APPLICATION OF ENZYME TO BIOREMEDIATE WATER FROM ETHYLENE DICHLORIDE AND OIL

BY

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This thesis, written by **MOHAMMAD** ABDULLAH MOHAMMMAD NAGGAZ JABER under the direction his thesis advisor and approved by his thesis committee, has been presented and accepted by the Dean of Graduate Studies, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN ENVIRONMENTAL SCIENCE.**

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DEDICATION

I would like to dedicate this achievement to my father, my late mother (God rest her soul) and to my wife, who took care of my home and my children during my study at KFUPM. I am sure that without her support this work would not be achieved.

In addition, I dedicate it to my children: Ghala, Sama and Moua'ad. They were my sunshine that inspired me and made me pushed myself to the limits to finish and be next to them.

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Gratitude and praise be to Almighty Allah for making it possible for me to accomplish this work successfully.

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I wish to express my thanks to all faculty members and staff of Earth Science and Chemistry Department for their support. Acknowledgement is due to the KFUPM for supporting the scientific research.

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LIST OF ABBREVIATIONS

H.C Hydro Carbon

EDC: Ethylene Dichloride

GC/FID Gas Chromatograph/ Flame Ionization Detector

GC/MS Gas Chromatograph / Mass Spectrometer

pH power of Hydrogen (negative log of hydrogen ion concentration in water-based solution.

KFUPM : King Fahd University of Petroleum and Minerals

C.O Crude Oil

US : United States

EPA: Environmental Protection Agency

VCM : Vinyl Chloride Monomer

PVC : Polyvinyl Chloride

USD: United State Dollar

U.K: United Kingdom

UV: Ultra Violet

DNAPL: Dense Non-Aqueous Phase Liquids

PAH: Polycyclic Aromatic Hydrocarbons

VOC: volatile organic compounds

TCE: trichloroethene

DCE: dichloroethene

KFUPM : King Fahad University Of Petroleum And Minerals

ABSTRACT

Full Name	Mohammad ||A. M. Ben Naggaz Jaber
Thesis Title	Application of Enzyme to Bioremediate Water From Ethylene Dichloride, Oil
Major Field	Environmental Science
Date of Degree	Aug. 2012

This project was designed at laboratory scale to study the influence of using three commercial enzymes as a bioremediation tool to treat/ recover ground water from EDC and Oil. The project designed at the laboratory to simulate the real environmental condition of ground water at semi aquifer geological structure. The objective of using enzymes is to increase the bioremediation process to treat ground water from EDC and oil by increasing the activities of the microorganisms which exist in the natural water. EDC and oil contaminate ground water as results of leaching through soil due the industrial accidents which were recorded during the previous 30 years. Due to the low Solubility of EDC in water (0.869 g/100 ml at 20ºC) and its high density, it dominated in the low layers of water and stay for long time before it treated naturally. On the other hand, oil take also long time for natural remediation.

The project/ main experiment were monitored for 8 weeks while the confirmation experiment was monitored for 6 weeks. Several parameters were monitored physically such as color and odor, while the peak areas of EDC and oil were analyzed on weekly basis by means of the GC/MS and GC/FID. Results showed that the influence of enzymes to enhance the reduction rate of the EDC, oil was promising. Two experiments conducted during the project, the 1st experiment was conducted to test the reduction rate of the EDC and oil in the presence of the three products under the designed conditions, while the 2nd experiment was conducted at lower scale to confirm the results of the main experiment. The scope of the 2nd experiment was to test the reduction rate of the EDC only under two temperatures i.e. 25°C and 37°C. As a result of the two experiments, the overall average reduction rate of the three environmental conditions was about 5% for the main experiment and 7% for the confirmation experiment at the same environmental conditions. The reduction rate of the pollutants at the 1st two weeks was high comparing with the subsequent weeks, where it reach in some times 15%- 20 % from the initial concentration. The concentration of the pollutants increased again due the breakdown of the pollutants component. One of finding is the formation of EDC layer at the bottom of the test tubes under the water and oil layers, this finding lead to a result that the EDC will settle at the bottom layers of earth crust (soil). The

physical properties of the sample i.e. color, odor did not change due to the introduction of the enzymes which means it is safer than the use of the chemical products. The pH of water was slightly moved from the acidic and bases situation to the neutral pH, which means that the enzymes help to neutralize the water pH. The effect of pH was not significant on the reduction rate of the pollutant, this result is not in line with the results of the previous studies. The results of the main experiments where the test tubes were sealed show that as the temperature and dose of the enzyme decreased as the reduction rate increase. The reduction rate at (4 °C, and 0.5 ml) was 6%, while at (37 °C and 2 ml), the reduction rate was 3% and the reduction rate was 5% at 25 °C and 1 ml dosage of enzyme. For the confirmation experiment where the test tubes were also sealed, the average reduction rate were between 3% and 5% also for the same conditions of the main experiment, however the reduction rate of the open test tubes were high comparing with the closed test tubes (between 18% and 37%). The reduction rate of the sample at 37 °C and 2 ml was 33%, while the average reduction rate of samples at 25 °C and with the same dose was 18%. The availability of the microorganisms was tested at the beginning of the two experiment and at the end of the experiment, and it shows that microorganisms are available at the beginning and at the end of the experiment.

The study provided some recommendation to enhance the research on the bioremediation of water by means of enzymes rather than Bacteria.

Finally by the end of the project, the following points were founded:

1. The project proved that the concept of using enzymes for the water treatment is a valid concept and it requires more studies from scientists and industries. The concept shows promising results need which require more improvements.
2. The use of enzymes is safer for environment since there are no consequences or byproduct as result of the treatment and recovery process and acceptable from human since there is no introduction of any more artificial products to treat the sources of drink and irrigation.
3. The efficiency of enzymes to treated water from tested pollutants (H.C and EDC) defer from product to the other, also it depends on the presence of Oxygen. The following table shows the overall reduction rate of pollutants in the tested samples under anaerobic conditions:

PRODUCT	AVERAGE REDUCTION RATE
P1	4.4%
P2	4.2%
P3	4.0%

4. The presence of oxygen (aerobic conditions), and as the temperature increase leads to speed up the process of treating water from the pollutants.

ملخص الرسالة

الاسم الكامل: محمد عبدالله محمد بن نقاز جابر

عنوان الرسالة: تطبيقات استخدام الانزيمات لمعالجة المياة حيوياً من مادة الايثلين دايكولورايد والزيت والشحوم الصناعية.

التخصص: علوم بيئة

تاريخ الدرجة العلمية: أغسطس 2012

يتناول مشروع الدراسة البحثية (**تطبيقات استخدام الإنزيمات لمعالجة المياه حيوياً من مادة الايثلين دايكولورايد (EDC) والزيت والشحوم الصناعية**) امكانية استخدام الانزيمات لتحفيز الكائنات الحية الدقيقة والتي تعيش في المياه الجوفية وذلك لزيادة كفاءتها وذلك لمعاجلة المياه الملوثة بالمواد الهيدروكربونية المذكورة في عنوان الرسالة بدلاً من استخدام البكتيريا والمعالجة الكيميائية. وقد تم تصميم والتخطيط للتجربة بهدف محاكاة الواقع الفعلي للظروف الجيولوجية والجوية الخاصة بالمياه الجوفية. وقد استخدم في الدراسة ثلاث من المنتجات العالمية المعروفة في مجال المعالجة الحيوية للمياه الملوثة وخاصاً المياه الملوثة بالزيوت البترولية في ضل توافر الكائنات الحية الدقيقة في المياه الجوفية . ويعد المصدر الرئيس لتلوث المياه الجوفية بالزيوت البترولية ومادة الـ(EDC) هو الحوادث الصناعية والتسريبات النفطية خصوصا عن النقل من مكان الى اخر . ومن الملاحظ ان مادة الـ (EDC) عادةً ما تستقر في قاع المياه الجوفية وتكون ملاصقة للأتربة التي تحتوي المياه وذلك بسبب انخفاض نسبة ذوبانها في المياه (0.869 g/100 ml at 20ºC) وارتفاع كثافتها. في حين ان الزيوت البترولية تطفو على سطح الماء بسبب انخفاض كثافتها. تم تصميم التجربة لقياس نسبة تدني المواد الملوثة للمياه عند استخدام ثلاث انواع من الانزيمات التجارية المحفزة للكائنات الحية الدقيقة (كل نوع من الإنزيمات على انفراد) على أن

تخضع لثلاث درجات حرارة مختلفة (4 درجات مئوية و 25 درجة مئوية و 37 درجة مئوية). وتحت كل درجة حرارة تم تجهيز مجموعة من العينات والتي كانت تحتوي على جرعات مختلفة من المنتجات الثلاثة (1%، 2%، 4%) من اجمالي حجم انبوب الاختبار)، اضافة الى اخضاع العينات لدرجات مختلفة من الحمضية والقلوية (pH) (4، 7، 10). وقد تم عمل تجربتين الاولى او الرئيسية حيث كانت مدة مراقبة العينات ثمانية اسابع . في حين ان التجربة الثانية (كان الهدف منها تأكيد النتائج وصلاحية اجراءات التحاليل) والتي كانت مدة مراقبة العينات 6 اسابيع. استخدم لتحليل العينات الوسائل النظرية وذلك لمراقبة التغيرات الفيزيائية مثل اللون والرائحة , في حين استخدم جهازي الـ(GC/MS & GC/FID) لمراقبة التدني في مستوى انخفاض الملوثات وذلك بشكل اسبوعي. وقد اظهرت نتائج التجربة قدرة الانزيمات على تخفيف تركيز الملوثات الهدروكرب ونيه بنسب تتراوح بين 5% (في التجربة الرئيسية) و 7% في التجربة الثانية. وقد كان من اهم النتائج عدم تأثير درجة الحمضية على عملية المعالجة في جميع العينات المستخدمة. كما ان نسبة انخفاض معدل المواد الملوثة كان يعتمد على درجة الحرارة ومقدار الجرعات المستخدمة للمعالجة من الانزيمات. حيث لوحظ انه كلما انخفضت درجة الحرارة وارتفع معدل الجرعة كلما كانت نسبة انخفاض المواد الملوثة أكبر حيث كانت نسبة تدني مستوى المواد الملوثة بمقدار 6% عند درجة حرارة 4 درجات مئوية وجرعة 2 ملل. وعلى العكس في حال ارتفاع درجة الحرارة وانخفاض ألجرعة فعند درجة حرارة 37 درجة مئوية وجرعة 0.5 ملل كانت نسبة انخفاض المواد الملوثة 3%. اما يخص نتائج التجربة الثانية فقد كانت مماثلة في النتائج للتجربة الاولى فيما يخص العينات المحكمة الاغلاق. اما ما يخص العينات الغير مغلقة والتي كانت تحت الظروف الهوائية (aerobic conditions) فقد كانت النتائج معاكسة للنتائج الخاصة بالعينات المغلقة . حيث لوحظ ارتفاع نسبة انخفاض المواد الملوثة عند ارتفاع درجة الحرارة وارتفاع معدل الجرعة حيث كانت نسبة انخفاض

الملوثات في درجة حرارة 37 درجة مؤوية وجرعة 2 ملل 33%. وانخفاض معدل نسبة تدني المواد الملوثة (18%) في درجة حرارة الغرفة وفي حال كانت الجرعة 0.5 ملل. ولكن بشكل عام تكون نسبة تدني الملوثات في كلتا الحالتين الخاصة بالعينات المفتوحة هي ضعف افضل حالات الانخفاض في نسبة الملوثات في العينات المغلقة . ومن الملاحظات الهامة هو عدم تغير لون او رائحة العينة مع مرور وقت التجربة . كما لوحظ ان نسبة انخفاض الملوثات في الاسبوعين الاولى من التجربة كانت عالية والتي تصل الى 20% في حين تتدنى في الاسابيع اللاحقة.

وبذلك اثبتت الدراسة نجاح وفاعلية مبدأ استخدام الانزيمات كمواد للمعالجة الحيوية بدلاً من استخدام المعالجة الكيميائية. وقد اعطت الدراسة نقطة انطلاق لباحثين في المستقبل لإجراء دراسات اوسع في ضل الظروف الجيولوجية المحيطة بالمياه الجوفية الخاضعة للدراسة اضافة الى بعض التوصيات الخاصة بتحسين اداء الانزيمات عملياً.

خرج المشروع في نهاية بالنتائج الرئيسية التالية:

1. اثبت المشروع نجاح استخدام الإنزيمات كوسيط لعملية معالجة المياه من المواد الملوثة (H.C and EDC). وقد خرجت التجربة المعملية بنتائج مشجعة ولكنها تحتاج إلى مزيد من الدراسة والبحث العلمي من قبل العلماء المختصين بالمجال البيئي والشركات العاملة في هذا المجال.
2. يعتر استخدام الإنزيمات كمادة محفزة للكائنات الحية الدقيقة التي تتغذى على المواد الملوثة ا من واسلم للبيئة وللصحة العامة، حيث لم تظهر أي مواد أو مخرجات ثانوية مضرة بالبيئة أو الصحة العامة، بالإضافة إلى أن النتائج النهائية أظهرت اتجاه درجة الحمضية إلى الاعتدال بعد أن كانت درجة الحمضية عالية أو منخفظه في بداية التجربة.

3. فعالية الإنزيمات المحفزة للكائنات الحية الدقيقة لمعالجة المياه من المواد الملوثة، تختلف من منتج الى آخر . ويوضح الجدول التالي متوسط نسبة انخفاض المواد الملوثة بنهاية التجربة العلمية في ظل محدودية الأكسجين خلال فترة التجربة:

PRODUCT	AVERAGE REDUCTION RATE
P1	4.4%
P2	4.2%
P3	4.0%

4. يعتبر توفر الأكسجين وارتفاع درجة الحرارة من أهم العوامل المساعدة على تسريع عملية المعالجة الحيوية للمياه الملوثة.

CHAPTER 1
INTRODUCTION

1.1 BACKGROUND

1.1.1 OIL/ CRUDE OIL

Crude oil /Petroleum is a naturally occurring flammable liquid consisting of a complex mixture of hydrocarbons and other liquid organic compounds, that are found in geologic formations beneath the Earth's surface. A fossil fuel, it is formed when large quantities of dead organisms are buried underneath sedimentary rock and undergo intense heat and pressure(40).

Petroleum is recovered mostly through oil drilling. This comes after the studies of structural geology, sedimentary basin analysis, reservoir characterization It is refined and separated, most easily by boiling point, into a large number of consumer products, from petrol (or gasoline) and kerosene to asphalt and chemical reagents(40),(48).

Petroleum is a fossil fuel derived from ancient fossilized organic materials. Vast quantities of these remains settled to sea or lake bottoms, mixing with sediments and being buried under anoxic conditions as further layers settled to the sea or lake bed, intense heat and pressure built up in the lower regions. This process caused the organic matter to change, first into a waxy material known as kerosene and then with more heat into liquid and gaseous hydrocarbons.

The largest volume products of the oil industry are fuel oil and petrol. Petroleum is also the raw material for many chemical products. Oil is vital to many industries,

and is of importance to the maintenance of industrialized civilization itself. Oil accounts for a large percentage of the world's energy consumption, ranging from a low of 32 per cent for Europe and Asia, up to a high of 53 per cent for the Middle East, South and Central America (44%), Africa (41%), and North America (40%). The world at large consumes 30 billion barrels of oil per year(40) , (34), (44).

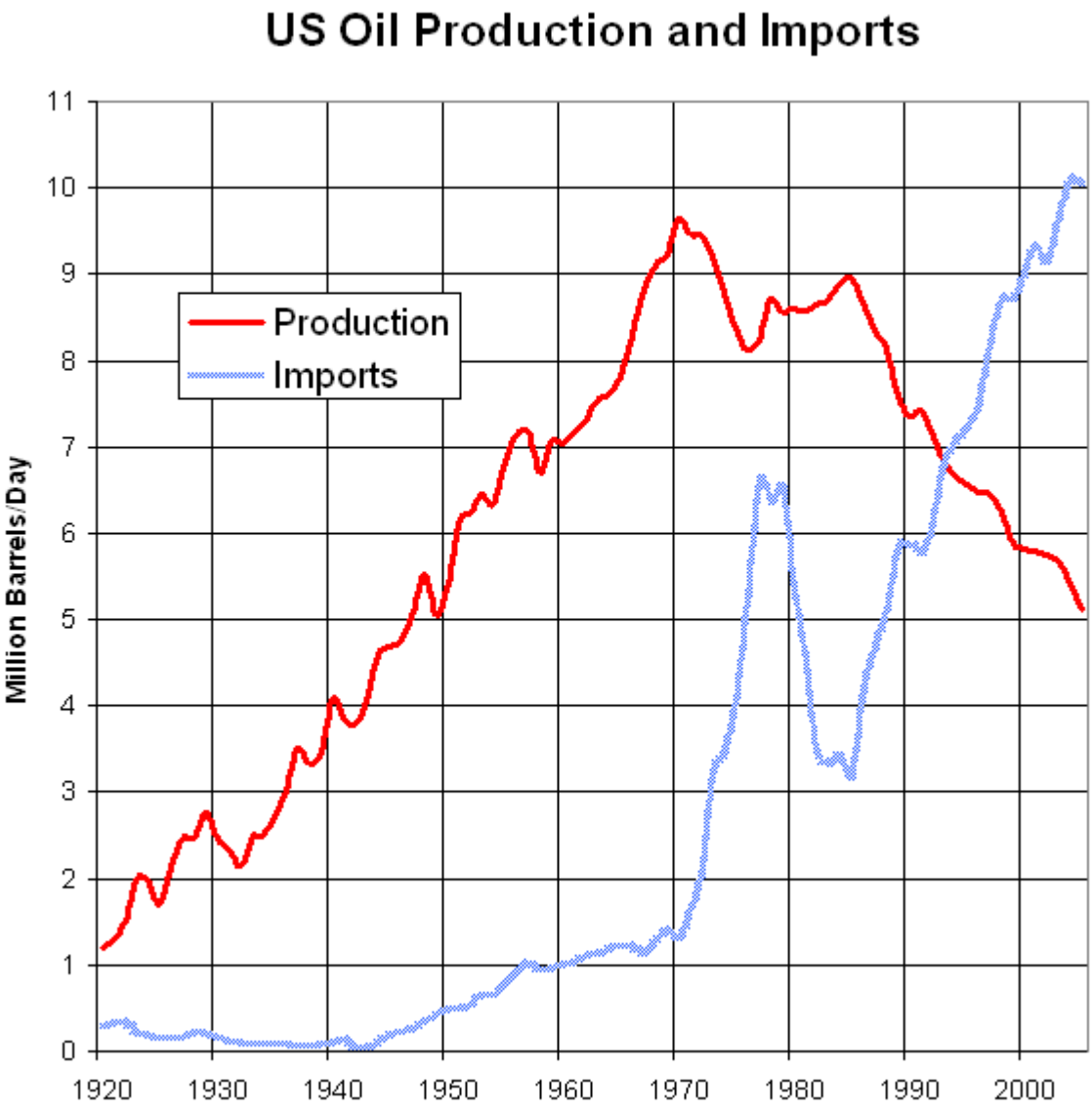


Figure 1.1 US oil production and import from1920 to 2000

Source: U.S. Energy Information Administration

Oil is used as fuel for engine motors such as cars, airplane and also it used to produce other products such as lubricants, wax, sulfur (45).

The main accidents of oil are the oil spill where 40% of the accidents occurred during the loading or discharging. The top 20 major oil spills recorded between 1970 and 2002, 95% occurred in the 1970s, 1980s and 1990s, and only 5% occurred in the 2000s. A number of these incidents, despite their large size, caused little or no environmental damage as the oil was spill some distance offshore and did not impact coastlines. Most of the recorded accidents, the longest radiance time that spilled oil appears to have had in the marine and costal environment was less than a decade(36), (49).

The major ecological impact has come at the time of the spill occur within the first few months after that most oil has been reduced to tarry residues or was chemically detectable by sediments organisms. The short term impact of a major spill can be devastating to the organisms in the immediate vicinity including shellfish, finfish and waterfowl (36). Another impact of oil to the environment occurred due to the burning of fusel fuel which results in the globule warming (36).

1.1.2 ETHYLENE DICHLORIDE (EDC)

1,2-Dichloroethane (DCE) or "Dutch oil" in old chemistry, commonly known by its old name of ethylene dichloride (EDC). EDC was introduced to as a new chemical product in 1794 by a group of scientists is used to produce vinyl chloride

monomer (VCM). It is a colorless liquid with a chloroform odor. 1,2-Dichloroethane is also used generally as an intermediate for other organic chemical compounds and as a solvent (19); (40).

Figure 1.1 Chemical formula of 1,2-dichloroethane (EDC)

EDC Production is primarily achieved through the iron(III) chloride-catalyzed reaction of ethane (ethylene) and chlorine.

$$H2C=CH2 + Cl2 \rightarrow ClCH2\text{-}CH2Cl$$

1,2-dichloroethane is also generated by the copper(II) chloride-catalyzed "oxychlorination" of ethylene:

$$2\ H2C=CH2 + 4\ HCl + O2 \rightarrow 2\ ClCH2\text{-}CH2Cl + 2\ H2O$$

In principle, it can be prepared by the chlorination of ethane and, less directly, from ethanol (34); (40).

The ethylene dichloride (EDC) industry relies largely on the total PVC demand as it contributes substantially to the EDC consumption expansion. The projected annual growth rate of the EDC market is expected to reach up to 3.5-4% in the coming 5 years. The global ethylene dichloride production is expected to grow at a

CAGR of 2.3 percent from 2009 to 2020. According to the Global Business Intelligence (GBI) Research report "Ethylene Dichloride (EDC) Global Supply Dynamics to 2020" offered by Research and Markets, the global ethylene dichloride production is expected to grow at a Compound Annual Growth Rate (CAGR) of 2.3 percent from 2009 to 2020. In 2000, global EDC production was 27.2 million tons and grew at a CAGR of 0.1 from 2000 to 2009. Global EDC capacity in 2009 was 42.6 million tons (19); (42).

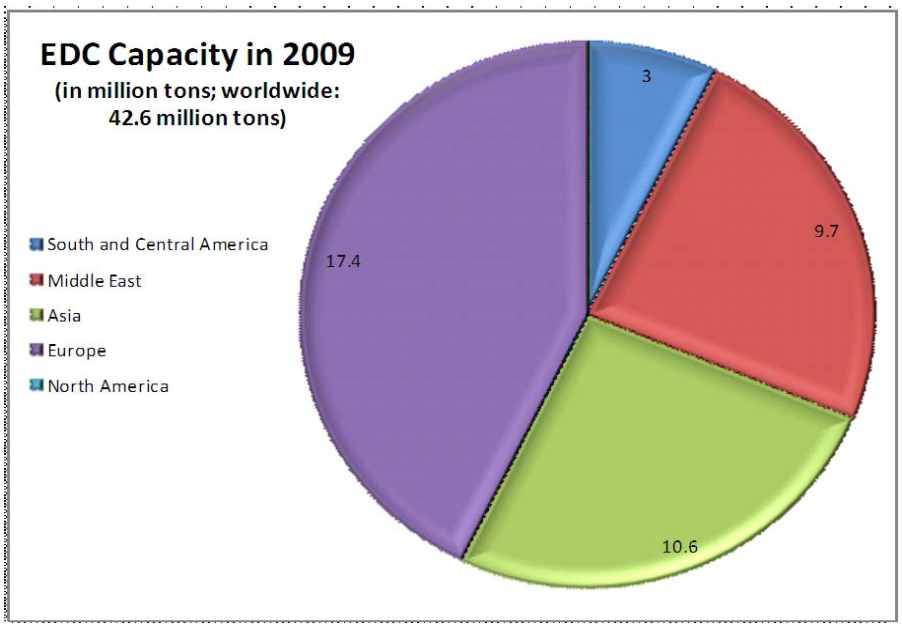


Figure 1.3 EDC capacity in 2009 Source: www.process-worldwide.com/management/markets_industries/articles/299124/

Ethylene dichloride is a heavy, oily, liquid which burns with a smoky flame. Usually it is colorless but it will darken in the presence of air, moisture, and light. It has a pleasant chloroform-like odor and irritating vapors. Ethylene dichloride is

slightly soluble in water and miscible with alcohol, chloroform. When in heated water, ethylene dichloride will corrode iron and other metals (38); (9).

Table 1.1 summarize the physical and chemical properties of EDC as classified by US-EPA and different MSDS.

EDC was commonly used as a gasoline additive to scavenge inorganic lead compounds. The transition to the use of lead-free gasoline has essentially eliminated the use of EDC as a fuel additive (46); (42); (40). With approximately 95% of the world's consumption of the EDC is used in the VCM with hydrogen chloride as a byproduct. The hydrogen chloride can be re-used in the production of more EDC via the oxychlorination (42).

