海洋性細菌デレヤ・マリナの脂質組成は、脂肪酸組成のみならずリン脂質組成も共に培養の物理的条件によって変化する

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要旨

海洋性細菌デレヤ・マリナの脂質は、小量の中性脂質と大量のリン脂質から成る。リン脂質はホスファチジルエタノールアミン（PE）とホスファチジルグリセロール（PG）がその主なものである。脂肪酸組成は共通しており、炭素数16、18の飽和脂肪酸、16、18の不飽和脂肪酸（モノエン酸）、および17、19のシクロプロパン脂肪酸酸である。種々の培養条件でデレヤ・マリナを培養すると、培養温度、培地中の人工海水の塩分濃度、および培地のpH等の物理的因子によって、デレヤ・マリナの膜脂質組成が変化することを認めた。即ち、膜脂質の脂肪酸組成（飽和脂肪酸、不飽和脂肪酸、シクロプロパン脂肪酸の割合）のみならずリン脂質組成（PE/PG比）も変わることにより、デレヤ・マリナが細胞膜の膜の流動性を一定に維持していることが推定された。

EFFECT OF PHYSICAL FACTORS ON BOTH THE PHOSPHOLIPID AND FATTY ACID COMPOSITION OF THE MARINE BACTERIUM DELEYA MARINA

MARIA ESTELA RIVEROS MEAURIO*

ABSTRACT

The purpose of this research is to study the effect of temperature, salinity and pH on the lipid composition of marine bacterium, Deleya marina. The synopsis of the physical properties of the lipid was presented and they may behave under different environmental conditions was discussed.

Cultivation of temperature was varied from 10°C to 34°C. The pH of the medium was varied from 6.5 to 8.8. The salinity of the medium was also varied at levels from 0.5 to 4.0 times the concentration of sea water.

The main phospholipids found in Deleya marina were phosphatidylethanolamine (PE) and phosphatidylglicerol (PG) and others unknown lipids. The major fatty acids of PE and PG were saturated (16:0 , 18;0) monoenoic (16:1 , 18:1) and cyclopropanoic (17:0 ,19:0) acids.

Not only fatty acid composition but also phospholipid composition of the menbranous lipid from the bacterium Deleya marina, is affected by physical factors such as cultivation temperature, salinity and pH presented.

INTRODUCTION

Bacterial fatty acids usually consist of simpler saturated (SFA) and monoenoic (UFA) components but also contain odd-numbered branched chain and cyclopropane acids (CFA). Also polyunsaturated acids have been found in the lipids from some bacteria and marine organisms. Fatty acids with an odd-numbered of carbon atoms occur in significant amounts in many marine organisms, too. The marine bacterium, Deleya marina is gram negative, and characteristic in cyclopropanoic fatty acid content.

There has been a big question that "How microorganisms adapt themselves to the environmental changes ?" There were many reports about the relationship between FA composition and physical factors, or physicochemical property of PL and physical factors, or PL composition and physical factors.

Temperature dependent change of UFA/SFA ratio has been known well. The increase of UFA/SFA ratio at lower temperature is elucidated as the microbial adaptation for the maintenance of membrane fluidity (Wada, '89).

Salinity dependent change of CFA composition was also reported and discussed in the view point of the lipid phase regulation (Monteoliva-Sanchez, M. '88). As to the physicochemical

property of phospholipid (PL). The effect of pH was considered as the effect of hydratation/dehydratation of the lipid group.

The effect of cations condensing effect was also discussed as to the change of PG/PE ratio by divalent cations such as Mg^{2+} and Ca^{2+}. The change of PE/PG ratio was reported as temperature condition was varied. But the result and considerations were isolated and seemed to be fragmentary. Then we tried to set up the unified view of these knowledges about the relationship between the lipid composition and environmental physical factors.

EXPERIMENTS

CULTIVATION OF THE BACTERIUM

The first step of this research work consisted in the preparation of the medium for cultivation .

We have basically used the medium as follows: glucose 1% polypeptone 0.1% yeast extract 0.0 2% in artificial sea water. The pH of the medium was adjusted by addition of 1N HCL or 1N NaOH before sterilization.

Small fluctuations in pH were corrected by the addition of 1N HCL or 1N NaOH to maintain the pH at the required optimum level during the fermentation process.

To study the salinity effects the samples were distributed

in 500 ml a shaking flask and the salinity was varied from 0.5 to 4.0 times the salt concentration. The incubation temperature was at 22°C and agitated at 200 rpm.

Temperature and pH effects were observed using a 5 lit. jar fermenter the agitation speed was 500 rpm . The pH and the temperature conditions were varied from 6.5 to 8.8 and from 10°C to 34°C, respectively.

LIPID EXTRACTION

The cells were harvested by using centrifugation at 700 rpm for 15 minutes at 4°c. Whole cells were washed with about 100 ml of 0.9% NaCl solution (once) and centrifuged again then they were decanted. The sample was blended with 50 ml of chloroform - methanol (1:2) and 20 ml glass beads and they were disrupted at 100 rpm for 5 minutes at 4°C in a homogenizer (model NISSEI - AM 7)the blended material was filtered in a vacuum and the solid remaining was resuspended in chloroform - methanol (1:2) and was homogenized again this procedure was carried out twice.

The combined filtrates were transferred to a cylinder, water was added and the mixture was shaken thoroughly. The aqueous supernatant was drawn of by aspiration, and the solvent was removed on a rotary vacuum evaporator with water bath (model BM - 42). The bacterial cells were reconstituted with a few drops of chloroform - methanol (1:2) and transferred to a clean minivi-

al suitable for evaporation so the solvent was removed by evaporation with a stream of nitrogen . The lipid extracts obtained were stored until analyzed.

SEPARATION OF PHOSPHOLIPDS FROM THE EXTRACT BY TLC

The sample to be analyzed was reconstituted with a small amount of chloroform - methanol (1:2) and then spotted at the bottom of the plate and the plate then placed in a tank containing the eluting solvent (chloroform - methanol - water 65-25-4 v/v) TLC plates used were 0.25 mm in thickness & 20 cm X 20 cm square pre coated with silica - gel without fluorescent indicator. The solvent moves up the plate by capillary action. When it nears the tops of the plate the plate is removed from the tank and dried to removed the remaining water.

Lipids became visible as brown spots after the plate is left for a few minutes in a tank of iodine vapor. After that the plates were sprayed with a solution of H_2SO_4 13% and $CuSO_4$ 3% and the lipids were visible as a dark brown spots by heating the plates at 140°C for 10 minutes. The spots density were measured with a Shimadzu chromato scanner CS - 930.

OBTAINING OF PE & PG EXTRACT AND DCS

Lipids extract obtained was reconstituted with 300 microliter of chloroform - methanol (1:2) and is spotted in line at the bottom of the plate. After finishing the chromatography process the plate is removed from the tank and dried with a stream of nitrogen and left in a tank of iodine vapor until the lipids fraction became visible to observant eye.

After that is removed of the tank and with a pencil is marked the PE & PG fraction outline.

Then were made the followings steps:

1- Scraping of the plate, the PE and PG fraction were transferred to a conical flask .

2- The sample was blended with 30 ml of chloroform - methanol (1:2) and mixed again in the ultrasonic bath for a few minutes.

3- The were filtered using funnel and filter paper.

4- Evaporation of the solvent with rotary vacuum evaporator.

5- After evaporation was added a few ml of chloroform-methanol (1:2) and transferred into minivials suitable for evaporation with a stream of nitrogen.

6- The PE and PG extract obtained from pH and temperature experiment were blended again with 100 microliter of chloroform-methanol (1:2) They were put 30 microliter in the standard aluminum sample pans which were weighed before putting the sample. After drying with stream of nitrogen they were blended with 5

microliter of water and 5 microliter of glycerine. The aluminum pans were sealed and measured in the DSC 7 differential scanning calorimeter to observe the phase transition temperature.

RESULTS AND DISCUSSION

Deleya marina possesses relatively simple lipid composition.(G C Burguete). Phospholipid were the major lipid, with small amount of neutral lipid.

The FA composition of the neutral lipid was almost the same as phospholipid. Therefore, we considered only phospholipid in this report.

EFFECT OF TEMPERATURE

The figure 1 shows the effect of temperature on growth at each temperature. As temperature was raised the growth became faster. The lipid were extracted at the stationary phase.

The figures 2 and 3 show the effect of temperature on FA compositions of PE and PG respectively. The lines are Regression lines. As temperature was raised, CFA and SFA were increased and UFA was decreased. Correlation coefficients are 0,98-0,99 (SFA & CFA) and - 0,98 (UFA).

PE and PG showed the similar change.

The figure 4 shows the effect of temperature on PE/PG ratio. The line is also the regression line, correlation coefficient is 0,76. Then, we may say, as temperature is raised, PE/PG ratio decreased.

DISCUSSION

These result could be elucidated as follows: As temperature is raised, desaturase is inactivated, and CFA synthetase is activate. They, main phase transition temperature (Tm) of both PE and PG are increase. On the other hand, PE/PG ratio is decreased. Therefore, as a whole, Tm of the membrane is kept constant.

Previously, to elucidate the increase in PE amount, Th (lamella to hexagonal II phase transition temperature) lowering effect was discussed. However, we think that Th is insufficient for the elucidation of these results, because (1) Th is far above the experimental temperature, (2) FA composition is not included in that elucidation.

EFFECT OF SALINITY

The figure 5 shows growth curves at each salinity. Salinity is expressed as relative value to the standard concentration, 3.3%, Temperature was kept at 22 C. Initial pH was 7.8.

The bacterium seems to be grown well at lower salinity. But at 0.2, growth got worse and at 0, no growth was observed,although these data at the lowest salinity is not shown here.

Lipids were also extracted at the stationary phase. The figure 6 and 7 show the effects of salinity on fatty acid composition of PE and PG, respectively. The lines are regression lines. Correlation coefficients of SFA, UFA, and CFA are 0.69 &- 0.42 & 0.35, and 0.76 & 0.72, respectively. Although correlation coefficients of UFA are relatively low, we say that FA composition is affected by salinity of the medium. Besides, PE and PG show the similar results.

The figure 8 shows the effect of salinity on the PE/PG ratio that is decreased as salinity is!increased.Correlation coefficient is - 0.76.

DISCUSSION

The FA composition was changed to lower the Tm. The true meaning of this charge is not known. However, by the so called "cation condensing effect", Tm of PL, especially Tm of PG is greatly raised at higher salinity. Then, PE/PG ratio decreased at higher salinity, and Tm of membrane was assumed to be kept constant.

EFFECT OF pH

The figure 9 shows the effect of pH on growth. pH and salinity were kept at 7.8 and 3.3%, respectively. Significant changes in the content of any fatty acid as a function of pH were observered in Deleya marine.Lipid were extracted at the stationary phase.

The figure 10 and 11 show the effect of pH on FA composition of PE and PG respectively. The lines are the regression lines. It showed the slight increase of CFA and slight decrease of SFA and no change of UFA. However, correlation coefficients of SFA, UFA, and CFA are -0.2, -0.21 & -0.1, and 0.68 & 0.23 respectively, then we may say that FA composition was not affected by the pH condition of the medium. PE and PG showed the similar change.

The figure 12 shows the effect of pH on PE and PG ratio.Correlation coefficient is o.95.
The PE/PG ratio is decreased when the pH is lowered.

DISCUSSION

The effect of pH on FA composition is small. Instead, as previously reported, phosphate part of PL was partly protonated and dehydrated, thus, Tm was raised, as pH was lowered by increasing pH, phosphate part was ionized and likely to be hydrated, and Tm was decreased. Then, PE/PG ratio decreased at lower pH

and increased at higher pH and Tm Of membrane was assumed to kept constant.

Tm ANALYSIS BY DSC

To certify our consideration about the results, measurement of Tm is necessary. Typical results is shown in figure 13. As peaks of the phospholipid is small and broad, and appers quite near to the large H2O peak, it is difficult to determine the main phase transition temperature (Tm). Therefore we are continuing this work to overcome some difficulties in Tm measurement.

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Scheme 1. Experimental Procedure

I. Cultivation of the bacterium

↓

Lipid extraction

↓

II. Phospholipid analysis by TLC-densitometry

↓

Separation of PE & PG

↓

III. FA analysis by GC

(IV) Phase transition temp. determination by DSC

Scheme 2. Cultivation Conditions

◇*Deleya marina* (ATCC 25374) Gr.neg.

◇Medium composition

Glucose	1%
Peptone	0.1%
Yeast extracts	0.02%
Artificial sea water	1L

◇Physical factors of culture

	Temp	pH	Salinity
◆[a]	10 ℃～34 ℃	7.8	1(ca.3.3%)
◆[b]	22 ℃	7.8(init.)	0.5～4.0
◆[a]	22 ℃	6.4～8.8	1

