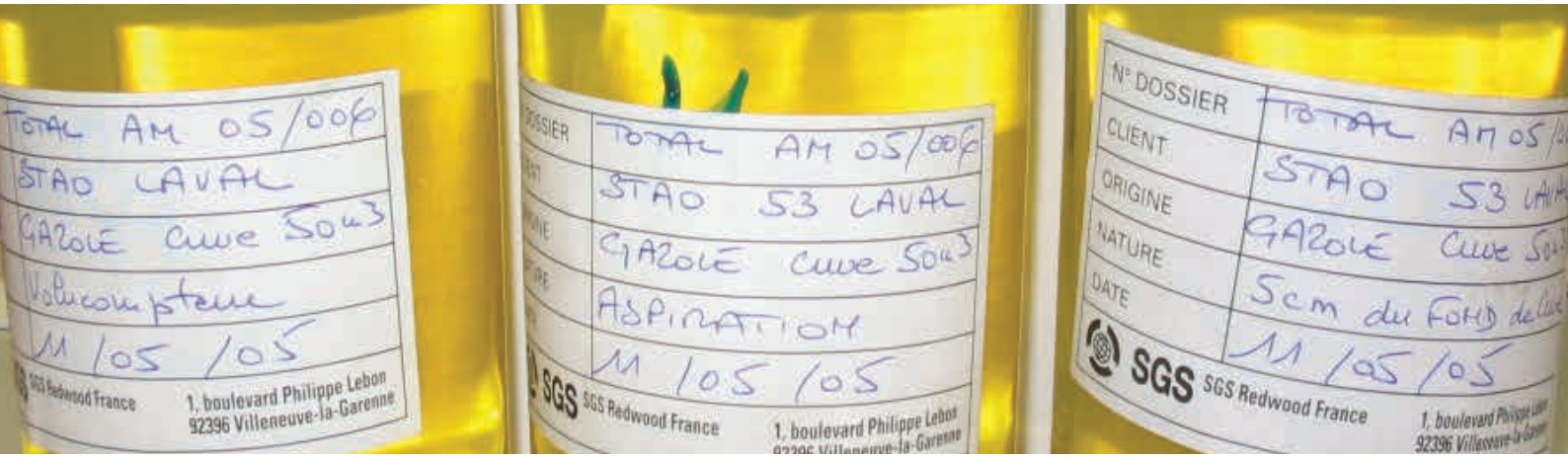
XBEE
Enzyme Fuel Technology

INTERTEK CALEB BRETT
Microbiological study in Diesel oil



Intertek Analyses Chalon realized some tests on the beginning of 2006 to demonstrate the efficiency of the XBEE enzyme biotechnology to eliminate the contamination in a heavily polluted road diesel fuel. Results in CFU (Colony forming Unit).

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Intertek petroleum industry services include laboratory testing, cargo inspection, R&D, materials analysis, asset integrity management, corrosion control, safety, and much more.

BACTERIA			
	D Day	D Day+15	D Day +60
XBEE Diesel 1:4,000	$1,5.10^8$ (150,000,000)	$2,6.10^7$ (26,000,000)	$3,4.10^4$ (34,000)
XBEE Diesel 1:500	$1,5.10^8$ (150,000,000)	$2,2.10^7$ (22,000,000)	$3,3.10^4$ (33,000)

YEAST			
	D Day	D Day+15	D Day +60
XBEE Diesel 1:4,000	$1,0.10^6$ (1,000,000)	$1,0.10^6$ (1,000,000)	$4,0.10^4$ (40,000)
XBEE Diesel 1:500	$1,0.10^6$ (1,000,000)	$5,0.10^5$ (500,000)	$4,0.10^3$ (4,000)

MOLD			
	D Day	D Day+15	D Day +60
XBEE Diesel 1:4,000	$1,4.10^7$ (14,000,000)	$2,7.10^7$ (27,000,000)	$2,0.10^4$ (20,000)
XBEE Diesel 1:500	$1,4.10^7$ (14,000,000)	$1,5.10^7$ (15,000,000)	$4,0.10^4$ (40,000)

LEAN & GREEN
The XBEE Enzyme Fuel Technology has been awarded the Lean & Green Tools Certificate.

