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Determine the optimal conditions for the growth of kytococcuc sedentarius bacteria when analyzing plastic waste and identify some of the decomposition products

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Abstract---This study aimed to determine the optimal conditions for the growth of *Kytococcuc sedentarius* bacteria isolated from landfill soil in Anbar province using nylon bags as the only source of carbon and energy, used in liquid nylon bag medium in experiments to determine optimal conditions for growth, the best pH at 7 PH, the best temperature at 30 °C, the best vaccine volume was at 2 ml / 100 ml, and the best incubation period was after 4 One week, applying all these optimal growth conditions for bacterial isolation, the decomposition rate of nylon bags used in the study was 43.2%.

***Keywords*---**Kytococcuc sedentarius, environmental problem, environmental conditions.

Introduction

Plastic has become an important and complementary element in our daily lives, as the amount of annual human consumption of plastic is increasing significantly, and this great use is attributed to several factors including resistance, ease of design and use, in addition to the low cost (Edmund *et.al.*, 2019), although plastic was initially assumed to be harmless but in recent years

plastic has become a cause of many problems in the environment (environmental pollution with plastic waste). It is considered one of the most important factors of environmental pollution especially in the aquatic environment if it causes sabotage of the aquatic environment as well as adverse adverse effects in wildlifeProshad *et.al.*, 2018 Kershaw and Rochman, 2015), the distribution of plastic waste is associated with human growth The increase in population has led to an increase in demand for plastic yk and plastic products (Chaudhry, 2018), according to Verschoo (2015) Plastic waste that is used only once causes a global environmental problem and plastic waste in nature decomposes into microplastics and its presence in the environment is a problem because it is more resistant and contains toxic and carcinogenic chemicals, and because living organisms consume it they will affect the environment. Biodegradability is the process of decomposition of large polymer molecules by groups of organisms and some of them break down the polymer chain into smaller olegomer molecules (oligomers) and monomers (Tokiwa *et.al.*, 2009), the rate of biodegradability is strongly influenced by several properties of the polymer and environmental factors such as ultraviolet radiation, pH, temperature, humidity and enzyme properties (Ahmed *et.al.,* 2018), Biodegradability of plastics can be enhanced by improving the growth of microorganisms responsible for the decomposition process as well as genetic improvement of these microorganisms and trying to reach the highest production of enzymes responsible for decomposition Biodegradation is carried out by various microbes such as bacteria and fungi (Muhamad *et.al.,* 2015).

Most studies have shown that many genera of bacteria are able to decompose plastics of all kinds (Nanda, and Sahu, 2010), in addition to bacteria yeast can dissolveplastic polymers using their enzymes (Bhardwaj *et.al.*, 2012), There are many studies that confirm that the biodegradability of plastics by bacteria can be a promising biological treatment strategy for polluting ecosystems (Yoshida *et.al.,* 2016), bacteria need food-rich environments as well as a small part of inorganic matter to grow, some of which need large amounts of oxygen and others die in the presence of oxygen because of their different reductionist oxidative abilities and this is the marked variation in the needs of these Organisms from food and air, researchers have created many ways to develop these organisms in laboratories, each method of which suits each group or type of these organisms and in order to grow bacteria in the laboratory I use what is called environments, which contain the nutritional needs necessary to develop each group according to its needs of food and these environments are either liquid, solid or semi-solid, The ideal requirements for the growth of bacteria vary greatly depending on the types of bacteria. Bacteria show the greatest diversity among all living things in their ability to live in different environments (Arnoldini *et.al.,* 2018) .

Materials and working methods:

Preparationof the bacterial vaccine :

Prepare 100 ml of nutrient broth medium prepared by adding 13 g of this medium in 1000 ml distilled water, using a 250 ml beaker, adjusting the pH to 7 and sterilizing the repellent for 15 minutes at a temperature of 121 °C o and a pressure of 15 lb/ng ^{2}, After cooling the medium to 30 m^{o} pollinating the medium

with pure colonies of bacterial isolation elected by means of a sterile carrier and then leaving the medium in the incubator for 24 hours, growth is observed through the opacity formed in the medium with uniform density 3×10-6 colonies / ml of *Kytococcuc sedentarius* bacteria.

