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RESEARCH ARTICLE

OPTIMIZATION of MICROBIAL CONSORTIA in the DEGRADATION of BIODIESEL EFFLUENT from *JATROPHA CURCUS*

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ABSTRACT

The utilization of biodiesel produced from *Jatropha curcus* as renewable energy is relatively new area of research. The waste generated during biodiesel production may cause serious impact on the soil. The present study was aimed at optimizing microbial consortia in the degradation of biodiesel effluent (BDE). A portion of land (loamy soil) measuring 1.5 m by 1.5 m was polluted with BDE for 28 days. Standard microbiological and chemical methods were used to determine BDE utilizing organisms and physicochemical properties of the soil. The result of the total heterotrophic microbial counts from BDE polluted site at 0 to 28 days revealed significance $p<0.0001$, $p<0.001$ and $p<0.01$ for bacterial, fungal and yeast counts. Percentage occurrence of bacterial isolates from BDE polluted soil showed high values for *Bacillus subtilis* (28.95%), *Pseudomonas aeruginosa* (21.05%), *Staphylococcus epidermidis* (18.42%), *Staphylococcus aureus* (15.79%). *Aspergillus niger* (20.63%) and *Saccharomyces kluyveri* (14.29%) also recorded highest occurrence for fungi and yeast isolates respectively. Performance level for the growth rate of bacterial isolates in BDE showed highest against *Staphylococcus epidermidis* (OD 1.6 at day 4), *Bacillus subtilis* (OD 1.5 at day 6) and *Pseudomonas aeruginosa* (OD 1.4 at day 4). The result from this study revealed the effect of depth in the degradation capacity of consortia microorganisms in BDE polluted soil.

Keywords: *Jatropha curcus*, biodiesel effluent (BDE), microbial consortia, optimization, degradation

1. INTRODUCTION

The world is shifting to renewable source of energy due to green-house gas emission into the atmosphere**.** Biodiesel from *Jatropha curcas* (JC) has widely been explored by researchers as alternative source of bioenergy. Biodiesel from JC provides an alternative to petroleum energy balance, which burns with less harmful emission, low sulphur and carbon dioxide, biodegradable, nontoxic, and environmentally beneficial in terms of cost and efficiency [1]. Large amount of waste are generated during the process of

biodiesel production [2], which in turn pollute the receiving soil where biodiesel plants are situated in the form of effluent. These wastes may pose serious environmental impact to the ecosystem.

Wastewater from alkali-catalyzed biodiesel production process is alkaline in nature with a high content of oil and grease, and low content of nitrogen and phosporus [2]. Biodiesel effluent (BDE) are reported to contain high biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolve solute (TDS), glycerol and residual fat, oil and grease [3].

Studies on BDE treatment revealed several approaches which include electrochemical coagulation [4, 5], advanced oxidation process [3], dissolved air flotation [6], and integrated treatment process [7]. These methods used so far were characterized with one disadvantage or another. Electrochemical coagulation generates metal hydroxide in solution which further pollutes the wastewater; advanced oxidation method leads to formation of intermediate compounds that may be more harmful and difficult to degrade. The biological methods used involve the utilization of already established pure culture of microorganisms for the treatment of BDE [8, 9]. Information on the degradation potential of BDE using native microorganisms in the soil is relatively scarce. Given the enormous environmental impact BDE has on the environment, this study is aimed at optimizing microbial consortia in the soil for the degradation of BDE.

2. MATERIALS AND METHODS

2.1. Preparation of Contaminated Site.

A portion of land (Loamy Soil) measuring 1.5 m by 1.5 m was marked out for contamination with BDE. The site was polluted with 20 dm^3 of biodiesel effluent and allowed to stay for seven (7) days. Prior to pollution, soil samples from the marked portion of land was analysed to ascertain the indigenous soil microflorals (control). After contamination, soil samples were collected at depth 0-10 cm, 10-20 cm and 20-30 cm for 28 days in an interval of 7 days.

2.2. Characterization of Soil Samples

The characterization of soil such as moisture content, clay, sand, and silt content, organic matter, soil pH, exchangeable capacity, micro and macro nutrient were determined following the method according to Cresswell and Hamilton [10].

2.3. Enumeration and Isolation of Microbial Isolates (pre and post contamination).

Serial dilution was carried out according to the method of Ishak *et al*. [11]. A 1 cm^3 aliquot from 10^{-4} dilution factor was plated in triplicates for bacteria (Nutrient agar), fungal (Potato dextrose agar) and yeast (Yeats extract agar). 0.5 g chloramphenicol was introduced to potato dextrose and yeast extract agar to inhibit bacteria growth. Colony forming unit per gram (cfu/g) was calculated following the methods of Onifade and Abubakar [12].