[a] 5 L Stirred tank, 0.5 VVM, 300rpm

[b] 1 L Shaking flask, 200rpm

Fig.1 Effect of Temperature on Growth

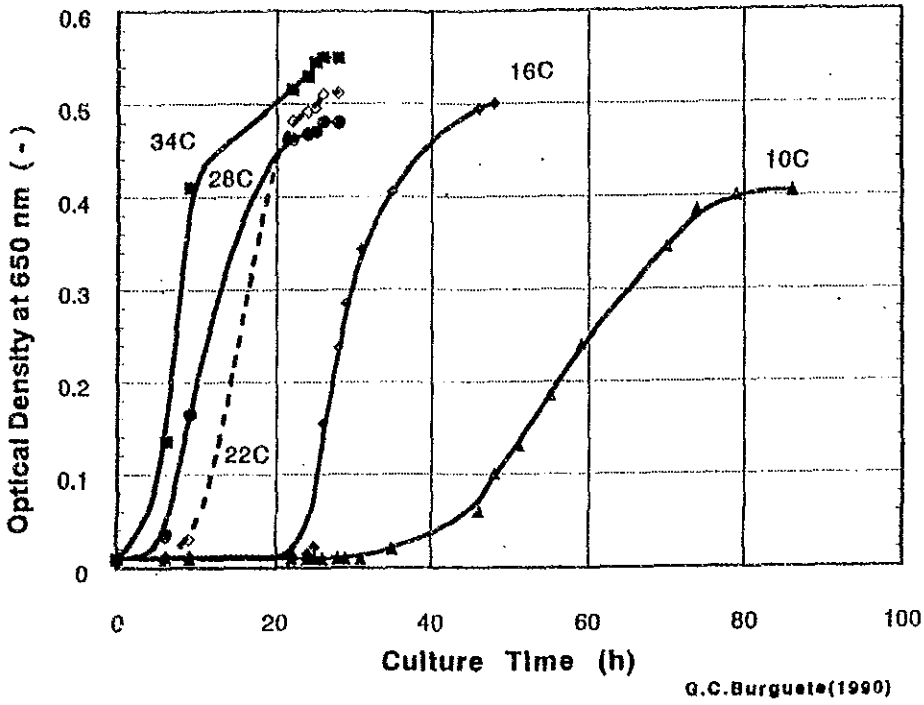


Fig.2 Effect of Temp. on FA Composition of PE

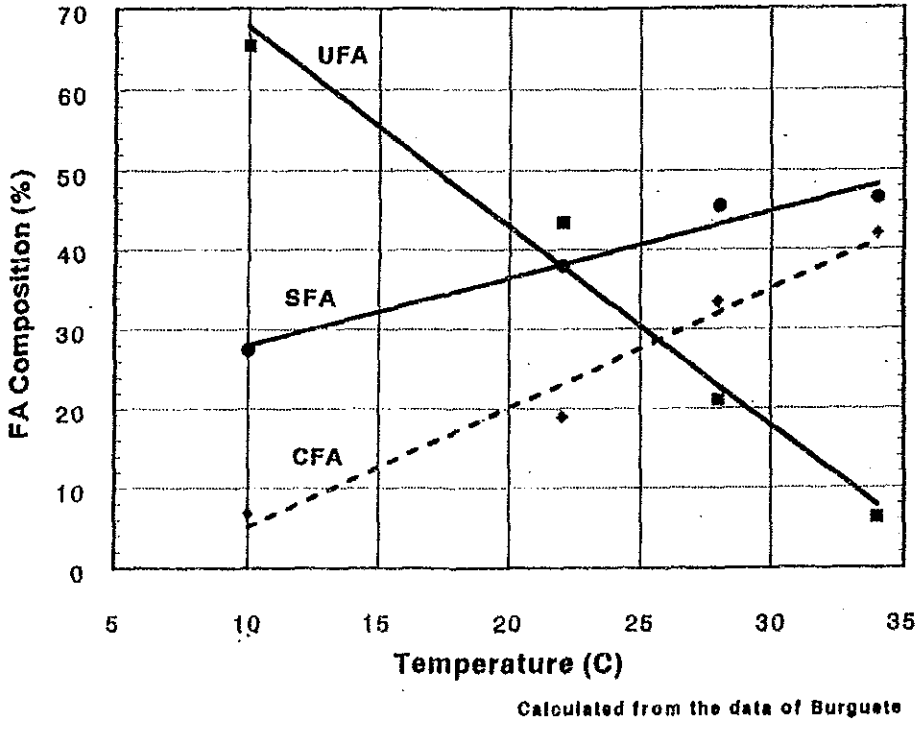
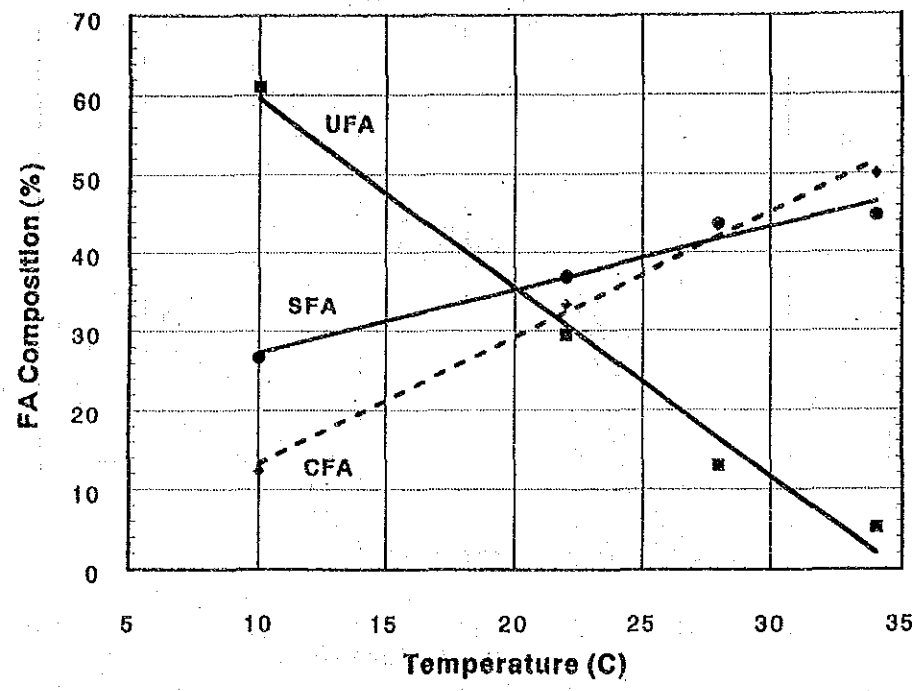
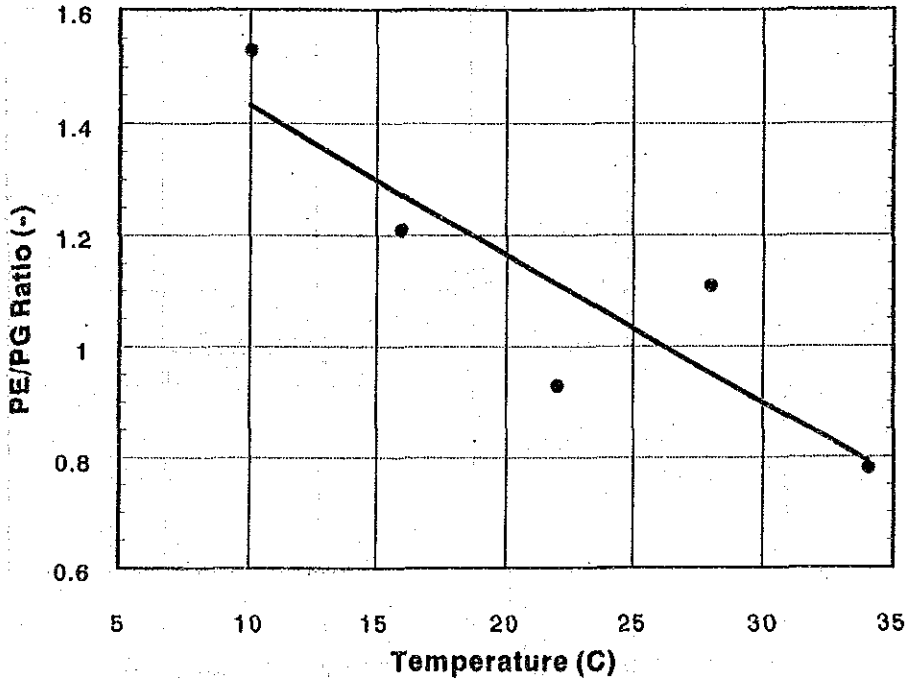


Fig.3 Effect of Temp. on FA Composition of PG



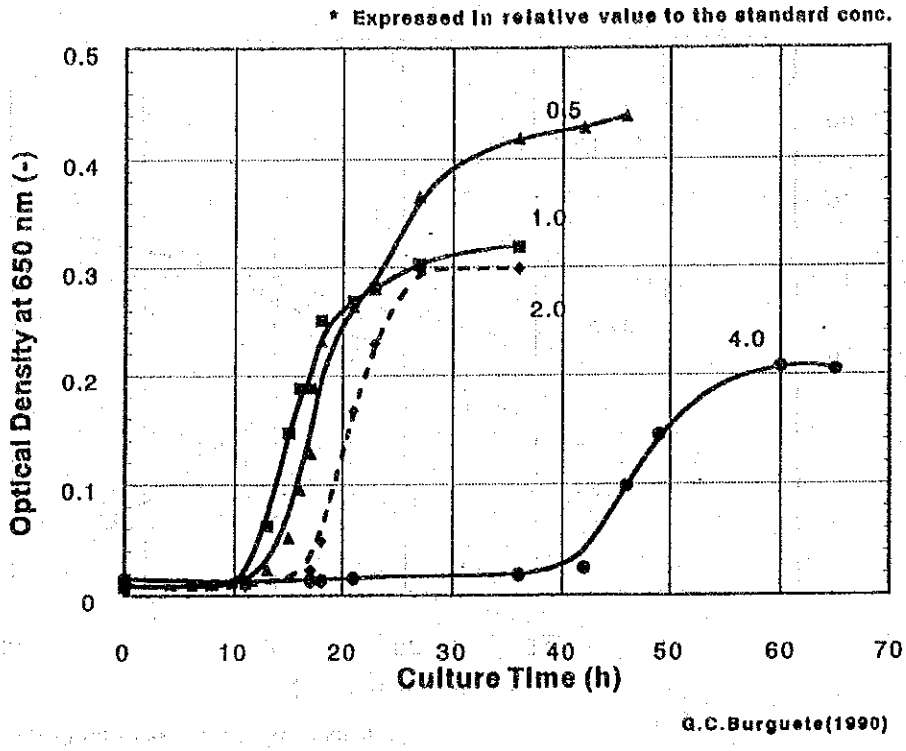
Calculated from the data of Burguete

Fig.4 Effect of Temperature on PE/PG Ratio



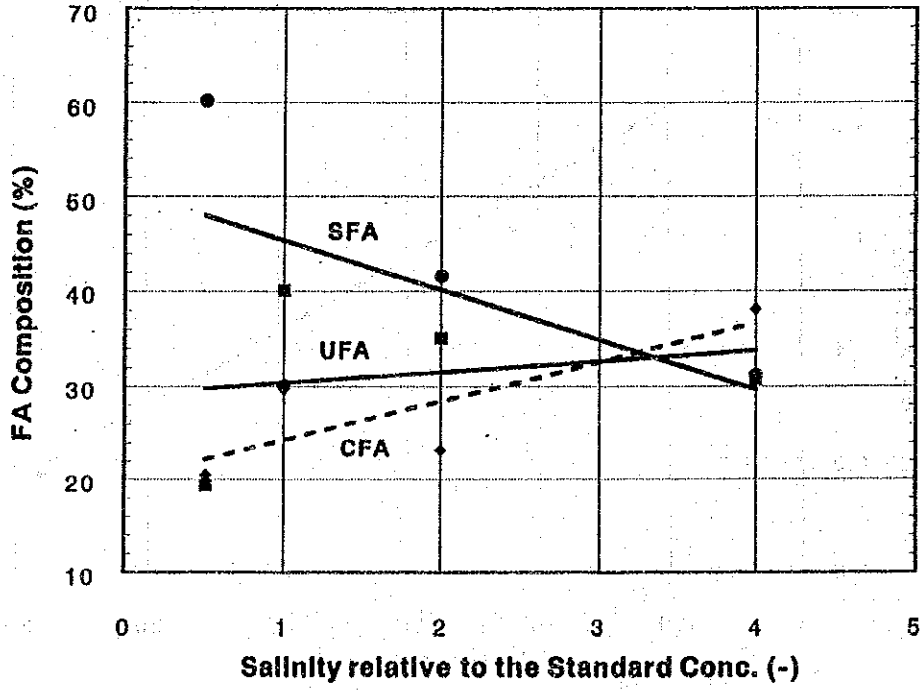
Calculated from the data of Burguete

Fig.5 Effect of Salinity* on Growth



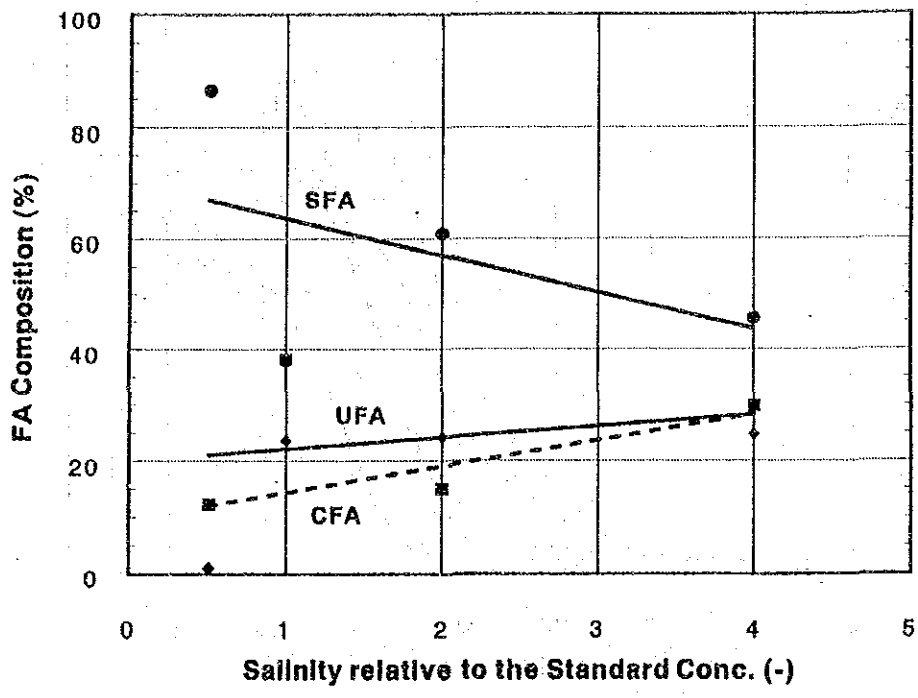
Q.C.Burguete(1990)

Fig.6 Effect of Salinity on FA Composition of PE



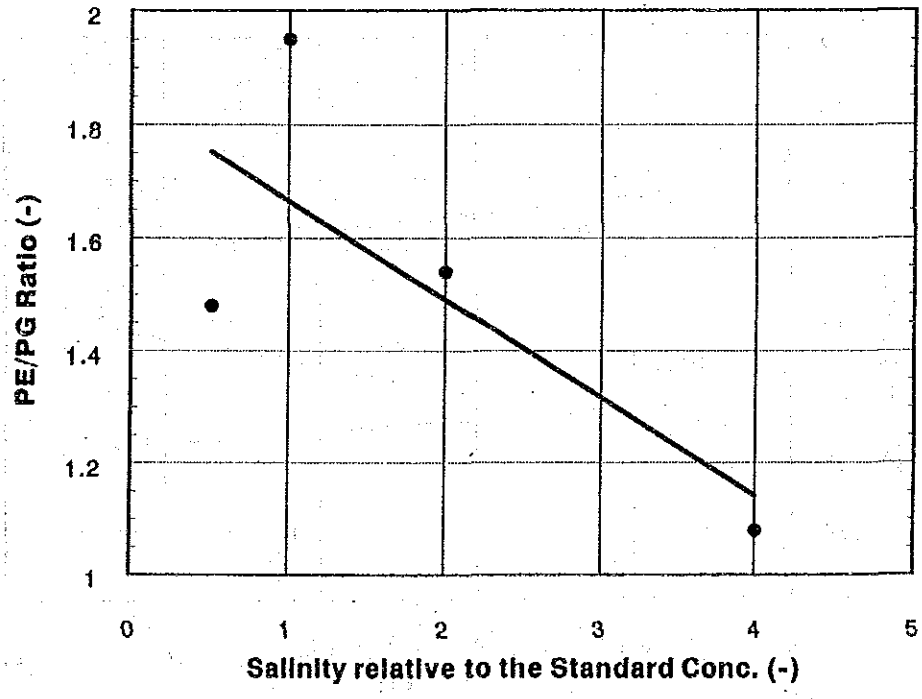
Calculated from the data of Burguete

Fig.7 Effect of Salinity on FA Composition of PG



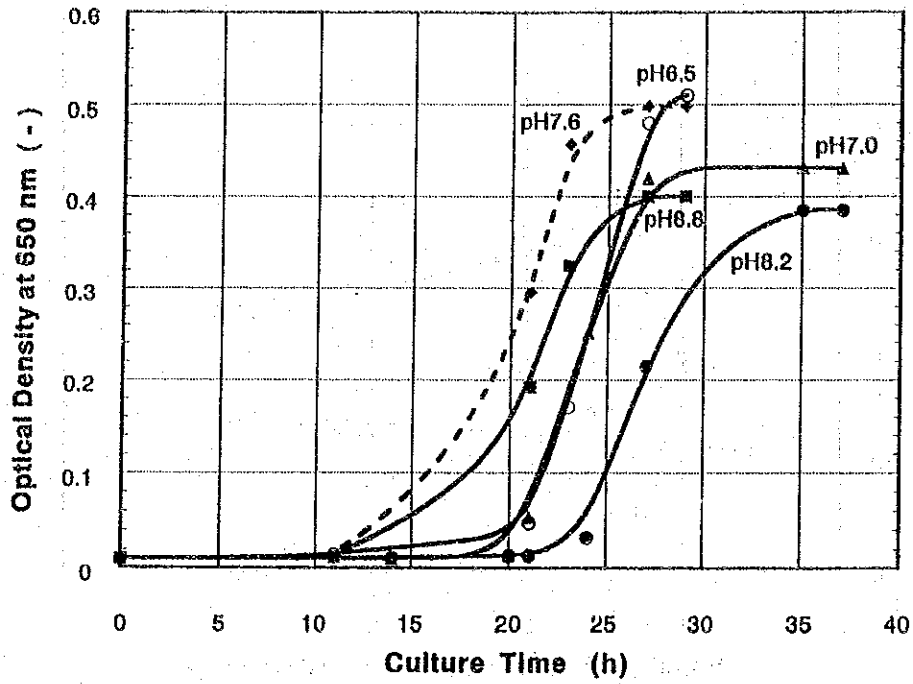
Calculated from the data of Burguete

Fig.8 Effect of Salinity on PE/PG Ratio



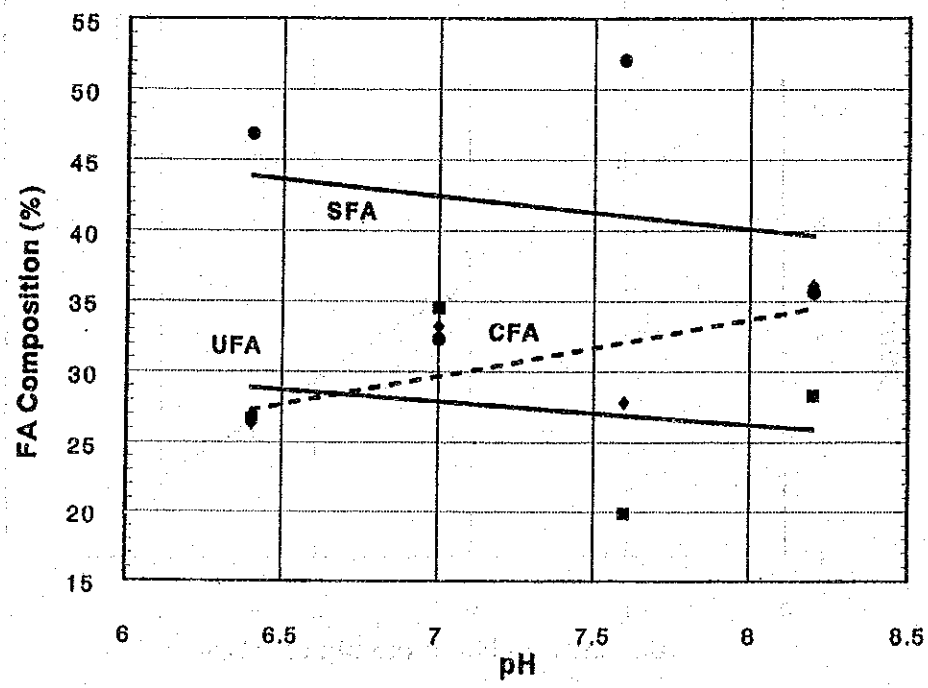
Calculated from the data of Burguete

Fig.9 Effect of pH on Growth



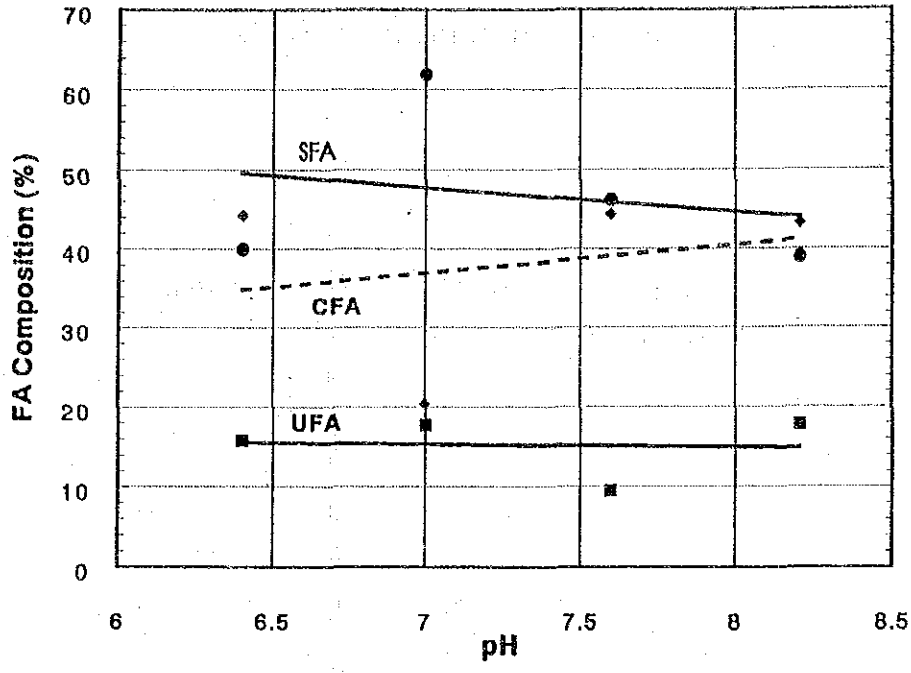
G.C.Burguete(1990)

