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Basic Biodegradability Assessments (Aerobic /Anaerobic)

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التقييمات الأساسية للتحلل الحيوي (الهوائية/اللاهوائية)

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Abstract:
Biodegradation is a biological process when the organic compounds are broken down into smaller compounds and the responsible for this process is the enzymes, which are produced by microorganism that is living in the environment. The biodegradation is possible to occur in presence of oxygen (aerobic) or without oxygen (anaerobic), but the final product of the degradation is often carbon dioxide or methane. In this study Sample was used is grass. The sample went through a number of chemical analysis test to know the organic and inorganic contents. These tests are Total Dry Matter by Oven Drying for Overnight at 100°C. Total Ash in Forages. ADF (determination of acidic detergent fibers), NDF (determination of neuronal detergent fibers), ADL (Determination of Acid Detergent Lignin), Kjeldahl method to measure crude protein and nitrogen in the sample. Anaerobic digestion was carried out using a simple method to investigate if the sample is able to degrade and produce CH4 and CO2. GC was used to measure the quantity of gas produced. Measured the pH for each bottle. The results shows the percentages of total DM, total of ashes, NDF, ADF, ADL, where % DM (27.19) , Ashes % (12.97) ,%NDF (65.33), %ADF(56) ,%ADL(8) also shows the percentages of crude protein and nitrogen which determined by Kjeldahl Method, where the average of crude protein was 50.03% and nitrogen was 8.00% ,shows the reading of the volume of gas produced over nine days.

Keywords: Biodegradation, Aerobic Anaerobic, Microorganism, Organic Originating.

الملخص
التحلل الحيوي هو عملية بيولوجية يتم فيها تحلل المركبات العضوية إلى مركبات أصغر والمسؤول عن هذه العملية هي الإنزيمات التي تنتجها الكائنات الحية الدقيقة التي تعيش في البيئة. من الممكن أن يحدث التحلل الحيوي في وجود الأكسجين (الهوائي) أو بدون الأكسجين (اللاهوائي)، ولكن المنتج النهائي للتحلل غالبًا ما يكون ثاني أكسيد الكربون أو الميثان. في هذه الدراسة تم استخدام عينة من العشب. خضعت العينة لعدد من اختبارات التحليل الكيميائي لمعرفة محتواها العضوي وغير العضوي. هذه الاختبارات عبارة عن إجمالي المادة الجافة عن طريق التجفيف بالفرن طوال الليل عند درجة حرارة 100 درجة مئوية. إجمالي الرماد في الأعلاف. NDF (تحديد ألياف المنظفات العصبية) ADF (تحديد ألياف المنظفات الحمضية) ، ADL (تحديد حمض المنظفات اللجنين) ، طريقة كلداهل لقياس البروتين الخام والنيتروجين في العينة. تم إجراء الهضم اللاهوائي باستخدام طريقة بسيطة لمعرفة ما إذا كانت العينة قادرة على التحلل وإنتاج الميثان وثاني أكسيد الكربون. تم استخدام GC لقياس كمية الغاز المنتجة. قياس الرقم الهيدروجيني لكل زجاجة. تظهر النتائج نسب إجمالي DM، إجمالي الرماد، NDF، ADF، ADL، حيث % DM (27.19)، Ashes % (12.97)، %NDF (65.33)، %ADF (56)، %ADL (8) أيضاً تبين نسب البروتين الخام والنيتروجين التي تم تحديدها بطريقة كلداهل، حيث بلغ متوسط البروتين الخام 50.03% والنيتروجين 8.00%، وتبين قراءة حجم الغاز المنتج خلال تسعة أيام.

الكلمات المفتاحية: التحلل الحيوي، اللاهوائية الهوائية، الكائنات الحية الدقيقة، المنشأ العضوي.

