

Kinetics of Bioremediation of Oil Contaminated Water Dispersed by Environment-Friendly Bacteria (*Pseudomonas aeruginosa*) and Fungi (*Aspergillus niger*)

Onoh Ikechukwu Maxwell^{1*}, Mbah Gordian Onyebuchukwu¹, Okeke Elochukwu Chinonso¹, Igwilo Christopher Nnaemeka², Eze Kenneth Afamefuna¹

¹Department of Chemical Engineering, Enugu State University of Science and Technology, ESUT, Enugu, Nigeria ²Department of Science Laboratory Technology, Federal College of Agriculture, P.M.B., Ishiagu, Nigeria Email: *maxwell.onoh@esut.edu.ng

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Abstract

The comparative effectiveness of remediating water polluted with crude oil, using environment-friendly bacteria (Pseudomonas aeruginosa) and fungi (Aspergillus niger) were investigated. The samples were separately treated with Aspergillus niger and Pseudomonas aeruginosa. The bioremediation kinetic efficiency for these systems was studied. At the end of the bioremediation periods, the oil and grease content of the samples decreased from 47.0 mg/L in the untreated sample to 7.0 mg/L after 20 days when inoculated with bacteria while the sample inoculated with fungi decreased to 10.0 mg/L. Post analysis when inoculated with bacteria showed a fall in the value of the biological oxygen demand (BOD) from 73.84 mg/L to 33.28 mg/L after 20 days, while, the fungi inoculated sample showed a reduction from 73.84 mg/L to 38.48 mg/L. The biodegradation process with the bacteria was consistent with the pseudo-first-order model with a rate constant of 0.0891 day⁻¹, while the biodegradation process with the fungi was consistent with the first order reaction model with a rate constant of 0.422 day⁻¹. The degree of degradation after the 20th day of inoculation with Pseudomonas aeruginosa was 85.11%, while with Aspergillus niger was 78.72%. Thus, the results obtained showed that, Pseudomonas aeruginosa performed better than Aspergillus niger. The bioremediation data with fungi fitted the first-order model, while that of the bacteria fitted the pseudo-first-order model. Therefore, the data obtained in this study could be applied in the design of a bioremediation system for potential application to remediation of crude oil polluted water.

Keywords

Bioremediation, *Aspergillus niger*, *Pseudomonas aeruginosa*, Crude Oil, Oil Contaminated Water, Kinetics

1. Introduction

Industrialization is very crucial in the economic advancement of any country. Industries however, have been observed to be the principal donors of ecosystem pollution globally [1]. Pollution is the introduction of contaminants or pollutants into the natural environment that results in undesirable modification making an ecosystem unwholesome and intolerable to humans and biomes [1] [2].

Global pollution is increasing due to the variations in natural and anthropogenic activities leading to contamination of various terrestrial and aquatic ecosystems with heavy metals, inorganic and organic compounds. Controlled and uncontrolled discharge of solid and liquid wastes, use of agricultural fertilizers, herbicides, insecticides and sewage disposal, explosives and accidental or intentional spillages, are some of the main contributors of alarmingly increased content of various contaminants in the biosphere. Industries such as textiles, electroplating, tannaries and refinaries are recognised as a serious environmental threat all over the world [3].

According to [2], the petroleum sector represents one of the most hidden risks to the environment because it affects ecosystems, which in turn affects all other living things, including humans. Based on the aforementioned issue, the principal prevalent and perilous concern of petroleum industry undertakings is environmental devastation. Crude oil is one of the important energy sources that have tremendous use in transportation, industries, domestic and other daily human activities [4]. However, the widespread distribution of these resources and its over exploitation have resulted in serious challenges, leading to harmful impacts on the environment and human health owing to their mutagenic, carcinogenic and toxic properties [5].

The environmental effects of this pollution are increasing greenhouse effect; acid rain; biodiversity harm; air, soil, and water deprived quality [6]. Environmental pollution by oil spill has occurred in different parts of the world in both aquatic and terrestrial environments. Most of these spills are caused by activities such as drilling, refining, and transportation of crude oil products [7]. Most of these spills are associated with negligence and sabotage, corrosion of pipes, and oil tanker accidents [8].