Fig.10 Effect of pH on FA Composition of PE



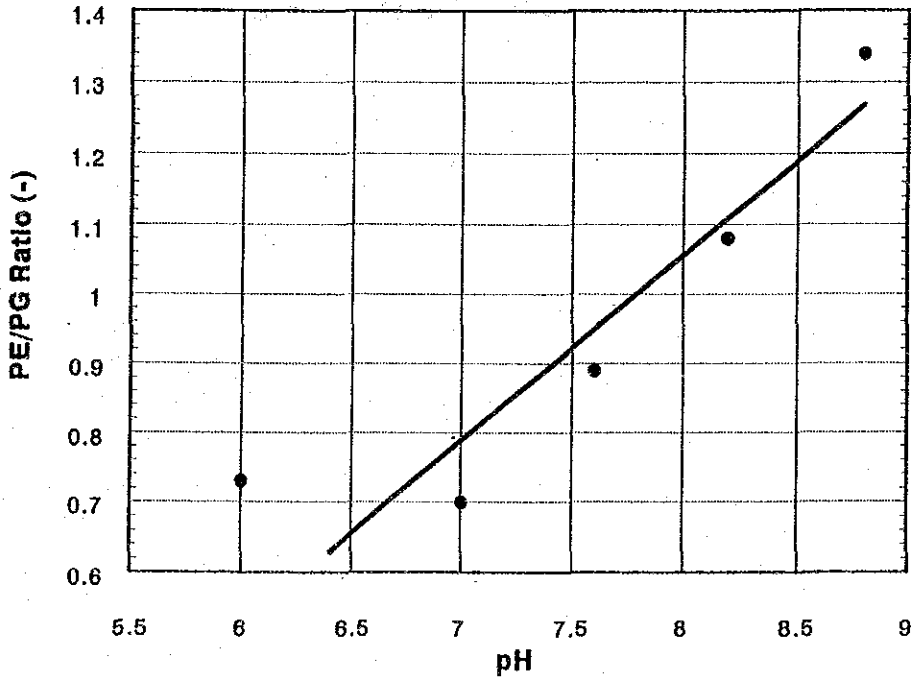
Calculated from the data of Burguete

Fig.11 Effect of pH on FA Composition of PG



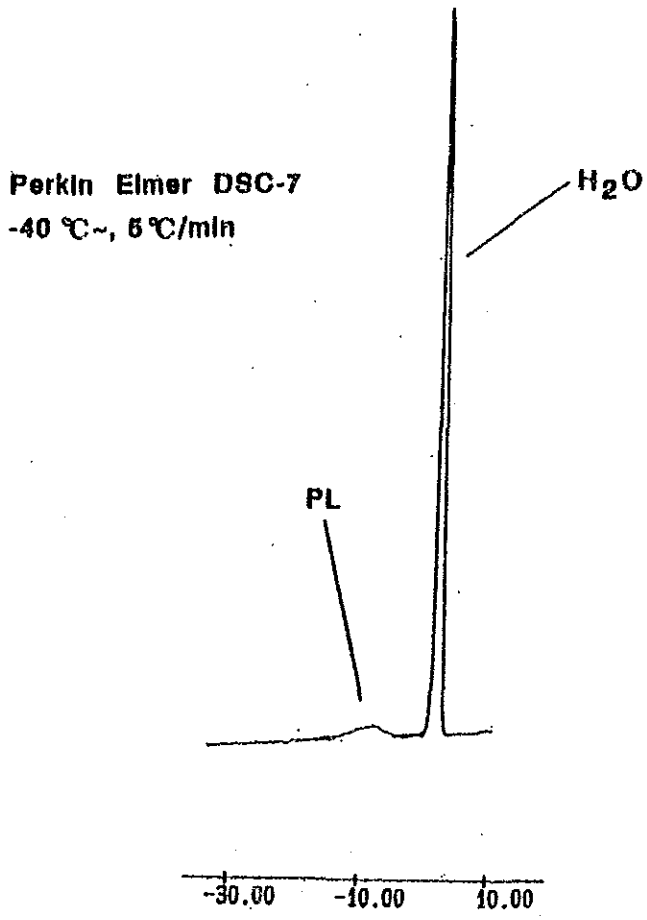
Calculated from the data of Burguete

Fig.12 Effect of pH on PE/PG Ratio



Calculated from the data of Burguete

Fig.13 Tm Analysis by DSC



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T S U K U B A - J A P A N

第１０回化学技術研究コース 報告書要旨
研究課題：環境計測技術と評価
研修者　：ビクトル　(Victor Eugenio Barreto Medina)

産業革命は鉱工業の急激な発展を促し、人間社会を変化させることによって、それまでより、自然に対してより強く影響を与えるようになった。さらに近年になって急激な産業の発展と世界人口の増加によって単に地域の問題ではなく、国際的視点からの地球環境汚染が問題になっている。

水質汚濁の原因には種々の人類の活動がある。１）鉱業；金属、石炭、硫黄、石油、岩石・珪酸塩　２）農業；肥料、殺虫剤、農薬　３）畜産及び漁業；し尿、非利用部分、処理廃液　４）人間生活；生活廃液、し尿、廃熱など　５）工業；食品、化学、パルプ、繊維、製紙、石油、洗濯、皮革、製鉄、非鉄、セラミックス、めっき、ソーダ工業など。　水質汚濁の主な項目としてはＢＯＤ、ＳＳ、油分、酸・アルカリ、アンモニア、フェノール、硫化物、色度、臭気、界面活性剤、熱などがある。また、汚染源の立地条件によっても環境への影響は異なる。

水質測定の技術は分析機器と分析手段の発達により大きな進歩をし、簡易な方法により迅速に再現性のある結果が得られるようになった。しかし、実際に応用するときは分析方法の全体、適用の範囲などを考慮し、より適した方法を採用する必要がある。試料の採取においては地域の特殊性、状況、季節、天候、時間、潮汐、風向、地形、深さなどを正しく把握し、測定項目や測定目的に最適な採取、前処理、保存をしなければならない。

ｐＨはガラス電極で測定する。懸濁物はフィルターで濾して重量法で、濁度は光学的方法で測定する。一般的化学的成分の測定には滴定法、重量法が、微量化学成分の測定には吸光光度法、原子吸光法、ガスクロマト法などが用いられる。

前処理法として塩酸による分解、硝酸による分解、溶媒抽出法、酸化・還元、蒸留などによって各成分の分離や選択的反応のための準備がおこなわれる。

分析方法各論として隣化合物、窒素化合物（アンモニウム、亜硝酸、硝酸イオン、有機窒素）、酸消費量、アルカリ消費量、硬度、カルシウム、マグネシウム、クロム、シリカの分析法を列挙した。

廃水の処理には一次処理（固形、懸濁固体の除去）、二次処理（溶解、懸濁有機物の除去）、三次処理（窒素、隣、微細懸濁物除去・微量有機物除去・溶解無機塩の除去）がある。

かって日本は水に恵まれた国であったが経済発展のために選択の余地の無い高度な工業化の発展をし、同時に大量の廃水による環境の汚染を起こした。クローズドシステムがこの相反する問題を解く鍵である。廃水の三次処理を行い水の再利用が行われるようになるだろう。いかにして簡易に、経済的に水の再利用がおこなわれるかなど、現在の世界には多くの問題が残されている。

Abstract

The present final report includes the following: In the first part, estudies of cause abaut water pollution, in the secund part water samples of lake, river and sea, and their analysis chemical respectively, in the last part the waste water treatment.

INTRODUCTION

Mankind began to influence nature actively from the time when various forms of primitive industries including the agriculture, weaving and the manufacture of metal tools were started several thousand years ago, but from the time of the industrial revolution which took place some 250 years ago, mankind started to influence its environment more actively than ever before. The development of the coal mining industry and the invention of the steam engine have accelerated the growth of various industries, which on the other hand have contributed not only to the improvement in de quality of the life of mankind but also to the rapid increase of population. Such rapid growth of industries since the industrial revolution has been conspicuously influencing the natural environment, but we have preoccupied with endless pursuit of the abundance in our daily living supported by the modern industries and did not realize that the knowledge of sciences and technologies should have been used for the conservation of the natural environment. Rather, the people who have lived in the age of the rising industrialization were proud of the black smoke rising into the air from stacks of the factories as the symbol of the prosperity of the community or the country. Today, However, the increase in the world population with the progress of civilization has been so great that it is described as the population explosion, and such as the shortage of food supply in cities. The world environmental pollution by the industries in many countries has emerged in recent years as the problem that calls for action by the individual countries from international standpoint; Because there are no national boundaries when environmental pollution or destruction are progressing gradually throughout the world.

Water Pollution Sources

water pollution is a phenomenon that is characterized by the deterioration of the quality of land water (rivers, lakes, and ground water) or sea water as a result of various human activities. Pollution which is currently in progress in our modern society industries such as mining, agriculture, stock breeding, fisheries, forestry, urban human activities, manufacturing industry construction works and various tertiary industries.

Classify waste water according to their physical and chemical properties:

1- water pollution by mining industry:

The characteristics of waste water from mining industries varies greatly depending on the king of mine for instance.

a)metal mining industry
b)coal mining industry
c)sulfur mine
d)petroleum and natural gas drilling industries
e)stone-quarrying and clay-supply industries

2-water pollution by agriculture:

Agriculture has been eviction of water pollution in many instances. But sometimes, it is also responsible for polluting water. Water pollution caused by agriculture is mainly and outcome of fertilizers and agricultural chemicals such as insecticides and herbicides.

a) Water pollution by fertilizers:fertilizers given to crops are not always fully consumed, and peat of it remains in the soil by being absorbed by soil colloid, and influence the quality of underground, rivers, and sea water when it is dissolved.

b) Water pollution by agricultural chemicals: of de agriculture chemicals developed in the past, many of them are prohibited being used because of toxicity.

Nevertheless, those remaining in the environment are still factors that country to environmental pollution. In addition to above-mentioned pollution, there are instances of water

pollution caused by indirect effect of agricultural activities.

3-Water pollution by stock breeding and fisheries:
When cattle or house are fed on a big pasture, there is little possibility of the livestock causing water pollution because their excreta area spread widely all over the pasture. However, when livestock is fed intensively in a limited area it causes a number of problems.

4-Water pollution by urban activity:
municipal waste such as waste water, human waste, household waste, waste gas and waste heat are the causes of water pollution. the type of water pollution in city varies largely depending on the characteristics of the city, life style of inhabitants, degree of civilization, degree of development of sewage treatment plan plants or sewerage systems.

5-Water pollution by manufacturing industry:
Of water pollution with which human activities are concerned, the manufacturing industry is the largest in scale, and most diversified. The characteristics of the water pollution of manufacturing industry can be summarized as follows: a) Unlike the mining industry and agriculture, the discharging condition of waster water from manufacturing industries is relatively simple because it is rarely affected by complicated conditions of the natural environment. For example, waste water of the manufacturing industry is discharged concentratively from a fixed outlet, which is called "point source pollution." Besides waste water from the manufacturing industry is generated as a result of the production activity which is 100 % artificial, and thus the manufacturing industry can be considered as the source of water pollution which is most easy to catch and control in this point.

b) Source of water pollution from manufacturing industry are relatively easy to trace back through scientific method, but their control is not always easy because it involves complex social and political problems. Furthermore, tracing such point sources of pollution is not always easy because the interests of individual manufacturing companies can be

affected by pollution control measures.

c) In contact to the relatively simple form of discharging process, the composition and quality of waste water vary widely depending on the type and scale of individual factories, change from time to time, and its influence also takes a variety of forms.

d) The influence of waste water on the environment varies depending on the geographical location of the factory.

I- In the case of an industrial conglomerate or large scale industrial complex: the influence of waste water on the environment is extremely large, but as far as such manufacturing facilities are concentrated in one area based on the results of careful environmental assessment, waste water from these facilities can be controlled in the most ideal

II- In case factories are located in commercial or residential areas of a city: the location of this type is primarily undesirable. Actually, however, there are a number of such cases because of historical reasons, or geographical advantages concerning the purchase of raw materials, transportation of products, or access to the labor market.
In Japan, locations within easy access to the source of industrial water are decreasing in these areas. As a result, factories that recycle water are increasing; small scale manufacturing companies establish cooperative associations and are promoting the construction of joint waste water treatment facilities.

III- Location of the manufacturing factory is whether mountainous area, plain, or costal region is also and important factor to be considered in assessing the influence of waster water on the environment. Especially, in the case of a water-consuming type of manufacturing industry, its location has to be decided depending on accessibility to an industrial water source. On the other hand, the condition of the factory has to be discharged also has a great influence on the form and type of environmental pollution.

As discussed in the foregoing, the composition of industrial waste water is complex, and it varies largely

according to the difference in the manufacturing process even among the same type of manufacturing factories. furthermore, the manufacturing industry is constantly subject to the impact of technological innovations which occur at increasingly short intervals.

Quality of Waste Water According to the of Manufactory Industry

Quality of Waste water	Type Industry	B.O.D	S.S	PH	Quantity of waste water m^3/d	Other
1.Organic.high concentration	Food processing processed meat products " marine " " agricultural products. (canned goods.pickles etc) Beer Cooking Oil Starch	 300-600 200-2000 200-600 500-2000 150-1100 500-3000	 100-150 150-1000 120-200 250-1000 100-300 3000	 7 7 - 8.5 1 - 12 8 - 11 1 - 17 6 - 8	 50 - 100 200 - 5000 50 - 600 5000-10000 50 - 200 100 -1000	
	Chemical Industry Animal and Vegetal Oil and Fats. Drug	 100-2000 40-2500	 400-600 200-600	 4 - 9 2 - 11	 100-2000 1000-3000	
	Pulp Kraft pulp(KP) Sulfite pulp(SP) Semi-Chemical Pulp(SCP)	 300-700 300-500 500-2000	 40-80 50-300 200-600	 7 - 9 3.5-4.5 3 - 7	P/Lt Pulp 150-300 150-500 100-150	COD 500-1500 500-1600 10005000
II Organic. low concentration	Food Dairy products polished crops and flour	 50-350	 70-150	 6.5-11	 1000-6000	
	Textile Cotton.Spinning Dyeing and adjusting	 150-200 10-350	 60-800 20-250	 3.5-9 30-200	 100-1000	
	Paper making	150-200	250-600	8-9	500-2000	
	Chemical industry Organic industrial products	 100-300	 20-150	 1-13	 50-500	
	Petroleum Recycling of waste Oi	 20-200 20-200	 300-500 20-100	 2-8 1-13	 10-50 water draing in 10000 barrels/ day 50000 m3	
	Pulp Ground pulp(GP)	 100-150	 30-40	 6.5-7.5	 30-400	
	home laundry	90-410	140	8.7-11.5		oil cont 110

Table 2

III Organic. matter(containing toxic substances)	Leather tanning and dyeing	80-2500	50-3000	7-12	30-600	Cr and S
	Iron making blast furnace pig iron	3000-4000		9-9.5		CN,S,NH,
IV Inorganic ordinary waste water	Chemical industry Chemical fertilizers inorganic industrial products	-------- --------	50-150 1000-2000	1-4 1-9	100-1000 500-2000	
	Ceramic,porcelain clay and quarrying industries Glass Concrete products	20-70 -----	150-300 150-500	7-9 9-14	50-5000 100-300	
	Iron Marking Steel-making and rolling	Oil content of cold rolling	500-1000	3-8	7-12	water temp. 40-50 C
	Iron making by blast furnace		500-3000	7-8	10-15	
V Inorganic matter(containing toxic susbtance)	Nonferrous metal Primary refining	---------	500-3000	6-8	1000-3000	Heavy Metals
	Metal products electroplating	----------	----------	1-2	10-100	CN20-200 Cr40-150 Cu40-150
	Chemical Industry Electronic Caustic Soda	---------	---------	-----	-----	Hg

Mayor Causes and Source of Water Pollution

CAUSE	SOURCE (INDUSTRIES AND OTHERS)
B. O. D Content (organic matter)	Food processing industry(canned goods,meat and dairy product processing,marine product processing,starch processing)brewery,sugar making,wool processing(wool wasning) pulp and paper industry, municipal sewerage, night soil treatment plant.
Suspended solids (SS)	(organic SS) food processing, paper and leather industries, coal washing, municipal sewerage.(inorganic SS) ceramic industry, gravel yard, quarrying industry, mining industry.
Oil and fats	Food processing, petrochemical, iron and steel making, metal finishing, wool processing (wool washing) industries.
Acids or Alkalis	Mining, paper making, pulp, electroplating, metallurgical, chemical, textile and leather industries.
Amonia	Gas and coke plants.
Phenols	Gas and coke plants, synthetic resing and other organic chemical industries.
Sulfides	Pulp and gas industries, sewage treatment plants dyeing and leather industries.
Color	Dyestuff manufacturing, dyeing, paint manufacturing. leather, chemical,industries, night soil tratment plants.
Odor	Chemical, pulp, marine product processing, meat processing, sugar making, leather industries, sewage and night soil treatment plants.
Detergent (surface active agent)	Municipal sewage, textile industry, laundry.
High-temperature water	Steam and nuclear power plants.

