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*Full Length Research Paper*

# Organic amendment optimization for treatment of hydrocarbon contaminated soil using the chemical-biological stabilization process

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**Sugar cane cachasse was tested as an organic soil amendment at 0, 2, 4 and 9% (dry weight), for the remediation of hydrocarbon contaminated soil (with an average initial concentration of 14,356 mg/Kg), which had been pre-treated by the incorporation of 4% (dry weight) calcium hydroxide according to the chemical-biological stabilization treatment method. Remediation efficiency was measured in terms of overall hydrocarbon reduction, hydrocarbon stabilization, soil leachates, microbial activity, acute toxicity and biomass production in a tropical forage grass (*Brachiaria humidicola*). Compared to the control, the over all half life for hydrocarbon degradation was optimal with 2 - 4% cachasse, reducing the half life from 301 days to about 195 days. The treatment with 9% cachasse presented reduced respiration rates, probably due to fermentation conditions, and a longer half life. Hydrocarbon availability (versus stabilization), and thus potential toxicity and leachability, was lowest in the treatments with 4 and 9% cachasse. In these treatments, there were no methanol extractable hydrocarbons after 19 months, although the TPH concentration was 1,000 - 1,500 mg/kg. In less than four months, toxicity, as determined by the Microtox method, was reduced to regional background levels (Effective Concentration 50 > 100,000 mg/L), and soil leachates (TCLP) were reduced to < 1 mg/L in all treatments. Grass biomass production was related to the amendment concentration, being two to three times greater in the treatment with 9% cachasse during the major part of the treatment. According to these results, a 4% application rate is recommended to optimize the microbial response, with an additional 4% added after one year to further stimulate pasture growth.**

**Key words:** Soil remediation, petroleum, biodegradation, toxicity, biomass production, pasture.

## INTRODUCTION

In Mexico there are many sites which are contaminated with both organic and inorganic compounds, principally due to the activities of the mining and petrochemical industries, as well as the secret disposal of hazardous waste, and spills (Volke and Velasco, 2002).

The environmental impacts that accompany the inadequate management of these compounds include contamination of soil and aquifers due to vertical migration, degradation of the aesthetic value of the landscape, and horizontal migration due to the overspill of waste pits during heavy rains. These problems have led to social conflicts and complaints of possible impacts to agricultural land, as well as demands on the environmental authorities to address these problems. This has resulted in the recognition of the importance of developing useful technologies for the treatment of contaminated sites to achieve permissible criteria, and that these criteria be appropriate so that the biota is not affected (Domínguez, 2008).

Remediation technologies represent an alternative to land disposal of hazardous wastes, whose capacities and possibilities of success can vary greatly from site to site.

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**Abbreviations: TCLP,** Toxicity characteristic leaching; **TPH,** total petroleum hydrocarbons; **TOG,** total oil and grease.

Among all of the companies authorized to remediate soil in Mexico, more than one-half use biological methods, with wind rows and land farms being the most common types. Soil washing, chemical oxidation and physical separation constitute another important part of the remediation technologies employed (Volke and Velasco, 2002).

Biological treatment methods offer advantages to the physical and chemical treatment methods since the contaminants are generally destroyed, and the final products are usually not toxic (especially if the mineralization is complete). Also, biological methods are generally less expensive and do not require specialized equipment (Coyne, 2000).

However, the application of remediation technologies invented in developed countries may not be appropriate to developing countries, especially in tropical regions which have very different climatic, socio-economic, and cultural conditions. This is especially important with respect to the remediation of hydrocarbon contaminated sites since oil production in tropical and semitropical regions accounts for roughly one-fourth of total global production (Energy International Administration, 2008). Fortunately, tropical regions have a distinct advantage when it comes to biological methods, with high temperatures and humidity that favor biological reactions that can be used for bioremediation and phytoremediation.

During the last two decades, a new treatment method for the remediation of hydrocarbon contaminated soil has been developed, the chemical-biological stabilization method (Adams, 2004a, b; Guzmán et al*.,* 2004; Adams et al*.,* 2007; Adams and Guzmán, 2008). This recently patented technology (Adams, 2007, 2008) was developed in the southern Gulf of Mexico region using locally available materials, machinery and know-how for application in humid tropical and semitropical environments. The overall focus of this technology is not hydrocarbon concentration reduction, but rather restoration of soil fertility, toxicity elimination and soil leachate reduction. It consists of partially stabilizing the soil with calcium hydroxide to limit hydrocarbon migration and improve soil structure, followed by the application of an organic amendment to further improve soil conditions and also stimulate microbial activity. Both mineralization and humification are stimulated using this method, with humification responsible for about one-third of the hydrocarbon reduction (Adams et al., 2007). Previously, several tests were done to optimize the chemical reagent dosage (Adams, 2004b), and to determine what kind of organic amendments are preferable. In the present study, the optimization of the organic amendment concentration was investigated.

