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BIODEGRADATION OF CASSAVA (*Manihot esculentus*) EFFLUENT USING WHITE ROT FUNGUS (*Pleurotus ostreatus*) AND BROWN ROT FUNGUS (*Gloeophyllum sepiarium*)

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ABSTRACT
The utilization of cassava effluent as a sole carbon and energy source by white rot fungus (*Pleurotus ostreatus)* and brown rot fungus (*Gloeophyllum sepiarium*) was examined. The microorganisms were unable to utilize 100% unsterilized and sterilized effluent. The test organisms utilized 10% effluent in minimal salt medium (MSM) with *P. ostreatus* having a mean growth of 1.42 cm and *G. sepiarium*, 1.73 cm. When 2 g/l yeast was incorporated into 10% effluent in MSM, the mean growth increased to 1.97 cm for *P. ostreatus* and *G. sepiarium*, 1.89 cm. When the test organisms were incubated for 30 days at temperatures of 4 ^{0}C, 28 ^{0}C and 37 ^{0}C, *P ostreatus* had a growth of 0.96, 4.4 and 0.5 cm respectively and *G. sepiarium*, 0.63, 2.18 and 0.5 cm. When the pH of the medium was adjusted to 6, 8 and 10, *P. ostreatus* had mean growth of 2.77, 3.14 and 2.63 cm respectively and *G. sepiarium*, 1.74, 2.16 and 1.01 cm. The rate of biodegradation by these organisms was determined using $C0_2$ evolution method. *P. ostreatus* had a mean growth rate of 21.0 while *G. sepiarium*, 6.0. The results of this study show that these organisms can degrade cassava effluents, pH 8 appeared to be their optimum pH and 28 ^{0}C appeared to be their optimum temperature for biodegradation.

KEYWORDS: biodegradation, cassava effluent, $C0_2$ evolution, *Pleurotus ostreatus, Gleophylum sepiratus*

INTRODUCTION

Cassava (*Manihot esculentus*) is a staple food of nearly one billion people in Africa, South America, Asia and the Pacific (Food and Agriculture Organization, FAO, 2004, Australian National University, ANU, 2007). By its nature, cassava processing for starch extraction produces large amounts of effluent high in organic content, suspended solids, visible dust waste (FAO, 2004) and hydrocyanic acid (Arimoro *et al*., 2008). When this water is released directly into streams and rivers, residual starch can cause rapid growth of bacteria, resulting in oxygen depletion and detrimental effects on aquatic life (FAO, 2004, ANU, 2007).

Biodegradation is used to describe the complete mineralization of complex inorganic compound to simpler ones like $C0_2$, H_20, $N0_3$ and other inorganic compounds by microorganisms (Atlas and Bartha, 1998). White rot fungus have been used to degrade humic acids, lignin (Grinhut *et al.*, 2011), diazinon and methomyl pesticides (Nyakundi, *et al.*, 2011). The objectives of this research therefore are to biodegrade cassava effluents using *P. ostreatus* and *G. sepiarium* and compare the biodegradable ability of the two organisms.

MATERIALS AND METHODS

Study Area: The study area of this study is cassava processing industry located at Mobil metropolis in Minna Niger State, Nigeria.

Effluent: Cassava effluent was obtained from a cassava processing industry in the study area. The effluent was collected with a clean container inside two sterile five liter gallon from the effluent outlet section of the factory. It was transported and stored for one hour in the refrigerator of Microbiology laboratory, Federal University of Technology, Minna, Niger State, Nigeria.

Soil samples: Two hundred grams (200 g) of soil samples used were obtained 1m to the effluent source. Another 200 g of soil samples was obtained 100m from the effluent source.

Microorganisms: The test organisms used for this study were white rot fungus (*Pleurotus ostreatus*) and brown rot fungus (*Gloeophyllum sepiarium*) obtained from a stock culture from Microbiology laboratory of the Federal University of Technology, Minna, Niger State, Nigeria.

Utilization of unsterilized and sterilized 100% cassava effluent by *P. ostreatus* and *G. sepiarium*
One hundred milliliter (100 ml) of unsterilized cassava effluent was distributed into ten 250 ml Erlenmeyer flask and *P. ostreatus* was aseptically inoculated into the first five of the flask while *G. sepiarium* was inoculated into the remaining five flasks. The inoculated flasks were incubated at 28 ^{0}C for thirty days.