Water Pollution Control:

The purpose of waste water treatment is to obtain clean effluent by removing the pollutants from the waste water, or by decomposing the pollutants in the water to make them lose their properties as pollutants.

Measurement of Water Pollution:

factory effluent and public water such as rivers, lake and sea water, the technology of water pollution measurement has been remarkably advanced by the recent development and expansion of various analyzing device, which have resulted in the success of the rapid and energy-saving processes of improved precision. It goes without saying that it is necessary to adopt advanced sophisticated methods. At the some time, it is necessary to be always aware of the scope together with the range capable for application as to the proper judgement for the use of methods.

Sampling:

Of factory effluent is made at drains or at a paint where sampling of water having a similar quality possible. In case the drain runs into a wasteway, a watergate is constructed to prevent the river water or sea water from flowing upstream and tounifi the quality of the water whereby the flooding water is sampled. It is necessary to adopt a method of sampling in accordance with the geographical and climatical environment of each country.

Rivers:

The investigation must made including the periods or seasons
when the volume of water is small and when irrigation (or other water utilization)is being carried out it should be the time when the water quality is stable after a long spell of fine weather.

Places of sampling include:

Irrigation or other water utilization points, a point fully mixed with polluted water inflow and the point before its inflow, a point joined and fully mixed with the branch stream and the point before its inflow, a point joining with running water, and a reference point used as the point to maintain the environmental reference and to grasp the condition of achievement, etc.

The sampling must be made at the level of the flow center, which is the point of maximum velocity, or the sulfate right above it, However, the sampling must also be made at both banks of river, depending on the running condition.

Lakes and reservoirs:

Sampling must be mode at the point indulin;the center of the lake, irrigating point, the point mixed up with polluted water and river water inflow, the out flowing point of the lake water, and the reference point.

As to level of sampling, sulfate layer water must be sampled in the circulation season, while the water in the respective layer at every depth partition of 5 to 10 m must be sampled in the stratification season.

Sea:

The level of sampling must be, as a principle, the sulfate layer, which is 0.5 m below the sea sulfate. And the meddle layer, which is 2 m below the sea sulfate. In case the depth of waters less than 5 m, only the sulfate layer must be sampled while the lower layer must also be sampled if necessary when the depth of water is more than 10 m.

Method of sampling: to sample flowing water or the surface water, sample containers must be used to collect the sample directly. To sample the water from the elevated position, a ladle or a polypropylene bucket with rope must be used.

In case the purpose is to sample the water at different layers, the Heyroth samples, the vandorn sample or the kitahara sample must be used.

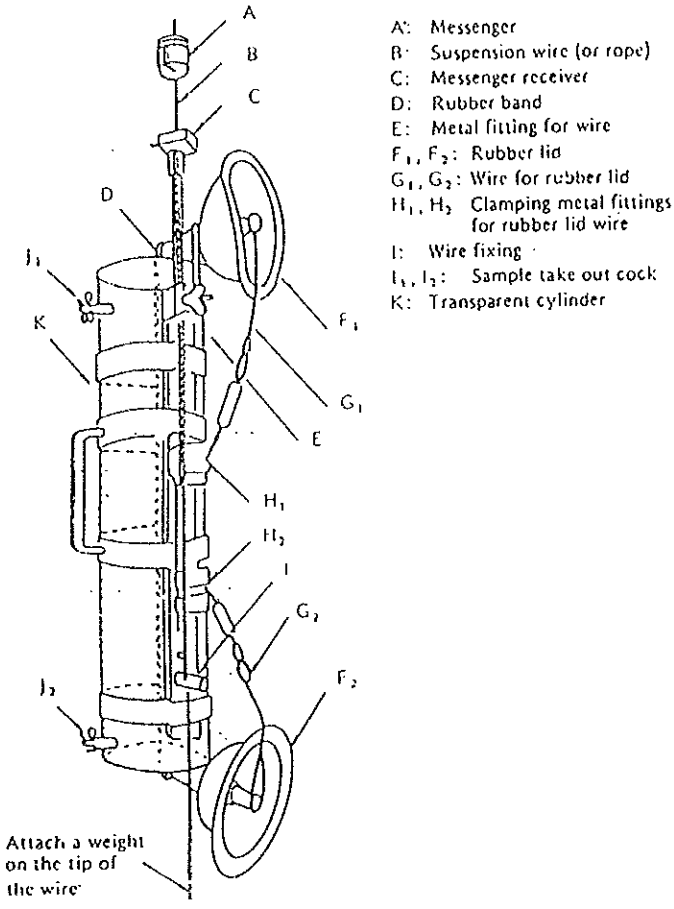

Fig. 1 Vandorn water sampler

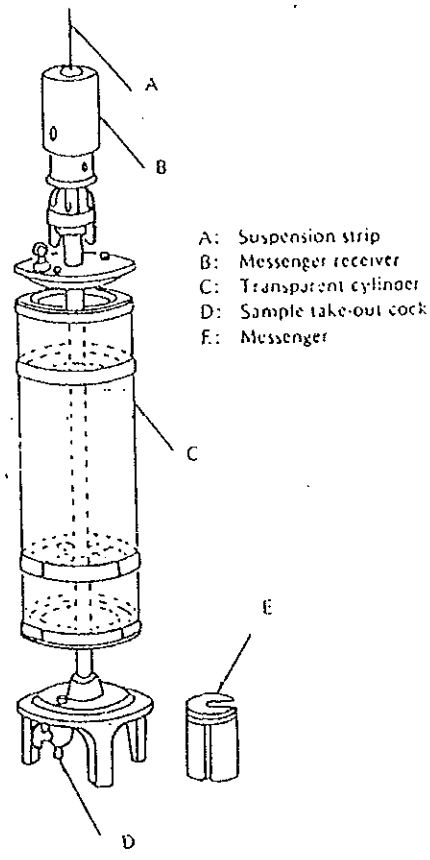

Fig. 2 Kitahara water sampler

Methods of Examination

General test: pH (hydrogen ion concentration index)

pH is the common logarithm of the inverse number of the molar concentration of hydrogen ion, the activity to be exact.

pH=-log[H+];[H+] is the hydrogen ion concentration(mol/l).

there are two pH measurement methods, one is the colorimetric method using a pH indicator or test paper, and the other is the electrical method using a glass electrode.

The colorimetric method is economical and simple to use, but it is often disturbed by the color, turbidity, salt, concentration, colloidal substance, residual chlorine, oxidizing and reducing substance. So, the electrode method is recommendable.

Suspended Solids: Use glass fiber filter paper membrane. filter with a pore size of 1 u m to filter the sample under suction, then wash with water. After that, dry the filter paper for two hours a 105 to 110°C. the weight is measured, followed by the cooling-off in the desiccator. The size of the object in this test ranges from 1 u m to 2 mm. The amount of the sample must be set to contain more than 5 mg the solids at one test. Normally, 200 ml of sample is sufficient.

Turbidity: the degree of water turbidity is measured by various optical methods. In all cases, the comparative value is shown in relation to the standard solution. Since none of the methods are intended to measure the absolute value of a specific substance, the analytical results are different according to the kind of method or the standard substance adopted. For instance, it will be expressed like transmission light turbidity 35 (kaolin).

turbidity standard solution(kaolin standard solution) a solution with a fixed amount of refined kaolin dispersed in water. When kaolin is 1 mg/l, the turbidity is 1.

Analysis of Various Chemical Components

Various chemical components in water generally require measurement of lower concentrations, thus often necessitating instrumental analysis. In many cases, the following methods are particularly used: Spectrophotometry

Atomic Absorption Spectrometry

Gas Chromatography

Pretreatment

In the analysis of metal components, it is necessary to carry aut, in principle, pretreatment for the purpose of making a solution of the subject components though decomposition of the suspended solids and metal complex, or decomposing the co-existing organic matter.

The selection of the method, however must depend on the condition of the sample and the purpose of the test.

1-Boiling with hydrochloric acid or nitric acid: to a 100 ml sample water is added 5 ml of the acid and then boiled for

short time. In case it contains suspended matter, it is concentrated down to about 15 ml.

2-Decomposition with nitric acid and perchloric acid,or with nitric acid and sulfuric acid: this method is applied when the sample contains organic matter. In case it is more difficult to decompose the sample, it is suggested that the method be adopted in which the sample is evaporated by heating in an electric oven, then it is dissolved in hydrochloric acid; or the method be adopted in which the sample is fused with sodium carbonate.

3-Copper,Zinc,lead,Cadmium: These four kinds of metal elements have similar chemical characteristics, so that they can be determined in a common method. The sample water pretreated is subjected to either one of the solvent extraction methods to separate or concentrate the metals. After back extraction of the metals, or the volatization of the solvent followed by the wet decomposition of the remaining metal complex, these metal elements in the solution are analyzed by atomic absorption spectrometry.

a)Selective extraction using dithizone: Any one of these four metal elements produces colored complex with dithizone, so dithizone is used in the spectrophotometry for each element. This operation can also be adopted in the pretreatment for atomic absorption spectrometry to selectively extract each metal element, In other words.

Copper: Extracted from a weak acidic solution (hydrochloric acid + phosphoric acid) using dithizone-chloroform solution. Zinc: Extracted from a solution of pH 7 in the presence of citrate and thiosulfate.

Lead: Extracted from a solution of pH 8.5 to 10 in the presence of citrate and potassium cyanide.

Cadmium: Extracted from a solution of pH 12 in the presence of citrate and sodium cyanide. Since the copper-dithizone complex is extremely stable after the extraction, the back extraction using acid is difficult. Therefore, the dithizone complex is acid-decomposed to make an inorganic solution after expelling the organic solvent by evaporation.

All the other three elements can be back extracted using hydrochloric acid(2+100), so any one of them is subjected to atomic absorption spectrometry after extracting back into an aqueous solution.

b)Simultaneous extraction using dithizone: these metals are simultaneously extracted from a weak alkali solution using dithizone-chloroform solution. When the extracted chloroform solution is shaken with hydrochloric acid(2+100), zinc, lead and cadmium are stripper into the water layer. On the other hand, copper, as well as mercury, nickel and cobalt, remain in the chloroform layer. This is treated similarly as item(a).

c)Extract diethyldithiocarbamate(DDTC): When DDTC extraction method is applied for a solution of pH 9, many metals including the above metal elements are separated. butyl acetate or methylisobuthylketone(MIBK)is used as the extracting solvent. The organic extract is heated to expel the solvent. After decomposing the residue using acid, the sample solution is prepared.

4-Iron,Manganese: Since these two metal elements exist in abundance on earth, Environmental regulation demands to determine these metals which exist in dissolved state in nature only. Immediately after sampling, the sample is filtered and atomic absorption spectrometry is applied to the filtrate. When a large quantity of silica exists in the sample, the negative error is indicated so that it may be necessary to add about 100 to 200 mg/l of calcium or magnesium as the releasing agent. Iron can also be determined by 1,10-phenanthroline method and manganese by spectrophotometry using potassium periodate.

5-Chromium: Estimation of chromium is divided into total chromium and hexavalent chromium.

a)Total chromium: the sample solution is treated with sulfuric acid and is made to hexavalent chromium totally using potassium permanganate. Then, it is reacted with diphenylcarbazide to develop color to estimated by spectrophotometry.

b)Hexavalent chromium: This can be estimated by a method similar to the above only omitting the acid treatment. Other than this, it is possible to adopt atomic absorption spectrometry.

6-Arsenic: Spectrophotometry using silver diethydithiocabamate, arsenic is oxidized using potassium permanganate and co-precipitated with ferric hydroxide. the precipitate is dissolved into sulfuric-hydrochloric acid. Then, potassium iodide, stannous chloride and zinc metal are added. as a result, arsine (AsH_3) is generated, which is then absorbed into a brucine-chloroform solution of silver diethyldithiocarbamate. After that, the absorbance of the formed red purple color is measured.

7- Mercury: Total mercury and alkylmercury compounds are estimated.

a)Total Mercury: Three methods are available. Each of then is an atomic absorption spectrometry using a special atomizer. Reduction-vaporization method the sample is treated with sulfuric-nitric acid, potassium permanganate and potassium persulfate for oxidation (95°C, 2 hrs) so that all are changed into mercury (II). After reducing excessive permanganate, stannous chloride is added to reduce mercury into metal mercury, then subjectected to atomic absorption spectrometry of closed circulation method or of open aerating method.
Heating-vaporization method in sulfuric acid solution, mercury is extracted as dithizone complex into chloroform. And a fixed amount of the extract is taken and the solvent is evaporated. This is then subjected to atomic absorption spectrometry of heating vaporization method.

Amalgamation method mercury reduced and vaporized, is collected onto gold or silver as amalgam. This is then heated to generate the mercury vapor again for atomic absorption spectrometry. This method is adopted to analyze a very small quantity of mercury.

Non-Metal Components

Phosphorous Components

Phosphorous is not a regulated substance at present. However, it is strongly required to reduce its discharge in relation to the eutrophication problem in closed water area. Phosphorous in water exists also in the form of inorganic phosphates such as orthosphate and pyrophosphate, as well as organic phosphorous compounds. It is therefore necessary in the determination of total phosphorous that the sample be treated to white fume using sulfuric acid-nitric or hydrochloric acid-nitric acid in advance and changed to orthophosphate. Besides, there may be cases when hydrolyzable phosphorous compounds are estimated by boiling with strong acid for a long time.

Orthophosphate is determined in the following methods: A method in which heteropoly acid is formed by adding ammonium molybdate and sulfuric acid to the sample, and by reducing this with stannous chloride, to form molybdenum blue; The molybdenum blue formed is extracted into organic solvent; Or a method in which heteropoly acid is extracted and then molybdenum blue is formed in the organic phase.

Nitrogen compounds:

Same as in the case of phosphorous, nitrogen compounds are related to eutrophication, although not regulated at present. Nitrogen compounds exist in many forms in water and are determined according to the classification shown

1) Ammonium ion (NH_4+)
2) Nitrite ion (NO_2-)
3) Nitrate ion (NO_3-)
4) Organic nitrogen
5) Total nitrogen

Ammonium ion:

Ammonium is separated and collected by steam distillation. In case the concentration of collected ammonium ion is high, the neutralization titration or the ion selective electrode method is applied, while the indophenol method is used when it is low. In the indophenol method, spectrophotometry is adopted

for analysis utilizing the fact that ammonium ion reacts with phenol under the existence of hypochlorite ion to form indophenol blue. In addition, there is a method. That has been used for a long time called Nessler's method. However, this method is not popular in Japan at present because it utilizes a mercury compound as reagent.

Nitrite ion :

Is determined by the 1-naphthylamine method. Sulfanilic acid is added to the sample for diazotation. then 1-naphthylamine or N-(1-naphthyl) ethylenediamine is added to form a red azo-compound.

Nitrate ion:

Brucine-sulfanilic acid method is used. Brucine-sulfanilic acid is added to the sample water and is made to react with strong sulfuric acid. As a result, a yellow color is formed, which is then determined by spectrophotometry. Besides this, there is a method using devarda's alloy. Recently, a cadmium-copper column reducing method is used for tests of water supply sources. In this method, the sample water is passed through a column filled with copper-coated cadmium particles. Nitrate is reduced to nitrite for the following estimation.

Organic nitrogen:

This is estimated by the Kjeldahl method. under the existence of copper or mercury catalyst, sulfuric acid-potassium sulfate is added to the sample water and the solution is heated. Then, ammonia, generated as the result of decomposition of organic substances, is collected and determined. In this method, nitrite and nitrate are not included in determination.

Alkalinity (pH 4.8): acid demand.

Reagent

a) Methyl red-bromocresol green mixed solution, dissolve 0.02 g of methyl red and 0.1 g of bromocresol green in 100 ml of ethanol(95%).

b) 1/10 N Sulfuric acid solution, add and mix 3 ml of sulfuric acid in 100 ml of water, after cool dilute anode adjust the volume to 1 l with water.

Normalization:

Heart 40 - 60 min. Sodium carbonate at 500 - 600°C, After cool in a desiccator, dissolve 1.325 g of the sodium carbonate in water and adjust the volume to 250 ml. Take 20 ml of the solution in a beaker, add 3 - 5 drop of mixed indicator. and titrate with 1/10 N standard sulfuric acid solution. When the color is change to graypurple, boil and remove the carbon dioxide. After cool, continue titrate until the color change to gray-purple. Calculate factor of the standard solution.

F= a*b/100*20/250*1/(x*0.0053).

a= weight of sodium carbonate g

b= content of sodium carbonate %

x= volume of demanded standard sulfuric acid solution ml

0.0053 = seoretical demand of unhydrus sodium carbonate demand for 1 ml of 1/10 N sulfuric acid solution.

c) 1/50 N standard sulfuric acid solution, remove 250 ml of the 1/10 standard sulfuric acid solution to 1 l of volumetric flask and adjust the volume to 1 l.

