows thermophilic organism has maximum cellulase and protease activity. Thermophilic Bacillus species shows maximum degradation as compared to other mesophilic organism and fungi on lab scale and in our experimental studies indicate that fungus is the best degrader.

Table 10. Biodegradation of Carbohydrate and protein contents by isolated fungus.

Contents	Before degradation	After degradation	
		Mucor	Rhizopus
Carbohydrate (mM/50)	0.9	0.2	0.1
Protein (mg/50)	16	13	12

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Declarations:

Conflict of interest: The authors declare that they have no conflict of interest.

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التحلل البولوجى لمخلفات الطعام عن طرق الكائنات الحة الدقيقة المعتدله والمُحبة للحرارة في مدينه دهوك

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الخلاصة

تعتبر نفايات المطبخ من أصعب القضايا العالمية ذات التأثير السلبي على البيئة والصحة. يحتوى على كميات كبيرة من السليلوز والنفايات العضوية الأخرى التى تصبح بيئات مثالية لنمو الميكروبات المسببة للأمراض ومنتجاتها السامة التى قد تصل إلى الأراضي الزراعية ونظام المياه. كحل وسط، تم عزل وحفظ بعض الميكروبات فى ظل ظروف معينة لتقليل هدر الطعام وإدارته. فى الدراسة الحالية ،كائن حي دقيق معزول من مخلفات القمامة والتى تم جمعها من مطابخ مدينة دهوك. تم عزل نوعين من البكتريا والفطريات المتوسطة وبكتيريا محبة للحرارة. تم تأكيد جميع العزلات من خلال الخصائص المورفولوجية والكيميائية الحيوية وتم تحديدها حتى مستوى الجنس. أظهرت النتائج أن الكائنات الحية الدقيقة الوسيطة كانت نوعين من الفطريات ينتميان إلى جنس *Mucor* و *Rhizopus* وبكتيريا واحدة تنتمي إلى *Staphylococcus spp.* بالإضافة إلى أحد الكائنات الحية الدقيقة المحبة للحرارة تنتمي إلى *Bacillusspp.* تم تقدير نشاط الانزيمات للعزلات واظهرت ان البكتيريا *Bacillus spp.* له نشاط السليولاز والبروتياز والكتلاز بينما *Staphylococcus spp.* له نشاط الأنزيم البروتينى والكتلاز فقط. *Mucor* و *Rhizopus spp.* لديهم نشاط السليلاز والكتلاز فقط. لتقدير مستوى تدهور القمامة. تم تقدير مستوى تحلل القمامة من خلال قياس وزن العينة قبل وبعد التحلل وكذلك الكشف عن محتوى البروتين والكربوهيدرات باستخدام طريقة فولين لورى وحمض الساليسيليك ثنائي النيترو وحمض الكبريتيك والفينول. أظهرت دراسة مقارنة أن كلا من الكائنات الدقيقة المحبة للحرارة (*Mucor*) والكائنات الحية الدقيقة المحبة للحرارة (*Bacillus*) أظهرت أفضل مادة تاثيرا مقارنة بالمكورات العنقودية و *Rhizopus* .

الكلمات الدالة: التحلل البيولوجي؛ الكائنات الحيه الدقيقه المحبه للحرارة؛ الكائنات الحيه الدقيقه المعتدله للحرارة؛ نشاط الإنزيم ؛ مخلفات الطعام.

التمويل: لايوجد.

بيان توفر البيانات: جميع البيانات الداعمة لنتائج الدراسة المقدمة يمكن طلبها من المؤلف المسؤول.

اقرارات:

تضارب المصالح: يقر المؤلفون أنه ليس لديهم تضارب في المصالح.

الموافقة الأخلاقية: لم يتم نشر المخطوطة أو تقديمها لمجلة أخرى، كما أنها ليست قيد المراجعة.