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Studying the optimal conditions for the biodegradation process of plastic materials by Pseudomonas aeruginosa

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ABSTRACT

Monoester is produced as a consequence of the ester bond hydrolysis mechanism used by microorganisms to degrade Polyhydroxyalkanoates (PHAs). Following its breakdown, this monoester yields alcohol and phthalic acid (PA). The current work was aimed to study the ability of P. aergiounas to degradation of hydrocarbons after determining the optimal conditions for the process. A sample of plastic water bottles was obtained from local marketplaces in Babylon city. Swabs were collected from the rumen of cows, sheep, and goats and immediately sent to the laboratory for culture. The swabs were placed on blood agar and subjected to incubation at a temperature of 37°C for a duration of 24 hours. Pseudomonas aeruginosa isolates were classified based on their morphological and biochemical features. The present investigation revealed the optimal conditions for bacterial growth and cell proliferation. The current study demonstrates variations in cell counts at different pH levels (5, 6, 7, and 8) and temperatures (23, 30, 37, and 44) under varying plastic concentrations. The optimal circumstances for P. aeruginosa were a pH of 7, a concentration of 1ml, and a temperature of 37°C. The results demonstrate the capacity of bacteria to degrade by quantifying the viable bacterial population that grows on MSM. The viable count of Pseudomonas first grew on the first day and subsequently declined during the course of the experiment. The percentage of biodegradation for P. aeruginosa was 59.31%. It is concluded from the current study that *P. aeruginosa* isolated from the rumen of some ruminants (cows, sheep and goats) has the ability to biodegradation of plastic materials.

Keywords: *P. aeruginosa*; Biodegradation; plastics; **LDPE**.

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1. Introduction

Plastics are synthetic polymers consisting of elongated chains. Over fifty years ago, synthetic polymers began to replace natural materials in nearly all fields, and today, plastics have become an essential component of our daily lives. Over time, the stability and durability of plastics have consistently been enhanced, making them synonymous with materials that are highly resistant to various environmental factors. The term 'plastic' originates from the Greek word "plastikos," which signifies the capability to be shaped into diverse forms and sizes [1-2]. Microorganisms, including bacteria and fungi, participate in the breakdown of both natural and manmade plastics. The biodegradation of plastics occurs actively in various soil circumstances based on their qualities, as different microorganisms responsible for the degradation process vary and have their specific optimal development conditions in the soil. Plastics can serve as suitable materials for heterotrophic bacteria to grow on [3-4]. Biodegradation is influenced by various aspects, such as the properties of the polymer, the type of organism involved, and the method of pretreatment. The degradation of polymers is influenced by various factors, including their mobility, crystallinity, molecular weight, functional groups, substituents, and the presence of plasticizers or additives [2, 5]. During degradation, the polymer undergoes a process of conversion into its constituent monomers, which are then mineralized. The majority of polymers are too large to pass through cellular membranes. Therefore, they need to be depolymerized into smaller monomers before they can be absorbed and biodegraded. Various environmental factors such as heating, cooling, freezing, thawing, wetting, or drying can potentially harm the mechanical properties of the polymer, leading to issues such as cracking. Fungal growth on polymers can lead to localized swelling and rupture as the fungus infiltrate the solid polymer material. Microbial enzymes can depolymerize synthetic polymers, such as poly(caprolactone), resulting in the absorption of monomers into microbial cells and subsequent biodegradation [6-10]. So, the current work was aimed to study the ability of P. aergiounas to degradation of hydrocarbons after determining the optimal conditions for the process.

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2. Materials and Methods

2.1. Swabs

For culture, swabs were extracted from the rumen of goats, sheep, and cows and brought straight to the lab. The swabs were incubated at 37°C for 24 hours after being cultivated on blood, Cetrimide, and MacConkey agars.

2.2. Identifying Bacteria

A diagnosis of bacteria was made using the following criteria:

2.3. Media attributes and morphological diagnostics

After a 24-hour incubation period at 37.0°C, the P. aeruginosa colonies growing on blood agar and MacConkey agar were identified based on their culture characteristics.

2.4. Microscopic analysis

Bacterial colonies were discovered by examining the morphological features of the bacterial cells under a microscope, particularly how they interacted with the gram stain, which shows the organization and structure of the bacterial cells.

2.5. Motility test and biochemical reaction

Numerous biochemical tests were performed to identify and diagnose bacteria, including the methyl red, citrate, urease, voges-proskauer, catalase, oxidase, KIA, and indole test.