Treatment

a)Take 100 ml of sample solution, add 3 - 5 drop of mixed indicator solution.

b)Titrate with 1/50 N standard sulfuric acid solution, until color change blue to gray-purple (pH 8).

c)calculate the acid demand (ph.8) with following equation.

i- mg equivalence

A=a*f*1/50*1000/V

A: Acid demand(pH 8)(mg equivalent/l)

a: Titrate volume of N/50 standard sulfuric acid ml.

f: Factor of N/50 standard sulfuric acid solution.

(factor of 1/10 N sulfuric acid solution)

V: volume of sample ml.

ii-mg $CaCo_3$/l

B=a*f*1000/V*1

B:acid demand mg $CaCo_3$/l

1:equivalent value of the N/50 sulfuric acid solution.

Acid Demand / Alkalinity (pH 8.3)

Reagent

a) Phenolphalein solution (0.5w/v%), dissolve 0.5 g phenolthalein in 50 ml of ethanol, dilute to 100 ml with water. Adjust the pH 8.3 with 1/50 N sodium hydroxide until the color is slightly red.

Treatment

Take 100 ml of sample solution in a beaker, add 3 or 5 drops of phenolphalein solution.

Titrate with standard N/50 sulfuric acid solution until red color disappear.

Calculate the acid demand (Ph 8.3) by follows equation.

i) mg equivalence

$$A = a*f*1/50*1000/V$$

ii) mg $CaCo_3/l$

$$B = a*f*1000/V*1$$

Discussion of Terms

The terms, acidity and alkalinity, as used in water analysis may not be in accord with generally accepted terminology with a neutral point at pH 7. In water analysis a pH of about 4.5 is frequently the end point for titration of alkalinity and a pH of about 8.2 for acidity.

In addition to free hydroxide, alkalinity may be produced by anions that to hydrolyze; there include carbonate, bicarbonate, silicate, phosphate, borate, arsenate, illuminate, possibly fluoride, and contain organic anions in waste waters, all the effects due to there anions are lumped together in an alkalinity analysis.

Hardness

1. Total hardness: H= b*1000/V*1
   H: Total hardness (mg $CaCo_3$/l)
   b: Demanded 0.01 mol/l EDTA sol. (ml)
   V: Sample (ml)
   1: Factor of 1 ml EDTA sol. for mg $CaCo_3$.

2- Calcium Hardness H (Ca)= a*1000/V*1

3- Magnesium Hardness H (Mg)= (b/V-a/V(Ca))*1000*1

Calcium Titration method by EDTA

Reagent:
-Potassium hydroxide solution 50 w/v % water.
-KCN solution 10 w/v in water.
-Hydroxylammonium hydrochloride solution 10 w/v % in water.
-NANA diluted crystal.
-0.01 mol/l EDTA solution.
Treatment
-Take a aliquot of sample solution (contain <mgr of Ca), dilute to about 50 ml with water.
-Add 4 ml of KOH solution and stand about 5 min.
-Add 5 to 6 drop of KCN 10% sol. ,0.5 ml of hydroxy ammonium 10 % solution and mix.
-Add NANA diluted crystals and teetered with EDTA solution. to change the color red purple to blue.
-Calculate Ca consecration with following equation.

C= a*1000/V*0.4

C: concentration (mg Ca/l)
a: Demanded EDTA solution ml.
V: Sample ml
0.4: Comparable value of Ca quantity (mg Ca/l) for 1ml of EDTA sol.

Magnesium Titillation by EDTA

Reagent

-Ammonium chloride ammonia buffer solution, dissolve 67.5 g of ammonium chloride in 570 ml of ammonia water and dilute to 1 l.

-KCN solution 10 w/v % in water.

-Hydroxylammonium hydrochloride solution 10 w/v % in water.

-Eriochrome blue black T solution 0.5 w/v % aqueous solution.

-0.01 mol/l EDTA solution.

Treatment

-Take a aliquot of sample solution, dilute to about 50 ml with water.

-Add 4 ml of NH_3-NH_4Cl buffer solution.

-Add 5 to 6 drop of KCN sol.,0.5 ml of hydroxy ammonium solution and mix.

-Add 1 or 2 drops BT solution and titrate to change the color red purple to blue.

Calculate

$Mg=(b-a)*1000/V*0.243$

a: demands of EDTA solution (ml) for Ca titration.

b) demands of EDTA solution (ml) for Ca,Mg titration

Atomic Absorption Analysis

Standard Solution

Ag : Take 1.575 g of Ag NO3 after dried at 110°C, dissolve in water and adjust the volume to 1 l.

Al: Dissolve 1.000 g of metallic aluminum in slightly excess of dilute to 1 l with water. Dissolve 1.754 g of aluminum potassium sulfate in 0.25 mol/l diluted hydrochloric acid to adjust 1 l.

As : Dissolve 1.321 g of arsenic trioxide in 2 ml of 1 mol/l NaOH solution, After dilute with water acidify with diluted hydrochloric acid, adjust the volume to 1 l.

Ba : Dissolve 1.779 g $BaCl_2$ in water and adjust its the volume to 1 l.

Be : Dissolve 1 g of metallic beryllium with 10 % hydrochloric acid by heat, after cool, adjust to volume 1 l.
Ca : Dissolve 2.497 g of $CaCO_3$ in slightly excess of hydrochloric acid, dilute to 1 l.
Cd : Dissolve 1.000 g of metallic cadmium in dilute nitric acid. Dry on the water bath, add 5 ml of HCl(1mol/l), dry up . Add diluted Hcl and water adjust to 1 l.

Determination of Sulfate Ion By Barium Chromate Method

Reagent

Barium cromate suspended solution, 2.5 g of barium chromate in 200 ml mixture (100 ml acetic acid (1+15) and 100 ml HCl (1+500)), shake well, and stock in polyethylene bottle.
-Calcium contain ammonia solution, dissolve 1.85 g of calcium chlorate in 500 ml ammonia solution (1gr Ca/ml), keep in CO_2 defended polyethylene bottle.
-Sulfate ion standard (0.1 mg SO_2/ml), treat potassium sulfate at 800°C until constant, and dissolve 1.815 g of the crystal and adjust volume to 1 l. When the determination dilute 10 time.
-Barium Chromate dissolve 8 g of potassium chromate in 800 ml of water and warm to 70-80°C. Add little by little 100 ml of barium chloride warm solution ($BaCl_2.2H_2O$/100ml H_2O) until upper solution is little yellow color and precipitate barium chromate. wash 2 or 3 times the precipitate by decantation with 500 ml hot water, centrifuge 2 or 3 times with cold water. Add 100 ml HCl(1+5) and dissolve by heat (if there ware precipitates insoluble, filter), add water to 700 ml, warm to 70 or 80 °C, add ammonia solution (1+6) until changing point of bromo-thymol blue and precipitate barium chromate. Repeat the treatment and purify the precipitate. Dry 1hr. at 105 - 110°C grind to fine power. Keep in the little reagent bottle.

Treatment

Take 10 ml sample solution in 50 ml beaker, add 4 ml of barium chromate suspended solution, stand 2-3 min. Add gently 1 ml of ethyl alcohol, shake 1 min. And stand 10 min. Centrifuge and take in 10 mm photemetric cell the upper solution and measure absorbance at 370 nm.

Colorimetric Phosphate determination by 1- Amino- 2- Naphthol-4- Sulfonic Acid

Determination range: PO_4(3-) 0.02 at 0.75 mg

Reagent

1)Ammonium molybdate solution: Dissolve 3.2 g of ammonium molybdate tetra-hydrate in 20 ml of water,transfer in sulfuric acid (25+30), after cool, add 0.35 ml of HNO_3. Stock in refrigerator. the solution useable in 1 week.

2)1-Amino-2-nafthol-4-sulfonic acid: Dissolve 0.5 g of 1-Amino-2- nafthol-4-sulfonic acid and 2 g of sodium sulfite (anhydrous) in 50 ml of water. In another, dissolve 20 g of sodium hydrogen sulfite in 120 ml of water, mix both water and add water to 200 ml. Stock in refrigerator. the solution usuable 1 week.

Original standard solution of phosphoric acid (0.1 mg PO_4 ml):

Dry potassium dihydrogenhosophate about 3 hs. at 110°C dissolve 0.1433 g of the crystals in water, transfer 1 l volumetric flask, and adjust the volume.

Standard solution of phosphoric acid: Transfer 20 ml of the original standard solution to 200 ml volume with water.

Treatment

Take sample (contain 0.02 to 0.75 ml as PO_4) to 50 ml volumetric flask, dilute 40 ml with water.

Add 2 ml of ammonium molybdate solution and mix, add 2 ml of 1-amino-2-naphtol-4-sulfonic acid solution, adjust the volume.

After 5 min., measure the absorption at 810 nm.

Collect the absorbance with blank test.

Calculate phosphate concentration.

CHROMIUM (VI)

Reagents

a)Sulfuric acid (1+9).

b)Ethanol (95%)

c)Diphenylcarzid solution (1 w/v %): dissolve 0.5 g diphenyl carbazide in 25 ml acetone and add 25 ml of water. The solution can use about 1 week.

d)Chromium standard solution (0.005 mg Cr/ml: dissolve 1.24 g potassium dichromate in water, remove to 1l volumetric flask and adjust the volume. take just 10 ml of the solution to 1l volumetric flask and dilute to measure line.

Treatment

a)Take sample solution (contain 0.002 to 0.05 mg of Cr(VI) for two (A and B) beakers. Neutralize with sulfuric acid (1+35) or sodium hydroxide solution (4w/v%).

b)Transfer the solution in beaker A to a 50 ml volumetric flask A and add 2.5 ml of sulfuric acid (1+9)

c)Add 2.5 ml sulfuric acid (1+9) to the solution in beaker B, add a little of ethanol(95%), reduce the chromium(VI) to chromium(III) and remove the ethanol. After cool trasfer to 50 ml volumetric flask B.

d)Keep the flasks at about 15 C, add 1 ml of diphenylcarbazide solution (1w/v%) to each flasks. Shake immediately. adjust the volume with water and stand 5 min.

e)Measure absorption of the solution A at 540 nm, as reference is the solution in flask B.

SILICA IN WATER SAMPLE

Silica is clasificated as ionic silica, dissolved silica, colloidal silica and total silica. Those are expressed as SiO2.

Ionic Silica: React with ammonium molybdate and made hetero-molybdate. Compound.

Molybdena Yellow Method: Determination method of ionic silica by yellow hetero-molybdate.

Range of determination: 0.1 to 1.0 mg SiO2.

Standard deviation: 2 to 10 %.

Reagent:

Keep all of the reagents in the poli-ethylene bottles.

a) Water: To make the reagent or to treat, use distrated water.

b) Ammonium Molybdate (10w/v%): Dissolve 21.2 g of ammonium molybdate in water and dilute to 200 ml.

c) HCl (1+1).

d) Oxalic Acid solution: Dissolve 20 g oxalic acid in water and dilute to 200 ml.

e) Silica Standard Stock Solution: Dry one hr. fine silica power at 700 to 800°C. Take 0.5 g and mix well with 4 g of sodium Carbonate in Pt crucible and then fuse 30 min. After cool dissolve with water, transfer to volumetric flask and adjust the volume.

f) Silica Standard (0.1mg SiO_2/ml) Standard Solution: Take 20 ml of silica standard stock solution to 200 ml volumetric flask and dilute with water.

Treatment

a) Filter sample water, take 50 ml of the sample in 50 ml tube.

b) Add 1 ml of HCl (1+1) and 2 ml of ammonium molybdate solution and then stand 5 min.

c) Add 1.5 ml oxalic acid solution, shake and stand 1 min.*

d) Measure absorbance at 410 to 450 nm.

e) As blank test, take 50 ml of pure water and treat similar and correct the absorbance.

f) Calculate the quantity the calculation curb.

* If there is not phosphate ion, did not add the oxalic acid solution. Because the absorbance decrease in this case, therefor, take the time correct.

CALCULATION CURVE

Take 1 to 10 ml of silica standard solution (0.01 mg Si/ml).

Add water to total volume are 50 ml. adjust temperature to 20°C and treatment and measure the absorbance. a) to f) Make the calibration curve.

WASTE WATER TREATMENT PROCESS

Primary Treatment:

1-Removal of massive solid.
2-Removal of suspended solid.

Secondary Treatment:

1-Removal of soluble and suspended organic materials.

Tertiary Treatment:

1-Removal of nitrogen,phosphorous and very fine SS.
2-Removal of very small amount of organic matter.
3-Removal of soluble inorganic salts.

CHEMICAL INDUSTRY

Waste water contains various organic compounds metals used as catalyst and acid and alkali

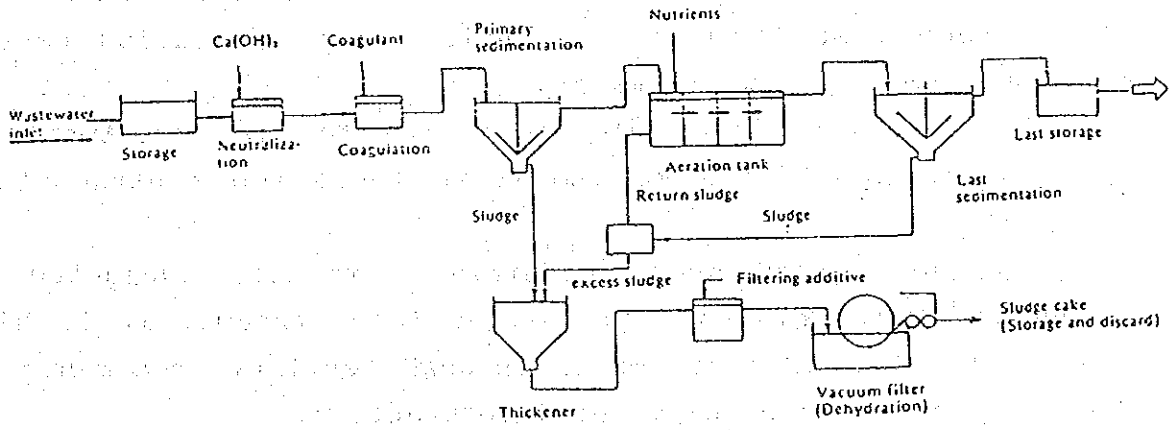

Fig. 2 Chemical industry (Waste water contains various organic compounds, heavy metals used as catalyst and acid and alkali.)

CHEMICAL INDUSTRY

Waste water contains various organic compounds metals used as catalyst and acid and alkali

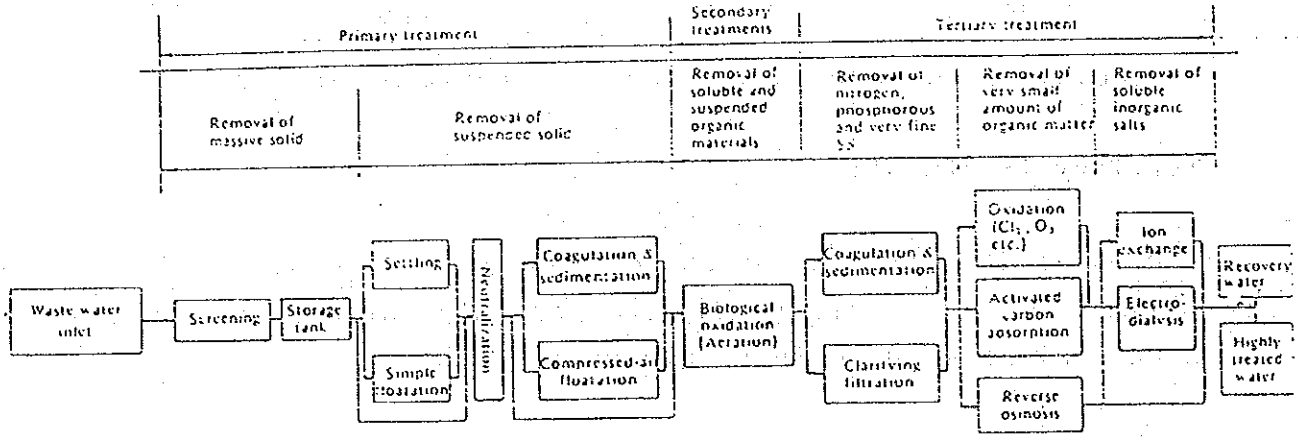

Fig. 1 Outline of waste water treatment process

Summary

In the past, Japan was a land endowed with much water. After the second world war, however, in order to survive we had no choice but to grow as a highly industrialized country and have developed along this line. As a result, industrial water and, at the same time, from severe environmental pollution caused by a huge amount of waste water.

The establishment of closed systems for the treatment of waste water provides a key to solve these two contradictory factors.

The day has passed when industrial effluent, subjected to secondary treatments, was discharged into streams. It is high time that reuse of waste water through tertiary treatments be further promoted and fully put into practice.

As to how simply and economical should waste water be reused, there remain many difficult problems to be solved.

in this world.

Acknowledgment

I wish to express may deepest gratitude to Dr. T. Okubo of process research and development division of N.I.M.C.R. for his helpful advice and valuable assistance during the period of this individual training course. Thanks also all the N.I.M.C.R. staff for their warm cooperation, special thanks to our coordinator Miss K. Ito for her kindness and help during may stay in Japan.
Finally, I want to thank the government of Japan for the financial support and for giving me the opportunity to be trained in Japan.

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Photocatalytic Degradation of Aromatic Sulfonic Acids in TiO_2 Aqueous Suspensions

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Thonburi, Bangkok 10140, Thailand.