## MATERIALS AND METHODS

### Soil and cell preparation

A mixture of generally clayey waste soil derived from samples taken in Tabasco State and the southern part of Veracruz state (Mexico) was used for these tests. This material was leftover soil from sample analyses from the Bioremediation Laboratory of the Universidad Juárez Autónoma de Tabasco (Juarez Autonomous University of Tabasco). It had an initial total hydrocarbon concentration of 14,300 mg/Kg (dry weight), principally weathered hydrocarbons. 40 kg of this material was placed in treatment cells with dimensions of 40 × 40 and 40 cm deep.

### Chemical-biological stabilization

4% of $Ca(OH)_2$ (dry weight basis) was added to the soil in the treatment cells and mixed thoroughly (chemical treatment phase). Three days later, sugar cane filtrate waste (cachasse) was added to the treatment cells at 2, 4 and 9% on a dry weight basis and mixed into the soil (biological treatment phase). A control was also added which consisted of contaminated soil which had been treated with 4% $Ca(OH)_2$ but to which no cachasse was added. Fifteen days later, the cells were planted with seeds of humidicola grass (*Brachiaria humidicola*). In each treatment cell 15 evenly spaced, small holes were opened to a depth of 1 cm and five seeds were placed in each hole. The holes were covered with soil and the cells were watered periodically during the initial two months of the study to maintain soil humidity.

### Monitoring

Samples were collected from the cells prior to the application of the treatments and after 117, 402, 539 and 567 days. Soil was collected as a core using a split spoon sampler 10 cm in diameter and 20 cm long.

### Acute toxicity

Soil extracts were prepared with deionized water, using 10 g of soil in 100 ml of solution and mixing vigorously for 1 min. Subsequently the mixture was let to settle for 24 h. The extract was decanted and filtered using a 0.2 µm nylon Millipore filter. The filtrate was analyzed using a Microbics model 500 analyzer (Microbics Corp.; Carlsbad, California) based on the method in the Mexican norm NMX-AA-112-1995-SCFI (SECOFI, 1996).

### pH

Soil pH was measured in a mixture of 1:2.5 of dry soil to water using an Orion 3 STAR pH meter previously calibrated to pH 4 and 7 according to the Mexican norm NOM-053-SEMARNAT-1993 (SEMARNAT, 1993).

### Hydrocarbon concentration

The hydrocarbon concentration was analyzed by two methods (EPA, 1997), the field based turbidimetric method (EPA 9074) and the infrared spectrophotometric method (EPA 418.1).

### EPA Method 9074 (PetroFLAG)

Two grams of field moist soil was extracted with 30 ml of methanol. The solution was filtered using a 0.2 µm nylon Millipore filter, and the filtrate was added to a proprietary reagent solution (Dexsil Corp., 1997) which produces turbidity in the presence of hydrocarbons, according to the manufacturer's suggestions. The turbidity

was measured using a PetroFLAG hydrocarbon analyzer and the turbidity compacted to a standard supplied by the manufacturer (Adams and Ramírez, 1999).

#### EPA method 418.1

2 g of oven dried soil was extracted with 25 ml of perchloroethylene in the presence of 0.5 g of anhydrous sodium sulfate and 1.5 g of silica gel. The solvent recovered was analyzed in an Infracal TOG/TPH analyzer (Wilks Enterprise, South Norwalk, Connecticut, USA). The absorbance was correlated to the hydrocarbon concentration by means of a calibration curve with diesel.

#### Biological respiration

Respiration was measured using the Stotzky (1965) method, by placing 200 g of moist soil in a glass jar. On top of the soil a small beaker containing 10 ml of 2 N KOH was placed as a $CO_2$ trap, making sure that the top of the beaker was slightly above the surface of the soil. Samples were incubated for 18 h at 30°C. Subsequently, the KOH solution was removed from the jar and 25 ml of 2 N $BaCl_2$ was added to this solution. The mixture was titrated adding three drops of phenolphthalene solution (1 in 96% ethanol) which produced a violet color. A 0.5 N solution of HCl was slowly added using a burette until the solution turned clear. In this test three clean gravel blanks were also tested to determine the amount of KOH consumed by non-biological (atmospheric) $CO_2$.

