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Physiochemical Analysis of Ground Water of Selected Area of Kaithal City (Haryana) India

D. P. Gupta[a*], Sunita[a] & J. P. Saharan[b]

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ABSTRACT: Ground water samples were collected from different locations in the radius of 25 km. of Kaithal city, Haryana(India). These water samples from 20 sampling points of Kaithal were analyzed for their physicochemical characteristics. Laboratory tests were performed for the analysis of samples for pH, Colour, Odour, Hardness, Chloride, Alkalinity, TDS etc. On comparing the results against drinking water quality standards laid by Indian Council of Medical Research (ICMR) and World Health Organization (WHO), it is found that some of the water samples are non-potable for human being due to high concentration of one or the other parameter. The usefulness of these parameters in predicting ground water quality characteristics were discussed. Thus an attempt has been made to find the quality of ground water in and around Kaithal City town, suitable for drinking purposes or not. [Researcher. 2009;1(2):1-5]. (ISSN: 1553-9865).

Key Words: Water quality parameters, physiochemical study, pollution study, drinking water.

INTRODUCTION

The quality of ground water depends on various chemical constituents and their concentration, which are mostly derived from the geological data of the particular region. Ground water occurs in weathered portion, along the joints and fractures of the rocks. In fact, industrial waste and the municipal solid waste have emerged as one of the leading cause of pollution of surface and ground water. In many parts of the country available water is rendered non-potable because of the presence of heavy metal in excess. The situation gets worsened during the summer season due to water scarcity and rain water discharge. Contamination of water resources available for household and drinking purposes with heavy elements, metal ions and harmful microorganisms is one of the serious major health problems. The recent research in Haryana(India)[1] concluded that it is the high rate of exploration then its recharging, inappropriate dumping of solid and liquid wastes, lack of strict enforcement of law and loose governance are the cause of deterioration of ground water quality. Thus there is a need to look for some useful indicators, both chemical and physical, which can be used to monitor both drinking water operation and performance. Kaithal City area comprises different types of Archaeancrystalline formations. At present there is no major industry in and around the study area, yet household waste water and garbage (municipal sewage) are directly discharged into the area. The water supply for human consumption is often directly sourced from ground water without biochemical treatment and the level of pollution has become a cause for major concern. The water used for drinking purpose should be free from toxic elements, living and non-living organisms and excessive amount of minerals that may be harmful to health. Keeping this in focus, the quality aspects of ground water in Kaithal City area were analyzed for general water quality. Hence, it is highly essential to examine the presence of toxic substances in distribution water for potable purpose before it is used for drinking.

EXPERIMENTAL

Sample collection

Water samples from the selected sites were collected during Sept2007-March 2008 and taken in pre-cleaned polyethylene bottles. The samples after collection were immediately placed in dark boxes and processed within 6 h of collection.

Physico-chemical analysis

The collected samples were analyzed for major physical and chemical water quality parameters like pH**,** Electrical conductivity (EC), Total Dissolved solids (TDS), total hardness (TH), Ca^{2+}**,** Mg^{2+}, as per the method Assessment of Ground Water Quality described in "Standard methods for the examination of water and wastewater American Public Health Association (APHA)[2].The parameters present in the water sample can be calculated by using various methods[3-4] . The pH of all the water samples was determined

using a pH meter(Model no 101 E, Systonic). Electrical conductivity was measured using a conductivity meter. The chloride, total hardness and total alkalinity were estimated by the standard methods of water and waste water[5-7].

RESULTS AND DISCUSSION

A total of 20 water samples from hand pumps, tube wells and Govt. supply used by people of Kaithal City were collected in clean polythene bottles and brought to the laboratory. The samples were chemically preserved by the addition of 3-5 ml concentrated HNO_3 per litre of the sample. The temperature, pH, conductivity and dissolved solids of the water samples were determined on the spot using a thermometer; pH meter, conductometer and TDS meter. Various standard methods (APHA-AWWA-WPCF, 1995; HMSO, 1986)[2] were used for the determination of other parameters. Total alkalinity was determined by visual titration method using methyl orange and phenolphthalein as indicator. Total hardness and calcium were measured by EDTA titrimetric method using EBT indicator respectively. Chloride was determined by Argentometric method using potassium chromate indicator. The chemical data were complied further to know location wise distribution. The data revealed that there were considerable variations in the examined samples from different sources with respect to their chemical characteristics. The results indicate that the quality of water considerably varies from location to location. The underground water is characterized by a relatively constant pH of around 6.0 and 6.9 in the samples collected from Friends Colony and HCTM College. Water sample with low pH(6.0) attributed to the discharge of acidic water into these sources by the agricultural and domestic activities. Samples collected form the Sampling points Sector 20 Area (8.23) and Old Anaj Mandi Area(7.38) were slightly basic which can be seen from its pH and alkalinity values. In fact 98% of all world ground water are dominated by Calcium and bicarbonate ions due to lime stone weathering in the catchments and under ground water beds[3]. In water buffered by the presence of bicarbonate, carbonate and hydroxyl ions, this temperature effect is modified. Though pH has no direct effect on the human health, all the biochemical reactions are sensitive to variation of pH. For most reaction as well as for human beings, pH value 7.0 is considered as best and ideal. In the present study pH value of water samples varied in a narrow range within the permissible limits in all sources. The pH is of the utmost importance in determining the corrosivity of water. In general, the lower the value of pH, the higher the level of corrosion. It has been observed that in some cases decrease in pH is accompanied by the increase in bicarbonate, carbonate and hydroxyl ions. Decrease in pH can be caused by the increase in the amount of organic carbon, total carbonate by the use of sewage.

pH was positively correlated with electrical conductance and total alkalinity. The EC values were found higher at Sector 20 Area due to concentrated colloids in canal water and dissolved salts in Old Anaj Mandi. Very low conductivity was found at sampling points Chandana gate & Vashno Mata Mandir Area. The values of salts such as Ca, Mg, Cl^- and SO_4^{2-} suggested that the surface water sources posses' very low components, while underground water sources which are far away from surface ground water or Dam water source were found to possess higher amounts of components. Conductance is a function of water, hence a standard temperature, usually 25^0C, is specified in reporting conductivity. Higher the concentration of electrolytes in water the more is its electrical conductance.

In the present study, electrical conductance was highest in water sample of by Karnal By pass area and Sector 20 Area. It is well known that the conductance of water increases with salts. Total dissolved solids and conductivity can be used to delineate each other. Conductivity is proportional to the dissolved solids. Both showed analogous trend in seasonal variation[3].

The water samples from Chandana gate, Nursing Colony Area, Anaj Mandi area and old Anaj mandi Area were found to possess high TDS value when compared with the tolerance limit of 1500 ppm. Among this, the water sample from near old Anaj mandi Area having TDS in the order of 2900 ppm. The TDS was found to be low for the water samples collected from the Friends Colony and HCTM College. Samples from the sources Public Club Area, Hospital Area and water Works area showed low level of TDS of the range less than 250 ppm, which indicates that the recharging of under ground water through either rain water or by the water from near by canals.

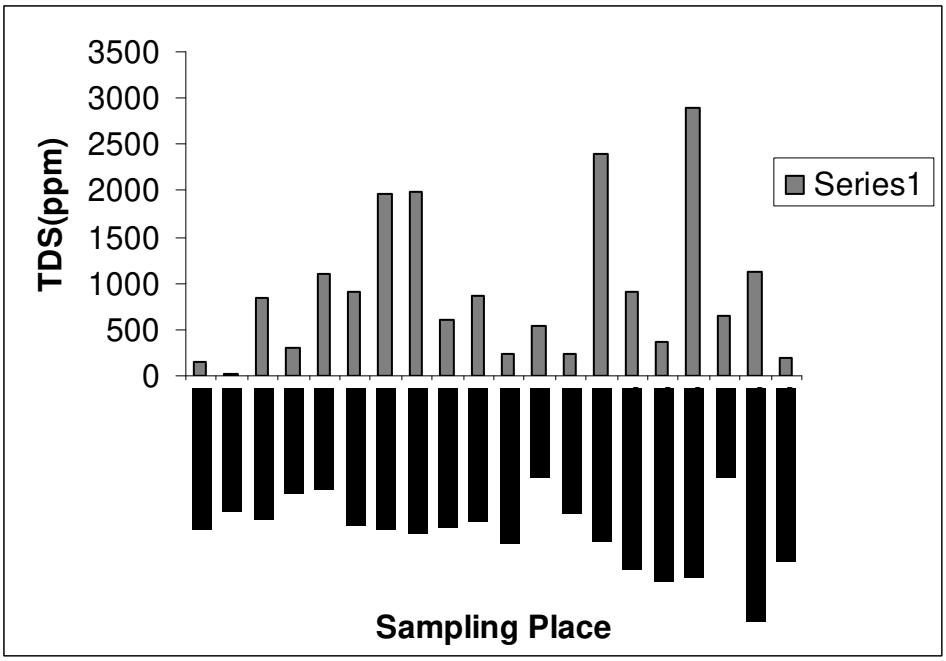

Figure: 1 TDS (ppm) values of various water samples collected in Kaithal City

The possibilities of dissolution of rockery minerals are very low. The Dissolved Oxygen of the water samples varies from 4.0 to 8.8 ppm from the all sampling locations.

Alkalinity in terms of HCO_3^- of all these water samples ranged from 0.0- 1930 mg/L respectively. Chloride contents of these water samples ranged from 35-875 mg/L respectively. Calcium of water samples ranged from 25- 425 mg/L, respectively. Magnesium content of all water samples ranged from 35-485 mg/L. Total alkalinity is a measure of the ability of the water to neutralize acids. The constituents of alkalinity in neutral system include mainly carbonate, bicarbonate, hydroxide and other components which may contribute to alkalinity are $H_2BO_3^{2-}$, HPO_4^{2-} and HS^-. These compounds result from dissolution mineral substances in the soil and atmosphere [4-6].

Alkalinity is a big problem for industries also, as alkaline water if used in boilers for steam generation may lead to precipitation of sludge, deposition of scales and cause caustic embrittlement. This study also indicates that any industry establishment in this area must have alkalinity treatment plant prior to use of ground water or should go for some alternate water source.

Water hardness is the traditional measure of the capacity of water to react with soap, hard water requiring considerably more soap to produce lather. Hardness is one of the very important properties of ground water from utility point of view for different purposes. In the present study water was very hard and crossed the permissible limits. It is well known that hardness is not caused by a single substance but by a variety of dissolved polyvalent metallic ions, predominantly calcium and magnesium cation, although other cation likes barium, iron, manganese, strontium and zinc also contribute. The high concentration of total hardness in water samples may be due to dissolution of polyvalent metallic ions from sedimentary rocks, seepage and run off from soil. As we know calcium and magnesium, are the two principal ions. The concentration of total hardness in drinking water sources ranged between 75 and 1110 mg/L (Nawlakhe; 1995)[7], (Sastry *et al*)[8] also reported water samples from ponds, wells and hand pumps were very hard ranging from 222.8-1094.4 mg/L. In old Anaj Mandi Area tube well and well water showed high concentration of total hardness.

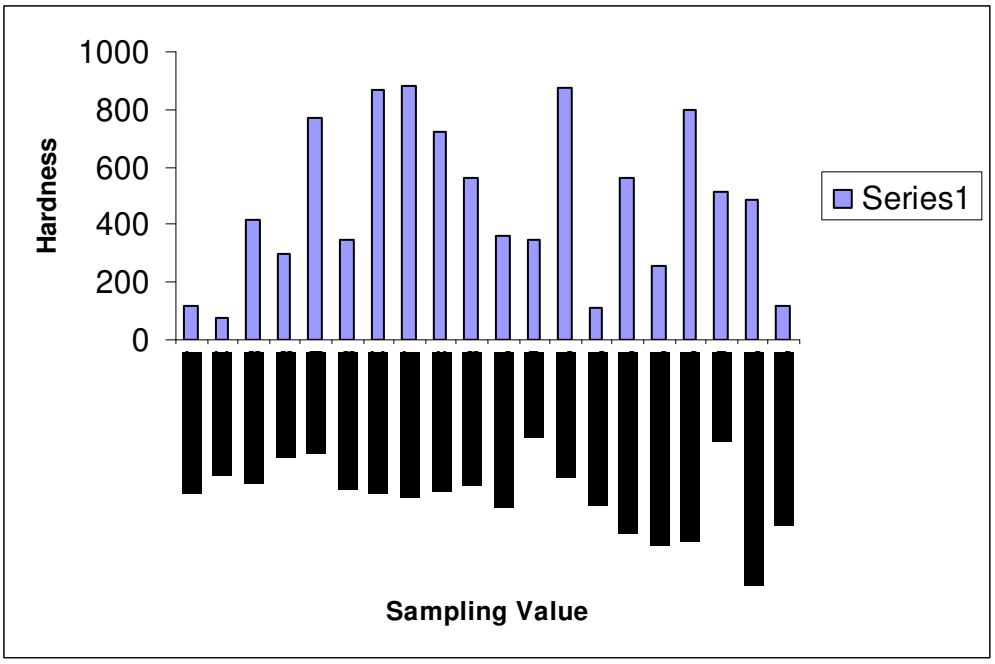

Figure: 2 Hardness (ppm) values of various water samples collected in Kaithal City Town

In the present study total hardness was positively correlated with fluoride, chloride, calcium and magnesium. The strong correlation-ship between these parameters could be due to changes in land use namely deforestation, disruption in internal sources of hardness and alkalinity, climatic factor or industrialization.

As calcium and magnesium are directly related to hardness and hence combined in discussion. The acceptable limits for calcium and magnesium for domestic use are 75 ppm and 30 ppm, respectively, in ground water. Whereas in case of non-availability of water sources, calcium up to 200 ppm could be accepted.

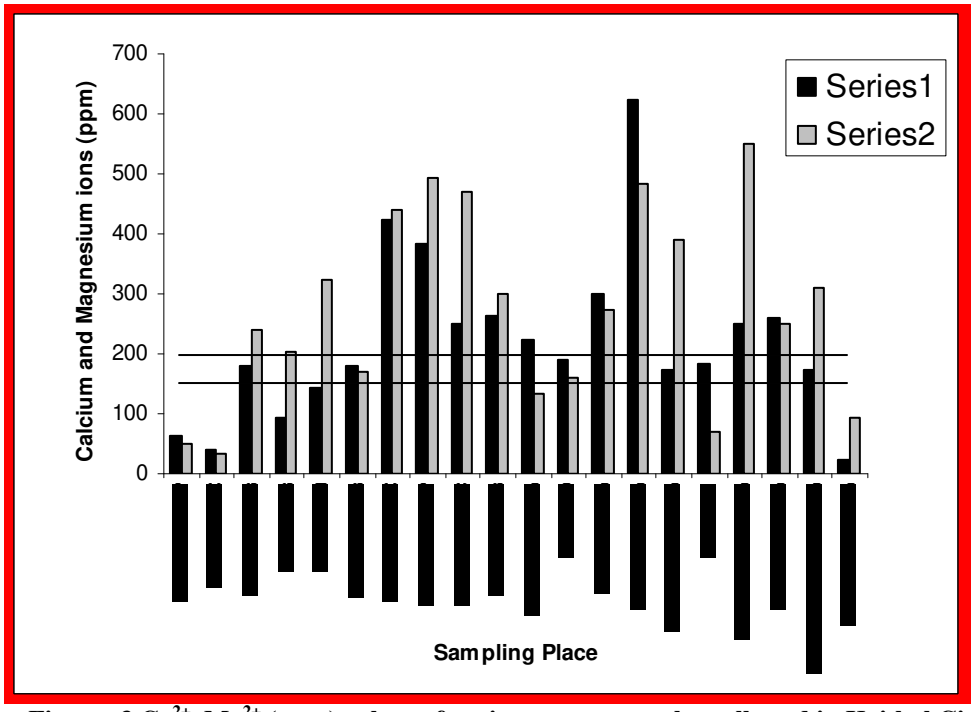

Figure: 3 Ca^{2+}, Mg^{2+} (ppm) values of various water samples collected in Kaithal City

Chloride ions are very high in water of Anaj mandi area and very less in HCTM water sample. The results are given in figure 4

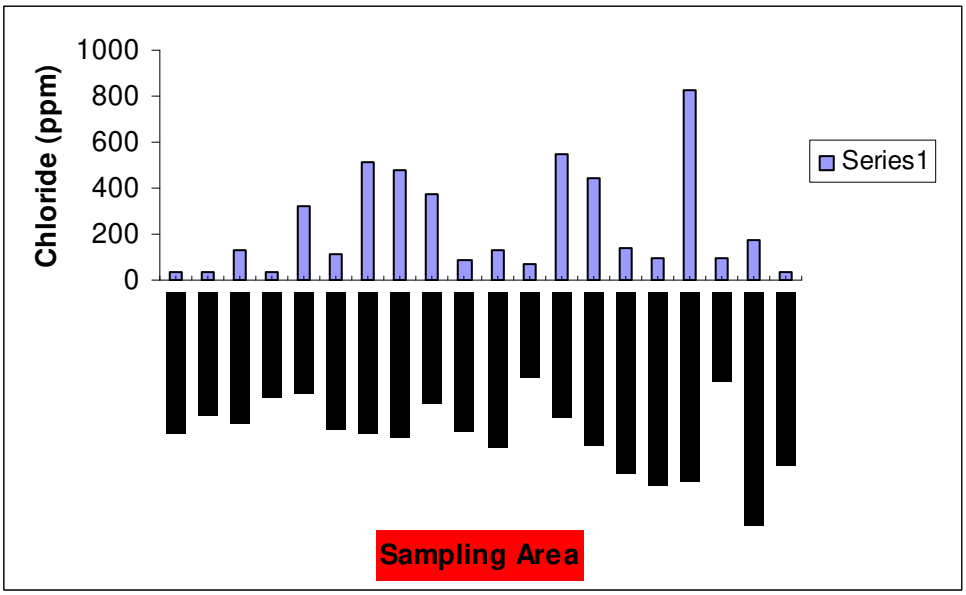

Figure: 4 Chloride (ppm) values of various water samples collected in Kaithal City.

Conclusions

The ground water which were taken from the various places of in and around Kaithal City town were analyzed and the analysis reports that the water quality parameters like pH, EC, Cl^-,TDS ,Ca^{2+}, Mg^{2+} and Hardness lies within the maximum permissible limit prescribed by WHO and ICMR. Except few parameters like DO, few samples were reported with lower DO than the permissible level, but this value does not have any impact for the water to use for drinking purpose. ***According to this report, the ground water in and around HCTM College, Water Works Area are suitable for drinking, agriculture and industries and really it is not harmful to human beings.***

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Achieving the Millennium Development Goals: An Assessment of Water and Sanitation Intervention of the Ikaram Millennium Village, Nigeria.

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Abstract: This article describes an assessment of water and sanitation intervention of the Ikaram-Ibaram Millennium village project as efforts towards achieving the Millennium development goals. [Researcher. 2009; 1(2):6-13]. (ISSN: 1553-9865)

Introduction

The millennium development goals are series of eight time-bound development goals that seek to address issues of poverty, education, equality, health and the environment, to be achieved by the year 2015. They were agreed by the International community at the United Nations Millennium Summit, held in New York in September 2000. To address these challenges, all member countries of the United Nations signed the Millennium Declaration in September 2000, which laid out quantified, targeted goals-the Millennium Development Goals (MDGs) – to halve extreme poverty in its many forms by 2015. In January 2005, the UN Millennium Project, commissioned by the UN Secretary General, recommended an action plan detailing what needs to be done and how to achieve the MDGs. The report identified practical strategies to eradicate poverty by scaling up investments in infrastructure and human capital while promoting gender equality and environmental sustainability. Sub-saharan Africa is the region most off-track on the MDGs; the Millennium Project estimates that a typical country in sub-Saharan Africa will need to significantly increase public investments to approximately $75-$80 per capita by 2006, rising to $125-$160 by 2015, in order to meet the goals. In typical rural community, the required investments average US$110 per capita/year over 5-10 year period.

Between 1990 and 2001, the number of people in sub Saharan Africa living on less than $1 a day rose from 227 million to 313 million with one-third of the population below the minimum level of nourishment and many countries including Nigeria crippled by disease, drought and poor infrastructure. Thus the millennium village project (MVP) was inaugurated in 2004 as a direct response to this growing crisis. The Ikaram-Ibaram Millennium Village Project is been executed with support from the Earth Institute –Columbia University, The Millennium Promise New York, UNDP Nigeria and Ondo State Government of Nigeria.

The importance of safe water in poverty alleviation and socio-economic development cannot be overemphasized. Access to safe drinking water and adequate sanitation are part of the Millennium Development Goals of reducing poverty by the year 2015. Safe water has been described as water that meets the National Standard for Drinking Water Quality for Nigeria (FMWR, 2004). However, abnormally low levels of access to clean water by a large proportion of humanity have been reported. Worldwide, about 2 billion people struggle daily for access to clean and sufficient water (Smith and Marin, 2005). Africa is the region that suffers most from inadequate access to water supply. Yahaya (2004) reported that in Africa, only 62 percent of the populations have access to potable water supply (compared with 82 percent Worldwide, 81 percent in Asia and 85 percent in Latin America). Furthermore, of 55 countries in the world whose domestic water use is below 50 litres per capita per day, 35 are in Africa. In Nigeria, 52 percent of the population does not have access to safe drinking water (UNDP 2006; UNICEF 2007). Improved access to safe drinking water is a prerequisite to poverty reduction. Access to safe drinking water prevents the spread of water-borne and sanitation-related diseases. Lack of access to safe water and adequate sanitation services especially in developing countries often result in about two million infant death annually.(Cosgrove and Rijsberman, 2003; Gomez and Nakat, 2002; The World Bank, 2001).
This paper therefore seeks to discuss efforts at achieving the water and sanitation MDGs in Ikaram, Nigeria.

Scope of the MVP Concept

The Millennium Villages Initiative aims to establish a rigorous proof of concept for implementing the practical interventions needed to achieve the MDGs in rural Africa Over five year time frame. It seeks

to scientifically demonstrate and document low cost, and integrated community based intervention geared towards empowering rural areas in order to enable them achieve the MDGs. Figure 1. Shows location of Ikaram-Ibaram Millennium Village Project (MVP) and other Millennium Villages in Africa.

The scope of the MVP covers the eight MDG goals through nine objectives as stated below.

To eliminate hunger and malnutrition in the villages by increasing production, access and utilization of nutritious foods, with a special focus on improving nutritional status of pregnant women, nursing mothers and infants under two (MDG 1)

To improve livelihoods of women and men and increase their incomes for both on and off-farm activities beyond extreme poverty (MDG 2)

To ensure full attendance to primary schools for both boys and girls and eliminate gender disparity in schools (MDG 2) and 3).

To improve access to medical services, especially focused on improving women's health and drastically reducing child and maternal mortality (MDG 4 and 5)

To decrease rate of infection of HIV/AIDS, malaria, tuberculosis and other major diseases; and increase access to essential medicines such as antiretroviral medication (MDG 6).

To integrate the principles of sustainable development into village programs to reverse the loss of environmental resources and enhance ecosystem services (MDG 7).

To increase access to energy, clean air, water and sanitation for households, schools and medical services (MDG 7).

To eliminate the digital divide by making available the benefits of communication technologies, especially access to the internet and mobile telephone services (MDG 8)

To record with scientific rigor and accuracy the inputs, costs and results of the investments and interventions implemented in each Millennium Village and examine opportunities for scaling up lessons learned.

For the purpose of this paper, focus will be limited to the water and sanitation aspect of the MDG goals.

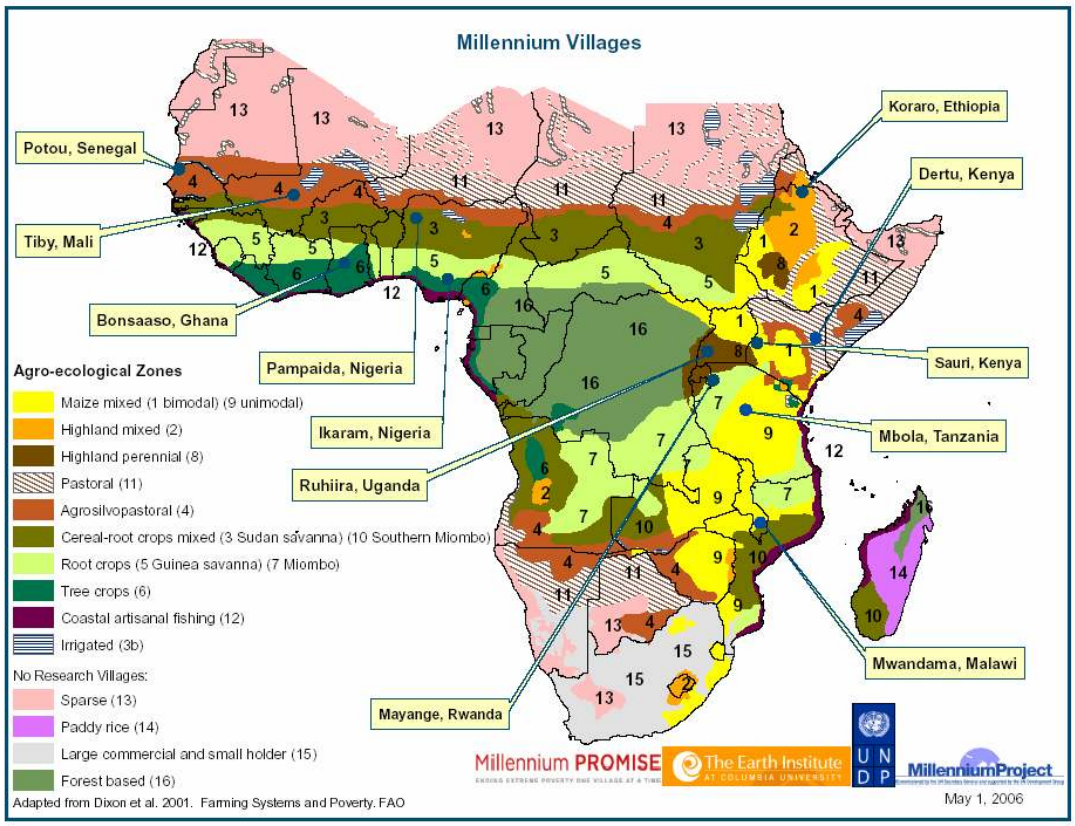

Fig. 1: Locations of Millennium Villages in Africa

Description of the Ikaram-Ibaram Millennium Villages

The Ikaram –Ibaram Millennium Village is located in Akoko North West Local Government Area of Ondo State Southwest Nigeria (Figure 2). It is located approximately 400km from Lagos, 9km north of Oke-Agbe, and 100km from Akure, the Ondo State capital. The Ikaram-Ibaram Millennium Village in Nigeria was established in May 2006 with generous support from the Japanese Government to UNDP and a gift from Sara Miller McCune. The Ikaram community is the primary research site in the Ondo State project with an estimated population of 4,417 people. However, Ikaram is one of several contiguous and densely packed communities that make up the larger Millennium Cluster Village. Combined, these communities – Ikaram, Ibaram, Iyani, Ase, Erusu, Gegegede and Ajowa – have a population of 18,307 people. The cluster village is referred to as Ikaram-Ibaram to simplify matters and because the communities are all located in the same local government area. Because the MVP seeks to use an integrated and holistic approach and because of the contiguous nature of the overlapping Ikaram-Ibaram communities, interventions targeted for Ikaram have also played a crucial role in supporting 'cluster-wide' initiatives, being leveraged for positive effect on the overall population.