2.4. Characterization of Microbial Isolates

Phenotypic characterization of all the microbial isolates was carried out to identify the soil microflorals [13, 14] . The percentage occurrence of the identified isolates was obtained according to the method by Okechi *et al*. [15].

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2.5 Microbial optimization for growth rate of isolates

Microbial growth rate determination was carried out following the methods described by Lapinskiene *et al*. [16] and Obayori *et al.* [17]. Replicate conical flasks containing 50 cm^3 of biodiesel effluent with 10 cm^3 of mineral salt medium (MSM) were prepared. The flasks were autoclaved for 15 minutes, and after cooling, 1cm^3 of inoculated broth of each microorganism was added to the different flasks and incubated at room temperature for a period of 14 days. Flasks containing the wastewater and MSM only (un-inoculated) served as controls. The increase in cell density was determined by measuring the optical density (OD) at 600 nm using a UV/Vis spectrophotometer (Model T70). The microbial isolates that showed appreciable growth were selected and used for the biodegradation of biodiesel wastewater.

Table 1. Physiochemical properties of polluted and unpolluted soil samples.

Parameters	**Before Pollution**			**After Pollution**		
	0-10cm	**10-20cm**	**20-30cm**	**0-10cm**	**10-20cm**	**20-30cm**
pH	5.96	5.83	6.33	6.12	7.98	7.92
EC (µs/cm)	138	175	117	152	206	144
BD (g/cm^3)	1.21	1.22	1.27	1.21	1.21	1.28
MC (%)	20.85	23.10	23.09	27.31	24.64	23.85
E.A (meq/100g)	0.20	0.24	0.21	0.18	0.22	0.20
E.B (meq/100g)	5.03	4.24	3.38	7.58	6.41	5.34
CEC (meq/100g)	5.58	5.64	4.58	5.16	5.93	5.81
OC (%)	0.92	1.14	0.96	0.91	1.11	0.97
OM (%)	1.59	1.97	1.66	1.57	1.92	1.68
Clay (%)	17.16	23.10	15.16	17.16	23.10	15.16
Silt (%)	4.27	4.36	3.24	4.27	4.36	3.24
Sand (%)	78.57	72.54	81.60	78.57	72.54	81.60

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Table 2. Macro-element in the polluted and unpolluted soil samples.

Soil Sample	**Depth (cm)**	**Total-nitrogen (mg/100g)**	**Total-phosphorus (mg/100g)**	**Total-potassium (meq/100g)**
Before Pollution	0-10	1.68	3.17	0.22
	10-20	1.12	2.56	0.18
	20-30	1.68	3.80	0.18
After Pollution	0-10	1.68	3.06	1.23
	10-20	1.14	2.36	0.11
	20-30	1.14	2.71	0.15

Table 3. Total heterotrophic microbial count from Biodiesel effluent polluted soil at different depths.

Sampling Period (Day)	**Depths of soil (cm)**	**Bacterial Counts (cfu/g) x 10^4**	**Fungal Count (cfu/g) x 10^4**	**Yeast Count (cfu/g) x 10^5**
Control (unpolluted Soil)	0 - 10	2.30±0.72	9.70±0.80	1.60±0.15
	10 - 20	2.40±0.54	8.80±0.82	1.70±0.10
	20 - 30	1.20±0.61	1.19±1.36	0.32±0.02
1 – 7	0 - 10	6.0±0.59	0.32±0.05	0.35±0.02
	10 - 20	6.9±0.40	0.26±0.03	1.18±0.18
	20 - 30	2.6±0.91	0.42±0.04	4.00±0.05
8 – 14	0 - 10	12.5±0.90	0.30±0.05	0.80±0.04
	10 - 20	8.30±0.21	0.40±0.05	0.20±0.15
	20 - 30	7.00±0.25	0.34±0.02	0.12±0.01

15 – 21	0 - 10	5.50±0.21	9.00±0.49	0.96±0.02
	10 - 20	4.00±1.57	8.50±0.50	0.90±0.12
	20 - 30	2.10±0.45	7.50±0.31	0.15±0.15
22 – 28	0 - 10	3.70±0.66	6.30±0.64	0.07±0.01
	10 - 20	4.10±2.21	4.50±0.76	0.06±0.02
	20 - 30	1.80±0.61	6.80±0.41	0.01±0.03
p-values		0.000	0.001	0.01

Values in triplicate, Mean±Standard Deviation

Table 4. Frequency of occurrence of bacterial isolates.