Introduction

Biodegradation is a biological process in which organic compounds are broken down into smaller compounds. The enzymes responsible for this process are produced by microorganisms living in the environment. Biodegradation can occur in the presence of oxygen (aerobic) or without oxygen (anaerobic), but the final product of the degradation is often carbon dioxide or methane. The materials that can be degraded are the organic materials such as plants or animals or the substances which are organic originating or unnatural matter that is like the plant and animal characters and can be used by microorganisms. The biodegradation phenomenon occurs in the presence of oxygen, wherein numerous organic substances are typically decomposed under aerobic conditions by microorganisms known as aerobes. These aerobic bacteria utilize oxygen to oxidize various substrates, including carbohydrates and lipids, to harness energy via a mechanism referred to as cellular respiration. Prior to this, the established glucose molecules undergo catabolism into two smaller entities, a process that transpires within the cytoplasmic matrix of the aerobes. Subsequently, these diminutive molecules are transported to the mitochondria, where aerobic respiration commences. The process of molecular degradation is facilitated by oxygen, resulting in the formation of water and carbon dioxide, concomitantly releasing energy. Aerobic process does not produce pungent gases, also is responsible for the improvement of the environment of the workers and keep the pathogenic in check. It is the biodegradation process when the oxygen does not exist; since this way helps to reduce the volume of input material it has been used to treat and biodegradable the waste water sludge, also it is known as a source of renewable energy of using biogas to replace fossil fuels is possible because it is rich in methane and carbon dioxide. Moreover, nutrient-rich solids can be used as fertilizer after digestion (1).

The early phases of this step occur extracellularly because the large insoluble polymers cannot enter the cell. Typically, only a small number of bacteria are able to produce the necessary enzyme for the breakdown of very large molecules; as the number of bacteria that can do so increases, the size of the molecules will decrease. There are a number of organisms in anaerobic digestion, which are identified by lipid hydrolysis. *Clostridia* and *micrococci* are shown to the responsible for producing the mainly extracellular lipases in anaerobic digesters, the main function of these enzymes is to break down the triglycerides to result in fatty acids and glycerol as reaction products. In the context of anaerobic digestion, there exists an extensive variety of extracellular proteases, with some demonstrating a high degree of specificity, while others possess the capability to degrade a limited array of proteins and peptides. These proteases exhibit functionality across a pH range of 5 to 11; however, the specific enzymes that exhibit activity within the pH range of 7 to 8 are frequently zinc-containing metalloproteins that can be inhibited by potent chelating agents such as EDTA. Additionally, certain other proteases display pronounced sensitivity to organophosphorus compounds. (2)

As examples of polysaccharides in anaerobic digestion, cellulose, hemicelluloses, and starch are degraded first by extracellular hydrolases formed by different bacterial genera. Anaerobic digestion is also capable of degrading pectin and dextran. Amino acids and sugars consider being the most readily fermentable substrate, but some anaerobic organism has the ability to ferment alkanoic acids, purines and pyrimidines. The result of fermenting the sugars is usually alcohols. In the fermentation process of amino acids there is an important intermediate called pyruvate, this process considers being the most important step in terms of quantity and acetate is the main final product.

Volatile fatty acids (VFAs) serve as crucial intermediates in the process of anaerobic digestion. This group of compounds includes acetate, propionate, butyrate, caproate, caprylate, valerate, and heptanoate. The oxidation of long-chain alkanoic acids leads to the production of acetate, a process characterized by the removal of two carbon fragments from the terminal end of the molecule during each cycle. The anaerobic β-oxidation of alkanoic acids generates significant amounts of NADH2. Additionally, carbon dioxide and molecular hydrogen are by-products of fermentation, which play a vital role in anaerobic digestion. (3)

The thought for many years that most or all the final products of the fermentative can turn into methane directly, take as example, the reaction which is supposed to be performed in pure culture of (methanobacillus omelianskii) is illustrated as:

$$2CH_3CH_2OH+CO_2\text{-----------}\ 2CH_3COOH+CH_4$$

However (M. Omelianskii) is shown in recent time to be containing a very close symbiotic association of two strict anaerobes. Acetate and hydrogen are formed from the first member of this association

$$CH_3CH_2OH+H_2O\text{---------------}2CH_3COOH+2H_2$$

Due to the characterization of acetate as the most intricate organic precursor for direct conversion into methane, this process, along with the relevant microorganisms, is collectively referred to as the obligatory hydrogen-producing acetogenic (OHPA) bacteria, which represents a crucial component of the treatment methodology.
Even though acetate is one of the important substrates, there is only one species which can generate methane from it.