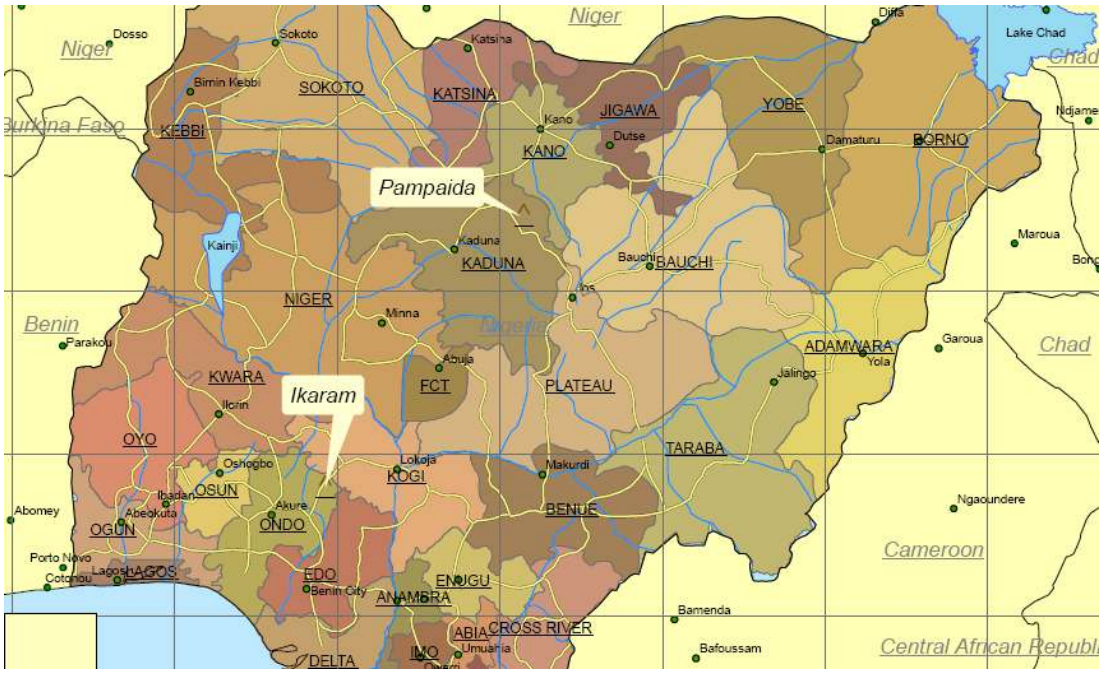

Fig. 2. Map of Nigeria Showing Location of Ikaram Millennium Village.

Project Methodology

The project adopts a participatory and demand driven approach to problem identification, solution planning and project implementation and management.

Needs Assessment and Participatory Appraisal:

Needs assessments of local community facilities and infrastructure to identify areas of critical need and opportunities for quick-impact initiatives was conducted. Important baseline data which included a detailed demographic survey of the entire cluster, water and sanitation baseline study and a socio-economic survey were obtained. Data entry and analysis covering all these studies was done to facilitate comprehensive development strategies and intervention plans.

In order to optimize the level of community involvement, the village baseline assessment was conducted in close consultation with local leaders and community groups. As a result, the community quickly committed to improve their capacity for action by establishing democratically elected committees which liaises with the MV team to advise and consult on development priorities for the village.

Fig. 3: Cross section of community members during a participatory meeting.

Several participatory meetings were held in order to establish Water and sanitation committees (WASHCOMs) which will focus on the water and sanitation infrastructures, technology options, operation, management and record keeping. Participation was all inclusive to ensure involvement and participation of women, men and youth (Fig. 3). In addition, two members from the committee were appointed to act as representatives of the sector in the MVP Central Committee. This committee will be a key decision making organ in planning, implementing and monitoring project activities within the cluster.

Access to potable water is severely compromised with too few boreholes and pumps to serve the community, especially during the dry season when the boreholes do not function. Even during the rainy season, when the boreholes are operational, the water is frequently not clean enough to drink. This results in villagers having to travel long distances to collect water. An immediate goal therefore is to provide the village cluster with a viable water supply. A first step to reaching this goal is the implementation of a comprehensive study followed by detailed analysis to determine suitable locations for the establishment of safe, reliable boreholes. To this end, baseline survey was carried out on existing water points and sanitation facilities to assess: number and type of existing water points in the Cluster; in terms of viability, quality and accessibility. The baseline survey brought clearer picture of water situation in the cluster. It reveals that the critical areas of water needs are in the health centres and schools where water does not exist.

Table 1: Analysis of pre intervention water Survey of Ikaram-Ibaram MVP

S/N	SUB VILLAGE & POPULATION	WATER POINT	TOTAL No.	TOTAL FUNCTIONAL/PERRENIAL (IN THE CASE OF WELLS) WATER POINTS
1	IKARAM	MPBH	8	3
	4,982	SLPBH	4	3
		MBH	3	1
		WELL	185	78
2	ASE	MPBH	1	1
		SLPBH	0	0
	72	MBH	1	0
		WELL	1	1
3	IBARAM	MPBH	4	4
		SLPBH	2	1
	613	MBH	2	1
		WELL	19	3
4	IYANI	MPBH	1	1
		SLPBH	1	0
	514	MBH	3	2
		WELL	15	9
5	GEDEGEDE	MPBH	4	3
		SLPBH	1	1
	995	MBH	0	0
		WELL	15	1
6	ERUSU	MPBH	6	2
		SLPBH	2	1
	3,067	MBH	10	6
		WELL	126	52
7	AJOWA	MPBH	10	7
		SLPBH	3	0
	8,064	MBH	9	6
		WELL	377	187

Key: MPBH=Manual powered borehole, SLPBH=Solar powered borehole, MBH=Motorized borehole.

The following results were obtained from the survey

A ratio of 257 people per functional drinking water point was established. The failure rate of existing water points is very high. 25% for Solar powered boreholes with gravity tanks and standpipes and 38% for hand pump fitted boreholes

A large number of people make use of unsafe water points – 738 Numbers of unprotected hand dug wells of which only 331 (44.9%) are perennial.

For sanitation, only 398 toilet facilities were available for use by the 18, 307 inhabitants of the cluster village. These comprise of 206 flush toilets, 19 VIP toilets and 173 pit latrines.

Moving Forward

Focusing on schools and health centers, the MVP has supported the cluster villages with provision of hand pump fitted boreholes in 13 schools, and 4 motorized boreholes in health centers across the cluster and 1 motorized borehole for use at the proposed office of the MVP. This support is to improve the water supply situation of the cluster villages. This was achieved through

1. An in depth geophysical survey conducted to determine viable VES points for drilling of borehole.
2. Contract awarded for the borehole construction
3. Monitoring of drilling and construction activities for quality assurance.
4. Installation of hand pumps in schools and submersible pumps in health centers to make boreholes operational.
5. Establishment of environmental Health Clubs in Schools was also carried out to ensure hygiene and education education through child to child methodology.

Fig 4 (a)

Fig 4 (b)

Fig.4: Water supply source to L.A. Primary School, Ajowa (a) before and (b) after intervention

Fig. 5 (a)

Fig. 5 (b)

Fig. 5: Water supply source to Ikaram Basic Health Centre (a) before and (b) after intervention.

The transformation of the village and the confidence of villagers in their ability to take charge of a brighter future become more tangible as each week pass and more are accomplished. It is projected that the output in the next few years will improve greatly and the overall infrastructural capacity of the village is improving steadily – particularly in the areas of water and sanitation.

Proposed Water and Sanitation Intervention

The following have been proposed in order to support the Ikaram cluster villages to achieve the water and sanitation MDG goals.

1. Construction of Ventilate Improve Pit (VIP) latrines in all 16 Primary schools.
2. Rehabilitation of non functional boreholes in the cluster.
3. Training of Community members on construction of Sanitation Platforms (SANPLAT) and basic environmental sanitation and hygiene.
4. Capacity building for local government water and sanitation technicians for effective water and sanitation management in the cluster
5. Construction of hand dug wells in cluster Fulani communities
6. Establishment of Environmental Health clubs in all 16 primary schools
7. Training of primary school, health institution and the community on the operation and maintenance techniques for water and sanitation infrastructure
8. Hand wash campaign to promote hygiene and hand washing at critical periods.
9. Quarterly monitoring of water quality and treatment in the cluster.
10. Adoption and promotion of drip irrigation for production of high value crops.

Conclusion

Our generation has the unprecedented opportunity to end extreme poverty throughout the world by 2025. Achieving the Millennium development goals by 2015 will be a crucial step along the way. However this requires a holistic approach through demand responsiveness and participatory planning. Our support towards these laudable and achievable targets will solve our quest for a more peaceful and prosperous world. It will be a worthwhile legacy for our children to inherit a poverty free world where there is adequate provision of water and sustainable sanitation. The transformation of the village and the confidence of villagers in their ability to take charge of a brighter future become more tangible as each week pass and more are accomplished. It is projected that the output in the next few years will improve greatly and the overall infrastructural capacity of the village is improving steadily – particularly in the areas of water and sanitation.

Acknowledgement

The authors sincerely acknowledge the efforts and contributions of all members of the Ikaram-Ibaram MVP team towards this piece of work.

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Heteroblastic Expression In Leaves Of *Phyllanthus Urinaria* Linn.

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Abstract: Heteroblasty is a stair-case phenomenon in some plant life. The juvenile stage through heteroblastic development attains the adult stage. The present article deals with morphological changes observed in leaves of *Phyllanthus urinaria* Linn. from juvenile stage to adult stage. This is the first report of heteroblastic development in *Phyllanthus urinaria* as well as for the genus *Phyllanthus*. [Researcher. 2009;1(2):14-16]. (ISSN: 1553-9865).

Key words: Heteroblasty, morphology, eophylls, *Phyllanthus urinaria*.

Introduction

Phyllanthus urinaria Linn., commonly known as chamber-bitter or stone-breaker, belongs to the genus *Phyllanthus* of family Euphorbiaceae. This is a widely used medicinal herb in jaundice, liver and urinary disorders and is often confused with other herbaceous species of *Phyllanthus*.

In many plants, there is a gradual transition from juvenile traits, present soon after germination, to stable adult traits acquired by the time flowering commences, called heteroblastic development. Several experimental studies were made by Goebel (1889, 1928), Day (1998) regarding heteroblastic development. This phenomenon can be investigated from different view points, e.g. morphological, anatomical, genetical, physiological and environmental (Allsopp, 1965). The change in leaf morphology is one of the main components of this heteroblastic development. As such, the present study involves changes in morphological parameters of leaves of *Phyllanthus urinaria* Linn. The changes in leaf shape and size, appearance of trichomes and phyllotaxy or arrangemet of leaves of seedling and adult plants have been considered for study.

Materials and Methods

Seeds of *Phyllanthus urinaria* Linn. were collected from natural habitats of different parts of West Bengal, India and grown in the Nursery of the Experimental Botanic Garden of the Department of Botany, University of Calcutta. The developmental stages of seedlings were recorded and studied till 30th leaves stage. Moreover, the seedlings of the investigated taxon were also collected from the natural habitats in order to arrive at constancy of characters by comparing with the raised ones. The herbarium sheets were prepared and the voucher specimens were deposited in Calcutta University Herbarium (CUH), Kolkata.

Results and Discussion

The morphology of the leaves of angiosperms exhibits remarkable diversity. The changes in the morphology of the leaves are undoubtedly the most conspicuous feature of heteroblastic development in the vascular plants. In the simplest case (as is the case with most of the *Phyllanthus* species), there is merely increase in size of the leaves, without any change in form. However, in *Phyllanthus urinaria*, irrespective of any form changes, an increase in leaf size (both length and width) to a maximum (8th eophyll) is followed by a subsequent fall in the terminal portions (mature leaves) of the shoot. This change in leaf size is clearly illustrated in the fig. I.

Leaf shape is the most obvious trait that changes during heteroblastic development. In *Phyllanthus urinaria,* apart from size changes, there are progressive changes in leaf shape from node to node (fig. I). The cotyledons (regarded as paracotyledons due to foliar and photosynthetic nature) are oblong in shape with round apex and obtuse base. The cotyledons are followed by juvenile leaves termed as eophylls. These eophylls are developed up to 8th node and characterized by widely obovate leaves with faintly mucronate to rounded apex and acute, asymmetric base. First lateral branch emerges from the seventh node and bears narrow obovate leaves. The subsequent branches bear mature leaves characterized by oblong shape. These mature leaves have distinctly mucronate tip and obtuse, asymmetric oblique base.

In addition to changes in leaf shape, the ontogeny of a plant frequently involves changes in the phyllotaxy. In *P. urinaria*, the first two leaves are subopposite and at right angle to paracotyledons. Third Leaf is shifted laterally somewhat, thus initiating a spiral system which continues till 7-8th juvenile leaves

(eophylls). After this lateral branch develops with emergence of leaves in alternate distichous manner. This change in phyllotaxy of leaves on lateral branch may be associated with onset of flowering. The first two leaves, after the cotyledons, have a decussate arrangement was also shown by Van Iterson (1907) in many dicotyledons with whorled leaves. This change from decussate to spiral pattern provide support to Goebel's idea (1928) that the spiral arrangement is always derived from the decussate in dicotyledons.

The appearance of trichomes is also evident in *Phyllanthus urinaria*. The leaves on branches bear multicellular hairs intramarginally but the seedling leaves (paracotyledons and eophylls) are devoid of hairs. The study of impact of such type of heteroblastic development on the genotype may reveal interesting result.

This is the first report of heteroblastic development in *Phyllanthus urinaria* as well as for the genus *Phyllanthus*. This heteroblastic development of leaves serves as marker character and will help in identification of this taxon, from other closely related species of genus *Phyllanthus*, at juvenile stage i.e. much before flowering and fruiting stages which, in turn, can also be utilized in conservation of this medicinally important plants. Further, the knowledge of heteroblastic development in *Phyllanthus urinaria* can be of importance in morphological and physiological studies, and, it may provide a relatively simple interpretation of a wide range of experimental data for which varied and often somewhat strained explanations have been put forward.

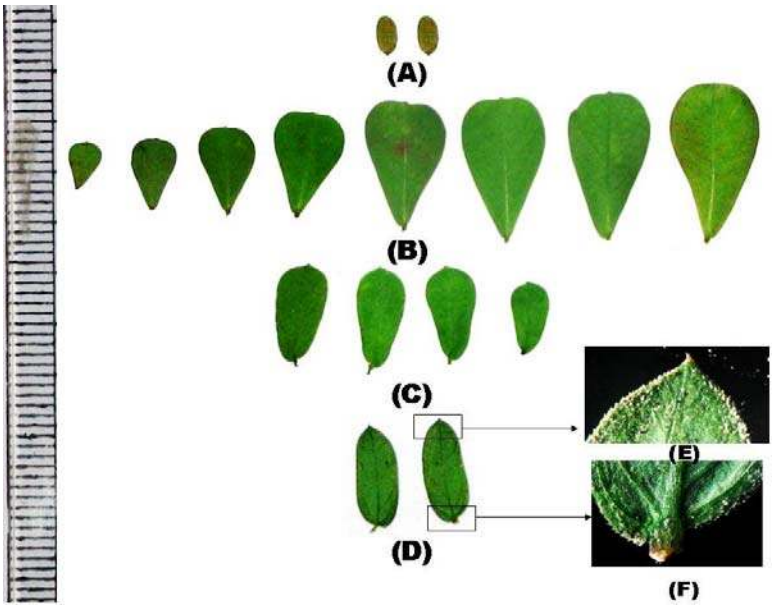

Fig. I: Heteroblasty in leaves of *Phyllanthus urinaria* Linn.
(A- Cotyledons, B – first eight juvenile leaves (eophylls), C – leaves on lateral branches, D – mature leaves, E – apex and margin of mature leaf, F – base of mature leaf)

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Kinetics of Biological Reduction of Chemical Oxygen Demand from Petroleum Refinery Wastewater

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ABSTRACT: Petroleum refinery wastewater contains various hazardous contaminants which need to be removed before being discharged into the environment. Chemical oxygen demand (COD) is the parameter that measures the level of pollutants in wastewater. Biological method is one of the effective, cheaper and environmentally friendly methods of removing these pollutants. In this work, *Bacillus subtilis* and *Micrococcus luteus* isolated from Kaduna petroleum refinery, Nigeria, were employed in the COD reduction. *Bacillus subtilis* reduce COD by 82.9% while *Micrococcus luteus* reduced it by 76.5%. 76.5%. The COD reduction process from the refinery wastewater is described by Michaelis-Menten kinetics whereby it was initially zero order at high substrate concentration and then changed to first order at moderate to low substrate concentration. The rate constants for the COD reduction by *Bacillus subtilis* were 29.37 mg/l.d and 0.0654 d^{-1} for the zero order and first order regions respectively, whereas the reduction by *Micrococcus luteus* had constants 30.4 mg/l.d and 0.0.0776 d^{-1} for the zero order and first order regions respectively. This work showed that the two bacteria used have the potential to be utilized in COD reduction processes. [Researcher. 2009; 1(2): 17-23]. (ISSN: 1553-9865).

Key words: Biological; Chemical oxygen demand; kinetics; Petroleum Refinery

INTRODUCTION

Water is employed in the refinery processes for cooling system, distillation, hydro-treating, desalting, equipment flushing and tank drains (Abdulkarim *et al.,* 2005). Therefore, Wastewater released from petroleum and petrochemical industries contain hazardous substances due to the number of sources it came into contact with (Lentech, 2008; Hazardous substance research centers, U.S.A, 2003). The wastewater is characterized by presence of toxic organics (phenols, aromatics and polycyclic aromatics), heavy metals (lead, chromium and cadmium) and in-organics (sulphides) which need to be removed before discharge.

Biological treatment is preferred over physicochemical as the former is cost effective, efficient and environmentally friendly (Ojo, 2006). The wastewater may be treated by physicochemical or biological methods. The most rational way of decontamination of the environment loaded with petroleum derivatives is an application of methods based mainly on metabolic activity of microorganisms (Bogusiawska *et al.,* 2005). Bacteria that have prior exposure and adaptation to petroleum hydrocarbons exhibits higher biodegradation rate (Leahy and Colwell, 1990). Biotreatment is assessed by increase in biomass growth and reduction in the substrate. *Bacillus subtilis* and *Micrococcus luteus* have been found to have high biomass growth and substrate reduction among group of bacteria isolated from petroleum contaminated wastewater (Hamza *et al.,* 2008)

Chemical oxygen demand (COD) which measures the oxygen equivalence of the constituents in the wastewater is used as a measure of substrate composition (Tchobanoglous *et al.,* 2003). The COD and BOD are gross overall indicators of sewage composition and they therefore do provide a measure which relates to the potential environmental damage of a wastewater. Though, COD has advantage over BOD of being measurable in about 2 hours by conventional methods or in a few minutes using sophisticated instruments (Bailey and Ollis, 1986), if large amount of chemicals enter wastewater chemical reaction occur which consume large amount of oxygen (i.e high COD). On the other hand, if the oxygen level of water drops too much fish and other aquatic life may not survive (Wisconsin department of natural resources, 2006).

A variety of microbial growth and biodegradation kinetics models have been used for removing both organic and inorganic materials from aqueous solution. Such model allows the prediction of amount of chemicals that remain at a given time and the calculation of the time required to reduce chemicals to a certain concentration (Okpokwasili and Nweke, 2005). Knowledge of bio-kinetics is also essential for biological wastewater system design and optimization of operational conditions (Nakhla *et al.,* 2005). Biokinetic coefficients are usually obtained either by observing substrate depletion with time in batch

experiments, and then fitting the data with an appropriate model or by respirometric studies (Morgenroth et al., 2002).

Sand bioreactors could achieve 90% COD removal from sanitary sewer overflow with peat having the lowest of 75% COD removal (Tao *et. al*., 2007). Up-flow anaerobic sludge blanket reactor (UASB) reactor was demonstrated to have, COD removal efficiency of 75-85% at a Hydraulic Retention Time (HRT) of 5 days with an influent COD concentration of about 40 gL-1 and Organic Loading Rate (OLR) = 7-8 g CODL-1.d-1 (Sobhi *et al*., 2005). Many industrial wastewaters, including food processing wastes, invariably contain colloidal and particulate organics that undergo hydrolysis prior to biodegradation. Morgenroth et al. (2002) have reviewed the kinetic modeling of hydrolysis in municipal wastewater treatment and noted that the most widely used kinetic model was first order with respect to particulate substrate. Biodegradation kinetics of high oil and grease rendering wastewater was found to obey Monods and Haldane kinetic model (Nakhla, 2005).

This work is aimed at obtaining the kinetics of COD removal using bacteria in a suspended growth biological system.

MATERIALS AND METHODS

Sample Collection

The wastewater for this study was collected using sterile plastic containers at the bio-filter (unit for the removal of organic substrates) inlet of Kaduna Petroleum Refinery, Nigeria. The wastewater was preserved at low temperature before the commencement of the experiment.

Isolation and Identification of the Bacteria

The source *Bacillus subtilis* and *Micrococcus luteus* used in this experiment from the biofilter inlet and effluents wastewater samples of Kaduna Petroleum Refinery, Kaduna, Nigeria. The two bacteria were isolated by spread plate technique; they were further identified by their morphological features, gram reactions, motility, fermentation reactions and enzyme production (Hamza *et al.*, 2008).

Refinery Wastewater Treatment and COD Determination

The COD was determined by reactor digestion method (Hach Company manual, 2007). The wastewater samples (4.5 liters) were poured into two suspended growth plastic bioreactor (6.8 liters). Pure cultures of *Bacillus subtilis* and *Micrococcus luteus* were inoculated into the two bioreactors. Air was supplied into the bioreactor through a sparger. The biotreatment studies was conducted at room temperature (30-35 ^{0}C). The substrate removal was measured by monitoring the COD reduction at the interval of two days.

RESULTS AND DISCUSSIONS

The biomass growth was indicated by increase in the optical density of the cultured sample (Figure 1). The substrate consumption was measured by reduction in the chemical oxygen demand (Figure 2).

The two isolates were able to grow in wastewater cultured samples, this indicate their ability to be utilized substrates therein as source of nutrients (Oboh *et al.,* 2006). The optical density of *Micrococcus* increases from 0.12 to a maximum of 0.85 while that of *Bacillus subtilis* increases from 0.13 to 0.69. None of the bacterial culture exhibit lag phase (Figure 1). This could be attributed to their prior exposure and adaptation to petroleum contaminated environment (Leahy and Colwell, 1990). *Micrococcus luteus* exhibits all the other three phases (Figure 1): exponential, stationary and death phase. *Bacillus subtilis* exhibits only two phases (Exponential and Stationary). This could be due to the ability of *Bacillus* to form endospores which enables it to withstand deleterious conditions such as radiation, heat, cold and lack of nutrients (Lindquist, 2007).

The substrate consumption was indicated by the reduction in chemical oxygen demand (COD). The COD was reduced from 595 to 102 mg/l by *Bacillus subtilis* and it was reduced from 595 to 140 mg/l by *Micrococcus luteus* (Figure 2). It is obvious that the rate of COD removal is divided into rapid removal and moderate removal stage (Guan bao *et al*., 2003). The results showed that the two bacteria are capable of reducing the COD from refinery wastewater.

The kinetics of the COD reduction was determined by fitting the COD data trough different kinetic models. The data fitted into zero and first order kinetic models as shown in Figures 3 to 6.