Utilization of 10% cassava effluent in minimal salt medium by *P. ostreatus* and *G. sepiarium*
Ten percent cassava effluent in minimal salt medium (NH_4N0_3-2.78g, KH_2P0_4-0.98g, K_2HP0_4-0.7g, $ZnS0_4$-0.01g, $MnS0_4.4H_20$-0.005g, $CaCl_2.2H_20$-0.05g, $CaCl_2.6H_20$-0.001g, thiamine hydrochloride- 0.001g, agar 20g/1000ml) was sterilized using autoclave at 121 ^{0}C for 15 minutes. It was distributed into Petri dishes and allowed to gel. Duplicate of the plate was inoculated with the test organisms and then incubated at 28 ^{0}C for 30 days. The growth was determined using mycelia extension method at each successive day as described by Smith (1977).

Effect of yeast incorporated into 10% cassava effluent in minimal salt medium by *P. ostreatus* and *G. sepiarium*
Two gram per liter (2 g/l) of yeast was incorporated into 10% cassava effluent in MSM and was sterilized using autoclave at 121 ^{0}C for 15 minutes. It was distributed into Petri dishes and allowed to gel. Duplicate of the plate was inoculated with the test organisms and then incubated at 28 ^{0}C for 30 days. The growth was determined using mycelia extension method at each successive day as described by Smith (1977).

Determination of optimum temperature for the growth of P. *ostreatus* and *G. sepiarium* in 10% cassava effluent in minimal salt medium

Ten percent (10%) cassava effluent in MSM was sterilized using autoclave at 121 ^{0}C for 15 minutes. It was distributed into Petri dishes and allowed to gel. Duplicate of the plate was inoculated with the test organisms and then incubated at 4 ^{0}C, 28 ^{0}C and 37 ^{0}C for 30 days. The growth was determined using mycelia extension method at each successive day as described by Smith (1977).

Determination of optimum pH for the growth of P. *ostreatus* and *G. sepiarium* in 10% cassava effluent in minimal salt medium

The pH of 10% cassava effluent in MSM was adjusted using dilute Sodium Hydroxide, NaOH and Tetraoxosulphate IV acid, H_2S0_4 to pH 6, 8, and 10. It was sterilized using autoclave at 121 ^{0}C for 15 minutes. It was distributed into Petri dishes and allowed to gel. Duplicate of the plate was inoculated with the test organisms and then incubated at 28 ^{0}C for 30 days. The growth was determined using mycelia extension method at each successive day as described by Smith (1977).

Determination of the rate of biodegradation
The rate of biodegradation was determined using $C0_2$ evolution method as described by Cornfield (1961).

RESULTS
The test organisms were unable to utilize 100% unsterilized cassava effluent. The organisms became nonviable and the culture dried up within the thirty days incubation period. The same was recorded when the test organisms were inoculated into 100% sterilized cassava effluent.

Figure 1 shows the growth of *P. ostreatus* and *G. sepiarium* in 10% cassava effluent in MSM for a period of 30 days. The growth ranges from 0.5 to 2.4 cm for *P. ostreatus* and 0.5 to 2.5 cm for *G. sepiarium*. The mean growth for *P. ostreatus* was 1.42 cm while the mean growth for *G. sepiarium* was 1.73 cm.