ABSTRACT

Aqueous suspensions of titanium dioxide(anatase) containing aromatic sulfonic acids such as sodium benzenesulfonate,p- toluene, p- nitro, p- hydroxy and p- chloro substituted benzenesulfonic acids have been illuminated with light at $\lambda > 310$ nm under a variety of experimental conditionds. The reactions followed the first order kinetics. The effect of initial concentration, temperature and substituent groups are examined. Degradation rate decreases with Hammett constant, which indicates the electrophilic nature of degradation reaction. The two intermediate products of the photodegradation of sodium benzenesulfonate are p-phenolsulfonic acid and 2,5- hydroxyquinonesulfonic acid. The reaction mechanisms, based on OH attack on the aromatic ring, are proposed.

Introduction

Semiconductor materials are currently of much interest in the view point of solar energy utilization. They have been applied not only to produce useful chemicals but also to convert pollutants in waste streams to less harmful form. Photocatalytic degradation of water organic pollutants appears to be a promising method, in particular when using titanium dioxide as a photosensitive material. Research in this field has recently been reviewed.[1] References 2-14 indicate papers published since.

The aromatic sulfonic acids constitute a category of water pollutants. Their presence in water stems principally from industry. They are mainly used as intermediate in the aromatic chemistry especially in the dye and dyeing auxillary industry. Many of them can not be degraded by biological treatment. For example, biodecomposition of sodium dodecyl-benzenesulfonate (DBS) requires two days; branched isomers of DBS are not biodegraded even after a week of exposure to bacteria. The photocatalytic decomposition process of aromatic sulfonic acid has not been clarified in detail yet.

In the present work, the photocatalytic degradation of sodium benzenesulfonate was studied at several semiconductor powders. For aqueous suspension of anatase, effects of illumination characteristics, initial concentration and temperature on the reaction rate were investigated. The effect of substituents, e.g.nitro, chloro, on the rate of degradation and complete mineralization have been compared.

Experimental Section

Material. As photocatalyst, TiO_2 (anatase and rutile) were obtained from Aldrich. Sodium benzenesulfonate(reagent grade, Kanto Chemical Co.), p- toluene sulfonic acid sodium salt(reagent grade, Wako), p- chlorobenzene sulfonic acid(reagent grade,Wako), p- nitro-benzenesulfonic acid (reagent grade, TCI), p- phenol sulfonic acid sodium salt (reagent grade,

TCI) were used as supplied. Deionised water was used to prepare aqueous solutions. All solvents and other chemicals were reagent or spectrophotometric grade and were used without further purification.

Method. Solutions of 1.00×10^{-3} M benzenesulfonic acid were used unless otherwise indicated. The majority of the reaction was carried out in a photochemical reactor cell which was circulated via a pump.

In typical experiments, 70.00 mg of catalyst powder was suspended in 25.00 cm^{-3} of aqueous solution in the Pyrex glass cell. The cell, while stirring, was illuminated for a specific period by 500 W super-high pressure mercury lamp through water filter having Pyrex glass window. Infrared ray and ultraviolet ray below 310 nm were thus eliminated. The reactor cell was thermostated at specific temperature during irradiation. Subsequently, the sample was filtered through a 0.45 μm Millipore membrane and the filtrate was analyzed for the organics by HPLC techniques. The $SO_4^=$ anion was analyzed by ion chromatography. To monitor the evolution of CO_2, the cell was sealed with a butyl gumseptum and aluminium cap.

Analyzes. The disappearance of sodium benzenesulfonate and the appearance of the various intermediate products were followed by reverse-phase HPLC chromatography on a Schimadzu LC-9A chromatograph equiped with Hitachi L-400 UV detector. The detection wavelength was 260 nm and the column was a Crestpak C18S. The mobile phase was a mixture of methanol and 0.01 M KH_2PO_4. Identification of the eluting compounds was made by comparing the retention times with those of pure samples.

The appearance of sulfate ions in solution was monitored by ion chromatography using JASCO 880-PU HPLC chromatography equiped with a Shodex IC I-613 ion exchange column and a conductivity detector. The eluent was a mixture of 1.00 mM phathalic acid, 0.35 mM tetrabutly ammoniumhydroxide and 0.5% tetrahydrofuran.

The evolution of carbondioxide was monitored with Shimadzu GC-6AM equiped with an active carbon 80/100 mesh column and a FID detector after catalytic conversion to methane.

Total Organic Carbon was determined via a Shimadzu TOC-500 total organic carbon analyzer. It was measured before and after irradiation.

Results

1. Photocatalytic Activity of Semiconductors

Figure 1. shows reduction of sodium benzenesulfonate concentration with reaction time for aqueous suspension of several semiconductors. As shown in Figure 1., the disappearrance of sodium benzenesulfonate in the absence of semiconductor powder was very slow therefore direct photolysis was excluded in the experiments. The photocatalytic activity of semi - conductors was compared by using the values of the initial rate (r_o) and half-life ($t_{1/2}$); shown in Table 1. It was in the order of TP-2 > anatase > Pt/TiO_2 > rutile.

Among these semiconductors, TP-2 was the best catalyst but the reaction was too fast to study the kinetics by the procedure explained in Experimental Section. The experiments mentioned below were , therefore, carried out using TiO_2 anatase.

2. Kinetics of the Photocatalytic Degradation of Sodium Benzenesulfonate. Comparison with other substituted benzenesulfonic acids.

The rate of the photocatalytic degradation of sodium benzenesulfonate depends on various parameters such as type of photocatalyst, initial concentration and temperature.

The disappearance of sodium benzenesulfonate(Figure 2.)in the presence of TiO_2 is faster than in its absence. The same results were also found for the substituted benzene sulfonic acids. The photocatalytic disappearance of these compounds at $\lambda > 310$ nm(Figure 2-6)

Figure 1. Comparison of the photocatalytic activities of semiconductors

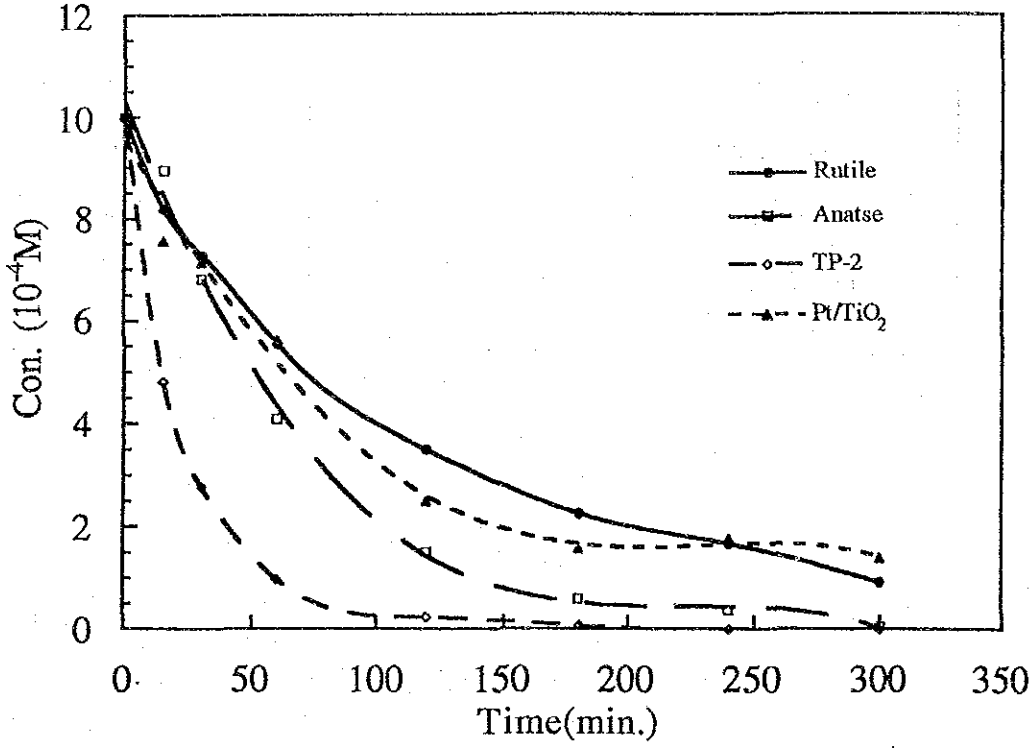


Table 1. Photocatalytic Activites of Semiconductors

Semiconductor	$t_{1/2}$ / min	r_o / $mMhr^{-1}$
none	-	-
TiO_2 / Rutile	72.0	0.600
TiO_2 /Anatase	48.0	0.760
Pt / TiO_2	66.0	0.680
TP - 2	15.0	2.325

Figure 2. Degradation of sodium benzenesulfonate at 25 °C

(B : with TiO_2, $\lambda > 310$ nm ; C : without TiO_2, $\lambda > 310$ nm ;

D : with TiO_2, without illumination)

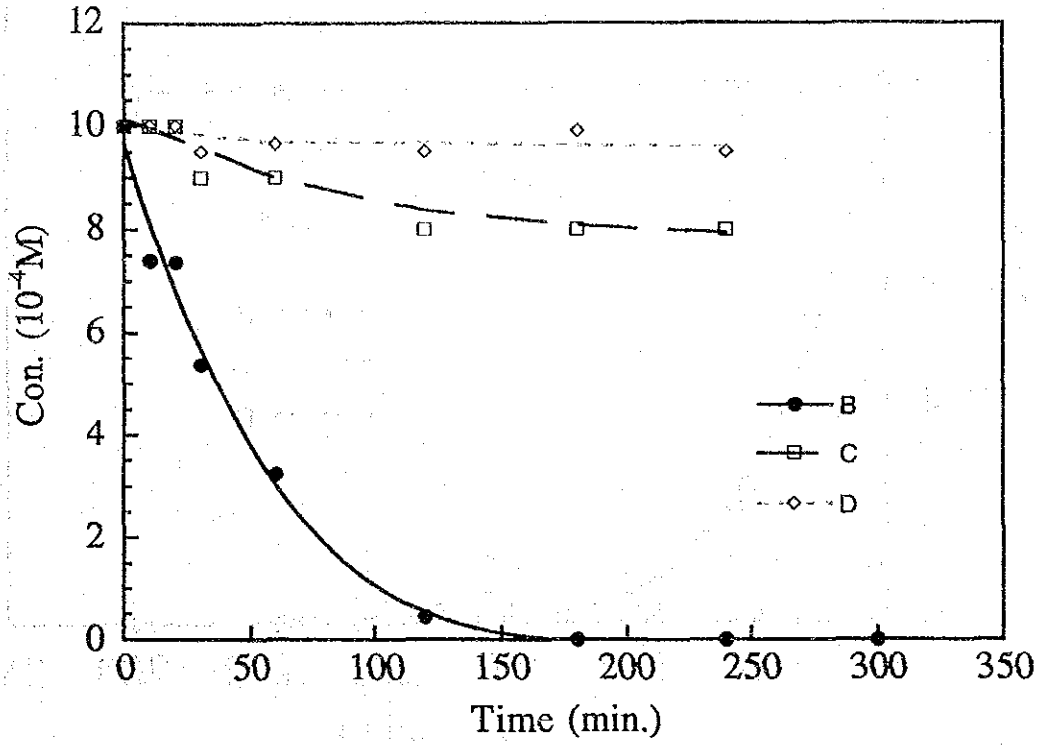


Figure 3. Degradation of p- nitrobenzenesulfonic acid at 25 °C

(B : with TiO_2 , $\lambda > 310$ nm ; C : without TiO_2 , $\lambda > 310$ nm ;

D : with TiO_2 , without illumination)

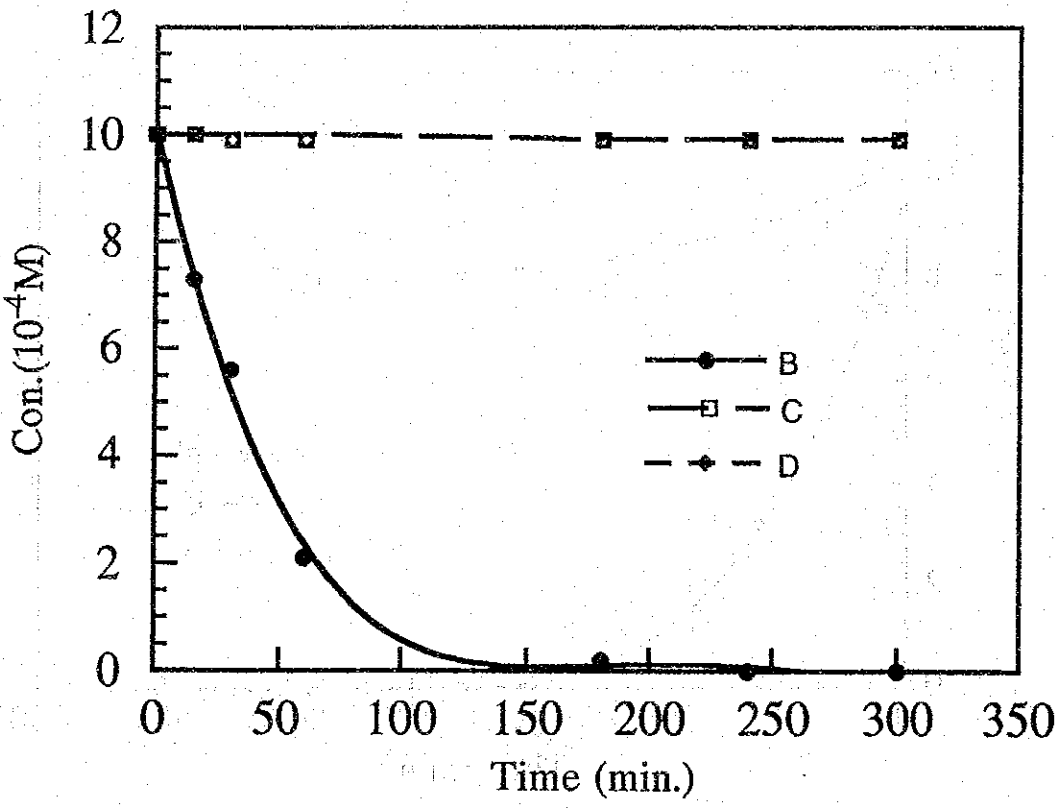


Figure 4. Degradation of p- phenolsulfonic acid sodium salt at 25 °C
(B : with TiO_2, $\lambda > 310$ nm ; C : with TiO_2 $\lambda > 310$ nm ;
D : with TiO_2, without illumination)

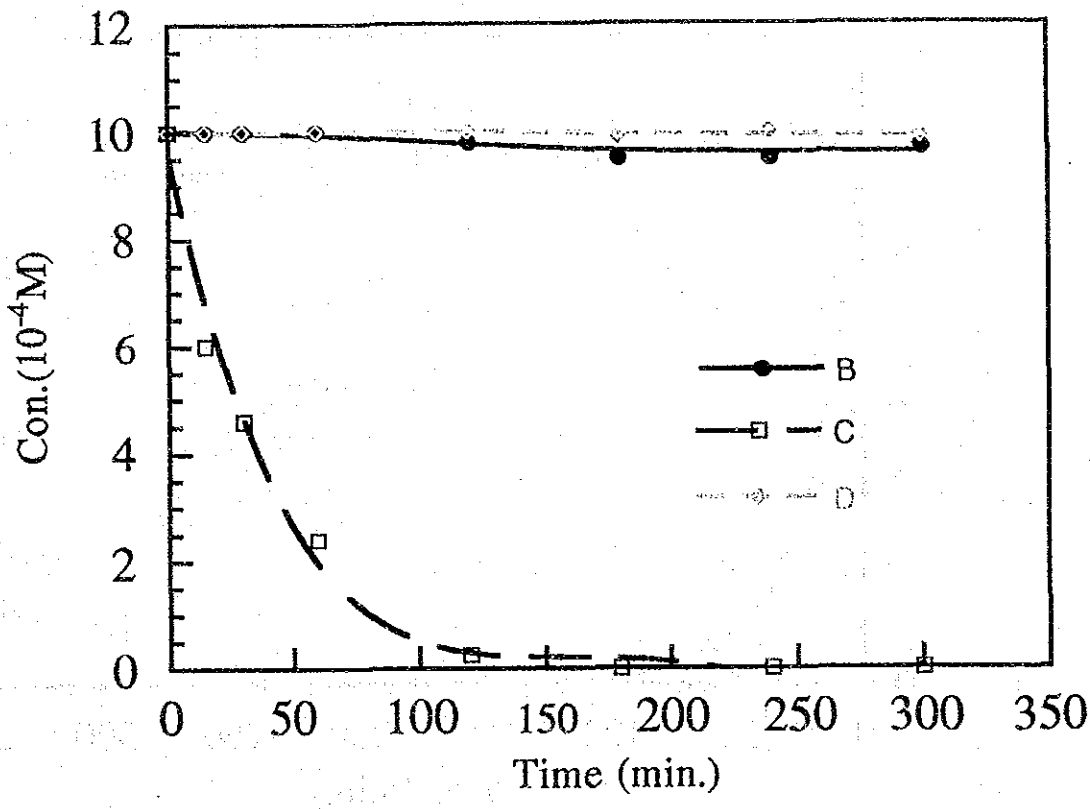


Figure 5. Degradation of p- toluenesulfonic acid sodium salt at 25 °C
(B : with TiO_2 , $\lambda > 310$ nm ; C : without TiO_2 , $\lambda > 310$ nm ;
D : with TiO_2, without illumination)