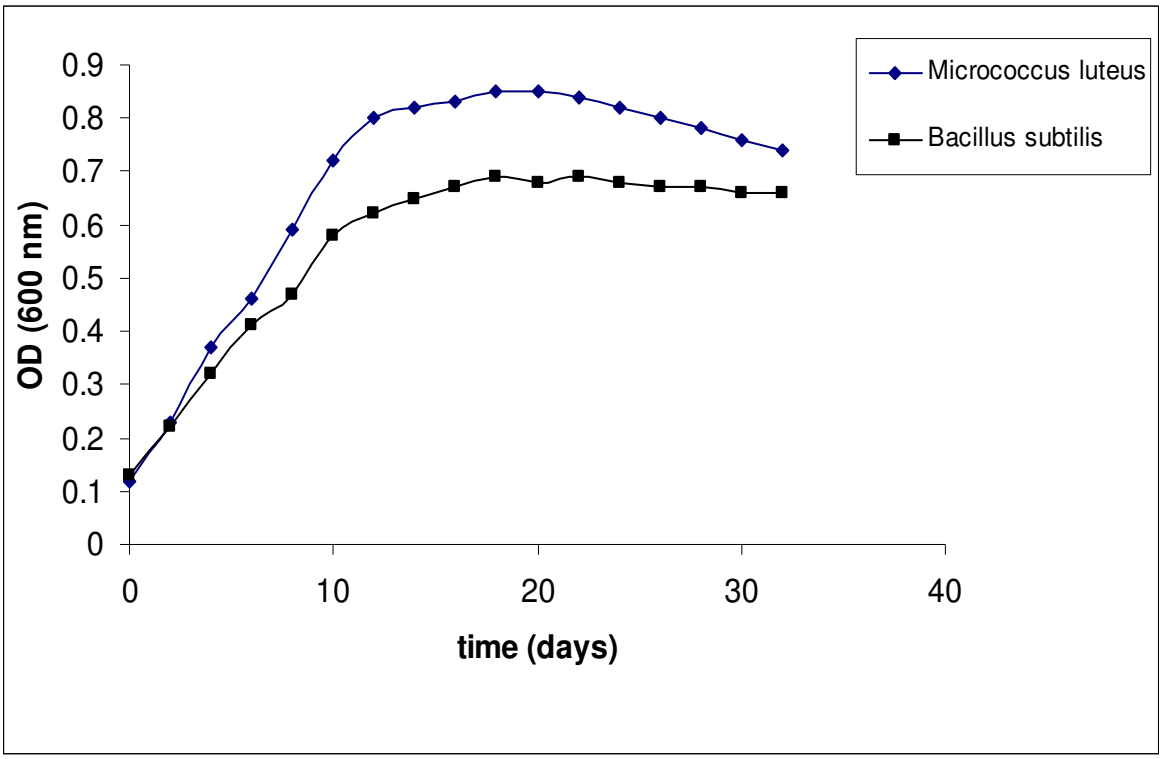

Figure 1: Growth Profile of *Bacillus subtilis* and *Micrococcus luteus* on Refinery Wastewater

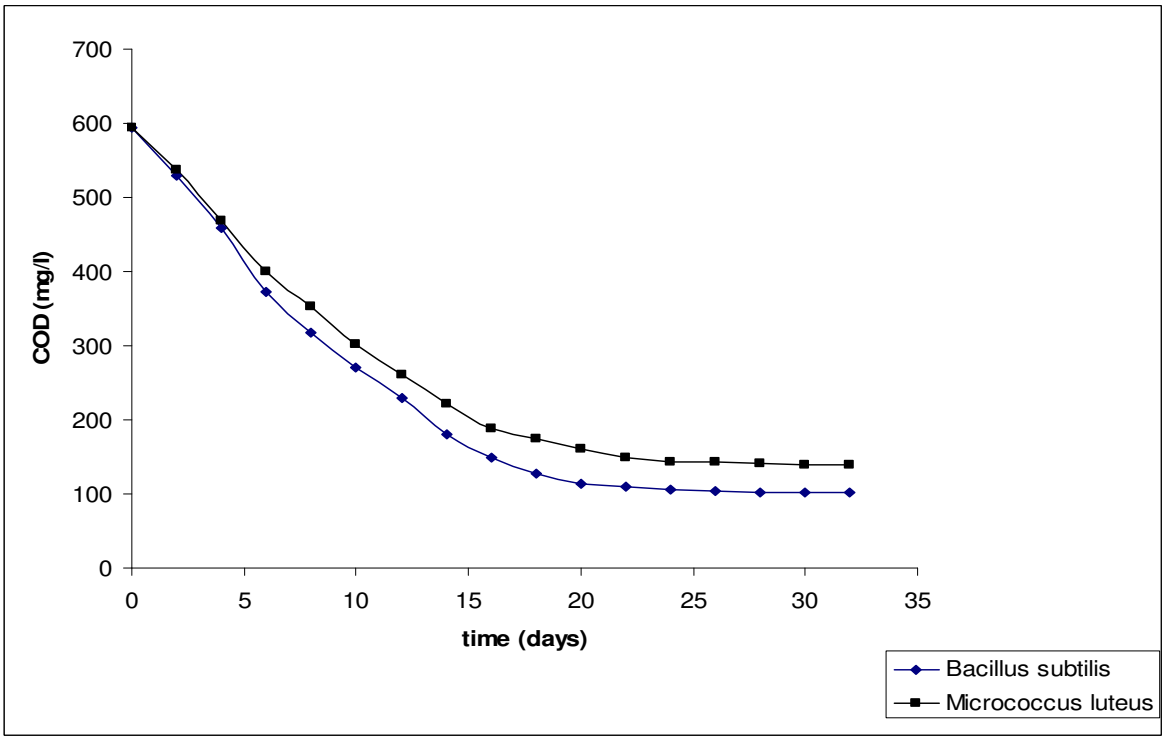

Figure 2: COD Reduction by *Bacillus subtilis* and *Micrococcus luteus*

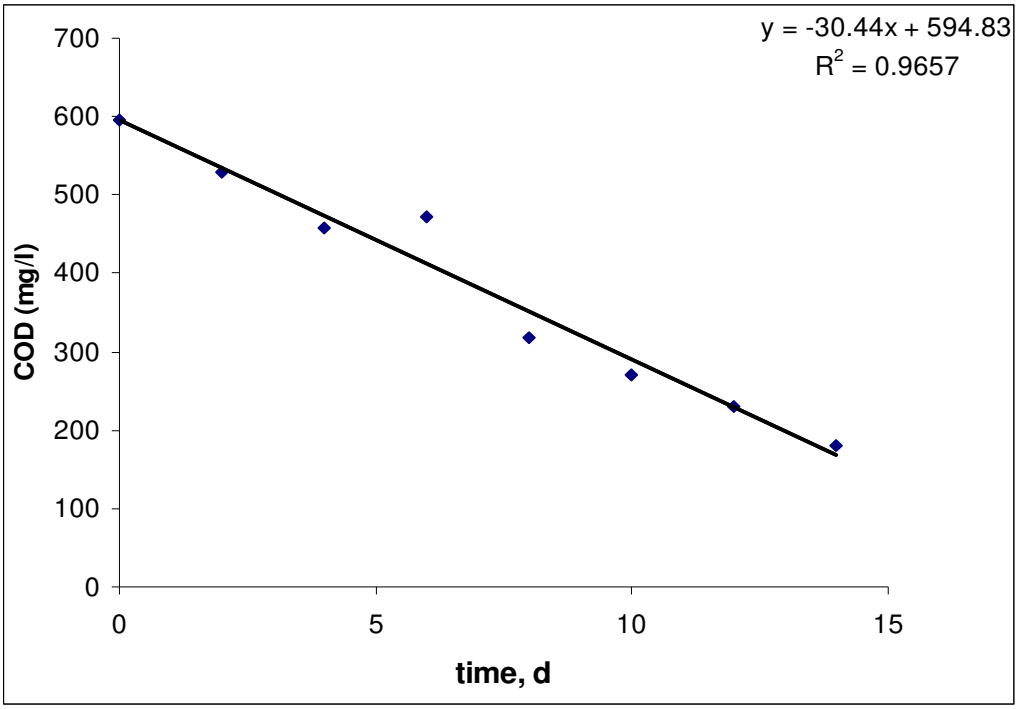

Figure 3: Testing Zero Order Kinetics for COD Removal by *Micrococcus luteus*

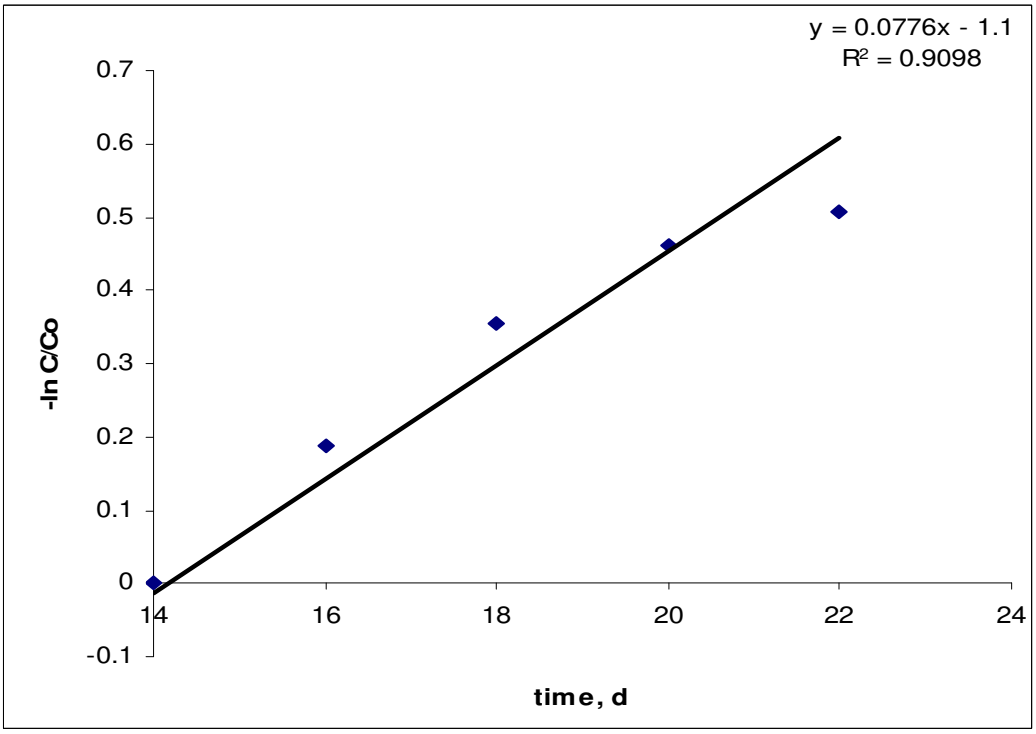

Figure 4: Testing First Order Kinetics for COD Removal by *Micrococcus luteus*

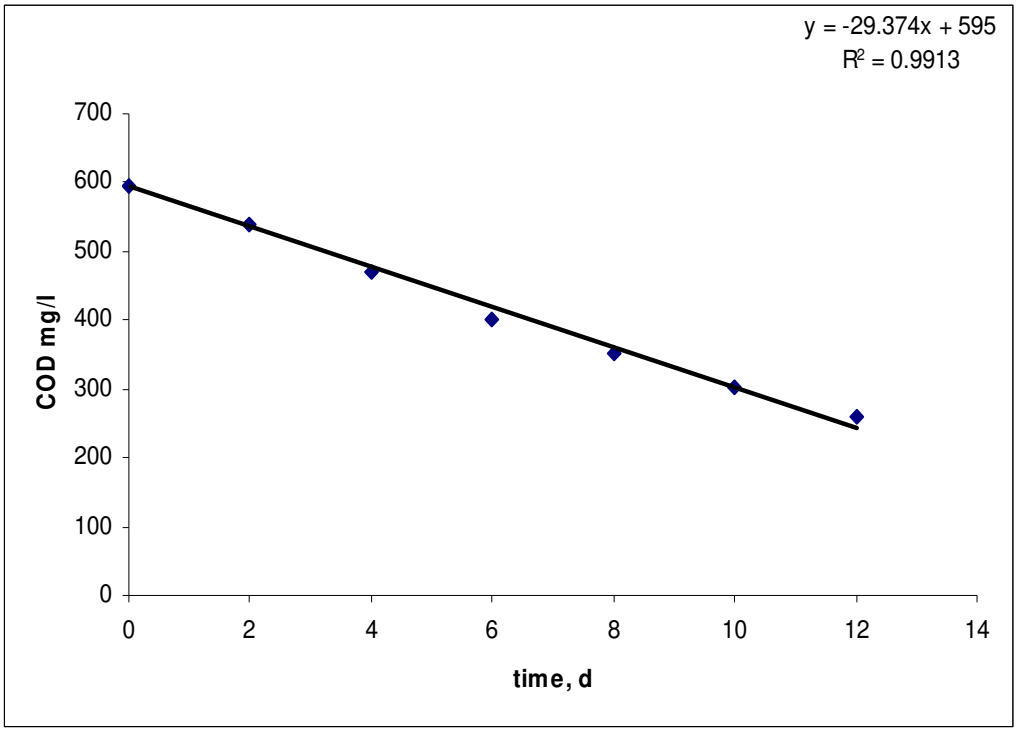

Figure 5: Testing Zero Order Kinetics for COD Removal by *Bacillus subtilis*

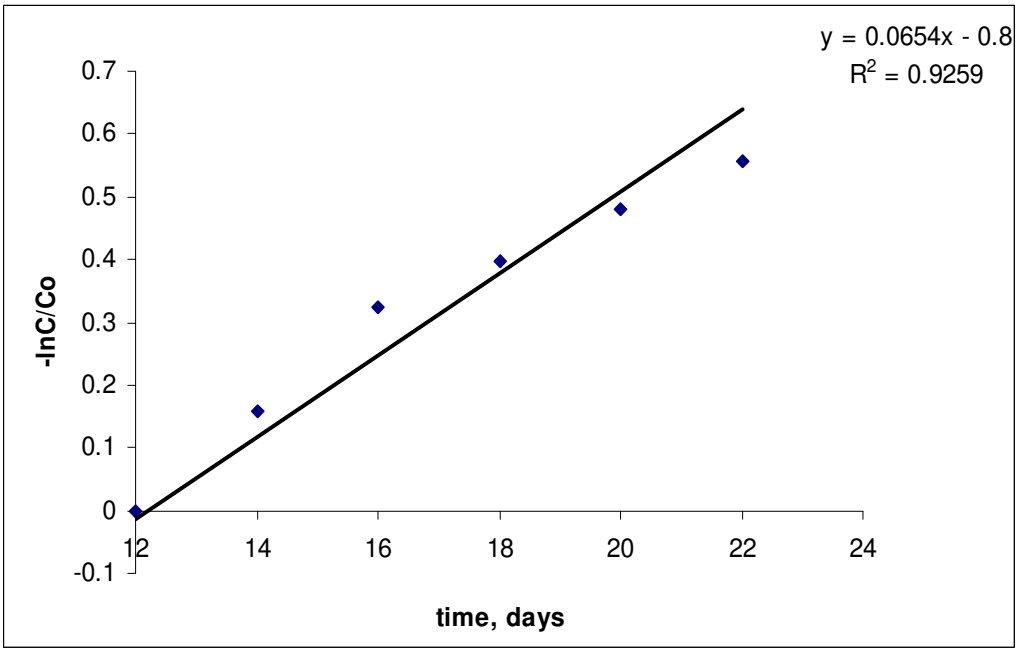

Figure 6: Testing First Order Kinetics for COD Removal by *Bacillus subtilis*

Micrococcus rate of COD removal is zero order from 595 to 181 mg/l (Figure 3), and then first order from 181 to 109 mg/l (Figure 4). *Bacillus subtilis* rate of COD removal is zero order initially at high substrate concentration (595 to 260mg/l) (Figure 5), and then first order subsequently at moderate to low COD concentration (260 to 149 mg/l) (Figure 6). It is obvious that the rate of COD removal is divided into rapid removal and moderate removal stage (Guan bao *et al*., 2003).

Substituting the value of the constants (rate constant, k and initial COD concentration, C_o) from the above plots into the integrated rate expression for zero order (equation 1) and for first order (equation 2), the kinetic models were obtained (Table 1)

$C = C_o - kt$ 1

$C = C_o e^{-kt}$ 2

Table 1: Summary of COD Removal Kinetic Models

Parameter	**Order**	
	Zero	**First**
COD removal (Micrococcus luteus)	594.8 – 30.4t	$181e^{-0.0776t}$
COD removal (Bacillus subtilis)	595.4-29.3745t	$260e^{-0.0654t}$

In all of the above cases, it can be observed that the rate of substrate reduction is zero order initially at high substrate concentration and then first order at low substrate concentration. This substrate reduction behaviour is exhibited by data that obeys Michaelis Meten kinetics (Okpowasili, 2005 and Levenspiel, 1999). The reason for this behaviour was because at high substrate concentration every site of the organism is saturated with the substrate that made the rate to be constant (i.e. zero order). As the substrate concentration decreased only few available site of the organism was covered and that made the rate of reaction to be proportional to the substrate concentration i.e. first order (waterloo, 2006).

CONCLUSIONS

From the results obtained, it showed that *Bacillus subtilis* and *Micrococcus luteus* have high ability to reduce chemical oxygen demand from refinery wastewater. The amount COD removal by *Bacillus subtilis* and *Micrococcus luteus* were respectively 82.9% and 76.5. The kinetics of the COD reduction obeys Michaelis Menten kinetics, meaning it is zero order at high substrate concentration and first order at low substrate concentration.

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1/10/2009

Effects of Soil Temperature Pattern on the Performance of Cucumber Intercrop with Maize in a Tropical Wet-and-Dry Climate of Nigeria

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ABSTRACT

Maize and cucumber were intercropped in simple randomized complete block design (RCBD) with three replicates in two field trials in early and late planting seasons of 2004. The result showed that mean soil temperature of 33°C and 31°C at 5 and 10cm during late season could be said to have enhanced the productivity of cucumber yield by about 50% compared to early season with mean soil temperature of 30°C and 29°C at 5cm and 10cm below soil surface. The mean cucumber yield of 9t/ha and 6.1t/ha for mono and mixed crop respectively during early season trial was significantly lower ($P<0.05$) than the mean cucumber yield of 15.34t/ha and 12.34t/ha for late season. [Researcher. 2009;1(2):24-36]. (ISSN: 1553-9865).

Keywords: Soil Temperature Pattern; Cucumber Intercrop; Maize; Nigeria

INTRODUCTION

In most parts of the tropical wet and dry climate, thermal meteorological parameters such soil and air temperatures are part of critical considerations in crop production and they are equally important component of the plant environment in a tropical wet-and-dry climate. For instance timing of phenological events had been confirmed to be clearly correlated with different climatic factors such as air temperature and soil temperature. (Wielgolaski, 2001). Also, many studies agreed that accumulated temperature was recognized as the main factor influencing year-to-year variation in major plants physiological phases (Galan et al., 2001; Schaber and Badeck, 2003).). Consequently, the present study sought to investigate the effects of soil temperature pattern on the performance of two grown crops (Maize and Cucumber) in the study area using the inter-cropping system which occupies a great percentage of cultivated land in West Africa (Wahua, 1986).

Maize and cucumber are two important food crops in Nigeria as well as in other parts of the world. Over the years maize has been useful as a food, construction material, and medicinal or decorative plant. With the industrial development, it increasingly became an industrial raw material for the production of starch, gluten, oil, flour, alcohol and lignocelluloses for further processing into a whole range of products and byproducts. On the other hand, cucumber is a major fruit vegetable that is eaten raw (in salad) or cooked. It can also be put in vinegar; the crop serves as a major source of vitamins for people in developing countries. (Ayotamuno et al, 2000)

MATERIALS AND METHODS

Study area

Location of the experiment

The experiment was conducted at the University of Agriculture Abeokuta along Alabata road in Odeda local government area of Ogun state, south western Nigeria. The field experiment was conducted during the early and late growing season of 2004 at the Agro Meteorological Teaching and Research Farm land located adjacent to the meteorological station within the vicinity of the College of Environmental Resources Management University of Agriculture (7° 15¹ N, 3° 25¹E). The location map of the study area is shown in Figure 1.

Planting and crop maintenance

Maize cultivars, Suwan1(M1) and TZ Comp4 (M2) and cucumber were intercropped in two field trails in early and late planting season of 2004. Early planting was done in May 2004 while late planting was done in August 2004 planting season, usually after the establishment of rains. According to Stern *et al.* (1981) the time of establishment of the rains is marked by the period when two days were not followed by a

continuously dry period of say 5, 7 or 10days. Between three and four seeds of maize and cucumber were planted at a depth of 2.5cm in each stand and the stands on the row for maize were spaced at 2.5cm between stands while between stands for cucumber were spaced at 50cm.
Three weeks after planting the maize and cucumber seedling were thinned to two and one per stand respectively following the recommended thinning procedure (Kowal and Andrews, 1973) and this resulted in a plant population of 26,666plantha^{-1} and 13,333plantha^{-1} for maize and cucumber, respectively. The plots were hand hoed and weeded manually at 3 and 6 weeks after planting. All plots received a basal dressing of 70kg N/ha urea.

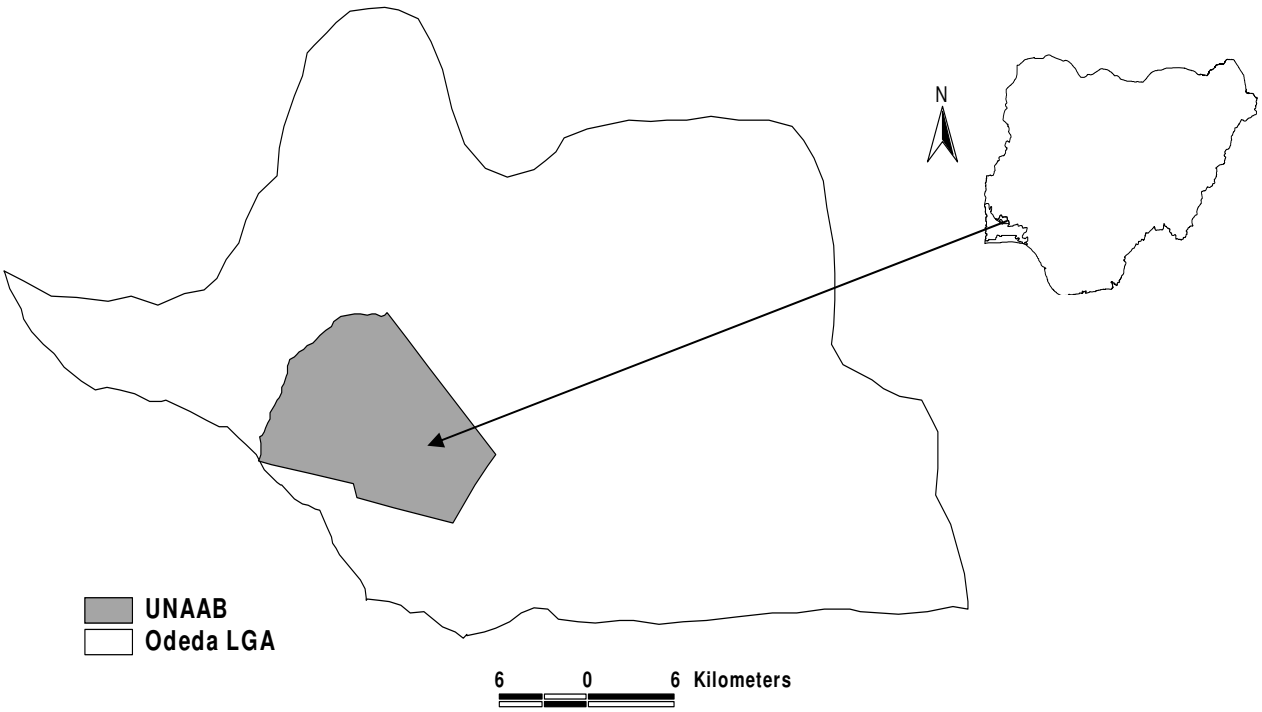

Fig.1: Location of University of Agriculture, Abeokuta within Odeda Local Govt. Area in Ogun State, Southwest Nigeria.

Lay out of experimental plot

Experimental plots were arranged in simple randomized complete block design (RCBD) with three replicates for early and late growing seasons in 2004. Cropping systems, early and late planting seasons as the main factors. Each plot was 5 x 2.25m with 1m walking path between the adjacent plots.

Data collection and analysis
During the phenological stages, data were collected on soil temperature at 5 and 10cm from the soil surface along with other agrometeorological data from the meteorological station adjacent to the experimental plots except soil temperature that were measured on the experimental plots. Data were also collected on some important phenological crop growth parameters and yield characters.

Growth parameters
Plant height: This was taken from a sample of four plants (maize) tagged within the two central rows of each plot. The mean from the four plants was then determined.

Leaf area: The leaf area shall be determined by the non destructive length x width method described by Saxena and Singh, (1985) using the relation: Leaf area = 0.75 (length x width), where 0.75 is a constant. Five leaves were measured for each treatment plot and the mean leaf area determined. The leaf area for cucumber was measured by the girth system whereby graph sheets were used to trace the area (surface) of five leaves in each treatment plot and the mean determined.

Days to 50% flowering: The effect of treatment on the flowering period of maize and cucumber were measured by recording the days from planting to when 50% of both plants flowered.

Yield parameters

Fruit length: The lengths of five fruits weighed were measured and the mean determined.

Fruit weight: Five cucumber fruits from each plot were weighed separately and the mean determined. Also, weekly harvest totals were recorded for analysis.