EDC also can be used as a good solvent. EDC is used as paint remover. As a useful 'building block' reagent, it is used as an intermediate in the production of various organic compounds. In the laboratory it is used as a source of chlorine, with elimination of ethene and chloride. Historically, EDC was used as an anti-knock additive in leaded fuels (37); (38).

The total emissions of ethylene dichloride from stationary sources in California are estimated to be to be at least 26,000 pounds per year (43).

Table 1.1 Physical and chemical properties of EDC

PHYSICAL PROPERTIES		CHEMICAL PROPERTIES	
Description	Clear, colorless, oily liquid	Molecular formula	C2H4Cl2
Density	1.2351 g/cm3 @ 20ºC	Molecular weight	98.97 g/mol
Boiling point	57.4ºC		
Melting point	-96.9ºC		
Vapor pressure	64 torr @ 20ºC		
Solubility	Slightly soluble in water (0.869 g/100 ml at 20ºC); miscible with alcohol; soluble in ordinary organic solvents		

Sources:www.sabic.com/corporate/ar/productsandservices/chemicals/edc.aspx (December 14, 2012).

From 1990 till 2011, 27 accidents were recorded in the US. These accidents resulted from processing failures (50%), transportation accidents (40%), pipeline/ storage leakage (10%). The affected sphere from these accidents of the environment is the soil (about 70%), while atmosphere is affected with about 15% and the rest for water phase (rivers and ground water). These accidents resulted in no fatalities/ injuries. However, these accidents resulted in loss of about USD 58,000,000. This amount includes the direct cost (immediate corrective actions, shut down of factories, equipment repair, etc…) and indirect cost (investigation, environmental remediation, failure repair, consultation, penalties, etc…) (42).

The U.S. EPA and The International Agency for Research on Cancer have classified EDC in Group B2: Probable human carcinogen. The U.S. Department of Health and Human Services states that EDC is "reasonably anticipated to be a human carcinogen" in its "Report on Carcinogens,". Other public health agencies list EDC as a probable human cancer-causing agent. The State of California lists EDC as a chemical "known to the state to cause cancer." In addition, EDC exposure can result in serious and permanent damage to the heart, central nervous system, liver, kidneys, lungs, gastrointestinal system, eyes, and skin, and commonly results in depression, memory loss, and adverse personality changes (43).

Probable human exposure to EDC occurs through inhalation and ingestion. EDC is toxic (especially by inhalation due to its high vapor pressure), highly flammable, and carcinogenic. Its high solubility and 50-year half-life in anoxic aquifers make it a perennial pollutant and health risk that is very expensive to treat conventionally, requiring a method of bioremediation (19).

The U.S. EPA estimates that if an individual were to breathe air containing ethylene dichloride at 0.04 μg/m3, over a lifetime, that person would theoretically have no more than a 1 in 1 million increased chance of developing cancer (43); (19).

1.2 OBJECTIVES

The main objectives of the study are:

1. To study the performance of three commercial Enzymes to reduce the concentration of EDC, O&G in water in three environmental conditions (high temperature, room temperature and low temperature) & compare its performance with performance of other enzymes at the same environmental conditions.
2. To compare the water quality before and after the bioremediation for each enzyme and compare it with the national and international regulations.
3. Suggest solutions to increase the performance of Enzyme and maintain water quality.

CHAPTER 2

LITERATURE REVIEW

The quality of life on the earth is linked to the overall of environment quality. Previously, people believed that they had an unlimited abundance of land and resources, however in these days; the resources show our carelessness and negligence in using them(7). Pollution of the biosphere with toxic elements has been accelerated dramatically since the beginning of the industrial revolution. Toxic metal pollution of water has become a major environmental problem. Due to their non-biodegradable nature and bio magnification through the food chain, heavy metals are adversely affecting the human health. The primary sources of these pollutants are the burning of fossil fuels, mining, municipal wastes, including sewage, fertilizers, pesticides, and industrial effluents (17).

Vast number of pollutants and waste materials (chemical waste) are disposed into the environment per annum. Approximately 6 x 10^6 chemical compounds have been synthesized. More than 450 million kilograms of toxins are released globally in air and water. The contaminants causing ecological problems leading to imbalance in nature is of global concern. The environmentalists around the world are trying to overcome it by several means. The traditional methods of treating the contaminated sites are physical, chemical and thermal processes (7),(3).

Below a summary of the latest litterers dealt with the treatment techniques of chemical pollutants in the water environment.

2.1 Treatment techniques used for chemical waste and oil spill in water environment

Contaminated water environments could be treated using any of physical, chemical and biological techniques singly or in combination in order to achieve a safe contamination level (55). The choice of treatment techniques depends on many factors such as the characteristics of the site, the type of contaminants, the cost, the time constraints, among other factors (43); (55); (56).

The physical and chemical remediation techniques are often used together and they include air sparging, electrokinetics, pump and treat, permeable reactive barriers, ultraviolet-oxidation method, adsorption/membrane filtration, etc. while the biological techniques include natural attenuation, phytoremediation and bioremediation (57); (55); (56).

Vapor extraction is a cost-effective remediation technique that has been demonstrated to be successfully used for the treatment of volatile organic compounds (VOCs) from the vadose (unsaturated) zone. However, it has been considered inadequate to be solely used in the remediation of spills involving that was found in the saturated zone. Air sparging has been found to be effective in the remediation of DNAPLs present in the saturated zone (24).

Electrokinetics has been used as a remediation technique for the treatment of heavy metals, radionuclides, and organic contaminants found in the saturated and unsaturated zone (43); (47), (48). It has also been reported to be successfully used for the remediation of salt-impacted groundwater generated from produced water spills (49).

The pump and treat techniques are among the most widely remediation technologies for the treatment of contaminated groundwater. Palmer and Fish evaluated the effectiveness of this technique at Superfund sites and stated that the effectiveness of the method is affected by the lengthy period of time required to achieve cleanup and the great cost of cleanup. They then suggested the use of chemical enhancement methods for remediation of certain sites (50).

A low-cost alternative to the use of pump and treat is the use of permeable reactive barriers which are installed across the flow path of contaminant. The permeable reactive barriers have been used for the remediation of organic contaminants to a very large extent however, their use for inorganic contaminants are not as extensive. The successful use of this technique for up to 99.9% removal of Uranium, an inorganic contaminant, was demonstrated by EPA (43).

A wide variety of organic and explosive contaminants as well as microorganisms such as Salmonella and Escherichia Coli have been reported to have been treated using the ultraviolet-oxidation method. UV/oxidation is a destruction process that treats contaminants through the synergistic action of high intensity UV light alone, or in combination with oxidants such as ozone and hydrogen peroxide (47); (51).

The use of adsorption technologies is well established for the treatment of contaminants. Different types of adsorbent materials have been successfully used for remediation. For example, the use of organo-clay as an adsorbent material for petroleum hydrocarbons is well studied (59). Activated carbon is another adsorbent that has been extensively used for remediation and it is one of the best adsorbents used for organic pollutants and explosives (60). Frank and McMullen (1996) developed a new technology that combined adsorption with filtration which was used to remove chromium from groundwater and wastewater. In this technology, Granular activated carbon (GAC) filtration was used to remove chromium in its trivalent and hexavalent states.

Natural attenuation relates to all natural processes that work together to limit and contain the spread of contamination (60). It is often relied upon to clean up residual contamination after the initial remediation action. Since the subject of this project is the use of enzymes to treat contaminated water from chemical pollutants, the remaining part of this chapter will focus in water treatment by means of the biological techniques from oil spill and EDC accidents.

2.2. Water Bioremediation

Bioremediation is defined as the use of microorganisms for the treatment of contamination either by degradation or transformation (2), (52) and comprehensively defined bioremediation as the technique of accelerating the natural biological degradation process of organic contaminants by microorganisms

through the supply of nutrients to contaminated environments or through the manipulation of the contaminated media by supplying air or controlling the temperature which then breaks the contaminants into harmless or less-harmful substances. It is also defined as the process which uses microorganisms to break down contaminants under controlled conditions into less harmful forms or to levels below the established concentration limits (7). It is also defined as the technique of eliminating, attenuating or transforming contaminants using biological processes (3).

The concept of using microorganisms for degradation is not new because there was evidence of compost piles which involved microorganisms as far back as 600 BC by Romains to treat wastewater, and in 1891, there was the establishment of the first biological sewage treatment plant in Sussex, UK (53). Since the 1940s, it had been known that microorganisms were able to degrade petroleum hydrocarbons through the studies conducted by microbiologists (52); (5). The first commercial use of a bioremediation system was in 1972 to clean up a Sun Oil pipeline spill in Ambler, Pennsylvania (National Research Council (47). Since 1972, bioremediation has become a well-developed way of cleaning up different contaminants. A survey prepared by the Environmental Protection Agency in 1992 received information on 240 cases of bioremediation in the United States (Alexander 249). Most of these cases involved treating contaminated soil or groundwater.

The basic idea that both lower as well as higher plants can be used for environmental remediation is certainly very old. For example, the knowledge that aquatic and semi-aquatic vascular plants can take up Pb, Cu, Cd, Fe, and Hg from contaminated solution has been around for a long time. Recently, other aquatic macrophytes have been reported as hyperaccumulators of Cr, Cd, Mn, Hg, Pb, Fe, and Cu. These aquatic plants can tolerate high levels of heavy metal concentration by sequestering them. (Bioremediation of contaminated water bodies)

Chapelle (1999) reported that bioremediation of petroleum hydrocarbons became an accepted remediation technology in the early 1970s and was actively considered as a remedial strategy to clean up the petroleum hydrocarbon-contaminated ground water system at the Whitemarsh Township site in Pennsylvania. However, bioremediation did not become known to a broader public in the United States as a technology for cleaning up oil-contaminated shorelines until in the late 1980s, and this attention was as a result of the Exxon Valdez oil spill of 1989 in Prince William Sound, Alaska (52). The word "bioremediation" however, did not appear in peer-reviewed scientific literature until 1987 (53) and in the years since 1989, articles on bioremediation have been found in scientific journals, trade journals for hazardous waste and environmental industries, and in the popular press (52).

Bioremediation systems are run under both aerobic and anaerobic conditions; however, most are under aerobic conditions (3). Bioremediation processes are carried out by bacteria, fungi and algae which are ubiquitously distributed in the soil and water environments (33); (53). When evaluating the use of bioremediation

for cleanup, there are certain critical factors that must be considered. First consideration is given to the factor relating to the contaminant itself, such as the magnitude or extent of contamination, the toxicity and concentrations of contaminants, the degradability of the contaminants and the mobility of contaminants (2); (3). Consideration is also given to the proximity of human and environmental receptors to the contaminants and the risks posed by the contaminants as well as the ability to properly monitor the process of bioremediation (2).

Bioremediation has been used for the treatment of recalcitrant organic compounds such as polycyclic aromatic hydrocarbons (PAHs); benzene, toluene, ethylbenzene, and xylenes (BTEX); and pesticides and herbicides; explosives (43) and chlorinated volatile organic compounds (VOCs) such as tetrachloroethene (PCE), trichloroethene (TCE), and dichloroethene (DCE). It has also been used for the treatment of chlorinated aliphatic compounds such as vinyl chloride and dichloroethane (DCA) (38); cyanide (21); metalloids and heavy metals (57); (58).

Bioremediation has been applied to different types of contaminated environments and environmental conditions. It has been applied to contaminated soil, sediment, sludge, wetland, surface water, ground water, waste water, ocean (4); (54); (32) and even cold environments (27); (11); (28); (29).

2.3. Types of Bioremediation

On the basis of whether the contamination is treated on-site or off-site, bioremediation can be broadly classified into two, which are in situ bioremediation and ex situ bioremediation (26). In situ bioremediation involves treatment of the contaminants at the site of contamination whereas; ex situ bioremediation involves the treatment of contaminated material after it has been physically removed from the site of contamination. On the basis of the fate of the contaminants, there are three classifications; which are biotransformation, biodegradation, and mineralization (53). Biotransformation involves the conversion of contaminants into less or nonhazardous form; biodegradation involves the breakdown of contaminants into smaller parts; while mineralization is the complete biodegradation of organic contaminants into inorganic constituents such as carbon dioxide or water. Bioremediation can also be classified on the basis of whether there is human intervention or not into two broad categories; which are intrinsic bioremediation and engineered bioremediation (2); (5). In intrinsic bioremediation, the natural microbial processes are allowed to degrade the contaminants; whereas in engineered bioremediation, the microbial activities are enhanced by certain processes such as biostimulation or bioaugmentation. Biostimulation has to do with the addition of materials such as nutrients, oxygen or other electron donors and acceptors to the contamination site in order to increase the population or activity of naturally occurring microorganisms available for bioremediation; bioaugmentation,

on the other hand, involves the addition of microorganisms to the existing ones in order to enhance the degradation (53).

2.4. Advantages and Limitations of Bioremediation

The use of bioremediation has numerous advantages (2); (26); (54) which include:

- It is natural process and is therefore perceived by the public as an acceptable waste treatment process for contaminated material.
- It is good tool for complete destruction of a wide variety of contaminants. Moreover, the results of the bioremediation process usually are harmless.
- No need to transfer the contaminated meads from sphere to another sphere, the process will tack place in the same place.
- Possibility of complete conversion of contaminants to nontoxic byproducts.
- Bioremediation requires minimum mechanical equipment/ analysis and mentoring.
- Bioremediation requires lower cost when compared to other remediation technologies.
- It has relative ease of implementation
- It is nonintrusive, therefore allowing for continued use of site
- It has greater public acceptance because it does not disturb the natural

surroundings of the site

- It can be used in combination with other physical or chemical treatment methods.

Bioremediation has its own limitations (disadvantages), however these disadvantages are accepted in some cases comparing with the disadvantages of other types of remediation. The main disadvantages are (2); (26); (54):

- It may be difficult to control and may require more extensive monitoring. Since the bioremediation products will spread over the contaminated site and the nearby sites.

- It may not reduce concentration of contaminants to the required levels depending on the environmental conditions and contamination factors.

- It requires more time than conventional treatment methods, the time includes the preparation, operation and closing the bioremediation process.

- In some cases, microbial metabolism of contaminants may produce toxic products which have higher impact to the environment than the parent compounds.

2.5. Factors affecting Bioremediation

The control and optimization of bioremediation processes is a complex system of many factors Scientists divided the factors affecting bioremediation into scientific

factors, non-scientific factors and regulatory. The scientific factors include the sources of energy for microorganisms, the bioavailability of contaminants, and the bioactivity and biochemical activities of the microorganisms. The non-scientific factors include regulatory factors, research and technical factors, human resource factor, and economy and liability factor. (26).

Factors affecting bioremediation can also be broadly divided into two which are biological factors and environmental factors (2). The biological factors include the rates and extent of contaminant degradation; general indicators and microbial physiological factors such as nutrient availability and C:N:P ratios; and effects of temperature, moisture and pH. The environmental factors include the geologic and hydrogeologic factors, bioavalability, soil matric potential, and redox potential (2). Other important factors affecting the bioremediation process include: the existence of a microbial population capable of degrading the pollutants; the availability of contaminants to the microbial population (7).

In general, bioremediation process takes long time, more sensitive and required more control in the cold environment due to the complexity and variability of climate, population, permafrost, and environmental sensitivity in the cold regions. However, hydrocarbons can be degraded by microorganisms when major factors, such as nutrient availability (including oxygen), organic compound bioavailability, and temperatures are optimized (29).

2.6. Enzymatic Bioremediation

Owing to the limitations of microbial bioremediation by factors such as nutrient level of contamination site, low bioavailability of contaminants, aeration and because microbial bioremediation relies on the growth of microorganisms to metabolize the contaminants which makes it to be generally slow (23); enzymatic bioremediation has been borne as an alternative technique to offer support to microbial bioremediation (10). Enzymatic bioremediation involves the use of enzymes rather the microorganisms to degrade contaminants. Microorganisms that degrade contaminants use enzymes to carry out the degradation and this makes the concept of enzymatic bioremediation an extension of microbial bioremediation; since it just requires identifying the enzymes responsible for the degradation (10). For example, the degradation of trichlorophenol (TCP) by bacteria is initiated by the enzymes FADH2-utilizing monooxygenases (Pieper et al., 2004); enzymatic bioremediation of TCP is much more rapid than its microbial remediation. Enzymatic bioremediation is advantageous because it can be used to treat recalcitrant contaminants, it can be operated at high and low contaminant concentrations over a wide range of pH, temperature and salinity (2); (23).

Both in situ and ex situ bioremediation can be enhanced with enzymatic processes; however, in situ enzymatic bioremediation may be less effective because of the difficulty of providing optimum environmental conditions (16). Hydrolases, dehalogenases, transferases and oxidoreductases are the most representative enzymatic classes used for the remediation of contamination (23). Members of

these classes of enzymes that have been extensively used for bioremediation include phosphotriesterases, carbohydrases, amidases, proteases, depolymerase, mono- or di-oxygenases, reductases, dehalogenases, cytochrome P450 monoxygenases, peroxidases and phenoloxidases (16).

Enzymatic bioremediation has very numerous applications and has been used extensively for the treatment of a wide range of contaminants. Sutherland developed enzymes that were used for the treatment of pesticide residues resulting from agricultural production and processing industries. Chlorinated triazine herbicides, for example, atrazine contamination from agricultural activities have also been reported to have been successfully treated using free enzymes (33). Ligninolytic enzymes from white-rot fungi have been used for the treatment of oil-contaminated soil and to degrade polycyclic aromatic hydrocarbons (16); (12). Enzymatic bioremediation has also been used for the treatment of waste water from different industries (12). It has been used for the detoxification of organophosphorus, carbamate and pyrethroid insecticides (13); (23). It has also been used for the remediation of petroleum products (25), phenols and polychlorinated biphenyls (16).

Many organizations and companies are into production of commercial enzymes used for bioremediation. The Commonwealth Scientific and Industrial Research Organisation (CSIRO) developed enzymes that were used for the treatment of off-farm water contaminated by atrazine and pesticide residues (CSIRO, 2009). The CSIRO's enzyme-based products known as Landguard are claimed to be able to

remove 90 %organophosphates from irrigation wastewater in 10 minutes. EZ-Enzyme is another commercial enzyme that has been extensively implicated for the bioremediation of organic pollutant (1).

2.7. Oil and Ethylene dichloride accidents

From 1990 till 2011, 27 accidents were recorded in the US. These accidents resulted from processing failures (50%), transportation accidents (40%), pipeline/ storage leakage (10%).

Oil accidents in the form of oil spills are inevitable events at all phases of oil and gas field development. Oil transportation by tankers and pipelines has the largest percentage of accidental oil input into the sea while drilling and production activities have minimal contribution (36). A reduction in the total number and volume of oil spillage tankers has been observed since 1970s, though, this does not necessarily indicate a general downward in the number of oil spill (9). Improved production technologies and safety training of personnel have dramatically reduced accidental spills from platforms to about 3% of petroleum inputs worldwide (62). In USA the overall, oil spillage down inland waterways under EPA response jurisdiction spill prevention programs and regulation. The oil spills of more than 500 gallons were decreased from 1982 to 2012 to more than 60 %.(Trend Analysis of oil spill).

Oil spill accidents have negative environmental impacts and show one of the most complex and dynamic patterns of pollutant distribution and impact in the marine

environment (20). These impacts include impacts on seabirds, benthic organisms and fishes (49). In addition to the economical and life style of people impacts of oil spills, the oil spill have major impacts on the seabirds life cycle, benthic organisms under marine environment and beneath ground water.

The majority of EDC accidents accrued due to transportation (80%) while the remaining reported accidents accrued due to operational failure. (17), (18).

A number of accidents involving ethylene dichloride have been reported as far back as 1967 in every part of the world (FACTS). One of these EDC accidents involved an explosion where 13 employees and 23 co-workers were injured (OSHA).

The long term impact to human health and water quality is the major concerns of EDC accidents.

CHAPTER 3

MATERIAL AND METHODS

This experiment was conducted to test the redaction rate of two pollutants i.e. EDC and HC that lays in the range between C20 to C40. The preparation and designed of the experiment based on:

1. The academic experience of the theses committee.
2. Filed experience of different projects in Jubail industrial area.
3. Bioremediation work procedures that received from different manufacturers.

3.1 Materials:

The following material has been used to conduct the experiment:

- 82 Plastic Test Tubes (50 ml)

  This type of test tubes is used to provide good visibility to the contents of the samples and avoid the broken in case if the set fail from the shakers.

- pH Meter.

  The pH meter will be used to observe the change in the acidity of the samples according to the experimental procedures at the beginning of the experiment and at the end.

- 2 Shakers

  The shaker was used to keep continues movement of the samples over the observation period.

Figure 3.1 shaker

- Refrigerator

  The refrigerator was used to keep the samples at 4 °C.

- Environmental chamber

  The environmental chamber was used to keep the samples at 34°C.

- GC/ MS with Sampler GC 80 and GC/ FID with Autosampler 7693

  The GC/MS and GC/FID were used to measure the peak areas of the EDC and HC in the 82 samples. More details about GC/MS and GC/FID are in section 3.3.

- Balance (Electronic)

  The electronic balance was used to help in the identification of the dose of the solid Bioremediate product

- Microscope.
- Sterilized petridish
- 1000 vials (5 ml).

3.2 Solutions and chemicals:

The following chemicals have been used through the experiment:

- Light Arab oil
- Ethylene dichloride
- Distilled water.
- Fresh water.
- Enzymes (commercial bioremediation products).
- NaOH
- HCl

3.3 Instruments

- Gas Chromatograph/ Flame Ionization Detector (GC/FID) (Agilent Technologies Model 7890A) Figure 3.2

  The Flame Ionization Detector (FID) is the most widely and successfully used Gas Chromatographic (GC). FID detector used to analyzing volatile hydrocarbons and other carbon containing compounds. GC /FID first developed in 1957 by scientists working for the Commonwealth Scientific and Industrial Research Organization in Melbourne, Australia. Many in the industry believe its sensitivity is so powerful it is without parallel among Gas Chromatographic (GC) detectors.

Since the FID is mass sensitive, not concentration sensitive, changes in carrier gas flow rate have little effect on the detector response. It is preferred for general hydrocarbon analysis, with a detection range from 0.1ppm to almost 100%.

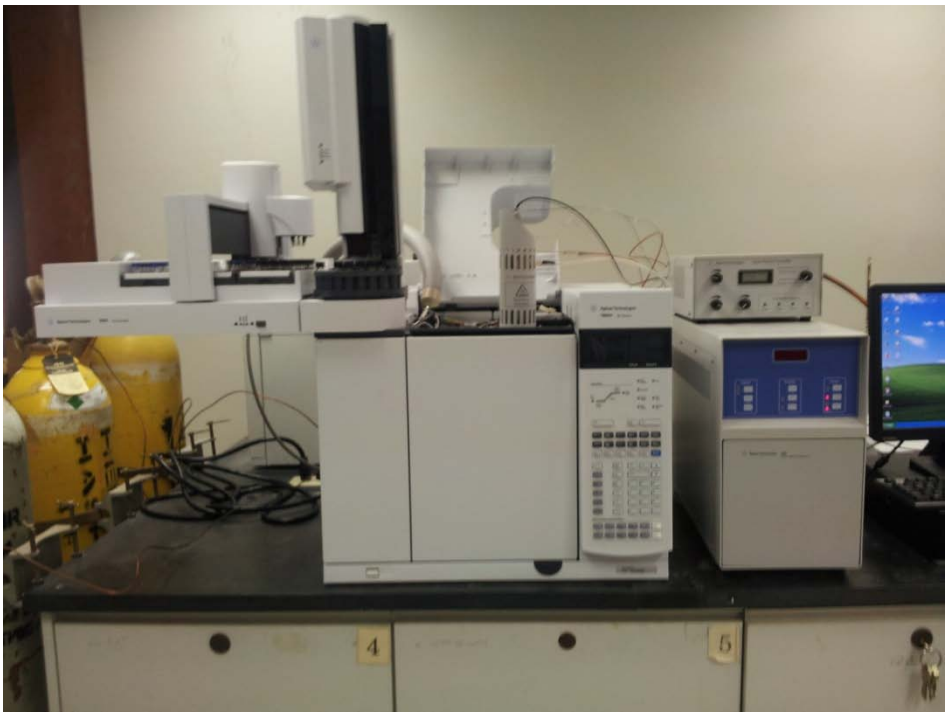


Figure 3.2 Gas Chromatograph Model 7890A/ Flame Ionization Detector (GC/FID) (Agilent Technologies)

- Gas Chromatograph / Mass Spectrometer GC/MS Agilent Technologies Model 7890A) Figure 3.3
- GC/MS is a method that combines the features of gas chromatography and mass spectrometry to identify different substances within a test sample. GC/MS has been widely accepted as a "gold standard" for chemical identification of volatile and semi-volatile organic compounds in mixtures, drug detection, environmental analysis, explosives investigation, and

identification of unknown samples. Additionally, it can identify trace elements in materials that were previously thought go undetected by other technologies.