#### Hydrocarbons in leachates

A TCLP type extraction technique was used to obtain soil leachates, at a pH of 5 according to Mexican norm NOM-SEMARNAT-053-1993 (SEMARNAT, 1993). 10 g of dry soil was added to a 200 ml Erlenmeyer flask to which was added 96.5 ml of extraction solution (5.7 ml glacial acetic acid and 64.3 ml of 1*N* NaOH in 1 L of deionized water). The mixture was adjusted to pH 4.98 ± 0.05. The solution was mixed at 180 RPM for 18 h. The mixture was then left to settle and 50 ml of supernatant was extracted with an equivalent volume of perchloroethylene, mixing for five minutes. Subsequently, the solvent was recovered and evaporated in a small porcelain dish. The residue was resuspended in 7 ml of perchloroethylene and the absorbance measured using the Infracal analyzer as described previously.

#### Biomass production

The humidicola grass was cut periodically leaving 3 cm above the soil according to Alvarez (2006). Subsequently, the cuttings were dried in an oven at 60°C and weighed.

## RESULTS AND DISCUSSION

### Hydrocarbon reduction

Since the initial concentration of petroleum hydrocarbons varied slightly between treatments (11,870–17,842 mg/Kg), the data were normalized to initial concentrations in each treatment, to be able to compare between treatments. In Figure 1, all treatments resulted in considerable reductions, this being 93% for the treatments with 2 and 4% cachasse, and 89% for the treatment with 9%. In the control, a reduction of 80% was obtained. These reductions roughly correspond to exponential decay functions with half lives of 301 days in the control (R = 0.9473), 188 days in the treatment with 2% cachasse (R = 0.9074), 201 days in the treatment with 4% cachasse (R = 0.8769), and 232 days in the treatment with 9% cachasse (R = 0.9075). The percentage of total hydrocarbons extractable with methanol initially was 78 – 100%, which was reduced to 55% in the control, and 43% in the treatment with two percent cachasse. In the treatments with 4 and 9% cachasse, no methanol extractable hydrocarbons were present at the end of the study, although the final TPH concentrations were 1,030 and 1,460 mg/Kg, respectively. Table 1 shows the concentration of methanol extractable hydrocarbons.

### Acute toxicity and soil leachates

Toxicity and leachates are both factors which are related to hydrocarbon availability versus stabilization. The toxicity of all samples during the tests were considerably low, at or below regional background levels (EC50 ≥ 100,000 mg/L, TU ≤ 10). No relationship was observed between hydrocarbon concentration and toxicity in this study, probably due to the fact that the soil used in this study was weathered prior to the remediation tests. Table 2 shows acute toxicity in treatments.

Table 3 shows the hydrocarbon concentration in TCLP leachates. The initial concentration of TCLP leachates was between 4.5 and 5.6 mg/L. Within the first four months, leachates were reduced to < 1 mg/L and remainned at this level during the remainder of the treatment period.

### Microbial respiration

The respiration rates observed throughout the study are shown in Figure 2. As seen in Figure 2, the initial respiration rates in the treatments with 0, 2 and 4% cachasse were directly related to the concentration (R = 0.96), ranging from 1.4-16.4 $mgC_{CO2}$/Kg/h, whereas the respiration rate in the treatment with 9% cachasse was relatively low (9.2 $mgC_{CO2}$/Kg/h) for the amount of organic carbon added. In the control, slight increases or reductions in the initial value were observed but generally staying in the rage of 2 - 4 $mgC_{CO2}$/Kg/h. In the other treatments the respiration rates generally slowed down with time, probably due to the consumption of the primary carbon source (cachasse), with all treatments slowing down to the control value of 2 - 4 $mgC_{CO2}$/Kg/h.

### Biomass production

The production of biomass is shown in Figure 3. In Figure 3, it becomes apparent that the biomass in the control