$$CH_3COOH\text{-------------}CH_4+CO_2$$

Methanol (CH_3OH) can be used by acetoclastic organism Methanosarcina barkeri as substrate. some of the methanogens have the ability to use formaldehyde (HCHO) and the known oxidation of hydrogen by carbon dioxide. is used to obtain energy as it is illustrated in the following reaction (4).

$$4H_2+CO_2\text{------------}\ CH_4+2H_2O$$

this reaction which forms the basis of the unity of an otherwise fairly diverse group of bacteria however, about twice as much methane is generated from acetate than from the reduction of carbon dioxide in anaerobic digestion.

This biochemical reaction acts as a basic mechanism to support the unification of a collection of other very diverse bacteria, but it should be noted that, while carbon dioxide is reduced during anaerobic digestion, about twice the amount of methane is generated by the degradation of acetate.

There are three groups of tests according to the OECD, the Organization for Economic Cooperation and Development, these tests described by:

- Assessment of ready biodegradability or screening involves evaluating inherent biodegradability through simulation methods.

There are six methods that enable the screening of chemicals for ready biodegradability in an aerobic aqueous medium: closed bottle, manometric respirometry, carbon dioxide (CO2) evolution (modified Sturm test), dissolved organic carbon (DOC) die-away, modified OECD screening—DOC die away, and MIT Ministry of International Trade and Industry—Japan. The purpose of screening tests is to determine a compound's ability to degrade naturally without encountering any issues. Although these assays are frequently used to identify substances that are easily biodegradable from others, they are not frequently utilized to evaluate the rate of breakdown in biological systems. For that reason, when it gives negative results the inherent (potential) biodegradability tests will be necessary because this type of tests will provide information relating to the biodegradability of a matter. Because of the similarities between the natural process and the experimental conditions, the Zahn–Wellens procedure, for instance, is a test that examines the chemical pathway in an activated sludge treatment plant. Simulation test methods are used in the end-run to verify whether the results of the earlier tests were positive or negative. (5)

The main aim of neutralization of the sludge and its properties is the goal of sludge digestion such as odours also reduce its ability to putrescent in addition to reducing microbial activity which leads to reducing the number of pathogens. By removing 30–40% of the solid's gas during sludge digestion, the amount of organic matter is decreased, making the stabilized anaerobic product easier to handle and dispose of. The BOD of the final product will not be contributed because the final anaerobic metabolism is gas consequently the aeration is not needed to achieve a BOD reduction. This is one of the advantages of anaerobic treatment because the aeration process is expensive, furthermore the aerobic is limited and depends on the oxygen transfer rate as the BOD rises.
Polysaccharides such as cellulose, starch and hemicellulose consist most of the weight of dry matter, cellulose consists of an unlimited number of glucose molecules joined to each other by (1-4) glycosidic bonds to create a long chain. Hemicellulose consists of branched polymers of arabinose, galactose, mannose, xylose and glucose, Hemicellulose is responsible for solidity of cell wall where hemicellulose links of cellulose fibrils to form microfibrils which consider to be the main content of the cell wall, furthermore, hemicellulose joined to Lignin to form a complex web of bonds to be responsible for structure strength which plays important role to reduce the microbial degradation. Cellulose is strongly associated with hemicellulose, lignin and another substance, was the prevailing belief that most of the cellulose are degraded in aerobic environment but recent study proven that there is a large amount of cellulose degraded under anaerobic condition. Anaerobic digestion of cellulose happens by attachment of cellulolytic bacteria of the fiber substrate, allowing the series of cellulolytic enzymes associated with the membrane of the bacterial attack and degrade the individual cellulose molecules (6). There are a number of enzymes which responsible for the degradation of cellulose, three of them are mentioned in some studies, these enzymes are endoglucanases, cellobiohydrolases, and b-glycosidase. Endoglucanases plays a role in cleaving cellulose molecular This action allows the cellobiohydrolases to cleave the cellobiose subunits additional step to degraded cellobiose subunits to glucose monomers by the action of the enzyme b-glycosidase (7).
Numerous industries, including those that produce pulp, leather, and textiles, produce wastewater that is thought to contain a significant amount of cellulose. High levels of suspended solids and organic matter are typically found in wastewater. Although the conventional activated sludge methods are not entirely effective in removing these concentrations, they are still the most widely used method for treating wastewater with high concentrations. Even though they are expensive, a variety of physiochemical processes with high filtration are used to treat wastewater that contains a lot of cellulose. By installing membranes into the aerobic tank containing activated sludge, for instance, a membrane bioreactor (NBR) process is created. Other removal efficiencies include ozonation, coagulation, precipitation, adsorption, chemical oxidization, and membrane improvement of treatment methods that solely focus on altering conventional activated sludge processes. (8)(9)

