Comparison of biostimulation and bioaugmentation for remediation of soil contaminated with spent motor oil

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ABSTRACT: Aerobic fixed bed bioreactors were used to study and compare biostimulation and bioaugmentation for remediation of soil contaminated with spent motor oil. Bioaugmentation using consortium of bacteria and biostimulation using inorganic fertilizer and potassium dihydrogen orthophosphate were investigated. The bioremediation indicators used were the oil and grease content removals, total heterotrophic bacteria counts and carbon dioxide respiration rates. Results showed that biodegradations were very effective with 50, 66 and 75 % oil and grease content removal efficiencies for control, bioaugmentation and biostimulation respectively after ten weeks. Carbon dioxide respiration followed similar pattern as the oil and grease content removals. Biostimulation option has the highest carbon dioxide generation (6 249 mg/kg) and the control with the least (4 276 mg/kg). Therefore, the biostimulation option can be used to develop a realistic treatment technology for soils contaminated with spent motor oil.

Keywords: *Bacterial count; Bioremediation; Carbon dioxide respiration; Closed system; Fixed bed bioreactor; Oil and grease content*

INTRODUCTION

The quality of life on earth is linked, inextricably, to the overall quality of the environment. Releases of persistent, bioaccumulative and toxic chemicals have a detrimental impact on human health and the environment. These contaminants find their way into the tissues of plants, animals and human beings by the movement of hazardous constituents in the environment. Contaminated lands generally result from past industrial activities when awareness of the health and environmental effects connected with the production, use, and disposal of hazardous substances were less recognized than today (Vidali, 2001). The problem is worldwide, but more severe in the developing countries where there were no effective regulatory policies on the environment. It is now widely recognized that contaminated land is a potential threat to human health, and its continual discovery over recent years has lead to international efforts to remedy many of these sites, either as a response to the risk of adverse health or environmental effects caused by contamination or to enable the site to be redeveloped for use. Petroleum contaminants are typical examples of these hazardous constituents. Soil contaminated with petroleum products can be defined as any earthen material or artificial fill that has human or natural alteration of its physical, chemical, biological or radiological integrity resulting from the introduction of crude oil, any fraction or derivative thereof (such as gasoline, diesel, or motor oil), or oil-based product. (Dhanasekaran *et al.*, 2009; Chukwuma *et al.*, 2010; Refaat, 2010) Because of these alterations and potential threat to public health and the environment, some forms of remediation become imperative. Physicochemical technologies have been used for ages for the treatment or disposal of soils contaminated with petroleum products (Adams *et al.*, 2009; Yousefi Kebria *et al.*, 2009). These technologies include: thermal treatment, dig and dump method, chemical method,

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separation techniques and stabilization/solidification technology. However, the physicochemical technologies have their limitations (Less and Senior, 1995; Vidali, 2001). These limitations include: they are expensive to implement at full scale, they are not environmentally friendly, their technologies are complex and they lead to destruction of soil texture and characteristics. (Zhang *et al.*, 2009) Furthermore, the physicochemical technologies do not always result in complete neutralization of pollutants (Yerushalmi *et al.,* 2003). Due to limitations of the physicochemical technologies stated above, great deals of literature have reported that bioremediation technologies are alternatives and or supplements to these technologies. This is because of their cost effectiveness, environmental friendliness, simplicity in technology and conservation of soil texture and characteristics (Vidali, 2001; Yerushalmi *et al.,* 2003; Adams and Guzman-Osorio, 2008; Fouépé *et al.*, 2009). Oil spills from the refineries, industries, filling stations, loading and pumping stations, petroleum products depots, during transportation and at auto-mechanics workshops all contribute to soil contamination, and actually make up a larger percentage of polluted ground in the world versus those contaminated by catastrophic spills. The dig and dump method of disposing these oil polluted lands is expensive and only transfer the contamination from one place to another. This disposal technique is very prominent in the developing countries, where there were no effective regulatory policies on the environment. In no doubt, the dig and dump practice has led to the contamination of thousands of other sites remote from their place of initial contamination and therefore, urgent actions need to be taken for environmental safety in general and of public health importance. In most developing countries, oil spills at auto-mechanic workshops have been left uncared for over the years and its continuous accumulation is of serious environmental concern because of the hazard associated with it. For instance, spent motor oil disposed off improperly contains potentially toxic substances; such as benzene (carcinogens), lead, arsenic, zinc and cadmium, which can seep into the water table and contaminate ground water (Igwe *et al.,* 2008; Shah *et al.*, 2009). It consequently results in serious health hazard such as anemia and tremors, which can cause death. Bioremediation is the naturally occurring process by which microorganisms transform environmental contaminants into harmless end-products. It is a promising, innovative and cost effective technology for