2.6. Identification of bacteria isolates via VITEK2

For microbiological identification, VITEK 2, the most recent colorimetric technology generation, is the industry standard. Procedure: In compliance with the instructions given by the manufacturer, Biomerieux, the following procedures were carried out.

2.7. Making Low-Density Polyethylene (LDPE) Powder

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To completely dissolve low-density polyethylene (LDPE) sheets, they were submerged in xylene and heated for fifteen minutes. After that, the residue was physically crushed while gloves were worn. After being allowed to evaporate, the crushed residue was dried for the entire night at 60 °C in a hot air oven. Room temperature was used to preserve the resulting powder [11].

2.8. Effect of pH

The pH of the fermentation medium was changed to the values 5, 6, 7, and 8. Following autoclaving, 1% cleaned sterile plastic materials were added to the flasks. In triplicate flasks, discs of bacterial specie from a seven-day culture of *P. aergiounas* were inoculated. Control flasks with the above medium but without tested bacterial species were prepared. Flasks were incubated for 7 days at 37°C for P. aergiounas (the best incubation temperatures from the previous test).

2.9. Determination of dry weight of the residual polyethylene (PE).

The weight loss of plastic material was determined in two ways, the conical flask test method.

2.9.1. Conical flask test method

Pre-weighed 100 mg polyethylene discs were aseptically transferred to a conical flask holding 100 ml of culture broth medium inoculated with fungal strains [12]. In the microbe-free media, plastic discs were employed as a control. Separate flasks were retained for each treatment and placed in a static incubator. After three weeks, the plastic discs were collected, cleaned with distilled water, shade-dried, and weighed to determine their final weight. The weight loss of the plastics was calculated using the data obtained.

3. Results & Discussion

3.1. Distributed of study samples

The present study involved the collection of 115 swab samples from the rumen of cows, sheep, and goats as shown in "**Table 1"**. The study revealed that 21 out of the total samples, accounting for 18.3%, tested positive for the development of *P. aeruginosa* when cultured on optimum media such

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as blood agar and MacConkey agar. Out of the 96 samples, 94 (81.0%) showed negative results for bacterial growth.

Table 1. Distributed of study samples according to UTI.

	No. (%) +ve culture	No. (%) -ve culture	Total No.(%)	P value
Number	*21(18.3%)*	*94(81.0%)*	*115(100.0%)*	*0.001*

3.2. Identification

"Figure 1" displays the morphology and size of Pseudomonas aeruginosa isolates on MacConkey and Cetrimide agars, specifically for the initial isolate. The bacterial isolates belonging to the genus were identified based on microscopic features, specifically the Gram stain reaction. Furthermore, colony features such as colony color, texture, metallic luster, and pigment production were employed to identify isolates and genera. The laboratory assays used to detect the presence of Pseudomonas aeruginosa using biochemical means. The results of the urease, indole test, M-R test, lactose fermentation, and V-P test were all negative, whereas the oxidase, citrate, motility, and catalase tests were all positive.