Sampling Period (Day)	***Pseudomonas aeruginosa***	***Staphylococcus aureus***	***Staphylococcus epidermidis***	***Bacillus Subtilis***	***Micrococcus letus***	***Proteus mirabilis***	**% Occurrence of total isolates**
Control (unpolluted Soil)	2	1	1	1	1	1	18.42
7	2	1	1	3	1	1	23.68
14	1	2	2	3	2	0	26.32
21	1	2	2	2	0	0	18.42
28	2	0	1	2	0	0	13.16
% occurrence of each isolates	21.05	15.79	18.42	28.95	10.53	5.26	100

Table 5. Percentage frequency of occurrence of fungal/yeast isolates.

Sampling Period (Day)	**Fungi**					**Yeast**			
	Aspergillus niger	***Mucor* spp.**	***Aepergillus tamarii***	***Pennicillium notatum***	***Aspergilllus flavus***	***Saccharomyces kluyveri***	***Saccharomyces exiguus***	***Saccharomyces unisporus***	**% Occurrence of total isolates**
Control (unpolluted Soil)	3	2	3	2	1	2	2	1	25.40
7	2	2	2	1	1	2	1	2	20.63
14	3	2	2	1	2	2	1	1	22.22
21	3	1	0	1	1	2	2	2	19.05
28	2	1	1	0	0	1	1	2	12.70
% occurrence of each isolates	20.63	12.70	12.70	7.94	7.94	14.29	11.10	12.70	100

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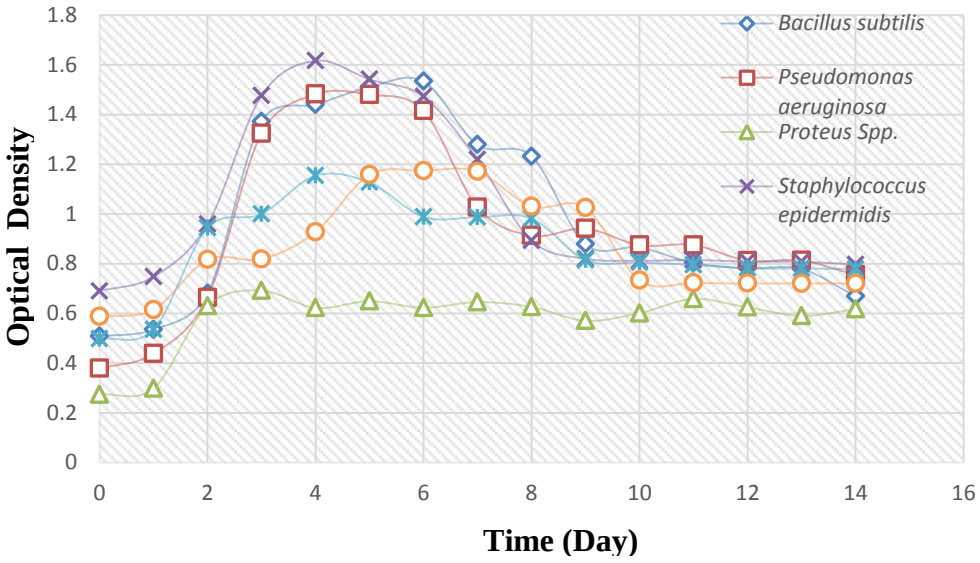

Figure 1. Growth curve of bacterial isolates in BDF wastewater.

