Environment Canada
Environnement Canada

Canada Centre For Inland Waters
Centre Canadien Des Eaux Intérieures

COMPARISON OF ROTARY SHAKERS AND CYCLONE FERMENTORS IN THE BIODEGRADATION OF PETROLEUM

By: D. Liu and W.M.J. STrachan

UNPUBLISHED REPORT
RAPPORT NON PUBLIE

COMPARISON OF ROTARY SHAKERS AND CYCLONE FERMENTORS IN THE BIODEGRADATION OF PETROLEUM

By: D. Liu and W.M.J. STrachan

May 22, 1979

Submitted: Canadian Research

COMPARISON OF ROTARY SHAKERS AND CYCLONE FERMENTORS IN THE BIODEGRADATION OF PETROLEUM

by

LIU, D. and W. M. J. STRACHAN

NATIONAL WATER RESEARCH INSTITUTE

CANADA CENTRE FOR INLAND WATERS

BURLINGTON, ONTARIO, CANADA

L7R 4A6

INTRODUCTION

The fate of man-made substances in the environment is an area of international concern since the disappearance, persistence and transformation of chemicals determines their usefulness, potential hazard and hence, to a degree, their utilization in commerce. Chemical, physical and biological factors all affect a substance's fate in the environment but microbial action is generally considered to be the most important one responsible for its degradation. Thus, knowledge of the microbiological processes involved in the metabolism of these man-made substances would yield useful information concerning their persistence in the natural environment.

Due to the many variables involved, it is impractical to study the biodegradation of such substances in all natural environments and consequently various laboratory procedures have been developed for the assessment of a substance's biodegradability[2-8]. However, a review of this literature discloses that during such biodegradation studies, there is generally a failure to appreciate the importance of the biodegradation apparatus in which the microorganisms carry out their work. Culturing in a rotary shaker is widely used in the biodegradation study; the performance of this culture technique[2,8] was therefore compared with the cyclone fermentor used in our laboratory. Since the majority of the problem-causing chemicals are lipophilic in nature, i.e., water

insoluble substances, kerosene was arbitrarily chosen as the test substance in the present experiments to examine the effect of the biodegradation apparatus on the rate of petroleum degradation.

MATERIALS AND METHODS

Organisms and growth medium

The organism and growth medium used in these experiments have been previously reported[4]. The medium was sterilized for 20 min at 121°C. Kerosene (Fisher, odorless) and an aqueous solution of Indulin C (a thiolignin) were sterilized separately for 15 min at 121°C and added aseptically to the sterilized basal mineral medium to give a final concentration of 5000 and 750 ppm, respectively.

Inoculum

An active, 2% (v/v) bacterial inoculum (~2 x 10^8 cells ml^{-1}) was obtained from the third consecutive culture transfer with Pseudomonas desmolytica in its logarithmic phase of growth in the kerosene-mineral medium. This inoculum was used in assessing both the rotary shaker and the fermentory systems.

Rotary shake culture

Duplicate cultures were prepared in 1000 ml Erlenmeyer flasks containing 500 ml of kerosene-growth medium. The cultures were incubated at 30°C on a New Brunswick rotary shaker operating at 220 strokes min^{-1} in a 2.5 cm circular orbit. Aliquots of 30 ml of culture suspension were withdrawn aseptically at the required

time intervals for the analysis of bacterial dry weight and pH measurement of the culture broth.

Cyclone fermentor batch culture

The cyclone fermentors used in these experiments were exactly the same as reported before except that they were operated in the batch mode[4]. Twelve hundred milliliters of kerosene-mineral medium were placed, aseptically, into a sterile cyclone fermentor and inoculated with 2% (24 ml) inoculum. The fermentors were maintained at 30°C and sterile air was supplied to the fermentors at 1.8 liters min^{-1}. At various time intervals 30 ml of culture suspension were withdrawn aseptically for pH measurement and bacterial dry weight determination.

Dry weight

The bacteria were removed from the culture suspension by centrifugation at 20,000 x g for 20 min. The resulting pellet was washed with 0.05 M phosphate buffer (pH 7.4) and two aliquots of distilled water. The cell pellet was taken up in 5 ml of distilled water, dried for 16 hours at 105°C and weighed.

pH measurement

Following the removal of the bacterial cells by centrifugation, the pH of the supernatant of the fermentation broth was measured directly with a pH meter.