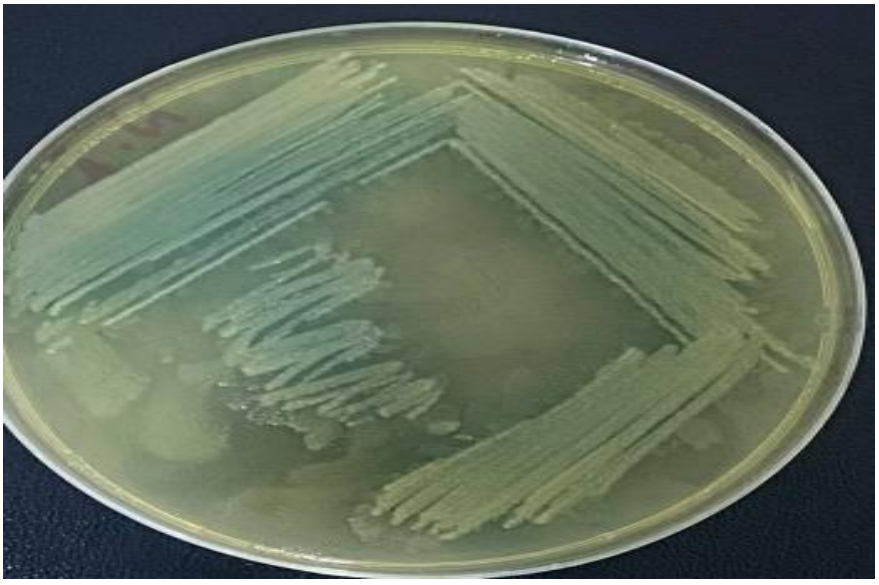

Figure 1. ***P. aeruginosa colonies on Cetrimide agar.***

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3.3.Optimum conditions

3.3.1. Plastic powder concentration

The culture mediums, which were supplemented with several amounts of plastic powder (0.5%, 1%, 1.5%, and 2%), were cultured at a temperature of 37°C and a pH of 7 for a duration of 4 days. The **"Figures 2-5"** illustrate the different rates at which the plastic powder was consumed. At a plastic powder concentration of 0.5%, pseudomonas exhibited a consumption rate of 2.5% on the first day, which continued to climb until the fourth day, reaching 19.3%. At a concentration of 1%, pseudomonas exhibited an initial consumption rate of 4.5% on the first day, which continued to climb until the fourth day, reaching 37.3%. At a concentration of 1.5%, the consumption rate of pseudomonas was observed to be 3.7% on the first day, and this percentage continued to climb until the fourth day, reaching 26.6%. At a concentration of 2%, the consumption rate of pseudomonas was initially 1.6% on the first day, and this percentage continued to climb until the fourth day, reaching 13.2%. The concentration of Pseudomonas exhibited an initial consumption rate of 1.1% on the first day, which progressively increased until the fourth day, reaching a value of 9.2%.