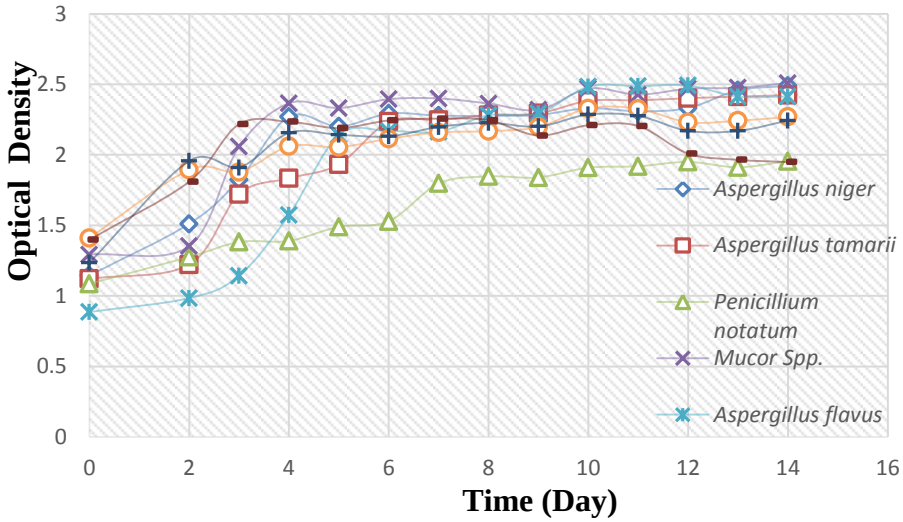

Figure 2. Growth curve of fungal isolates in BDF wastewater

3.RESULTS AND DISCUSSION

3.1. Physicochemical Properties of Polluted and Unpolluted Soil Samples

The physicochemical properties of the polluted and unpolluted soil samples were investigated (Table 1) to determine effect of physicochemical properties for the effective degradation of BDE. The pH, EC, BD, MC values increased as the depth of soil increased. The result of mid-pH values after pollution with BDE is in agreement with the reports of Siles *et al*. [18], who averred that acidification phase of BDE may be attributed to coagulation/flocculation process. There were no significant changes in the E.A, E.B and CEC values of soil with increased depth profile. There was low reduction recorded in OC (%), OM (%), Clay (%), Silt (%) values, with progressive increase in sand (%). The result of the physicochemical studies support the claim that depth of soil and physicochemical properties affect population and metabolic activity of soil microflora as revealed in this study. Progressive decrease in soil macro-elements, total N, total P and total K was recorded.

3.2. Microbial Counts of Biodiesel Effluent Polluted Soil at Different Depths

The total heterotrophic microbial count from biodiesel effluent polluted soil at different depths recorded varied counts for bacterial, fungal and yeast with the sampling period under review. Depth of soil 0 to 10 cm recorded high population counts for bacterial, fungal and yeast, followed by 10 to 20 cm depth, while 20 to 30 cm recorded low microbial counts. As the depth of the soil increased, the population counts of the soil microflora decreased. There was significant reduction in population count for the fungal and yeast after pollution with BDE. The result of the total heterotrophic microbial counts from BDE polluted site at 0 to 28 days recorded significance $p<0.0001$, $p<0.001$ and $p<0.01$ for bacterial, fungal and yeast counts respectively.

Several study have reported soil microbiome in degradation of BDE [19, 16] after first week of pollution. Frequency of occurrence of bacterial isolates recorded high occurrence against *Bacillus Subtilis* (28.95 %), and low occurrence against *Proteus mirabilis* (5.26 %). For occurrence of fungal isolate *Aspergillus niger* (20.63 %) recorded the highest and *Pennicillium notatum* (7.94 %) lowest; yeast isolates recorded highest against *Saccharomyces kluyveri* (14.29 %) and lowest against *Saccharomyces unisporus* (12.70%). The result of this study revealed high bacterial presence compared to fungal and yeast. The presence of the bacterial in high percentage may be attributed to the ubiquitous nature of bacteria population.

3.3. Biodegradation Potential of Microbial Consortia from Soil Polluted with BDE

The degradation of biodiesel result in the production of mineral diesel that are easily absorbed by soil microflora's [20, 21, 16, 22]. Growth curve of bacterial isolates in BDE wastewater (Figure 1) revealed highest population density against *Staphylococcus epidermidis* (OD 1.6 at day 4), *Bacillus Subtilis* (OD 1.5 at day 6) and *Pseudomonas aeruginosa* (OD 1.4 at day 4). Lowest population density was confirmed against *Proteus* sp. and *Micrococcus letus.* Notable exponential growth rate was recorded between day 2 and day 7, while progressive decrease occurred between days 8 to 16. This study showed that active biodegradation of BDE occurs between days 0 to day 7.

The growth rate curve of fungal isolates in BDE wastewater (Figure 2) recorded lag phased between day 0 and day 4 for all the fungal isolates under review. Constant progressive exponential growth rate was

recorded against *Aspergillus niger*, *Aspergillus tamari*, *Mucor* sp. *Aspergillus flavus* and *Saccharomyces exiguus* at OD 1 to 1.5. *Penicillum notatum* recorded lowest exponential growth at OD 1.5 (day 4 and day 14). Unlike the bacterial species, fungal specials requires longer period for successive biodegradation.

4. CONCLUSION

The utilization of biodiesel produced from *Jatropha curcus* as renewable energy may cause significant impact to the receiving soil owing to the waste generated during this process. This study showed the effect of depth on the degradation capacity of consortia microorganisms such as *Staphylococcus epidermidis*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. Bacterial isolates recorded shortest time of degradation compared to fungal isolates with up to 14 days degradation. In order to obtain successive degradation of BDE, optimization of consortia microorganisms is required to achieve better results.

5. STATISTICAL ANALYSIS

Descriptive statistics and one-way analysis of variance (ANOVA) was employed in this study using statistical package SPSS version 22.0.

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The authors declare that there was no conflict of interest in the course of this study.

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