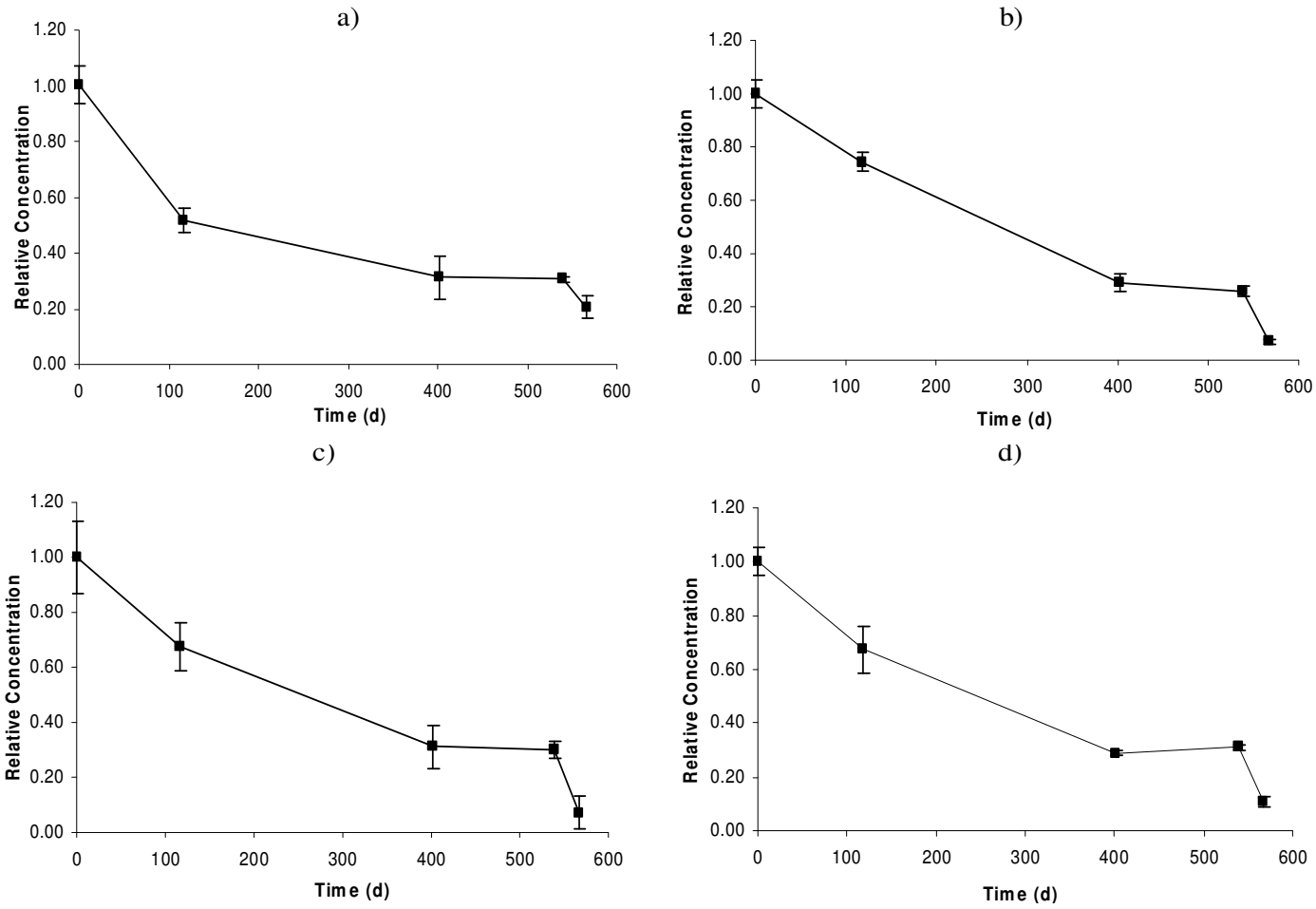


**Figure 1.** Relative hydrocarbon concentration during the treatments. Extraction with tetrachloroethylene; a) control, b) 2% cachasse; c) 4% cachasse and d) 9% cachasse.

**Table 1.** Concentration of methanol extractable hydrocarbons (EPA 9074).

| Treatment (% cachasse) | Methanol extractable hydrocarbons (mg/Kg) | | | | |
|---|---|---|---|---|---|
| | 0 day | 117 days | 402 days | 539 days | 567 days |
| Control (0) | 14,100±954 | 4,515±417 | 3,074±341 | 2,304±388 | 1,481±751 |
| 2 | 13,921±1431 | 1,3276±622 | 3,253±1230 | 3,325±893 | 593±867 |
| 4 | 13,491±667 | 1,2167±1290 | 1,535±107 | 2,269±593 | 0 |
| 9 | 12,847±3895 | 4,649±702 | 944±738 | 2,698±732 | 0 |

**Table 2.** Acute toxicity in treatments.

| Treatment (% cachasse) | Acute toxicity (Toxicity units*, TU) | | | | |
|---|---|---|---|---|---|
| | 0 day | 117 days | 402 days | 539 days | 567 days |
| Control (0) | 10.0 ± 0.0* | 9.7 ± 4.2 | 11.1 ± 1.5 | 10.0 ± 0.0 | 10.0 ± 0.0 |
| 2 | 10.0 ± 0.0 | 10.1 ± 0.1 | 10.0 ± 0.0 | 10.0 ± 0.0 | 11.0 ± 9.0 |
| 4 | 10.0 ± 0.0 | 10.3 ± 0.5 | 10.0 ± 0.0 | 10.0 ± 0.0 | 10.0 ± 0.0 |
| 9 | 10.0 ± 0.0 | 10.0 ± 0.0 | 12.4 ± 7.0 | 10.0 ± 0.0 | 9.0 ± 1.2 |

*1 TU =1/(Effective Concentration 50), where EC50 is expressed as a fraction; A value of 10 UT, which corresponds to the regional background level, was assigned to samples which presented toxicity too low to quantify according to this method.