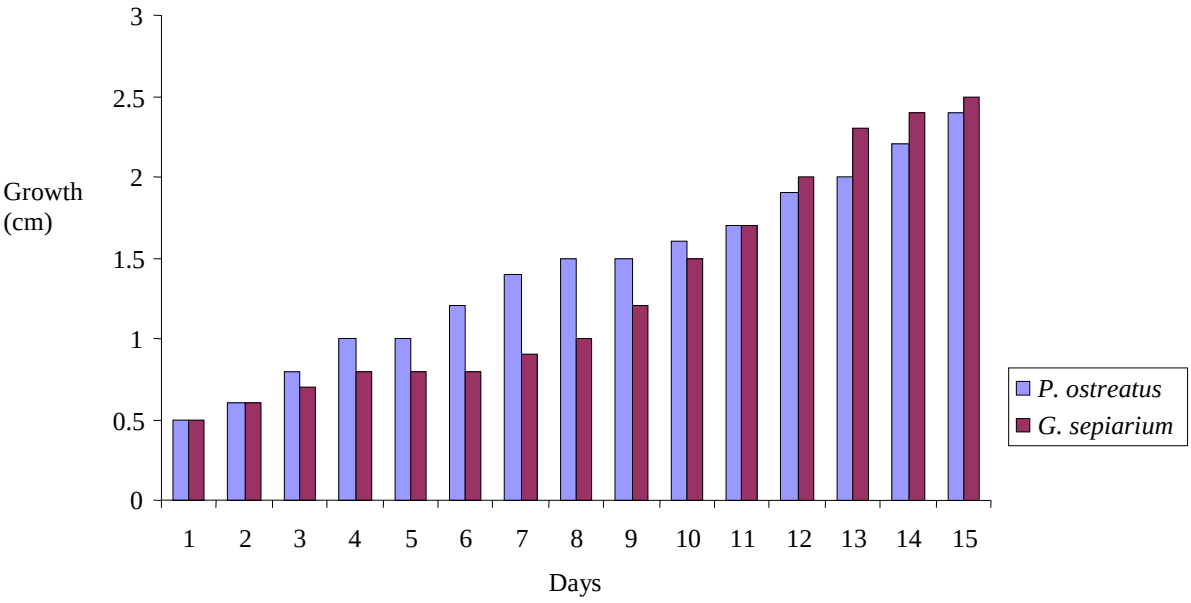

Fig. 1: Utilization of 10% cassava effluent in MSM by *P. ostreatus* and *G. sepiarium*

Figure 2 shows the growth of *P. ostreatus* and *G. sepiarium* in yeast incorporated in 10% cassava effluent in MSM for a period of 30 days. The growth ranges from 0.5 to 3.5 cm for *P. ostreatus* and 0.5 to 3.2 cm for *G. sepiarium*. The mean growth for *P. ostreatus* was 1.97 cm while the mean growth for *G. sepiarium* was 1.89 cm.

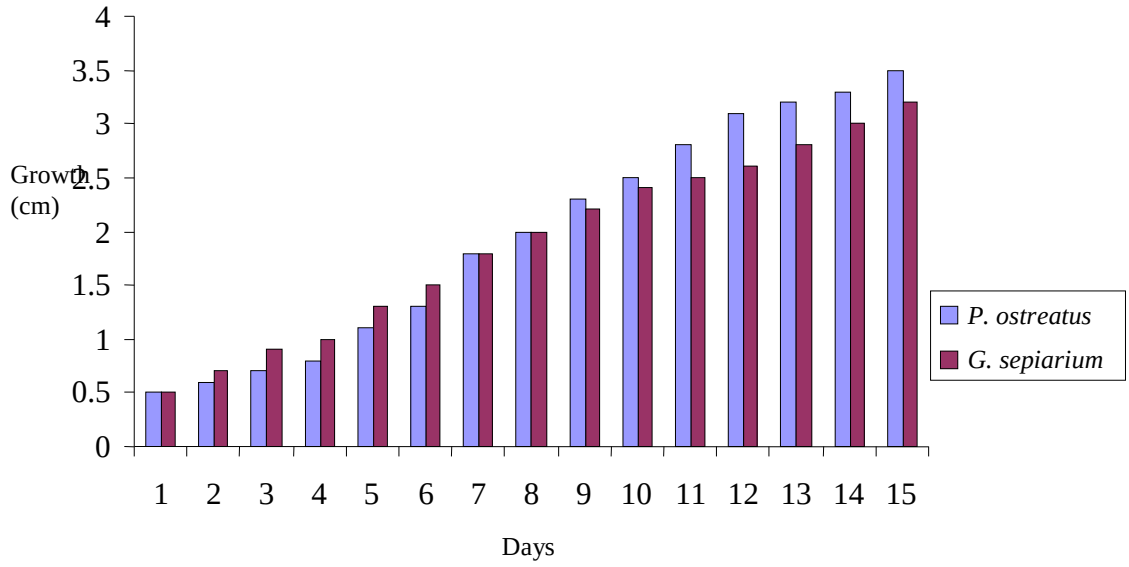

Fig. 2: Utilization of yeast incorporated into 10% cassava effluent in MSM by *P. ostreatus* and *G. sepiarium*

Figure 3 shows the growth of *P. ostreatus* and *G. sepiarium* in 10% cassava effluent in MSM for a period of 30 days at 4 ^{0}C incubation temperature. The growth ranges from 0.5 to 1.6 cm for *P. ostreatus* and 0.5 to 0.8 cm for *G. sepiarium*. The mean growth for *P. ostreatus* was 0.96 cm while the mean growth for *G. sepiarium* was 0.63 cm.