Calculation of the percentage of plastic consumption :

The gravimetric method was used to calculate the percentage of plastic consumption according to (Arafa, 2003) **by calculating the amount of plastic residual** by taking a sterile filter paper type Whatman filter paper (No.1) and taking the medium after the **incubation and filtered through the aforementioned infusion sheet and after the filtration process the remaining plastic was washed from the decomposition well to get rid of the remnants of the medium and living mass and put the remaining plastic in the** ovenoven **at a temperature** of 60° **C for** 24 **An hour to dry it well from moisture after which the remaining plastic was isolated and weighed with a sensitive scale and calculated the weight of the plastic before and after the incubation and know the difference in the weight of the decomposed plastic as the weight loss criterion was used as evidence of the plastic analysis process and according to the following equation:**

D= (A-B/A) x100%

=A plastic weight before (kidney) brood

=B plastic weight after brood

=D **Percentage of decomposing plastic**

Optimization of optimal environmental conditions for the growth of selected plastic decomposing bacterial isolate:

The liquid medium containing nylon bag residues of0.35 **mg/**100 **mL medium was used as the sole source of carbon and energy prepared in the laboratory** by dissolving substances 500 mg of K_2HPO 4, 400 mg of KH_2PO_4, 100 mg of NaCl and 20 mg of $CaCl_2$ and 200 mg of $(NH\ 4)_2$ SO 4, 20 mg of MgSO 4 , 12 mg of FeSO 4 and 100 mg of $MnSO_4$ in 1000 ml of distilled water **In identifying the factors influencing the analysis of plastics, where 250 ml conical decoctions** were used and placed in **each of them** 100 **ml of liquid medium and sterilized the decanters in the repellent, the media was then inoculated with the bacterial isolation used and incubated in the incubator at a temperature of** 30°C **o for** 2 **A week using different criteria, including pH, temperature, vaccine size, incubation type and incubation duration, after which the consumption rate was estimated at each step using the weight loss criterion as evidence of plastic analysis, according to** Arafa, 2003**.**
pH

Adjust the pH of the growth medium to each of the following pH numbers: (6,7, 8, 9, 10) **to determine the optimal pH for plastic analysis in the liquid medium.**

Vaccine size

Different vaccine sizes were used for the purposes of pollinating the liquid medium with bacterial isolation to test the effect of the volume of the added vaccine in the analysis of plastics and the volume of the vaccine used was (0.5,1,2,3,4 **) ml /** 100 **ml medium.**

temperature

Different temperatures were tested to determine the optimum temperature for plastic analysis in the liquid medium and the temperatures were (20 , 25, 30, 35, 40, 45 **) C.**

Duration of the hug

The hydrolyzed isolation of plastic was tested on the liquid medium containing nylon bags as the sole source of carbon and at the temperature, pH and volume of the vaccine determined in the previous steps and for different incubation periods were (1, 2, 3, 4 **) weeks to get the best incubation period for plastic analysis.**

Cuddle Type

After using the pH, temperature, volume of the vaccine and the optimal incubation duration obtained in the previous vertebrae of plastic-decomposing bacteria in the center of liquid nylon bags, two types of incubation were used (fixed incubator and vibrating incubator).

Results and discussion:

Study of optimal environmental conditions for the growth of elected bacterial isolation :

pH :

The results shown in Figure 1 showed that *Kytococcuc sedentarius* bacteria have a greater ability to analyze plastics at pH 7 compared to the rest of the figures and by 37.6% followed by pH 8 with a decomposition of34.6% for 2 weeks incubation.

This result is attributed to the fact that microbiology has the ability to withstand a wide range of pH and that the pH of the medium often tends to change during the fermentation period so the pH (Silva-Sánchez *et.al) must be controlled.* , , that pH affects the decomposition medium and in the enzymatic processes and the transport of nutrients and other substances through the cell membrane and that the increased growth of bacterial cells in the medium leads to a change in pH in the decomposition medium during the incubation period. Patil and Bagde, (2015), the biodegradability of plastics is greatly influenced by environmental conditions and that the low pH in the acidic environment slows down the biodegradability of plastics by bacteria that usually tend to the neutral pH as well

as its height and this is related to the insulation environment of Tamnou *et.al.,* (2021).

