

OSE II Use and Testing in Antarctica

Correspondence between the CEO of the OSEI Corporation Mr. Steven Pedigo and Dr. Walter McCormack of the University of Buenos Aires Argentina below.

The testing with OSE II on fuel from generators showed that OSE II caused the growth of microbes, and the chart is below as well. Since OSE II detoxifies the hydrocarbons and the bacteria shows growth then the fuel would have been remediated over time. The TPH tests were destroyed in a shipwreck, however the observations by the scientist stated there was great promise with OSE II, since they could see a change in the composition of the fuel, and the fuels smell was eliminated in a very short time. Once the fuels smell has depleted, soon after this occurrence the fuels flammability would have been eliminated as fire fighters who have used OSE II for years can attest to with LED meters. This was a good use for OSE II, to show it can be effective in cold temperatures.



Since this test OSE II has been used successfully in Alaska repeatedly, in Norway, and Canada. See emails below.

Argentinian Station Antarctica

From: "Walter Mac Cormack" <<u>wmac@ffyb.uba.ar</u>> To: <oseicorp@msn.com> Subject: request for Antarctic bioremediation Date: Thu, 2 Nov 2006 17:26:49 -0300

Dear Sir:

We are a university research team from Argentina working in the field of bioremediation of contaminated soils in Antarctica. Our aim is to develop technology for the bioremediation of hydrocarbon contaminated soils in cold areas. Next austral summer we are planning to test different sources of nutrients for improving the elimination of hydrocarbons from soils. We are very interested in using your product OSE II as a nutrients source able to stimulate the natural bacterial flora of Antarctic soils and if it works, to continue using it in the future.

We would like to know whether are you interested in collaborating with our research activities by giving us a small quantity of OSE II (enough for the treatment of 300 kg of contaminated soil) for the experiments we will perform in our Antarctic expedition that starts next December. This way we could test the efficiency of your product and consider to use it in the future assays.

Looking forward receiving your answer as soon as possible.

Regards,

Dr Walter Mac Cormack Facultad de Farmacia y Bioquímica Universidad de Buenos Aires Cátedra de Biotecnología Junin 956 6º piso (C1113AAD) Buenos Aires, Argentina ----- Original Message -----From: "OSEI CORP" <oseicorp@msn.com> To: <<mark>wmac@ffyb.uba.ar</mark>> Sent: Wednesday, November 08, 2006 3:54 PM Subject: RE: request for Antarctic bioremediation

Dear Mr. Walter Mac Cormack,

We are very interested in sending you enough product in order for your University group to test OSE II. We will send you a protocol, and OSE II. Please let us know which University you are associated with, and give us your phone number and address, and we will get the product and information sent to you. Please correspond with me direct since I invented the product and would like to be close to all of your operation at stevenosei@msn.com.

I will await your response phone number and address.

Sincerely, Steven Pedigo Chairman OSEI Corporation From: "Walter Mac Cormack" <<mark>wmac@ffyb.uba.ar</mark>> To: "OSEI CORP" <oseicorp@msn.com> Subject: Re: request for Antarctic bioremediation Date: Sun, 26 Nov 2006 03:41:58 -0300

Dear Steven Pedigo:

We have received the sample of OSE II and the instructions. Thank you very much for your kindness. In a few days we will depart to Antarctica and test your product during the Antarctic summer. We will contact you as soon as we have some result. Of course, we are open to any questions that you require. Thank you again and we will contact you soon.