**Table 3.** Hydrocarbon concentration in TCLP leachates (EPA 418.1).

| Treatment (% cachasse) | TPH in leachates (TCLP mg/L) | | | | |
|---|---|---|---|---|---|
| | 0 day | 117 days | 402 days | 539 days | 567 days |
| Control (0) | 5.6 ± 0.7 | 1.4 ± 0.6 | 1.4 ± 0.7 | 0.8 ± 0.2 | 0.6 ± 0.1 |
| 2 | 4.5 ± 0.3 | 0.9 ± 0.1 | 0.6 ± 0.4 | 0.8 ± 0.1 | 0.9 ± 0.0 |
| 4 | 5.0 ± 0.5 | 1.1 ± 0.1 | 0.5 ± 0.2 | 0.9 ± 0.2 | 0.8 ± 0.1 |
| 9 | 5.1 ± 1.2 | 1.1 ± 0.4 | 0.7 ± 0.2 | 1.2 ± 0.7 | 0.7 ± 0.3 |

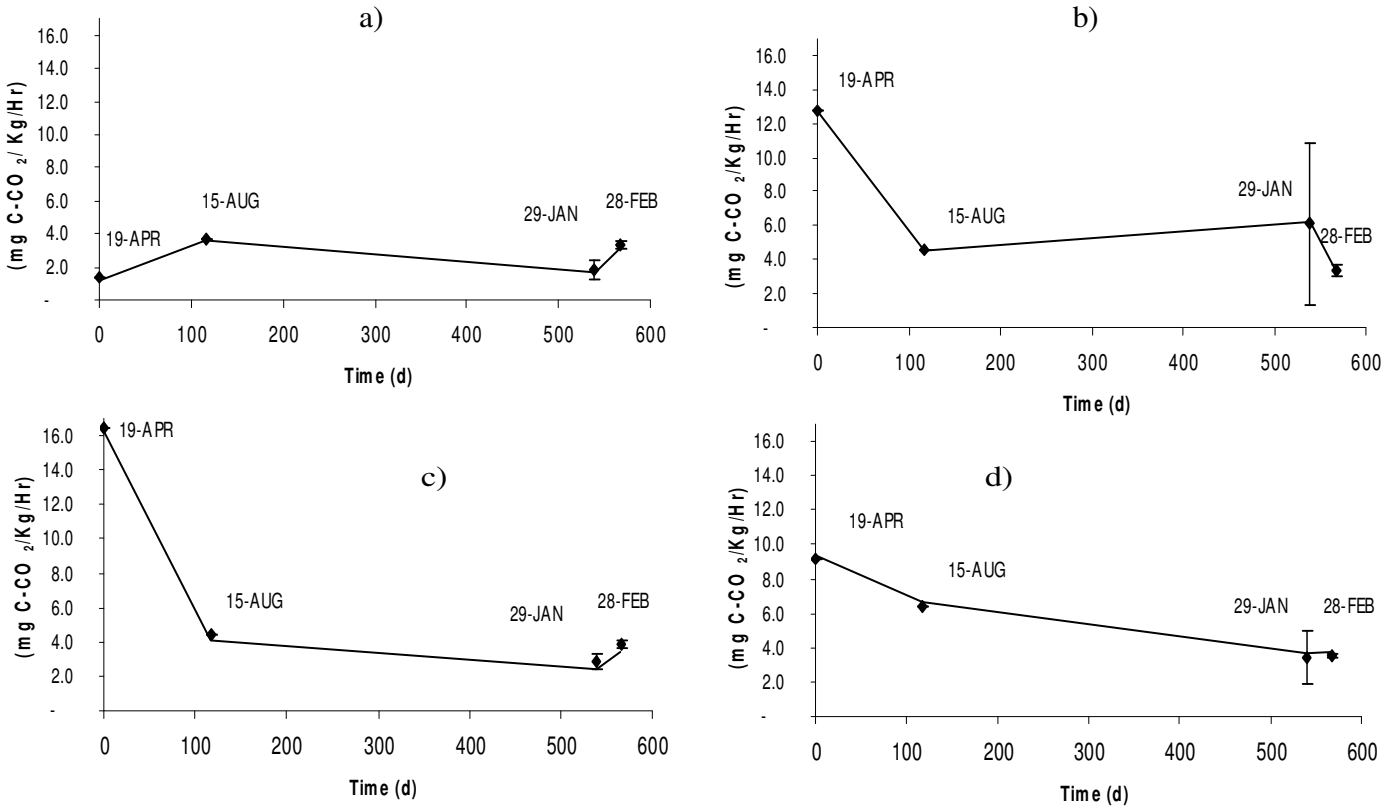


**Figure 2.** Relation between respiration rates and cachasse concentration in each treatment; a) control, b) 2% cachasse, c) 4% cachasse and d) 9% cachasse.