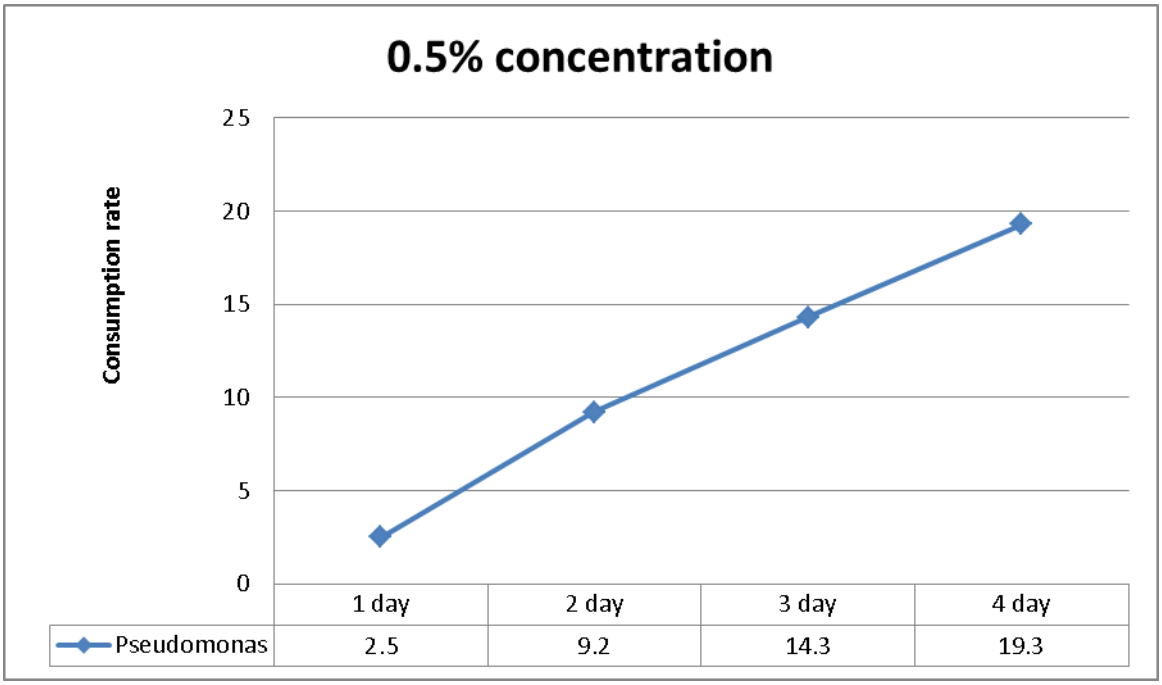

Figure 2. ***Consumption rate at 0.5 % concentration*****.**

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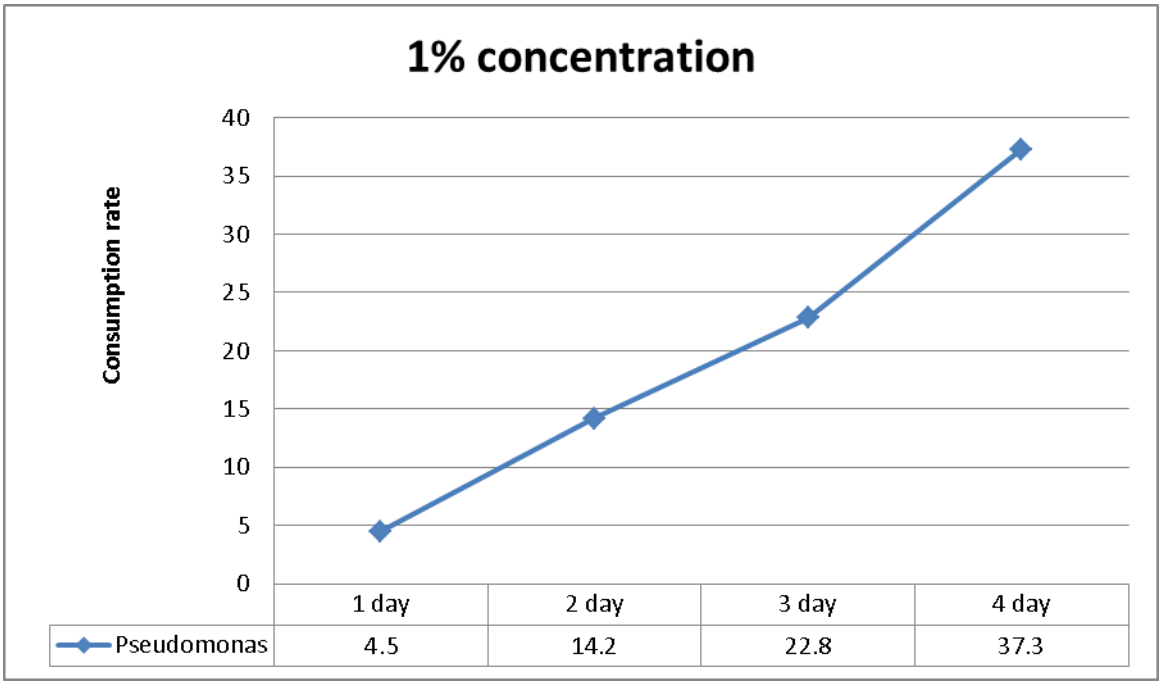