Material and Methods

Sample preparation:

The forages sample was collected and refridged then dried by oven for overnight. Apparatus and Materials:

- Forced – air drying oven set at 100C.

- Analytical electronic balance
- Aluminum dish (crucibles)
- Desiccators.
- Muffle furnace with pyrometric controller.
- Refluxing apparatus.
- 600 ml Berzelius beakers
- Volumetric flasks
- 1 ml beakers.
- Glass rod.

Reagents:

- NDF solution per liter.
  - 18.6 g EDTA
  - 4.56 g disodium hydrogen phosphate (anhydrous)
  - 30 g sodium dodecyl sulphate
  - 6.81g di-sodium tetraborate decahydrate
  - 10 ml 2-ethoxy ethanol

  The pH of the solution should be within 6.9-7.1.
- ADF solution per liter.
  - 36 g Hexadecyltrimethylammonium Bromide.
  - 50 ml (1N) sulfuric acid technical grade.
- ADL solution.
  - 95% sulphuric acid.

-Total Dry Matter by Oven Drying for Overnight at 100C:

- Crucibles were dried at 100C for overnight then removed to and let cool to room temperature in desiccators.
- The closest crucibles were weighed 0.001g, (W_1) removing one at a time from desiccators and keeping desiccators closed between dishes removable.
- 2 g of sample was weighed (W) and added to the dishes and the weight of the dishes and the sample were recorded (W_2)
- The samples were inserted into preheated oven at 100C and left to dry overnight.
- The samples were moved to desiccators and allowed to cool then the dishes and dried samples were weighed and the weight was recorded (W_3)

***-Total Ash in Forages*:**

1- The crucibles that have been dried overnight at 100C were moved from the oven to desiccators and allowed to cool, and the crucibles weight was recorded.

2- 2 g of sample was weighted into crucible and the weight was recorded.

3- The sample was turned to ashes in furnace at 500C for 2 hours.

4- Crucibles were allowed to cool in furnace to less than 200C then placed in desiccators the weight of crucible and ash was recorded (W_4)

-Determination of Neural Detergent Fiber (NDF):

1- The sample dried overnight at 100C and ground to pass a 1mm sieve.

2- Thoroughly the sample was mixed and 1.0 g of sample weighted.

3- Approximately 90 ml of NDF solution was measured into 500 ml reflux container.

4-0.5 g of sodium sulphatic was added.

5-The sample was added and the weighing boat rinsed with 10 ml of NDF solution.

6-The mixture heated to the boiling point then the heat reduced once the mixture started boiling and refluxed for 60 minutes.

7-gooch crucibles that have been dried at 100C overnight then the weight recorded.

8- The sample filtered through Gooch Crucible, the fiber washed with hot water and re-filtered twice then washed with acetone and re-filtered twice.

9- Crucible dried overnight at 100C and the weight recorded.

-Determination of Acid Detergent Fiber:

1 - The sample dried overnight at 100C and ground to pass a 1mm sieve.

2 - Thoroughly the sample was mixed and 1.0 g of sample weighted

3 - Approximately 90 ml of ADF solution was measured into 500 ml reflux container.

4-The sample was added and the weighing boat rinsed with 10 ml of NDF solution.

5-The mixture heated to the boiling point then the heat reduced once the mixture started boiling and refluxed for 60 minutes.

6-gooch crucibles that have been dried at 100C overnight then the weight recorded.