The damage caused by the toxicity of crude oil to organ systems may be immediate or it may take months or years. Environmental degradation occasioned by crude oil exploration and exploitation activities in Nigeria has become predominant and has gained international attention [9]. According to oil spill data obtained from the department of petroleum resources [10], a total of 6194 oil spills occurred between 1976 and 2001. This reported spills accounted for about three million barrels of crude oil dumped into the environment in this period. More than 70% of these spills were not recovered, 69% of these spills occurred at off-shore, while a quarter was in swamps, 6% were spilled on shore [9]. According to a report by [11], the quantity of crude oil spilled into the environment in the first eight months of 2020 in Niger Delta, stood at 3346.94 barrels, which is equivalent to 532,078 liters. According to the report, 19505.07 barrels of crude oil were spilled into the environment in this region in 2019 [11].

Different techniques are applied either *in situ* or *ex situ* for eliminating toxic substances of oil spill. Several techniques, such as mechanical, burying in secure landfills, evaporation, composting, dispersion, absorption, washing, solvent extraction, incineration, bioventing, biopile and the application of dispersants have been employed to remediate crude oil contaminated soil [12] [13]. However, these techniques are costly, inefficient, non-biodegradable, noxious to plants and animals, and can lead to incomplete decomposition of contaminants [12] [14]. Among the different techniques available, bioremediation has been chosen for this research. According to [15], marine micro-organisms consume spilled oil as a primary energy source in a method known as bioremediation.

Bioremediation is currently a very promising technology for the treatment of sites contaminated with petroleum hydrocarbons. This method is typically more cost-effective compared to other physicochemical options [16]. Bioremediation is the natural process of oil degradation by indigenous micro-organisms. There are hundreds of species (including fungi, algae, and bacteria) capable of degrading oil in water and/or soil [15]. Bioremediation is therefore, a natural process that relies on microbes to break down the pollutants in water sources [16] [17]. Bioremediation for oil spills is a technique that uses microbes to eliminate contamination of hydrocarbons from water and soil, thereby making them safe for aquatic and terrestrial species [3]. During bioremediation process, there is a total mineralization of organic pollutants (contaminants) into CO_2 , H_2O , inorganic compounds, and cell protein [18].

Kinetics of bioremediation process however, can be evaluated in two ways: 1) the first concerns with the factors influencing the amount of transformed compounds with time and 2) the other approach seeks the types of curves describing the transformation and determines which of them fits the degradation of the given compounds by the microbial culture [19] [20]. Studies of biodegradation kinetics in a natural environment are often empiric, reflecting only the basic level of knowledge about the microbial population and its activity in the given environment [19] [21]. In chemical and biochemical processes, kinetic models are necessary because, process performance is explained [22].

Numerous studies on the biodegradation process have been focused on various factors that directly or indirectly, influence the amount of hydrocarbon compounds transformed over time and provide the rate of degradation as well as curves that, describe the transformation (referred to as kinetics) of any given compounds by the various bacterial microbes [14] [16] but, studies using environment-friendly bacteria (*Pseudomonas aeruginosa*) and fungi (*Aspergillus niger*) are not well documented. Hence, it is necessary to close these knowledge gaps. This study therefore evaluated the kinetics of bioremediation of oil contaminated water dispersed by environment-friendly bacteria (*Pseudomonas aeruginosa*) and fungi (*Aspergillus niger*).

2. Materials and Methods

2.1. Sampling

The oil contaminated water sample was collected from Calabar River in Okodia, Bayelsa State, Nigeria (Latitude: 4°56'59.99"N, Longitude: 8°19'18.00"E) and stored in an air tight container at room temperature. Sample characteristics and nutrients were measured using standard methods for examination of water and wastewater [23]. The bacterial culture, isolations, and identification were done at the pharmaceutical microbiology laboratory of the Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. Identification of the bacterial isolates was accomplished by the observation of colonial characteristics (shape, size, color, surface appearance, and texture), Gram reaction, and biochemical tests. The characterization of the isolates was performed, by employing the gram staining reaction, catalase test, citrate test, sugar fermentation test, coagulase test, motility test, oxidase test, urease test, indole test, methyl red, and vogesproskauer test as described by Bergey's Manual of determinative bacteriology, 9th edition (1994).