Figure 3. ***Consumption rate at 1 % concentration.***

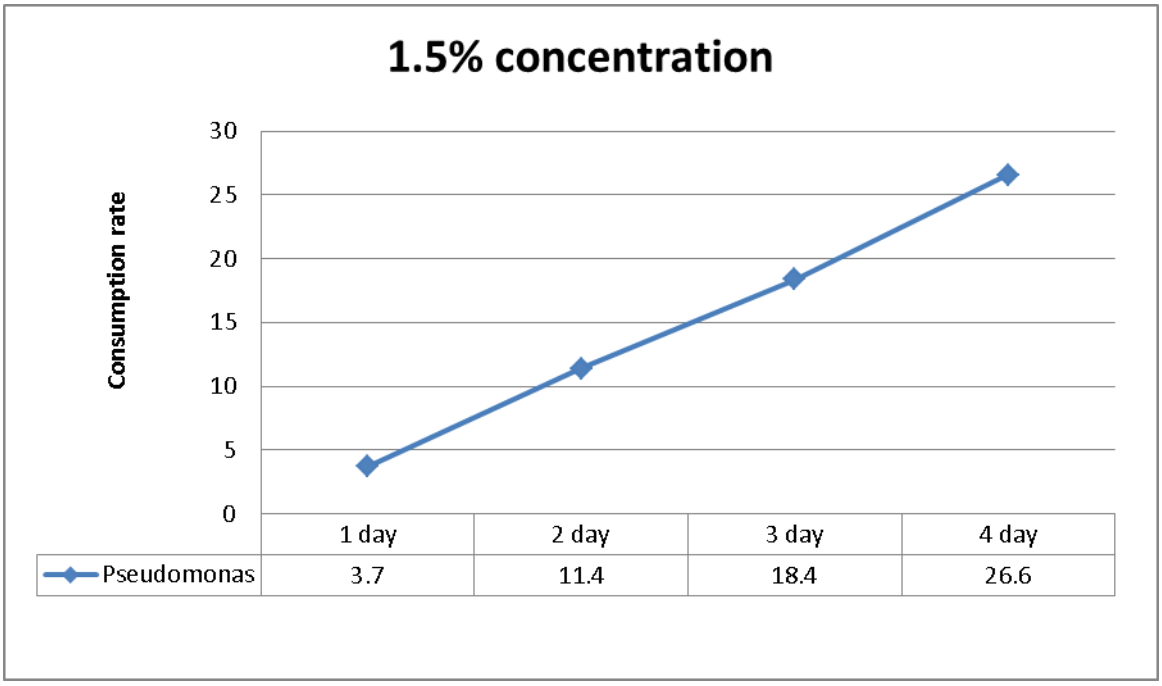

Figure 4. ***Consumption rate at 1.5 % concentration.***

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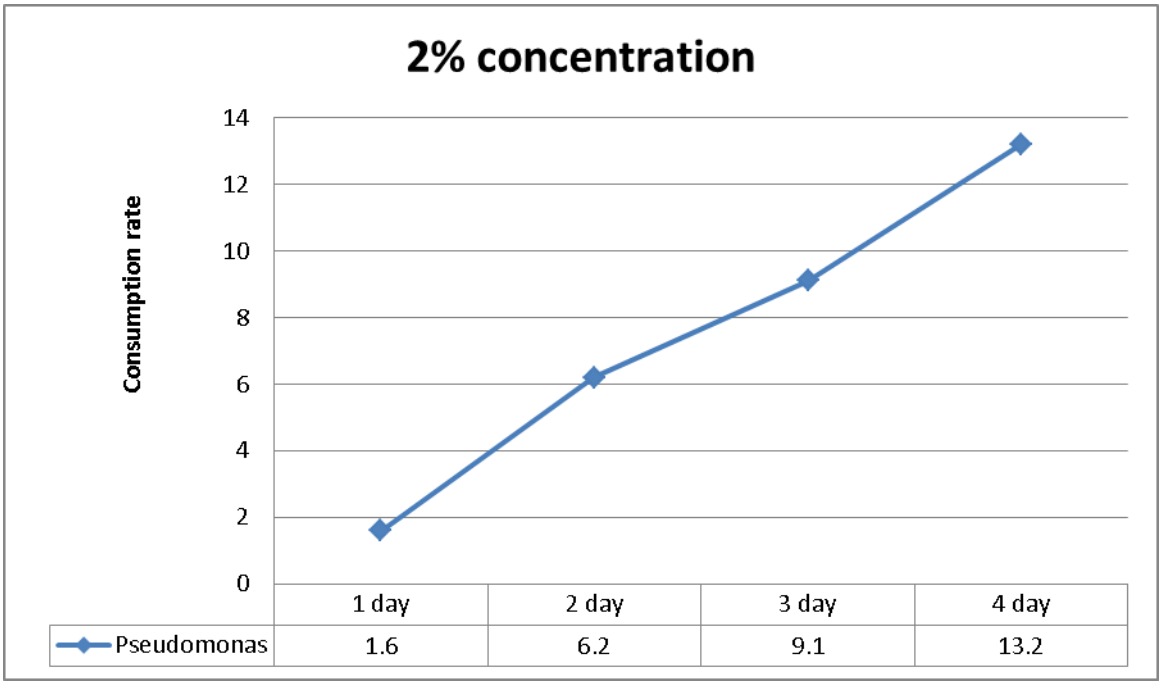

Figure 5. ***Consumption rate at 1.5 % concentration.***

3.3.2. Effect of pH

Bacterial isolates were cultivated on Minimal Salt Medium (MSM) supplemented with a 1% concentration of plastic powder. The cultures were kept at a temperature of 37°C and subjected to varying pH levels (5, 6, 7, and 8). **"Figures 6-9"** illustrate the pace at which bacteria isolates consume plastic. At a pH of 5, the consumption rate of pseudomonas was initially 1.2% on the first day, and this percentage continued to climb until the fourth day, reaching 7.2%. At a pH of 6, the consumption rate of pseudomonas was initially 4.8% on the first day, and this percentage continued to climb until the fourth day, reaching 26.8%. At a pH of 7, the consumption rate of pseudomonas was initially 6.9% on the first day, and this percentage continued to climb until the fourth day, reaching 43.3%. At a pH of 8, the consumption rate of pseudomonas was initially 2.8% on the first day and steadily increased until the fourth day, reaching 17.6% [12], who said that the ideal pH for bacterial development is 7 [13], stated that the most favorable utilization of phenol by pseudomonas takes place at a pH of 7. Furthermore, pH has a crucial influence in the proliferation of microorganisms. The pH directly affects the metabolism process of microorganisms and can cause disruptions in this process [14-15].