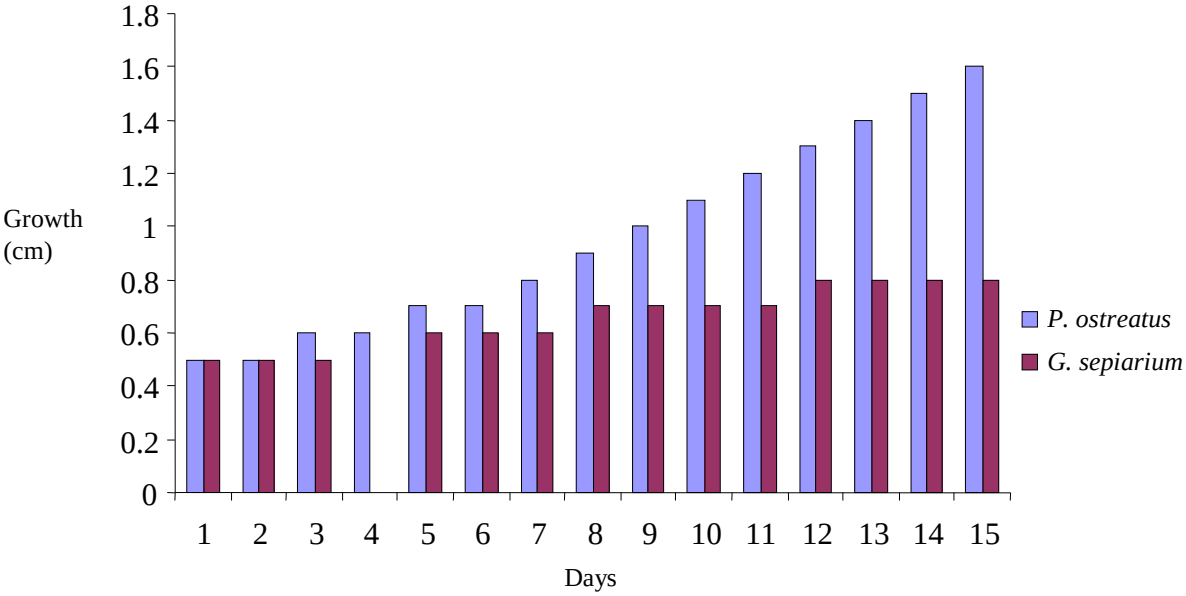

Fig. 3: Growth of *P. ostreatus* and *G. sepiarium* in 10% cassava effluent in MSM at 4 ^{0}C

Fig. 4 shows the growth of *P. ostreatus* and *G. sepiarium* in 10% cassava effluent in MSM for a period of 30 days at 28 ^{0}C incubation temperature. The growth ranges from 0.5 to 12 cm for *P. ostreatus* and 0.5 to 4.2 cm for *G. sepiarium*. The mean growth for *P. ostreatus* was 4.40 cm while the mean growth for *G. sepiarium* was 2.18 cm.

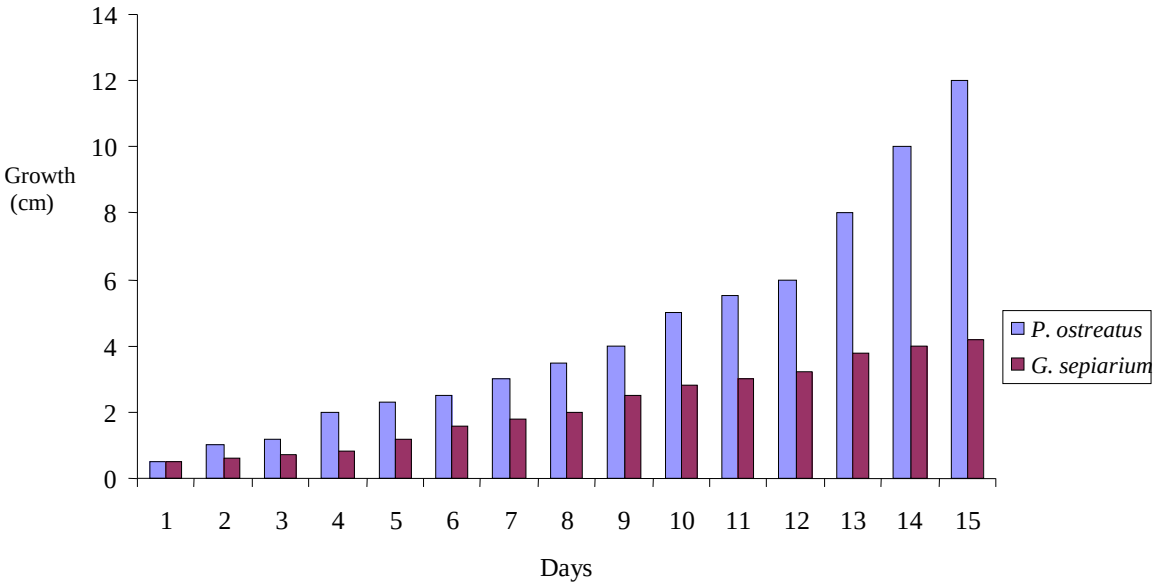

Fig. 4: Growth of *P. ostreatus* and *G. sepiarium* in 10% cassava effluent in MSM at 28 ^{0}C

Fig. 5 shows the growth of *P. ostreatus* and *G. sepiarium* in 10% cassava effluent in MSM for a period of 30 days at 37 ^{0}C incubation temperature. The growth ranges from 0.5 to 0.5 cm for *P. ostreatus* and 0.5 to 0.5 cm for *G. sepiarium.* The mean growth for *P. ostreatus* was 0.5 cm while the mean growth for *G. sepiarium* was 0.5 cm.