Fresh cob weight: Five fresh maize cobs from each plot were weighed separately and the mean determined. This was done to give a good estimate of the total yield of fresh maize.

Weight of seeds per cob: Five maize cobs from each plot were allowed to dry in the field to 14% moisture content and then harvested. The seeds were removed from the cobs and weighed. The mean was then determined.

Data analysis

Data collected were subjected to analysis of variance (ANOVA) to evaluate the effects of seasonal variations and their interactions on the response variables. The significant difference of treatment means were determined using least significance difference (LSD) at 5% level of probability (Steel and Torrie, 1988).

RESULTS

Hydrothermal characteristics during the early cropping season 2004

Decadal rainfall and air temperature for the early season were related to the main phases of vegetative growth and reproductive development of maize-cucumber in Fig 2. The peak rainfall (150mm) was observed at about 30 days after planting and this period coincide with the plant establishment and vegetative stages during the early life of maize and flowering stage of cucumber plants revealing that moisture was not limiting at these critical stages of plant life. Another lesser peak of 69.8mm was also observed at about 80 days after planting which also showed that at maturity stage of the maize plants life moisture stress was also not pronounced while cucumber had completed its life cycle and no more on the field. The critical rainfall for maximum yield at these periods could be said to range between 150-69.8mm. Temperature also differed slightly during maize-cucumber growth stages in the early cropping season of 2004. Minimum and maximum temperature varied between 22 – 24^{o}C and 29 - 32^{o}C respectively. Temperature was higher during planting, establishment and early vegetative growth (32^{o}C –29^{o}C) than during reproductive growth in the early cropping season. (24^{o}C-22^{o}C). The cumulative amount of rainfall for the period between sowing and 50% flowering was 324.6mm, accordingly the rainfall for the period between 50% flowering and maturity (reproductive phase) was 261.4mm in the early season crops. Minimum and maximum temperature of 23^{o}C and 32^{o}C were recorded in the early season at planting.

Hydrothermal characteristics during the late cropping season 2004

As shown in Figure 3 late season rainfall trends revealed lowest rainfall amount was observed for the period between 3rd and 9th decades corresponding to 30days after planting to 90days after planting (16mm to 64.6mm) with peak rainfall at 50days after planting (5th decade) (114.9mm). This implies that moisture stress at the later part of plants life was less pronounced than that at the initial stage of plants life during the late growing season. Temperature differed slightly in the late cropping season. Minimum and maximum temperature varied between 21^{o}C-23^{o}C and 28 ^{o}C -32 ^{o}C respectively. Temperature was low during

planting, establishment and early growth than during flowering, i.e 21°C -22 °C and 29°C – 32 °C respectively. The amount of rainfall for period between sowing and 50% flowering was 176.30mm. Accordingly, the rainfall for the period between 50% flowering and maturity (reproductive phases) was 214.10mm. Rainfall was therefore much larger during both vegetative and reproductive phase in the early season than late-season. The late-season can be defined as dry since the rainfall curve remains below the maximum temperature curve for most periods during both the vegetative and flowering phases as shown in figure 2. The minimum and maximum temperatures at planting in the late-season were 22°C and 28°C respectively.

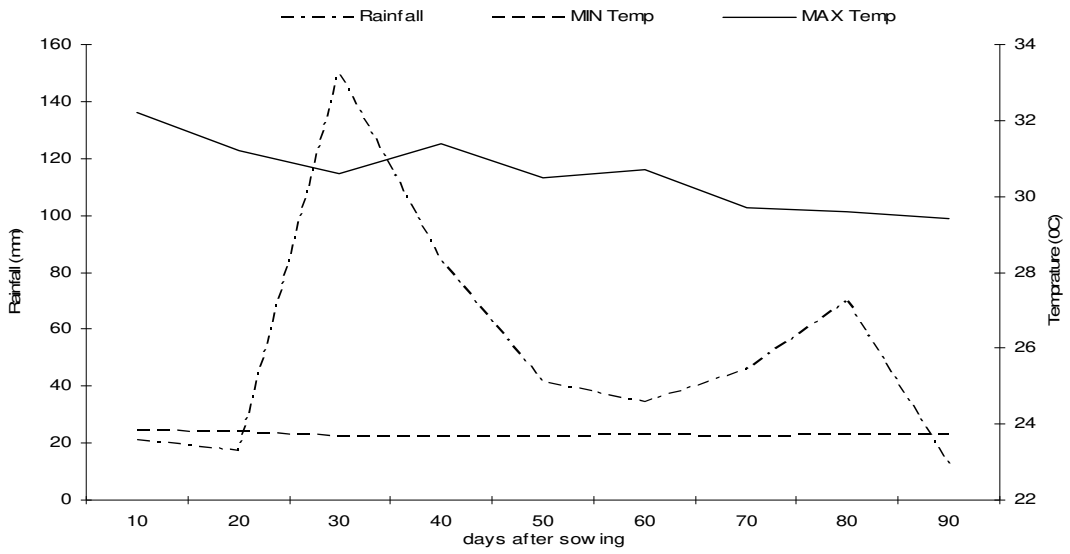

Figure 2: Decadal rainfall, minimum and maximum air temperatures during the growth of cucumber/maize at Alabata, UNAAB in the early season (May – July) of 2004

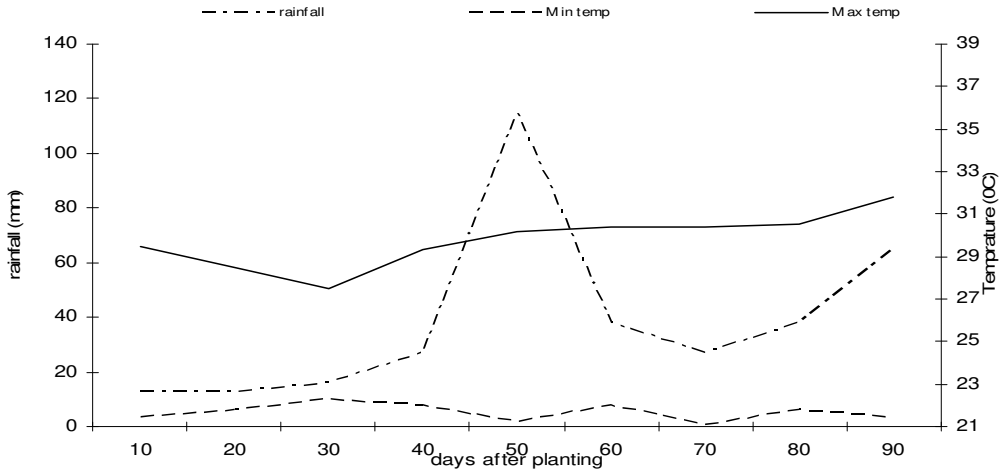

Figure 3: Decadal rainfall, minimum and maximum air temperatures during the growth of cucumber/maize at Alabata, UNAAB in the late season (August – October) of 2004.

Figure 4 shows the amount of rainfall at each phenological stage. Rainfall at pre-sowing period, establishment, flowering, 50% maturity and first harvest for early season were 27mm, 11mm, 324.6mm, 84.5mm respectively and 76.8mm against 12.6mm, 16mm, 206.5mm, 64.6mm and 37.8mm respectively for

late season . This showed that early season moisture maize-cucumber plants experience no moisture deficiency throughout the entire period.

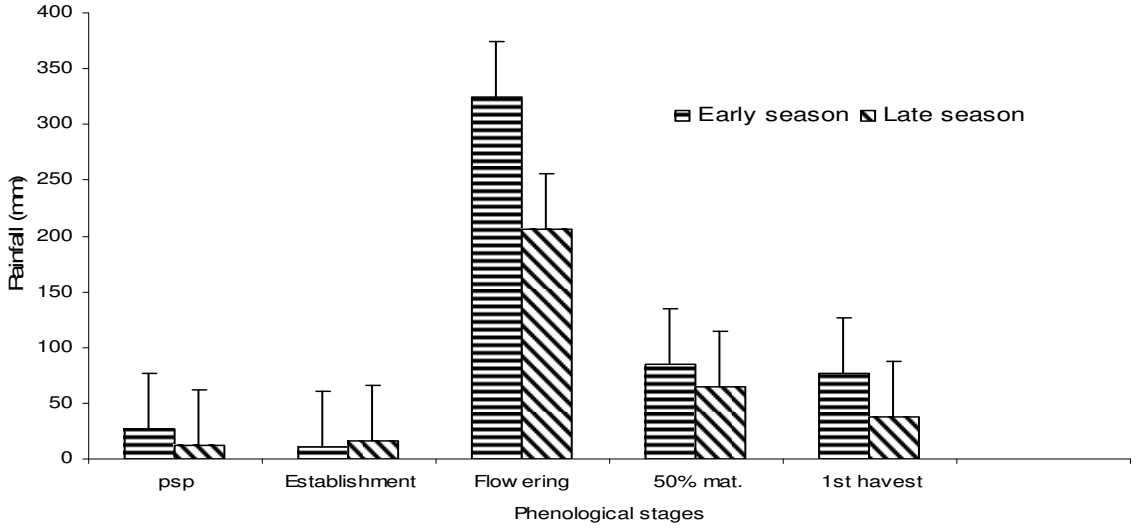

Figure 4: Rainfall at major phenological stages during the growth of cucumber/maize at Alabata, UNAAB in 2004 cropping season

Figures 5 and 6 shows soil temperature at 10cm below soil surface during the growth of cucmber/maize at Alabata, UNAAB in 2004 cropping season. Soil temperature did not vary significantly under both mono and mixed cropping. Highest temperature (32°C) was recorded at vegetative and flowering stages followed by that at maturity stage of about 26°C. Soil temperature was however higher at the early stage than at later stage of the plant life. This means that soil heat flux was higher at vegetative and flowering stages than at maturity stage, this is an indication that water absorption rate was higher at these periods.

Figures 7 and 8 shows soil temperature at 5cm during the growth of cucumber /maize at Alabata, UNAAB in 2004 cropping season. Soil temperature on both mono and mixed cropping was similar at all sampling occasions. During the early season slight difference started at vegetative stage and flowering stage. Late season soil temperature at 5cm was almost the same on both mono and mixed cropping especially at sowing, establishment and vegetative stages, slight difference was observed around flowering stage and this was maintained till harvest. The result showed that mean soil temperature of 33°C and 31°C at 5 and 10 cm during late season may be said to have enhanced the productivity of cucumber crop.

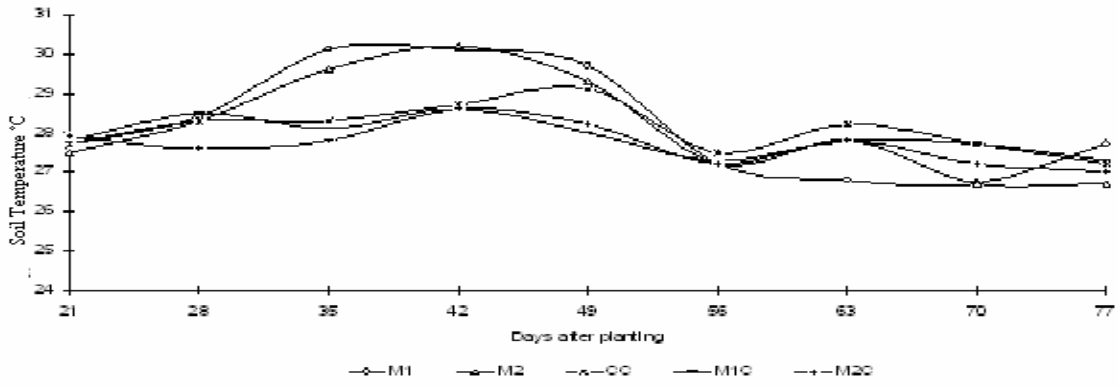

Figure 5: Soil temperature at 10cm depth during early season of 2004

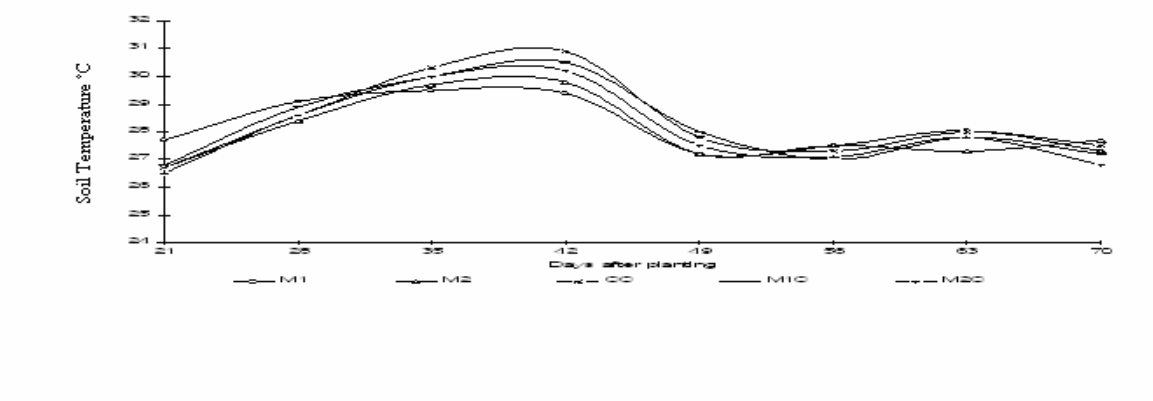

Figure 6 : Soil temperature at 10cm depth during late season of 2004

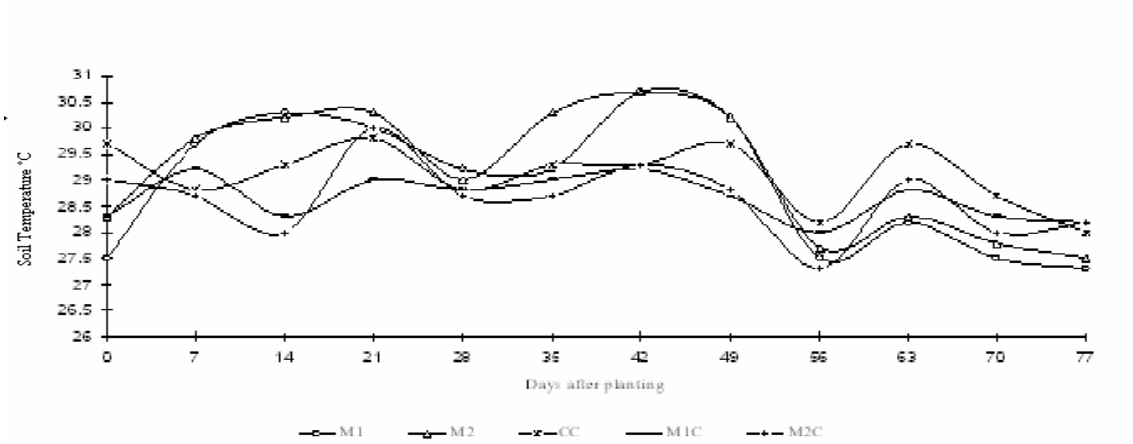

Figure7 : Soil temperature at 5cm depth during early season of 2004

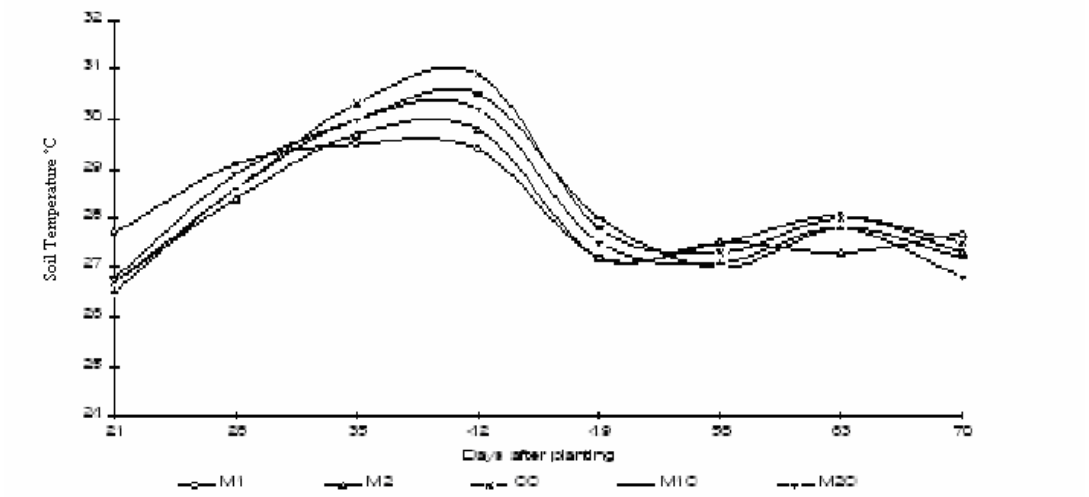

Figure 8 : Soil temperature at 5cm depth during late season of 2004

Growth Parameters

Leaf area of Maize (Early season)

Leaf area of maize in monoculture and mixed stands at 3, 4, 5, 6, 7, and 8 weeks after planting for both early and late season is presented in Table 1. The table shows difference between the leaf area of the maize cultivar Suwan-1 in monoculture and mixed stand at 3 weeks after planting while the difference was not statistically significant in the leaf area of TZCOMP4. The difference was not significant at 4, 5, 7 and 8 weeks after planting. Cultivar TZECOMP4 had a much bigger leaf area than its counterpart Suwan-1 in both monoculture and mixed cropping at all the sampling periods. In monoculture from 3 weeks after planting to 8 weeks after planting the leaf area of TZECOMP4 ranged between 230.94cm^2 to 535.00cm^2 while it was between 224.77cm^2 to 518.04c.m^2 for the leaf area of cultivar Suwan-1. In mixed stand the leaf area of TZECOMP4 ranged between 217.82cm^2 to 522.82 cm^2 while for Suwan-1 it ranged between 180.15cm^2 to 476.67cm^2. Also in monoculture, TZCOMP4 reached its largest value (535.12cm^2) at 7 weeks after planting while Suwan-1 attained peak value of (518.04cm^2) at 8 weeks after planting. However, in mixed stand Suwan-1 attained peak (477.00cm^2) at 8 weeks after planting while TZECOMP4 attained peak (524.82cm^2) at 8weeks after planting.

Late season

Table 1 also shows that the difference in leaf area of maize in monoculture and mixed stand was not significant in all the sampling occasions. Cultivar TZECOMP4 (M2) had a much bigger leaf area than its counterpart Suwan-1(M1) in both monoculture and maize/cucumber mixtures at all the sampling periods. In monoculture from 3weeks after planting to 8weeks after planting the leaf area of TZECOMP4 (M2) ranged between 150.59cm^2 to 636.91cm^2 while it was between 129.64cm^2 to 588.61cm^2 for the leaf area of cultivar Suwan-1 (M1). In TZECOMP4-cucumber mixtures (M2C) it ranged between 182.56cm^2 to 686.42cm^2 while for Suwan-1-cucumber mixture (M1C) ranged between 128.04cm^2 to 588.61cm^2. Also in monoculture, TZCOMP4 (M2) reached its largest value (636.91cm^2) at 6weeks after planting while suwan-1(M1) attained peak value (588.61cm^2) at 7 weeks after planting. However, in mixed stand suwan-1(M1C) attained peak (588.61cm^2) at 7 weeks after planting so also is TZECOMP4 (M2) attained peak (686.42cm^2) at 7 weeks after planting.

Table 1: Effect of seasons and cropping system on the leaf area (cm ^{2}) of maize at Alabata (UNAAB), 2004 cropping season

EARLY SEASON						**LATE SEASON**						
Cropping system	**3***	**4***	**5***	**6***	**7***	**8***	**3***	**4***	**5***	**6***	**7***	**8***
M1	225	241	300	486	444	518	130	309	519	572	589	574
M2	231	258	374	491	535	530	151	332	580	637	614	613
M1C	180	208	321	398	448	477	128	338	550	567	589	546
M2C	218	234	314	445	504	523	183	364	645	683	686	670
LSD (0.05)	35**	55	77	94**	96	61	57	60	129	120	99	127

* Week After Planting M1. Sole Maize (Suwan 1) M2- Sole Maize (TZComp 4)
M1C- Suwan 1/ Cucumber M2C- Tzcomp4/ Cucumber **- significant

Plant height (Early season)

Presented in Table 2 is plant height of maize in monoculture and maize/cucumber mixtures at 3, 4, 5, and 7 weeks after planting. The difference was not significant at 3, 4, and 5 weeks after planting. In monoculture from 3weeks after planting to 7weeks after planting the plant height of TZECOMP4 (M2) ranged between 58.00cm to 201.58cm while it was between 55.72cm to 186.67cm for the plant height of cultivar Suwan-1(M1). In mixed stand from 3weeks after planting to 7weeks after planting the plant height of cultivar TZECOMP4 (M2C) ranged between 57.78cm to 170.83cm while for Suwan-1(M1C) it ranged between 57.77cm to 192.17cm. Also in monoculture, TZCOMP4 (M2) and Suwan-1(M1) reached peak value of 201.58cm and 186.67cm respectively at 7weeks after planting. In mixed stand Suwan-1(M1C) attained peak (192.17cm) while TZECOMP4 (M2C) attained peak (170.83cm) also at 7weeks after planting.

Late season

The result on Table 2 also shows no significant difference in plant height of both Suwan-1(M1) and TZCOMP4 (M2) in monoculture and maize/cucumber mixtures at all observation points. Cultivar Suwan-1(M1) was generally taller than its counterpart TZCOMP4 (M2) in monoculture except for the 4th week whereas TZCOMP4 was higher in mixed cropping than Suwan-1 at all the sampling occasions. In monoculture from 3weeks after planting to 7weeks after planting the plant height of TZECOMP4 (M2) ranged between 28.37cm to 207.48cm while it was between 32.80cm to 216.03cm for the plant height of cultivar Suwan-1(M1). In maize/cucumber mixtures the plant height of cultivar TZECOMP4 (M2C) ranged between 32.83cm to 211.30cm while for Suwan-1(M1C) it ranged between 28.03cm to 200.77cm. Also in monoculture, TZCOMP4 (M2) and Suwan-1(M1) reached peak value of 207.48cm and 216.03cm respectively at 7weeks after planting. In mixed stand suwan-1(M1) attained peak (200.77cm) while TZECOMP4 (M2) attained peak (211.30cm) also at 7weeks after planting.

Yield parameters

Weight of maize seeds / cob

Early season weight of seeds / cob of the two maize cultivars (Table 3) were not significantly different in sole maize and maize-cucumber intercropped. Grain yield/ ear of Suwan-1 mixed with cucumber (M1C) had the highest amount (107g) while sole Suwan-1 (M1) had lowest (84g). Between these extremes are sole TZCOMP4 (M2) and TZCOMP4 mixed with cucumber (M2C) of 93g and 91g respectively. Late season grain yield/ ear of TZCOMP4 mixed with cucumber (M2C) had the highest amount (72g) while sole Suwan-1 (M1) had lowest (58g) followed by sole TZCOMP4 (M2) (66g) and Suwan-1 mixed with cucumber (M1C) of 61g. Differences of means were not significant at $P<0.05$.

Fresh maize cob weight

Early season cob weight is another maize yield character presented in Table 3. Suwan-1 mixed with cucumber (M1C) had highest cob weight (263g) followed by sole Suwan-1 (M1) (253g) and sole TZCOMP4 (M2) (240g) while TZCOOMP4 mixed with cucumber (M2C) had lowest of 203g. Late season yield of TZCOMP4 mixed with cucumber (M2C) had highest cob weight (266g) followed by Suwan-1-cucumber mixtures (M1C) (263g) and sole TZCOMP4 (M2) (250g) while sole Suwan-1 (M1) had lowest of 224g.

Cucumber fresh fruit characters

Early season fruits yield revealed that the variation in the fruit weight of cucumber was significantly different at $P<0.05$ with fruits from sole cucumber (CC) having much bigger fruit weight (296g) followed by cucumber-TZCOMP4 mixtures (M2C) (200g) while cucumber-Suwan-1 mixtures (M1C) has lowest fruit weight of 167g (Table 4). Fruit Length is another fruit character showed in Table 4. Fresh fruit from sole cucumber (CC) plot was highest (21cm) followed by fruit from Cucumber-Suwan-1 mixtures (M1C) (15cm) while its counterpart; cucumber-TZCOMP4 mixtures (M2C) had lowest fruit length of 13cm. The differences in fruit length between cucumber sole and cucumber-maize mixtures was significant at $P<0.05$.

Late season

Late season fruit yield in Table 4 showed that the variation in the fruit weight of cucumber was not significantly different (P<0.05) with fruits from sole cucumber (CC) having bigger fruits (321g) followed by cucumber-Suwan-1 mixtures (M1C) (292g) while cucumber-TZCOMP4 mixtures (M2C) has lowest fruit weight of 271g.
Fruit length is another fruit character shown in Table 4. Fresh fruit from sole cucumber (CC) plot also maintained highest (25cm) followed by fruit from cucumber-TZCOMP4 mixtures (M2C) (18cm) while its counterpart; cucumber-Suwan-1 mixtures (M1C) had lowest length fruit of 18cm. The differences in length fruit between cucumber sole and cucumber-maize mixtures was significant at P<0.05.