This finding is consistent with Sriyapai *et.al.,* (2018), which obtained the highest decomposition ratio between the two pH digits (8-6).

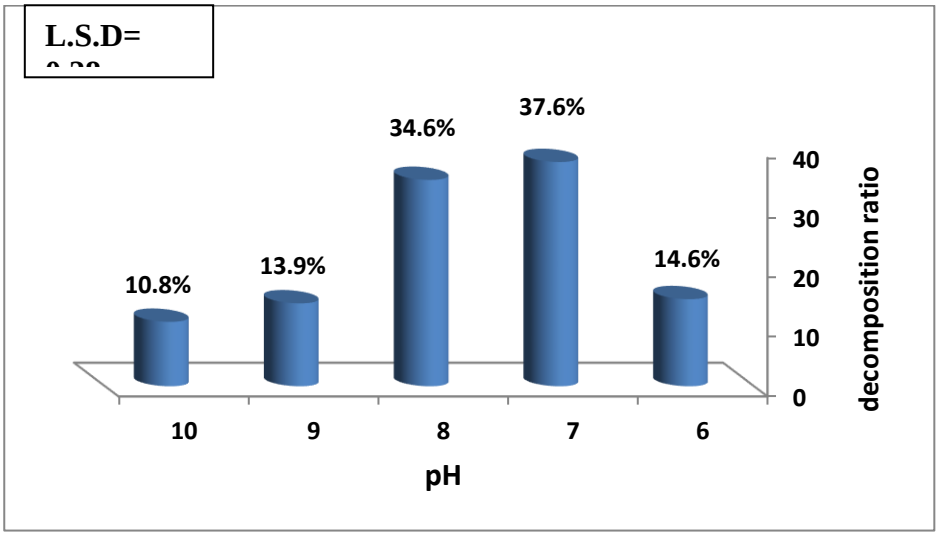

Figure 1 shows the effect of pH on plastic decomposition

Vaccine size:

The results shown in Figure (2) showed that *Kytococcuc sedentarius* bacteria have a greater ability to analyze plastics at the volume of the vaccine 2 ml / 100 ml medium gave the highest percentage of decomposition among other volumes and by 37.4% followed by the volume of 1 ml with a decomposition rate of 37%, and the decrease in the percentage was observed when the volume of the vaccine decreased or increased further**.**

The low degradation rate at the small volumes of the vaccine is due to the fact that the low concentration of the vaccine is insufficient to give a sufficient number of bacterial isolation and therefore reflects negatively on the decomposition rate, while the decrease in the percentage of decomposition with the increase in the volume of the vaccine used may be due to the state of intense competition for nutrient exploitation and accumulation of metabolic substances in the medium (Cantu-Jungles and Hamaker (2020).

This finding differs from Muhonja *et.al.,* (2018) that the best vaccine volume for plastic analysis was when using a volume of 1 mL/200 mL medium and this is different from what we got.