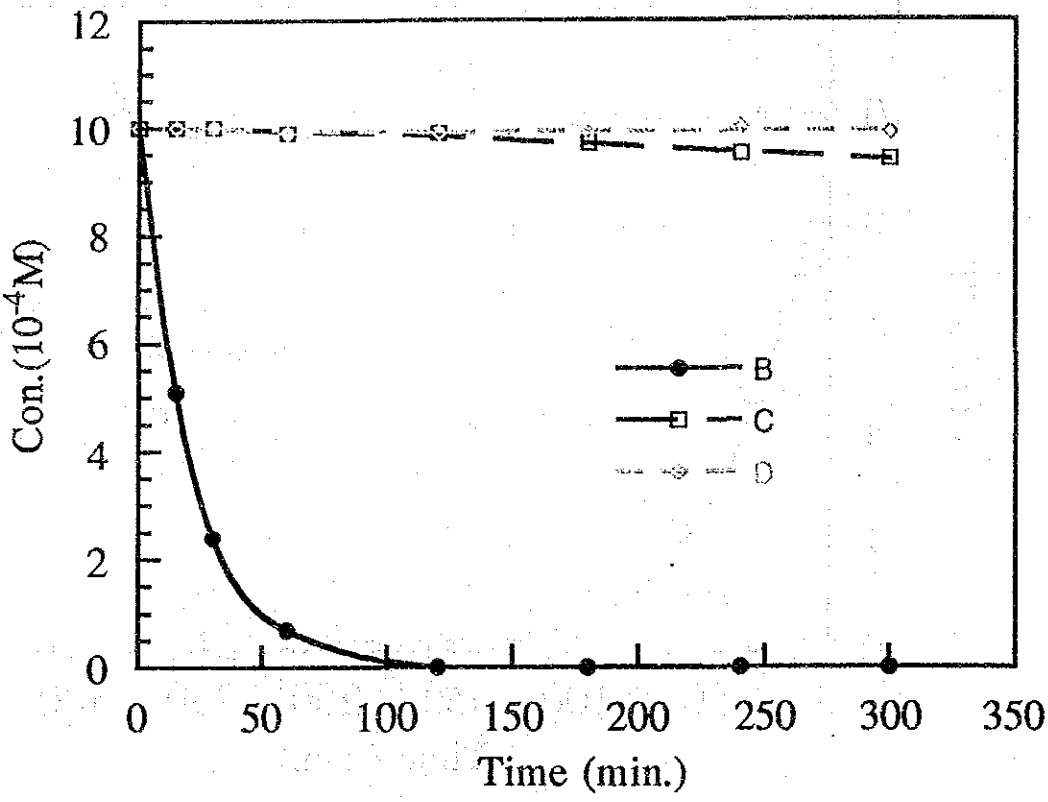


Figure 6. Degradation of p- chlorobenzenesulfonic acid at 25 °C
(B : with TiO_2 , $\lambda > 310$ nm ; C : without TiO_2 , $\lambda > 310$ nm ;
D : with TiO_2, , without illumination)

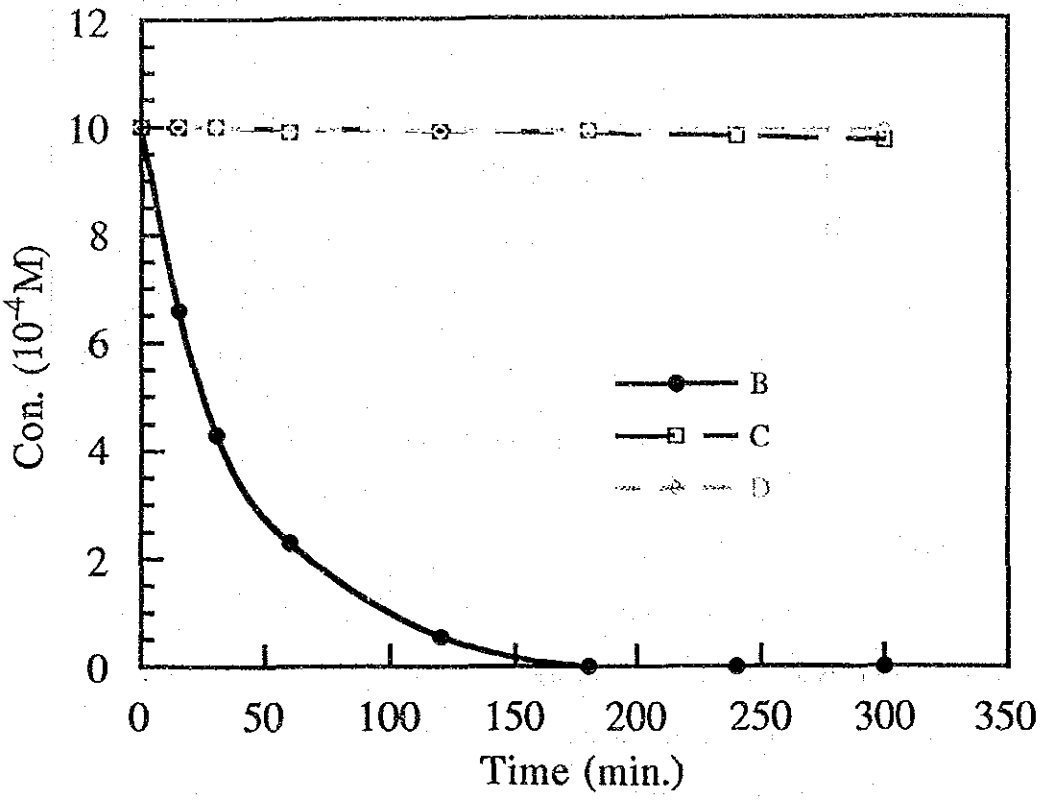


Table 2. Initial Rates of Photocatalytic Degradation (r_0) and Illumination Times Required to Decompose 50 % ($t_{1/2}$) of the Pollutants at 25 °C

Pollutant [a]	r_0 / mMhr^{-1}	$t_{1/2}$ / min
I	1.651	36.0
II	0.959	42.0
III	1.472	24.0
IV	1.773	12.0
V	1.144	18.0

[a] I : Sodium benzenesulfonate

II : p- nitrobenzenesulfonic acid

III : p- phenolsulfonic acid sodium salt

IV : p- toluenesulfonic acid sodium salt

V : p- chlorobenzenesulfonic acid

apparently follows a first-order kinetic law for the initial concentration chosen. None of these aromatic sulfonic acids is adsorped in the presence of TiO_2 in the dark. Table 2. sums up some kinetic results of the photocatalytic degradation of aromatic sulfonic acids over TiO_2.

3. Effect of Various Parameters on Sodium Benzenzsulfonate Photocatalytic Degradation

3.1 Effect of the Initial Concentration

Figure 7(A) illustrates the effect of altering the initial concentration of sodium benzenesulfonate on the initial rate of degradation. The linear transform plotted as shown in Figure 7(B) gives $k = 2.65 \times 10^3$ Mhr^{-1}. The data follow the Langmuir-Hinshelwood relationship $r_o = kKC_o / (1+KC_o)$ where k is the rate constant of sodium benzenesulfonate.

3.2 Effect of Temperature

The increase in r_o with increasing temperature is observed. To determine the activation energy, equation(1) has been applied.

$$\frac{d\ln r_o}{dt} = \frac{E - \lambda}{RT^2} = \frac{E_a}{RT^2} \qquad (1)$$

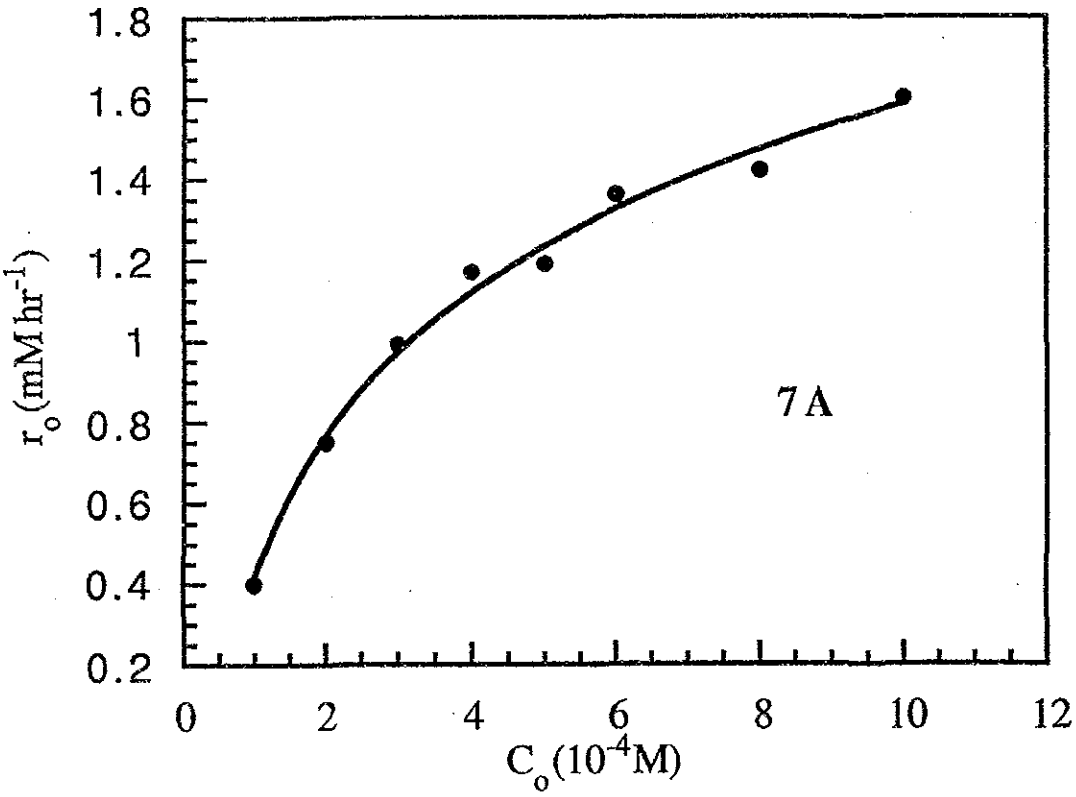
where E_a is the apparent activation energy,

E is the true activation energy, and λ is the heat of adsorption of the reaction.

The plot of $\ln r_o$ as a function of T, shows in Figure 8. , gives the value of $E_a = 15.65$ k $Jmol^{-1}$ at 25 °C. This value is a little larger than values reported for phenol[15] and salicylic[16] acid (10 and 11 $kJmol^{-1}$, respectively). Matthews pointed out that these values are close to that for OH radical reaction

Figure 7. (A) Plot of the initial rate, r_o , of sodium benzenesulfonate photocatalytic degradation against the initial concentration, C_o.

(B) Linear transform of (A).



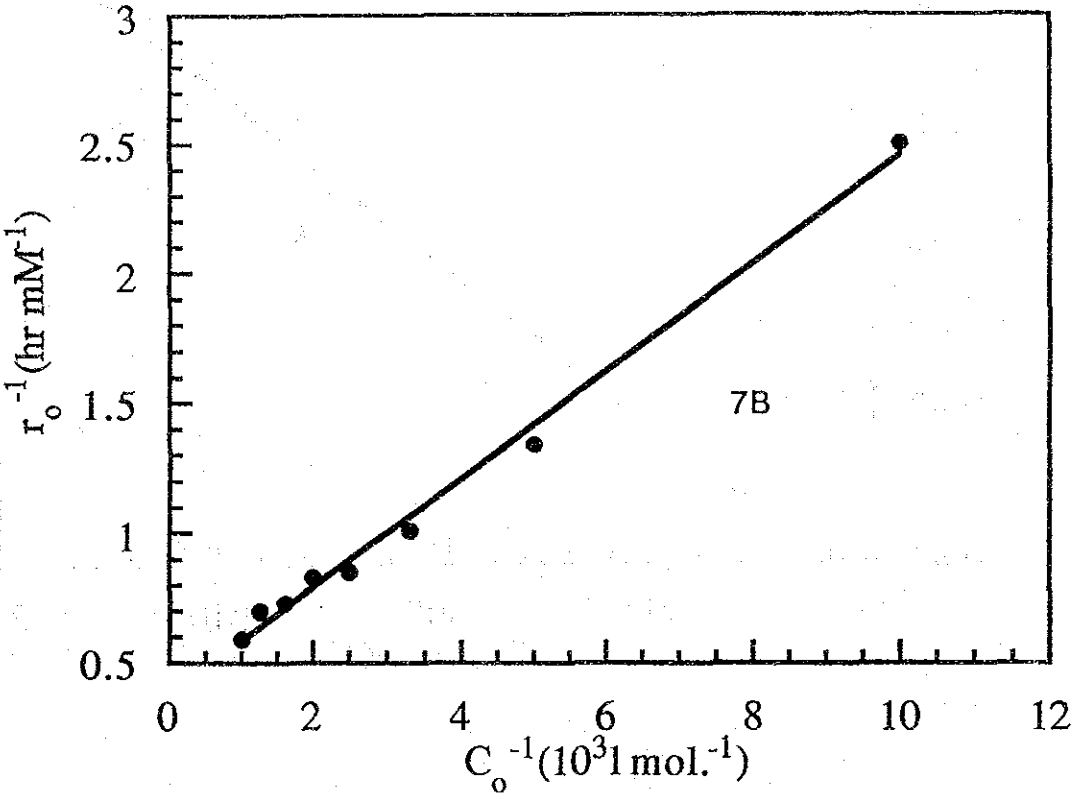
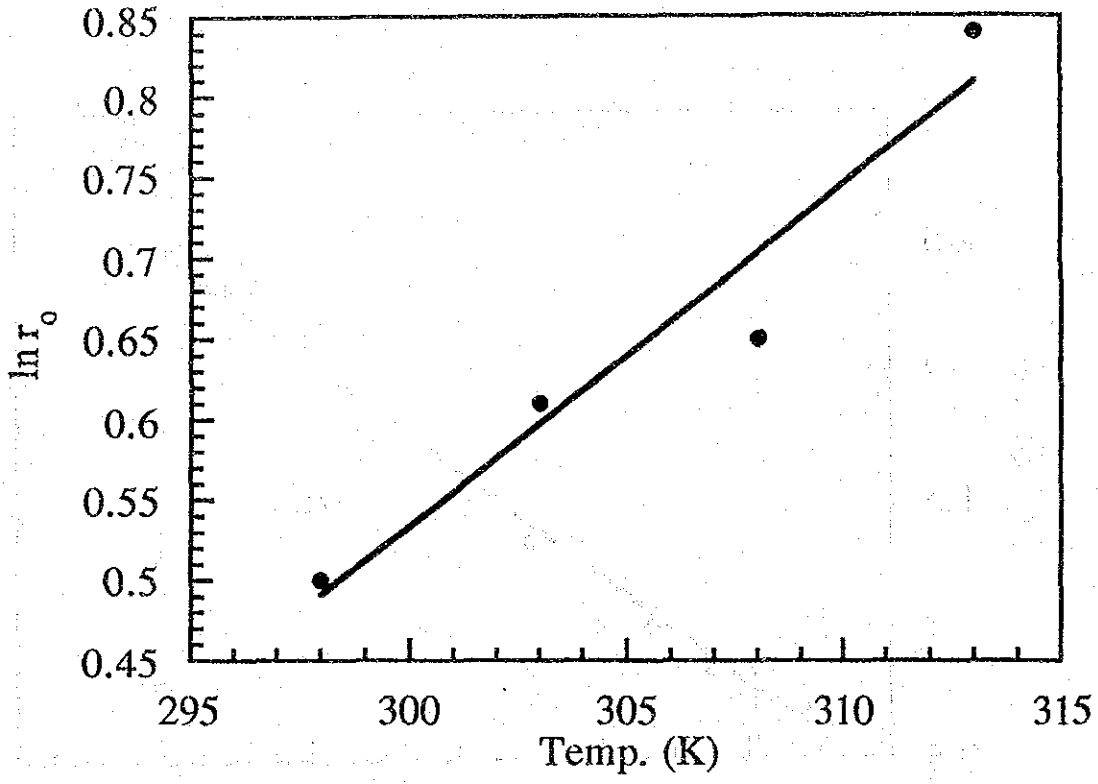

3
2.5
2
1.5
1
0.5
r_o^{-1}(hr mM^{-1})
7B
0
2
4
6
8
10
12
C_o^{-1}(10^3l mol.$^{-1}$)

Figure 8. Plot of ln r_o against temperature



4. Formation of $SO_4^{=}$, H^+ ion and CO_2 During Sodium Benzenesulfonate Degradation

Illumination of suspensions of TiO_2 powders in aqueous benzenesulfonate solutions leads to the formation of $SO_4^{=}$, H^+ ion and CO_2. Figure 9. shows the formation of $SO_4^{=}$ for the mineralization of benzenesulfonate. Formation of $SO_4^{=}$ follows the degradation of the parent substrate sodium benzenesulfonate.

The TOC and the evolution of CO_2 during sodium benzenesulfonate degradation were found to be a function of irradiation time. Illumination of suspensions of TiO_2 powders in aqueous sodium benzenesulfonate solution results in a significant loss of TOC. These measurements demonstrate that sodium benzenesulfonate can be completely mineralized.