Figure 9 shows yield in t/ha of maize across the seasons. Early season maize yield of suwan-1 mixed with cucumber (M1C) has the highest grain yield (5.69t/ha) followed by sole TZCOMP4 (M2) (4.98t/ha) then TZCOMP4 mixed with cucumber (M2C) had 4.48t/ha while sole suwan-1 (M1) had lowest of 4.5t/ha. However, late season maize yield of TZCOMP4 mixed with cucumber (M2C) has the highest grain yield (3.81t/ha) followed by sole TZCOMP4 (M2) (3.50t/ha) and suwan-1-cucumber mixtures (M1C) (3.25t/ha) while sole suwan-1 (M1) had lowest yield of 3.10t/ha

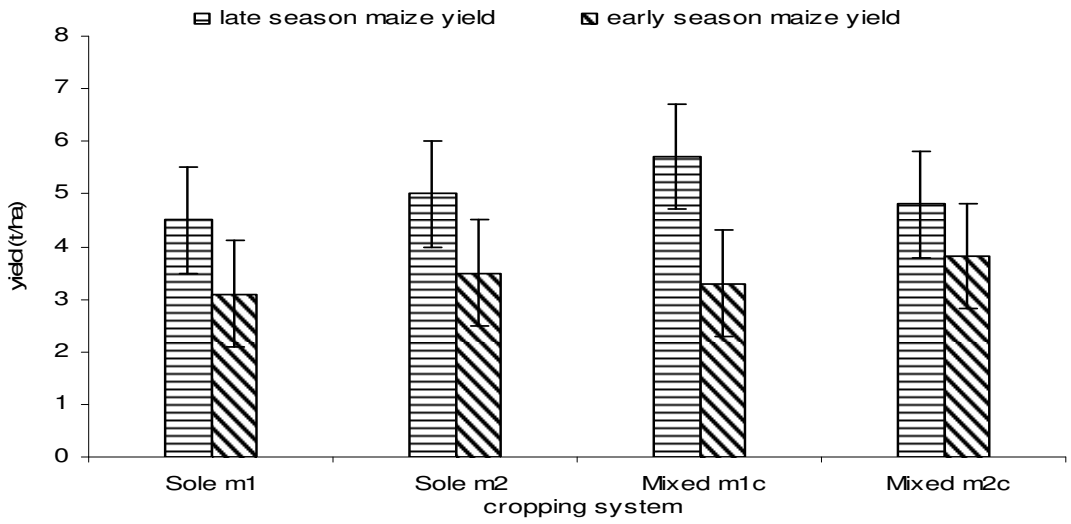

Figure 9: Maize yield under mono and maize-cucumber mixtures

Figure 10 shows fresh cucumber yield in t/ha across the seasons. Early season cucumber yield of sole cucumber (CC) has the highest yield (13.53t/ha) followed by cucumber mixed with TZCOMP4 (M2C) (6.08t/ha) while cucumber mixed with Suwan-1 (M1C) had lowest yield of 3.09t/ha. Similar trend was observed during late season cucumber yield with sole cucumber (CC) having the highest yield (23.01t/ha) followed by cucumber mixed with TZCOMP4 (M2C) (10.33t/ha) while cucumber mixed with Suwan-1 (M1C) had lowest of 8.92t/ha. Generally cucumber yield in the late season was higher than the early season yield across both mono and mixed culture.

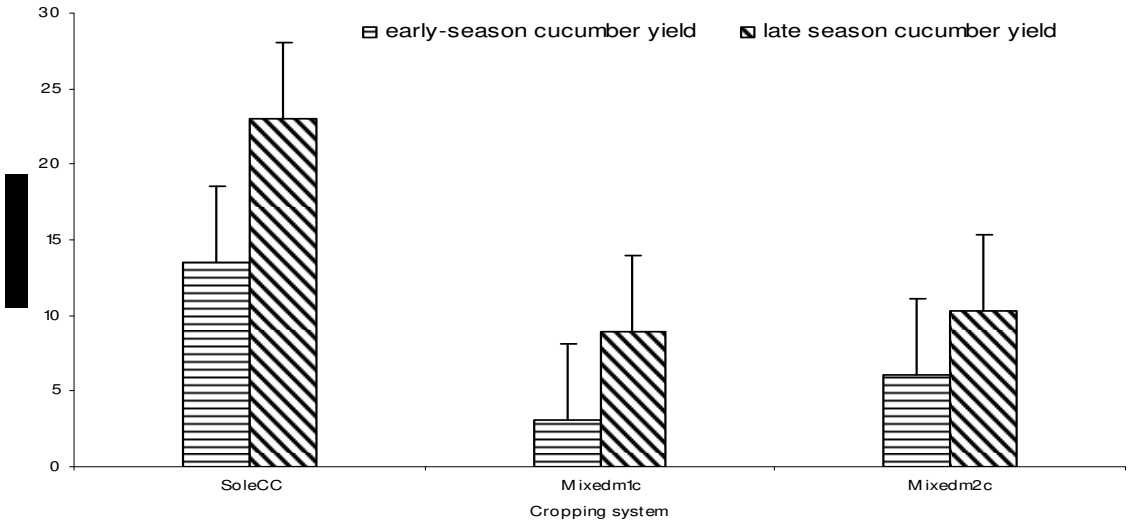

Figure 10: Cucumber yield under mono and maize-cucumber mixtures

Table 2: Effect of season and cropping system on the plant height of maize at Alabata, (UNAAB) 2004 cropping season

	EARLY SEASON					LATE SEASON				
Cropping system	3*	4*	5*	6*	7*	3*	4*	5*	6*	7*
M1	56	72	101	141	187	33	72	109	152	216
M2	58	81	103	149	202	28	78	105	146	207
M1C	58	73	105	167	192	28	72	103	143	201
M2C	58	66	90	122	171	33	81	119	160	211
LSD (0.05)	5	18	21	56**	29**	7	13	19	23	17

Table 3: Maize yield characters in mixed and monoculture

	EARLY SEASON		LATE SEASON	
Cropping system	Weight of seeds / cob (g)	Fresh Cob Weight (g)	Weight of seeds / cob (g)	Fresh Cob Weight(g)
M1	84	253	58	224
M2	93	240	66	350
M1C	107	263	61	263
M2C	91	203	72	266
LSD (0.05)	27	68	17	131

Table 4: Cucumber yield characters in mixed and monoculture

	EARLY SEASON		LATE SEASON	
Cropping system	Fruit Weight (g)	Fruit Length cm)	Fruit Weight (g)	Length Fruit(cm)
CC	296	21	321	25
M1C	167	15	292	18
M2C	200	13	271	18
LSD (0.05)	98**	5**	55	5**

DISCUSSION

The present study agreed with Ayotamuno et al. (2000) that though many factors serve to limit crop growth including soil types, nutrient contents, and climate, water has been observed to be the principal yield limiting factor. The early season rainfall amount was higher across most of the phenological stages than their corresponding amount recorded during the late season period. This pattern was favourable to maize plant but not too favourable for cucumber as it does not require high humidity for its optimal performance.

Minimum temperature for both the early and late planting season falls within the optimal temperature range required by cucurbits as this is in agreement with the work of Larkcom (1991) and Desai and Musmade (1998) at a minimum temperature of 18^{o}C during early growth is preferred with 24-27^{o}C being optimum. This range has been confirmed with prolific growth occurring at day / night temperatures of 28-35 / 20-25^{o}C and severe reduction in growth at night temperatures of 16^{o}C. Cucumber therefore requires heat to produce maximum yield replicate of which was prevalent during late cropping season. In both seasons, the effects of high daily mean temperatures were, greater on cucumber plants grown in monoculture than mixed stands, particularly the intercropped cucumber produced about the same numbers of fresh fruits during later stages of growth with the monoculture. It thus appears that the cool environment observed in mixtures with maize provided a favorable period conducive for cucumber flowers to open and probably for pollination and fruit formation to take place. The increased and prolonged vegetative growth of the late-season crops lengthened periods of flowering and fruiting, probably increased the assimilates available for fruit formation and development. Conversely, in the early-season crops the more shortened vegetative growth period resulting in fewer leaves and smaller leaf area possibly decreased radiation interception and photosynthesis. Also, the shorter periods of flowering and fruiting in the early-season crops probably decreased the amount of assimilate available for reproduction growth.
Furthermore, the yields of fresh edible fruit of cucumber in both cropping systems were higher in the late-season crops than in the early-season crops perhaps due to higher mean soil temperature of 33°C and 31°C at 5 and 10 cm during late season which enhanced the productivity of cucumber yield by about 50% compared with early season when mean soil temperature was 30°C and 29°C at 5 and 10 cm respectively. However, relatively lower yield of cucumber in early season can be attributed to heavy rainfall that coincided with flowering stage thereby leading to flower abortion. Thus the maximum economic returns from cucumber in either monoculture or mixed stand were greater in late-season crops than early-season-crops. The seasons of cultivation therefore, need to be taken into account when growing cucumber for maximum edible fruit and economic returns.

Intercropping with cucumber did not affect phenological growth stages (i.e. vegetative growth, flowering and fruiting) of the cucumber and growth and grain yield of associated maize in both seasons. This may be probably due to the differences in the stages of growth and development in relation to resources requirement and utilization of both crops. Cucumber had largely reached physiological maturity before growth of the maize was maximal. Similar observation was made by Olasantan and Bello (2004) on intercropping okra with cassava. Moreover, maize was able to grow properly after cucumber harvest to fully benefit from full sunlight, extra residual soil nutrient and moisture. Part of nutrient removed by cucumber during crop association would also be released to the soil by decomposition of cucumber residue for maize to use.

Furthermore, there are reasonable ecological benefits in intercropping cucumber with maize in relation to soil environment modification and weed control. Growing cucumber between maize rows suppressed weed growth and maintained cooler and moister soil and canopy environment in the maize. This may have been due to the ground cover provided by the associated cucumber, which reduced radiant flux to the soil surface and minimized water loss by evaporation during the day, and the inversion of soil temperature at night (Olasanatan, 1988). Such environmental conditions favored growth and fruit formation in intercropped cucumber when planting was later, particularly during late -season crops in 2004. It seems that the associated cucumber largely utilized the solar radiation, water and nutrients, which presumably otherwise would have been wasted and / or used by weeds in the maize inter-row space. Growing cucumber between maize rows thus appears to be an effective complementary biological method for weed control, soil and canopy environment improvement, judicious land use and increasing land productivity. It also generates income for resource-poor farmers and improves starch-based diets of the people.
In conclusion, the result indicated that the pattern in the distribution of hydrothermal parameters led to reduction in the maize yield during the late season and early season cucumber yield for both sole and mixed crop.

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The Effects Of Coupling Repetitive Motion Tasks With A Manually-Stressed Work Environment

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ABSTRACT
This paper presents an ergonomic evaluation conducted at a bottling plant in Ilorin, Nigeria. Virtually, all complaints from employees are from two key areas in the plant which ranged from occupational disorders such as carpal tunnel syndrome to other ailments of components of the upper extremities. A five-step regimen method was used to achieve desired solution. The occupational disorders were predictable due to the synergistic effects associated with repetitive production and hand intensive stress. Engineering improvements coupled with simple workstation modifications and an employee wellness program was recommended. [Researcher. 2009;1(2):37-40]. (ISSN: 1553-9865).

Key words: carpal tunnel syndrome, engineering improvements, repetitive motion tasks, wellness program

INTRODUCTION

The increase in concerns for ergonomic issues in the workplace is well founded; as it is related to work/machine interaction, workstation design, working position; the suitability of instruments to the physical and physiological characteristics of the workers, psychological factors and environmental conditions (heat, cold, noise, air pollution) which may affect workplaces (workstations) and affect the health of the workers (Bazroy, et al. 2003, World Health Organisation,1998; Ajimotokan, 2008). Thus, ergonomics improvement tends to lower the physical demands of work tasks, thereby lowering the incidence and severity of the musculoskeletal injuries (Ajimotokan, 2008).

Scientific evidence has demonstrated the effectiveness of ergonomic interventions, both in controlled and uncontrolled studies, in reducing both the exposure to potential workplace risk factors and the prevalence of work-related musculoskeletal disorders (Westgaard and Winkel, 1997; Smith, et al. 1999).

With reference to the bureau of labor statistics, over 50% of all occupational illnesses reported were associated with repetitive motion trauma (Bureau of Labor Statistics, 1997). A repetitive motion injury is not an acute or short-term result of a one-time accident, but instead, the chronic result of gradual, repeated trauma. The three most common repetitive motion injuries are muscle, tendon, and nerve injuries (MacLeod, 2000; American Industrial Hygiene Association (AIHA), 2003).

Carpal tunnel syndrome (CTS is the term used to describe the set of symptoms that result when the median nerve in the wrist is damaged or compressed (AIHA, 2003; Handy and Lafreniere, 2006). The symptoms of CTS range from mild numbness or faint tingling to extreme pain and/or loss of the use of the thumb (Handy and Lafreniere, 2006; Canadian Center for Occupational Health and Safety (CCOHS), 2001). CTS has shown up in studies of workers in frozen food plants, pork processing plants, and among supermarket workers (Chaing, 1993; Fairris and Brenner, 2001; Moore and Garg, 2001; Osorio, 1994). By the inherent nature, light manufacturing operations such as buffing, grinding, cutting, and packing (as well as palletizing and depalletizing) could all result in prevalence of CTS cases.

Body postures determine which joints and muscles are used in an activity and the amount of force or stresses generated or tolerated (Putz-Anderson, 1998), because the skeleton is essentially a lever system, there is certain postures in which it can absorb force more easily than in others. Phrased another way, there are certain postures in which the body is more susceptible to injury. Extreme postures, combined with force and frequency, will cause damage more quickly than when the postures are more natural or neutral (Kerst, 2003). Thus, the combination of force, frequency, and posture (FFP) contributes to wear and tear injuries. Effects of repetitive motions coupled with the performance of the same tasks are increased only when awkward postures and forceful exertions are involved.

As little information exists on the effectiveness of preventive measures in reducing work-related injury rates, several studies have been conducted to examine specific risk factors for work-related injuries in an attempt to identify workers at higher risk (Brenner, 2004; Zwerling, et al. 1996).

Ergonomic problems like those realized in these key areas – bottling and maintenance departments were most likely explained by the premise that the exposures are a part of a complex multiple risk factor function. It could be that the evolving of the problem was likely due to the combination of synergistic

effects resulting from the existence of repetitive tasks under hot and dry and/or cold and humid conditions. For multiple risk exposures, the most effective solution is to reduce risk factors by carefully analyzing what was currently being done, and then come up with the best solution to ultimately benefit both the worker and the company (National Institute of Occupational Safety and Health (NIOSH), 1997).

The study presents ergonomics evaluation of a workstation environment, coupling both the effects of repetitive tasks and manual stress, conducted at a beverage bottling plant in Ilorin, north central Nigeria. While there were a few work areas that utilized automated equipment, the bottling and maintenance workstation was partly labour-intensive with a few key ergonomic problem areas.

MATERIALS AND METHODS

This randomized intervention trial study was conducted at a beverage bottling plant in Ilorin, Nigeria for a six-month period. The facility operates four shifts – three production shifts and a general shift; and the workers donned, at a minimum, long coats and anti-slip boots.

Chronologically, five types of interrelated data indicators were used, to determine whether musculoskeletal problems are present and whether coupling both repetitive jobs with manual stress conditions that pose a significant risk for such disorders exist. These include:

1. A critical review of the literature was made to identify comparable studies that had been performed by other researchers. The databases of Environmental, Health & Safety, PubMed, among others were utilized and manually searching key relevant journals and conference proceedings to research comparable studies.
2. A review of injury and illness investigation register was conducted to identify areas of ergonomics concern, to understand the magnitude, pattern and seriousness of injury and define opportunities for intervention.
3. Interviews were completed with 104 affected employees during the fifth and sixth months of this study. Typically, the workers were incognito interviewed on the root of the ergonomic problems at hand.
4. A job- or task-based worker survey examining the likelihood of excessive physical fatigue or discomfort can also be an important indicator of potential disorder concerns. These surveys, which were typically administered on a one-on-one basis to avoid group bias, focus on relating discomfort sources to specific jobs or tasks. These job survey results were prioritized by the frequency and severity of discomfort to workers.
5. The primary method of job or hazard analysis system for risk assessment based on the Occupational Safety & Health Administration (OSHA) factors - the observational qualitative worksheets was completed during a walk-through review of job or task to identify obvious ergonomic concerns. These worksheets, which helped to identify mismatches between applied force, frequency, and assumed postures, provides a systematic risk screening method for an ergonomic job analysis. Factors such as stress, posture, force, repetitiveness, temperature extremes and priorities for change were subjectively evaluated and given a number ranking or a yes/no or true/false response.

RESULTS

The literatures found were comprehensive though not exhaustive on ergonomic issues couple with manually-stressed work environments, but the studies that were sited assisted in the recognition, evaluation and control of potential ergonomic problems.

Injuries and work-related illness investigation register provide a historic look at occurrences of strains, sprains, and RTDs such as CTS, wrist tendonitis among other ailments. These injury and illness rates and incidents yielded valuable information about the types of traumatic disorders present and facilitated the prediction of potential future losses, stemming from the situation.

The employee interviews brought out several key points. While it appeared to have some bias, the information gathered from individual worker history and questionable facility processes was significant and credible. Most of the workers complaints range from mild numbness or faint tingling to extreme pain and/or loss of the use of the thumb, worker fatigue or report of related problem. A few complaints were directed at soreness in the wrist and hand, and other complaints were directed at soreness in the elbow and shoulder areas which seem to either cause or complicate existing discomfort.

From qualitative assessments, several bottling tasks from arrangement of bottles into the bottle washer (depalletizing) to bottle screening operation, beverage bottling and crowning, palletizing, etc have been categorized as high risk for developing disorders in at least one body area.

DISCUSSION

The findings of this randomized trial imply that an engineering improvement coupled with simple workstation modification and an employee wellness program can reduce ailments of components of the upper extremities and prevent musculoskeletal disorders among workers in bottling facilities.

The review of observed complaints depicted that most of the disorders had been carpal tunnel syndrome or similar ailments of the wrist or other components of the upper extremity (e.g., fingers, hand, arm, elbow, etc.).

The qualitative worksheets used assisted in identifying the major problematic workstations within each of the two work areas. Careful evaluation of the completed worksheets resulted in the identification of arrangement of bottles into the bottle washer and palletizing tasks in beverage bottling as the most ergonomically unsound. These are manual tasks involving excessive folding/unfolding of fingers and bending of the wrist(s) with a repetitive sequence completed in hardly a second duration. Other high-risk tasks identified included bottle screening workstation, beverage bottling and crowning workstation in the bottling department and painstaking manual routine maintenance of machineries in the plant by maintenance department employees. The fit between the work piece and the employee in these high-risk areas were within the guideline values for these jobs except for few defined improvements.

RECOMMENDATIONS

As a result of the ergonomic issues identified from the ergonomics evaluation of this facility, the following was a list of recommendations deem indispensable.

1. The distance to the work piece should be kept at a minimum so that the arm movements or extensions of more than 15 inches are minimized (AIHA, 2003; Handy and Lafreniere, 2006). While the workstations that violated this principle in these two areas of concern were few in numbers. Stool height issues were particularly noticed during the evaluation of the bottle screening operation.
2. For the conditions, it appeared that the production rate for beverage bottling was too fast, so it was recommended that a qualified ergonomist, industrial engineer, work physiologist or time study expert to conduct a time motion study at this workstation.
3. An employee wellness program should be employed that included finger/hand exercises aimed at alleviating conditions that lead to RTDs, and in particular, CTS. Simple stretching exercises should be performed before the shift begins and/or during the first 5-20 minutes of each shift and after the lunch break. This would facilitate the overall body blood circulation and aid in the warming up of the muscles. However, an ergonomics awareness program should be put into operation plant-wide. This program would provide training on ergonomic-related issues and be the vehicle to move the wellness program in the right direction.

CONCLUSIONS

The combination synergistic effects coupled with the repetitive motions in a manually-stressed work environments can lead to premature development of occupational disorders such as CTS. However, the execution of appropriate engineering improvements with simple workstation modification and an employee wellness program can help to alleviate the seriousness of such work-related conditions.

Before this study, this facility had about 17 individuals/ month with some degree of disability attributed to RTDs. As indication of success, only six individuals (in the following month) have been diagnosed with a disorder in this area. While the counteractive action instigated from the results of this work cannot be seen as the only reason for the desired achievements, at least, the impact has been incredibly beneficial. Successful ergonomics processes follow a systematic, proactive approach driven by risk reduction strategies (Kerst, 2001). Though reactive steps are the typical beginning point, proactive approaches should be instituted to prevent these kinds of problems from developing.

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Application Of Generalized Inverse Of A Matirx To Models Not Of Full Rank

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ABSTRACT: This paper considered the application of generalized inverse of a matrix to models not of full rank. In the recent paper (1), On the generalized inverse of a matrix, the generalized inverse matrix was applied to solution of systems of equations that are linearly dependent and unbalanced. This paper is an extensive study of (1). It deals with the application of generalized inverse of a matrix to models that are not full rank. [Researcher. 2009;1(2):41-53]. (ISSN: 1553-9865).

KEYWORDS: Generalized Inverse of a matrix, linear models, least square, Estimation, Variance, Full rank partitioning

INTRODUCTION

The work presented in this paper is an extension of the earlier paper [1]. Here the generalized inverse of a matrix is applied to models which are not of full rank in nature.

Generalized inverse of a matrix is a research area in mathematical statistics. We are all aware of the fact that every non-singular matrix A has a unique inverse denoted by A such that $AA^{-1} = A^{-1}A = I$ where I is the identity matrix. That is a matrix has an inverse only if it is square and if only if it is non-singular or in other words, if its columns (rows) are linearly independent.

In recent years needs have been felt in numerous areas of applied mathematics for some kind of partial inverse of a matrix that is singular or even rectangular, hence the beginning of the use of generalized inverse. The most familiar applications of matrices is to the solution of systems of simultaneous linear equation, and the application of generalized inverse is no exception. Generalized inverse is also applied to least squares estimate (LSE) in the study of linear models.

Various methods exist for solving systems of simultaneous linear equation; some of them are: (elimination method, row reduction method, backward substitution method etc). Require that the set of linear equations be linearly independent. What if the system of equations is linearly dependent? Generalized inverse is able to solve linearly dependent and unbalance system of equations.{See Paper[1]}

Generalized inverse are of great importance in its general application to non-square and square singular matrices. In the case that A is non-singular $G = A^{-1}$ and G is unique.
The fact that A has a generalized inverse even if it is singular or rectangular has particular applications in the problem of solving equations like

$$AX = Y$$

More- over, generalized inverse are of great importance in the study of linear models where least square estimate often leads to equation of the form

$$X^1 Xb = X^1 Y$$

This has to be expressed in the form

$$b = \left(X^1 X\right)^{-1} X^1 Y$$

But if $X^1 X$ is singular then $\left(X^1 X\right)^{-1}$ does not exist hence the use of generalized inverse to solve such system of equations is needed, which is the main objective of this study. In mathematics, a generalized inverse or pseudoinverse of a matrix *A* is a matrix that has some properties of the inverse matrix of *A* but not necessarily all of them. The term "the pseudoinverse" commonly means the Moore-Penrose pseudoinverse.

The purpose of constructing a generalized inverse is to obtain a matrix that can serve as the inverse in some sense for a wider class of matrices than invertible ones. Typically, the generalized inverse exists for an arbitrary matrix, and when a matrix has inverse, then its inverse and the generalized inverse are the same. Some generalized inverses can be defined in any mathematical structure that involves associative multiplication, that is, in a semi group.

The various kinds of generalized inverses include

- one-sided inverse, that is left inverse and right inverse
- Drazin inverse
- Group inverse
- Bott–Duffin inverse (in German)
- Moore-Penrose pseudoinverse

1.2 HISTORICAL BACKGROUND OF GENRALIZED INVERSE MATRIX

The concept of a generalized inverse seems to have been first mention in print in 1903 by Fredholm, where a particular generalized inverse called by him pseudo inverse as an integral operator was given. Several investigations have concerned themselves with the Generalized inverse matrices, notably among them were: Hurwitz (1912), He characterized all pseudo inverse and used the finite dimensionality of null operators of Fredholm operators, already implicit in Hilbert's discussions in 1904 of generalized Green functions were consequently studied by numerous authors, in particular, Myller (1906), Westfall (1909), Bounitzky (1909), Elliott (1928) , Reid (1931). Bjerhanmer (1951), Penrose (1951)

Relevant publications are the work done by Moore (1920), Siegel (1937), Tseng, Murray and Von Neumann (1936), Alkinson (1950), Adetunde et al; (2008).

2. ALGORITHM FOR THE GENERALIZED INVERSE OF A MATRIX

An algorithm for finding the generalized inverse of a matrix is as follows, according to Adetunde et al; (2008)

Step 1: in A of rank r, find any non-singular minor of order r call it M

Step 2: invert M and transpose the inverse (M)

Step3: in A replace each element of M by the corresponding element of (M)

That is a = M the (s,t) element of m, then replace a b M, the (t,s) element of M equivalent to the (s,t) element of the transpose of M

Step4: replace all the other elements of A by zero

Step 5: transpose the resulting matrix and the result is G a generalized inverse of A

2.1 PROPERTIES OF GENERALIZED INVERSE OF A MATRIX

If G is a generalized inverse of A then

- AGA = A
- G is not unique
- G is of order m x n if A is of oder n x m

If G is a generalized inverse of XX then

- G is also a generalized inverse of XX
- XGXX = X; that is , GX is a generalized inverse of X
- XGX is invariant to G
- XGX is symmetric, whether G is or not

3 APPLICATION OF GENERALIZED INVERSE TOMODELS NOT OF FULL RANK

The model we shall be dealing with is

$$Y = Xb + e$$

Where Y is an N x 1 vector of observations y_i

B is a P x 1 vector of parameters X is an N x P matrix of known values (in most cases 0's and 1's) and e is a vector of random error terms.