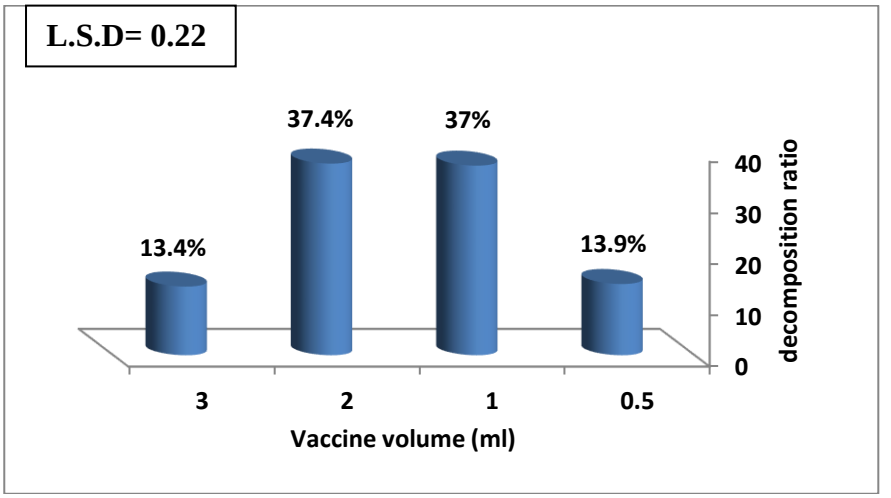

Figure 2 illustrates the effect of vaccine volume on plastic decomposition

Temperature:

Figure (3) shows the effect of brood temperature on the percentage of decomposition of the residues of nylon bags as the only source of carbon and energy for bacterial isolation at pH 7 and the volume of the vaccine 2 ml / 100ml medium and for the duration of incubation 2 weeks It was observed that the temperature of 30 ° C gave the highest percentage of decomposition among other temperatures, where the percentage of decomposition at 30° C is 37.6% followed by a temperature of 25 m with a decomposition rate of 14.6%.

These results are attributed to the fact that temperature is one of the important environmental factors that affect the biodegradability rate of plastics and the biodegradation test of plastics is usually performed at a low temperature in the range of 20-28 °C to support the growth of microorganisms that lead to the decomposition of plastics and this is related to the temperature of the insulation environment Pischedda *et.al.,* (2019). In another study A fourfold increase in the rate of biodegradation was achieved by increasing the incubation temperature from25 ° C –37 °C to a number of Šerá et.al industrial polymers ., (2020). High temperatures have detrimental effects on enzymes involved in plastic analysis, although there are enzymes that have a wide range of resistance and sensitivity to temperature (Pujari-Palmer *et.al* 2018).

This finding is consistent with the findings of Patil and Bagde (2015) which obtained the highest decomposition of PVA at a temperature of 30 °C.

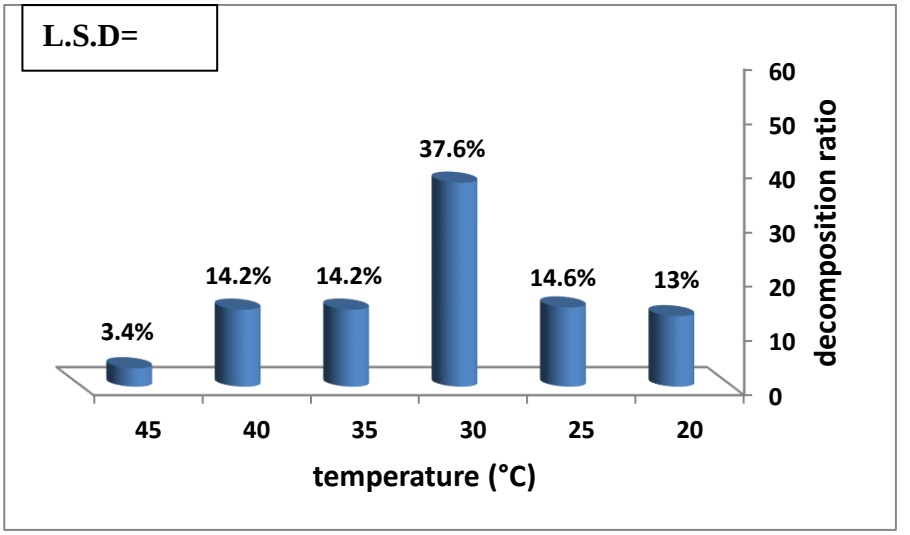

Figure 3 shows the effect of temperature on plastic decomposition
Incubation Type :

The effect of the type of incubation was studied in this study using fixed incubation and vibration, placing the liquid medium pollinated by bacterial isolation in a fixed incubator and a vibratory incubator, the results in Figure (4) showed that the percentage of consumption when using the vibratory incubator was the highest with a decomposition rate of 39.9%, while the incubation using the fixed incubator was 37%.