Intertek Analyses Chalon

Campus Industriel
Le Grand Chalon en Bourgogne
Route de Demigny BP 21
71102 Chalon Sur Saône Cedex
Tel 03 85 99 7279
Fax 03 85 99 7675
Email chalon@intertek.com
Web site www.intertek.-cb.com/

Rapport : IAC-R06-0007

Xbee
Avenue de Bielefeld Senne
29900 Concarneau

Chalon-sur-Saône le 28/04/2006

Contact : Monsieur Remy Penneg

N° Commande : 76/2829ANA
N° Devis : 2005-00001
Date Commande : 16/11/2005

ANALYSIS REPORT

Reference Microbiology Laboratory : File Id : 2005-00001

Login Date : 05/10/2005

Goal of the study :

- **Contaminated fuel (diesel) testing using IP 385/99 standard method.**
- **Effect of Xbee added at 1/4000 (volume) on the same contaminated fuel (diesel). Using IP 385/99 standard method.**
- **Effect of Xbee added at 1/500 (volume) on the same contaminated fuel (diesel). Using IP 385/99 standard method.**

Customer sample identification and description date of receiption in the laboratory:

- One sample of fuel (diesel) received 18/01/2006.The sample does not contain an aqueous phase.

Influence of XBee on contaminated diesel fuel

Sampling : bottom of diesel fuel storage tank in a gas station

Analytical Set up:

Method Standard IP 385/99 :
- Usage of Protocol B
- Culturing media used for the study are :

Gélose TSA : Trypcase Soja Agar (for bacteria counting.)
Gélose Sab : Sabouraud Gentamicine Chloramphénicol (for yeast and mold cultur and counting).

Protocol B : Dilution 0,1ml and 0,1ml N^{-1} and 0,1ml N^{-2} and 0,1ml N^{-3} and 0,1ml N^{-4} and finally inoculation of different culture media.

Incubation : 1 week for the growth of micro organisms at 25°C (+/- 2°C).
Total counting in each Petri dish .
Measurements are replicated to increase reliability of the counting . The reported results are the average of individual measurement .

Part 1 : Testing at reception of contaminated diesel samples (Day =0)

1- a Results :Total bacteria counting of samples as received :

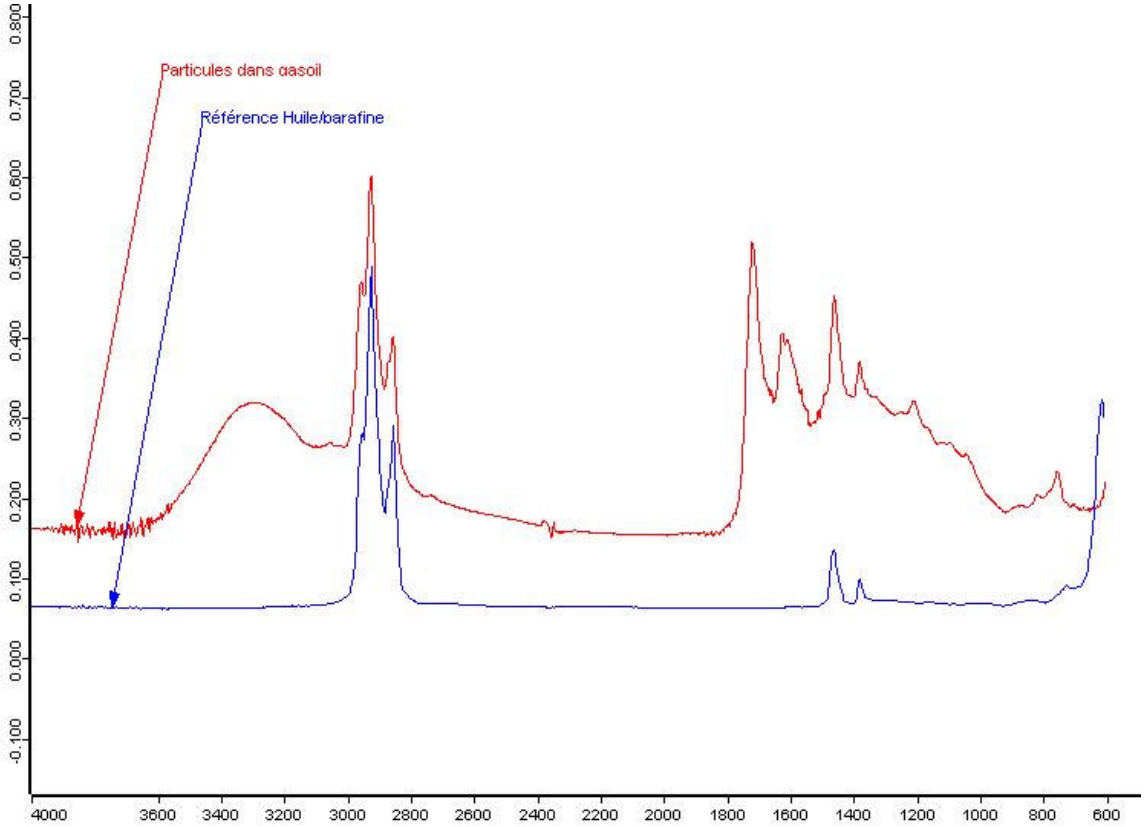
Method : Total viable counting (Methode Institut des pétroles : IP 385/99)