- The GC/MS is composed of two major building blocks: the gas chromatograph and the mass spectrometer. The gas chromatograph utilizes a capillary column which depends on the column's dimensions (length, diameter, film thickness) as well as the phase properties (e.g. 5% phenyl polysiloxane). The difference in the chemical properties between different molecules in a mixture will separate the molecules as the sample travels the length of the column. The molecules take different amounts of time (called the retention time) to come out of (elute from) the gas chromatograph, and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass to charge ratio.
- These two components, used together, allow a much finer degree of substance identification than either unit used separately. Combining the two processes reduces the possibility of error, as it is extremely unlikely that two different molecules will behave in the same way in both a gas chromatograph and a mass spectrometer. Therefore, when an identifying mass spectrum appears at a characteristic retention time in a GC/MS

analysis, it typically lends to increased certainty that the analytes of interest is in the sample.

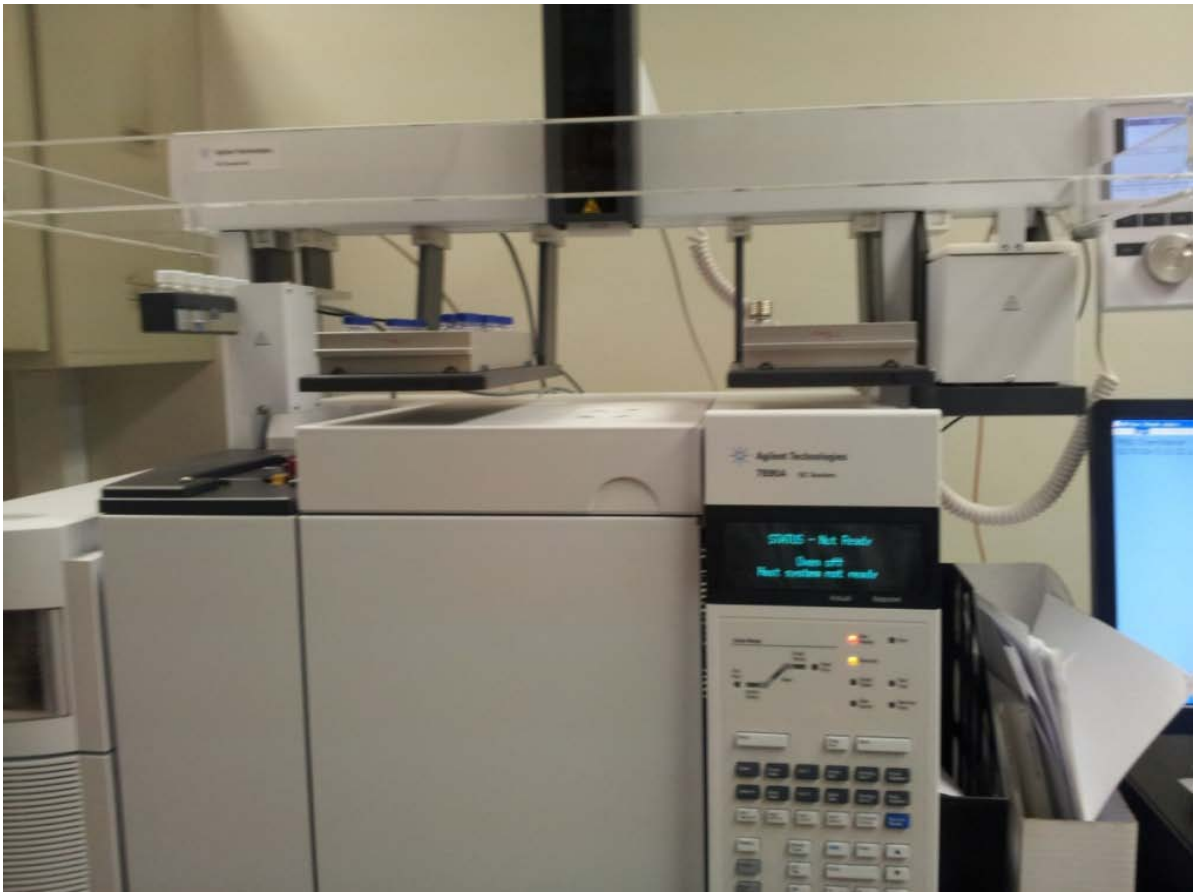


Figure 3.3 Gas Chromatograph Model 7890A / Mass Spectrometer 5975 inert MSD with Triple-Axis (GC/MS Agilent Technologies)

3.4 Samples Preparation

In order to test the objectives of the experiment, three types of water bioremediation products were selected to run the experiment. The products ware named as Product (1), Product (2) and Product (3). The products were selected based on the best practice in the petrochemical industry, brand of the product and producers / manufacturers experience in the bioremediations filed.

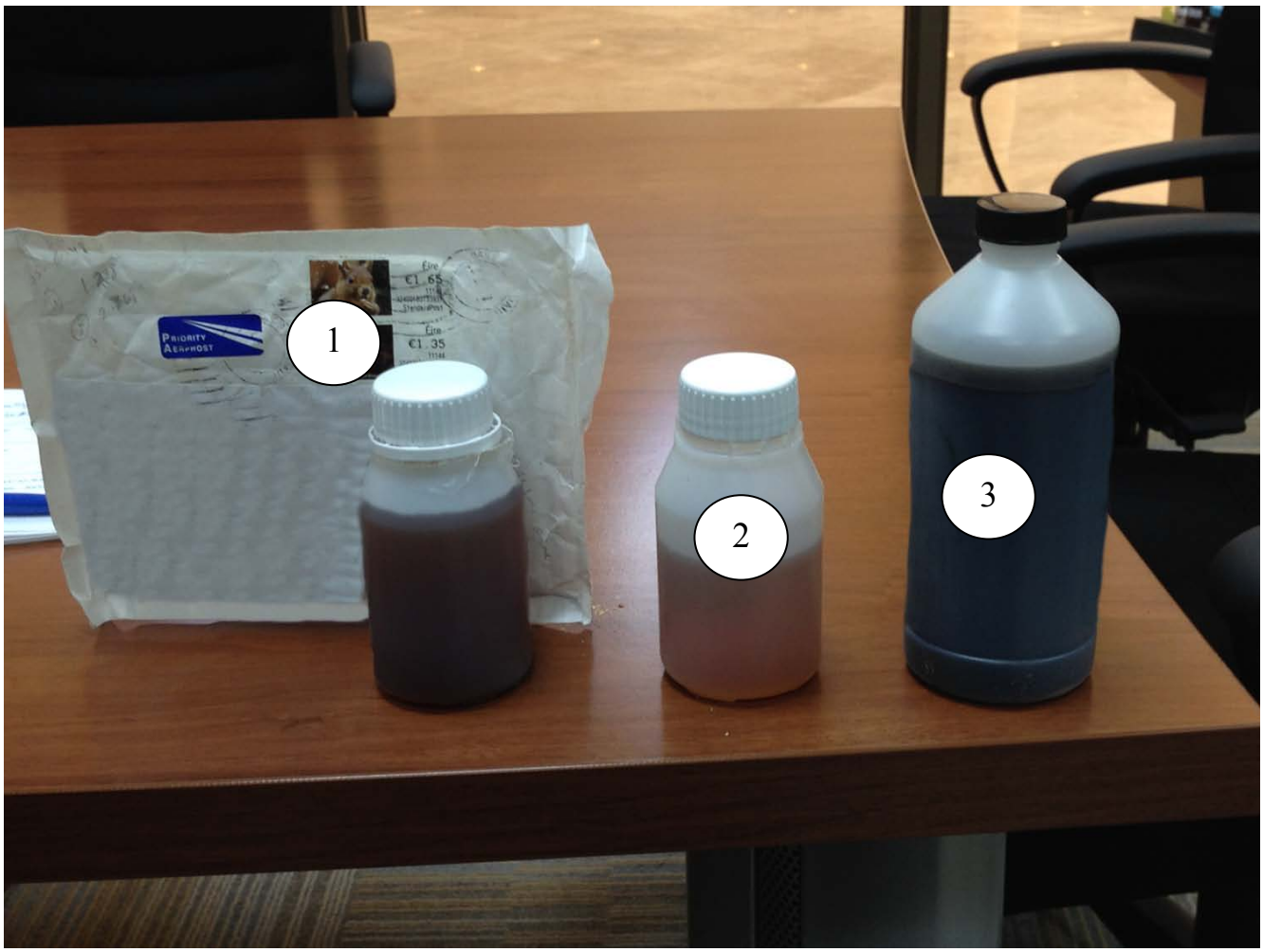


Figure 3.4 bioremediation products Product (1), Product (2) and Product (3)

Figure 3.5 shows the steps of the sample preparation for the experiment. Sample preparation some effort in order to avoid any confusion during the process of monitoring and the run of the experiment.

Figure 3.5 steps of sample preparation

Table 3.1 shows the general layout of the experiment and distribution of the samples at 4°C. The same distribution and setup was prepared for the other two environmental conditions (25 °C and 37 °C).

The environmental conditions were determined to be 4°C, 25°C and 37°C. The purpose of these settings is to simulate the real environmental conditions at different site of the world and to be in line with the previous studies.

Each environmental condition was subject to three levels of acidity, i.e. low pH (4), normal pH (7) and high pH (10). The intent of this step was to study the effect of pH on the degradation rate of the pollutants. Even in most of the previous studies, the pH was around the natural pH (7). However, they mention that their were a minor change in the pH after the bioremediation.

Figure 3.6 PH meter

For each pH three samples were prepared, each sample was doused with specific amount of the bioremediation products. The douses were 0.5 ml, 1.0 ml and 2.0 ml respectively. The doses were 1%, 2% and 4% of the total volume of the sample.

Table 3.1 sample preparation for 4°C, the same set up is used 25°C and 37 °C

TEMPERATURE	4 °C Temperature																									
bioremediation product	Product 1									Product 2									Product 3							
PH	pH (4)			pH (7)			pH (10)			pH (4)			pH (7)			pH (10)			pH (4)			pH (7)			pH (10)		
products concentration in ml (dose)	0.5	1.0	2.0	0.5	1.0	2.0	0.5	1.0	2.0	0.5	1.0	2.0	0.5	1.0	2.0	0.5	1.0	2.0	0.5	1.0	2.0	0.5	1.0	2.0	0.5	1.0	2.0

Since products (1) were in solid phase, the doses were prepared based on weight ratio, i.e. the average weight of the volume of Product (2) and Product (3). The objective of this step is to study the effect of the concentration of each bioremediation product in the bioremediation rate at each environmental condition at different level of acidity.

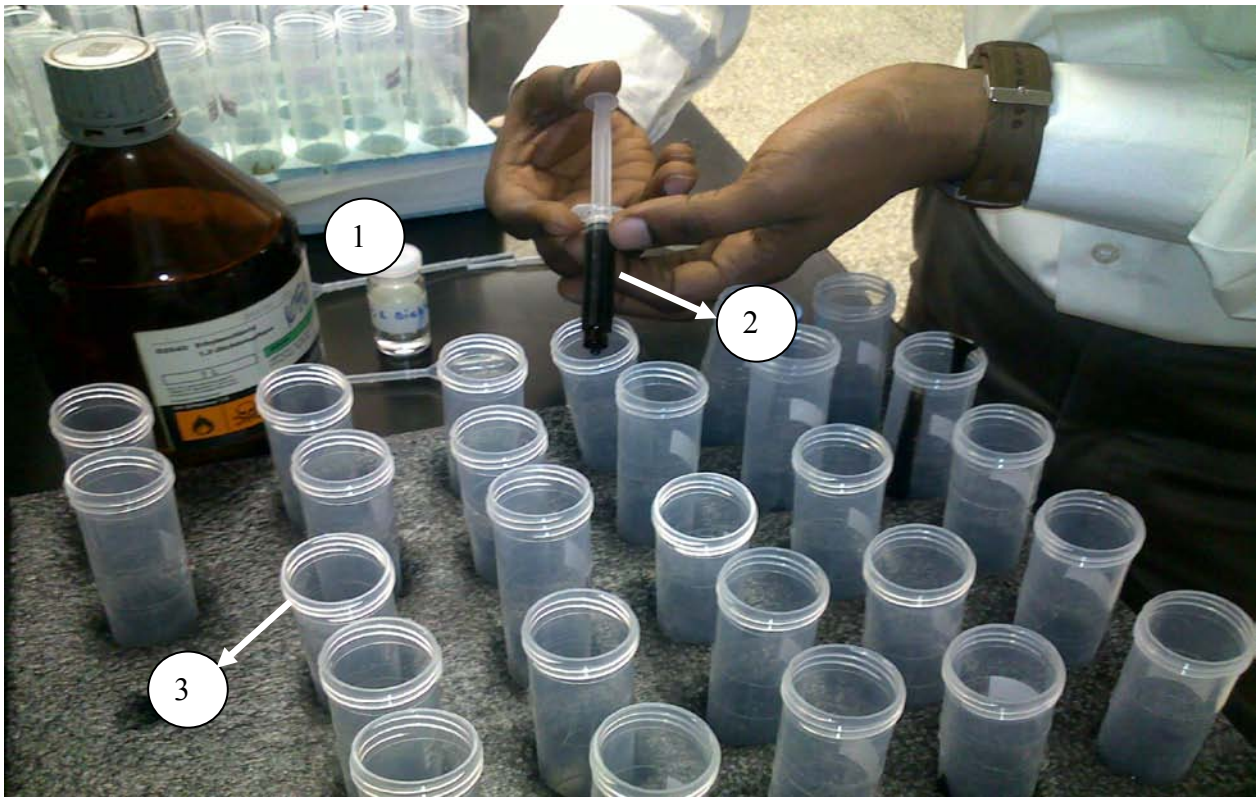


Figure 3.7 oil and EDC spick in the samples 1. EDC 2. Arab oil 3. Labeled test tubes

The samples were kept in semi anaerobic condition, i.e. the test tubes were opened every other two days. The purpose of this action is to simulate the environmental condition for groundwater (semi aquifer).

All sets of test tubes were kept under shaking by means of different techniques. The set of test tubes which were at room 25°C were shaken by means of shaker at rate of 5m/minutes, while the set of test tubes at 4°C and 37°C were shaken manually on daily basis.

Figure 3.8 samples of experiment at 4°C

Figure 3.9 samples of experiment at 37°C

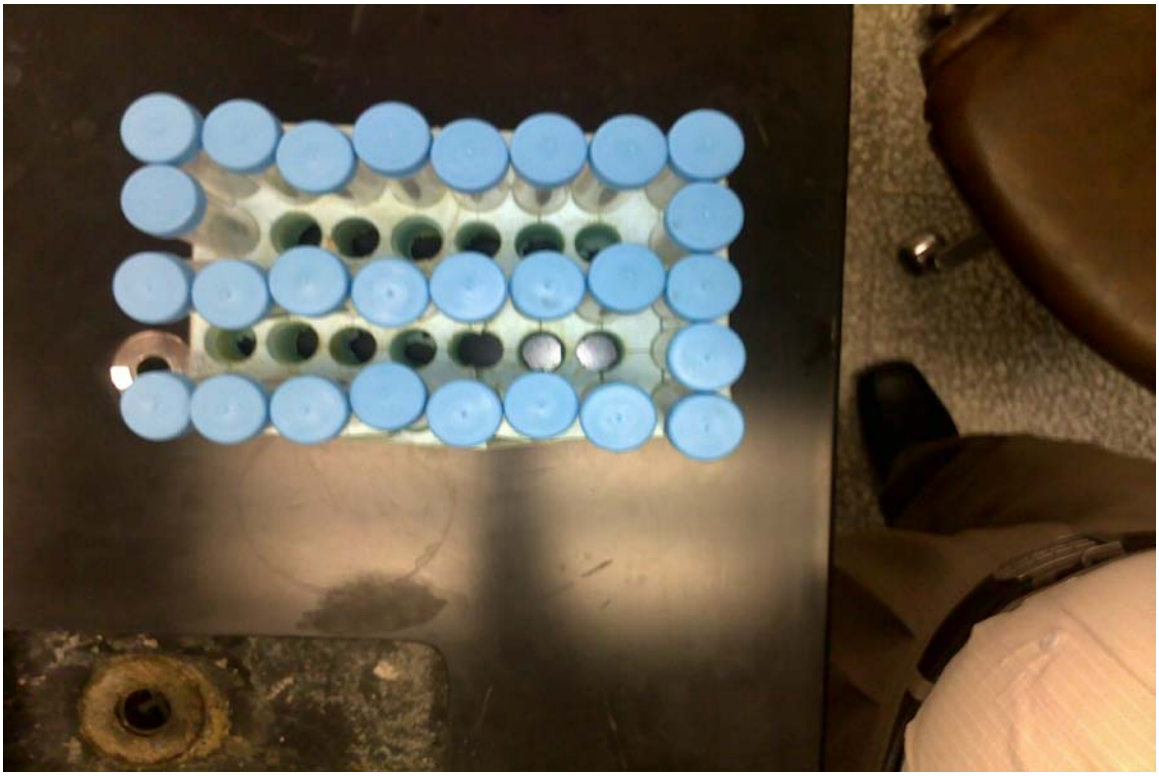


Figure 3.10 samples of experiment at 25°C

The total number of test tubes used for the analysis was 82, kept under observation for two months. The following parameters were monitored during the analysis period:

1. Changes in pH

   The pH was measured by the end of the experiment and compared with the initial seating as in table 3.1.

2. Odor
3. Reduction rate of EDC and oil.
4. Change in color
5. Behavior of the EDC and oil in the test tubes during the experiment.

The analysis was initially conducted by means of the GC/FID and the setting conditions of the GC/FID were described in Table 3.2.

Table 3.2 conditions of GC/FID during the analysis

PARAMETERS	CONDITIONS
Column type : nonpolar	J & W 10931-001 HP PONA : 325oC 50m x 200um x 0.5 um
Gas	Helium (He)
Column flow	1 mL/min
Injection volume	0.5uL
Solvent wash	n-Hexane chromasolv (Sigma-Aldrich)
Pre-injection solvent wash	4 times
Post-injection solvent wash	4 times
Sample wash	4 times
Sample pumps	6 times
MM inlet : temperature	250oC
MM inlet : pressure	32.417 psi
MM inlet : total flow	32 mL/min
MM inlet: septum purge flow	3 mL/min
Split flow	30:1
FID Detector: heater	250oC
FID Detector: H2 flow	40 mL/min
FID Detector: air flow	400 mL/min
FID Detector: make up flow (He)	9 mL/min
Temperature program	1. Start : 45oC, rate 20 o C/min 2. End : 200oC, hold time : 2 min
Run time	9.75 min
Post run temperature	45 o C
Post run time	3 min
Data rate/min peak width	20 Hz/0.01 min

In order to confirm results of analyiesd samples by means of FID , Gas Chromatograph / Mass Spectrometer GC/MS by Agilent Technologies was used. The unit contains 7890 GC System and 7693 Auto sampler. Since the sample consists of water, direct headspace sampling method was performed. The condition parameters are revealed in Table 3.3 including the headspace injection conditions.

Table 3.3 conditions of Headspace GC/MS during the analysis

PARAMETERS	CONDITIONS
Column type : nonpolar	Agilent 19091S – 433: 93.92873 HP-5MS 5% Phenyl Methyl Siloxane 325o C: 30m x 250um x 0.25 um
Gas	Helium (He)
Column flow	0.7 mL/min
Headspace Injection volume	250uL
Headspace Incubation Temp.	80oC
Headspace Agitation speed	250rpm
Headspace incubation time	5min
Headspace syringe flush time	2min
Headspace Syringe Temp.	80oC
Headspace Injection Speed	500uL/sec
Inlet : temperature	250 oC
Inlet : pressure	3.7586 psi
Inlet : total flow	103 mL/min
Inlet: septum purge flow	3 mL/min
Gas saver flow	20 mL/min
Purge flow to split vent	100 mL/min at 2min
Split Mode	splitless
MS heater transfer line	280oC
MS source temperature	230oC
MS quad temperature	150oC
MS method mode	scan
MS scan parameters	Low mass: 40; High mass: 550
MS method of ionization	Electron impact
EMV mode	Gain factor
Gain Factor	1.00
Resulting EMV voltage	1082
Oven Temperature program	1 Start : 45oC for 0min 2 6oC/min to 180oC for 2min 3 20oC/min to 250oC for 2min
Run time	30 min

3.5 Experimental Procedure.

The overall flow chart of the experiment process is shown in Figure 3.10.

The conditions of GC/MS prepared according to EPA 1997 Method 8260B which is used to determine volatile organic compounds in the waste water (39).

The whole sets of the experiment were prepared one day before the execution of the experiment as described in section 3.4. The general layout of the test tubes under each environmental condition is shown in table 3.1, Figures 3.7, Figures 3.8, Figures 3.9. The 4°C and 37°C were prepared by optimizing environmental chamber at 37 °C and refrigerator at 4 °C.

The volume of each test tube is 50 ml, each test tube containing 48 ml of water and 1.0 ml of oil and 1.0 ml of EDC. Then the bioremediation products were injected according to table 3.1.

When the setup of the experiment was completed, a sample from each test tube was collected to measure the initial peak areas of the oil and EDC in the water. The average initial peak area was about 130521.2 for all samples.

The samples were left in biology laboratory for two months, while the concentration of EDC and oil in each sample was measured on weekly basses by collecting about .05ml from each test tube and test the change in EDC and oil by means of GC/FID and Headspace GC/MS.

The results of analysis of each test tube were recorded on the tables 4.1, 4.2 and 4.3.

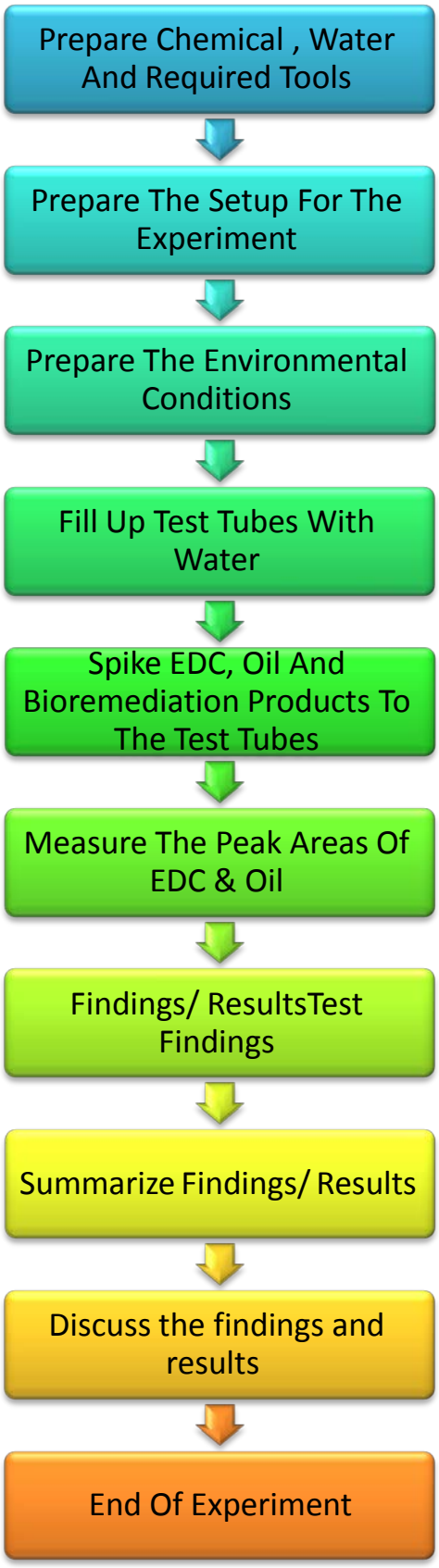


Figure 3.11 Experiment flow chart

IMPORTANT NOTE: Additional sample (control sample) was prepared at 25 °C to test the degradation rate of oil and EDC in water in the absence of bioremediation products. The sample analysis ware collected in different periods according to the following:

- Analysis of the First and second batches conducted every week after the set up of the experiment.
- Analysis of the third, fourth, fifth and sixth batches collected every two other weeks.

3.6 Observations

As mentioned on section 3.5, pH, odor, color of the samples and the concentrations of EDC and oil (pollutants) ware subjected to regular observations.

The color of the samples did not changes over eight weeks in addition to that the odor of the samples did not change from the original odor of the sample preparation.