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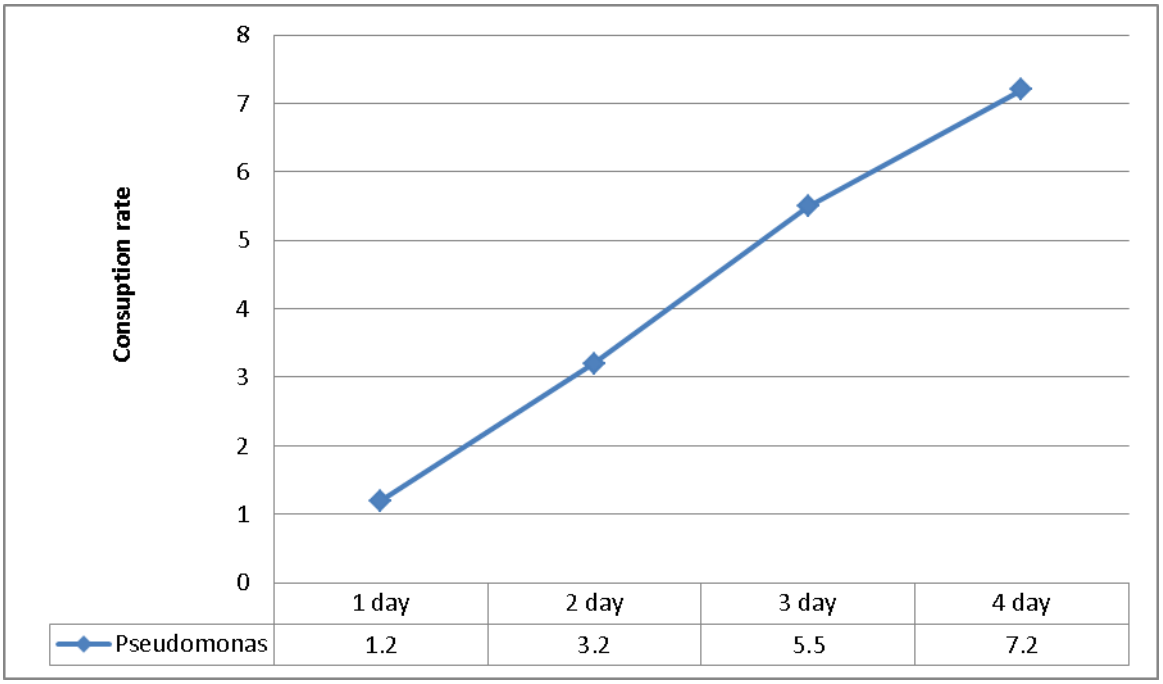