treatment and the treatments with 2 and 4% cachasse are similar during the earlier part of the study (up to 608 days) being in the range of 2 - 4 ton/Ha. The increases and decreases in biomass occur at the same time in these three treatments, and generally correspond to growing conditions, including temperature and rainfall, in this study which was carried out in the open. In contrast, the biomass observed in the treatment with 9% cachasse was much greater, being roughly two to three times greater during this same period (up to 608 days). In this treatment the biomass production gradually decreased down to 2 ton/Ha at 681 days. This is most likely related to the slow consumption of the cachasse originally added, and a corresponding slow deterioration of soil fertility (possible due to nutrient availability, water retention, and/or compaction). Even so, at the end of the study the biomass production in this treatment (9% cachasse) was still roughly double that observed in the other treatments (2.0 ton/Ha versus ~1.04 ton/Ha). The importance of soil conditioners for the improvement of biomass production was clearly shown in this treatment. This increase does not appear to be related to overall hydrocarbon concentration, toxicity, leachates of hydrocarbon stabilization, but in the overall improvement in soil fertility provided by this high application rate of an organic soil conditioner.

## Conclusion

In this study several factors were measured with respect to overall soil remediation, which had not beenconsidered in previous studies. Adams (2004b), Adams et al. (2005)

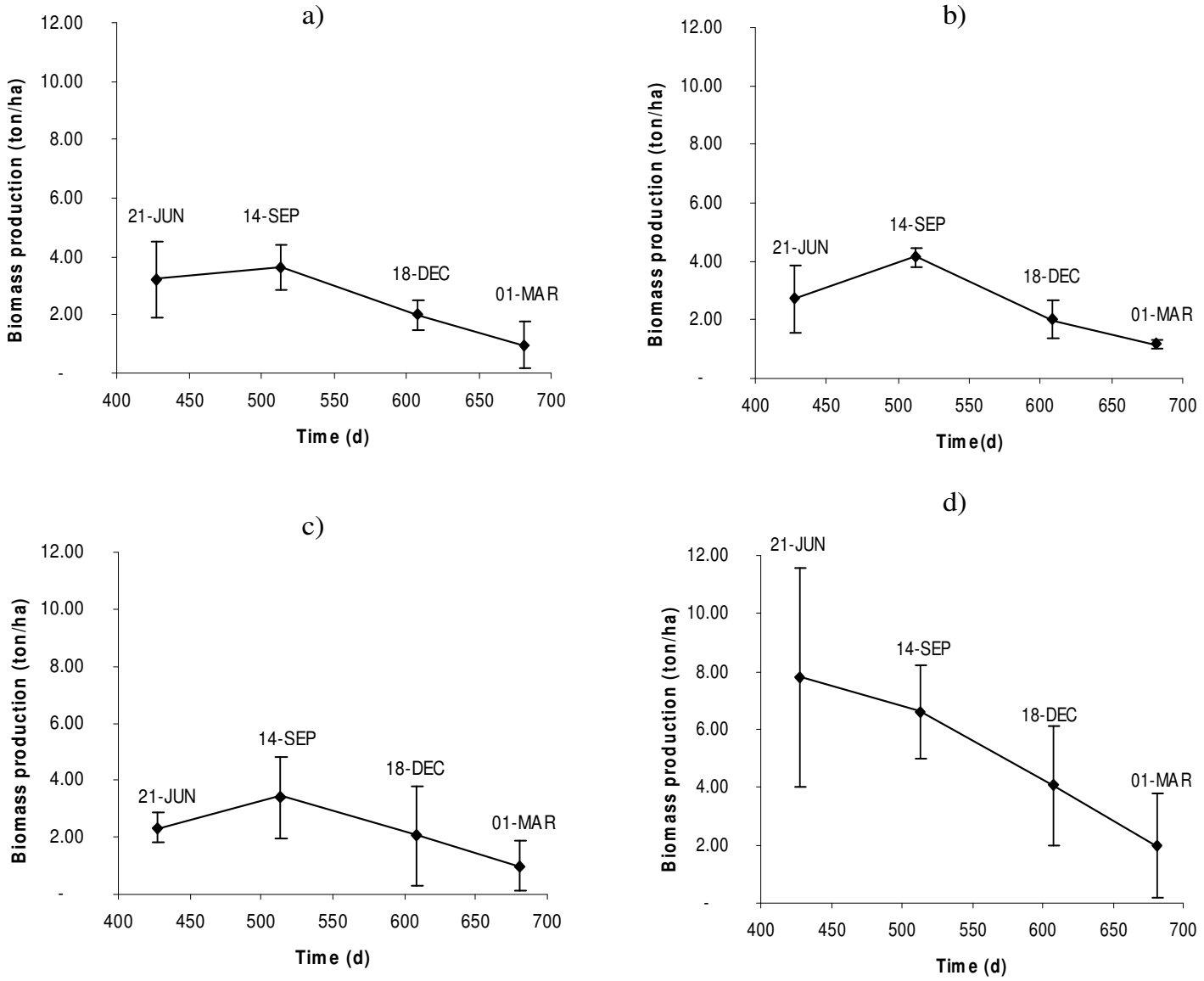


**Figure 3.** Relationship between biomass and cachasse concentration in each treatment. a) control, b) 2% cachasse, c) 4% cachasse and d) 9% cachasse.

and Adams and Guzmán-Osorio (2008) measured hydrocarbon reduction, toxicity and leachates but not microbial respiration of biomass production. In the present investigation, in addition to the total petroleum hydrocarbon concentration, the degree of hydrocarbon availability (as measured by the fraction of methanol extractable hydrocarbons and TCLP leachates) was also measured. Additionally, microbial respiration and pasture biomass production were also used as indicators of overall bio-treatment efficiency and soil restoration. Other researchers have also used microbial respiration rates as indicators of overall biodegradation and microbial activity (Atlas, 1986; Bartha and El-Din, 1993; Adams et al., 2002; Hershman and Temple, 1979). Likewise, the use of plant indicators for overall soil health in petroleum contaminated and remediated soils is well established (Rivera and Trujillo, 2004; Rivera et al., 2005).

Considering this combination of factors, the best treatment with respect to microbial respiration rate and hydrocarbon stabilization is the treatment with 4% cachasse. At the lower application rate of 2%, a slightly better half life was obtained, but only slightly better. Nonetheless, the best stabilizations were obtained at 4 and 9% cachasse, totally eliminating methanol extractable hydrocarbons at the end of the study.