2.2. Bioremediation Experimentation

The effectiveness of *Pseudomonas aeruginosa* and *Aspergillus niger* in improving the degradation of crude oil was carried out in a bioreactor.

2.2.1. Inoculation of the Test Micro-Organism into the Raw Sample

The raw sample was inoculated using an inoculating loop. The loop was sterilized by burning it in a Bunsen burner to kill any bacteria present. 2 mL of the standardized bacteria suspension was inoculated into 1.9 litres of the sample. The suspension was shaken vigorously for 10 minutes.

2.2.2. Preparation of Agar

28 g of hydrated nutrient agar powder was suspended in 100 mL of distilled water and kept to soak for 10 minutes. The suspension was homogenized by melting in a water bath at 100°C. 20 mL volume each was dispensed in bijou bottle. The bottles were made air tight. The nutrient agar was sterilized using an autoclave at 121°C for 15 min. The sterilized molten nutrient agar was kept at a temperature of 45°C until when used.

2.2.3. Determination of Cell Population

100 mL of distilled water was boiled and allowed to cool. 9 mL of it was with-

drawn and placed in four sterilized test tubes covered with cotton wool. The nutrient agar was poured into a petri dish and allowed to solidify. The dish was labelled with the name of the different microbes. Ten-fold serial dilution was done by taking 1 mL of the sample with a syringe and placing same into the first test tube containing 9 mL of the distilled water, making it a total of 10 mL. 1 mL was withdrawn from the first test tube and placed in the second test tube containing 9 mL of distilled water. 0.1 mL of the sample was dropped on each of the petri dish containing the nutrient agar. It was kept in the dark for 4 days.

$$Cell population (Cfu/mL) = \frac{Colony count on an agar plate}{Dilution factor \times Volume plated}$$
(1)

2.2.4. Total Acidity

50 mL of the sample was measured into each of the flasks and 2 drops of the indicator added.

This was then titrated with NaOH solution from the burette until the colour changed to light pink as the end point. The burette reading was recorded and the titration repeated twice.

Total Acidity(mg/L) =
$$\frac{\text{Titre value} \times \text{Normality of NaOH} \times 50 \times 1000}{\text{vol of sample used, mL}}$$
 (2)

2.2.5. Total Suspended Solids

The filter paper was weighed to obtain its weight, W_1 . 10 mL of the sample was filtered using the filter paper, which was then heated in an oven. The filter paper plus residue was dried to a constant weight, W_2 .

Total suspended solid was calculated using the formula

$$\Gamma SS = \frac{W_2 - W_1}{\text{vol of sample used, mL}} \times 1000$$
(3)

 W_1 = weight of filter paper

 W_2 = weight of filter paper + residue.

2.2.6. Total Dissolved Solids

The total dissolved solids present in the sample were determined by the Direct Reading Engineering Method (DREM) using the Hanna H19811-5 multi parametric meter. The probe was dipped into a beaker containing 10 mL of the sample and the TDS mode was activated. The value displayed was recorded.

2.2.7. Total Solids (TS)

The total solids were obtained by calculating the arithmetic sum of the total suspended solids and total dissolved solids.

$$TS = TSS + TDS$$

2.3. Kinetics of Bioremediation

The biodegradation kinetics was acquired using the decreasing concentration of the oil and grease content (OGC). The hydrocarbon compounds were biodegraded into carbon dioxide, water and energy (Hojae *et al.*, 2005). The basic reaction is

$$C_nH_n + O_2 \rightarrow CO_2 + H_2O + Energy$$

In order to determine the order of reaction of degradation of hydrocarbons, first order reaction and pseudo-first-order kinetic models were employed to fit the bioremediation data. First order reaction equation is given by [24] as:

$$\ln \frac{C_t}{C_o} = K_1 t \tag{4}$$

 C_o = initial concentration of OGC (mg/L)

 C_t = Remaining concentration of OGC (mg/L) at any time

 K_1 = First order reaction constant

t = time (day)

Pseudo-first-order reaction is given as (Wethasinghe et al., 2006):

$$\ln(\text{OGC}) = -K_i t + \ln(\text{OGC})_a \tag{5}$$

OGC = concentration of oil and grease content at time $(OGC)_o$ = initial concentration of oil and grease content t = time (day)

 K_i = order of reaction

3. Results and Discussion

3.1. Physico-Chemical Properties of Oil Contaminated Water, Fungi Treated Water and Bacteria Treated Water Samples

Table 1 shows the physic-chemical characteristics of the as-received sample of oil contaminated water. **Table 2** presents the physico-chemical characteristics of the water after bioremediation with bacteria (*Pseudomonas aeruginosa*), while **Table 3** presents the physico-chemical characteristics of the water after treatment with fungi (*Aspergillus niger*).