The following assumptions are made

$$e \approx (0, \sigma^2 I) \; and \; Y \approx \left(Xb, \sigma^2 I\right)$$

$\Rightarrow$ The random errors are distributed normally with a zero mean and constant variance

$\sigma^2 I \; and \; Y$ is also distributed normally with a mean of Xb and a constant variance $\sigma^2 I$. The normal equation corresponding to the model is given as

$$Y = Xb + e$$

which can be derived by the least squares method, to get

$$X^1 Xb = X^1 Y$$

Example

In an experiment to estimate the effect of type of plant on the weight of the maize fruit four different maize plants given the same condition recorded the following weight of its fruit at harvest as

Weight of 10 plants	Type 1	Type 2	Type 3	Type 4
	62	80	62	60
	71	75	75	
	83	45		
	90			
Total	306	200	137	60

To estimate the effect of the type of plant on the weight of plant we assume that the observation $y_{i,j}$ is the sum of four parts

$$Y_{i,j} = \nu + \alpha_I + e_{i,j}$$

Where

ν is the population mean of the weight of plant

α_I is the effect of the type I on weight

$e_{i,j}$ is the random error term peculiar to the observation $y_{i,j}$

To develop the normal equations, we write down 10 observations in terms of the equation of the model

$$62 = y_{11} = \nu + \alpha_I + e_{11}$$
$$71 = y_{12} = \nu + \alpha_I + e_{12}$$
$$83 = y_{13} = \nu + \alpha_I + e_{13}$$
$$90 = y_{14} = \nu + \alpha_I + e_{14}$$
$$80 = y_{21} = \nu + \alpha_I + e_{21}$$
$$75 = y_{22} = \nu + \alpha_I + e_{22}$$
$$45 = y_{23} = \nu + \alpha_I + e_{23}$$
$$62 = y_{31} = \nu + \alpha_I + e_{31}$$
$$75 = y_{32} = \nu + \alpha_I + e_{32}$$
$$60 = y_{41} = \nu + \alpha_I + e_{41}$$

This is written in matrix form as

$$\begin{pmatrix}62\\71\\83\\90\\80\\75\\45\\62\\75\\60\end{pmatrix} = \begin{pmatrix}y_{11}\\y_{12}\\y_{13}\\y_{14}\\y_{21}\\y_{22}\\y_{23}\\y_{31}\\y_{32}\\y_{41}\end{pmatrix} = \begin{pmatrix}1&1&0&0&0\\1&1&0&0&0\\1&1&0&0&0\\1&1&0&0&0\\1&0&1&0&0\\1&0&1&0&0\\1&0&1&0&0\\1&0&0&1&0\\1&0&0&1&0\\1&0&0&0&1\end{pmatrix}\begin{pmatrix}\mu\\\alpha_1\\\alpha_2\\\alpha_3\\\alpha_4\end{pmatrix} + \begin{pmatrix}e_{11}\\e_{12}\\e_{13}\\e_{14}\\e_{21}\\e_{22}\\e_{23}\\e_{31}\\e_{32}\\e_{41}\end{pmatrix}$$

$$Y = X \quad b + e$$

Y is the vector of observations, X is the incidence matrix and b is the vector of parameters to be considers

The normal equations corresponding to the model

$Y = Xb + e$ can be derived by least square to give

$$X^1Xb = X^1Y$$

$$X^1X = \begin{pmatrix}1&1&1&1&1&1&1&1&1&1\\1&1&1&1&0&0&0&0&0&0\\0&0&0&0&1&1&1&0&0&0\\0&0&0&0&0&0&0&1&1&0\end{pmatrix}\begin{pmatrix}1&1&0&0&0&0\\1&1&0&0&0&0\\1&1&0&0&0&0\\1&1&0&0&0&0\\1&0&1&0&0&0\\1&0&1&0&0&0\\1&0&1&0&0&0\\1&0&0&1&0&0\\1&0&0&1&0&0\\1&0&0&0&1&0\end{pmatrix} = \begin{pmatrix}10&4&3&2&1\\4&4&0&0&0\\3&0&3&0&0\\2&0&0&2&0\\1&0&0&0&1\end{pmatrix}$$

$$X^1Y = \begin{pmatrix} 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} 62 \\ 71 \\ 83 \\ 90 \\ 80 \\ 75 \\ 45 \\ 62 \\ 75 \\ 60 \end{pmatrix} = \begin{pmatrix} 703 \\ 306 \\ 200 \\ 137 \\ 60 \end{pmatrix}$$

Matrix X^1X has determinant equal to zero and hence not of full rank, therefore matrix X^1X has no unique inverse, hence the equation cannot be express as

$$b = \left(X^1X\right)^{-1}\left(X^1Y\right)$$

since $\left(X^1X\right)^{-1}$ does not exist.

This implies that the normal equation has no unique solution. To get one of the solution, we need to find any generalized inverse G of $\left(X^1X\right)$ and write the corresponding solution as

$$b^0 = GX^1Y$$

where G is a generalized inverse of X^1X

the notation b^0 *and not* b used in equation emphasizes that what is derived by solving is only a solution to the equations and not an estimator of b

choosing

$$G = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 \\ 0 & 1/4 & 0 & 0 & 0 \\ 0 & 0 & 1/3 & 0 & 0 \\ 0 & 0 & 0 & 1/2 & 0 \\ 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

$b^0 = GX^1Y$

$$\begin{pmatrix}\mu\\ \alpha_1\\ \alpha_2\\ \alpha_3\\ \alpha_4\end{pmatrix} = \begin{pmatrix}0 & 0 & 0 & 0 & 0\\ 0 & 1/4 & 0 & 0 & 0\\ 0 & 0 & 1/3 & 0 & 0\\ 0 & 0 & 0 & 1/2 & 0\\ 0 & 0 & 0 & 0 & 0\end{pmatrix}\begin{pmatrix}703\\ 306\\ 200\\ 137\\ 60\end{pmatrix} = \begin{pmatrix}0\\ 76.5\\ 66.7\\ 68.5\\ 60\end{pmatrix}$$

The expectation of b^0 is given as

$E(b^0) = GX^1E(Y)$

$E(Y) = Xb$

$\therefore E(b^0) = GX^1Xb$

$E(b^0) = Hb$

Where $H = GX^1X$ hence b^0 is an unbiased estimator of Hb but not of b

$$H = \begin{pmatrix}0 & 0 & 0 & 0 & 0\\ 0 & 1/4 & 0 & 0 & 0\\ 0 & 0 & 1/3 & 0 & 0\\ 0 & 0 & 0 & 1/2 & 0\end{pmatrix}\begin{pmatrix}10 & 4 & 3 & 2 & 1\\ 4 & 4 & 0 & 0 & 0\\ 3 & 0 & 3 & 0 & 0\\ 2 & 0 & 0 & 2 & 0\\ 1 & 0 & 0 & 0 & 0\end{pmatrix} = \begin{pmatrix}0 & 0 & 0 & 0 & 0\\ 1 & 1 & 0 & 0 & 0\\ 1 & 0 & 1 & 0 & 0\\ 1 & 0 & 0 & 1 & 0\\ 1 & 0 & 0 & 0 & 1\end{pmatrix}$$

The variance of b^0 is given as

$Var(b^0) = Var\left(GX^1Y\right)$

$= GX^1Var(Y)XG^1$

$= GX^1XG^1\sigma^2$

For a full rank model $Var(b) = (X^1X)^{-1}\sigma^2$, by an appropriate choice of G, $GX^1XG^1\sigma^2$ can reduce further to $G\sigma^2$

Estimating E(y)

Corresponding to the vector of observations y, we have the vectors of estimated expected values $E(\hat{y})$.

$$E(\hat{y}) \equiv \hat{y} = Xb^0 = XGX^1Y$$

This vectors is invariant to the choice of whatever generalized inverse of X^1 Xis used for G, because XGX^1

is invariant. This means that no matter what solution of the normal equations is used for b^0 the vector $\hat{y} = XGX^1Y$ will always be the same.

$$\hat{Y} = Xb$$

$$\begin{pmatrix} y_{11} \\ y_{12} \\ y_{13} \\ y_{14} \\ y_{21} \\ y_{22} \\ y_{23} \\ y_{31} \\ y_{32} \\ y_{41} \end{pmatrix} = \begin{pmatrix} 1 & 1 & 0 & 0 & 0 \\ 1 & 1 & 0 & 0 & 0 \\ 1 & 1 & 0 & 0 & 0 \\ 1 & 1 & 0 & 0 & 0 \\ 1 & 0 & 1 & 0 & 0 \\ 1 & 0 & 1 & 0 & 0 \\ 1 & 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 1 & 0 \\ 1 & 0 & 0 & 1 & 0 \\ 1 & 0 & 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} 0 \\ 76.5 \\ 66.7 \\ 68.5 \\ 60.0 \end{pmatrix} = \begin{pmatrix} 75.6 \\ 75.6 \\ 75.6 \\ 75.6 \\ 66.7 \\ 66.7 \\ 66.7 \\ 68.5 \\ 68.5 \\ 60.0 \end{pmatrix}$$

$\hat{y}$ *is the vector of* exp*ected values..*

To demonstrate the invariance of $\hat{y}$ to the choice of G. Consider

$$G = \begin{pmatrix} 1 & -1 & -1 & -1 & 0 \\ -1 & 5/4 & 1 & 1 & 0 \\ -1 & 1 & 4/3 & 1 & 0 \\ -1 & 1 & 1 & 3/2 & 0 \\ 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

H = GX^1X, hence we have

$$H = \begin{pmatrix} 1 & -1 & -1 & -1 & 0 \\ -1 & 5/4 & 1 & 1 & 0 \\ -1 & 1 & 4/3 & 1 & 0 \\ -1 & 1 & 1 & 3/2 & 0 \\ 0 & 0 & 0 & 0 & 0 \end{pmatrix} \begin{pmatrix} 10 & 4 & 3 & 2 & 1 \\ 4 & 4 & 0 & 0 & 0 \\ 3 & 0 & 3 & 0 & 0 \\ 2 & 0 & 0 & 2 & 0 \\ 1 & 0 & 0 & 0 & 1 \end{pmatrix} = \begin{pmatrix} 1 & 0 & 0 & 0 & 1 \\ 0 & 1 & 0 & 0 & -1 \\ 0 & 0 & 1 & 0 & -1 \\ 0 & 0 & 0 & 1 & -1 \\ 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

$$b^0 = GX^1Y = \begin{pmatrix} 1 & -1 & -1 & -1 & 0 \\ -1 & 5/4 & 1 & 1 & 0 \\ -1 & 1 & 4/3 & 1 & 0 \\ -1 & 1 & 1 & 3/2 & 0 \\ 0 & 0 & 0 & 0 & 0 \end{pmatrix} \begin{pmatrix} 703 \\ 306 \\ 200 \\ 137 \\ 60 \end{pmatrix} = \begin{pmatrix} 60 \\ 16.5 \\ 6.7 \\ 8.5 \\ 0 \end{pmatrix}$$

$$\hat{Y} = Xb^0$$

$$\begin{pmatrix} y_{11} \\ y_{12} \\ y_{13} \\ y_{14} \\ y_{21} \\ y_{22} \\ y_{23} \\ y_{31} \\ y_{41} \end{pmatrix} = \begin{pmatrix} 1 & 1 & 0 & 0 & 0 \\ 1 & 1 & 0 & 0 & 0 \\ 1 & 1 & 0 & 0 & 0 \\ 1 & 1 & 0 & 0 & 0 \\ 1 & 0 & 1 & 0 & 0 \\ 1 & 0 & 1 & 0 & 0 \\ 1 & 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 1 & 0 \\ 1 & 0 & 0 & 1 & 0 \\ 1 & 0 & 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} 60 \\ 16.5 \\ 6.7 \\ 8.5 \\ 0 \end{pmatrix} = \begin{pmatrix} 75.6 \\ 75.6 \\ 75.6 \\ 75.6 \\ 66.7 \\ 66.7 \\ 66.7 \\ 68.5 \\ 68.5 \\ 60 \end{pmatrix}$$

From the above results, it demonstrates that $\hat{y}$ is always the same no matter the G used. The sum of squares regression is also invariant to the choice of G

4 PARTITIONING THE TOTALL SUM OF SQUARES

Partitioning the total sum of square for the full rank model is the same for the model not of full rank. The only difference is that there is utility in corrected sums of squares and products of the x – variables.

$SS_T = Y^1Y - N\bar{Y}^2$

$SS_R = (b^0)^1 X^1Y - N\bar{Y}^2$

$SS_E = SS_T - SS_R$

$$Y^1Y = (62\ 71\ 83\ 90\ 80\ 75\ 45\ 62\ 75\ 60)\begin{pmatrix}62\\71\\83\\90\\80\\75\\45\\62\\75\\60\end{pmatrix} = 50993$$

$Y^1 Y = 50993$

$N = 10,\ \bar{Y} = 70.3$

$SS_T = 50993 - 10(70.3)^2$

$= 50993 - 10(4942.09)$

$= 50993 - 49420.9$

$SS_T = 1572.1$

$$b^0X^1 Y = (60\ \ 16.5\ \ 6.5\ \ 8.5\ \ 0)\begin{pmatrix}703\\306\\200\\137\\60\end{pmatrix} = 49733.5$$

$SS_R = 49733.5 - 49420.9$

$SS_R = 312.6$

SS_R is invariant to the choice of G, to show the invariance of SS_R , we consider

$$b^0 = \begin{pmatrix} 0 \\ 76.5 \\ 66.7 \\ 68.5 \\ 60 \end{pmatrix}$$

$$b^0 X^1 Y = (0 \quad 76.5 \quad 66.7 \quad 68.5 \quad 60) \begin{pmatrix} 703 \\ 306 \\ 200 \\ 137 \\ 60 \end{pmatrix} = 49733.5$$

$SS_T = 49733.5 - 49420.9$

$= 312.6$

Since SS_R is the same, no matter the G used we say that SS_R is invariant to G

$SS_E = SS_T - SS_R$

$= 1572.1 - 312.6$

$= 1259.5$

Test the hypothesis

$H_0 : Xb = 0$

$H_1 : Xb \neq 0$

For the full rank model the test hypothesis is

$H_0 : b = 0$

$H_1 : b \neq 0$

But for a model not of full rank b is not estimable, hence the hypothesis

$H_0 : b = 0$

$H_1 : b \neq 0$

Cannot be tested because b is a non-estimable function

ANOVA table

Source	Df	SS	MS	F
Regression	3	312.6	104.2	0.496
Residual Total	6 9	1259.5 1572.1	209.9	

$R^2 = 312.6/1572.1 = 0.199$

The total variation explained by the model is 19.9%, the overall model is not significant, meaning that the weight of the maize fruit does not depend on the type of maize plant.

SAS OUTPUT

CONCLUSION

In this paper, the method of generalized inverse had been applied on linear models which is not of full rank. Evidence has shown from our result that generalized inverse can not be overlooked since it plays a very important role in models not of full rank. Most importantly, the use of generalized inverse of a matrix enables us to solve systems of linear equations that are unbalance and linearly dependent easily.

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Energy Integration Of Crude Distillation Unit Using Pinch Analysis

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ABSTRACT: Energy Integration of Crude Pre-Heat Train of CDU I of Kaduna Refinery and Petrochemicals Company was carried out using Pinch Technology. Optimum minimum approach temperature of 15 ^{o}C was obtained for a total cost index of 0.208 Cost/s. The pinch point temperature was found to be 220 ^{o}C. The utilities targets for the minimum approach temperature were found to be 1.112 x 10^{8} kJ/hr and 1.018 x 10^{8} kJ/hr for hot and cold utilities respectively. A total number of heat exchangers required for maximum energy recovery is 38. Pinch analysis as an energy integration technique saves more energy and utilities cost than the traditional energy technique. [Researcher. 2009;1(2):54-66]. (ISSN: 1553-9865).

Keywords: Pinch Analysis, CDU, Energy Target, Maple, Composite Curve

1. INTRODUCTION

Pinch technology is a complete methodology derived from simple scientific principles by which it is possible to design new plants with reduced energy and capital costs as well as where the existing processes require modification to improve performance. An additional major advantage of the Pinch approach is that by simply analyzing the process data using its methodology, energy and other design targets are predicted such that it is possible to assess the consequences of a new design or a potential modification before embarking on actual implementation.

Pinch analysis originated in the petrochemical sector and is now being applied to solve a wide range of problems in mainstream chemical engineering.

Wherever heating and cooling of process materials take place, there is a potential opportunity. The technology, when applied with imagination, can affect reactor design, separator design and the overall process optimization in any plant. It has been applied to process problems that go far beyond energy conservation. It has been employed to solve problems as diverse as improving effluent quality, reducing emission, increasing product yield and debottlenecking, increasing throughput and improving the flexibility and safety of the process (Akande, 2008).

Energy saving in the Nigerian industrial sector has several possibilities, due to the fact that, almost all the industrial equipment stock in Nigeria were imported during the era of cheap energy. Consequently, they are inherently energy inefficient. Furthermore, given the fact that energy prices had been kept at a low level up to 1985, energy cost has not been a significant fraction of total production cost even for energy intensive industry like refineries in Nigeria. The improvement of energy efficiency can provide substantial benefit in general to all the sectors of the economy (Dayo, 1994).

The Crude Distillation Unit is a unit in Fuels section of Kaduna refining and Petrochemical Company (KRPC) where distillation of local crude into Naphtha, gasoline, kerosene, diesel and bottom residue is carried out.

Process integration using Pinch Technology offers a novel approach to generate targets for minimum energy consumption before heat recovery network design. The Pinch design can reveal opportunities to modify the core process to improve heat integration. Pinch Analysis is used to identify energy cost and heat exchanger network (HEN) capital cost targets for a process and recognizing the pinch point. The procedure first predicts, ahead of design, the minimum requirements of external energy, network area, and the number of units for a given process at the pinch point. Next a heat exchanger network design that satisfies these targets is synthesized. Finally the network is optimized by comparing energy cost and the capital cost of the network so that the total annual cost is minimized. Thus, the prime objective of energy integration is to achieve financial savings by better process heat integration (maximizing process-to-process heat recovery and reducing the external utility loads).

1.1 Pinch Technology

Most processes need to consume energy at one temperature level and reject it at another level. This is achieved using utilities. Energy is provided to a process using such utilities as steam, hot water, flue gas etc. it is rejected to cooling water, air, refrigerant or in heat recovery steam rising.

Heat recovery is used to reduce the utility cost of a process. Evaluation of heat recovery involves a balancing of utility against the capital cost of the heat recovery system. The utility cost not only depends upon the amount of energy consumed and rejected but on the utility actually used. Cooling water is cheaper than refrigerant, low pressure steam is cheaper than high pressure steam (Adefila, 2002).

1.2 Principle of pinch technology

Pinch analysis is a rigorous, structured approach that can be used on a wide range of process and site utility related problems. Such as lowering operating costs, de-bottlenecking processes, raising efficiency and reducing capital investment.

Looking at its application in terms of energy improvement, we start by considering the heat and material balance, of the process in question. The majority of processes consist of streams that need to be heated up and streams that need to be cooled down. For each stream that requires heating or cooling, there are two basic choices. The heat can either be exchanged between two process streams or it can be exchanged between the process and the utility system. A fundamental strength of pinch analysis is that it determines the most appropriate set of heat exchange matches. In doing so, it reduces the cost of hot and cold utilities by minimizing the cascade of heat from the expensive, high temperature region down to ambient and also from ambient down to expensive, sub ambient temperatures.

The power of pinch technology lies in two factors:

(i) Its ability to quickly evaluate the economics of heat recovery for a given process.

(ii) The guidance it provides regarding how a process can be modified in order to reduce associated utility needs and costs.

It is these two factors that attract the use of pinch technology to analyze and design the heat exchanger network of any system. Here, only the source and target temperature, heat capacity and mass flow rates of the process streams are required to carry out the analysis and it works on certain established principles or concepts such as Problem Table Calculation, Composite Curve, Grand Composite Curve, Super Target, Grid representation etc.

Targets

Targets are theoretical values that represent the ideal or perfect situation. They are very important as an analysis tool as it provides a comparison for how close the current design is to the optimal design.

Energy Targets

Energy targets are the minimum amount of utilities needed to satisfy the process stream requirements (Linnhoff and Parker, 1984). In pinch software, the energy target values are calculated depending on the Utility Load Allocation Method and pinch temperature. The hot and cold utility energy targets are both displayed.

Pinch Temperature

The pinch temperature is used in designing the optimal HEN by identifying the following:

- Impossible heat transfer between streams when the temperature difference between streams is equal or less than the pinch temperature.
- Unnecessary use of cold utility, when a cold utility is used to cool hot streams in the region above the pinch.
- Unnecessary use of hot utility, when a hot utility is used to heat cold streams in the region below the pinch.

Plots

Plots provide a visual analysis of key variables and trends for the heat integration in a given stream data. pinch software has a wide variety of plots available.

Composite Curve

A Composite Curve is a graphical combination (or composite) of all hot or cold process streams in a heat exchange network (Linnhoff and Vredeveld, 1984). The Composite Curve plot displays both the hot composite curve and cold composite curve on the same plot. The closest temperature difference between the hot and cold composite curves is know as the minimum approach temperature, ΔT_{min}. The composite curves are moved horizontally such that the minimum approach temperature on the plot equals the minimum approach temperature you specified.

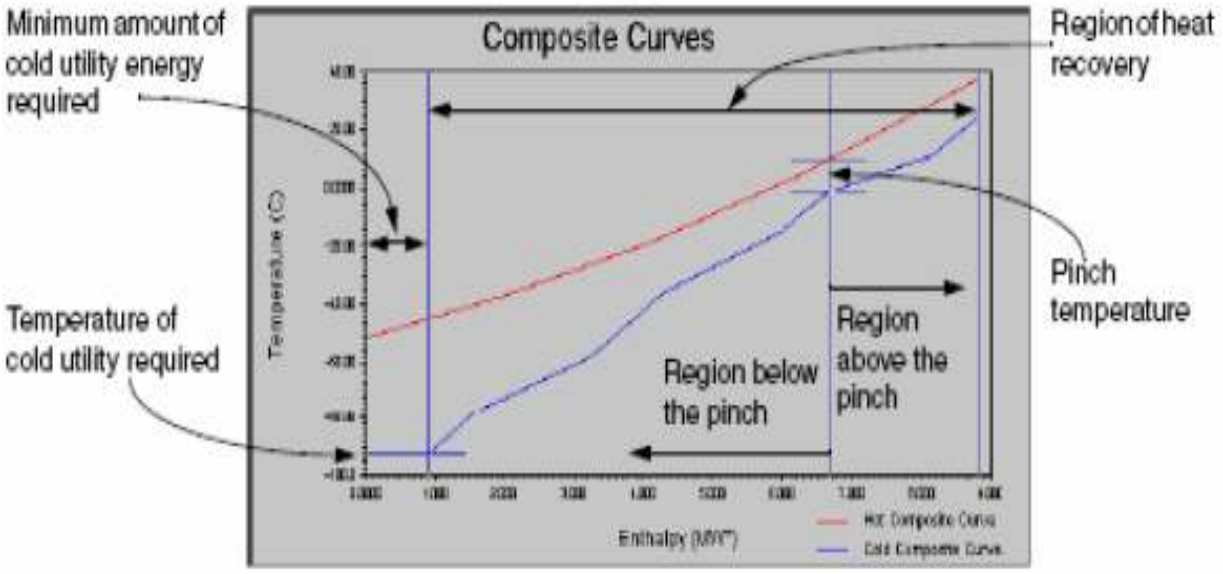

Figure 1: Composite Curve

Shifted Composite Curve

The Shifted Composite Curve is constructed the same way as the Composite Curve (Linnhoff et. al. 1982). However, the Hot Composite Curve (HCC) is shifted down by $\Delta T_{min/2}$ and the Cold Composite Curve (CCC) is shifted up by $\Delta T_{min/2}$. The following equations show how the shifted temperatures are calculated:

$$\text{Shifted Hot Stream Temperature} = \text{Unshifted Hot Stream Temperature} - \frac{\Delta T_{min}}{2} \quad (1)$$

$$\text{Shifted Cold Stream Temperature} = \text{Unshifted Cold Stream Temperature} + \frac{\Delta T_{min}}{2} \quad (2)$$

The result is that the hot and cold composite curves meet at the pinch location.

The figure below displays the unshifted and shifted composite curves. It can be observed that the two curves are shifted vertically.

Grand Composite Curve

The Grand Composite Curve is a plot of shifted temperatures versus the cascaded heat between each temperature interval (Linnhoff and Hindmarch, 1983). It shows the heat available in various temperature intervals and the net heat flow in the process (which is zero at the pinch). A grand composite curve sample is displayed in the figure below.