use in the cleanup of hazardous wastes. For bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products (usually carbon dioxide and water).

Bioremediation can only be effective, if the environmental conditions permit microbial growth and activity; its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate (Vidali, 2001; Nwuche and Ugoji, 2008; Nwuche and Ugoji, 2010). The extent of biodegradation is highly dependent on the toxicity and initial concentrations of the contaminants, their biodegradability, the properties of the contaminated soil, and the particular treatment system selected (Chambers, *et al.*, 1991; Less and Senior, 1995).

There are two approaches to bioremediation, the biostimulation and the bioaugmentation; biostimulation involves identifying and adjusting certain physical and chemical factors (such as soil temperature, pH, moisture content, nutrient content e.t.c) that may be impeding the rate of biodegradation of the contaminants by the indigenous microorganisms in the affected site. Once the factors have been adjusted appropriately, the contaminant biodegradation may proceed at appreciable and satisfactory rates (Chambers, *et al.*, 1991; Kosteck and Calabrese, 1991). On the other hand, the bioaugmentation involves the addition of highly concentrated and specialized populations of specific microbes into a contaminated site to enhance the rate of contaminant biodegradation in the affected soil or water because the density of contaminant-specific degraders will have been artificially increased (Chambers, *et al*., 1991; Kosteck and Calabrese, 1991; Malakootian *et al.*, 2009). Most work reported in the literature on the biological treatment of soil contaminated with used motor oil had been focused on the identification of microorganisms, which can be used to degrade used motor oil (Amund *et al*., 1987) and the use of plants for the degradation of used motor oil (Anoliefo and Edegbai, 2000). Besides, other works prior to this investigation were based on biological treatments of soil contaminated with spent motor oil in open microcosms (Bagherzadeh-Namazi *et al*., 2008; Abdulsalam and Omale, 2009). The objectives of this study were to examine the effectiveness of bioremediation of spent motor oil contaminated soil in a closed system and to compare the hydrocarbon removal efficiencies of spent motor oil contaminated soil between

the bioaugmentation and biostimulation approaches in order to develop a viable treatment technology. To our knowledge, this is the first experimental study, which investigated biodegradation of soil contaminated with spent motor oil in a closed aerobic fixed bed bioreactor. This study was conducted between September, 2008 and December, 2008 in Abukakar Tafawa Balewa University Bauchi-Nigeria.

MATERIALS AND METHODS
Materials
Sample collection
Top soil (0-16.4±0.3 cm) contaminated with spent motor oil with contamination history of about 40 years was collected from a typical auto-mechanics workshop (Baban Kaduna Auto-Mechanics Workshop) located at Tudun Wada in Zaria, Kaduna State-Nigeria and was transported at 4 °C to Bauchi (about five hours journey by road) where the experiments was conducted. The bacteria (*Pseudomonas aeruginosa* (Kiyohara *et al.*, 1992; Nnamchi *et al.*, 2006) and *Bacillus subtilis* (Abdulsalam

and Omale, 2009) and the Fertilizer (NPK 20:10:10) used were obtained from the National Veterinary Research Institute Vom, Plateau State-Nigeria and Bauchi Fertilizer Company Limited, Bauchi-Nigeria respectively. In addition, potassium dihydrogen orthophosphate (K_2HPO_4) used was of analytical grade.