In preliminary studies, Adams (2004a) found that the overall TPH degradation was very similar between 4 and 9% of organic amendment, and other factors such as pH, and toxicity, were also comparable. However, in that study, microbial respiration rates, hydrocarbon stabilization and

biomass production were not measured. With the new information provided in the present investigation we can confirm that there are no important long term differences in remediation results between the use of 4 and 9% cachasse. The use of 4% cachasse instead of 9% would appear to be more efficient in terms of the additional cost and logistic difficulties of using 9% cachasse versus 4%. Also, the treatment with 9% cachasse showed similar or slightly better stabilization than the treatment with 4%, and the microbial respiration was effectively inhibited during the early part of the study, probably due to the creation of fermentation conditions, and the overall biodegradation rate was reduced (increased half life). Due to these considerations, the application of 9% cachasse is not recommended.

One other important observation in the present study was that pasture biomass was definitively better in treatment with 9% cachasse than in any other treatment, thus showing a practical result of adding more organic conditioner, even though the biodegradation rate was not improved. To stimulate this pasture yield in the field, but at the same time avoiding low biodegradation rates, it is recommended to initially add only 4% cachasse, with an additional application after approximately one year, to further stimulate pasture production.

**REFERENCES**


Adams RH (2004a). Chemical-biological stabilization method for treatment of drilling cutting and hydrocarbon contaminated soil. In: 11th International Enviromental Petroleum Conference. Albuquerque N.M.

Adams RH (2004b). Chemical-biological stabilization of hydrocarbon contaminated soil and drilling cutting in tropical México. Land Contam. Reclam. 12(11): 349-361.

Adams RH (2007). Proceso de estabilización química-biológica para la remediación de suelo y recortes contaminados con aceites y derivados del petróleo: Patente No., Instituto Mexicano de la Propiedad Industrial, Mérida, Yucatán, México. p. 250821

Adams RH (2008). Chemical-biological stabilization process for repairing soils and cuttings contaminated with oils and petroleum derivatives. Patent No. U.S. Patent Office, Washington, D.C. p. 7, 413, 383.

Adams RH, Alvarez JA, Tinal OC, Guzman FJ (2005). Restoration of brine and oil contaminated marshlands by cationic exchange and chemical-biological stabilization. 12th International Environmental Petroleum Conference. Houston, Texas 7-11 Nov.

Adams RH, Domínguez VI, Vinalay L (2002). Evaluation of microbial respiration and ecotoxicity in contaminated soils representative of the pretroleum producing region of southeastern México. TERRA Latinoamericana, 20(3): 253-265.

