



# Evaluation of Acute Oral Toxicity of Potential Thermophillic Bacterial Consortia (TERIK) for Enhanced Oil Recovery

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## Abstract

Microbial enhanced oil recovery is an ecofriendly approach used to enhance the recovery of oil. In this investigation, formation water was collected from the oil wells of Gujarat. The formation water was enriched with the desired nutrients, to facilitate the growth of indigenous microbial species under reservoir conditions. The microbial species (TERIK) were morphologically analyzed through scanning electron microscopy. The acute oral toxicity of the microbial species was screened. Microbial species were appeared to be non-virulent and non-pathogenic. Hence, these species were appeared to be safe for field implementation.

## Introduction

Crude oil wells are producing formation water along with the oil, initially the oil was extracted out through indigenous pressure generated by water and gases. As the time increases, the pressure reduces and may affect the recovery of the oil. To overcome this scenario, different mechanical and chemical methods were employed, but are very expensive, energy consuming and not feasible. In the present study, we have used eco-friendly, non-pathogenic microbial approach. The water was enriched in specified M<sub>2</sub>X media to facilitate the growth of indigenous microbes. An indigenous microbe (TERIK) was analyzed morphologically through scanning electron microscopy. Sample was immersed in 2.5% glutaraldehyde and dehydrated with ethanol (10-100%). The sample was mounted on to a metal stub with double sided carbon tape. Micrographs of the specimens were captured digitally using digital image transfer recognition program Zeiss. Indigenous consortia administered by the oral route to the mice under EPA 712-C-96-322, OPPTS 885.3550 Guideline. Twelve mice, 6 male and 6 female were assigned to the dose groups, Control and Test. The test material was administered once by gavage to mice. The mice were fasted overnight and two hours after administration of the consortia [1-2]. Water was allowed ad libitum. At the end of the observation period the surviving experimental animals were sacrificed. Gross necropsy performed and all animals were carefully examined for the presence of any anaerobic species. This will help to implement the nonpathogenic microbe in the field without causing any environmental hazard.

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Received Date: 21 Mar 2017

Accepted Date: 30 May 2017

Published Date: 01 Jun 2017

### Citation:

Sharma N, Lavania M, kukreti V, Kumar S, Lal B. Evaluation of Acute Oral Toxicity of Potential Thermophillic Bacterial Consortia (TERIK) for Enhanced Oil Recovery. *Ann Pharmacol Pharm.* 2017; 2(11): 1058.

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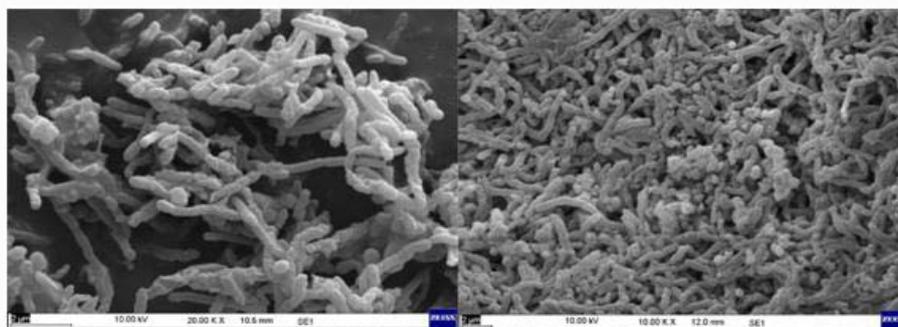
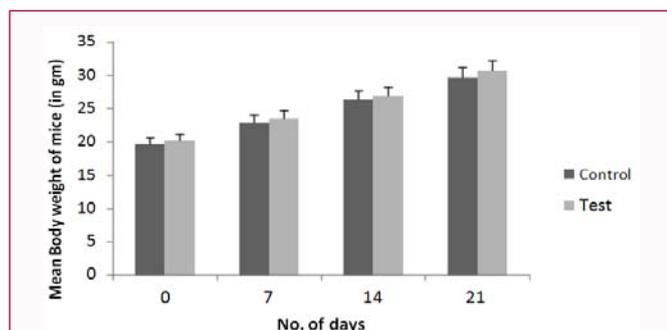


Figure 1: Morphology of the indigenous consortia TERIK (a) Scanning electron micrograph of five days grown culture in specified media; bar 2µm. (b) Twenty days grown culture clearly showing colorless network of bio-surfactant.



**Figure 2:** Mean body weight of mice in control and test mice. There was no statistically significant difference in the blood chemistry parameters- Glucose, SGPT (serum glutamate-pyruvate transaminase), BUN (Blood Urea Nitrogen), Total proteins, albumin and in hematological parameters- Hemoglobin, packed cell volume, red blood cells, white blood cells and prothrombin time (PT) in the test group when compared with the control group shown in table (1,2).

**Table 1:** Indicating blood chemistry parameter in control and test mice.

Group	Plasma Glucose (mg %)	Total Proteins (g %)	Albumin (g %)	Blood Urea Nitrogen (mg %)	SGPT (IU/L)
Control	97.53	6.53	4.35	31.4	39.6
Test	98.20	7.07	4.14	30.8	44.1

**Table 2:** Indicating hematological parameters in control and test mice. Hence, the microbial consortia (TERIK) were appeared to be safe for field implementation. These microbes can be used to reduce the permeability of high permeable zones of the reservoir and bring about the diversion of water flow path.

Group	Hb (Gram %)	Packed Cell Volume %	RBC (million/ cmm)	WBC (thousand/ cmm)	PT (sec)
Control	7.93	26.97	6.53	4.87	78.3
Test	8.83	29.33	6.64	6.13	79.3

## Results

### Morphology of consortia

The quantity of microbes formed on the 5<sup>th</sup> and 30<sup>th</sup> day was shown in Figure 1. The microphotograph indicated that the micro

-organisms primarily composed of rod and cocci. The sample was showing significant growth on the 5<sup>th</sup> day of incubation and on the 30<sup>th</sup> day colorless surfactant was covering the bacterial cells. As the incubation period increases, the cell number and its bi-product was also increased because the microbial cell was in exponential phase where the growth is balanced due to the presence of nutrients in the medium.

### Acute Oral Toxicity

Acute oral toxicity of the consortia was investigated with the intention to diagnose any possible adverse effect. TERIK consortia did not cause mortality in the treated Mice by oral route at the dose of 1.0 ml containing  $1.0 \times 10^6$  CFU. All the mice appeared normal and showed no clinical signs of intoxication after dosing till the end of the study. The body weight gain by the mice treated with the consortia was not found to be adversely affected during 21 day observation period [3]. The sample did not induce any gross pathological alterations in any of the mice, as evident during their necropsy. No live anaerobic bacteria as in test material were seen in any of the organs of the mice. The sample was found to be SAFE to mice by the oral route, at the dose level of 1.0 ml containing  $1.0 \times 10^6$  CFU. There was no statistical gain in the body weight of the mice in the control and test (Figure 2).

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