Methods
Process description
Each biodegradation investigation was carried out in an aerobic fixed bed bioreactor labelled TR1 (contro), TR3 (bioaugmentation), and TR6 (biostimulation). An amount of 1.5 kg of contaminated soil sample was inoculated into each of these bioreactors; this included, where appropriate, the various additives at room temperature of 2 133 CFU/g CS of consortium of *Pseudomonas aeruginosa* and *Bacillus subtilis* or 30.42 g of NPK 20:10:10 and 5.6 g of KH_2PO_4 to give a carbon : nitrogen : phosphorus molar ratio of 100:10:1 or heating at 121 °C. The bioreactors were completely closed in order to avoid CO_2 leakage to the
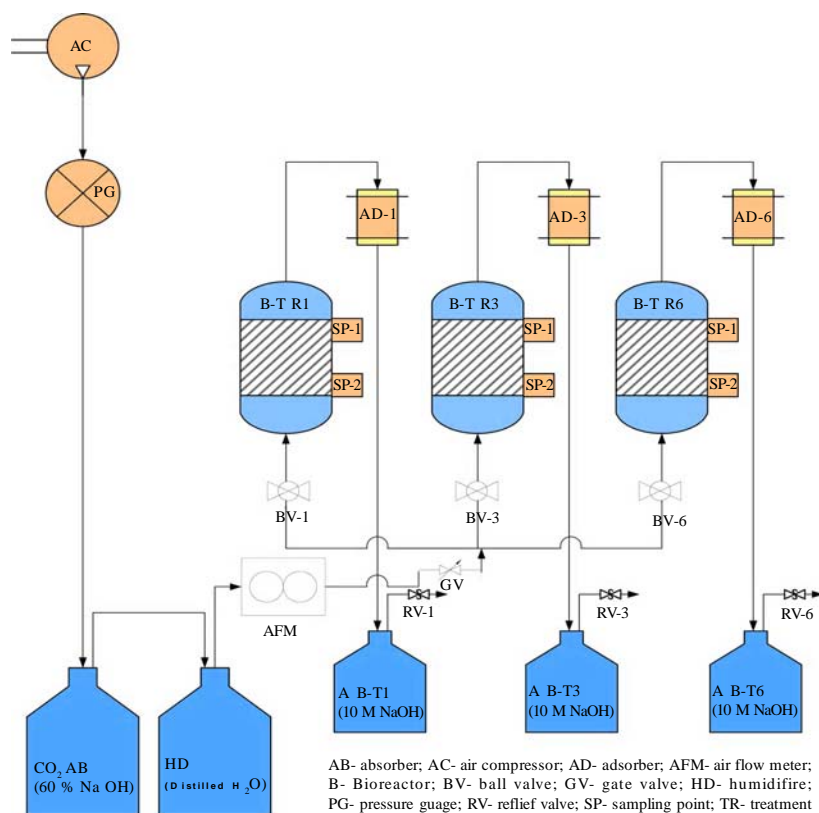

Fig. 1: Experimental Rig for the Rehabilitation of SCSMO in Aerobic Fixed Bed Bioreactors

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environment before passing into CO_2 traps. The process flow diagram is presented in Fig. 1. The absorber (AB) before the bioreactors contained a solution of 60 % (w/v) NaOH used to absorb CO_2 from the atmosphere and the humidifying unit (HD) to moisten the air before entering the bioreactors. The absorbers, after the bioreactors, contained 10 M solution of NaOH each meant to absorb the CO_2 generated from the bioremediation processes. The moisture content in all the treatments was set at 20 % (w/w) at the initiation of bioremediation. The airflow rate was maintained in all cases at an average rate of 10 L/hr using a flow meter for fourteen hours daily over the period of investigation. The progress of the biodegradation process was assessed by the measurement of the oil and grease content (O&G), the total heterotrophic bacteria counts (THBC) and physicochemical parameters in the microcosms on a weekly basis. In addition, the CO_2 respiration rates for each treatment were measured on 48 hourly basis.

Physicochemical and microbiological characteristics of contaminated soil

Physicochemical and microbiological properties of each treatment were determined by the following standard methods.(ASTM, 1982a and 1982b).

