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THE BIOLOGICAL WASTE WATER TREATMENT IN POST COVID-19 ERA BY PHOTOSYNTHETIC BACTERIA: *RHODOBACTER SPECIES*

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Abstract

The growth and use of microorganisms to treat waste water is a cheap and affordable method to treat waste water plant. This method can be efficiently maximized by the choice of microorganisms that have the ability to decontaminate water; hence it can be done by photosynthetic bacteria such as *Rhodobacter.* The *Rhodobacter species* was grown using corn waste water which is mostly seen as waste in this post covid-19 era. The isolates were gotten from Ubahudara stream and Ugutah Lake of Anambra and Imo state of Nigeria. The organisms were incubated at room temperature, and pH of 6.8, using tungsten lamp (150watts) for constant illumination at a distance of 50cm for 5 to 10days under an anaerobic condition. The result showed a visual sign of growth by turbidity and pigmentation. The test for motility showed that the organisms were motile. The Gram reaction test showed that they are Gram negative. Characterization showed that the organism utilizes glucose, lactate, fructose, citrate, malate, succinate, acetate and propionate and then left formate, benzoate and ethanol unutilized. The *Rhodobacter species* nitrogen production which occurred within four days interval was used to determine the growth of the organism on corn waste water as carbon source, therefore it showed the percentage of its nitrogen production as follows, 0.462%, 0.802%, 0.882%, 1.096% and 1.106% on the 1st, 5th, 9th, 13th and 17th day respectively.This showed that *Rhodobacter species* uses corn waste water as its carbon source thus decontamination of water in this Covid-19 era of economic hyper-inflation.

Keywords: *Rhodobacter species*, waste water treatment, Photosynthetic bacteria, Post covid-19 era, Corn waste water.

Introduction

The treatment of waste water biologically using microorganisms has a long history with mankind. This approach has shown to the world that it's a very effective and ecologically friendly process. (Liu *et al*., 2015). Its purpose in the treatment of waste water is to ensure pollutants removal from the natural ecosystem and water bodies especially when they have been polluted. Water bodies can be treated biologically or chemically, or even the combination of them. However, the treatments of this waste water biologically have greater advantages due to the fact that they are more reliable, cheap and the efficiency of the microbe of choice can be improved (Merugu *et al.*, 2014).

Also it is of great importance to create a benign environment as well as measures that are cost effective economically in other to keep under control these hazardous substances that causes pollution. Therefore great efforts for the improvement of waste water treatments have been made by imploring so many approaches which include Adsorption, Coagulation, Photocatalytic oxidation and biodegradation (Zhu *et al.*, 2016, Douglas *et al.*, 2016 & Li *et al.*, 2016). However, the effectiveness of these approaches are continually disturbed by some factors which include; stability, energy, efficiency as well as economy. Hence each of these approaches has its own merits and demerits, which made scientists to continue looking for approaches that can improve the treatment of waste water. The application of photosynthetic bacteria in the treatment of waste water has been extensively studied (Liu *et al*, 2015). Thus it is generally known that photosynthetic bacteria can bring about degradation of the harmful pollutants during their metabolic processes in an aqueous media. These bacteria have been found very effective and are ecologically friendly, which can help in the removal of phosphorous, nitrogen and carbon from wastewater (Idi *et al.*, 2015). Photosynthetic bacteria are of great importance and can be widely used in the control of environmental waste, pharmaceutical industry, restoration of the ecology, agriculture, chemical industry, aquaculture, animal husbandry. It can be applied in the treatment of waste water (Dalaei *et al.*, 2019) and remediation of heavy metals in the soil (Peng *et al.*, 2018).

These photosynthetic bacteria are prokaryotes that naturally exist in our environment and are capable of phototropic growth instead of photosynthetically. They are mostly known to use light energy as it's driver and then makes use of sulphides, organic carbon or hydrogen in the fixation of carbon dioxide required for anoxygenic form of photosynthesis, whereby the process as a whole neither need the presence of water (H_2O) as it's electron donor nor the release of oxygen (O_2) (Puyol *et al.*,2017). The *Rhodobacter specie* of the photosynthetic bacteria have a great diversity as a microorganism, whereby it have the great capability of several growth modes such as the anaerobic anoxygenic photosynthesis, aerobic respiration as well as fermentation (Agrawal, 2017). Thus it is of great importance in the treatment of wastewater in this post covid-19 era, because the covid-19 pandemic have impacted negatively on almost all African countries and their activities, also the outbreak was accompanied by the global economic shutdown and therefore there is a great need to utilize biological approaches which are cost effective and environmentally friendly to carry out the bioremediation of our environment by the treatment of the waste water. The purpose of this research is to determine the growth of *Rhodobacter species* using corn waste water as its substrate by monitoring its nitrogen production which will be used in waste water treatment.