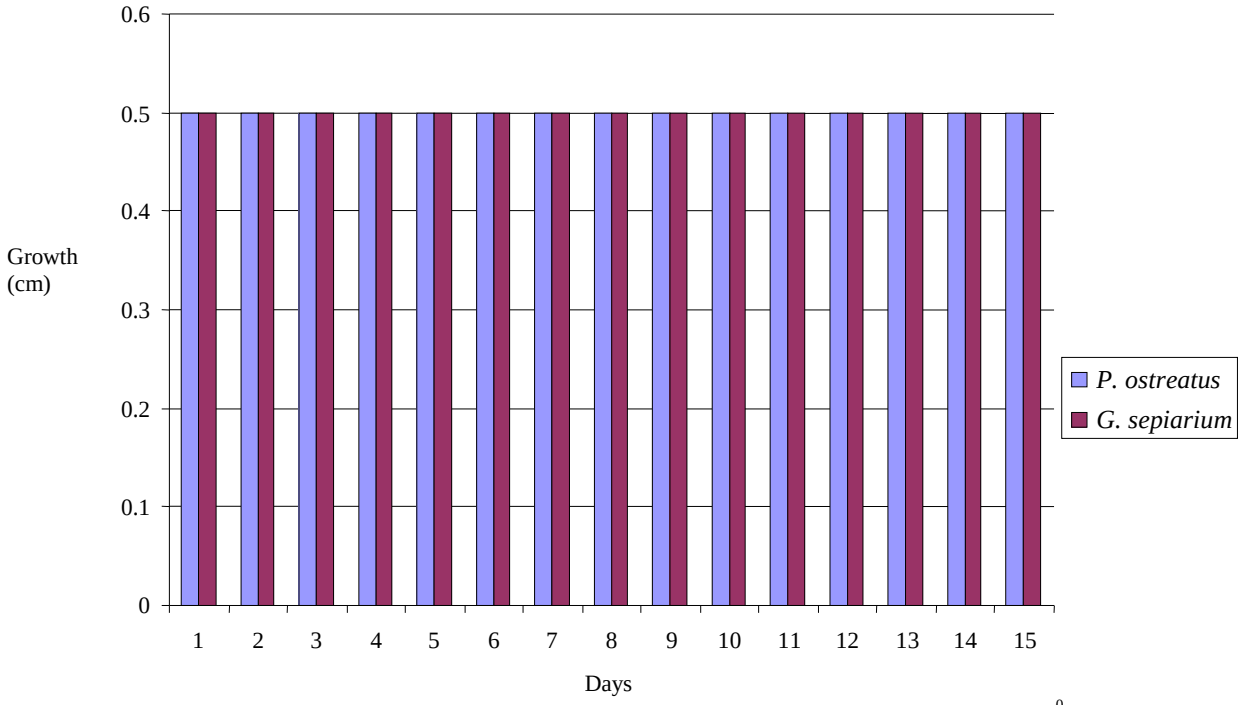

Fig. 5: Growth of *P. ostreatus* and *G. sepiarium* in cassava effluent in MSM at 37 ^{0}C

Figure 6 shows the growth of *P. ostreatus* and *G. sepiarium* in 10% cassava effluent in MSM for a period of 30 days at pH 6. The growth ranges from 0.5 to 6.3 cm for *P. ostreatus* and 0.5 to 2.6 cm for *G. sepiarium*. The mean growth for *P. ostreatus* was 2.77 cm while the mean growth for *G. sepiarium* was 1.74 cm.