- Particle size distribution was determined based on the Unified Soil Classification (ASTM-D 422-63).
- Bulk and particle densities were determined using the gravimetric analysis and soil porosities calculated from bulk and particle densities values (Brady and Weil, 1999)
- Soil pH was determined using the method of Bates (1954) and moisture contents by the ASTM-D2216
- Available phosphorus in soil samples was determined using the spectrophotometer (Ascobic acid molybdate method) and total nitrogen content by the Kjedahl method
- The temperature was measured using a digital thermometer.
- Indigenous bacteria identification was carried out by morphological and biochemical characterization of petroleum hydrocarbon utilizers following the methods of Buchanan and Gibbons (1974).
- Total heterotrophic bacteria counts (THBC) were carried out by employing the standard plate counting technique using nutrient agar.
- The CO_2 respiration rates were determined using the titrimetric analysis

Determination of O and G Content (Spectrophotometry Method)

Moist samples were collected aseptically from each bioreactor and air-dried for 48 h. 5 g of each air-dried sample were extracted by vigorous hand shaking for 3 min with 20 mL of toluene in a separation funnel. The mixtures were allowed to settle and the extracts were decanted into a volumetric flask and plug. The above procedure was repeated two times using 20 mL of toluene each time (Vu-Duc *et al.*, 2002). The total extracts were combined and diluted in the ratio 1:3 (extract: toluene), and then the absorbance of each sample was quantified using a CE 1020 (1000 Series) UV Spectrophotometer at 400 nm. Oil and Grease contents were extrapolated from a standard curve of absorbance (A400 nm) against concentration. Values of concentration obtained were multiplied by the dilution factor (DF) to give the actual concentration.

Organic Carbon in Soil Samples (Colorimetric Method)

An amount of 0.5 g of contaminated soil sample sieved through 2 mm sieve was weighed into a glass beaker, 10 mL of 1N $K_2Cr_2O_7$ was added to the glass. As well, 10 mL of concentrated H_2SO_4 was carefully added. The solution was shaken gently and allowed to cool at room temperature. The solution was washed into 100 mL volumetric flask and made to the mark with distilled water. In addition, a blank solution was prepared by adding 10 mL of 1N $K_2Cr_2O_7$ and 10 mL of concentrated H_2SO_4. 0, 2, 4, 6, 8 and 10 mL of 2 000 ppm sucrose solutions were pipette into different flasks, 10 mL of 1N $K_2Cr_2O_7$ and 10 mL of concentrated H_2SO_4 were added to each flask. Both the blank and standard solutions were washed into 100 mL flask and made to mark with distilled water. The standards now contain 40, 80, 120, 160, 200 ppm. The blank, standards and samples were read on a spectrophotometer at 600 nm. Samples concentrations were calculated using the following relation.

$$Organic\ carbon\ (ppm) = \left(\frac{average\ value\ of\ s\tan dard}{average\ value\ of\ absorbance} \right) \times (absorbance\ of\ sample) \times DF \qquad (1)$$

RESULTS AND DISCUSSION
Physicochemical and microbiological characteristics of contaminated soil

The physicochemical and microbiological characteristics of the contaminated soil were

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Table 1: Physicochemical and microbiological characteristics of SCSMO

Parameter	Characteristic/Value
Soil texture	Loamy Sand
Particle density (g/cm^3)	2.04±0.01
Bulk density (g/cm^3)	1.49±0.01
Soil porosity (%)	26.89±0.85
pH	7.43±0.01
O&G (g/kg)*10^2	1.41± 0.00
WC (%)	2.09±0.11
WAC (%)	34.13±0.02
OC (g/kg)*10^1	6.42±0.00
N (mg/kg)*10^3	1.40±0.00
P (mg/kg)	17.96±3.69
THBC (CFU/g) *10^8	2.60±0.52

Data presented are averages of triplicate determinations
SCSMO: soil contaminated with spent motor oil

determined and presented in Table 1. The high level of carbon content was due to the presence of spent motor oil in the soil, which is related to the oil and grease content. The value of the oil and grease content exceeds the safe limit of 500 mg/kg set by the Nigeria Ministry of the Environment and therefore, needs intervention for public safety and environmental health. The soil texture and pH value obtained were adequate for effective bioremediation (Vidali, 2001). In addition, results obtained for the microbiological analyses revealed that the THBC was above the minimum value of 10^5 required for effective bioremediation (Forsyth *et al.*, 1995). Hence, the numbers of indigenous bacteria in the test soil were adequate for effective bioremediation.