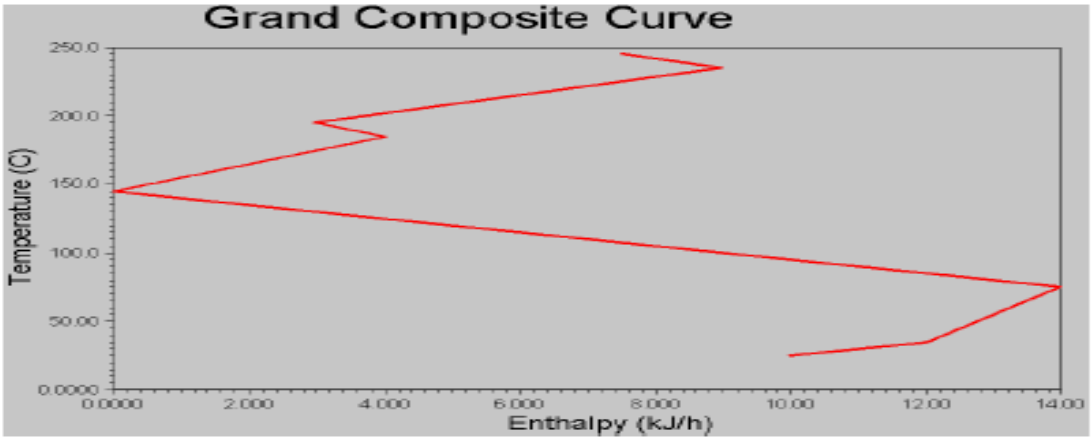

Figure 2: Grand Composite Curve

Degrees of Freedom

The value of the degrees of freedom indicates whether the HEN design can be controlled or not:

- $N_{DoF} < 0$ indicates that there is not enough manipulated variables in the HEN design and it is not possible to control all target temperatures.
- $N_{DoF} = 0$ indicates that there is enough manipulated variables in the HEN design to control the target streams' temperatures.
- $N_{DoF} > 0$ indicates that there is enough manipulated variables in the HEN design and you can implement more sophisticate control structures. The number of degrees of freedom is calculated using the following equation:

$$N_{DOF} = N_{mv} - N_{ts} \quad (3)$$

Area Targets

The area targets are the minimum amount of heat transfer area required for the hot and cold streams in a heat exchanger network (HEN) to achieve their specified temperature values. This equation is also known as the Uniform BATH Formula. The basic equation used to calculate the area target is:

$$A=\sum\left(\frac{1}{F_l\times\Delta T_{LM}}\right)_i\sum_j\left[\left(dT_h\times\sum_{jh}\left(\frac{MC_p}{h}\right)_{jh}+(dT_c)_i\sum_{jc}\left(\frac{MC_p}{h}\right)_{jc}\right)\right] \quad (4)$$

Number of Units Targets

Unit and shell targeting involve the calculation of the minimum number of units and shells in the heat exchanger network. The calculation is based on Euler's Network Theorem (Linnoff, 1983).

$$N_{u,\min}=N_i+N_l-N_i \quad (5)$$

Equation (6.26) is the minimum number of units in the heat exchanger network, not considering the existence of the pinch. The primary reason for calculating subsets is to simplify networks with a large number of streams. The minimum overall unit target can be expressed as:

$$N_{u,\min}=N_s-1 \quad (6)$$

Equation (6.27) is the minimum number of units in the heat exchanger network, not considering the existence of the pinch. In order to consider maximum energy recovery in the calculation of the minimum number of units in the heat exchanger network, the existence of the pinch must be considered.

The minimum number of units considering the pinch is calculated as follows:

$$N_{u,\min}=(N_A-1)+(N_B-1) \quad (7)$$

Cost Targets

The total annual cost of a heat exchanger network (HEN) is comprised of two parts: the capital cost and the operating cost.

- The operating cost network is the cost required to operate the process ($/yr).
- The capital cost of the network is a single investment required to build the heat exchanger network ($).

The target capital cost depends largely on how the area targets were calculated and what heat exchanger configurations are used in the HEN design.

2. METHODOLOGY

This section presents all the steps involved in the analysis, designing and optimization of Heat Exchangers Network of Thermal Hydrodealkylation Unit (CRU) of Kaduna Refining and Petrochemical Company. The procedures involved data extraction, process simulation and pinch analysis which are shown under Figure 3. The procedure involved analyzing of the existing Heat Exchangers Network of the Preheat train of the unit in order to extract all the necessary information required for the analysis. As mentioned earlier, the use of pinch technology in the energy conservation area remains the focus of this work.

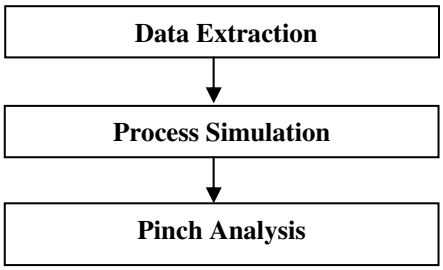

Figure 3: Steps involved in the energy integration of CRU unit of KRPC

2.1 Data Extraction

In the analysis of the existing network, a thorough study of the Process Flow Diagram (PFD) as shown in figure 8, Piping and Instrumentation Diagram (P&ID) and Laboratory analysis of the CDU feed and Products (Heavy Naphtha (VGO) and Atmospheric Gas Oil (AGO)) of CRU were carried out in order to extract all the necessary and available information require to carry out the process simulation of the CRU plant. The feed and product compositions of the laboratory analysis were used in carrying out the process simulation. The stream temperatures, mass flow rates, pressures were also extracted from PFD and P&ID for carrying out the pinch analysis as shown in Table 1, 2 and 3.

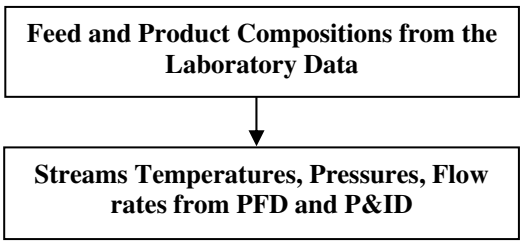

Figure 4: Data Extraction Steps

2.2 Process Simulation Procedure

Hysys Process Simulator was used for the process simulation of the plant streams. The source and target temperatures of all the streams, mass flow rates, feed and product compositions of the feed and product of the plant were used for obtaining the specific heat capacities and enthalpies of the streams.

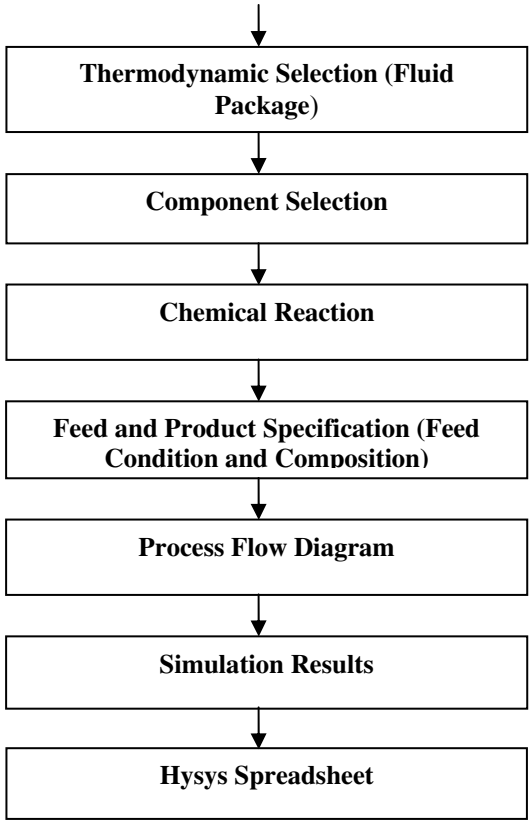

Figure 5: Process Simulation Steps using HYSYS

2.3 Maple Simulation Procedure

Maple procedure for carrying out pinch analysis is shown in figure 6.

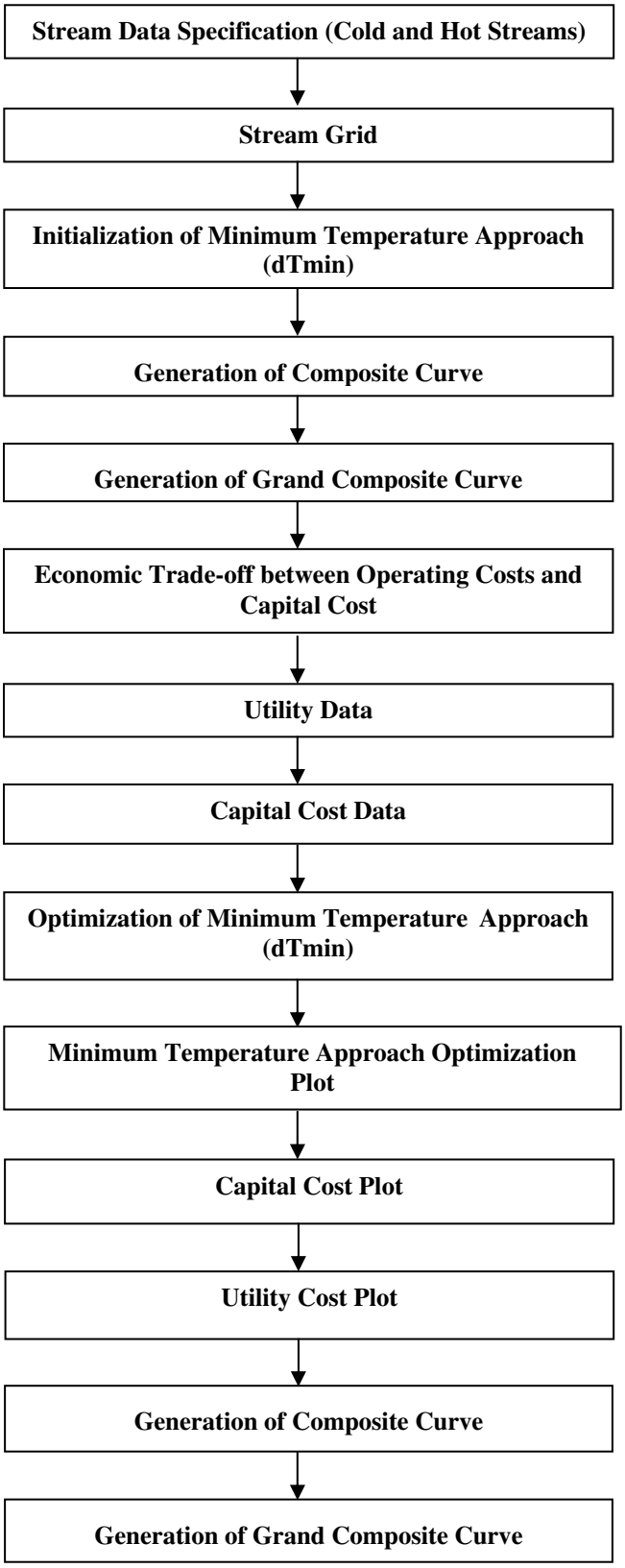

Figure 6: Pinch Analysis Simulation Procedure

3. RESULTS AND DISCUSSION

3.1 Data Extraction

Heat loads and temperatures for all the streams in the process are required for the heat integration carried out in this project. The Target and supply temperatures for the streams involved were identified as shown in Table 3. A furnace provides utility heating in the Crude Pre-Heat Train of CDU I Unit. The furnace design which was represented for fired heaters for the Pinch analysis as a heat sources as a single temperature that is hot enough to satisfy any anticipated heat load in the Unit. The air-cooling and water-cooling likewise can each be represented as heat sinks at a single temperature

3.2 Minimum Temperature Approach

In order to generate targets for minimum energy targets the ΔT_{min} value was set for the problem.

Table 2: Energy Target Results of Crude Pre-Heat Train of CDU I of Kaduna Refinery and Petrochemicals Company

Stream Name	Inlet Temperature	Inlet Temperature	Enthalpy
PA_3_Draw_To_PA_3_Return@COL1	319.4142597	244.0858396	36926963
WasteH2O_To_Cooled WasteH2O	73.23927034	40	819313.4
lowtemp crude_To_Preheat Crude	30	232.2222222	2.72E+08
Residue_To_Cooled Residue	347.2838075	45	2.15E+08
PA_2_Draw_To_PA_2_Return@COL1	263.5060272	180.1524082	36926963
PreFlashLiq_To_HotCrude	232.2225073	343.3333333	1.95E+08
AGO_To_Cooled AGO	297.3541067	110	13977836
Diesel_To_Cooled Diesel	248.0178225	50	45034887
Naphtha_To_Cooled Naphtha	73.23927034	40	6903304
Kerosene_To_Cooled Kerosene	231.7660237	120	19522251
PA_1_Draw_To_PA_1_Return@COL1	167.060092	69.55312558	58028085
To Condenser@COL1_TO_OffGas@COL1	146.6656862	73.23927034	65759664
KeroSS_ToReb@COL1_TO_Kerosene@COL1	226.1574774	231.7660237	7912951
TrimDuty@COL1	345.5907121	351.5295488	33391352

ΔT_{min}, or minimum temperature approach, is the smallest temperature difference that was allowed between hot and cold streams in the heat exchanger where counter-current flow was assumed.

This parameter reflects the trade-off between capital investment (which increases as the ΔT_{min} value gets smaller) and energy cost (which goes down as the ΔT_{min} value gets smaller). For the purpose of this project, typical ranges of ΔT_{min} values that have been found to represent the trade-off for each class of process have been used. Figure 8 shows the plot of total cost index against minimum temperature approach for Crude Pre-Heat Train of CDU I Unit. The plot shows that optimum minimum temperature approach desired is $15^{o}C$. This value of MTA was determined by parametric optimization.

Table 3: Energy Targets of Crude Pre-Heat Train of CDU I of Kaduna Refinery and Petrochemicals Company

Hot Utility (kJ/hr)	**Cold Utility (kJ/hr)**
1.12×10^8	1.018×10^8

Table 4: Pinch Analysis Targets of Crude Pre-Heat Train of CDU I of Kaduna Refinery and Petrochemicals Company

Pinch Analysis Targets	
Cost Index Targets	**Values**
Capital Cost (N)	9537410
Operating Cost (N/s)	0.112005
Total Annual Cost (N/s)	0.209418
Number of Unit Targets	Values
Total Minimum	19
Minimum for Maximum Energy Recovery	38
Shells	61
Energy Targets	**Values**
Heating	1.11E+08
Cooling	1.02E+08

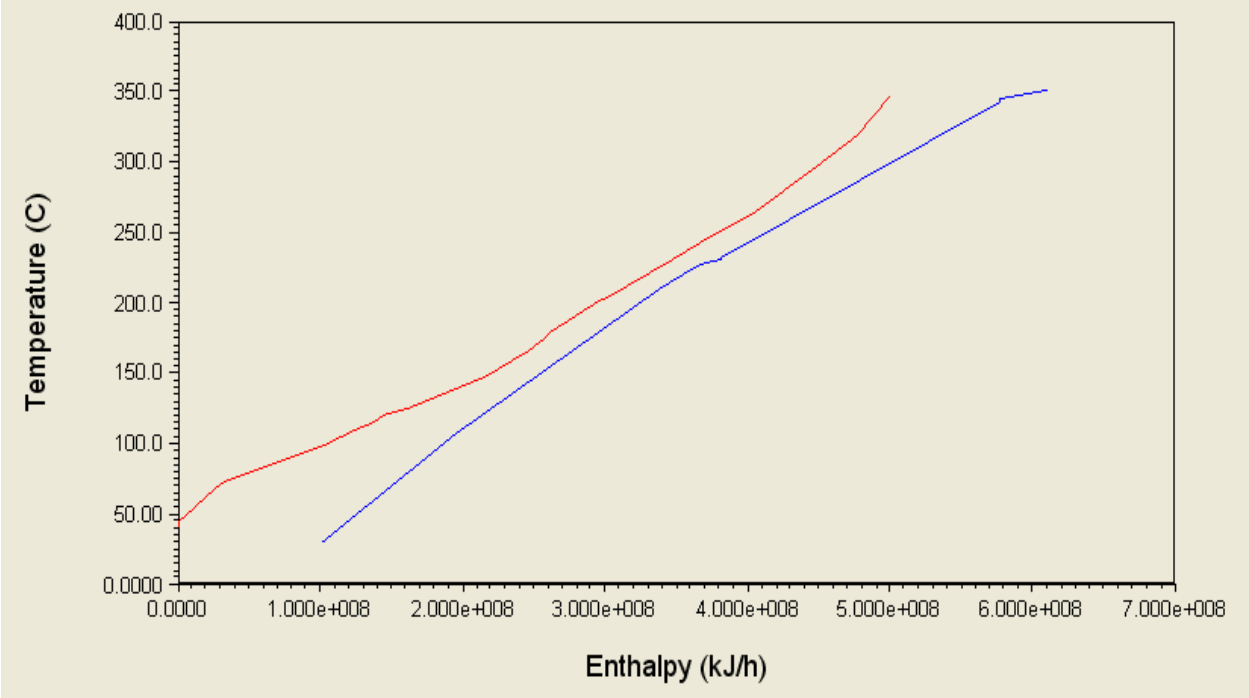

Figure 7: Shifted Composite Curve of Crude Pre-Heat Train of CDU I of Kaduna Refinery and Petrochemicals Company

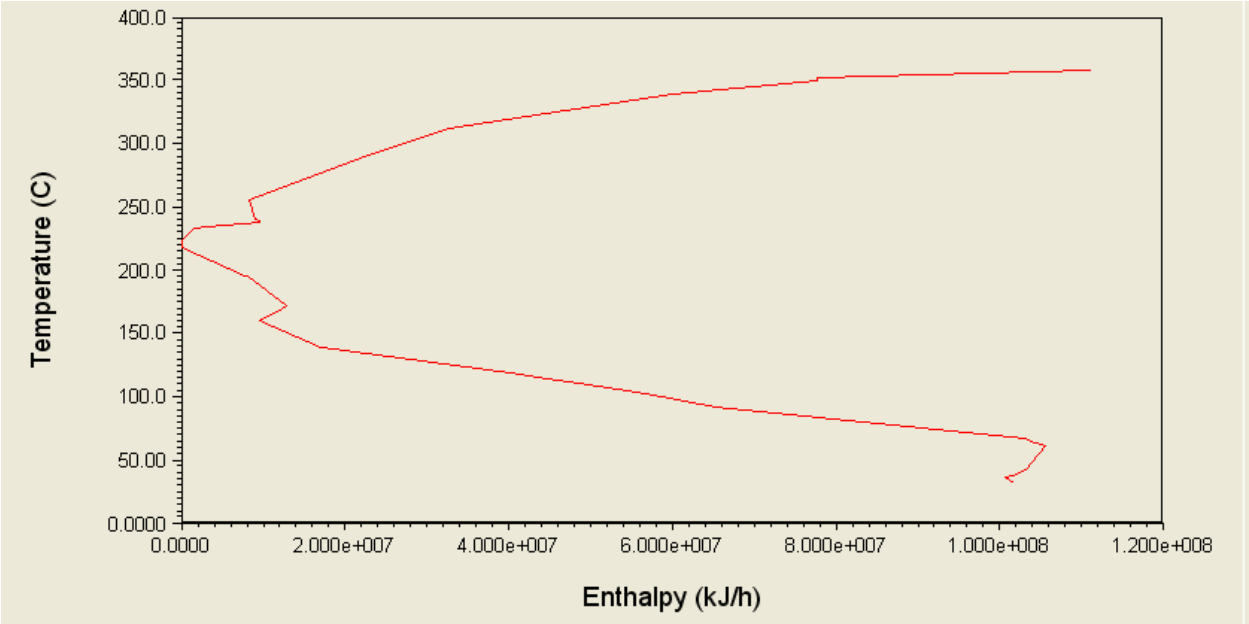

Figure 8: Grand Composite Curve of Crude Pre-Heat Train of CDU I of Kaduna Refinery and Petrochemicals Company

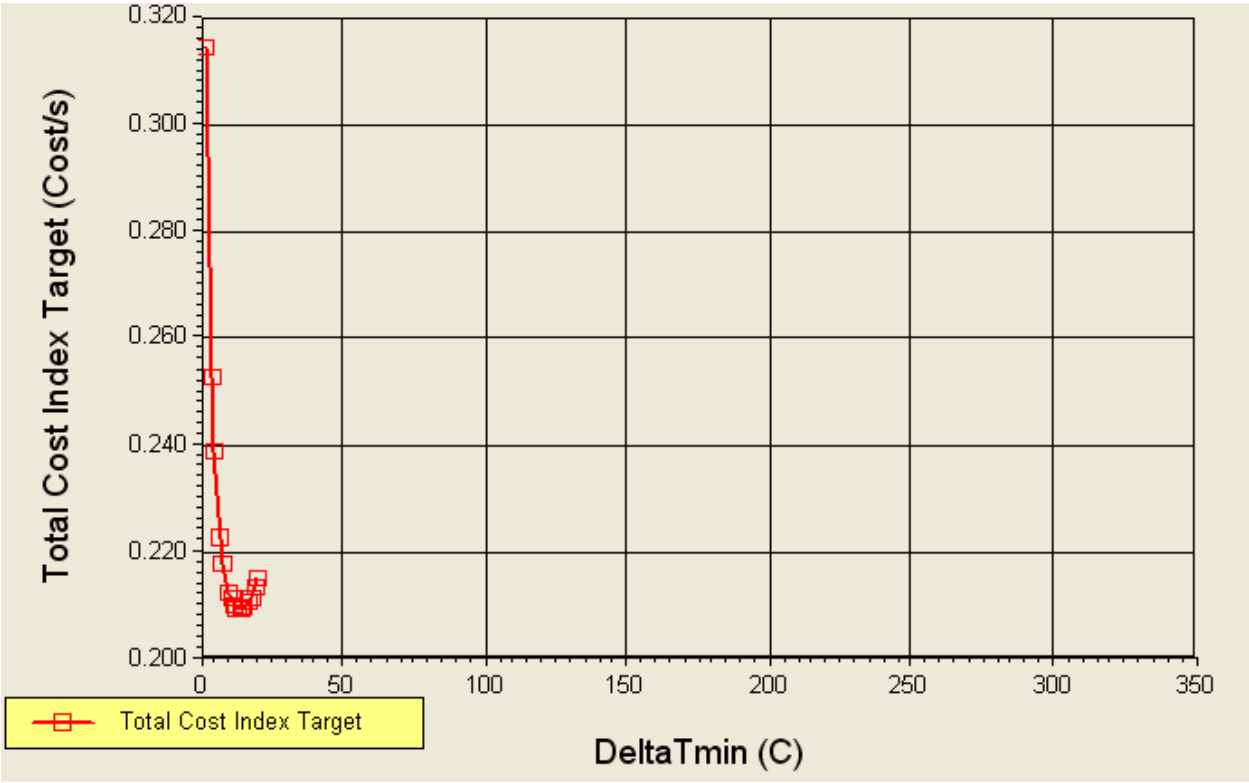

Figure 9: The Plot of Minimum Temperature Approach against Total Cost Index for Crude Pre-Heat Train of CDU I of KRPC.

3.3 Pinch Analysis Target Results

Figure 7 is the composite curve (temperature-enthalpy) profile of heat availability in the process (the "hot composite curve") and heat demands in the process (the "cold composite curve") together in a graphical representation. Figure 7, Table 2 and Table 3 shows that the heat available in the process is 1.12 x 108 kJ/hr while the heat demand in the process is 1.02 x 108 kJ/hr.. This shows that more heat is to be removed from the process than heat to be supplied to the system. Figure 8 (Grand composite Curve) shows that the Pinch temperature of the process is 370 ^{o}C.

The results show that the utility heating of the plant is slightly higher than the utility cooling of the plant. Therefore any utility cooling supplied to the process above the pinch temperature cannot be absorbed and will be rejected by the process to the heating utility, increasing the amount of heating utility required, hence waste of energy (hot utilities) by the Crude Pre-Heat Train of CDU I of Kaduna Refinery and Petrochemicals Company.

Table 4.3 shows that capital cost target of N 9,537,410 was obtained for the pinch analysis. Operating cost and total annual cost of N 0.112005/s and N 0.209418/s respectively were obtained for the energy target. The Heat Exchanger target shown in Table 3 also shows that total minimum number of heat exchangers required to meet the energy target is 19 while the minimum heat exchangers required for maximum energy recovery is 38.

4. CONCLUSIONS

The following conclusions may be drawn from the result of the analysis.