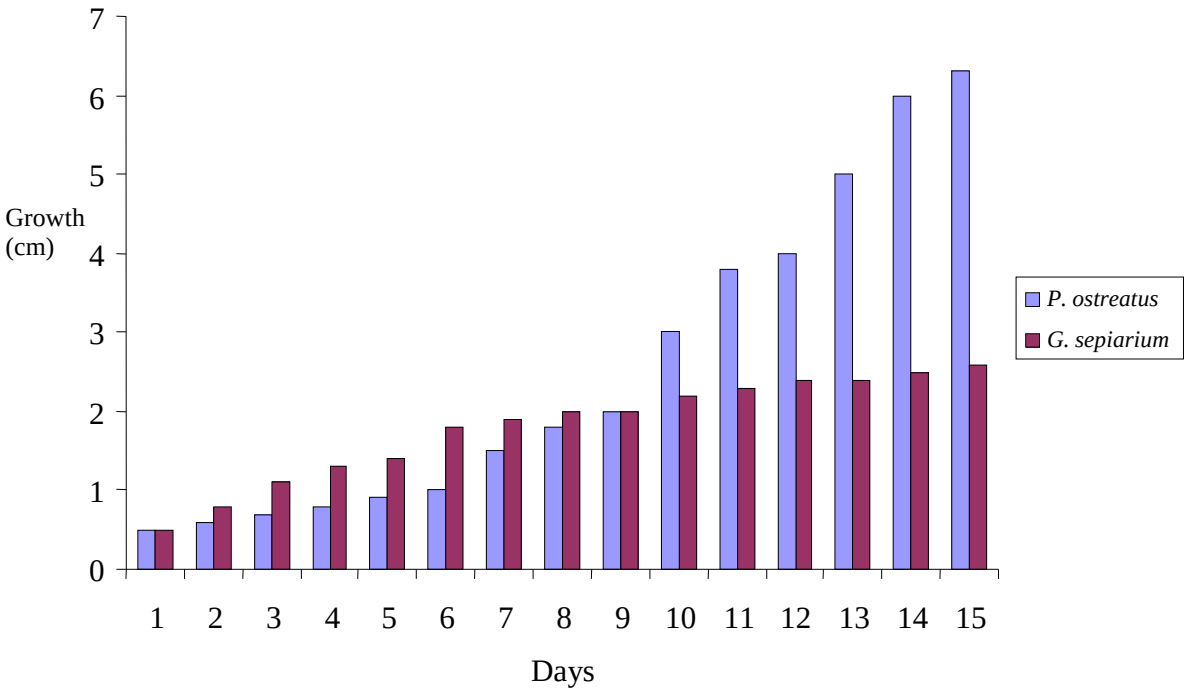

Fig. 6:Growth of *P. ostreatus* and *G. sepiarium* in 10 % cassava effluent in MSM at pH 6

Fig.7 shows the growth of *P. ostreatus* and *G. sepiarium* in 10% cassava effluent in MSM for a period of 30 days at pH 8. The growth ranges from 0.5 to 6.4 cm for *P. ostreatus* and 0.5 to 3.9 cm for *G. sepiarium*. The mean growth for *P. ostreatus* was 3.16 cm while the mean growth for *G. sepiarium* was 2.16 cm.

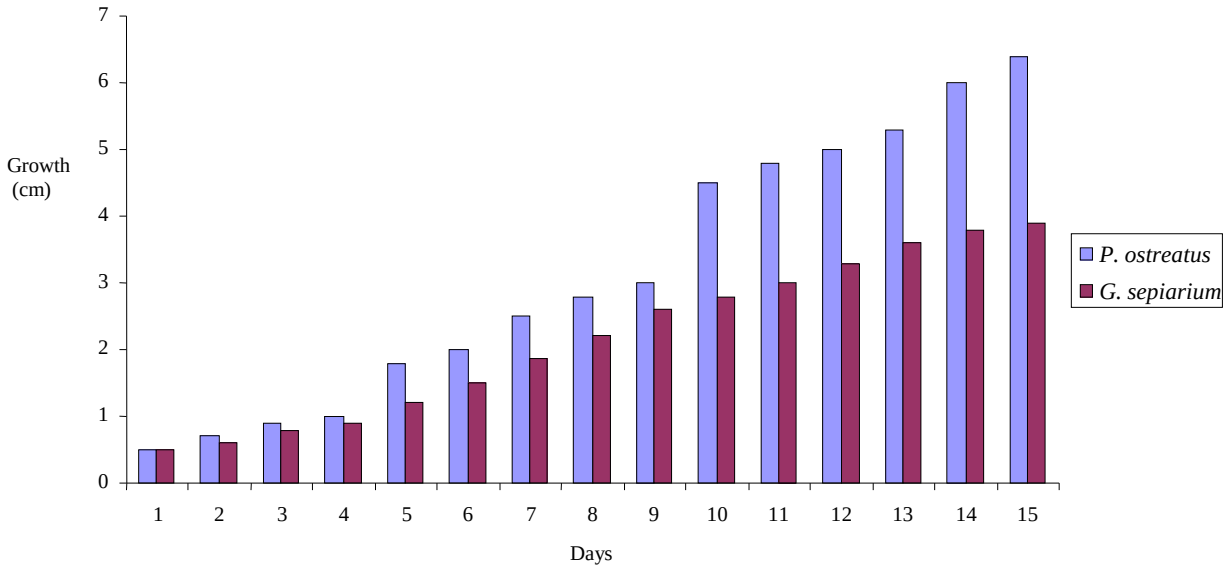

Fig. 7: Growth of *P. ostreatus* and *G. sepiarium* in 10% cassava effluent in MSM at pH 8

Fig. 8 shows the growth of *P. ostreatus* and *G. sepiarium* in 10% cassava effluent in MSM for a period of 30 days at pH 10. The growth ranges from 0.5 to 4.4 cm for *P. ostreatus* and 0.5 to 2.0 cm for *G. sepiarium*. The mean growth for *P. ostreatus* was 2.63 cm while the mean growth for *G. sepiarium* was 1.01 cm.