Environmental factors for treatments

Table 2 presents temperature, pH and moisture content ranges for the three treatments over the 70 days of investigation. Results showed that the temperature and pH were within the 10-40 °C (Irvine and Frost, 2003) and 5.5-8.5 (Chambers, *et al.*, 1991 and Less and Senior, 1995) required for effective bioremediation except for slight variation in pH over certain sampling episodes. Therefore, temperature and pH were not limiting factors in this study.

On the other hand, the moisture contents for the three treatments deviated significantly from the optimum range of 10-20 % (w/w), (Less and Senior, 1995); the low moisture contents observed can be attributed to the presence of oil in the soil, which resulted in the blockage of the pores, hence low water retention.

Oil and grease content (O and G) removal efficiencies

The removal efficiencies of O&G for the three treatments is presented in Table 2. From this table it can be seen that the biostimulation option (TR6) had the highest removal efficiency (75 %) over the period of study. The bioaugmentation (TR3) showed 66 % removal of the O and G content and the control (TR1) had the least of 50 %. These removal efficiencies fell within the range of 30-75 % reported by Chaineau *et al* (2002) for biodegradation of lubricating oil. The significant O and G removal observed for TR1 could be attributed to inadequate penetration of heat during the sterilization process as attested by the initial concentration of bacteria for TR1(Fig. 2) and the continuous supply of oxygen and water via the humidifying unit.

Total heterotrophic bacteria count

Results of total heterotrophic bacterial counts showed that the bacterial profiles obtained (Fig. 2) followed a typical growth pattern of microorganisms. It was also observed that TRI (control) had the least microbial population while TR3 and TR6 are competing in microbial populations. These observations correspond with the trend acquired for the O&G removal efficiencies obtained. Consequently, it is evident that the microbes utilize the carbon contained in spent motor oil for growth. In addition, bacteria species identified in the test soil were *Bacillus subtilis* and *Micrococcus leteus*. The identification of these species was in line with previous investigation on spent motor oil contaminated soil (Amund *et al.*, 1987) and in accordance with classification of Adams and Jackson (1996).

Table 2: Environmental Parameters and Oil and Grease Content (O and G) Removal Efficiencies for the Three Treatment Options

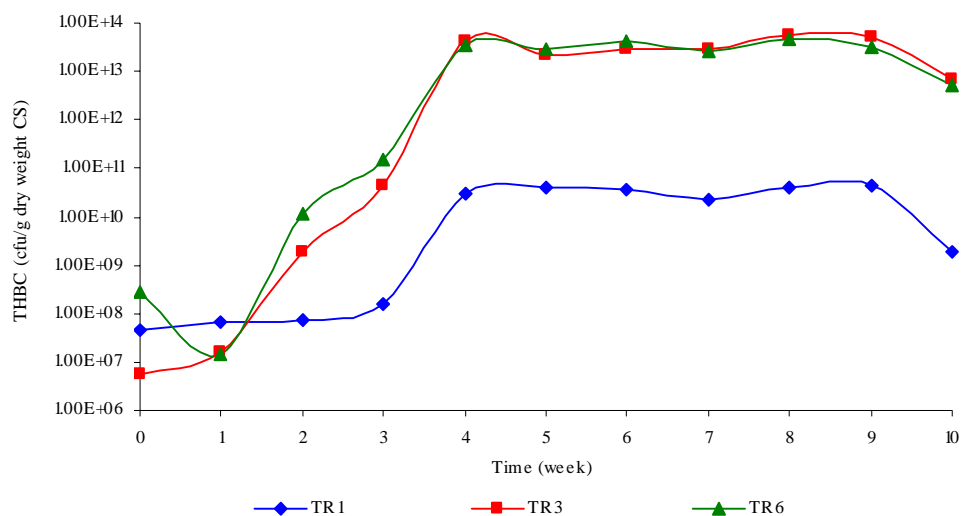| Treatment and Composition	Environmental Parameters			O and G (g/kg) efficiency		
	temp. (°C)	MC (%)	pH	day zero	after 70 days	(%)
TR1: SCSMO + HS + H$_2$0	27.00±0.5 - 30.60±0.4	0±0.0 - 22± 0.1	6.03±0.1 - 7.70±0.05	29.01±0.30	14.44±0.22	50
TR3: SCSMO + HS + H$_2$0 + EB	27.00±0.3 - 30.50±0.4	1±0.1 - 20±0.0	5.25±0.1 - 7.71±0.1	35.52±0.35	12.08±0.20	66
TR6: SCSMO + HS + H$_2$0 +NPK (20:20:20) + KH$_2$PO$_4$	26.60±0.2-31.00±0.0	5.2±0.1 - 20±0.2	5.50±0.05 - 8.85±0.03	38.59±0.24	9.83±0.15	75