Table 2 and Table 3 show the variation of dissolved oxygen (DO) contents of the oil contaminated water with time. It was evident that there was a steady and progressive decrease in the DO of the samples during the process of bioremediation. This is indicative that there was degradation of the crude oil in the contaminated water which made it possible for more air to permeate into the water. A decrease in the DO corresponds to the decrease in BOD of the contaminated water. The BOD directly affects the amount of dissolved oxygen in water bodies. The higher the BOD, the more easily oxygen is depleted in the water body. This results in less oxygen being available to aquatic life.

The tables also show the variation of pH of the polluted water with time in the course of bioremediation. The pH of bacteria inoculated sample increased from 5.8 to 7.4 after 20 days of inoculation, which was within the range of a normal pH for safe water, while that of the fungi inoculated sample increased from 5.8 to 5.9. The general results indicated that the pH of all samples tested increased with increase in bioremediation time. The steady rise in the pH within the pe-

riod of investigation is an indication that there was conversion of the hydrocarbons into less toxic and less acidic products. The pH of all samples was well within acceptable limits of 6 - 9 as stipulated by World Health Organisation standard for potable water.

S/N	Physico-Chemical Parameter	Analytical Values
1	pH	5.8
2	Total Dissolved Solid (TDS) mg/L	500
3	Conductivity (µs/cm)	800
4	Temperature (°C)	30.5
5	Acidity (mg/L)	170
6	Alkalinity (mg/L)	25
7	Biological Oxygen Demand (BOD) mg/L	73.84
8	Oil & Grease (mg/L)	47.00
9	Total Suspended Solid (TSS) mg/L	120
10	Chemical Oxygen Demand (COD) mg/L	160
11	Turbidity (mg/L)	12
12	Dissolved Oxygen (DO) mg/L	14.76
13	Total Solid (TSS) mg/L	620

 Table 1. Physico-chemical characterisation of the untreated sample of oil contaminated water.

Table 2. Bacteria (Pseudomonas aeruginosa) treated oil contaminated water sample.

Time (davs)	4	8	12	16	20
pH	7.1	7.3	7.0	7.2	7.4
TDS, mg/L	450	320	250	189	95
Conductivity, mg/L	650	580	500	460	215
Temperature, °C	25.5	27.7	26.4	28.7	28.3
Alkalinity, mg/L	10	6	3	2	2
Acidity, mg/L	130	100	80	70	50
Oil & Grease, mg/L	38.0	30.0	25.0	15.0	7.0
TSS, mg/L	105	90	70	65	40
COD, mg/L	120	100	90	60	49
DO, mg/L	9.15	8.11	7.49	7.07	6.66
BOD, mg/L	45.76	40.56	37.44	35.36	33.28
Turbidity, mg/L	10.5	9	7	6.5	4
TS, mg/L	555	410	320	214	135

Time (days)	4	8	12	16	20
pН	6.8	7.1	6.3	6.5	5.9
TDS, mg/L	490	400	323	290	150
Conductivity, mg/L	712	690	601	510	308
Temperature, °C	25.6	27.5	26.3	28.5	28.4
Alkalinity, mg/L	10	8	6	5	3
Acidity, mg/L	120	110	100	80	60
Oil & Grease, mg/L	42.0	38.0	30.0	20.0	10.0
TSS, mg/L	110	100	85	72	60
COD, mg/L	152	120	112	90	73
DO, mg/L	9.57	8.94	8.74	8.32	7.70
BOD, mg/L	47.84	44.72	43.68	41.60	38.48
Turbidity, mg/L	11	10	8.5	7.2	6
TS, mg/L	600	500	408	362	210

Table 3. Fungi (Aspergilus niger) treated oil contaminated water sample.