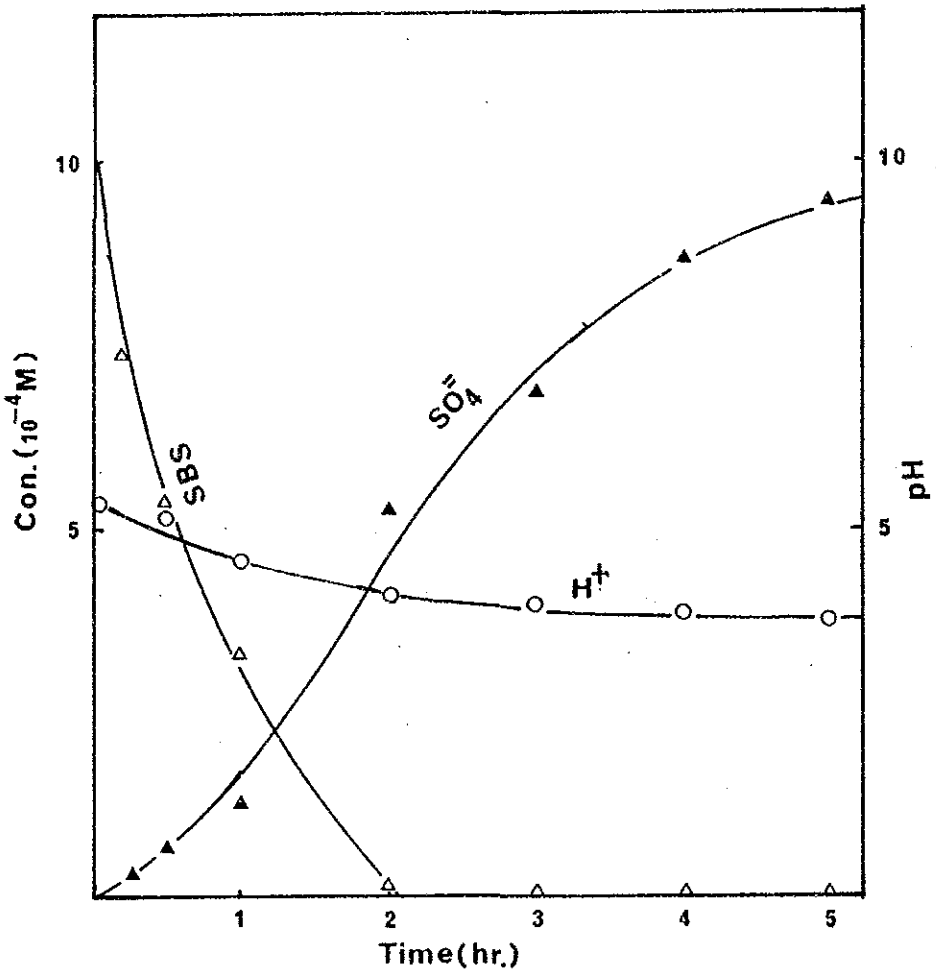
The pH decreases during the photocatalytic degradation of sodium benzenesulfonate, in agreement with total mineralization.

5. Intermediates

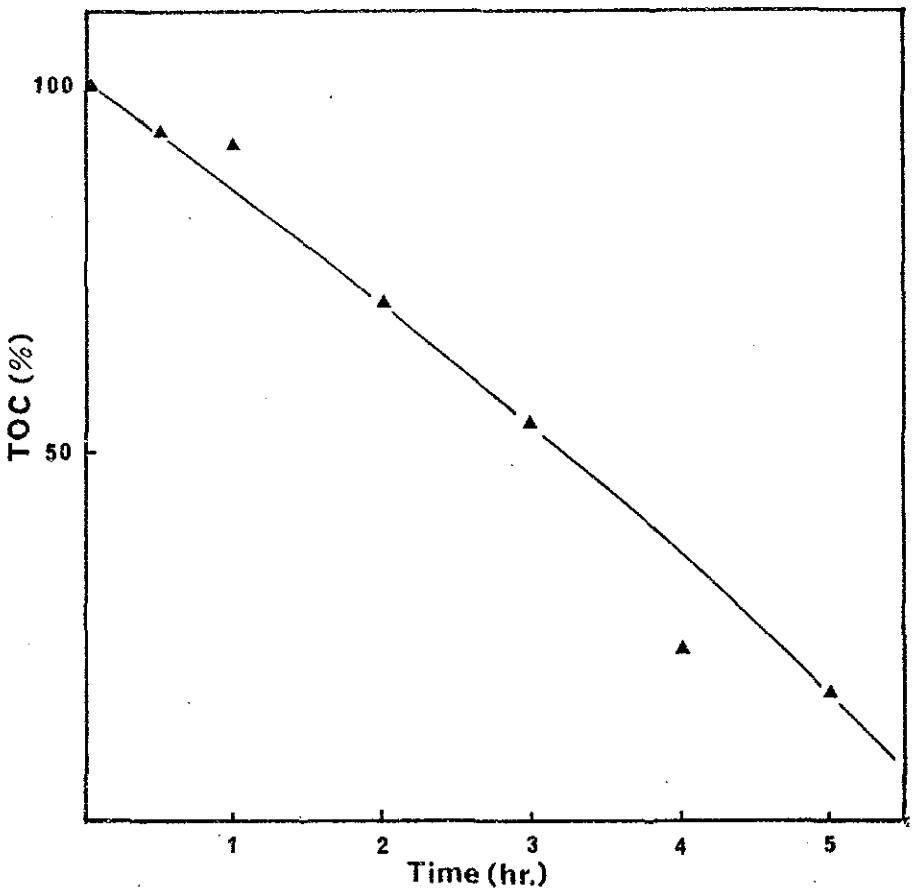
Liquid chromatography techniques permit identification of intermediate products from the photodegradation of organic substrates by comparing the retention times of these products with those from pure samples. In the case of sodium benzenesulfonate, four intermediates are detected by HPLC under the condition indicated. The two main ones are p- phenolsulfonic acid and 2,5- hydroxyquinonesulfonic acid. Identification of compounds resulting from the degra - dation of these two intermediates has not been succeeded.

Figure 9. (A) Plots showing the degradation of sodium benzenesulfonate(SBS), the formation of $SO_4^{=}$, and H^+ as a function of irradiation time.
(B) Plot showing TOC during the degradation of sodium benzenesulfonate.

(9A)



(9B)



Discussion

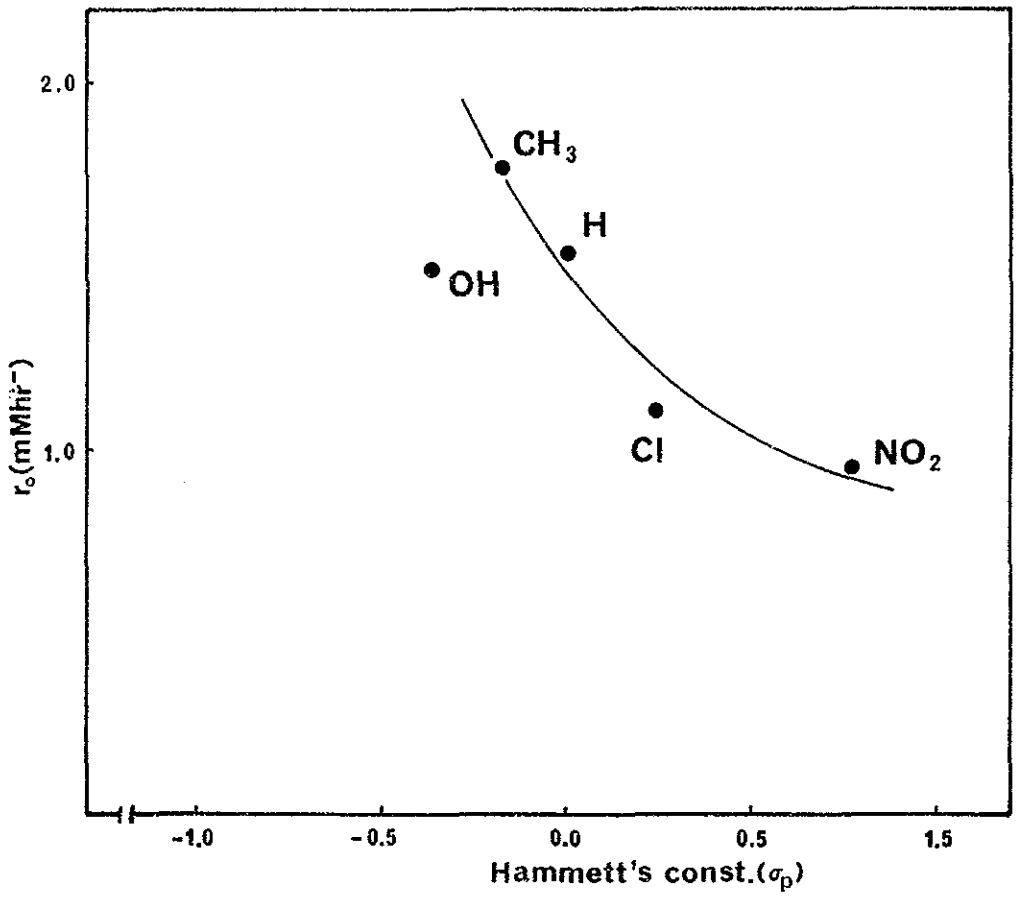
1. Kinetics of Photocatalytic Degradation of Sodium Benzenesulfonate

The presence of catalyst and illumination were found to be necessary for the degradation of sodium benzenenesulfonate. This sugests that the degradation is a photocatalytic process. Under the conditions employed, the initial rate of degradation depended on the initial concen - tration and temperature. The plot of r_0^{-1} as a function of C_0^{-1} yields a straight line as expected if r_0 obeys the Langmuir-Hinshelwood relationship, $r_0 = kKC_0 / (1+KC_0)$. From Figure 7(B), the value of $k = 2.65 \times 10^3$ Mhr^{-1} and $K = 1.818 \times 10^{-6}$ $lmol^{-1}$ can be calculated by assuming the simplest model. The increase in reaction rate with increasing temperature in the range 298-313 K is due to the increase adsorption of sodium benzenesulfonate.

The results in Table 2. reveals some interesting trends with respect to the substituents on the aromatic ring. The reactions are accelerated by electron - donating substituents and are retarded by electron - accepting substituents. The order of disappearance of the substituted aromatic sulfonic acids found in this work is $-CH_3 > -H > -OH > -Cl > -NO_2$

One of the important factors determining the degradation rate is the adsorption of reac- tant to TiO_2, as indicated by an excellent fit of the reaction kinetics to Langmuir - Hinshelwood equation (Figure 7). Below pH 5.5 in the absence of electrolyte, TiO_2 is positively charged[17]. Hence aromatic sulfonate is considered to adsorb to TiO_2 electrostaticaly and there may not be much difference in adsorbability among substituted aromaticsulfonates. Under this assumption the degradation rate was correlated with the electronegativity of substitutent group.As shown in Figure 10, the proportional relation was obtained between the initial rate and Hammett's sub - stituent constant. This result indicates the electrophilic nature of the photocatalytic degradation of aromatic compound.

Figure 10. Plot of the initial rate, r_o, of substituted aromatic sulfonic acid photodegradation versus the Hammett's substituent constant (σ_p).



2. Photodegradation Mechanisms

Irradiation of TiO_2 with light of energy higher than the band gap results in the creation of holes in the valence band and electrons in the conduction band:[18]

$$TiO_2 \text{ ----------> } TiO_2 \ (e^-_{cb} + h^+_{vb}) \qquad (1)$$

Trapping of the separated charges in shallow traps at the surface is also likely:[19]

$$e_{cb}^- \text{ ----------> } e_{tr}^- \qquad (2)$$

$$h_{vb}^+ \text{ ----------> } h_{tr}^+ \qquad (3)$$

In the presence of oxygen , the trapped reducing species may form superoxide radical anion:[19]

$$e_{tr}^- + O_2 \text{ ----------> } O_2^- \qquad (4)$$

whereas oxidisable species may react with the trapped holes:

$$h_{tr}^+ + X^- \text{ ----------> } X \qquad (5)$$

Alternatively the holes may oxidise water or hydroxyl ions on the surface of the particle to give OH radicals : [20,21]

$$h_{tr}^+ + H_2O \text{ ----------> } \dot{O}H + H^+ \qquad (6)$$

The hydroxylated intermediates which have been identified by HPLC methods indicate that the attack of the OH radical on the phenyl ring will be involved in the mechanism pathway. Formation of p- phenolsulphonic acid and 2,5- hydroxyquinonesulfonic acid can be explained by Scheme I and Scheme II.

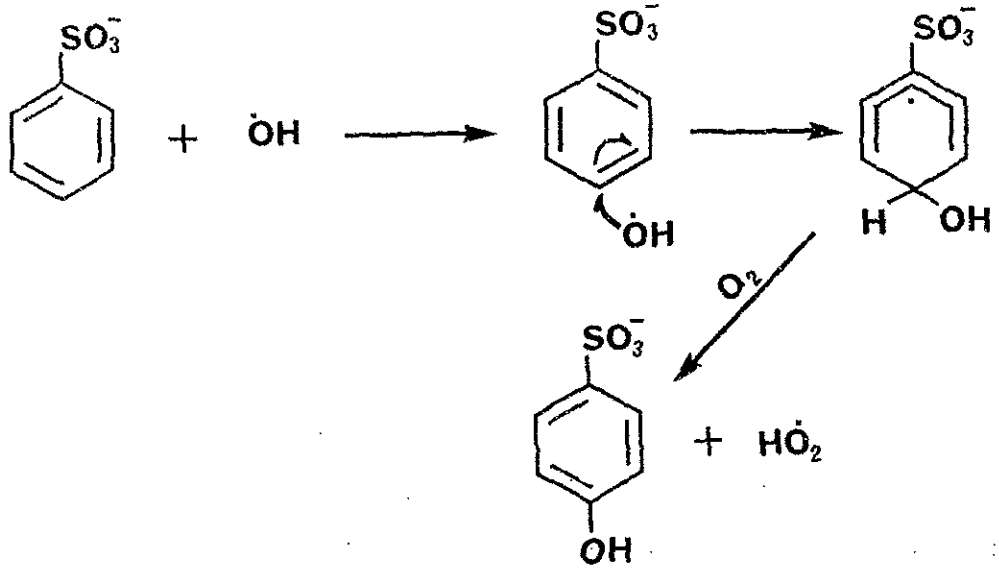
p- phenolsulfonic acid could result from the attack of an OH radical at the para position with respect to SO_3 group of sodium benzenesulfonate, as shown in Scheme I. The pathway for formation of 2,5- hydroxyquinonesulfonic acid is proposed in Scheme II in which the first step is the attack of OH radical at the ortho position.

Further oxidation leading to the formation of CO_2 is difficult to identify. A detailed mechasim for this photoreaction cannot be given on the basis of the present data.

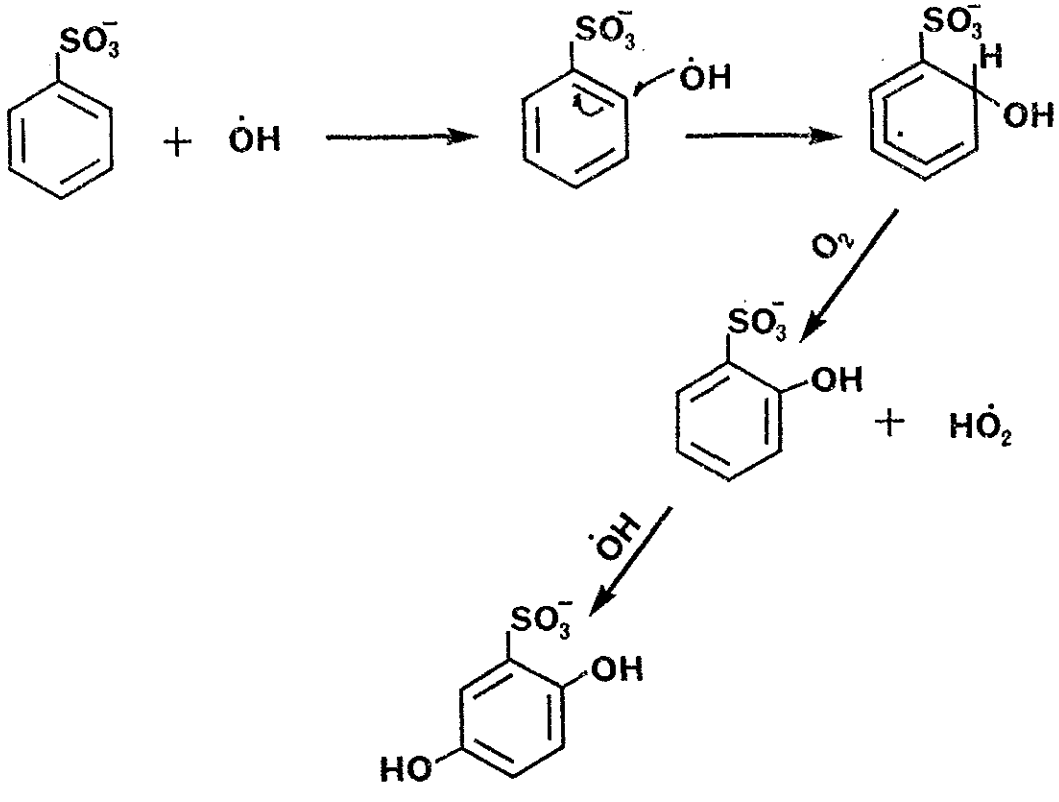
Conclusion

Aromatic sulfonic acids, examined in this work, can also be totally photomineralized to carbon dioxide with irradiation of aqueous, anatase TiO_2 suspensions. Titanium dioxide is inexpensive and can be reused. The rate of degradation is affected by the initial concentration, temperature and substituent groups. Unfortunately, only two aromatic intermediates have been identified,a complete detail of mechanism for this photoreaction cannot be given. Consequently, to explain the mechanism pathway, further experiment will be required.

Scheme I



Scheme II



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