We infer from these results that stirring and ventilation in liquid cultures leads to the provision of dissolved oxygen in the decomposition medium of the organism, especially if it is a microbiology that is compulsory ventilation and that stirring and ventilation leads to increased homogeneity of the components of the medium and increase the area to which bacteria are exposed, as ventilation is of great importance in increasing the growth and metabolism of bacterial cells and thus increasing their ability to decompose Kabore *et.al.,* 2015)) .

Patil and Bagde (2015) showed that the use of vibratory incubators gave a high decomposition rate using the isolates of bacteria of the genus Pseudomonas and the genus Bacillus and obtained a decomposition of plastics of 42% and 65% respectively.

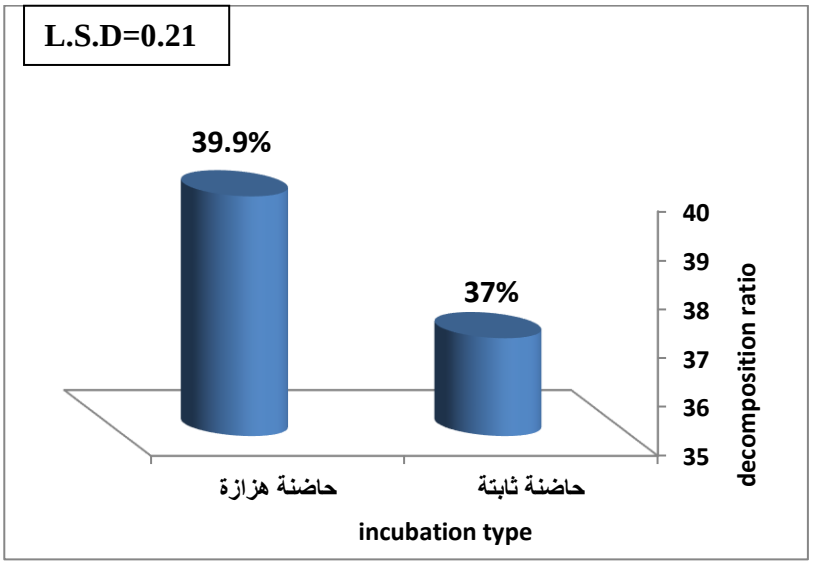

Figure 4 illustrates the effect of the brood type on plastic decomposition Duration of cuddling:

Figure 5 of *Kytococcus sedentarius* shows an increase in the decomposition rate of plastic waste with an increase in the duration of incubation and the highest percentage of decomposition after the incubation period of 4 weeks with a decomposition rate of 43.2% followed by the incubation period of 3 weeks and by 42.5%.

These results are attributed to the fact that the increase in the duration of incubation leads to an increase in bacterial activity on plastic parts due to the adhesion of microbes to the surface of the plastic and thus leads to the production of some plastic-decomposing enzymes outside the bacterial cell and this leads to a gradual decrease in the weight of the plastic in the middle, while when the duration of incubation decreases will get a lack of bacterial activity and therefore a lack of production of enzymes and this affects the process of biodegradation of plastic, **(Arutchelvi et.al., 2008).** This finding is almost consistent with the findings of Ariba Begum *et.al.,* (2015) that the best decomposition ratio after a one-month incubation period for the bacterium Desulfotomaculum and Pseudomonas is in the analysis of polyethylene cyst.