Alkalinity is a measure of the capacity of water to neutralise acids. The alkalinity of the sample decreased from 25 mg/L in the untreated sample to 2.0 mg/L after treatment with bacteria and to 3.0 mg/L after treatment with fungi. The acidity of the sample was 170 mg/L before inoculation of the micro-organisms. After inoculation, the acidity fell from 170 mg/L to 50 mg/L when inoculated with bacteria for duration of 20 days, while the sample inoculated with fungi reduced the acidity from 170 mg/L to 60 mg/L.

Total suspended solids (TDS) is a measure of the combined content of all inorganic and organic substances contained in a liquid, in molecular, ionic or micro-granular suspended form. The TDS showed a decrease from 500 mg/L when there was no micro-organism to 95 mg/L after 20 days of inoculation with bacteria, while the TDS also reduced from 500 mg/L to 150 mg/L in the fungi inoculated sample for 20 days.

Figures 1-5 depict the variation of oil and grease, BOD, total solids, total acidity and cell population with time.

From **Figure 1**, it was evident that *Pseudomonas aeruginosa* had a greater impact on bioremediation of the oil contaminated water than the *Aspergillus niger*. The results showed that a minimum of 20 days was required for the micro-organisms to biodegrade the hydrocarbons in the contaminated water to an acceptable level transforming them to less toxic substances such as CO_2 and H_2O . The oil and grease content of the sample decreased from 47.0 mg/L in the untreated sample to 7.0 mg/L after 20 days when inoculated with bacteria while, the sample inoculated with fungi decreased to 10.0 mg/L.

The biological oxygen demand (BOD) of the sample was observed to decrease in the course of bioremediation as shown in **Figure 2**. The pre-treatment analysis of the oil contaminated water showed that the BOD was 73.84 mg/L with high turbidity. Post analysis when inoculated with bacteria showed a fall in the value of the BOD from 73.84 mg/L to 33.28 mg/L after 20 days, while, the fungi inoculated sample showed a reduction from 73.84 mg/L to 38.48 mg/L. From the results it is evident that bioremediation occurred, reducing the BOD value of both samples. The reduction was more profound for the sample inoculated with *Pseudomonas aeruginosa*. The reduction in BOD could be attributed to the activities of the microbes in the contaminated water which converted the oil into less toxic substances as CO_2 and H_2O . The growth of the inoculated micro-organisms (bacteria and fungi) in the contaminated water was determined by calculating the microbial population every 4 days after inoculation for 20 days. **Figure 5** describes the growth of cell pollution in the oil contaminated water sample.



Figure 1. Variation of oil and grease with time (days).







Figure 3. Variation of total solid with time (days).







Figure 5. Variation of cell population with time (days).

3.2. Kinetics of Bioremediation

The kinetic degradation data obtained from this study was fitted to the first-order kinetics model (Equation (4)) in order to evaluate the rate constant of crude oil degradation in the various remediation treatments process shown in **Table 4** and from the plot of $\ln(C_t/C_o)$ against remediation time, t (**Figure 6**). [25] stated that increase in the biodegradation rate constants will result in faster rate of degradation.

Table 4 shows the data acquired for the first-order-reaction. The plot of $\ln(C_t/C_o)$ versus t gave a linear relationship, shown in **Figure 6**, from which k_1 was determined. From the regression coefficient, R² (0.8043), the first order kinetics did not properly fit this model.

3.2.1. Pseudo-First-Order Reaction Kinetics of Bioremediation Using Bacteria

Table 5 depicts the data for the pseudo-first-order reaction using Equation (5). The plot of $\ln(OGC)$ versus t was plotted as shown in **Figure 7**. It gave a linear relationship from which the slope and intercept of the graph was obtained as shown in the appendix. The regression coefficient, R² as calculated (R² = 0.9148) very well fits the bioremediation process.

Time (days)	$C_t (\mathrm{mg/L})$	C_t/C_o (mg/L)	ln C _t /C _o
4	38	0.8085	-0.21
8	30	0.6383	-0.45
12	25	0.5319	-0.63
16	15	0.3191	-1.14
20	7	0.1489	-1.90

 Table 4. First order reaction kinetics of oil & grease content for the bioremediation process using bacteria.





Time (day)	OGC (mg/L)	Ln(OGC)
0	47	3.85
4	38	3.64
8	30	3.40
12	25	3.22
16	15	2.71
20	7	1.20

Table 5. Pseudo-first-order reaction kinetics of oil & grease for the bioremediation process using bacteria.