Materials and Method

Technique for Sample Collection

The water samples used were collected from the flowing region of Ubahudara stream (located in Uli, Ihiala Local Government Area of Anambra State) between 10:00 am 10:30 am at a depth of 0.2m while those of Ugutah lake (located in Okwuege Akabor, Ugutah Local Government Area in Imo state) were collected from the flowing region of the lake between 11:30am-12:00pm at a depth of 0.2m with well labeled sterile sample bottles, using standard operating procedure (SOP) for water sample collection (Musselman, 2012). The samples were collected aseptically in triplicate and transported to the laboratory.

Formulation of Mineral Medium for Enrichment of *Rhodobacter Species*

The mineral medium for enrichment of *Rhodobacter species* comprises of the basal components KH_2PO_4, $MgSO_4$, $CaCl_2$, NH_4Cl_2, sodium succinate and yeast extract. These were measured using an electronic weighing balance.

Preparation of Media for Enrichment

The basal components were mixed together with 100mls of distilled water without trace salt solution, which was sterilized in an autoclave for 15 psi at 121^{0}C for 15 minutes and allowed to cool before adding the trace salt of 1.0ml, 2% $Fe_2SO_4.7H_2O$ of 0.5ml, which was then homogenized and the pH was read ranging from 6.8-7.2

The Characterization and Isolation of Bacteria Isolates

Isolation of Bacteria

The prepared medium was aseptically dispensed into six (6) different sterilized 150ml stoppered culture bottles which were well labeled. The water samples from Ubahudara stream and Ugutah Lake were used to inoculate the media (broth) by filling it to the brim. Vaseline was smeared on the mouth of the bottle which was then sealed with foil and masking tape in other to create an anaerobic condition. It was then incubated under constant illumination of light using a tungsten lamp (150 watts) at a distance of 50cm for 5-10 days at room temperature for possible growth.

The Characterization of Bacterial Isolates by the Utilization of Fermentative Sugars

To characterize the bacterial isolate, fermentative sugars such as acetate, formate, propionate, glucose, fructose, lactate, citrate, ethanol, succinate, malate, benzoate was used using the manufacturer's procedure. Then 1ml of the sterilized sugar solution was transferred into the test tubes containing the organism and was inoculated into the test tubes and incubated under constant light source for 1 to 8 days.

Sub-Culturing of the Bacteria Isolate

The enriched culture was streaked on the petri dish containing the solid medium (agar). An anaerobic condition was created and the plate was incubated at room temperature for 5 to 8 days under constant illumination of light for possible growth.

The Identification of Bacteria Isolates

To identify the bacteria isolate seen, test such as motility test and morphology by Gram staining and wet mount was carried out.

The Determination of the Total Nitrogen

Total nitrogen was determined using the formula:

$$Nitrogen\ (\%) = \frac{Titre\ value\ of\ sample\ \times 1.40\ \times total\ volume\ of\ sample}{1001\ \times sample\ volume\ for\ digestion\ (5ml) \times Aliquot\ volume\ distilled} \times \frac{100}{1}$$

Note: 1.40 is the nitrogen value equivalent to the 0.1m HCl used in titration.

Results

The result of this work shows that the isolation was carried out following the required procedure, thus observation was made from zero hour till when there was visual evidence of growth by pigment and turbidity as shown in Table 1. *The Rhodobacter species* was characterized using various fermentation sugars and shown in Table 2 using different sample from Ugutah Lake and Ubahudara stream with their control. The growth of organisms using succinate and corn waste water as carbon source at constant illumination using tungsten lamp (150 watts) was done as shown in Table 3 and 4.

The growth was monitored as it was determined by its nitrogen production as shown in Table 5 and 6 which was finally presented in a bar chart as shown in figure 1 and 2 respectively.

Table 1: Isolation of the *Rhodobacter*species from Ugutah Lake and Ubahudara stream.