RESULTS AND DISCUSSIONS

The growth curves of Pseudomonas desmolytica cultivated in kerosene-mineral medium and in kerosene-Indulin-mineral medium in cyclone fermentors and in rotary shake flasks are shown in Figure 1. These results indicate that the cyclone fermentors are more suitable in terms of cell yield for petroleum biodegradation than are the conventional rotary shakers. After 20 hours of incubation, the cell yield of P. desmolytica in kerosene-mineral medium in a cyclone fermentor had increased almost threefold over that in a rotary shake flask. The addition of Indulin to the kerosene-mineral medium resulted in an accelerated degradation in the fermentor to approximately the same extent. One explanation for the observed difference in performance of the two biodegradation apparatuses is that the fast flow rate experienced in the cyclone fermentor greatly increases the substrate availability to the microorganisms by enlarging the kerosene-water interfacial area. Another possibility is that more of the bacteria remain and divide within the medium in the cyclone fermentor, whereas a considerable percentage of the bacteria are removed from activity by accumulating as a ring at the air-water-vessel interface of the culture flasks in the rotary shaker. The rate controlling steps in hydrocarbon biodegradation have been reported to be determined by the transfer rate of hydrocarbon to the degrading microorganisms [1] and these experimental results not only agree with the findings of Atkinson and Newth, but also illustrate the importance of the apparatus when considering a biodegradability test.

When hydrocarbons are subject to microbial degradation, the major end products frequently found in the culture fluid are fatty acids, resulting in a lower pH in the growth medium. In the present study the sole source of carbon and energy for the microorganisms was the hydrocarbons, and therefore, measurement of the pH of culture fluid was followed as a convenient index of the rate of hydrocarbon degradation processes in the two biodegradation apparatuses. Figure 2 indicates again that the cyclone fermentor is superior in performance to the shaker flask in the biodegradation of hydrocarbons. With the aid of Indulin the acid production in the cyclone fermentor was very fast and the pH of the growth medium dropped from 6.9 to 3.75 within 13 hours, whereas the same medium in the shaker flask took 47 hours to reach this pH, indicating a hydrocarbon degradation rate in the cyclone fermentor 3.6 times faster than that in the shaker flask. This is comparable to the enhancement observed by following the cell yield and was probably due to the greater kerosene-water interface which reduces this growth-limiting factor which in turn governs the rate of petroleum degradation.

CONCLUSIONS

At the initiation of a biodegradation experiment, careful consideration must be given to the biodegradation apparatus so that microorganisms are given optimal conditions for the biodegradation processes. This study has shown that by using the

inexpensive cyclone fermentor in place of the standard gyratory shaker the bacterial growth rate and petroleum degradation rate in terms of acid production can be improved considerably. Consequently, the cyclone fermentor appears to offer convenience and speed and to be more suitable than the rotary shaker in the biodegradation of lipophilic substances requiring maximum contact between the microorganisms and the test substance. Other studies with this apparatus are currently underway to assess its reproducibility and the factors influencing its performance as a tool for a standard test of the degradability of new chemicals.

REFERENCES

1. Atkinson, J.H. and F.H. Newth. 1968. Microbiological transformation of hydrocarbons. in Microbiology. ed. P. Hepple. The Institute of Petroleum, London, pp. 35-45.

2. Gledhill, W.E. 1975. Biodegradation of 3,4,4'-trichlorocarbanilide, TCC, in sewage and activated sludge. Wat. Res. 9:649-654.

3. Kimerle, R.A. and R.D. Swisher. 1977. Laboratory continuous flow activated sludge unit with polyethylene porous filter candle. Wat. Res. 11:31-37.

4. Liu, D.L. and P.M. Townsley. 1972. Pulp mill lignin in continuous petroleum fermentation. J. Wat. Poll. Control Fed. 44:232-240.

5. Pitter, P. 1976. Determination of biological degradability of organic substances. Wat. Res. 10:231-235.

6. Saeger, V.W. and E.S. Tucker. 1976. Biodegradation of phthalic acid esters in river water and activated sludge. Appl. Environ. Microbiol. 31:29-34.

7. Sturm, R.N. 1973. Biodegradability of nonionic surfactants: Screening test for predicting rate and ultimate biodegradation. J. Amer. Oil Chemist Soc. 50:159-167.