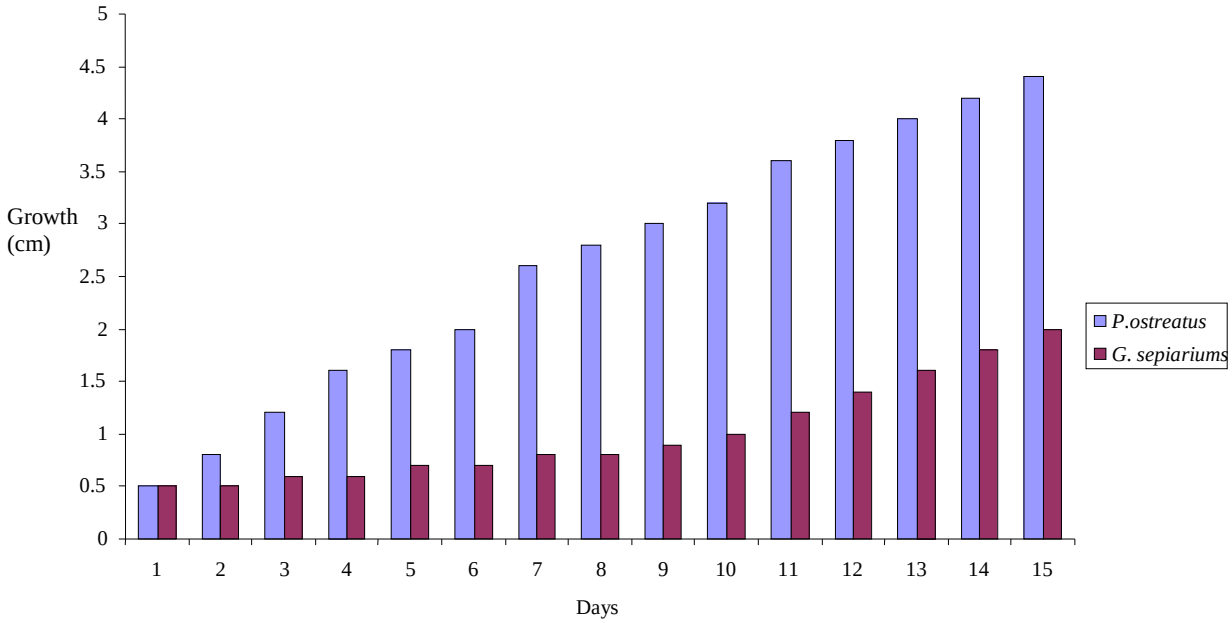

Fig. 8: Growth of *P. ostreatus* and *G. sepiarium* in 10% cassava effluent in MSM at pH 10

Figure 9 shows the rate of biodegradation of *P. ostreatus* and *G. sepiarium* using $C0_2$ evolution method. $C0_2$ evolved *for P. ostreatus* varied between 11 and 48.2 while that of *G. sepiarium* was between 2.2 and 13.2. The mean $C0_2$ evolution for *P. ostreatus* was 21.0 while the mean $C0_2$ evolution for *G. sepiarium* was 6.0.