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Fig. 2: Total heterotrophic bacteria counts in the three microcosms over the 70 days of investigation
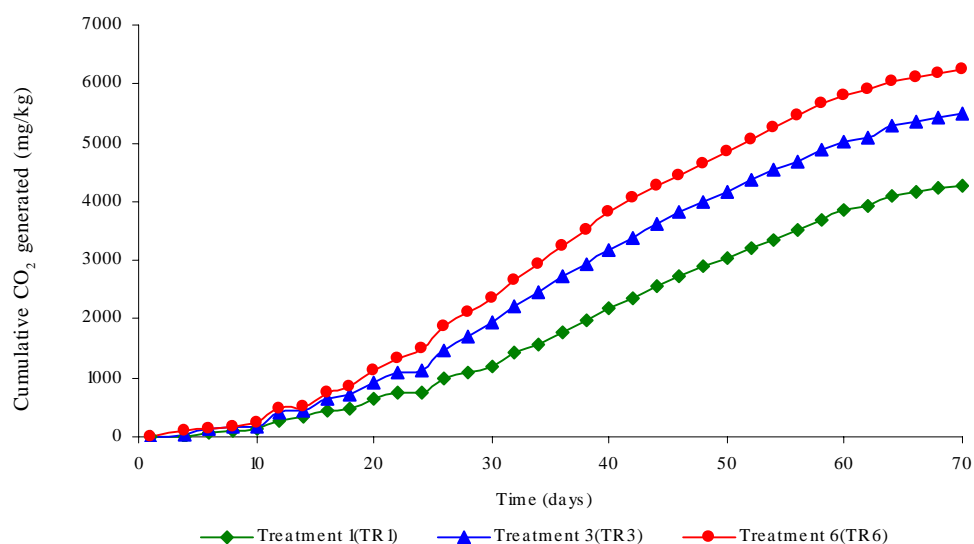

Fig. 3: Rate of Cumulative CO_2 Generation over a Period of 70 days

CO_2 generated from the bioremediation processes

The 48 hourly CO_2 generations allowed estimation of the cumulative CO_2 generations. The cumulative amount of CO_2 generated for each of the three treatments is presented in Fig. 3. Result showed that just as in the case of the O&G content, the biostimulation option (TR6) gave the best result (6 249 mg/kg), followed by the bioaugmentation option

(TR3) with 5 493 mg/kg and the control with the least of 4 276 mg/kg. The profiles obtained for the three treatments have similar features, which suggest that indigenous and added bacteria could be of the same genus (*Bacillus subtilis*).

In addition, these profiles can be divided into three periods as follows; (i) adaptation period, which is the period microorganisms used to adjust to their new

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environment or period where they synthesized the necessary enzymes for growth. In this period, there is little or no increased in the number of cells (this period lasted for the first 10 days of treatment); (ii) maximum oil degradation period (between 20-60 days) and (iii) decaying period (past 60 days). In the decaying period, the number of living microorganisms for each treatment reduced and rate of degradation slowed down. This could be likely linked to exhaustion in available nutrient in each treatment or the production of toxic metabolites.

CONCLUSIONS

From the results obtained in this research work, the following conclusions can be drawn: The petroleum removal efficiency for the spent motor can reach 75 % within 70 days of bioremediation in a closed system within the range of experimental conditions used in this study, based on the O and G removal efficiency and CO_2 generation, the biostimulation approach (TR6) gave the best result in this study and TR6 can be used to develop a safe and economical full-scale treatment technology for soils contaminated with spent motor oil.

ACKNOLEDGEMENT

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