Dr Walter Mac Cormack Facultad de Farmacia y Bioquímica Universidad de Buenos Aires Cátedra de Biotecnología Junin 956 6º piso (C1113AAD) Buenos Aires, Argentina



----- Original Message -----From: "steven pedigo" <stevenosei@msn.com> To: <<mark>wmac@ffyb.uba.ar</mark>> Sent: Tuesday, November 28, 2006 12:39 PM Subject: OSE II

Dear Walter Mac Cormack,

We appreciate your letting us know that OSE II is in your hands. We sent you a larger quantity than you needed, so you can do as much testing as you would like. We were also interested to see if it might be possible for you to put say a 6' in diameter of floating boom near the shore and test OSE II on oil on cold water as well. We are sure you will have success with OSE II on soil, since AT&T Alascom has used OSE II on spills in Alaska for the last 10 years. We have had numerous inquiries on using OSE II on cold water applications on water, and just have not had the time to test it. There are numerous groups interested in the North Sea especially. If it is possible to try some on a small amount of oil on water it would be great. If you develop any procedures utilizing OSE II in Antarctica, we will acknowledge your work in our literature as well. Will you have access to email while you are in Antarctica, and will you let us know what is going on from time to time at your leisure. Good luck, and if there are any questions let me know.

Thank You, Steven Pedigo



From: "Walter Mac Cormack" <<u>wmac@ffyb.uba.ar</u>> To: "steven pedigo" <stevenosei@msn.com> Subject: Re: OSE II Date: Wed, 29 Nov 2006 15:47:38 -0300

Dear Dr Pedigo:

Thank you very much for your letter. In relation to the test of OSE in a floating boom, I am afraid that it will be not possible as you suggest because the actual environmental legislation in Antarctica prevent the spill of contaminants in the environment and I think that the floating boom will be broken by the icebergs and small floating ice blocks that continuously are removed from the coastal glaciers existing in the Cove in which coast Jubany Station is located. Such ice blocks will destroy the boom in a few days, principally during windy days, when the surface is totally covered by moving iceblocks. As an alternative we could test the effect of OSE using a kind of container filled with Antarctic Sea water and exposed to the ambient temperature and other strong climate conditions. However, the container should be located on land, near the shoreline. Do you think that this test will be useful for you? Have you any other alternative assays?

Well, we will try to test your product on sea water in a way that avoid the spill of oil on the marine environment. We will wait for your suggestions. In addition, you could communicate with the responsible of the land experiments in Antarctica (Dr Lucas Ruberto) at any time to the following E-mail adress: lruberto@ffyb.uba.ar

Best regards Dr Walter Mac Cormack



----- Original Message -----From: "steven pedigo" <stevenosei@msn.com> To: <<mark>wmac@ffyb.uba.ar</mark>> Sent: Thursday, April 12, 2007 12:39 PM Subject: Re: OSE II

Dear Mr. Mac Cormack,

We were just interested in how things are working out with OSE II and your tests in Antarctica. When you get a chance, please let us know the status of the tests.

Sincerely, Steven Pedigo

This message was sent with High importance. Walter Mac Cormack <u>wmac@ffyb.uba.ar</u> Mon 4/30/2007, 2:00 PM

Dear Dr Pedigo:

Thank you very much for your interest. As you know we performed a long time assay in Antarctica that finished at the end of March. The effect of the presence of OSE II on hydrocarbon biodegradation was tested among other sources of nutrients. We tested the addition of inorganic salts, and a mix of fish meal and a surfactant (Brij) as alternatives systems and compared with an untreated system.