By the end of the experiment the following items were noted:

1. The average reduction rate of EDC and oil was (4%) in the presence of bioremediation product under all environmental conditions i.e. temperature, pH, different douses. Appendix A.
2. Minor change (less than 2%) in the pH toward the neutral pH.
3. The reduction rate of the closed test tubes at lower temperatures and high concentration of the enzyme was higher than the reduction rate of the closed test tubes at 37 °C and low concentrations of enzymes.

The findings will be discussed in more details in chapter 4.

From the previous observations, we can see that most of the results were not in line with the results of the studies conducted on the manufactures. On the other hand, they were in line with some of the academic studies which conducted on laboratory scale. See section 2.6

In order to confirm the previous results, an additional/ confirmation experiment prepared to ensure that these results are accurate and avoid any error during the analysis. Details of the preparation and results of the confirmation experiment will be discussed in section 3.7.

3.7 Confirmation Experiment

3.7.1 Experiment preparation

The purpose of the confirmation experiment is to confirm the results of the main experiment and validate the work procedure to serve the objective of the experiment, the thesis advisor and committee members decided to repeat the experiment in smaller scope.

The scope of the confirmation experiment was to test the degradation rate of the EDC ONLY in water 25°C and 37°C only at different pHs and under different doses. Table 3.3 shows the overall set up of the confirmation experiment.

The same materials which were used in the main experiment were also used in the confirmation experiment except the water where it was pond water collected from KFUPM lake. The samples of the confirmation experiment were prepared in similar way of the preparation of the main experiment. in order to ensure the availability of microorganisms in the water lake, a test of bacteria colonies was conducted by tacking some samples from the collected water in the sterilized Petridis.

Another set of samples was prepared with open test tubes (samples No. 3, No. 8 and No. 12) with water with normal pH7 at 25°C with 4 ml dose from each bioremediation product.

Finally one test tube (No.15) contains water with pH7, EDC and 2 ml of product 3 was left open at 37°C. The set up of the confirmation experiment was monitored for six weeks.

The samples were analyzed by means of the GC/MS only. Result of the analysis are shown in Table 3.5

Table 3.4 Set up of samples at room and hot temperature with different conditions

Sample/ Test Tube #	pH	Bioremediation Product	Temperature	Dose (ml)
1	7	P3	25 °C	2
2	7	P3	25 °C	4
4	4	P3	25 °C	2
5	7	P2	25 °C	2
6	7	P2	25 °C	4
7	4	P2	25 °C	4
9	7	P1	25 °C	2
10	7	P1	25 °C	4
11	4	P1	25 °C	4
13	7	NA	25 °C	NA
3	7	P3	37 °C	2
8	7	P2	37 °C	2
12	7	P1	37 °C	2

3.7.2 Observations of the second experiment:

The factors which were monitored during the main experiment were also monitored in the confirmation experiment. The observations of the confirmation experiment were similar to the observations of the main experiment in addition to the following:

1. Water of the open test tubes at 37°C and 25°C was evaporated. After four weeks, more than 75% of water in the test tube at 37°C was evaporated, while about 40% of the volume of the test tube at 25°C was evaporated.

2. The EDC form a liquid layer in the closed test tubes at 25°C.

3. The EDC form a semisolid layer in the closed test tubes at 37°C.

4. The EDC form a solid layer in the open test tube at 25°C and 37°C.

5. The degradation rate of EDC in the presence of the three bioremediation products in the second experiment is similar to the degradation rate in the main experiment i.e. between 4%-7%.

6. The average reduction rate of the closed test tubes was about 2% at 37°C while it was about 4% at 25°C.

7. The average reduction rate of the open test tubes was 33% at 37°C and about 18% at 25°C.

8. In general, the reduction rate of the open test tubes were higher than the reduction rate for the closed test tubes (samples)

The discussion of the findings of the conformation experiment will be discussed in more details in chapter 4.

CHAPTER 4
RESULTS AND DISCUSSIONS

4.1 INTRODUCTION

Tables 4.1, 4.2 and 4.3 shows the details of the results of the reduction rate of EDC and oil for the main experiment, while table 4.4 illustrates the summary of the reduction rate of the main experiment.

Tables 4.1 till table 4.4 and figures 4.1, 4.2 and 4.3 show that the concentrations of EDC and oil (HC in the range of C12-C40) ware reduced. The average reduction of these pollutants was 4%, which is more than the reduction rate of the control sample (2.5%).

From figures 4.1 till 4.3, bioremediation products worked to support the microorganisms to increase the degradation rate of the pollutants. The degradation rate at the first two weeks is higher than the degradation rate in the following weeks. With the passage of time, the peak areas of the EDC and Oil are lower than each initial peak areas.

Figures 4.1, 4.2 and 4.3 and graphs in index A show a jump in the second week of the experiment for the concentration of the pollutants, then followed by reduction in the concentrations. The behavior accrued due to the breakdown of the pollutants and metabolism of the microorganisms (38).

Another observation / result from Figures 4.1, 4.2 and 4.3 and graphs in index A, is the reduction rate at 4°C is higher than the reduction rate at 37°C (for closed test tubes – anaerobic conditions).

The following sections will discuss in more details the findings and results of the main experiment and confirmation experiment by forcing on the physical observations in addition to the observations analytical observations.

Table 4.1 Peak area results of analysis of P1.

Table 4.2 Peak area results of analysis of P2.

Table 4.3 Peak area results of analysis of P3.

Table 4.4 Average reduction rate of the three bioremediation products.

Table 4.5 Results of analysis in peak areas of EDC for the confirmation experiment.

SAMPLE	Batch 1	Batch 2	Batch 3	Batch 4	Batch 6
1	4711705290	4214865920	4501253863	4512101210	4511025232
2	5122967683	4563259842	4815475420	4801015866	4854152101
3	4901656765	3978658542	4325210132	4251129652	3991221554
4	5191304199	4758415386	4841521140	4854415210	4754854524
5	5041142542	4100145860	4011252310	4685475210	4600415223
6	5049808080	4042568524	4325211010	4754411010	4658545212
7	4956461828	4658562620	4401252231	4854412420	4754562521
8	4518485044	4045213253	3821542520	3715296213	3521424112
9	4977280780	4110142314	4421010205	4845214101	4754542411
10	4574388437	3854521230	4110123589	4452141322	4400125232
11	5102339951	4352141153	4210121521	4954524421	4854215212
12	5055505007	4011423658	4521556921	3659214662	3596582143
15	4435596607	3001200102	3312001523	2952126901	2425456010
Control (13)	4541074101	4494748562	4405211412	4400425326	4400052152

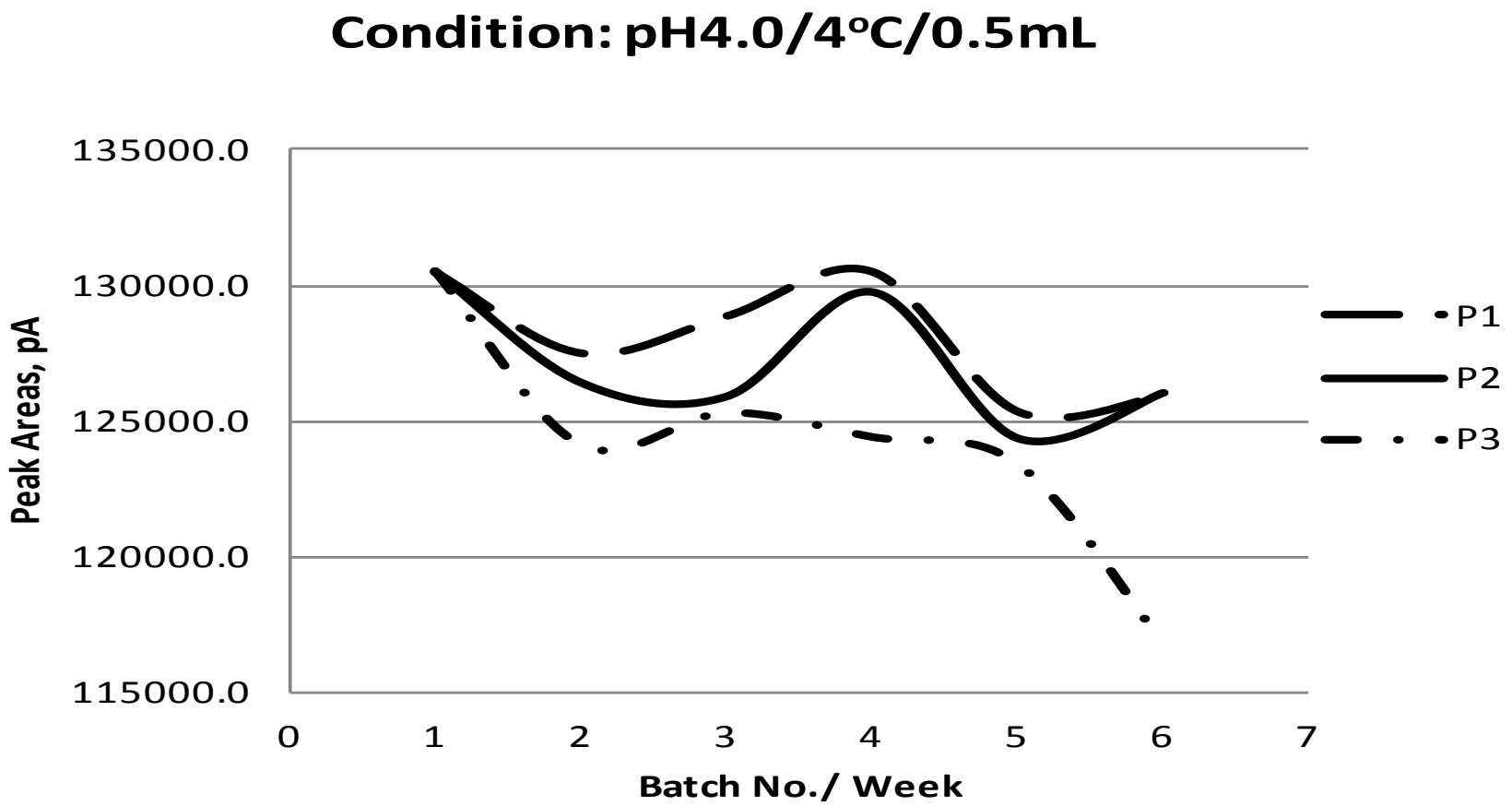


Figure 4.1 For the main experiment, reduction of EDC and oil pollutants on water after six weeks under the following conditions:

1. pH 4, 2. Temperature 4°C and 3. Dose 0.5 ml from the bioremediation products.

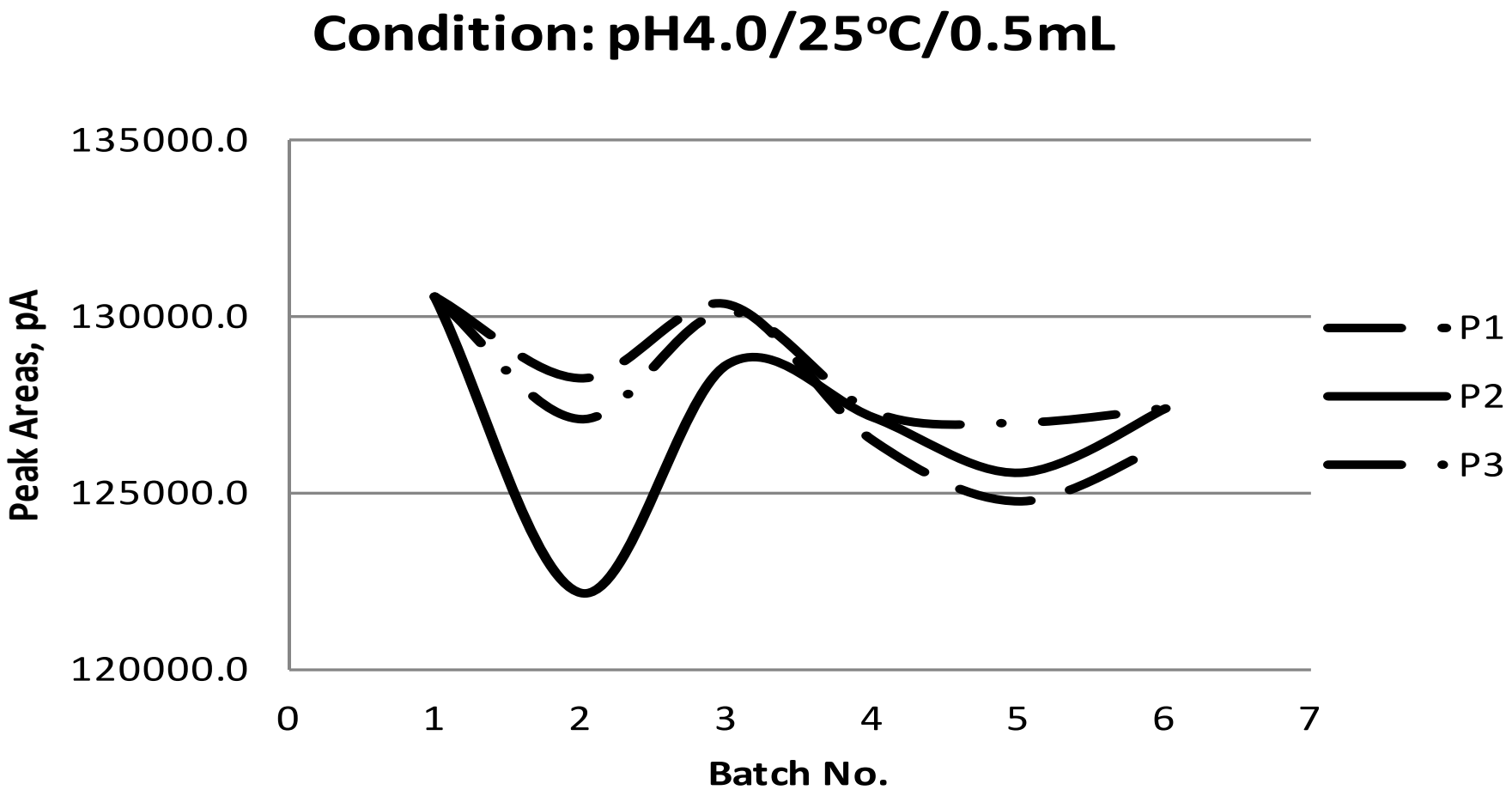


Figure 4.2 For the main experiment, reduction of EDC and oil pollutants on water after six weeks under the following conditions:

1. pH 4, 2. Temperature 25°C and 3. Dose 0.5 ml from the bioremediation products.

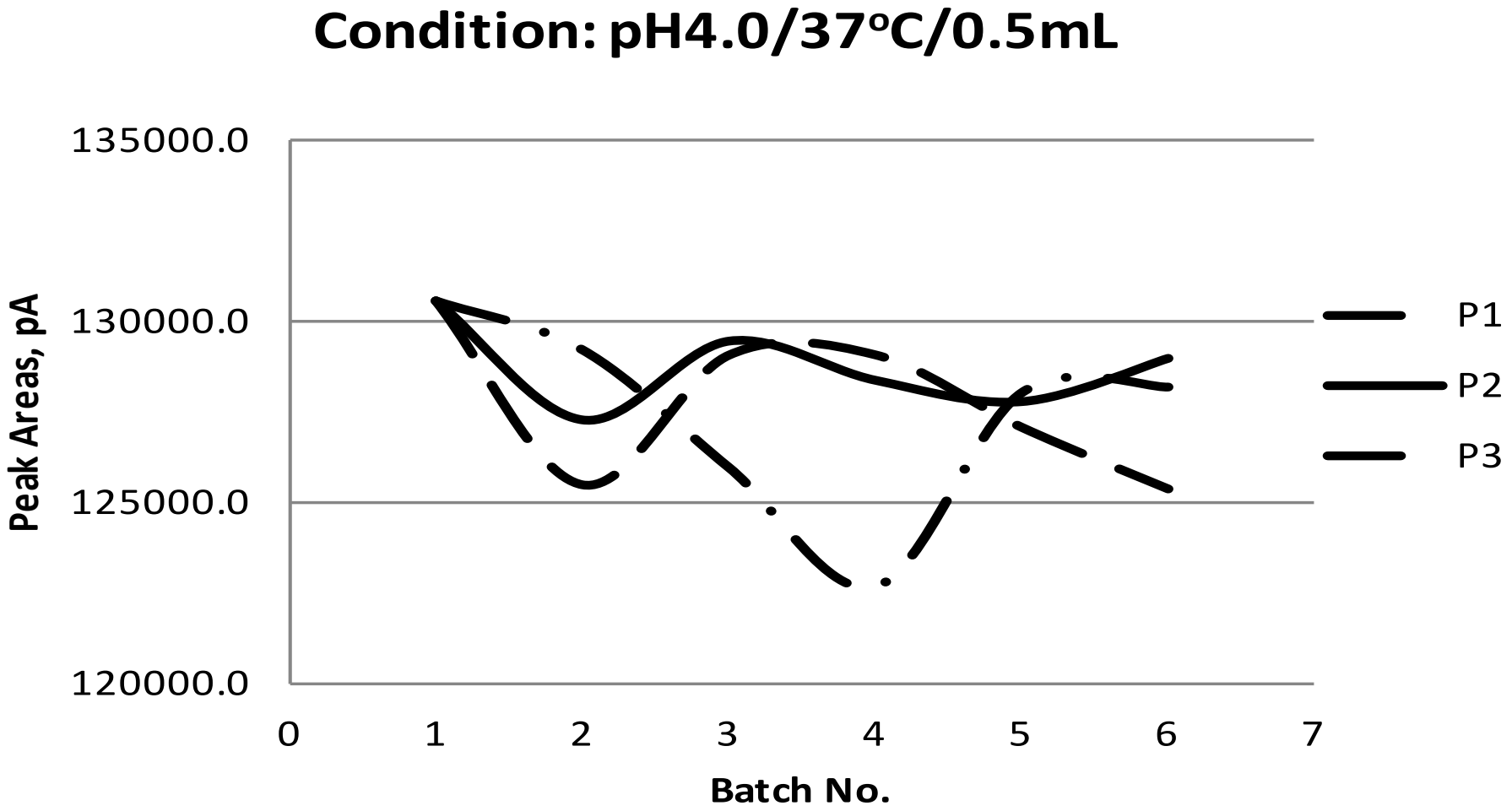

Condition: pH4.0/37°C/0.5mL
135000.0
130000.0
125000.0
120000.0
Peak Areas, pA
0
1
2
3
4
5
6
7
Batch No.
P1
P2
P3

Figure 4.3 For the main experiment, reduction of EDC and oil pollutants on water after six weeks under the following conditions:

1. pH 4, 2. Temperature 37°C and 3. Dose 0.5 ml from the bioremediation products.

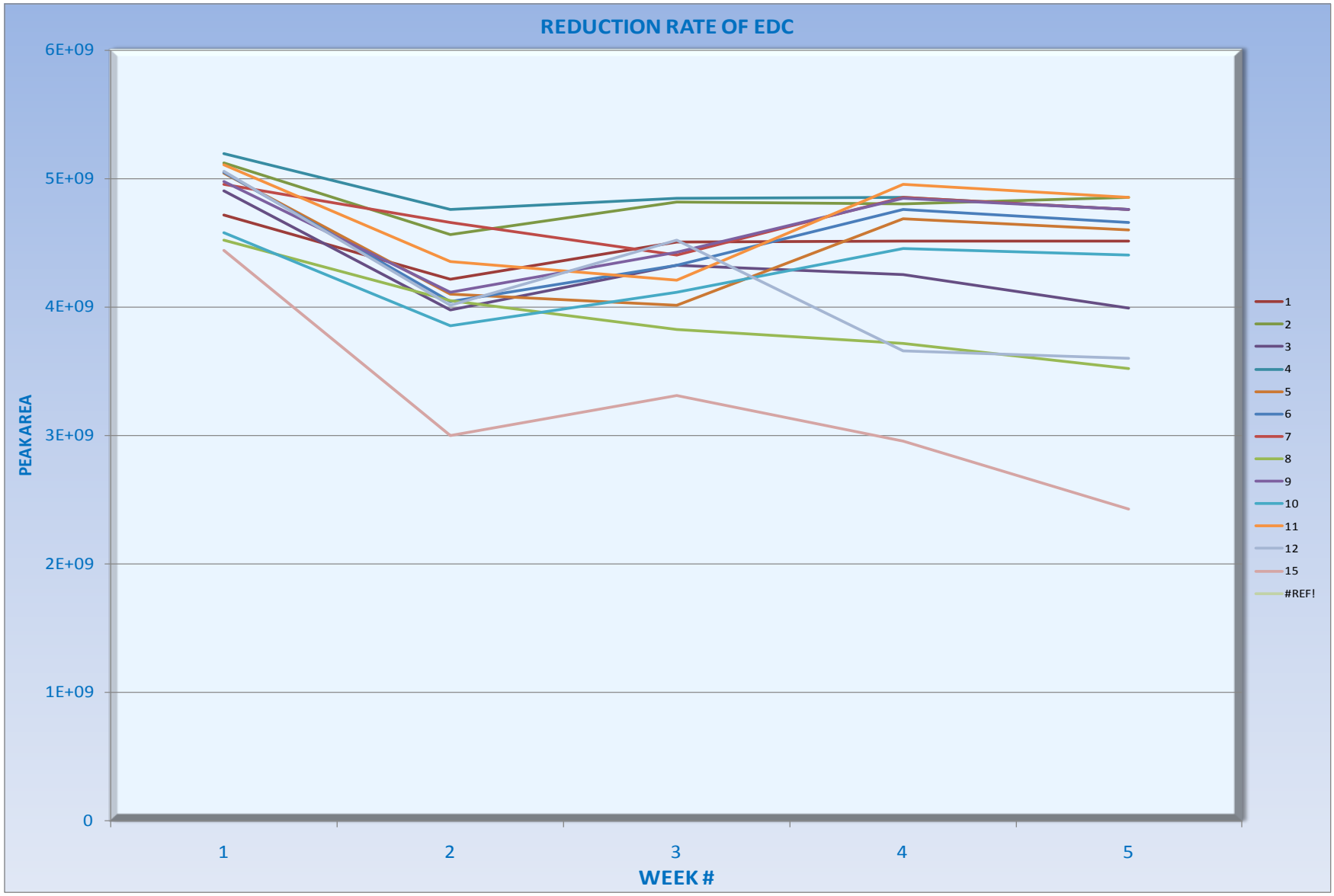


Figure 4.4 For the confirmation experiment, reduction of EDC on water after six weeks under the conditions on table 3.3

4.2 RESULTS OF THE MAIN EXPERIMENTS ANALYSIS:

As discussed in previous chapters, the samples of the main experiment and confirmation experiments were subject for physical monitoring and analysis for eight and six weeks respectively. pH, odor, sample colors were monitored on regular bases physically, while the concentration of EDC and oil (HCs in the range of C12-C40) in the samples were analyzed regularly by means of GC/FID and GC/MS. The main findings/results of the main experiment were:

1. No change in the color, odor of the samples during the monitoring period.
2. Referring to tables 4.1 to 4.3, figures 4.1 to 4.3 and appendix A, the overall reduction rate of EDC and oil in presence of bioremediation products was about 4%.
3. Figures 4.1, 4.2 and 4.3 and graphs in appendix A show that the reduction rate at 4°C is higher than the reduction rate at 37°C.
4. In the absence of shaking, EDC and oil forms two aqueous layers Figure 4.5 where EDC was formed at the bottom of the test tube while oil was formed between water and EDC.
5. Shaking of the samples during the experiment had low influence in the degradation rate.