Figure 6. ***Consumption rate at pH 5.***

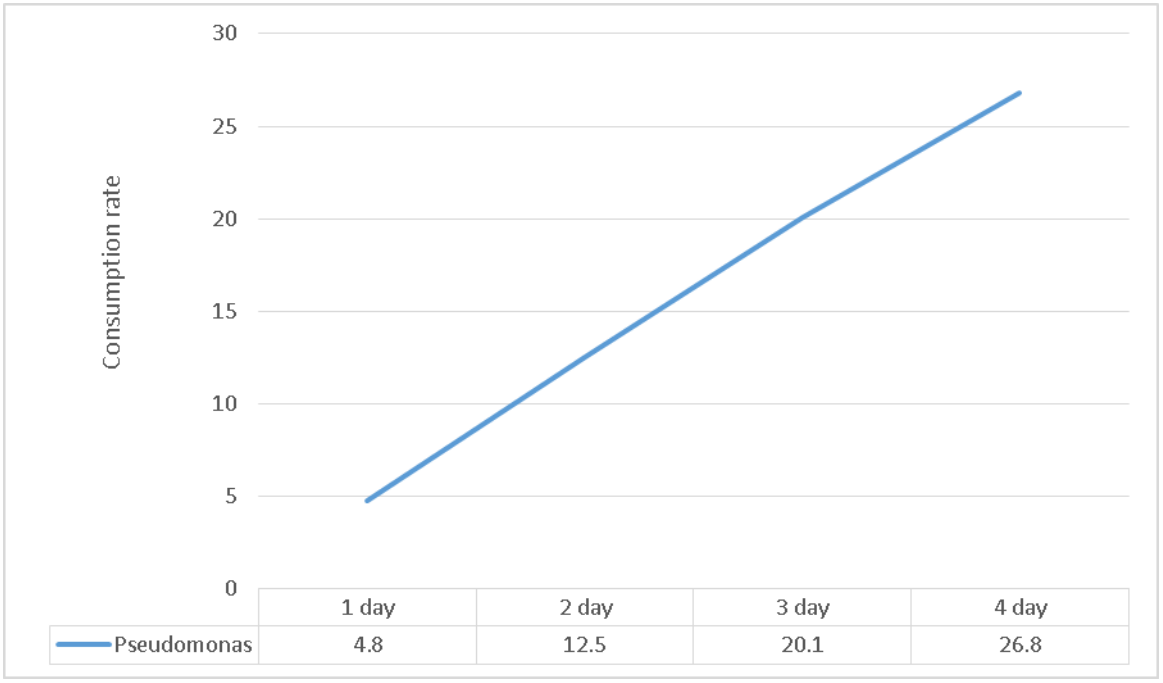

Figure 7. ***Consumption rate at pH 6.***

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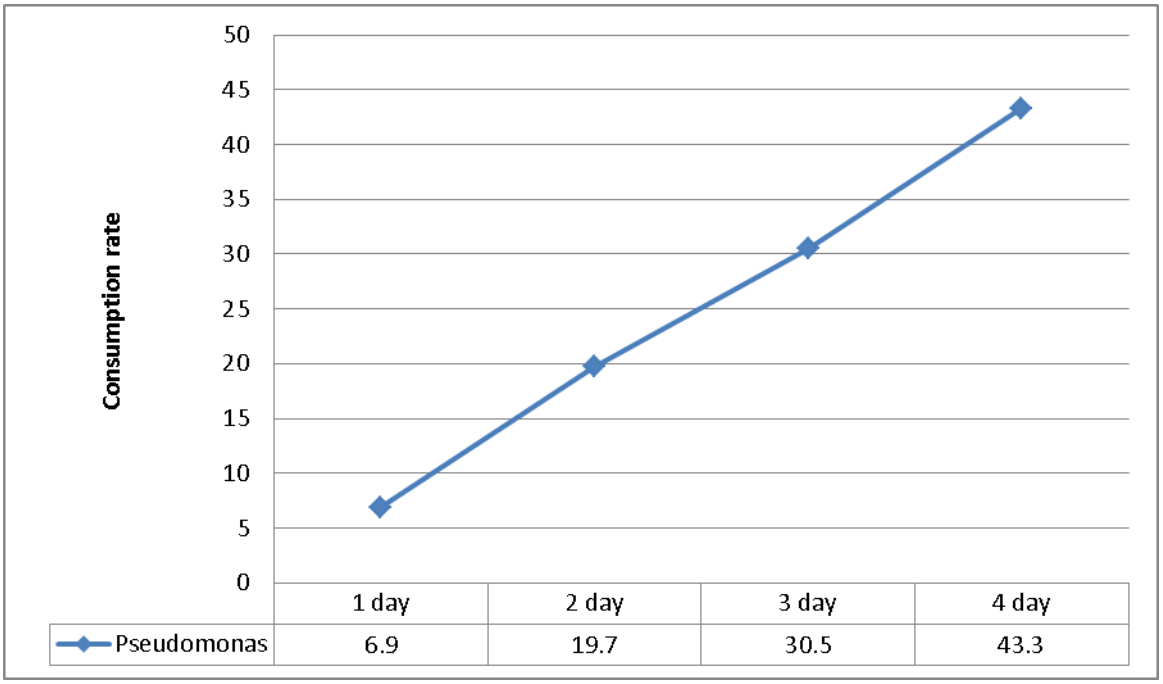