	Ugutah Lake		Ubahudara stream	
Days	Turbidity	Pigmentation	Turbidity	Pigmentation
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-
5	+	-	+	-
6	+	-	+	+
7	++	-	++	+
8	++	+	++	+
9	++	+	++	+

10	++	+	++	++

Key:

- = Absence of turbidity and pigments + = Appearance of turbidity and pigment
++ = increase in appearance of growths (turbidity) and pigmentation

Table 2: Characterization and Identification of *Rhodobacter* Species from Ubahudara stream and Ugutah Lake

Parameter	IsolateX	IsolateY
Colour	Orange brown	Yellowish brown
PH	6.6	6.9
Shape	ovoid/Rod	ovoid/Rod
Gram reaction	-	-
Motility test	+	+
Acetate	+	+
Formate	-	-
Propionate	-	+
Glucose	+	+
Fructose	+	+
Lactate	+	+
Citrate	+	+
Succinate	+	+
Malate	+	+
Benzoate	-	-
Ethanol	-	-
Possible species	*R.blasticus*	*R.capsulatus*

Key:

Isolates x = Isolate from Ubahudara stream; Y = Isolate from Ugutah Lake; + = possible test; - = Negative test

Table 3: The Growth of *Rhodobacter Species* Using Sodium Succinate as Carbon Source

Days	Growth (Turbidity)	Pigmentation
1	-	-

2	-	-
3	-	-
4	-	-
5	+	-
6	+	-
7	++	-
8	++	+
9	++	+
10	++	+

Key:

- = Absence of turbidity and pigments + = Appearance of turbidity and pigment
++ = increase in appearance of growths (turbidity and pigmentation)

Table 4: The Growth of *Rhodobacter Species* Using Corn Waste Water as Carbon Source

Days of Incubation	Growth (Turbidity)	Pigmentation
1	-	-
2	-	-
3	-	-
4	-	-
5	-	-
6	-	-
7	-	-
8	-	-
9	-	-
10	-	-
11	+	-
12	+	-
13	+	-
14	+	-

Key:

- = Absence of turbidity and pigments
+ = Appearance of turbidity and pigment

++ = increase in appearance of growths (turbidity and pigmentation)

Table 5: Total Nitrogen Determination of *Rhodobacter Species* using Corn Waste Water as Carbon Source.

% Nitrogen for the control (0.418)

Days	Cultures
1	0.462%
5	0.802%
9	0.882%
13	1.096%
17	1.106%

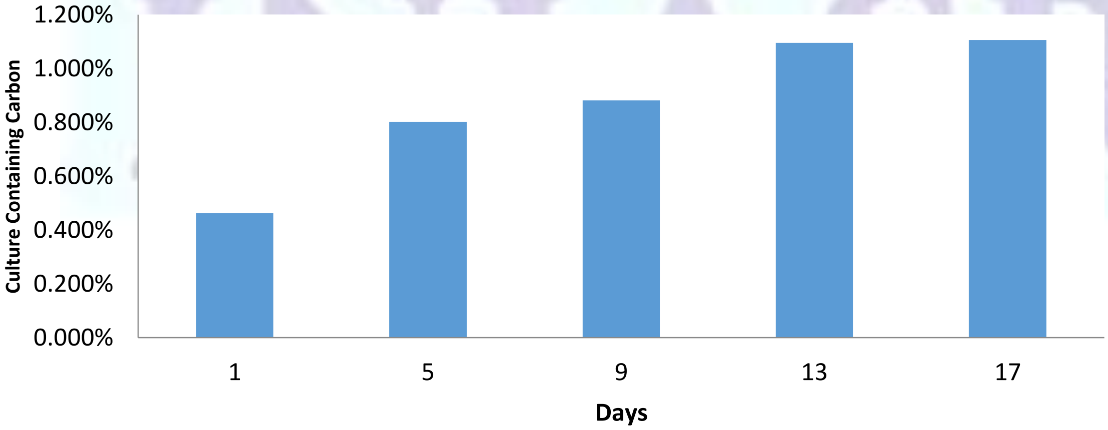

Fig 1: Bar Chart Showing Total Nitrogen Determination of the *Rhodobacter Species* using Corn Wastewater as Carbon Source

Table 6: Total Nitrogen Determination of *Rhodobacter species* using Succinate as Carbon

% Nitrogen for the control (0.918)

Days	Cultures containing succinate
1	0.962%
5	1.302%
9	1.382%
13	1.596%
17	1.606%