I dont know if you know about the accident (an uncontrollable fire) that the Argentinean Icebreaker (Almirante Irizar) suffered in April 10 when it came back to the continent. Unfortunately, all the samples obtained for chemical analysis in laboratories of Buenos Aires were in the ship. Samples to evaluate the level of hydrocarbons in each sample of each treatment were lost. This problem avoids a close comparison about the success of each treatment. I'm really sorry. You can imagine that this fire greatly affected our lines of research. However, bacteriological results were made in the field and we can infer some data about the efficiency of the treatments based on the level of total and hydrocarbon degrading bacteria in the different treatments. I am sending you a file with preliminary results of bacterial counts in the systems. As you can observe in the attached file (where we plotted the total (THAB) and the hydrocarbon-degrading bacteria (HDB) versus time in comparison with the control, OSE did enhance the total number of aerobic bacteria. There was an initial increase in all systems that are related to the preparation of the soil and is caused by the aeration and other activating factors. However, when the HDB were analyzed, a significant increase was evident compared with the control system (one order of magnitude higher approximately). This result could mean that although the OSE II did increase the total counts, it could also cause a turnover in the individual components of the bacterial flora, favoring the growth of the hydrocarbon-degrading fraction. Unfortunately, we have not the soil samples to confirm what happened with the hydrocarbon concentration in the samples. If you observe the other tested systems you can observe that both (addition of inorganic salts or fish meal + a commercial surfactant) seem to not work better than OSE in promoting bacterial growth. However, except for the system where fish meal was combined with a surfactant all the other systems had less total bacterial counts at the end of the assay compared with OSE. In addition when we observed the ratio HDB/TAHB system with OSE showed a slow but constant increase throughout the assay, reaching 80% at the end of the assay.

In conclusion, although OSE did increase bacterial counts, we can not discard that this product had been effective in remove of hydrocarbons from Antarctic soils. We have samples from the initial time (t=0) and we are trying to obtain from the Antarctic station, new samples from the microcosms systems in order to have a final-time samples. But independently of this possibility, our intention is to repeat the assay in the lab with Antarctic soil samples that we already required to the station crew.

We have not more OSE at the present time, because the remaining of the sample that you gently sent us was affected by the fire in the ship together with all the rest of our scientific materials and equipments. If you continue with your interest in this study I beg you that send us a new sample of the product in order to continue with the failed assay. Of course if we can rescue some of the samples, we will inform you about these samples and the results obtained from it. I hope that you understand the problem and can send a new sample of your product in order to continue with these study.

Sincerely yours

Dr Walter Mac Cormack





THAB: Total Heterotrophic aerobic bacteria OSE: Oil Spill eater II HDB: Hydrocarbon degrader bacteria Original Message -----From: "Walter Mac Cormack" <<mark>wmac@ffyb.uba.ar</mark>> To: "OSEI CORP" <oseicorp@msn.com> Sent: Thursday, November 09, 2006 4:09 PM Subject: Re: request for Antarctic bioremediation

Dear steven Pedigo:

Thank you very much for your fast and kind anwer. Excuse me for to omit our data in the previous E-mail. I am professor at Buenos Aires University (UBA) and we are working at the Industrial Microbiology and Biotechnology laboratory which depend on the School of Biochemistry of University of Buenos Aires. In addition, I am researcher of the Argentinean Antarctic Institute in the field of microbiology. For this reason, our main research line is development of bioremediation techniques at low temperature able to work well in the Antarctic Stations and, eventually, in any other hydrocarbon contaminated cold-soils. We have some biological systems (Antarctic bacterial consortia) which worked very well in previous studies at the laboratory level as well as in the field (in Antarctica). At this stage, as we commented to you in the previous E-mail, we are trying to optimize the nutrient addition required by the microorganisms. We are very interested in testing your product under Antarctic conditions. Of course, we will send you the results of the assay as fast as we had them and will mention the OSEI Corp. in case that a scientific publication emerges from these assays. Please, take into account that we are planning to carry out this assay between January and March (Antarctic summer). After that, samples will be sent to Buenos Aires for further analysis. Well Dr Pedigo, thank you again for your interest and we will wait for any answer that you consider pertinent. We hope that your product arrive to our lab before our departure, planned for December 20 approximately.

Please, as I will be absent of my lab until Thursday 16 in order to attend a scientific meeting I beg you that any E-mail during such period will include copy to one of my colleagues: Dr Lucas Ruberto (lruberto@ffyb.uba.ar).

Best regards

Dr Walter Mac Cormack

Our professional address is: Dr Walter Mac Cormack Cátedra de Biotecnología y Microbiologia Industrial Facultad de farmacia y Bioquímica, Universidad de Buenos Aires, Junin 956, 6º piso Buenos Aires (C1113AAD) Argentina