8. Tobin, R.S., Onuska, F.I., Brownlee, B.G., Anthony, D.H.J. and M.E. Comba. 1976. The application of an ether cleavage technique to a study of the biodegradation of a linear alcohol ethoxylate nonionic surfactant. Wat. Res. 10:529-535.

Figure 1. The rate of bacterial growth under various conditions.

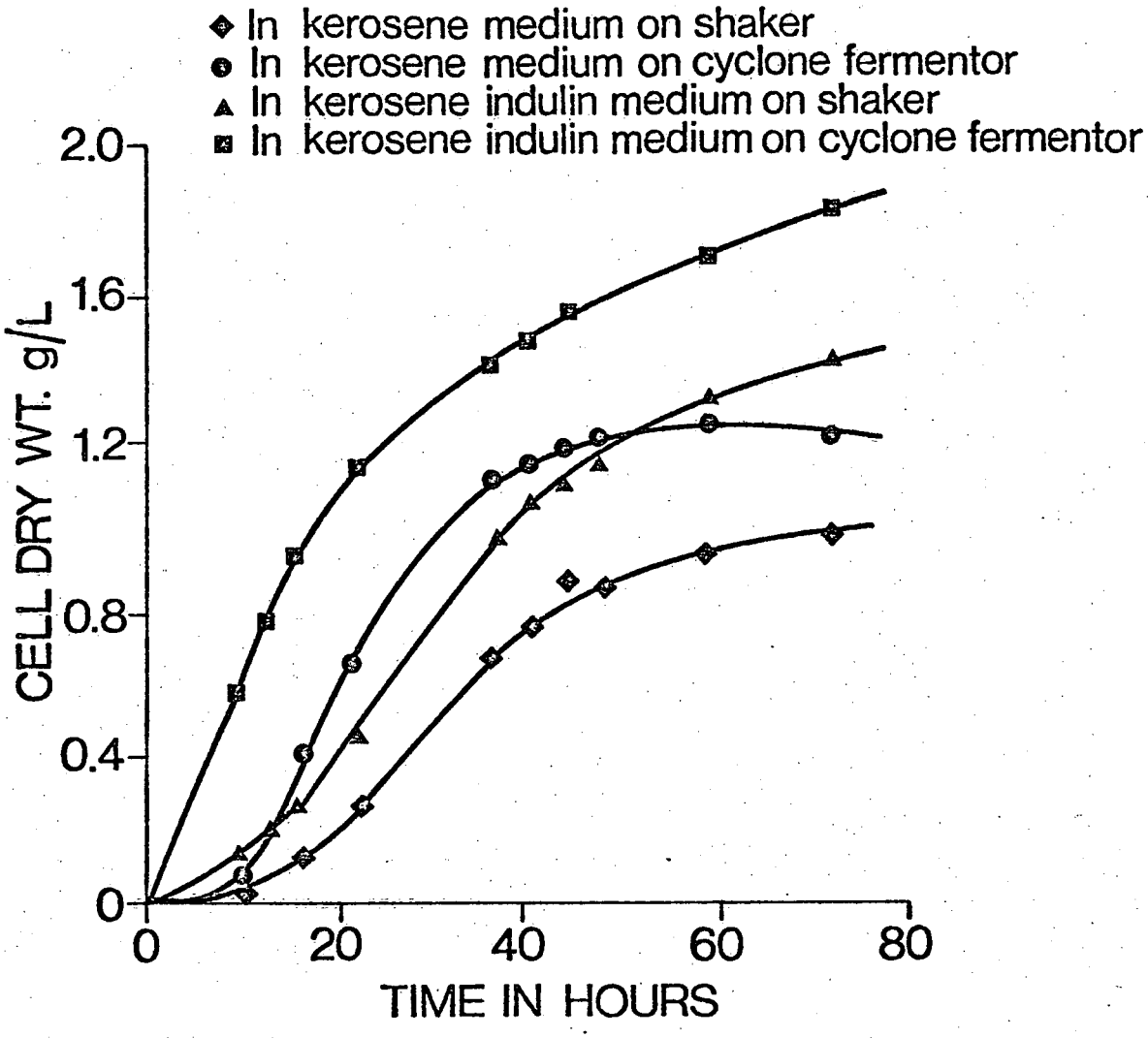
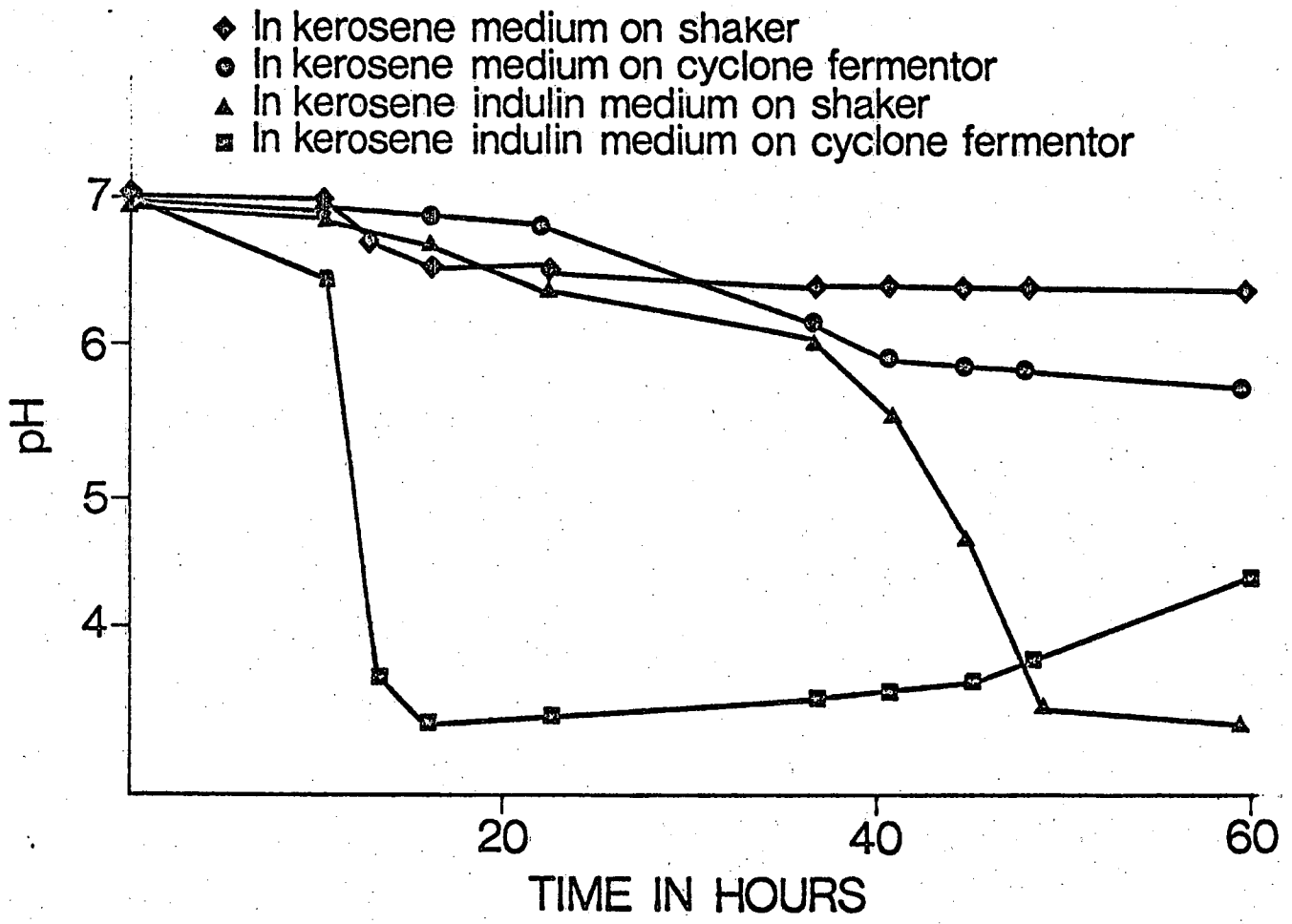

In kerosene medium on shaker
In kerosene medium on cyclone fermentor
In kerosene indulin medium on shaker
In kerosene indulin medium on cyclone fermentor
CELL DRY WT. g/L
2.0
1.6
1.2
0.8
0.4
0
0
20
40
60
80
TIME IN HOURS

Figure 2. Acid production by *P. desmolytica* under various growth conditions.


In kerosene medium on shaker
In kerosene medium on cyclone fermentor
In kerosene indulin medium on shaker
In kerosene indulin medium on cyclone fermentor
pH
7
6
5
4
20
40
60
TIME IN HOURS