Figure 8. ***Consumption rate at pH 7.***

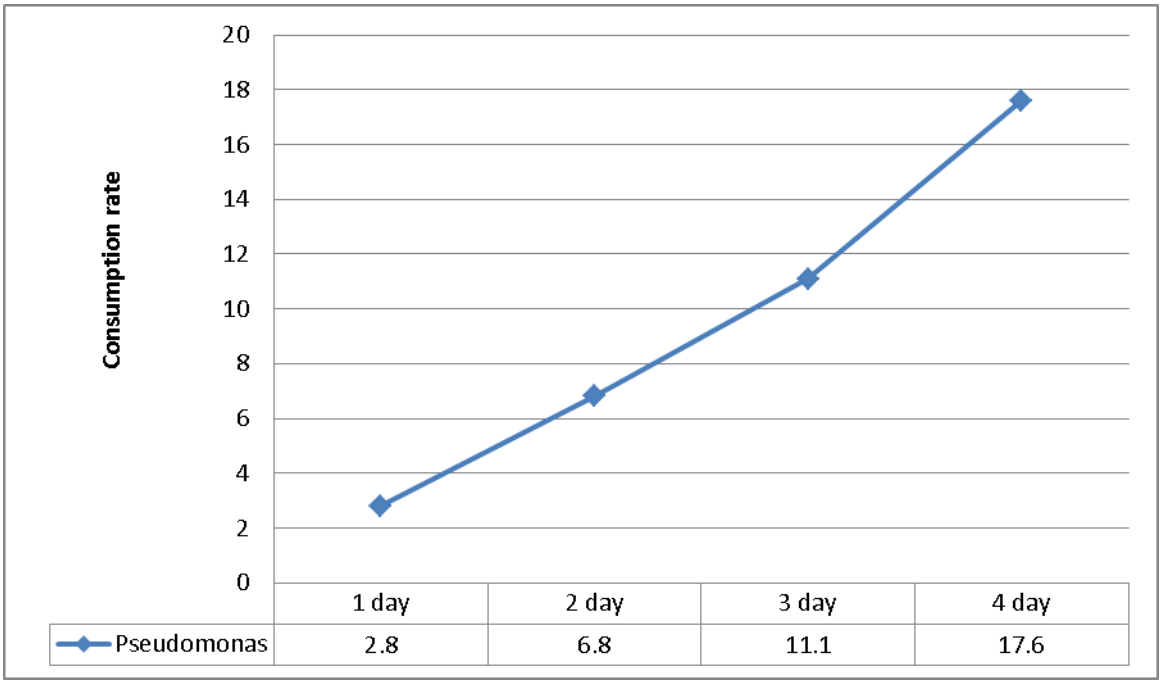

Figure 9. ***Consumption rate at pH 8.***

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3.4.Biodegradation of plastic percent

The findings of the current investigation demonstrate varying rates of biodegradation of plastic by Pseudomonas. **"Table 2"** displays the biodegradation percentage for pseudomonas, which is 59.31%. The degradation of plastic plays a crucial function in converting hydrocarbons into new materials that have less toxicity and environmental impact. The results demonstrate a significant level of degradation [16]. discussed the capacity of Pseudomonas to break down hydrocarbons and transform them into bio-emulsions. The capacity of Bacillus subtilis to break down various forms of plastic powder [17]. Mixed forms of bacteria are more effective at biodegrading hydrocarbons compared to single types of bacteria [18]. That pseudomonas achieved a biodegradation rate of 69% for hydrocarbons [19].

Table 2. Biodegradation percent of plastic.

Bacteria isolates	Initial weight of plastic powder oil	Weight of plastic powder that unconsumed	Weight of plastic powder that consumed by bacteria	Percent of plastic powder biodegradation
Pseudomonas	*0.671*	*0.273*	*0.398*	*59.31%*

It is concluded from the current study that P. aeruginosa isolated from the rumen of some ruminants (cows, sheep and goats) has the ability to biodegradation of plastic materials, and can be used to reduce the harmful effects of plastic materials on the environment.

4. Conclusions

It is concluded from the current study that *P. aeruginosa* isolated from the rumen of some ruminants (cows, sheep and goats) has the ability to biodegradation of plastic materials, and can be used to reduce the harmful effects of plastic materials on the environment.

5. Acknowledgements

Avoid the stilted expression, "One of us (R. B. G.) thanks..." Instead, try "R. B. G. thanks". Do NOT put sponsor acknowledgements in the unnumbered footnote on the first page, but at here.

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