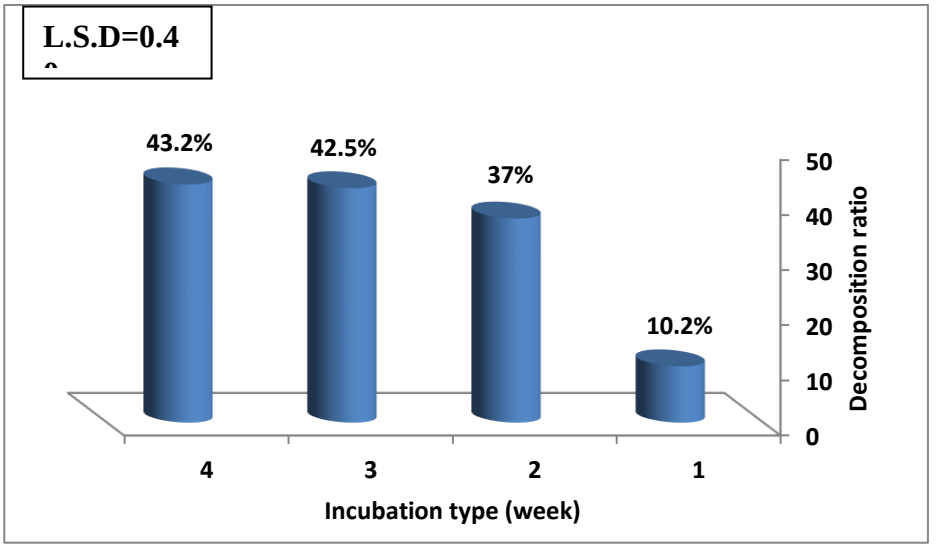

Figure 5 shows the effect of incubation duration on plastic decomposition Figure (6) Biodegradation of plastic underoptimal PH7, temperature **30 °C, vaccine volume 1 ml, incubation duration of 21 days)**

Some products decompose plastic waste microbially

Single-cell SCP protein

The living mass was separated from the decomposition medium after the filtering of the medium with filter sheets and separated from the remaining nylon bags and dried at a temperature of 65 ° C and the proportion of single-cell protein was 12.5% of the decomposition medium as one of the products of decomposition

Called a single cell protein, a single-cell protein is currently produced from a limited number of microbiology taking into account human consumption (Ritala *et.al.,* 2017). Abood *et. al.*, (2017) enables the use of the fungal and bacterial mixed farm system to produce single-celled proteins using garden thiel residues, in addition, the analysis of the protein produced has shown that it has nutritional value, so it can be used as animal feed, as well as can be considered as a basis for energy production by producing biofuels.

Table (1) Chemicals Produced by GC MA

Pronounced like t	Quality	Name	Area%	RT(min)	CAS Number
1	**53**	Cyclotetrasiloxane, octamethyl-	**1.79**	**6.671**	**000556-67-2**
2	**90**	Cyclotetrasiloxane, octamethyl-	**5.60**	**14.381**	**000540-97-6**
3	**90**	Dodecane	**1.59**	**17.935**	**000112-40-3**
4	**49**	Pentasiloxane, dodecamethyl-	**2.49**	**18.018**	**000141-63-9**
5	**64**	Pentacosane	**2.57**	**18.833**	**000629-99-2**
6	**83**	3',5'-diallyl-2',4'-dihydroxyacetophenone	**5.90**	**20.607**	**040815-80-3**
7	**35**	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15- hexadecamethyl-	**1.58**	**24.146**	**019095-24-0**
8	**98**	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dion	**15.61**	**25.703**	**000000-00-0**
9	**95**	Octadecane	**1.46**	**26.989**	**000593-45-3**
10	**94**	Eicosane	**13.08**	**32.77**	**000112-95-8**
11	**81**	Bis(2-ethylhexyl) phthalate	**8.52**	**34.736**	**000117-81-7**
12	**64**	5-Methylthio-7,8dihydro-6H-benzocyclohepta[2,1- e]pyrazolo[1,5-a]pyrimidine	**4.88**	**35.322**	**128039-58-7**
13	**52**	Ethane, 1-(4,4,4-trifluoro-1,3-dithiobutyl)-2-(3,3,3-trifluoro-1,2-dithiopropyl)-	**2.81**	**40.189**	**000000-00-0**
14	**35**	Silane,1,4-phenylenebis[trimethyl	**1.48**	**43.567**	**013183-70-5**
15	**43**	Cyclotrisiloxane, hexamethyl-	**1.75**	**45.02**	**000541-05-9**
16	**30**	1,3-dimethyl-4-azaphenanthrene	**1.96**	**45.445**	**000000-00-0**
17	**83**	1,3-Dioctadecyloxypropane	**9.52**	**46.338**	**085454-97-3**

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