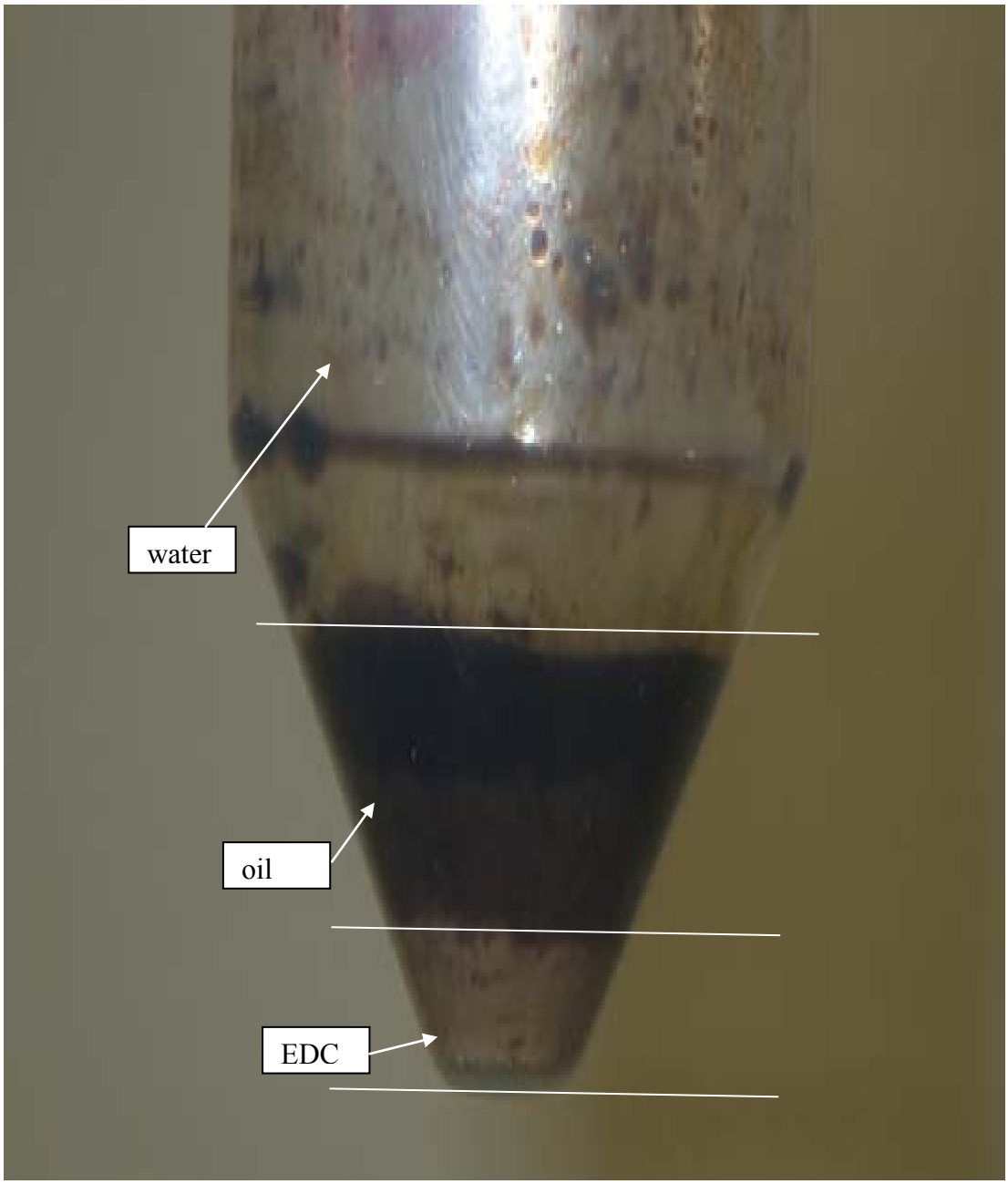


Figure 4.5 Formation of EDC and Oil layers in the sample.

4.3 DISCUSSION ABOUT THE RESULTS OF THE MAIN EXPERIMENTS ANALYSIS:

As result of the findings of the main experiment (section 4.2), and compare these results with the previous studies, we can find:

1. We see that there is no effect of the bioremediation products on the physical properties of the samples. this result proven that the effects of bioremediation method is safer than the use of the chemical remediation where the pH, odor and color of the samples changed from the natural conditions (2); (23). Moreover, use of enzyme is better tool for bioremediation if it is compeered with the use of bacteria as bioremediation product where unpleasant smells are generated after remediation is completed due to the death of bacteria as result of shortage of bacteria feed (26);(54).
2. In the case of anaerobic conditions, the reduction rate is increased as the temperature decrease. This result is similar to the results of the previous studies conducted at laboratory scale (29). This fact acquired due to the low movement of water contents at low temperatures.
3. There is no influence of the pH or the shaking on the reduction rate at anaerobic conditions. However, the pH after the introduction of enzymes had change slightly toward the normal pH. This give good indication about the effects of enzymes to improve water quality comparing with other remediation methods.

4. In the absence of circulation (shaking), the EDC and oil form distinguish layers which is easer for treatment.

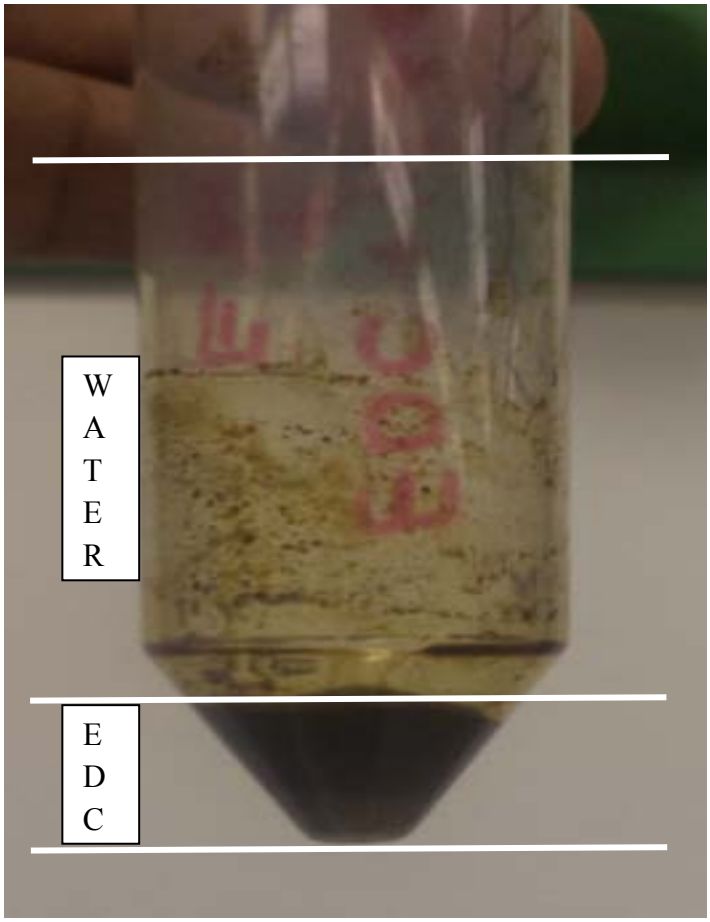
4.4 RESULTS OF THE CONFIRMATION EXPERIMENTS ANALYSIS:

Similar to the observations of the main experiment, pH, odor, colors of the samples were monitored regularly, while the peak area of EDC in the samples were analyzed on regular basis as discussed by means of Headspace GC/MS. Main findings of the confirmation experiment were:

1. No change in the color and odor of the samples during the monitoring period.
2. The overall reduction rate of EDC in the closed test tubes (anaerobic conditions), was 7%, table 4.5.
3. In the case of anaerobic condition a aqueous black color layer was formed, the layer was staked at the test tube layers.

Figure 4.6 Formation of EDC layer staking to the test tube wall.

4. The reduction rate of the closed test tubes (anaerobic conditions) is increased as the temperature decreased. The reduction rate at 25°C was 4% while it was 3% at 37°C.
5. In the case of the open test tubes (aerobic condition) the average reduction rate increased as temperature was increased. The reduction rate at 25°C was 18% while it was 33% at 37°C.
6. In the case of the open test tubes (aerobic condition) at 25°C, 40% of the water was evaporated while the quantity of EDC remains the same. A semi solid layer attached to the test tubes walls.



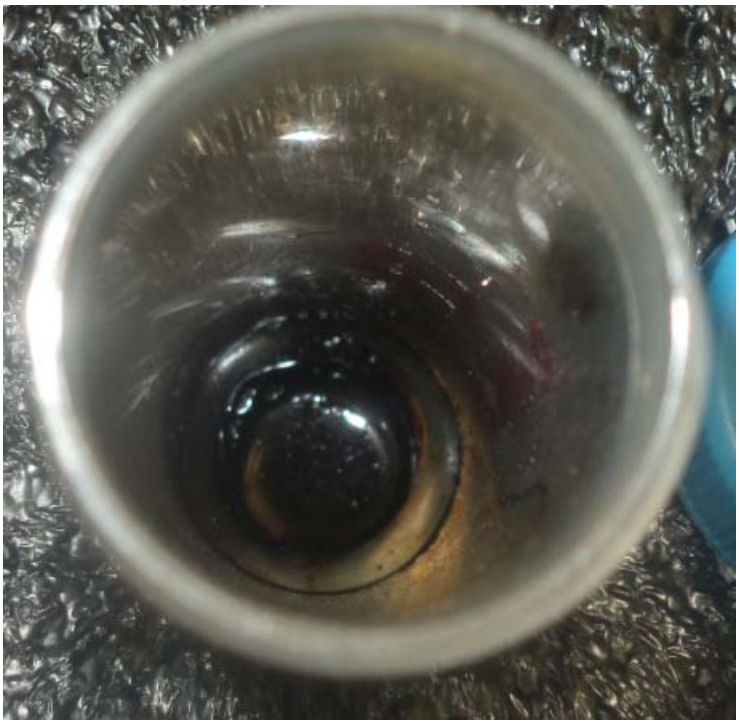


Figure 4.7 Evaporation of water in the open environmental conditions at 25°C and formation of small layer of EDC at the bottom of the test tube.

7. In the case of aerobic condition at 37°C, water was evaporated completely and a solid layer of EDC was formed.

Figure 4.8 Evaporation of water in the open environmental conditions at high temperature and formation of solid layer of EDC at the bottom of the test tube.

8. In the case of using product 1(which was solid form) in the aerobic conditions at 25°C a fungi was formed.

Figure 4.9 Formation of fungi at aerobic condition in room temperature.

4.5 DISCUSSION OF THE CONFIRMATION EXPERIMENTS ANALYSIS:

1. In the case of aerobic conditions, the reduction rate at high temperatures is higher than the reduction rate at low temperatures.
2. In aerobic conditions, EDC evaporates with water while oil remains at the bottom of the test tube.
3. Water quality improved in the case of aerobic conditions.

4.6 COMMON RESULTS OF THE BOTH EXPERIMENTS ANALYSIS:

1. In both cases (aerobic and anaerobic) there was a growth of microorganisms at the beginning and end of experiment.

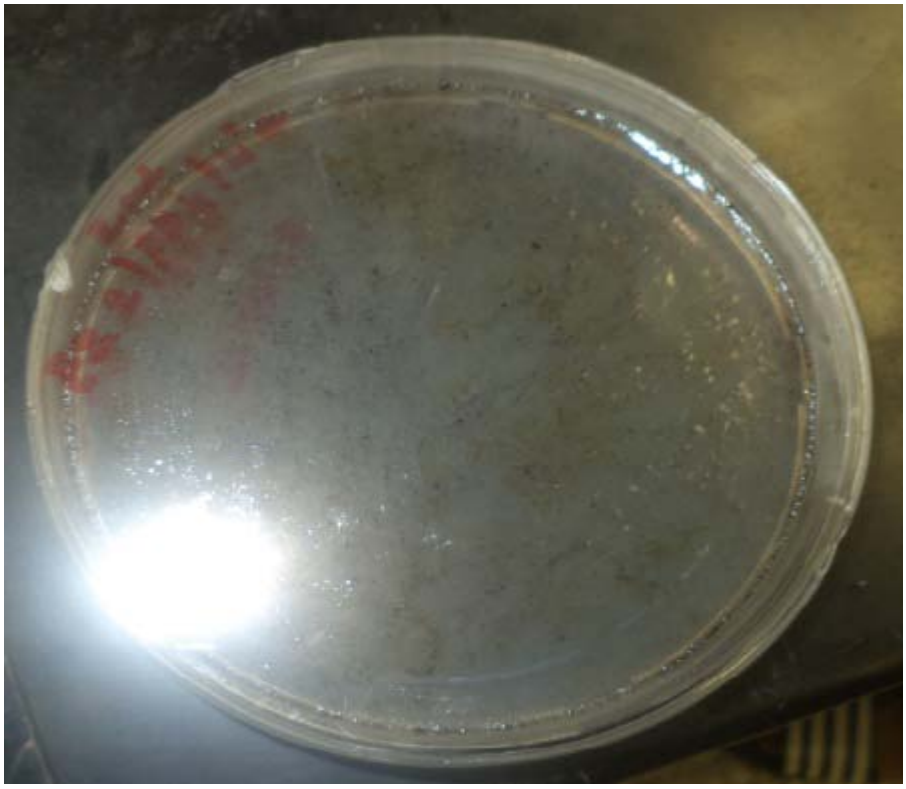


Figure 4.10 microorganisms Bacterial colony

2. The two experiments proven that enzymes improved the water quality (pH), in both aerobic and anaerobic conditions.
3. The degradation rate of the control sample in both cases (aerobic and anaerobic) was 5.7% and 2.5%.
4. The total degradation rate of all products was not in line with the data collected from manufacturers and suppliers, while it is in line with the previous scientific studies.
5. The effects of pH and dosage on the degradation rate are minimal.

6. The reduction rate at the closed environment increased as the temperature is decreased, while the reduction rate at open environment is increased as the temperature increased.
7. From table 4.6, we can find that P1 has the best performance to reduce the concentration of EDC and oil at anaerobic conditions.

Table 4.6 average reduction rate for each product during the main experiment

PRODUCT	AVERAGE REDUCTION RATE
P1	4.4%
P2	4.2%
P3	4.0%

CHAPTER 5
CONCLUSION

Enzymatic bioremediation is still a new field; almost all of the work thus far has been limited to bench studies. All these studies suggest that enzymatic processes can be an effective means for enhancing bioremediation, but more studies must be done under field conditions before large scale implementation. The study proven that the use of natural and artificial enzymes has been proven to be an effective means of enhancing bioremediation of the EDC and Oil from ground water in the presence of low amount of oxygen.

This study is considered as one of the first studies to investigate the ability of enzymes to support existing microorganisms in natural ground water to degrade the EDC and HC in the range of C12 to C40 from ground water biologically and return the water quality to the natural conditions. The aim of this study was to assess the potential for biodegradation of the mentioned pollutants by enhancing the activity of the microorganisms which were available naturally in the ground water. The activity of the existing microorganisms was enhanced by means of artificial enzymes from the local market.

The concept of use enzymes as bioremediation tool to reduce the concentration of pollutants depends on the increase the activity of microorganisms that exist in the natural environment (water or soil) by adding enzymes. So there is no introduction of new bacteria or organisms to the polluted area.

From the rustles of the study, we can see that the use of enzymes have been proven to be an effective means of enhancing the bioremediation for the EDC and oil components in the range of C12-C40 from ground water.

In the two experiment of this project – which was designed to simulate the ground water- the enzymes enhanced the water microorganisms to degrade the EDC and the oil components to lower concentrations within specific time, even thou the new concentrations were not within the allowable limits. However, it was good indications of the efficiency of the use of enzymes.

The study shows that the presence of oxygen and temperature plays important role in the degradation rate. At anaerobic conditions, as the temperature decreased, the degradation rate is increased if it is compared with other temperatures same environmental conditions. On the other hand, for the aerobic conditions, as the temperature increase, the degradation rate increased. In general the reduction rate of the pollutants in the aerobic conditions is twice the degradation rate at anaerobic conditions.

The other factor which was important in the degradation rate is the dosage of the enzyme, as the dosage of the enzymes is increased, the reduction rate also increased. In industrial applications, the optimum dose can be calculated depending on the other factors such as type of pollutants, magnitude, and water movement.

The effect of the pH was not clear in the results of the analysis. However, with the passage of time, the pH was changed from acidic and bass toward the neutral pH.

However, referring to previous studies, pH can be considered as one of the factors that enhance the degradation rate as pH is greater than 7 (neutral).

Finally, the concept of using enzymes in the water treatment is a valid concept and it requires more studies from scientists and industries. The concept shows promising results need some improvements; also it is safer for environment since there are no consequences or byproduct as of the treatment and recovery process and acceptable from human since there is no introduction of any more artificial products to treat the sources of drink and irrigation.

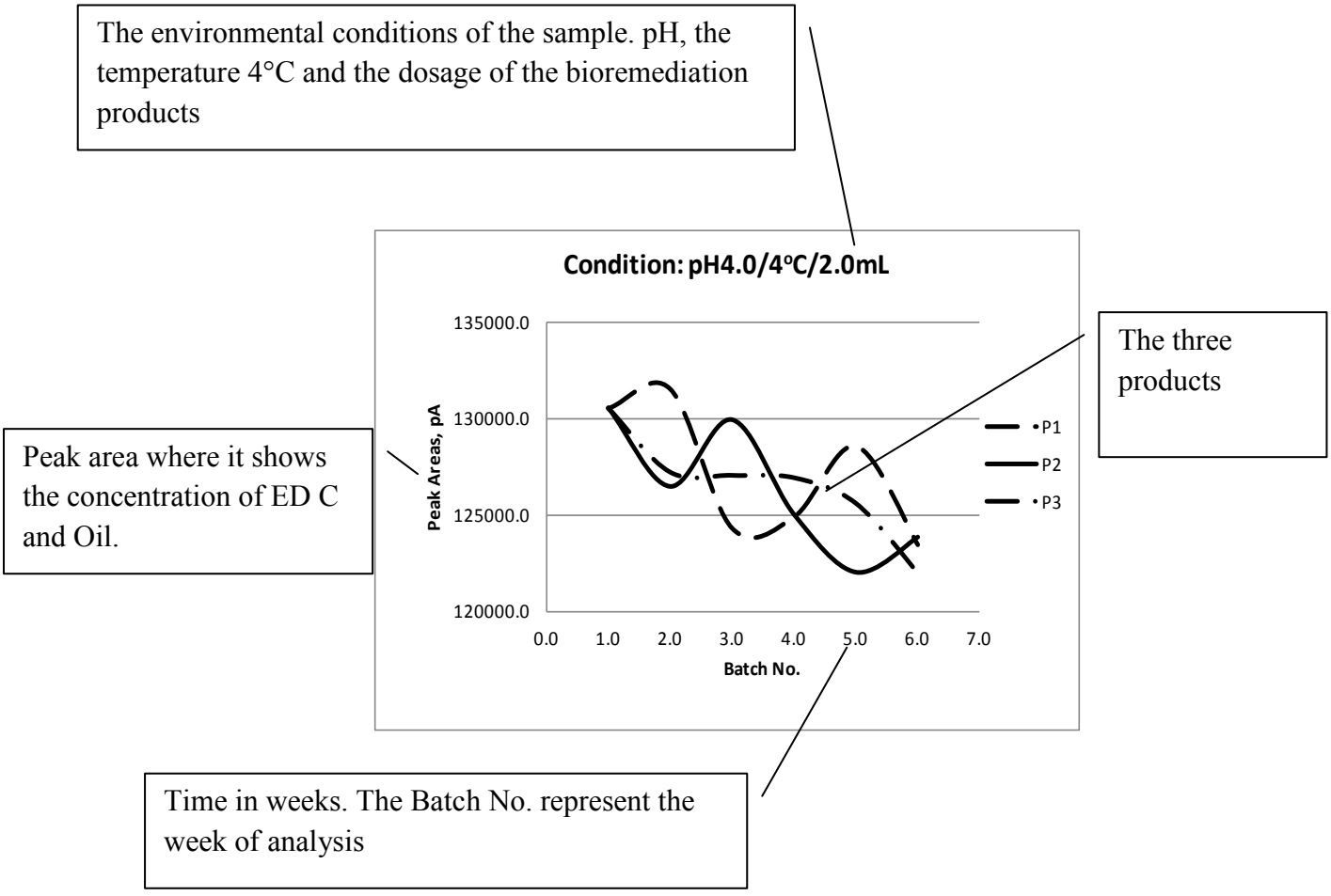
The study end up with the following main recommendations:

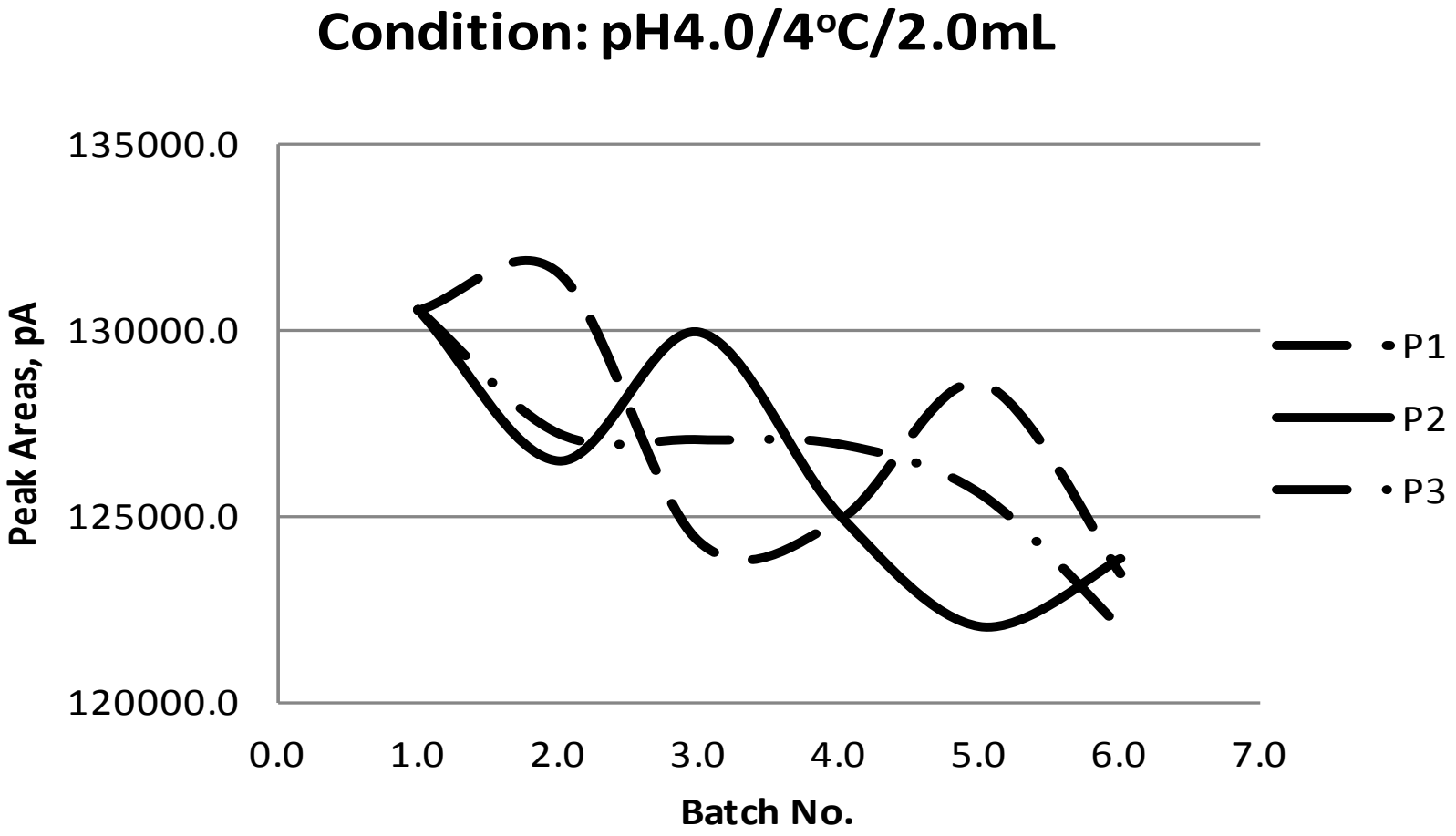
1. The use of enzymes for cleaning contaminated water is safer (from health and environmental point views) the use of chemical treatment or use bacterial method for remediation.
2. Universities, industrial companies and NGA shall encourage the scientific research in the application of enzymes as tool to reduces / eliminate the pollutants from water.
3. In the case of treating ground water with low amount of oxygen (anaerobic condition), it is recommended to:
    a. Circulate water on the aquifer in order to increase the presence of oxygen, if circulation is not applicable/ possible then,
    b. Conduct the treatment process at low temperature calamite (winter).

APPENDIX A

Results of analysis of the reduction rate of the EDC and oil. The reduction rate is measured by calculation of the peak area (pA), where the pA of EDC and pA of HC in the range of C12-C40 are combined together. The analysis was done via GC/MS and GC/FID. Some of the chromatograms and mass spectrum of the GC/FID and GC/FID are shown in APPENDIX B.

Detailed example of how to read the chart is shown below with illustration.