Sample description	**Bacteria** CFU/litre	**Yeast** CFU/litre	**Mold** CFU/litre	**Comments**
Diesel sample T =0	$1,5.10^8$	$1.0\ 10^6$	$1,4.10^7$	Strongly contaminated sample

***CFU** : Colony forming Unit (1 viable germ leads to the formation of one colony)*

1-b Results Chemical analysisi of the particulate contamination of samples as received

Technical method : Infra red Spectrometry and electron Microscopy.



The particles isolated by filtration in the contaminated diesel were analysed using infra red Microscopy and electron Microscopy (Xray Microanalysis using energy dispersive spectrometry EDS)

Infra RED : They are all of the same kind , they are a mixture of agglomerated particles of organic composition ,similar to paraffine (coming probably from byproduct from hydrocarbons chemically modified by micro organisms) .

EDX : Elemental analysis by Xray Energy dispersive spectrometry

EDX testing shows the presence of Carbon ,Oxygen ,Calcium ,Sodium , Sulfur and Iron . The Carbon is the major constituent of the particulates .

PART 2 : Study of biocontamination evolution (bacteria, yeast , mold) as a function of time after addition of XBEE

Completed testings:

Addition of XBee at concentration 1/4000 and 1/500 (volume/volume) in biocontaminated gasoil (see Part 1)

Analytical Protocol : IP 385/99 Total viable counting of bacteria , mold and yeast . cultivables

Day =0 : just after Xbee addition
After 15 days 5: After 15 days with Xbee concentrations (1/4000 and 1/500)
After 60 days: After 2 months with Xbee concentrations (1/4000 and 1/500)

Results reported as CFU :

***CFU** : Colony forming Unit (one viable germ leads to the formation of one colony)*

Bacteria				Yeast				Mold		
	Day = 0	**After 15 days**	**After 60 days**	**Day = 0**	**After 15 days**	**After 60 days**		**Day = 0**	**After 15 days**	**After 60 days**
Gazole Xbee 1 :4000	**$1,5.10^{8}$**	**$2,6.10^{7}$**	**$3,4.10^{4}$**	**$1,0.10^{6}$**	**$1,0.10^{6}$**	**$4,0.10^{4}$**		**$1,4.10^{7}$**	**$2,7.10^{7}$**	**$2,0.10^{4}$**
Gazole Xbee 1 :500	**$1,5.10^{8}$**	**$2,2.10^{7}$**	**$3,3.10^{4}$**	**$1,0.10^{6}$**	**$5,0.10^{5}$**	**$4,0.10^{3}$**		**$1,4.10^{7}$**	**$1,5.10^{7}$**	**$4,0.10^{4}$**
Gazole Xbee 1 :4000	**150 000 000**	**26 000 000**	**34 000**	**1 000 000**	**1 000 000**	**40 000**		**14 000 000**	**27 000 000**	**20 000**
Gazole Xbee 1 :500	**150 000 000**	**22 000 000**	**33 000**	**1 000 000**	**500 000**	**4 000**		**14 000 000**	**15 000 000**	**40 000**

Christine Vernier

Christian Gimenez