7- The sample filtered through Gooch Crucible, the fiber washed with hot water and re-filtered twice then washed with acetone and re-filtered twice.

8- Crucible dried overnight at 100C and the weight recorded

-Determination of Acid Detergent Lignin.

1- Gooch crucible with residue generated in ADF was placed in a large beaker and treated with 72% sulphuric acid.

2-The crucible was refill with 72% sulphuric acid hourly three times, then the crucible was fill with hot water and allowed draining.

3-Fibre washed with hot water and filter.

4- Crucibles dried at 100C in oven overnight, and then the weight was recorded.

5- Crucible contents ashed at 500-550C for 3 hours after that the weight was recorded.

- The Kjeldahl method:

1- Approximately 1.18 g of sample was weighted into a numbered kjeldahl tubes, and 2 kjeltabs were added and a few anti-bumbing granules was added then 20ml concentrated sulphuric acid

2-the tube placed into the digester rack and any empty places with empty tubes.

3- The tube rack placed into the digester and the instrument set.

4-when the digester stopped the tubes left to cool for 5 minutes then the tubes replaced in the holder.

5-the samples run on the Vapodest for analysis.

- Anaerobic digestion

- Twelve numbered bottles 500 ml prepared and fill of sludge water, linked to Graduated cylinder to collocate the produced gas from bottles, 200 ml of mineral media added to each bottle, different concentration of dried ground grass added to each 2 bottles as following.

-1 and 2 left as control (blank)

Added 0.25 % of cellulose to bottles number 3 and 4, 0.02% of sample added to bottles number 5 and 6, 0.25% of sample added to bottles number 7 and 8, 0.50% of sample added to bottles number 9 and 10, 1.0% of sample added to bottles number 11 and 12.

-The pH of the bottles measured; it was between (7.42 – 7.44).

-The volume of the gas produced collected over 9 days.

- *Gas Chromatography (GC*)

-The instrument set up and injected by 100 µL of nitrogen, CH_3, CO_2.

-100 µL was taken from each bottle and injected to the instrument, and the percentage of N2, CO_2 and CH3 measured.

- measured pH to each bottle.

Results and Discussions:

1-The calculation of total dry matter by oven drying for overnight at 100 and total ash in forages:

Table (1) The two replicates of percentages measurement of Dry matter (DM) and the percentage of Ashes of grass.

S. N.	Crucible Weight (g)		Sample Weight (g)		Crucible+ Sample weight (g)		Crucible+ Dried sample weight (g)		Crucible+ Ashes weight (g)		% DM		% Ashes	
N. R.	1	2	1	2	1	2	1	2	1	2	1	2	1	2
1	29.17	30.95	2.030	2.003	31.19	32.94	29.70	31.52	29.23	31.03	26.10	28.44	10.90	15.09
2	32.47	32.47	2.016	2.008	34.47	34.47	33.03	32.96	32.54	32.53	27.77	24.40	12.72	11.32
3	30.95	29.17	2.002	2.009	32.94	31.17	31.50	29.68	31.02	29.24	27.46	25.38	12.96	12.72
4	28.32	28.32	2.021	2.001	30.32	30.31	28.87	28.87	28.38	28.39	27.72	27.47	10.90	13.20
5	30.48	30.48	2.010	2.002	32.47	32.47	31.07	31.03	30.55	30.56	29.35	27.46	12.96	15.09
6	28.90	28.90	2.007	2.007	30.90	30.90	29.46	29.45	28.97	28.98	27.88	27.39	12.72	15.09
											27.63 %	26.75 %	12.19 %	13.75 %

The percentages were calculated according to the following equation:

% total DM = * 100

% total of Ash =

2-The calculation of neutral detergent fiber NDF:

24.26 – 24.05 = 0.21 g

23.80 - 23.34 = 0.46 g

0.21 +0.46 = 0.67 g = 67 %

Table (2) The replicate of the determination of NDF.