Figure 7. First order graph showing ln(OGC) against time for the bioremediation process of bacteria.

3.2.2. Degree of Degradation

The degree of degradation of the oil and grease content during the process of bioremediation was calculated and the data represented in **Table 6** and **Figure 8**.

3.2.3. First Order Reaction Kinetics of Bioremediation Using Fungi

Using the equation of the first order reaction kinetics (Equation (4)), a graph of $\ln(C_l/C_o)$ against t was plotted, it gave a linear graph with regression coefficients, R^2 as ($R^2 = 0.9018$) and a rate constant, K of 0.422 day⁻¹. Table 7 and Figure 9 show the results for the first-order-reaction of the bioremediation process with fungi.

3.2.4. Pseudo-First-Order Reaction Kinetics of Bioremediation Using Fungi

Table 8 depicts the data for the pseudo-first-order of reaction. The plot of $\ln(OGC)$ versus t was plotted using Equation (5) as shown in **Figure 10**. It gave a linear relationship from which the slope and intercept of the graph were obtained. The regression coefficient, R^2 as calculated is ($R^2 = 0.8714$).

3.2.5. Degree of Degradation of Remediation Using Fungi

The degree of degradation of the oil and grease content during the process of bioremediation was calculated and the data represented in Table 9 and Figure 11.

Time (day)	OGC (mg/L)	%D
4	38	19.15
8	30	36.17
12	25	46.81
16	15	68.09
20	7	85.11

Table 6. Degree of degradation (%D) of the oil & grease content for bacteria.



Figure 8. Plot of % degradation of OGC against time for the bioremediation process of bacteria.

 Table 7. First order reaction kinetics of oil & grease content for the bioremediation process using fungi.

Time (day)	$C_t (\mathrm{mg/L})$	$C_t / C_o (\mathrm{mg/L})$	$\ln C_t / C_o$
4	42	0.8936	-0.11
8	38	0.8085	-0.21
12	30	0.6383	-0.45
16	20	0.4255	-0.85
20	10	0.2128	-1.55



Figure 9. First order graph showing $\ln(C/C_o)$ against time for the bioremediation process of fungi.

Time (day)	OGC (mg/L)	ln(OGC)
0	47	3.85
4	42	3.74
8	38	3.64
12	30	3.40
16	20	3.00
20	10	2.30

 Table 8. Pseudo-first-order reaction kinetics of oil and grease for the bioremediation process using fungi.



Figure 10. First order graph showing ln(OGC) against time for the bioremediation process of fungi.

Table 9. Degree of degradation (%D) of the oil & grease content for fungi.

Time (day)	OGC (mg/L)	%D
4	42	10.64
8	38	19.15
12	30	36.17
16	20	57.45
20	10	78.72





Regression analysis was used to determine the conformity of the bioremediation data to kinetic models. This is a confirmation that the bioremediation process was effective and efficient in the clean-up of the contaminated water.

4. Conclusion

Bioremediation has proven to be an effective tool in the reduction of environmental contaminants, and restoring the affected environment back to its original condition. Pseudomonas aeruginosa performed better than Aspergillus niger in this bioremediation study. The oil and grease content of the sample decreased from 47.0 mg/L in the untreated sample to 7.0 mg/L after 20 days when inoculated with bacteria, while the sample inoculated with fungi decreased to 10.0 mg/L. Post analysis when inoculated with bacteria showed a fall in the value of the biological oxygen demand (BOD) from 73.84 mg/L to 33.28 mg/L after 20 days, while, the fungi inoculated sample showed a reduction from 73.84 mg/L to 38.48 mg/L. The biodegradation process with the bacteria was consistent with the pseudo-first-order model with a rate constant of 0.0891 day⁻¹, while the biodegradation process with the fungi was consistent with the first order reaction model with a rate constant of 0.422 day⁻¹. The degree of degradation after the 20th day of inoculation with Pseudomonas aeruginosa was 85.11%, while with Aspergillus niger was 78.72% as can be seen in Table 6 and Table 9 respectively. The bioremediation data with fungi fitted the first-order model, while that of the bacteria fitted the pseudo-first-order model.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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