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125000.0
120000.0
Peak Areas, pA
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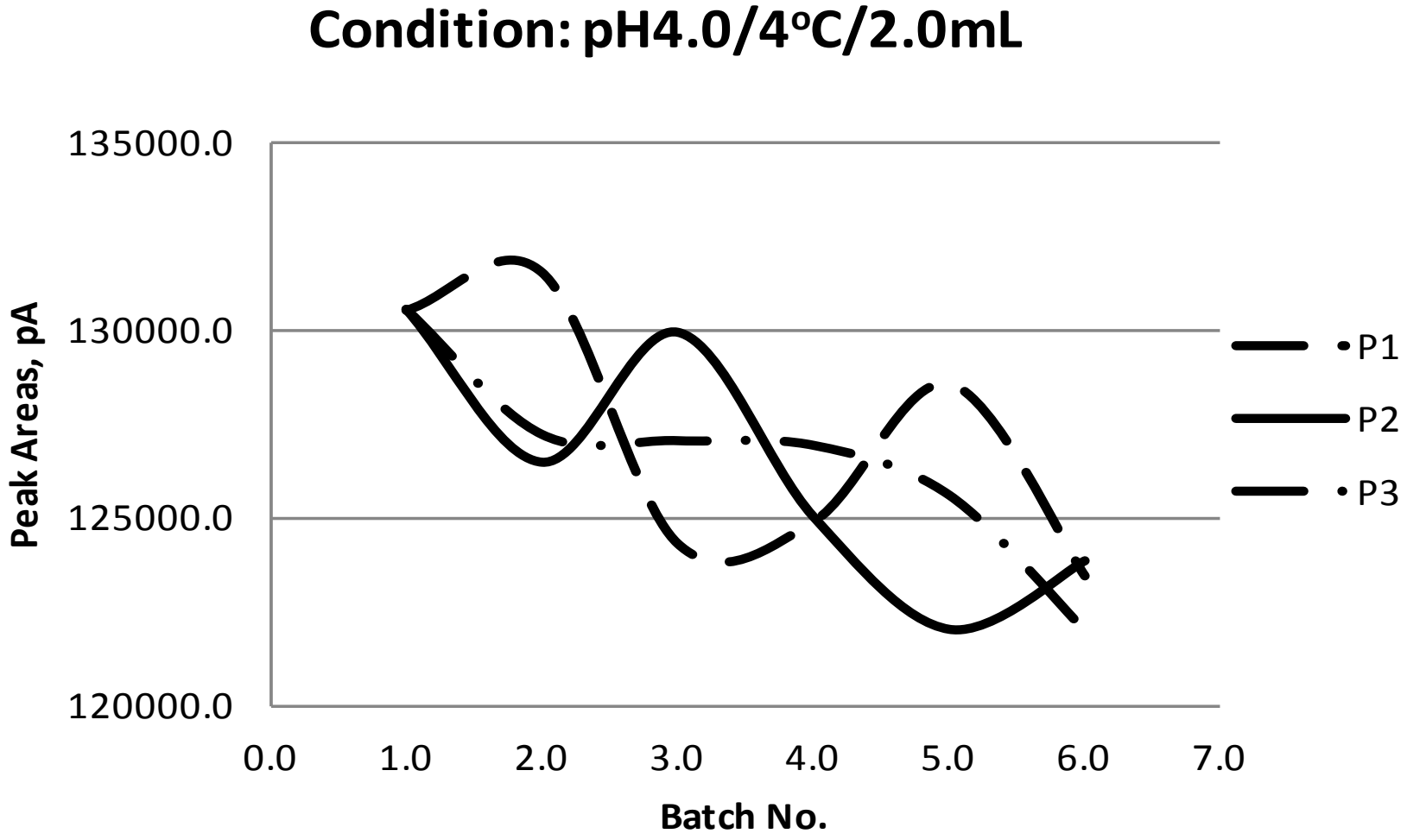
2.0
3.0
4.0
5.0
6.0
7.0
Batch No.
P1
P2
P3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Peak Areas, pA
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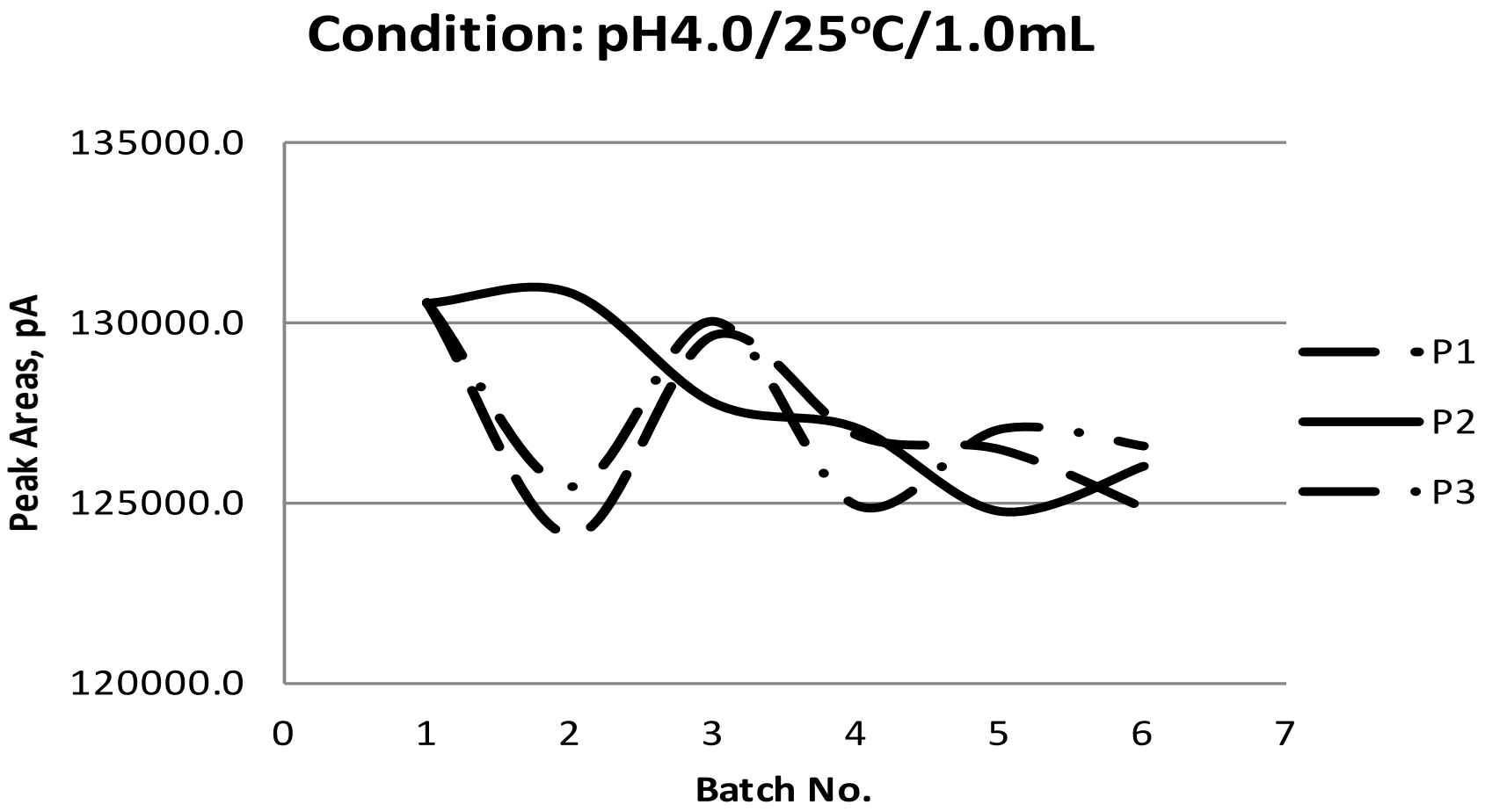
7.0
Batch No.
P1
P2
P3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1
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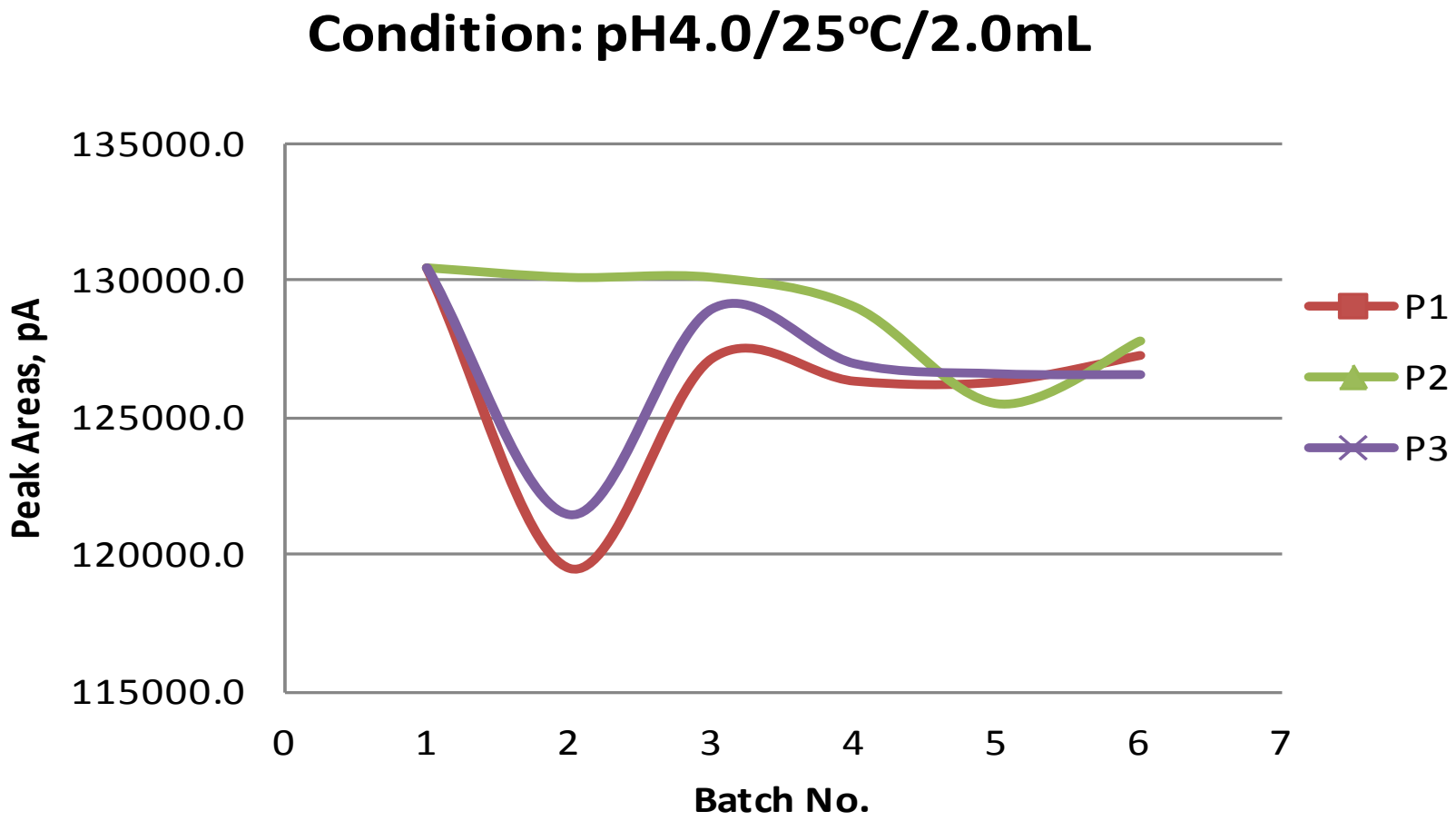
7
Batch No.
P1
P2
P3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125000.0
120000.0
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Peak Areas, pA
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1
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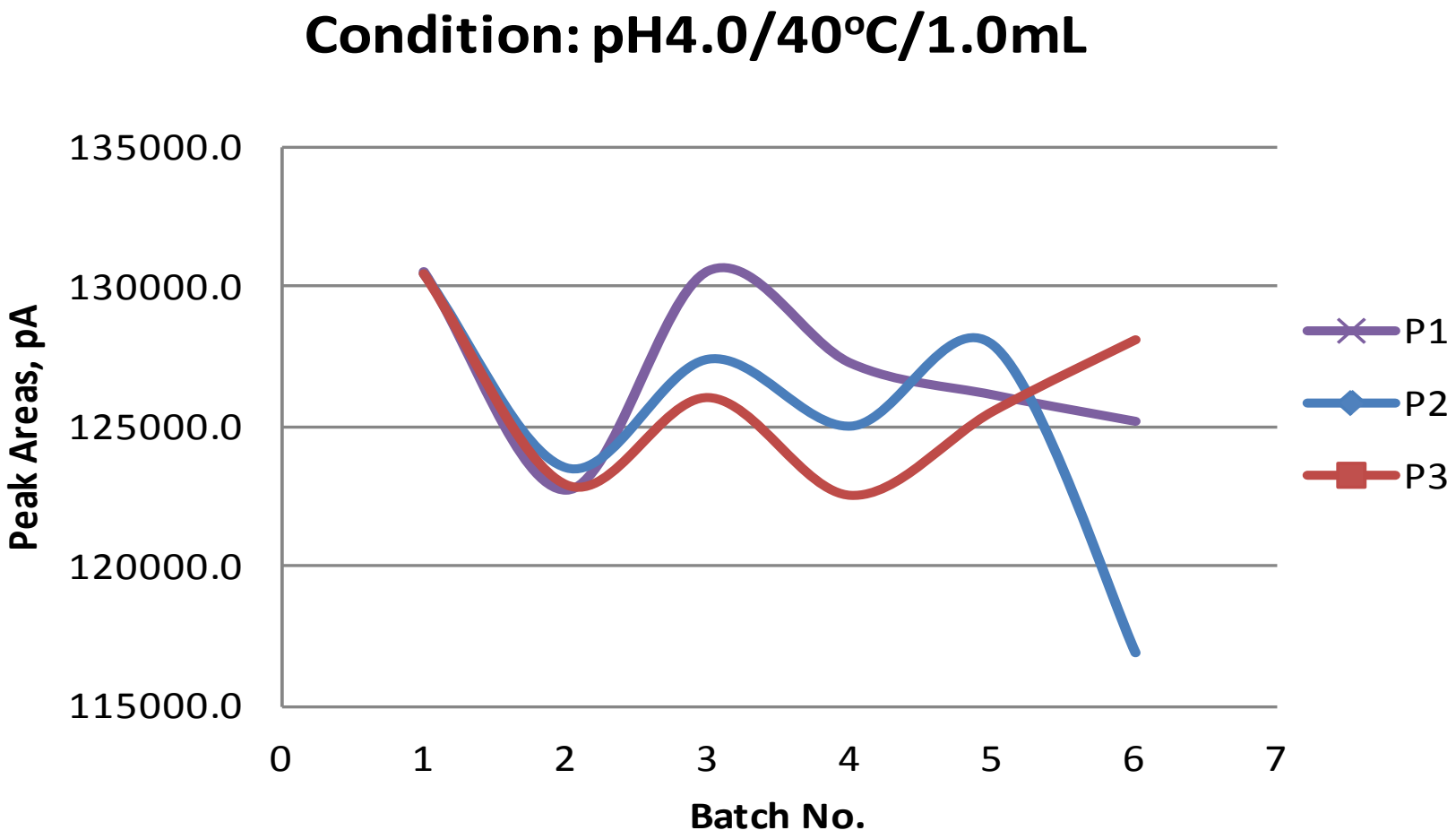
6
7
Batch No.
P1
P2
P3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Peak Areas, pA
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1
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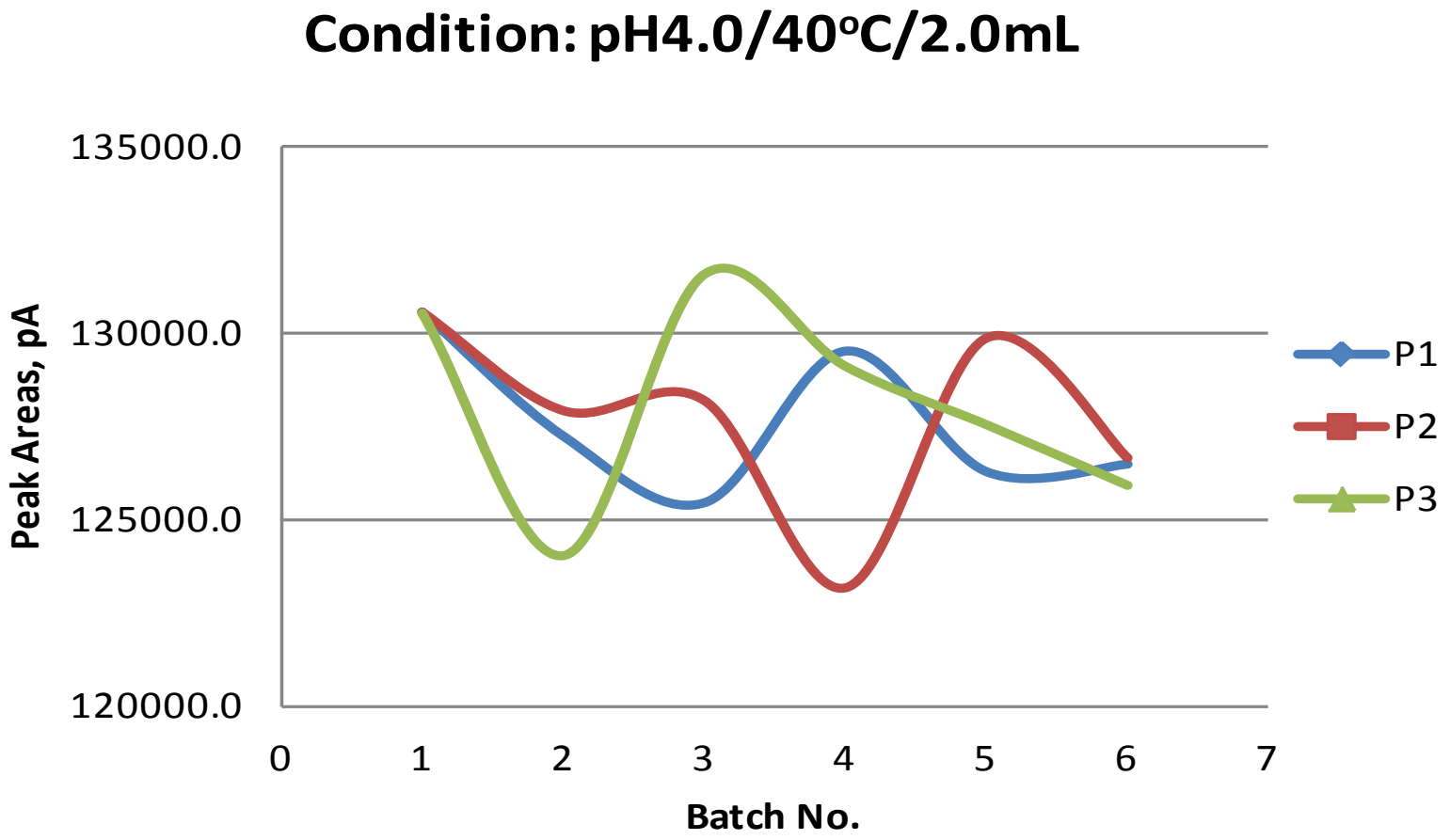
Batch No.
P1
P2
P3


Condition: pH4.0/40°C/2.0mL
135000.0
130000.0
125000.0
120000.0
Peak Areas, pA
0
1
2
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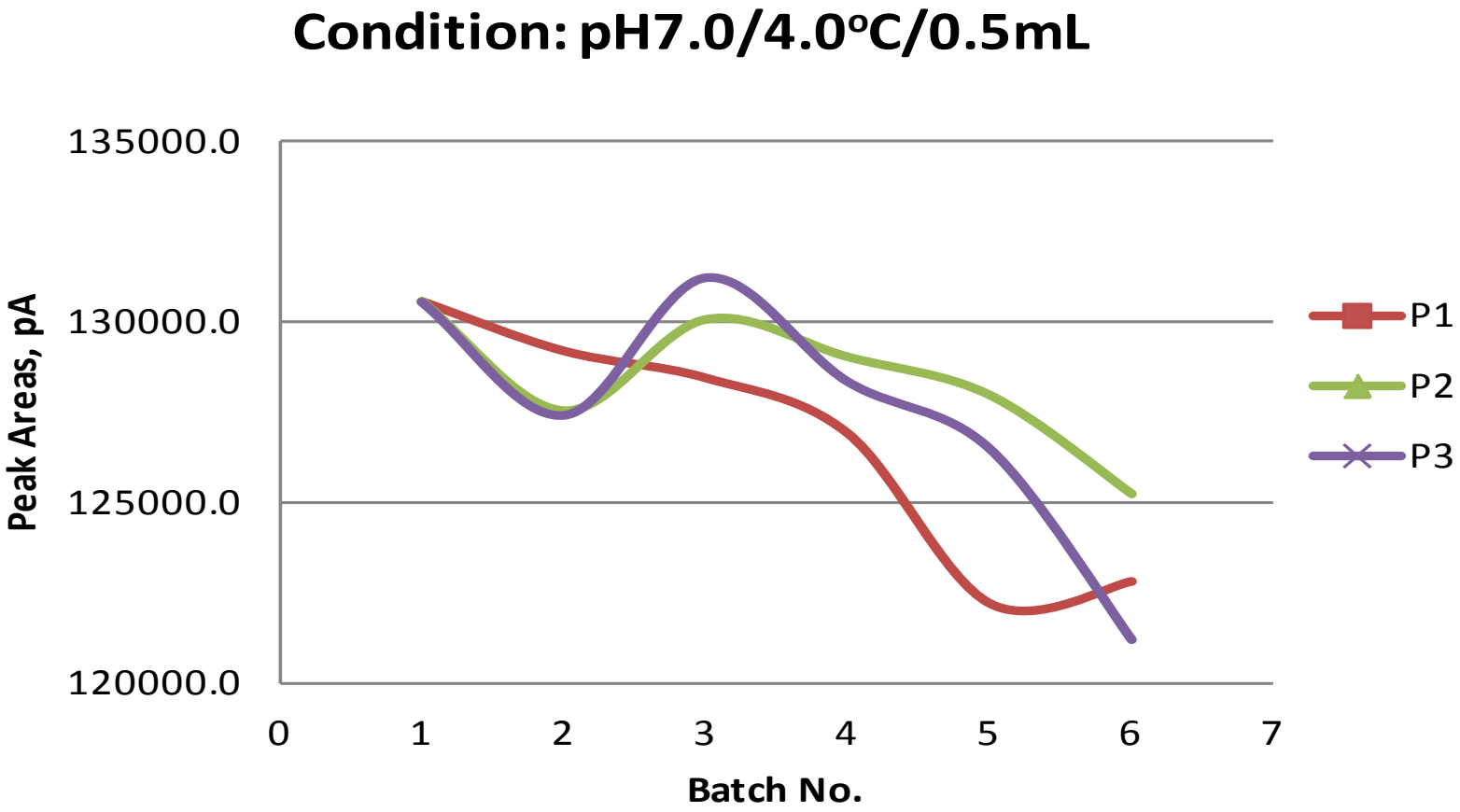
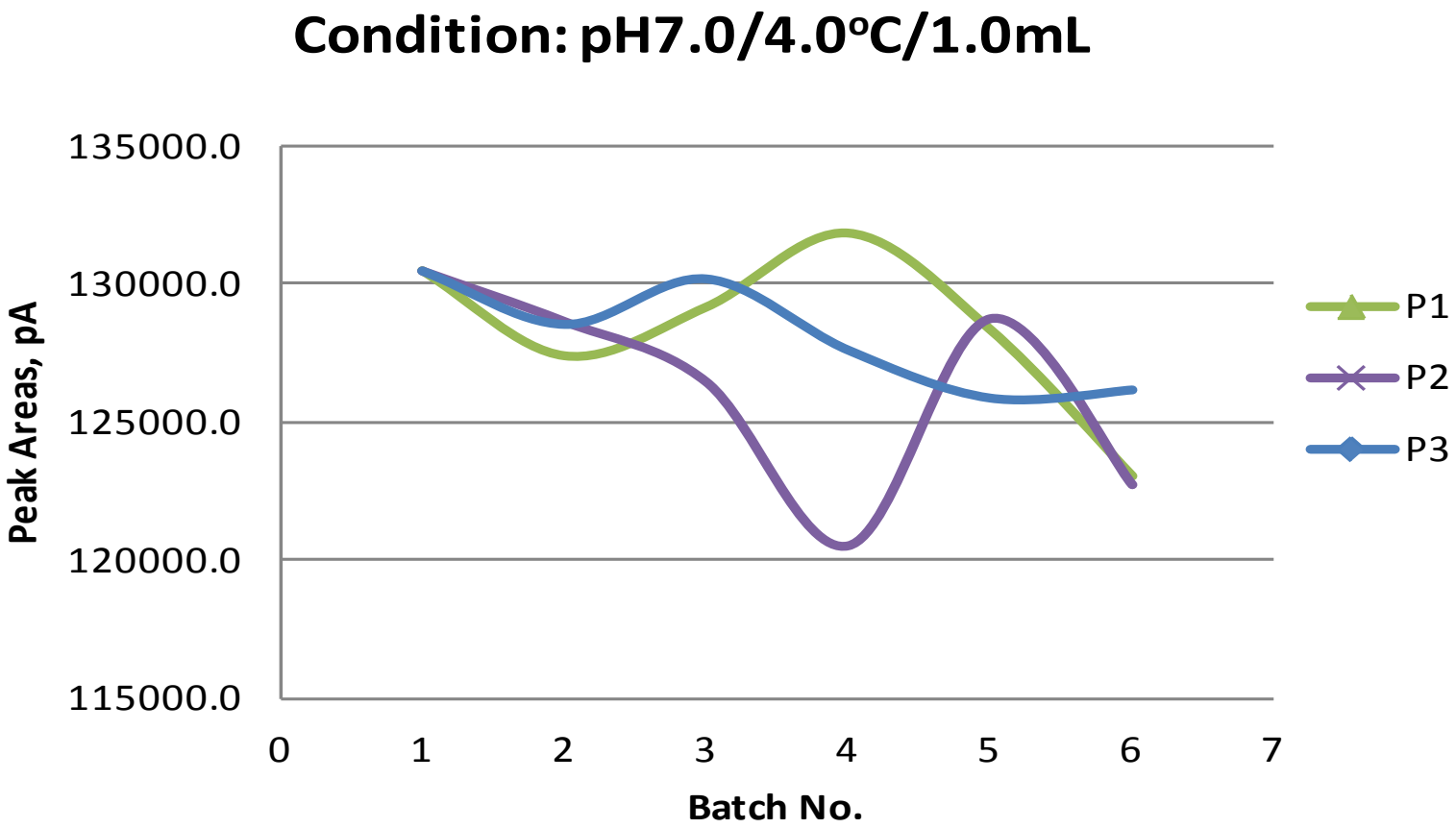
Batch No.
P1
P2
P3