Sample N.	Gooch crucible weight			Gooch crucible + dried sample weight		
	1	2	3	1	2	3
1	**24.05 g**	**24.06 g**	**24.06 g**	**24.26 g**	**24.25 g**	**24.53 g**
2	**23.34 g**	**23.35 g**	**21.74 g**	**23.80 g**	**23,83 g**	**21.82 g**
3			**20.99 g**			**21,06 g**

3-The calculation of acid detergent fiber ADF:

21.15- 20.99=0.16 g

22.50-22.09=0.41 g

0. 16+0.41= 0.57 =57%

Table (3) The replicate of the determination of ADF.

Sample N.	Gooch crucible weight			Gooch crucible + dried sample weight		
	1	2	3	1	2	3
1	**20.99 g**	**20.99 g**	**23.35 g**	**21.15 g**	**21.06 g**	**23.41 g**
2	**22.09 g**	**21.87 g**	**22.08 g**	**22.50 g**	**22.06 g**	**22.11 g**
3		**21.59 g**	**21.59 g**		**21.91 g**	**21.61 g**
4			**22.71 g**			**23.13 g**

4-The calculation of acid detergent lignin ADL

31.13 -31.0042 =0.12 g = 12 %

Table (4) The replicate of the determination of ADL.

Sample N.	Crucible+ sample weight			Crucible + Ash weight		
	1	2	3	1	2	3
1	**31.13 g**	**32.57 g**	**29.27 g**	**31.0042 g**	**32.57 g**	**29.20 g**

Table (5) The percentages of total DM, total of ashes, NDF, ADF, and ADL.

% Total DM	Total of Ashes	%NDF	%ADF	%ADL
27.63%	**12.19%**	**67%**	**57%**	**12%**
26.75%	**13.75%**	**67%**	**58%**	**5%**
		62%	**53%**	**7%**
27.19%	**12.97%**	**65.33%**	**56%**	**8%**

95-Determination of crude protein and nitrogen

-Kjeldahl Method.

Table (6) the percentages of crude protein and nitrogen which determined by Kjeldahl Method.

Sample N.	Sample weight	Volume (HCl) ml	% crude protein	% N
Blank	0	2.50	0	0
1	1.18 g	21.84	54%	8.64%
2	1.16 g	20.51	51.24%	8.19%
3	1.18 g	20.37	49.99%	7.99%
4	1.18 g	20.00	48.98%	7.83 %
5	1.18 g	20.03	49.06%	7.85%
6	1.18 g	19.26	46.96%	7.51%
			50.03%	8.00%

6-The results of anaerobic digestion:

Table (7) The reading of the volume of gas produced over nine days.

Time	V. blank	V. cellules.	V.0.02	V.0.25	V. 0.50	V. 1.0
0	0	0	0	0	0	0
1	210.24	208.12	104.06	168.83	163.52	101.93
2	231.47	232.54	123.17	211.3	273.95	310.05
3	260.14	270.76	142.28	277.13	390.75	467.2
4	284.56	327.03	174.13	402.42	609.49	744.39
5	312.16	440.64	201.73	483.12	771.46	897.61
8	374.8	733.71	268.62	599.92	939.23	1029.83
9	425.76	822.9	282.42	627.52	210.24	1106.28

Table (8) The reading of gas produced by each bottle minus the gas produced by blank bottle.

Time	V. cellules -V. blank	V.0.02-V.blank	V.0.25-V.blank	V.0.50-V.blank	V.1-V.blank
0	0	0	0	0	0
1	-2.12	-106.18	-41.41	-46.72	-108.31
2	1.07	-108.3	-20.17	42.48	78.58
3	10.62	-117.86	16.99	130.61	207.06
4	42.47	-110.43	117.86	324.93	459.83
5	128.48	-110.43	170.96	459.3	585.45
8	358.91	-106.18	225.12	564.43	655.03
9	397.14	-143.34	201.76	-215.52	680.52

The most common way to follow the pathway of measuring the outcomes of anaerobic biodegradation of organic compounds and the amount of gas produced from it such as (CH_4+CO_2) which consider being the common final products of the biodegradation of organic compounds.

In some research reported that about 10 to 40 ml of gas is normally produced by 10% sludge, taking in account the amount of sludge's organic matter and the retention period (10).