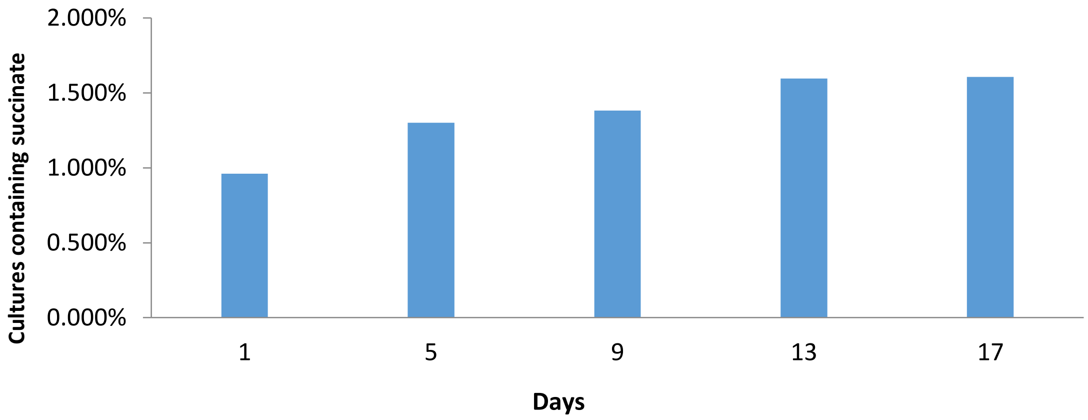

Fig 2: Bar Chart Showing Total Nitrogen Determination of the *Rhodobacter Species* using Succinate as Carbon Source

Discussion

The organism that was used for this study was isolated from Ubahudara stream and Ugutah lake of Anambra and Imo State respectively which was polluted by organic matters. Bacteria are distributed widely in so many habitats such as soil, ocean, fresh water and can be isolated always from these sources thereby it shows that the stream is a habitat for its isolation which is in accordance with Prasse *et al.*, (2015).

The inoculation of the enrichment media with the sample at pH 6.8 incubated under constant illumination of light using tungsten lamp, at temperature of 25^0C to 35^0C for 1 to 10 days yielded no turbidity and pigmentation on both lakes samples for the first four days. Observance of turbidity started on the 5th day with increase turbidity from the 7th to 10th day. On Ugutah lake, pigmentation was visible on the 8th day while that of Ubahudara stream started on the 6th day. This showed that succinate yeast extract was the main component of the media that supports the growth o*f Rhodobacter species* as shown in Table 1 which is in agreement with Stefania *et al.,* (2017).

The identification result showed that the *Rhodobacter species* is a Gram negative, spore forming and rod shaped bacteria as shown in Table 2. Also the result of the motility test showed that the organism is motile as also shown in Table 2 which agrees with Lu, *et al.*, (2019) explaining why it lives in an aquatic environment though it does not have flagellum. Fermentation test showed that organism found in Ugutah lake utilizes glucose, fructose, acetate, lactate, propionate, citrate, succinate and malate and it does not utilize formate, benzoate and ethanol while Ubahudara stream showed same fermentation result with the exception of propionate which was negative.

Rhodobacter assimilates succinate faster than corn waste water for its growth. Increase in growth rate with succinate occurred from the 6^{th} day of incubation while that of corn waste water started on the 11^{th} day of incubation as shown in Table 3 and 4 respectively. Pigmentation occurred with succinate from the 8^{th} day whereas no visible pigmentation was seen on corn waste water even at the 14^{th} day.

The growth of this organism using corn waste water and succinate as its carbon source was determined by monitoring its total nitrogen production. This occurs within five days (5) interval as shown in the Table 5 and 6. This shows that the organism utilizes succinate as its carbon source more than corn waste water with the evidence by its nitrogen production. The percentage of nitrogen produced on the first (1st), fifth (5th), ninth (9th), thirteen (13th) and seventeen (17th) day, were 0.962%, 1.302%, 1.382%, 1.596% and 1.606% respectively as shown in Table 6 as it uses succinate as carbon source; whereas the percentage nitrogen produced is 0.462%, 0.802%, 0.882%, 1.096% and 1.106% respectively as shown in Table 5 when it uses corn waste water as carbon source. This shows that corn waste water which is regarded as waste is of industrial importance for the growth of this organism. Thus the organism is of great importance to the environment, soil and plants because it can be of help in bioremediation and of great economic benefits (Idi, *et al*, 2015). It can also be used in single cell protein production (Vrati, 1984).

Conclusion

The magnitude at which COVID-19 spread across the globe and introduction of wide range of measures to control the pandemic, have resulted to hyper inflation to the world economy, hence alternative measures are being put in place to reduce the effects of economic inflation. Chemical wastewater treatment processes which includes chemical precipitation (coagulation, flocculation), ion exchange, neutralization, adsorption, and disinfection (chlorination/dechlorination, ozone, UV light) are usually expensive, hence the use of biological treatment methods which are cost effective. *Rhodobacter species* have a wide range of metabolic activities, with a great ability of growing in different growth conditions. Corn waste water was found to be supportive for the growth of *Rhodobacter species*, which can be used in the treatment of waste water in this post covid 19 era of economic instability.

Recommendation

The present study showed that *Rhodobacter species* is of great importance in bioremediation. Further studies can be made on the rate at which other photosynthetic bacteria decontaminates corn waste water, hence to know the best organism to employ during treatment of corn waste water.

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