Condition: pH7.0/4.0°C/0.5mL
135000.0
130000.0
125000.0
120000.0
Peak Areas, pA
0
1
2
3
4
5
6
7
Batch No.
P1
P2
P3


Condition: pH7.0/4.0°C/1.0mL
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130000.0
125000.0
120000.0
115000.0
Peak Areas, pA
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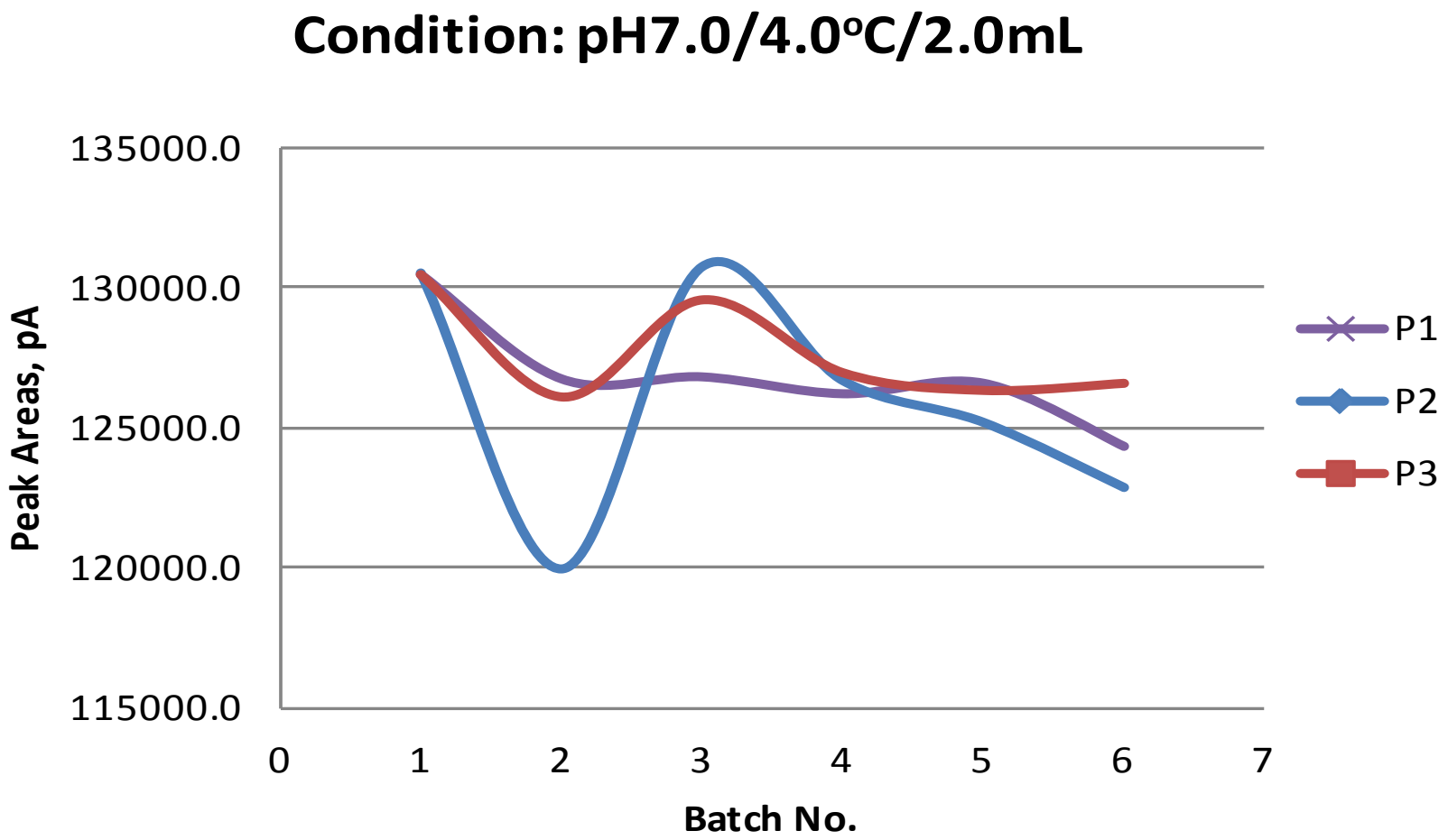
1
2
3
4
5
6
7
Batch No.
P1
P2
P3


Condition: pH7.0/4.0°C/2.0mL
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130000.0
125000.0
120000.0
115000.0
Peak Areas, pA
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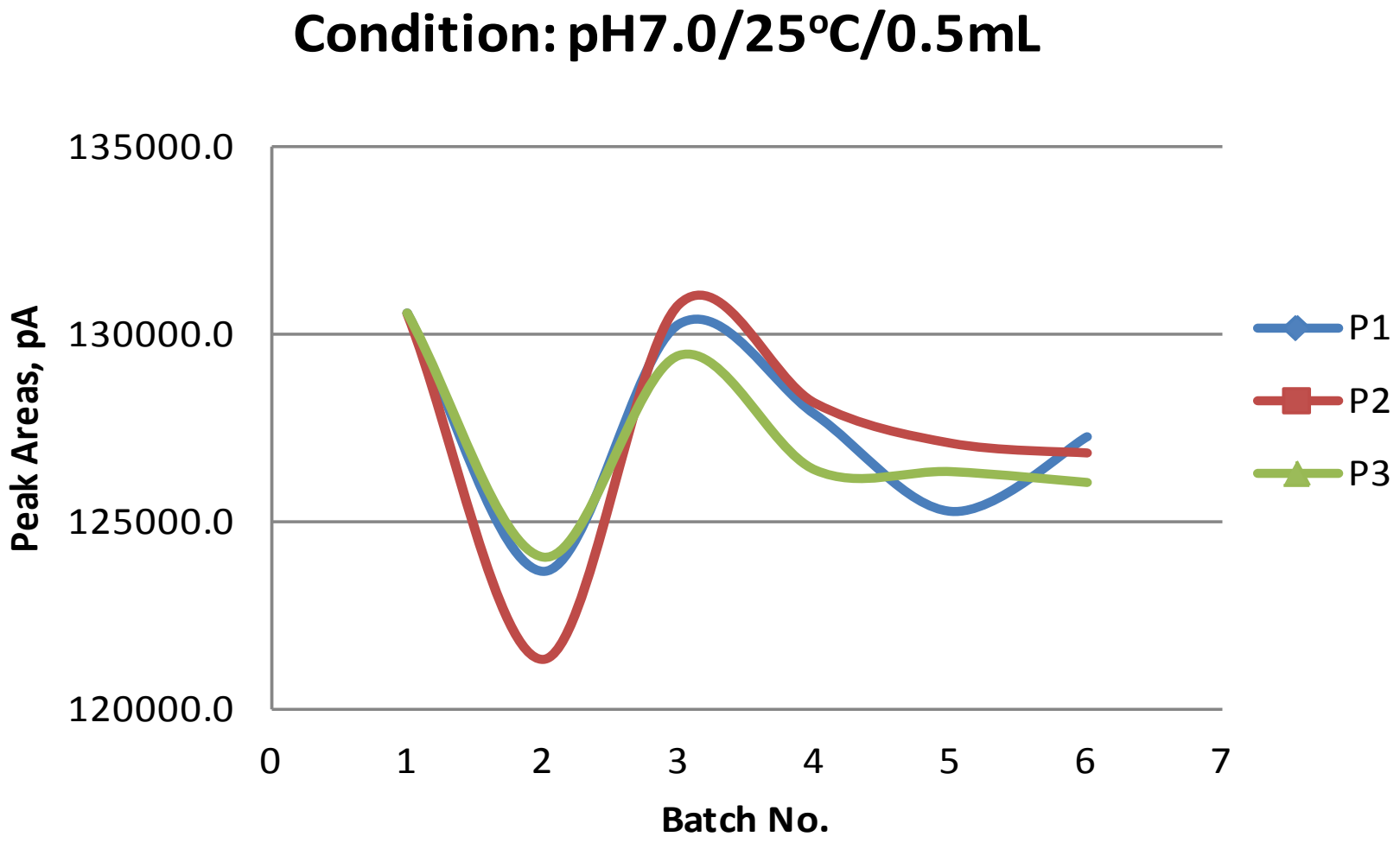
1
2
3
4
5
6
7
Batch No.
P1
P2
P3


Condition: pH7.0/25°C/0.5mL
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130000.0
125000.0
120000.0
Peak Areas, pA
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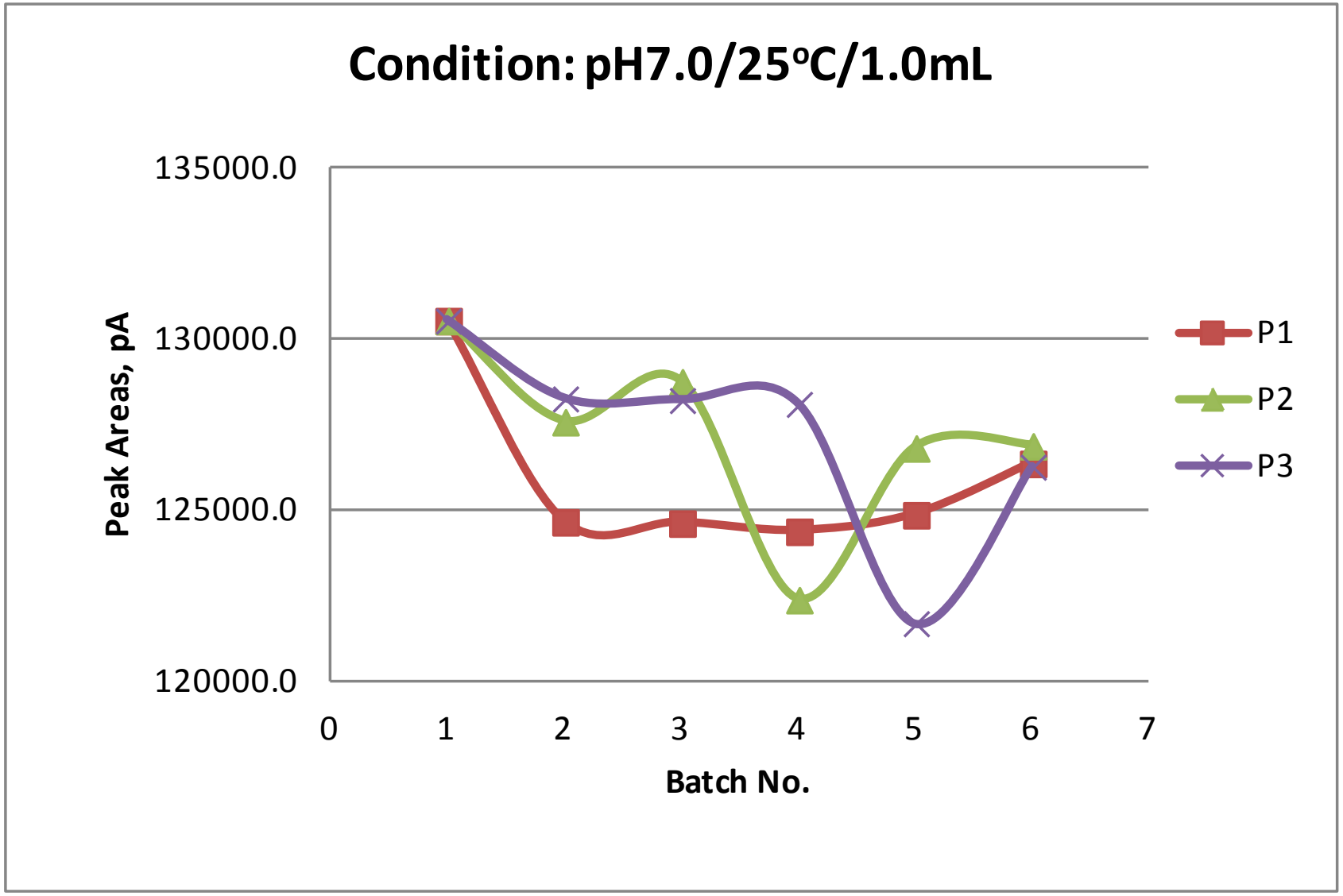
1
2
3
4
5
6
7
Batch No.
P1
P2
P3


Condition: pH7.0/25°C/1.0mL
135000.0
130000.0
125000.0
120000.0
Peak Areas, pA
0
1
2
3
4
5
6
7
Batch No.
P1
P2
P3

APPENDIX B

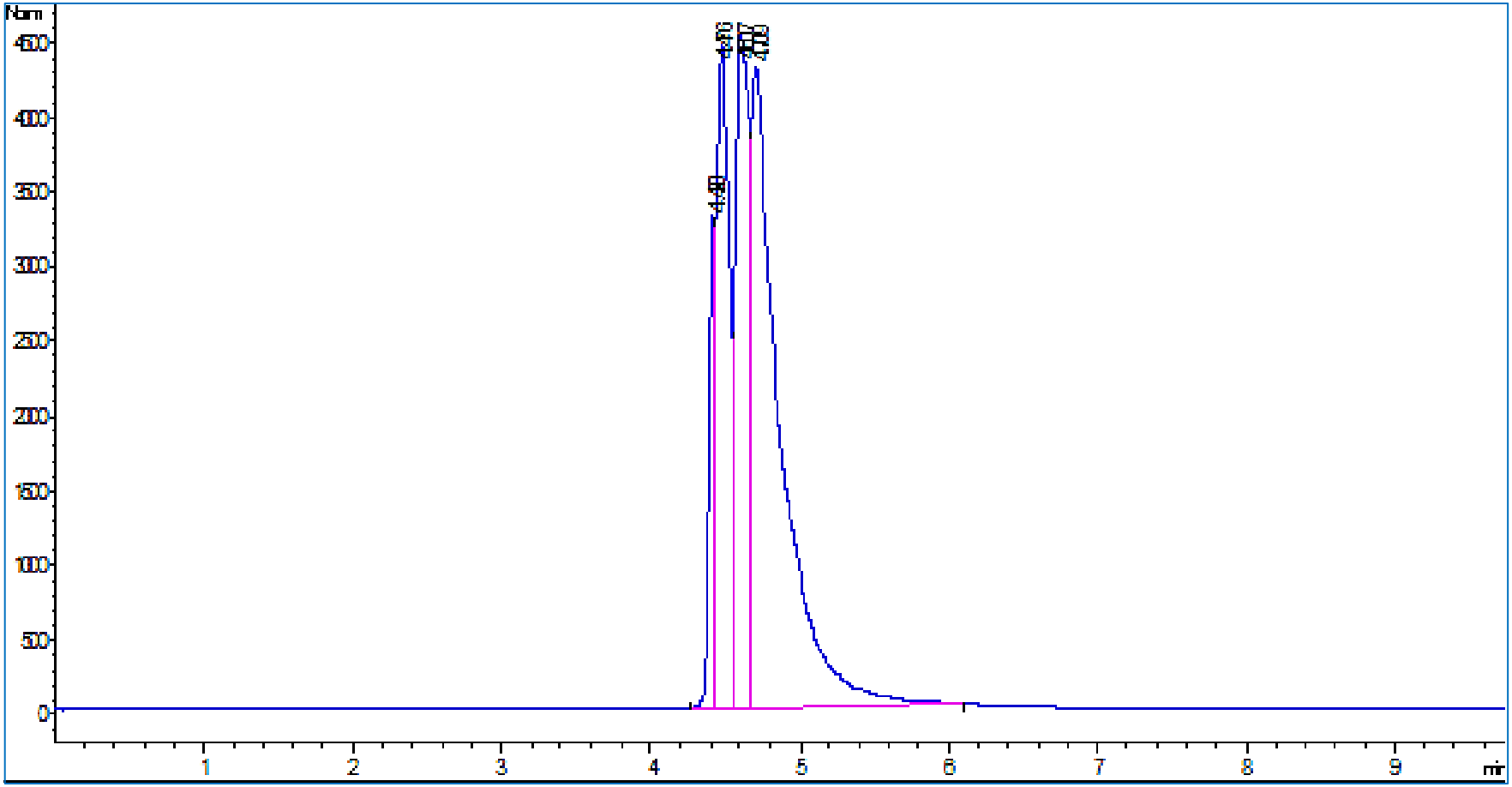


Figure 1 Chromatogram of P2/1.0/pH 4/40oC using GC-FID (pA vs min).

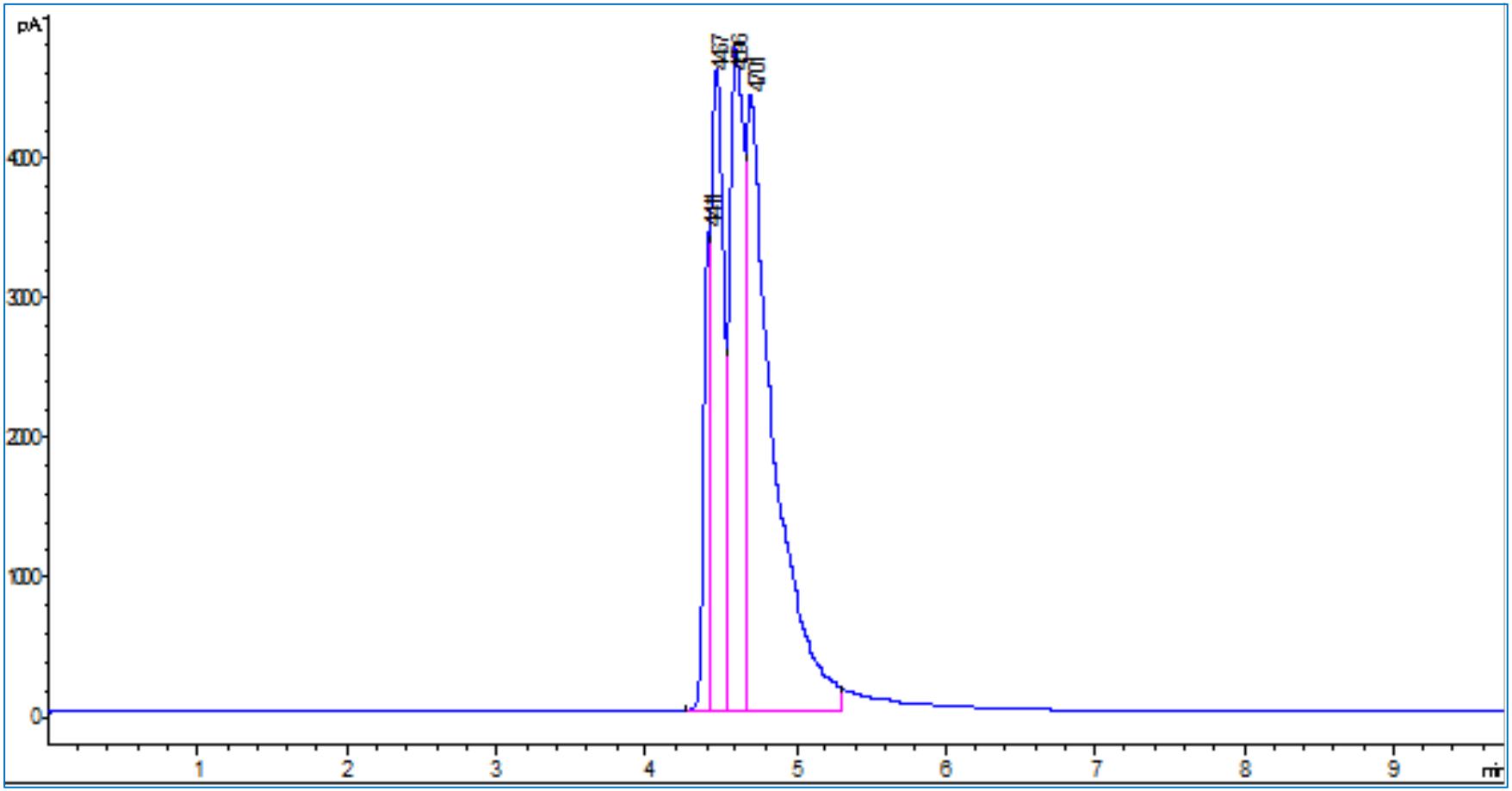


Figure 2 Chromatogram of P1/1.0/pH 4/40oC using GC-FID (pA vs min).

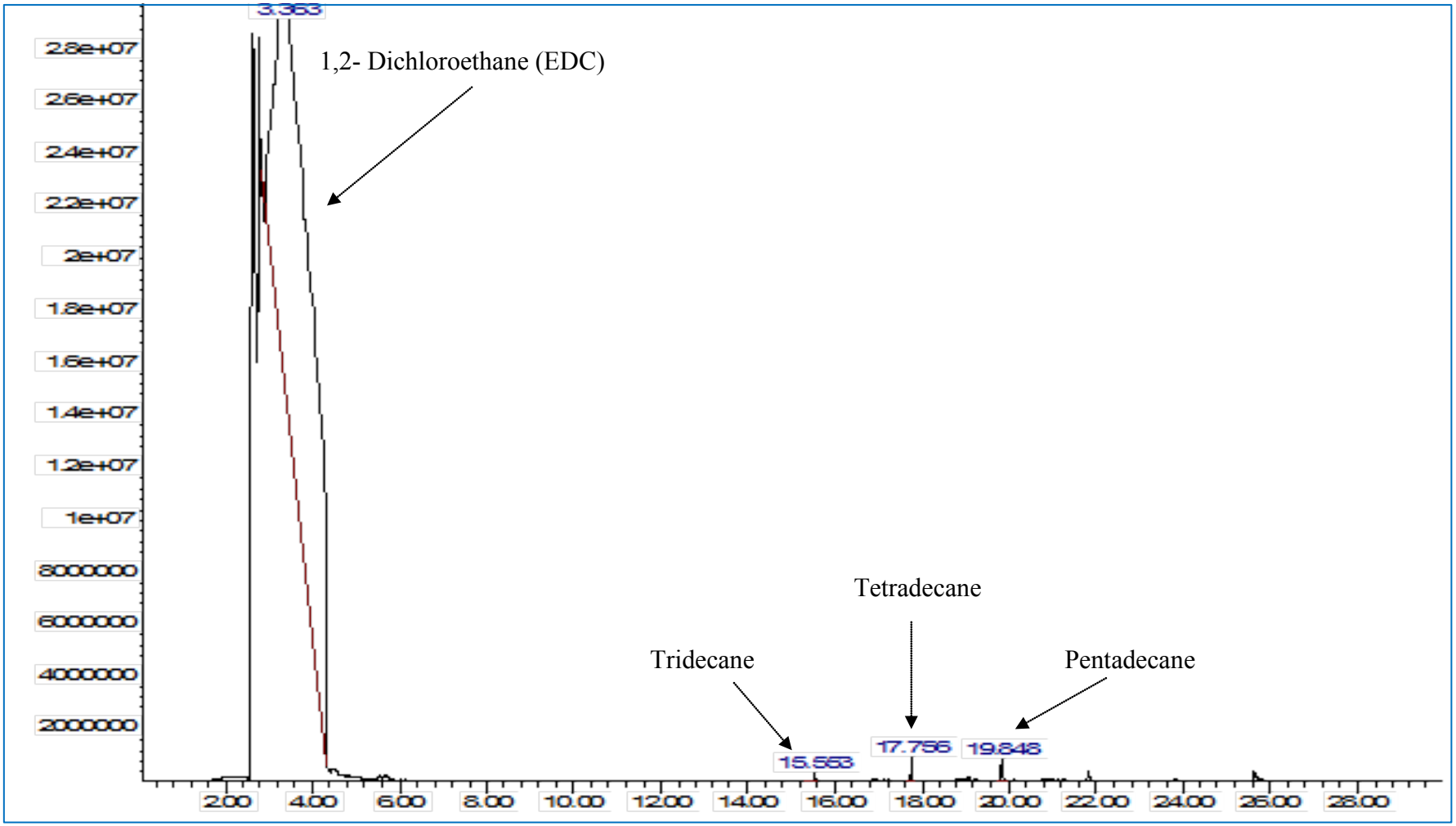


Figure 3 Chromatogram of P2/4mL/pH 7/ 25oC using GC-MS (abundance vs time).