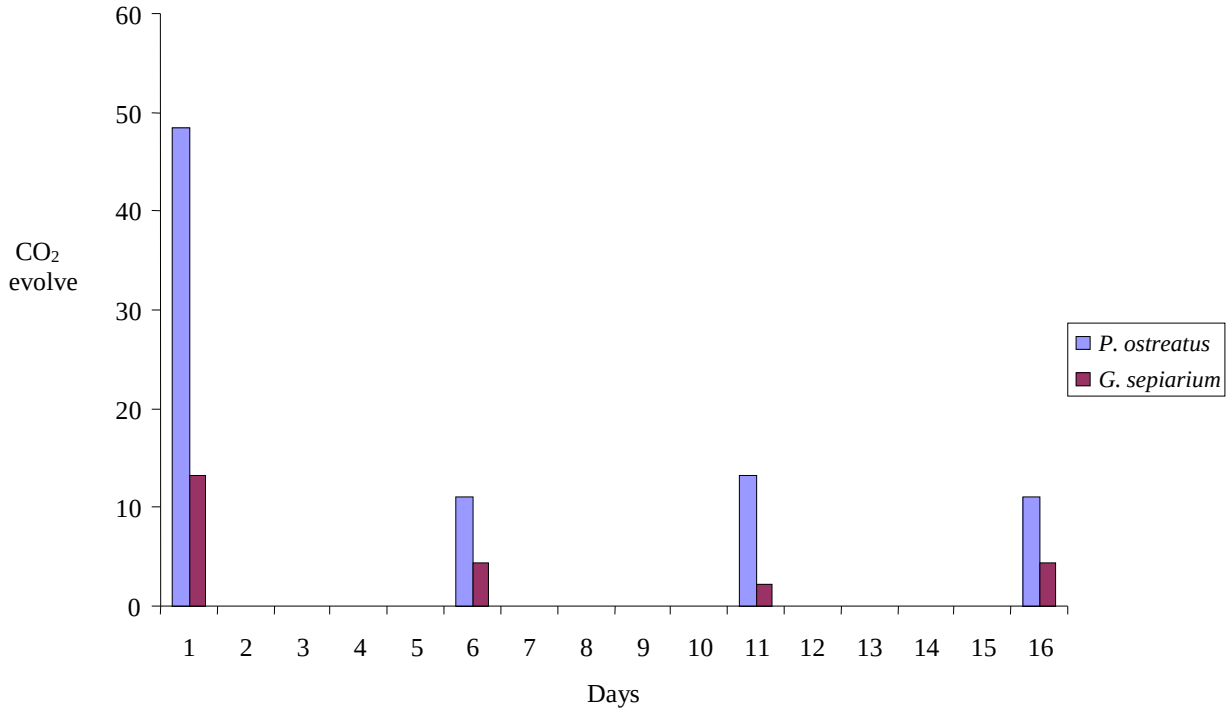

Fig. 9: Rate of biodegradation of *P. ostreatus* and *G. sepiarium* by $C0_2$ evolution method

DISCUSSION

Microorganisms are being explored for use to degrade effluent from industries. In this study, *P. ostreatus* and *G. sepiarium* were used to degrade cassava effluent. The ability of these organisms to degrade 100% effluent in both sterilized and unsterilized effluent proved abortive. This could be due to the high hydrocyanide constituents of cassava effluent. The test organisms were able to utilize 10% cassava effluent in minimal salt medium (MSM) with the effluent as a sole carbon source (Fig. 1). This shows that these organisms were able to hydrolyze the complex polysaccharides in the cassava effluent to simpler ones like $C0_2$, H_20, $N0_3$ and other inorganic compounds as a requirement for their growth and metabolisms possibly using their intracellular enzymes. When yeast was incorporated into the medium, the test organisms were able to better utilize the effluent for their growth and metabolism (Fig. 2).

The optimum temperature for the growth of these organisms was found to be 28 ^{0}C. This was evident by a higher mean growth of 4.40 cm and 2.18 cm for *P. ostreatus* and *G. sepiarium* respectively (Fig. 4) compared with a mean growth of 0.96 cm and 0.63 cm at 4 ^{0}C incubation temperature (Fig. 3) and no growth was observed at 37 ^{0}C (Fig. 5). This correspond to the findings of Nwokoye *et al*. (2009) reported that *P. ostreatus* grow optimally at 28^{0}C but in contrast with the reports of Zhi-Jiang *et al*. (2011) who stated that the optimum temperature for biodegradation of terephthalic acid by an isolated *Pseudomonas* sp. was 30^{0}C.

The optimum pH for the growth of these organisms was found to be pH 8. *P. ostreatus* had a mean growth of 3.16 cm at pH 8 (Fig. 7) and mean growth of 2.77 cm and 2.63 cm at pH 6 and 10 respectively (Fig. 6 and Fig. 8) while *G. sepiarium* had a mean growth of 2.16 cm at pH 8 (Fig. 7) and 1.74 cm and 1.01 cm at pH 6 and 10 respectively (Fig. 6 and Fig. 8). This was in contrast with the reports of Kılıç *et al.* 2011 who stated that the optimum pH for the biodegradation of phenol by *Synechocystis* sp. in media including triacontanol hormone was 6.5. Also Zhi-Jiang *et al*. (2011) stated that the optimal biodegradation of terephthalic acid by an isolated *Pseudomonas* sp was 7.0.

From the overall results, *P. ostreatus* had a higher potential and a higher rate of biodegradation (Fig. 9) in utilizing cassava effluent as a sole carbon and energy source than *G. sepiarium* at the various optimum conditions examined. Therefore, there should be improvement of ways for genetically improving the degrading ability of these two organisms and their ability to degrade other industrial effluents should be investigated.

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