The biodegradation process can be affected by the organic matter which present as total dry matter which consider to be the most important aspects according to some studies which reported that the content of the biodegradable organic lies between 70% to > 95% of dry matter content and the materials with percentage of dry matter less than 60% are not often worthy for anaerobic digestion (11).

According to the result that shown in table 4 the mean of total dry matter for grass is 27.19%, which consider being low percentage of dry matter and that may cause a reduction in the activity according to some researches otherwise the higher percentage of dry matter will record high gas production (10).

There is no doubt that there is a solid reason to favor a specific anaerobic medium. Except for K+, NH4, and Co, all mineral and metal nutrients should typically be present in sufficient amounts in ten percent sludge alone. But, as insurance, a more comprehensive medium is probably advised.

Conclusion

Organic compounds are broken down into smaller compounds during biodegradation. Microorganisms living in the environment produce enzymes that are responsible for this process. Biodegradation occurs with organic materials, in the presence of oxygen being broken down by aerobic bacteria known as aerobes. Through cellular respiration, these bacteria use oxygen to oxidize materials like sugars and fats in order to produce energy. In the aerobes' cytoplasm, the glucose molecules first break up into smaller ones. The smaller molecules then travel to the mitochondria, which are the site of aerobic respiration. O_2 guides the breakdown of molecules to generate water, carbon dioxide, and energy. The aerobic process does not generate foul odors, but it also helps to enhance the working environment and control the spread of pathogens. Carbon dioxide or methane is often the final result of biodegradation, whether it occurs aerobically or anaerobically. the anaerobic degradation accrues in more suitable conditions in the real environment. Consequently, the positive results do not mean that the sample is degradable. Also, the negative results should not be considered as evidence of the lack of anaerobic biodegradation. Additional tests are required to assess the abilities of anaerobic degradation.

References

[1] Milano, J., Ong, H. C., Masjuki, H. H., Chong, W. T., Lam, M. K., Loh, P. K., & Vellayan, V. (2016). Microalgae biofuels as an alternative to fossil fuel for power generation. Renewable and Sustainable Energy Reviews, 58, 180-197

[2] Elahi, A., Bukhari, D. A., Shamim, S., & Rehman, A. (2021). Plastics degradation by microbes: A sustainable approach. Journal of King Saud University-Science, 33(6), 101538

[3] Weimer, P. J. (2022). Degradation of cellulose and hemicellulose by ruminal microorganisms. Microorganisms, 10(12), 2345.

[4] Buan, N. R. (2018). Methanogens: pushing the boundaries of biology. Emerg Top Life Sci 2: 629–646.

[5] Mountfort, D. O., & Asher, R. A. (1978). Changes in proportions of acetate and carbon dioxide used as methane precursors during the anaerobic digestion of bovine waste. Applied and environmental microbiology, 35(4), 648-654.

[6] Dohányos, M., Zabranska, J., Kutil, J., & Jeníček, P. (2004). Improvement of anaerobic digestion of sludge. Water Science and Technology, 49(10), 89-96.

[7] Ku Kim S.,Lee T.,(2008)Degradation of lignocellulosic materials under sulfidogenic and methanogenic conditins, waste management (New York,N.Y.).Jan /2009,29(1),PP.224-227

[8] You S. J.,Wu D.C.,(2009) Potential for reuse of high cellulose containing wastewater after membrane bioreactor treatment ,Desalination,249(2),pp.721-728

[9] Al-Tohamy, R., Ali, S. S., Li, F., Okasha, K. M., Mahmoud, Y. A. G., Elsamahy, T., ... & Sun, J. (2022). A critical review on the treatment of dye-containing wastewater: Ecotoxicological and health concerns of textile dyes and possible remediation approaches for environmental safety. Ecotoxicology and Environmental Safety, 231, 113160.

[10] Shelton D. R.,Tiedje J. M.(1984)General Method for Determining Anaerobic Biodegradation Potential, Applied and Environmental Microbiology.April/1984,47(4),pp.850-857.

[11] Steffen,R.,Szolar,O.,Braun ,R.(1998)Feedstock for Anaerobic Digestion ,institute for Agrobiotechnology Tulln University of Agricultural Sciences , Vienna.