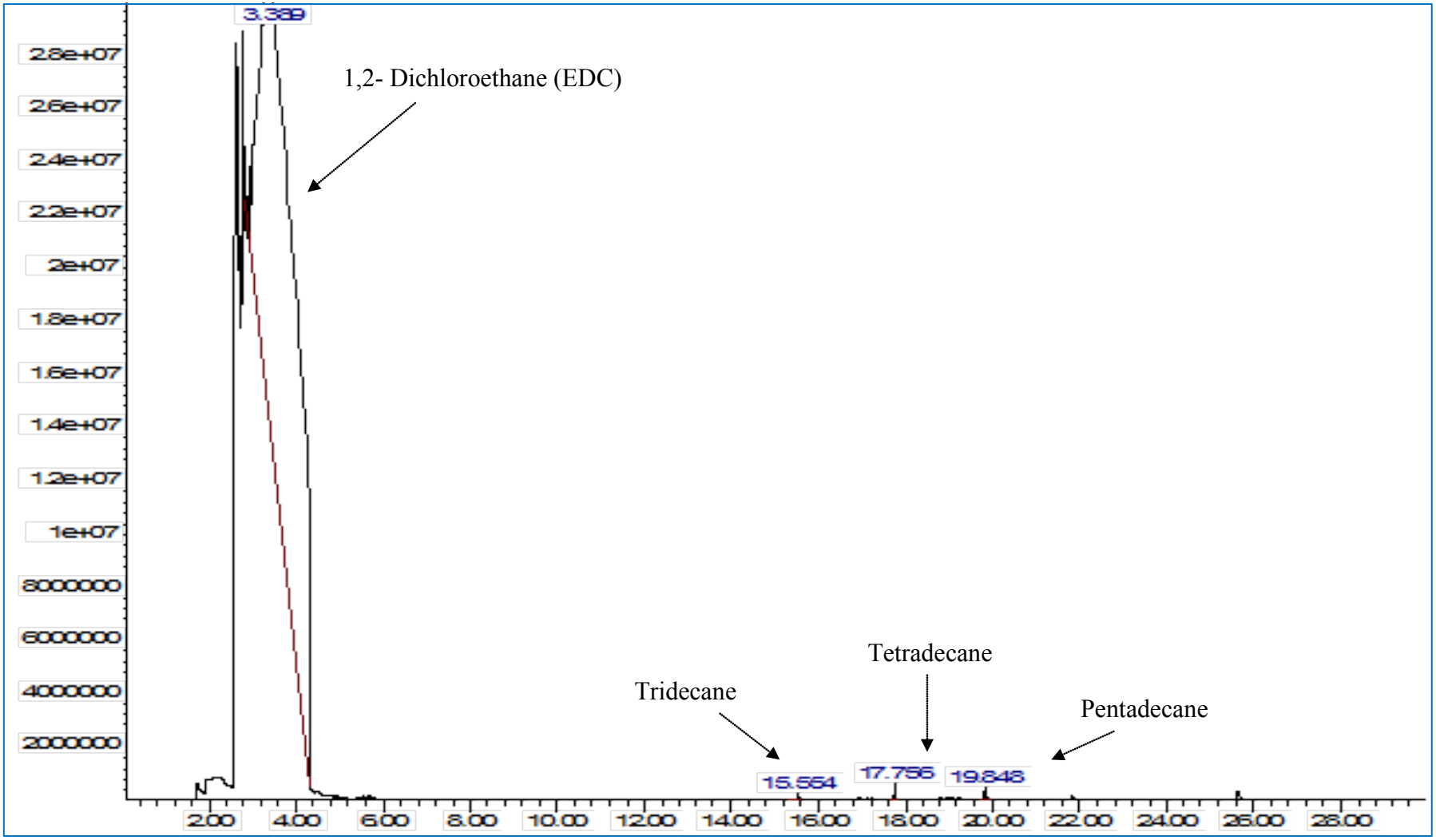


Figure 4 Chromatogram of EZ/4mL/pH 7/ 25oC using GC-MS (abundance vs time).

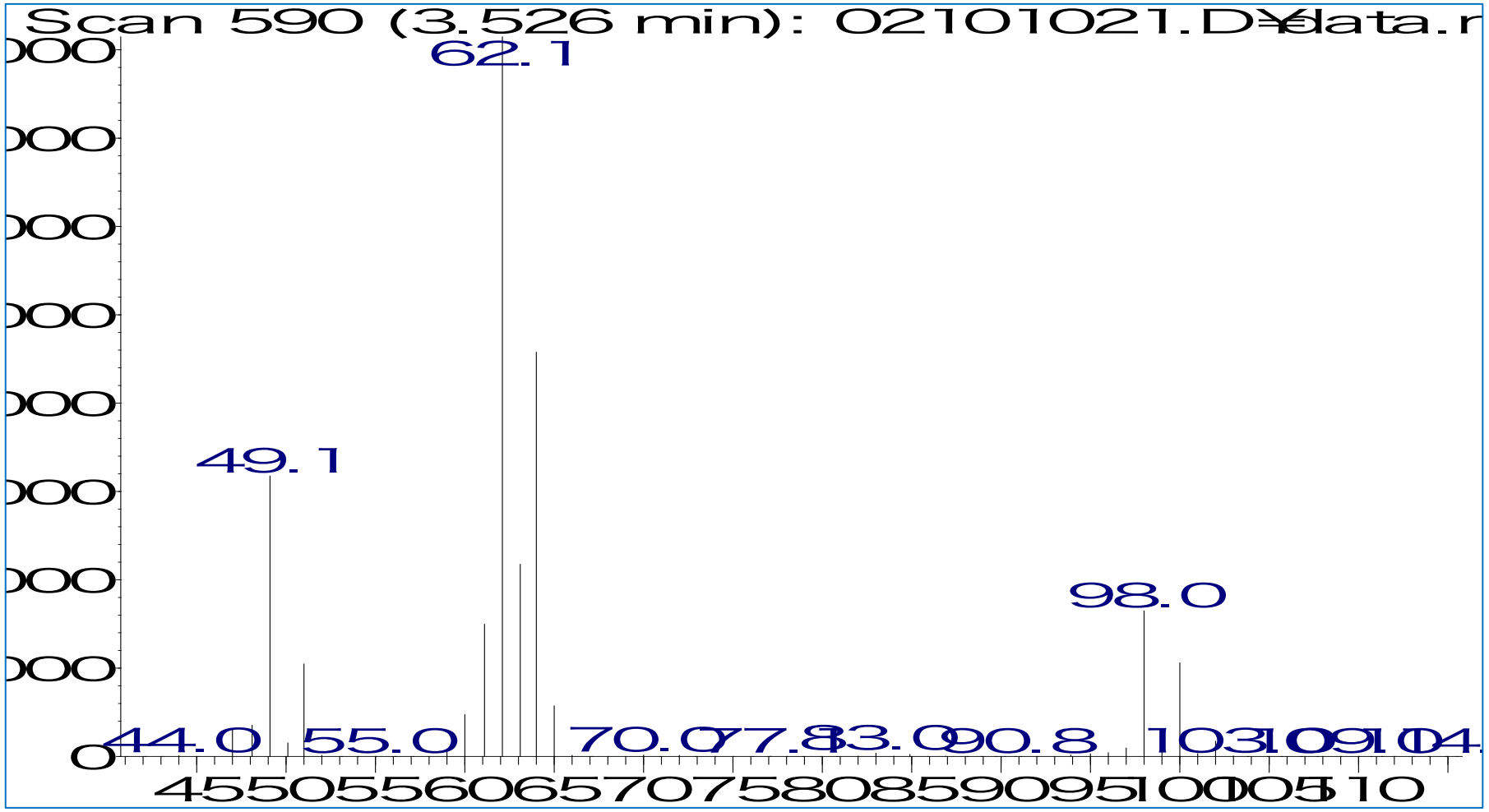


Figure 5 Mass spectrum of 1,2-Dichloroethane (EDC) for the sample (P2/4mL/pH 7/ 25oC) at 3.526minutes.

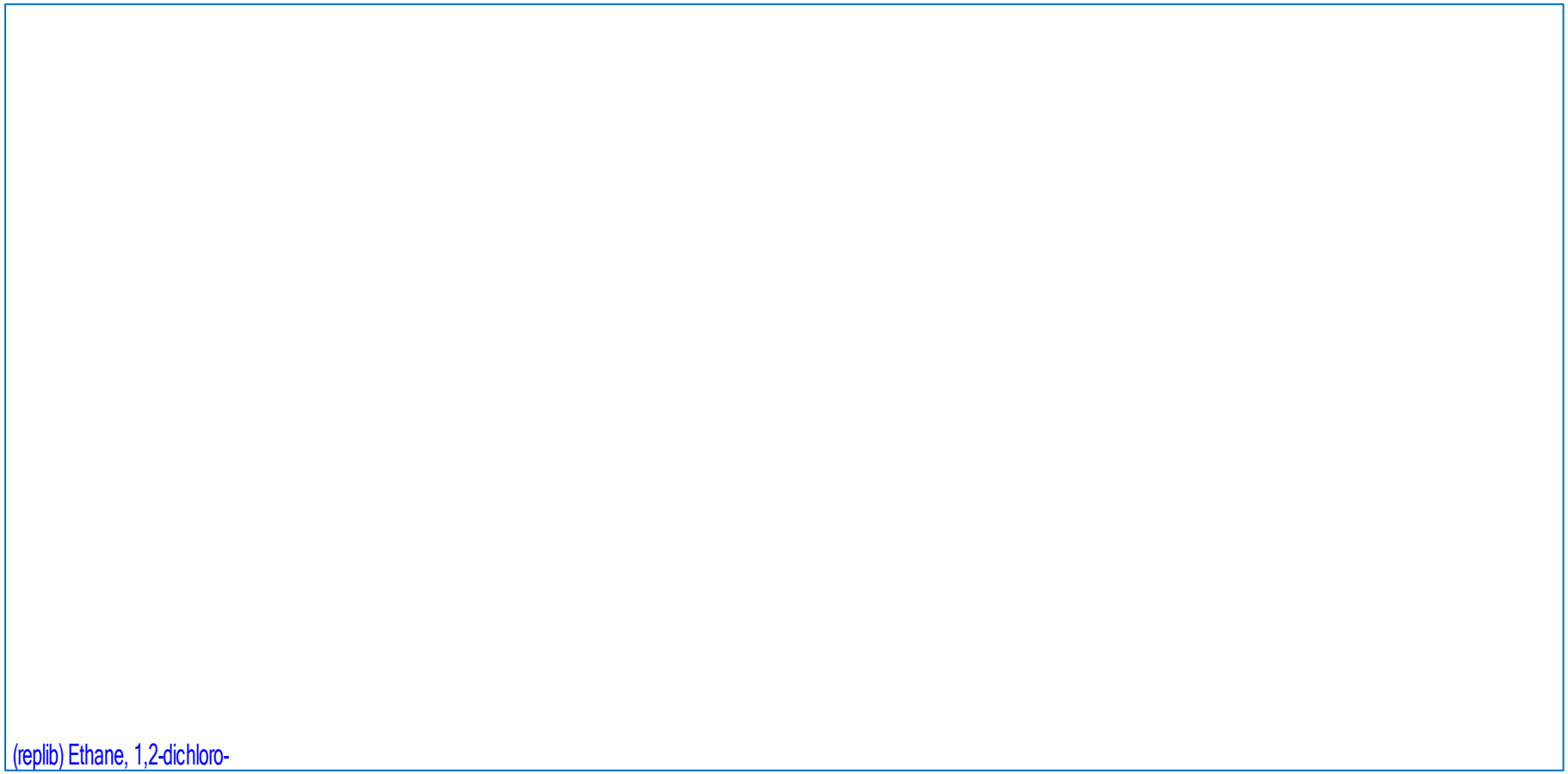


Figure 6 Library search mass spectrum which confirms presence of 1,2-Dichloroethane (EDC) at 3.526min for the sample P2/4mL/pH 7/ 25oC.

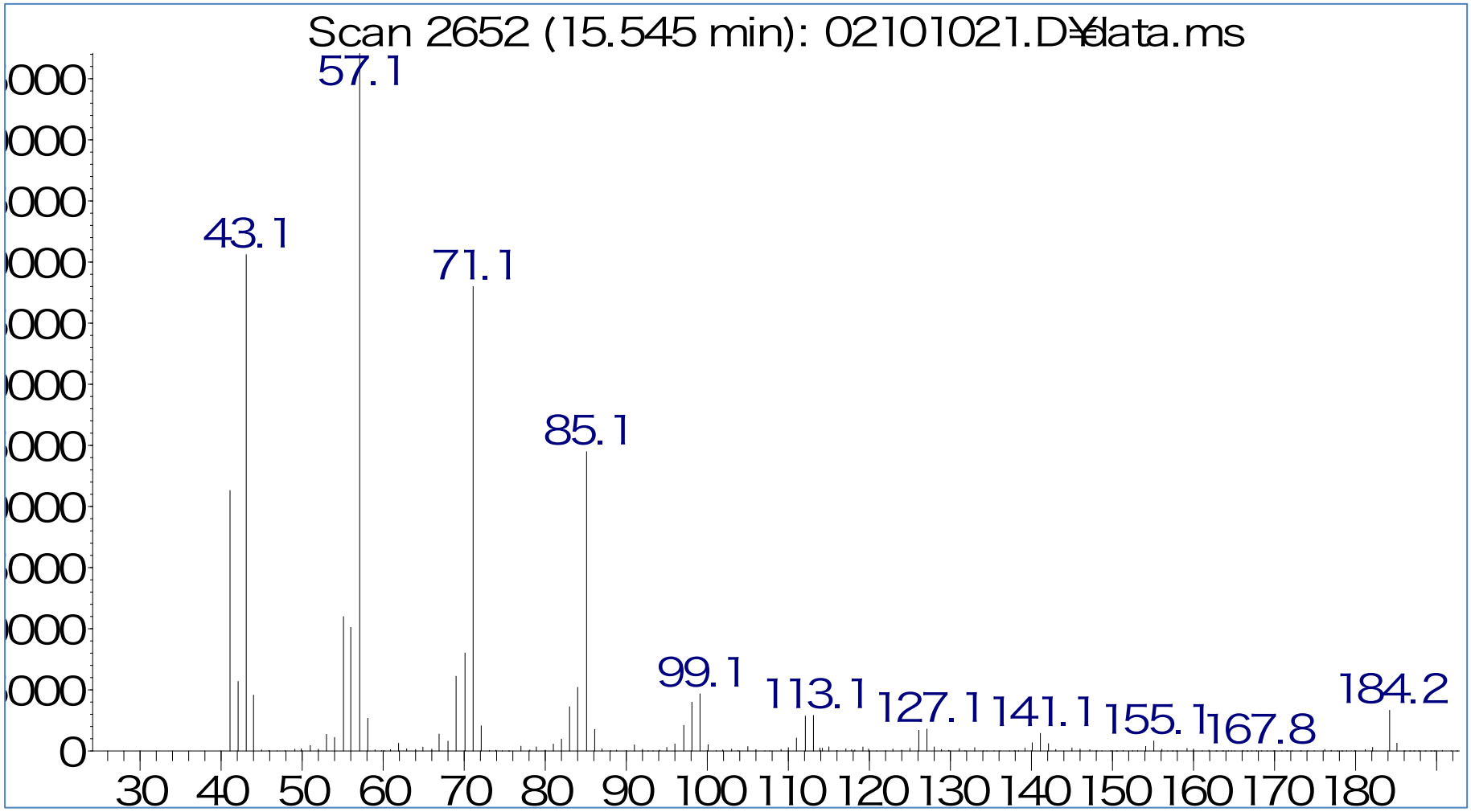


Figure 7 Mass spectrum of Tridecane at 15.545minutes for the sample P2/4mL/pH 7/ 25°C.

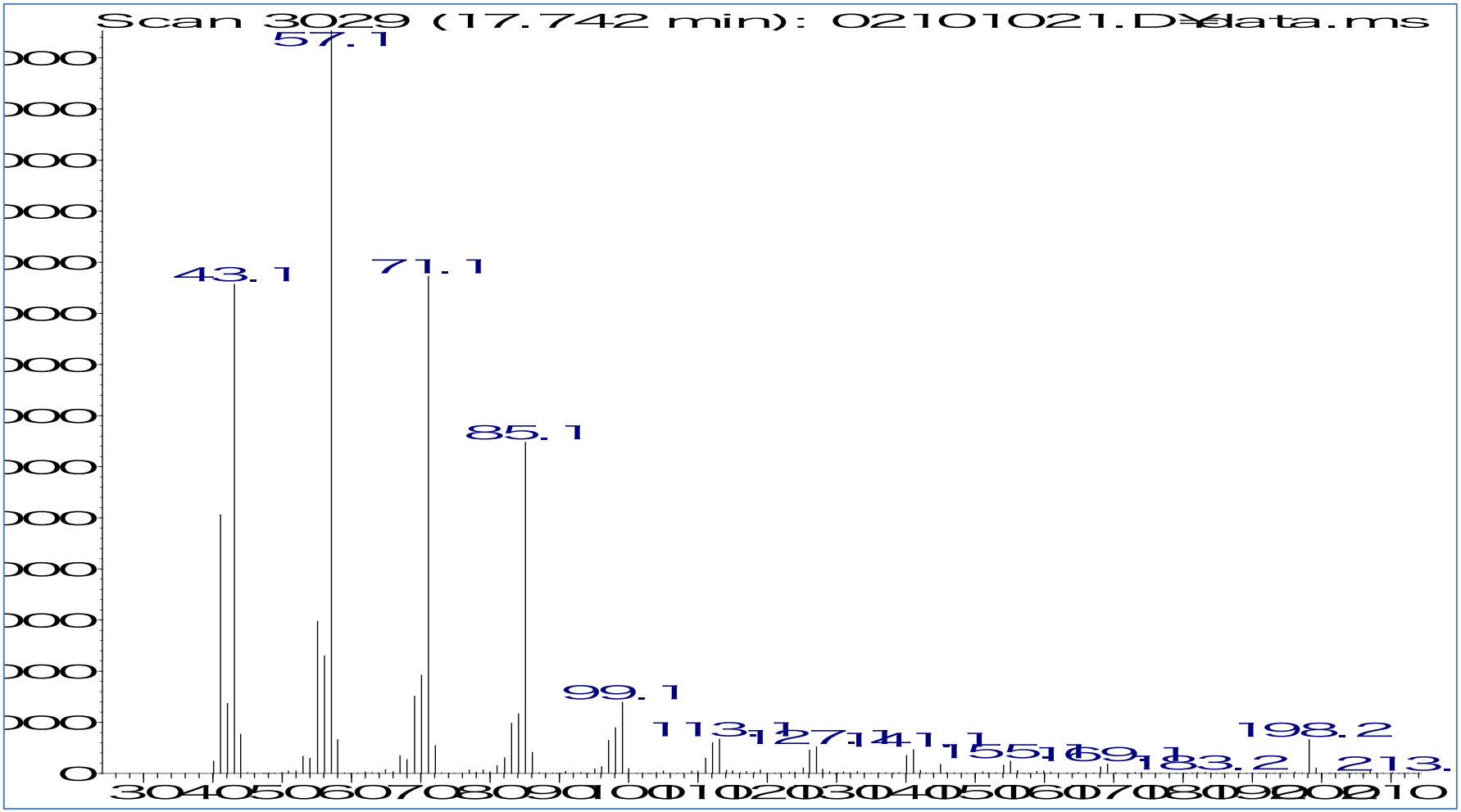


Figure 8 Mass spectrum of Tetradecane at 17.742minutes for the sample P2/4mL/pH 7/ 25oC.

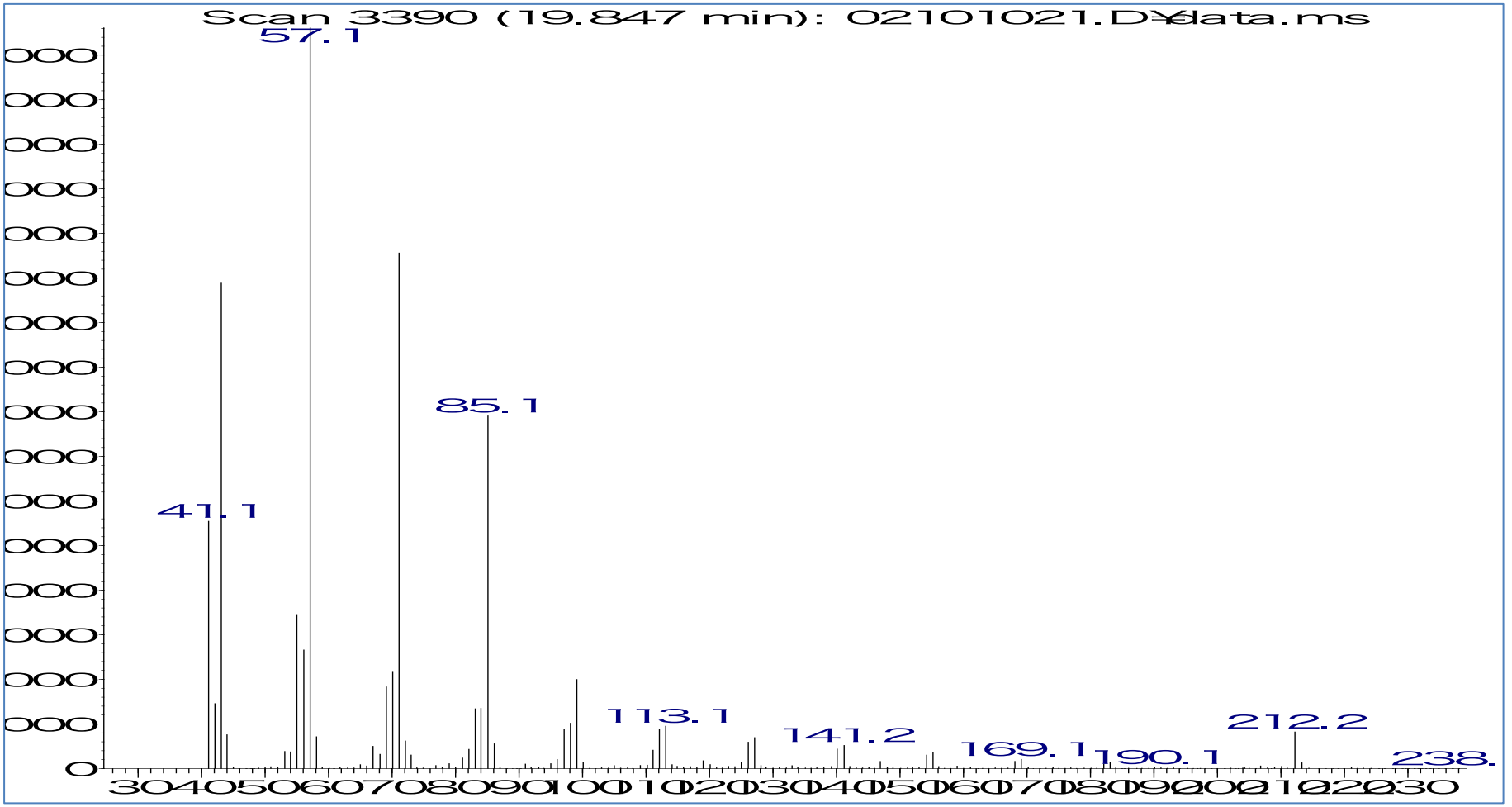


Figure 9 Mass spectrum of Pentadecane at 19.847minutes for the sample P2/4mL/pH 7/ 25^{o}C.

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