Adams RH, Guzmán FJ (2008). Evaluation of land farming and chemico-biological stabilization for treatment of heavily contaminated sediments in a tropical environment. Int. J. Environ. Sci. Technol. 5(2): 169-178.

Adams RH, Guzman FJ, Alvarez JA, Dominguez VI (2007). Long term fertility monitoring of soil treated by the chemical-biological stabilization method. 14th International Environmental Petroleum Conference. Houston, Texas, 6-9 Nov.

Adams RH, Ramírez AJ (1999). Optimización del método EPA 9074 como alternativa para análisis de hidrocarburos totales de petróleo, presentado en el VI Congreso Inter- Americano sobre el Medio Ambiente. Monterrey, Nuevo León, México, Red Interamericana para la Calidad Ambiental/Instituto Tecnológico de Educación Superior de Monterrey (ITESM).

Alvarez AL (2006). Determinación de producción primaria en el pasto humidícola (*Brachiaria humidícola*), con respecto a la concentración de hidrocarburos en el suelo de playas de presa agua de mina. Texistepec, Veracruz. Tesis de Licenciatura. División Académica de Ciencias Biológicas. Universidad Juárez Autónoma de Tabasco. p. 51.

Atlas RM (1986). Biodegradation of hydrocarbons in the environment, In: Enviromental biotechnology, reducing risk from environmental chemical through biotechnology (Omen GS Ed.). Plenum Press, New York.

Bartha R, El-Din NS (1993). Testing of some assumptions about biodegradability in soil as measured by carbon dioxide evolution. Appl. Environ. Microbiol. 59(4): 1202-1205.

Coyne M (2000). Microbiología del suelo: un enfoque exploratorio. 7ma. ed. Ed. Paraninfo. p. 416.

Dexsil Corporation (1997). Introduction to the PetroFLAG® Hydrocarbon Analysis System. Hamden, Connecticut, USA.

Domínguez VI (2008). Estudio del tratamiento de recortes de perforación mediante un sistema de desorción térmica a baja temperatura. (Tesis de Maestría), Tabasco, México, Universidad Juárez Autónoma de Tabasco.

Energy Information Administration (2008). International energy annual report. World Crude Oil Production, 1960-2008.

EPA (1997). Test methods for evaluating solid waste: Physical/chemical methods, Environmental Protection Agency, Publication No. EPA 530/SW-846.

Guzmán FJ, Adams RH, Ávila GJ (2004). Comparison of biostimulation, bioaugmentation and chemical-biological stabilization for remediation of hydrocarbon contaminated sediments, presented at the 11th International Environmental Petroleum Conference, Albuquerque, N. M., USA. (October 11-15).

Hershman LE, Temple KL (1979). Comparison of ATP, phosphatase, pectinolysase and respiration as indicators of microbial activity in reclaimed strip mine soils. Soil Sci. 127: 70-73.

Rivera-Cruz M, Trujillo N (2004). Estudio de toxicidad vegetal en suelos con petróleos Nuevo e intemperizado. Interciencia, 29(7): 369-376.

Rivera-Cruz M, Trujillo N, Miranda C, Maldonado CE (2005). Evaluación toxicologica de suelos contaminados con petróleos Nuevo e intemperizado mediante ensayos con leguminosas. Interciencia, 30: 326-331.

SECOFI (1996). Norma Mexicana NMX-AA-112-1995-SCFI, Secretaria de Comercio y Fomento Industrial, Análisis de agua y sedimento-evaluación de toxicidad aguda con Photobacterium phosphoreum-Método de prueba, México D.F., Dirección General de Normas, 6 marzo.

SEMARNAT (1993). Norma Oficial Mexicana NOM-053-SEMARNAT-1993. Que establece el procedimiento para llevar a cabo la prueba de extracción para determinar los constituyentes que hacen a un residuo peligroso por su toxicidad al ambiente. Secretaría de Medio Ambiente y Recursos Naturales, México, D.F., Diario Oficial de la Federación de fecha Julio.

SEMARNAT (2000). Norma Oficial Mexicana NOM-021-SEMARNAT-2000. Que establece las especificaciones de fertilidad, salinidad y clasificación de suelos, estudio, muestreo y análisis. Secretaría de Medio Ambiente y Recursos Naturales, México, D.F., Diario Oficial de la Federación de fecha Octubre.

Stotzky G (1965). Microbial respiration. In Black A (ed.). American Society of Agronomy. Madison, Wisconsin USA. Agronomy, 9: 1550-1569.

Volke S, Velasco JA (2002). Tecnologías de remediación para suelos contaminados. México: INE-SEMARNAT, Instituto Nacional de Ecología.