(i) Minimum approach temperature 15 ^{o}C was used to determine the energy target.
(ii) The pinch point was found to be 220 ^{o}C.
(iii) The utilities targets for the minimum approach temperature were found to 1.12 x 108 kJ/hr and 1.02 x 108 kJ/hr for hot and cold utilities respectively.
(iv) Pinch analysis as an energy integration technique saves more energy and utilities cost than the traditional energy technique

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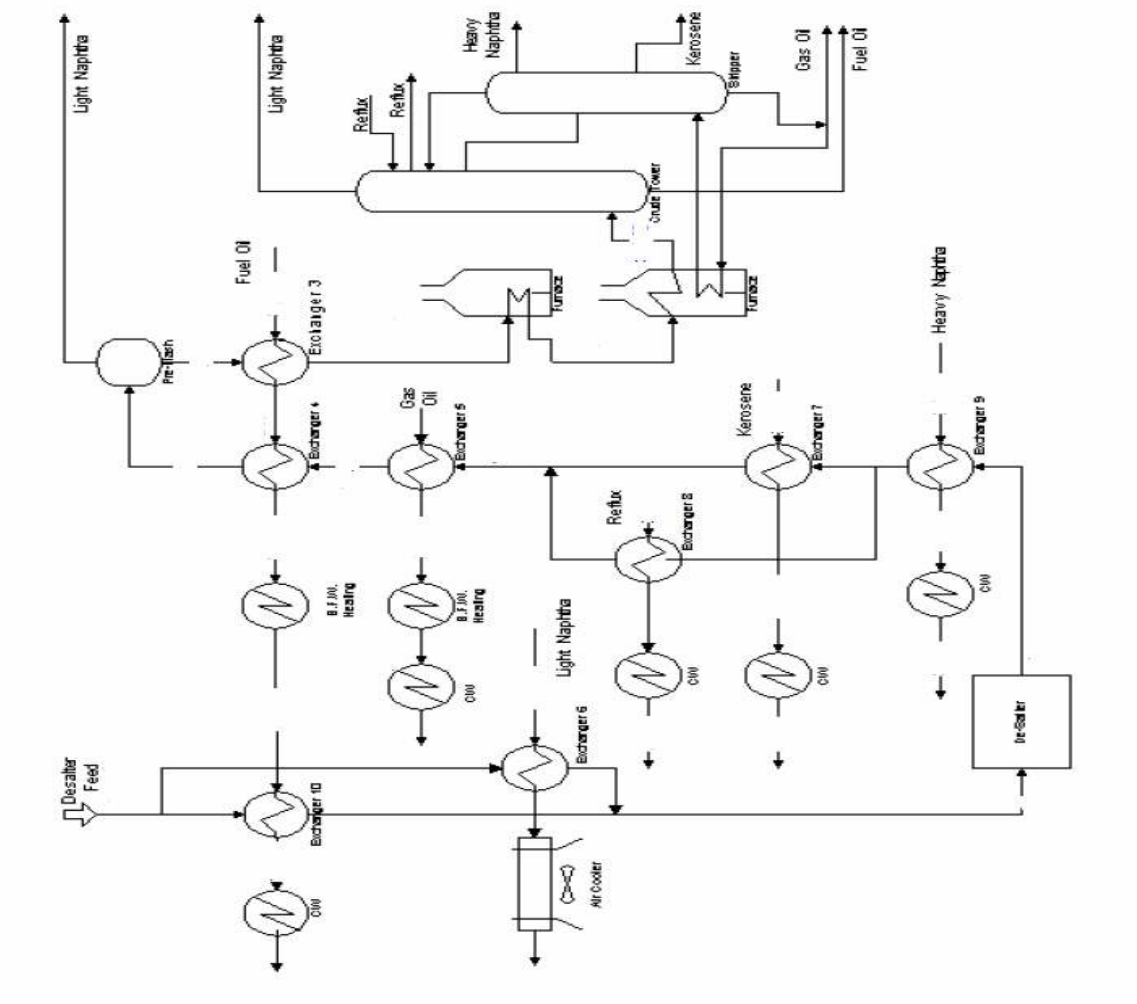
NOMENCLATURES

CRU	Thermal Hydro-dealkylation Unit
ΔT_{min}	Minimum Temperature Approach
HEN	Heat Exchanger Network
N_{DoF}	Number of Degree of Freedom
N_{ts}	the number of target streams
A	the target area
F_i	the correction factor accounting for non-counter current flow
ΔT_{LM}	The logarithmic mean temperature difference at each interval
i	denotes the i-th enthalpy internal
j	denotes the j-th stream
dT_h	The temperature change for the hot stream at each enthalpy interval
M	The mass flow rate of the stream
C_p	The specific heat capacity of the stream
h	The heat transfer of the stream
dT_c	The temperature change for the cold stream at each enthalpy interval
$N_{u,min}$	The unit target
N_s	The number of process and utility streams
N_{mv}	The number of manipulated variables
N_l	The number of heat exchanger loops
N_i	The number of independent systems
N_s	The number of process and utility streams
N_A	The number of process and utility stream above the pinch
N_B	The number of process and utility streams below the pinch
PFD	Process Flow Diagram
CDU 1	Crude Distillation Unit 1


Light Naphtha
Light Naphtha
Heavy Naphtha
Kerosene
Gas Oil
Fuel Oil
Reflux
Reflux
Stripper
Crude Tower
Fuel Oil
Furnace
Furnace
Heavy Naphtha
Pre Flash
Exchanger 3
Exchanger 4
Gas Oil
Exchanger 5
Kerosene
Exchanger 7
Exchanger 9
Reflux
Exchanger 8
B.F.W. Heating
B.F.W. Heating
CW
CW
CW
CW
Light Naphtha
Exchanger 6
Desalter Feed
Exchanger 10
Air Cooler
Desalter

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Western Blotting and ELISA Techniques

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Abstract: Western blotting and enzyme-linked immunosorbent assay (ELISA) are the two most useful and sensitive methods to measure the ng/ml to pg/ml ordered materials in the solution, such as serum, urine and tissue/cultured cell supernatant, and they are especially widely used in protein detection. Western blotting and ELISA have widely application in scientific researches, industry and medical practice. Besides the Western blotting, Northern blotting and Southern blotting are also useful in the biochemical application. This article describes the principle techniques for Western blotting and ELISA procedure. [Researcher. 2009;1(2):67-86]. (ISSN: 1553-9865).

Keywords: assay; enzyme-linked immunosorbent assay (ELISA); method; protein; SDS-polyacrylamide gel electrophoresis (SDS-PAGE); Western blotting

Abbreviation:
2-D, two dimensions
Ab, antibody
Ag, antigen
BCIP/NBT, 5-brono-4chloro-3-indolyl phosphate/nitro blue tetrazolium
ELISA, enzyme-linked immunosorbent assay
HRP, horseradish peroxidase
NP-40, Nonidet P-40
PBS, Phosphate-buffered saline
PMSF, phenylmethylsulfonyl fluoride
PVDF, polyvinylidene fluoride
SDS, sodium dodecyl sulfate
SDS-PAGE, SDS-polyacrylamide gel electrophoresis

1. Introduction

Western blotting and enzyme-linked immunosorbent assay (ELISA) are widely used in the protein detection (Savige, 1998). The name of Western blotting, it is also called Western blot. ELISA is the abbreviation of enzyme-linked immunosorbent assay (Ma and Shieh, 2006).

Since the inception of the protocol for protein transfer from an electrophoresed gel to a membrane by Towbin in 1979 (Towbin, 1979), protein blotting has evolved greatly (Kurien, 2006). Western blotting analysis can detect one protein in a solution that contains any number of proteins and giving the protein information (Dechend, 2006; Ma, 1994; 2004; Peter-Katalinic, 2005; Sakudo, 2006; Westermeier, 2005). Western blotting method is normally used with a high-quality antibody directed against a desired protein. First, separate the proteins using SDS-polyacrylamide gel electrophoresis (SDS-PAGE) is the beginning of the Western blotting. This separates the proteins by size. Second, transfer the protein from SDS-gel to a nitrocellulose membrane (electric transfer). Third, put the primary antibody on the membrane. Fourth, use the secondary antibody (this antibody should be an antibody-enzyme conjugate, e.g., horseradish peroxidase (HRP). Finally, use the dye and read the result. Recently, Knudson et al used Western blotting and ELISA to measure plasma endothelin-1 and it showed that plasma endothelin-1 concentrations were not different between control and prediabetic dogs. Also, they used Western blotting method and revealed a significant decrease in endothelin-A receptor protein in left circumflex coronary arteries (Knudson, 2006). The basic principle of an ELISA is to use an enzyme to detect the binding of antigen (Ag) antibody (Ab). The enzyme converts a colorless substrate

(chromogen) to a colored product, indicating the presence of Ag:Ab binding. An ELISA can be used to detect either the presence of Ags or Abs in a sample, depending on how the test is designed.

However, if a protein is degraded quickly, Western blotting and ELISA won't detect it well. In this case, radio-immune precipitation can be used for the protein detection. This article describes the principle techniques for Western blotting and ELISA procedure.

Polyvinylidene fluoride (PVDF) and nitrocellulose are the two membrane types most commonly used in Western blotting applications. PVDF was first introduced as a substrate by Millipore Corporation in 1985. Nitrocellulose membrane is a high quality membrane ideal for blotting of proteins and nucleic acids. The Nitrocellulose membrane is available in two pore sizes: 0.2 μm, transfer of low molecular weight proteins (<20 kDa) and nucleic acids (<300 bp); 0.45 μm, transfer of most proteins (>20 kDa) and nucleic acids (>300 bp). In our lab, we used nitrocellulose membrane.

2. Western Blotting

Western blotting is a method in molecular biology/biochemistry/immunogenetics to detect protein in a given sample of tissue homogenate or extract. It uses gel electrophoresis to separate denatured proteins by mass. The proteins are then transferred out of the gel and onto a membrane (typically nitrocellulose or PVDF membrane) and combine with antibodies specific to the protein. The secondary antiboy can be stained and pictured by a film. The film with the protein binds can be kept for a long time and scanned any time it needs to quantity the protein levels. As a result, researchers can examine the amount of protein in a given sample and compare levels between several groups. Other techniques also using antibodies allow detection of proteins in tissues and cells (immunocytochemistry).

The name Western blotting is a pun on the name Southern blotting, a technique for DNA detection and the detection of RNA is termed northern blotting.

2.1 Brief descriptions of the steps in a Western blotting

2.1.1 Tissue preparation

Typically, samples are taken from either tissue or from cell culture. The samples are cooled or frozen rapidly. They are homogenized using sonication or mechanical force. The resulting "whole-cell homogenate" or "whole-cell fraction" can be used as is, or subjected to centrifugation in a series of steps to isolate cytosolic (cell interior) and nuclear fractions. The prepared sample is then assayed for protein content so that a consistent amount of protein can be taken from each different sample.

Samples are boiled from one to five minutes in a buffer solution (e.g. Laemmli's buffer), containing dye, a sulfurous compound - typically beta-mercaptoethanol, and a detergent known as sodium dodecyl sulfate, or SDS. The boiling denatures the proteins, unfolding them completely. The SDS then surrounds the protein with a negative charge and the beta-mercaptoethanol prevents the reformation of disulfide bonds.

2.1.2 Gel electrophoresis

The proteins of the sample are separated according to molecular weight using gel electrophoresis. Gels have various formulations depending on the lab, molecular weight of the proteins of interest. Polyacrylamide gels are most common. Since the proteins travel only in one dimension along the gel, samples are loaded side-by-side into wells formed in the gel. Proteins are separated by mass into bands within each lane formed under the wells. One lane is reserved for a marker, or ladder, a commercially available mixture of proteins having defined molecular weights. Buffers and gels can be prepared by the researchers by bought from a company such as Bio-Rad.

It is also possible to use a 2-D gel (two dimensions) which spreads the proteins from a single sample out in two dimensions and proteins are separated according to isoelectric point (pH at which they have neutral net charge) in the first dimension, and according to their molecular weight in the second dimension.

2.1.3 Electronic Transfer

The polyacrylamide gel is good for separating of protein, but not suible for the staining and the further detecting. In order to make the proteins accessible to antibody detection, they are moved from within the gel onto a membrane made of nitrocellulose or PVDF. The membrane is placed face-to-face with the gel, and current is applied to large plates on either side. The charged proteins move from within the gel

onto the membrane while maintaining the organization they had within the gel. As a result of this "blotting" process, the proteins are exposed on a thin surface layer for detection. Both varieties of membrane are chosen for their non-specific protein binding properties (i.e. binds all proteins equally well). Protein binding is based upon hydrophobic interactions, as well as charged interactions between the membrane and protein. Nitrocellulose membranes are cheaper than PVDF, but are far more fragile and do not stand up well to repeated probings.

2.1.4 Blocking

Since the membrane has been chosen for its ability to bind protein, steps must be taken to prevent non-specific protein interactions between it and the antibody used for detection of the target protein. Blocking of non-specific binding is achieved by placing the membrane in a dilute solution of protein - typically bovine serum albumin (BSA) or non-fat dry milk, with a minute percentage of detergent such as Tween 20 or colloidal carbon.

2.1.5 Detection

During the detection process the membrane is probed for the protein of interest with antibodies, and links them to a reporter enzyme, which drives a colorimetric or photometric signal. For a variety of reasons, this traditionally takes place in a two-step process, although there are now one-step detection methods available for certain applications.

2.1.5.1 Two step

(1) Primary Antibody

Antibodies are generated when a host species or immune cell culture is exposed to the protein of interest. This is the primary antibody. After blocking, a dilute solution of primary antibody (generally between 0.5 and 5 micrograms/ml) is incubated with the membrane under gentle agitation. Typically, the solution is comprised of buffered saline solution with a small percentage of detergent, and sometimes with powdered milk or BSA. The antibody solution and the membrane can be sealed and incubated together for anywhere from 30 minutes to overnight. It can also be incubated at different temperatures, with warmer temperatures being associated with more binding (specific and non-specific).

(2) Secondary Antibody

After rinsing the membrane to remove unbound primary antibody, it is exposed to another antibody, directed at a species-specific portion of the primary antibody. This is known as a secondary antibody. The secondary antibody is usually linked to biotin or to a reporter enzyme such as alkaline phosphatase or horseradish peroxidase. This step confers an advantage in that several secondary antibodies will bind to one primary antibody, providing enhanced signal. Most commonly, a horseradish peroxidase-linked secondary is used in conjunction with a chemiluminescent agent, and the reaction product produces luminescence in proportion to the amount of protein. A sensitive sheet of photographic film is placed against the membrane, and exposure to the light from the reaction creates an image of the antibodies bound to the blot. As with the ELISPOT and ELISA procedures, the enzyme can be provided with a substrate molecule that will be converted by the enzyme to a colored reaction product that will be visible on the membrane. A third alternative is to use a radioactive label rather than an enzyme coupled to the secondary antibody, such as labeling an antibody-binding protein like *Staphylococcus* Protein A with a radioactive isotope of iodine. Since non-rediocticity methods are safer, quicker and cheaper, there are few groups to use the radioactive label method now .

2.1.5.2 One step

Historically, the probing process was performed in two steps because of the relative ease of producing primary and secondary antibodies in separate processes. This gives researchers and corporations huge advantages in terms of flexibility, and adds an amplification step to the detection process. Given the advent of high-throughput protein analysis and lower limits of detection, however, there has been interest in developing one-step probing systems that would allow the process to occur faster and with less consumables. This requires a probe antibody which both recognizes the protein of interest and contains a detectable label, probes which are often available for known protein tags. The primary probe is incubated with the membrane in a manner similar to that for the primary antibody in a two-step process, and then is ready for direct detection after a series of wash steps. It is possible to combine the detectable label to any primary antibody, but it costs more. This is suitble for that the antibody are needed in a big amount in the market.

2.1.6 Analysis

After the unbound probes are washed away, the Western blotting is ready for detection of the probes that are labeled and bound to the protein of

interest. In practical terms, not all westerns reveal protein only at one band in a membrane. Size approximations are taken by comparing the stained bands to that of the marker or ladder loaded during electrophoresis. The process is repeated for a structural protein, such as actin or tubulin, that should not change between samples. The amount of target protein is indexed to the structural protein to control between groups. This practice ensures correction for the amount of total protein on the membrane in case of errors or incomplete transfers.

2.1.6.1 Colorimetric detection

The colorimetric detection method depends on incubation of the Western blotting with a substrate that reacts with the reporter enzyme (such as alkaline phosphatase or horseradish peroxidase) that is bound to the secondary antibody. This converts the soluble dye into an insoluble form of a different colour that precipitates next to the enzyme and thereby stains the nitrocellulose membrane. Development of the blot is then stopped by washing away the soluble dye. Protein levels are evaluated through densitometry (how intense the stain is) or spectrophotometry.

2.1.6.2 Chemiluminescence

Chemiluminescent detection methods depend on incubation of the Western blotting with a substrate that will luminesce when exposed to the reporter on the secondary antibody. The light is then detected by photographic film, and more recently by CCD cameras which captures a digital image of the Western blotting. The image is analysed by densitometry, which evaluates the relative amount of protein staining and quantifies the results in terms of optical density. Newer software allows further data analysis such as molecular weight analysis if appropriate standards are used. The new reagent, enhanced chemiluminescent (ECL) detection is considered to be among the most sensitive detection methods for blotting analysis.

2.1.6.3 Radioactive detection

Radioactive method is more sensitive. Radioactive labels do not require enzyme substrates, but rather allow the placement of medical X-ray film directly against the western blot which develops as it is exposed to the label and creates dark regions which correspond to the protein bands of interest. The importance of radioactive detections methods is declining, because it is very expensive, health and safety risks are high and ECL provides a useful alternative.

2.1.6.4 Fluorescent detection

The fluorescently labeled probe is excited by light and the emission of the excitation is then detected by a photosensor such as CCD camera equipped which appropriate emission filters which captures a digital image of the Western blotting and allows further data analysis such molecular weight analysis and a quantitative Western blotting analysis. Fluorescence is considered to be among the most sensitive detection methods for blotting analysis.

2.1.7 Secondary probing

One major difference between nitrocellulose and PVDF membranes relates to the ability of each to support "stripping" antibodies off and reusing the membrane for subsequent antibody probes. While there are well-established protocols available for stripping nitrocellulose membranes, PVDF allows for easier stripping, and for more reuse before background noise limits experiments. Another difference is that, unlike nitrocellulose, PVDF must be soaked in 100% methanol or isopropanol before using. PVDF membranes also tend to be thicker and much more resistant to damage incurred by normal manipulation.

2.2 Tissue Sample Preparation

2.2.1 Isolate tissue (about 1 gram).
2.2.2 Put tissue in 3 volume of extract buffer.
2.2.3 Extract buffer (Table 1): The half-life of a 0.02 mM aqueous solution of PMSF is about 35 minutes at 8.0 pH. PMSF is usually stored as a 10 mM or 100 mM stock solution (1.74 or 17.4 mg/ml in isopropanol) at –20°C.
2.2.4 Homogenize sample under ice.
2.2.5 Centrifuge sample at 10,000 rpm for 10 minutes at 4°C, and collect the supernatant that contains the target protein for the measurement.
2.2.6 Keep supernatant at -70°C until usage.

2.3 SDS-PAGE [sodium dodecyl (lauryl) sulfate-polyacrylamide gel electrophoresis].

2.3.1 Use 12% SDS gel (or 5-15% depending on the sample). 12% SDS gel preparation is shown in Table 2 and an optional 12% SDS gel

preparation reagent amount is shown in Table 3.

2.3.2 Take 50 µl of sample and add an equal volume of 2 x SDS gel-loading buffer. 2 x SDS gel-loading buffer is shown in Table 4. 2 x SDS gel-loading buffer lacking dithiothreitol can be stored at room temperature. Dithiothreitol should then be added, just before the buffer is used, from a 1 M stock (Dissolve 3.09 g of dithiothreitol in 20 ml of 0.01 M sodium acetate (pH 5.2). Sterilize by filtration. Dispense into 1-ml aliquots and store at –20°C).

2.3.3 Boil the sample (in loading buffer) at 100°C for 3 – 5 minutes.

2.3.4 Load the sample for electrophoresis: 8 V/cm (6 x 8 = 48 volts) before the bromophenol blue (dye) front has moved into the resolving gel and 15 V/cm (6 x 15 = 90 volts) until the bromophenol blue reaches the bottom of the resolving gel.

2.4 **Electronic transfer and Immunological analysis**

2.4.1 Make the gel for transfer in transfer buffer: 0.65 mA/cm2 (about 100 volts) for 1.5 – 2 hours, or 30 volts overnight, on ice.

2.4.2 Western blotting transfer buffer (Table 5).

2.4.3 Block the filter with blocking buffer for 1 – 2 hours at room temperature (0.1 ml blocking solution per cm^2 filter), with gentle agitation on a platform shaker. Blocking solution is shown in Table 6 and Phosphate-buffered saline (PBS) (pH 7.4, 1000 ml) is shown in Table 7.

2.4.4 Discard blocking solution and immediately incubate filter with primary antibody.

2.4.5 Add 10 ml (0.1 ml of blocking solution per cm^2 of filter). Blocking solution is shown in Table 8.

2.4.6 Add 0.005 ml of primary antibody (1:2000) in to blocking solution.

2.4.7 Incubate at 4°C for 2 hours or overnight with gentle agitation on a platform shaker.

2.4.8 Discard blocking solution and wash filter 3 times (10 minutes each time) with 250 ml of PBS.

2.4.9 Incubate the filter with 150 mM NaCl, 50 mM Tris-HCl (pH 7.5) (phosphate-free, azide-free blocking solution) for 3 times for 10 minutes each time.

2.4.10 Immediately incubate the filter with secondary antibody.

2.4.11 Add 10 ml of phosphate-free, azide-free solution (150 mM NaCl, 50 mM Tris-HCl, 5% nonfat dry milk pH 7.5). Phosphate-free, azide-free blocking solution (pH 7.5, 1000 ml) is shown in Table 9.

2.4.12 Add 0.005 ml of secondary antibody solution (1:2000).

2.4.13 Incubate 1 – 2 hours at room temperature with gentle agitation.

2.4.14 Discard secondary and wash with 150 mM NaCl, 50 mM Tris-HCl (pH 7.5) (phosphate-free, azide-free solution) for 3 times for 10 minutes each time.

2.5 Alkaline phosphatase stain

2.5.1 Add 5 ml of the substrate 5-brono-4chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT) solution (Sigma).

2.5.2 Observe the filter for the blue color on the filter (about 20 minutes).

2.5.3 Discard BCIP/NBT solution when the bands are clear (about 20 minutes).

2.5.4 Immediately stop the enzymatic reaction by add water.

2.5.5 Cover the filter with plastic membrane and keep the filter. Analyze the blue bands and compare the color.

The half-life of a 0.02 mM aqueous solution of PMSF is about 35 minutes, at 8.0 pH. PMSF is usually stored as a 10 mM or 100 mM stock solution (1.74 or 17.4 mg/ml in isopropanol) at –20°C.

1X SDS gel-loading buffer lacking dithiothreitol can be stored at room temperature. Dithiothreitol should then be added, just before the buffer is used, from a 1 M stock (Dissolve 3.09 g of dithiothreitol in 20 ml of 0.01 M sodium acetate (pH 5.2). Sterilize by filtration. Dispense into 1-ml aliquots and store at –20°C).

Table 1. Extract buffer for Western blotting

50 mM Tris-HCl (pH 8.0) or 50 mM HEPES (pH 7.0)
150 mM NaCl
0.02% sodium azide
0.1% SDS
0.1 mg/ml phenylmethylsulfonyl fluoride (PMSF)
0.001 mg/ml aprotinin
1% Nonidet P-40 (NP-40) or 1% Triton X-100.

Table 2. 12% SDS gel preparation reagent amount (µl)

	Separating gel (12%)	Stacking gel (4%)
Water	1672	3020
Tris-HCl	1250 (1.5 M, pH 8.8)	1250 (0.5 M, pH 6.8)
SDS (10%)	50	50
Acr-Bis (30%)	2000	650
AP	25	25
TEMED	3	5
Sum	5000	5000

(AP is ammonium persulfate)

Table 3. Optional 12% SDS gel preparation reagent amount (µl)

	Separating gel (%12)	Stacking gel (4%)
Water	3344	3020
Tris-HCl	2500 (1.5 M, pH 8.8)	1250 (0.5 M, pH 6.8)
SDS (10%)	100	50
Acr-Bis (30%)	4000	650
AP	50	25
TEMED	6	5
Sum	10000	5000

(AP is Ammonium persulfate)

Table 4. 2 x SDS gel-loading buffer for Western blotting, 100 ml

62.5 mM Tris-HCl (pH 6.8), (Tris MW = 121.1, Sigma Catalog T-1503)
200 mM dithiothreitol
2% SDS (SDS MW = 288.38, Bio-Rad Catalog 161-0301)
0.01% bromophenol blue
0.25% glycerol

Table 5. 10 x SDS Running Buffer, pH 8.3, 1000 ml

Tris, 250 mM, 30.275 g (Tris MW = 121.1, Sigma Catalog T-1503)
Glycine, 1.92 M, 144.13 g (Glycine MW = 75.07, Bio-Rad Catalog 161-0717)
SDS, 1%, 10 g (SDS MW = 288.38, Bio-Rad Catalog 161-0301)

Table 6. Western blotting transfer buffer (1000 ml)

Chemicals	Concentration	Amount	M.W.
Tris	25 mM	3.03 g	121.1
Glycine	192 mM	14.41 g	75.07
Methanol	20%	200 ml	

Table 7. Phosphate-buffered saline (PBS), pH 7.4, 1000 ml (adjust to pH 7.4 with HCl)

NaCl, 150 mM, 8.77 g, MW = 58.44, Sigma Catalog S-9888
KCl, 2.7 mM, 0.2 g, MW = 74.55
Na_2HPO_4, 10.1 mM, 1.44 g, MW = 142.0
KH_2PO_4, 1.8 mM, 0.24 g, MW = 136.09

Table 8. Blocking solution, 100 ml, in 100 ml phosphate-buffered saline (PBS, pH 7.4)

Nonfat dried milk, 5%, 5 g
Antifoam A, 0.01%, 10 ml
Sodium azide, 0.02%, 20 mg
0.2 ml Tween 20
Sodium azide: 1 ml of 2% solution

Table 9. Blocking solution

Blocking solution, 10 ml, in PBS (pH 7.4)	
Nonfat dried milk	5%
Antifoam A	0.01%
Sodium azide	0.02%

Table 10. Phosphate-free, azide-free blocking solution (pH 7.5, 1000 ml)

NaCl	150 mM	8.766 g
Tris-HCl (pH 7.5)	50 mM	6.057 g
12 N HCl		about 3.35 ml
Nonfat dried milk	5% (w/v)	

2.6 Overall of Western blotting solutions (Table 11)

Table 11. Overall table of Western blotting solutions

Tissue Extract buffer, 100 ml
50 mM Tris-HCl (pH 8.0), 0.6 g (Or 50 mM HEPES (pH 7.0), 1.19 g)
150 mM NaCl, 0.88 g
0.02% sodium azide, 0.02 g
0.1% SDS, 0.1 g
0.1 mg/ml phenylmethylsulfonyl fluoride (PMSF), 0.01 g
0.001 mg/ml aprotinin, 0.1 mg
1% Nonidet P-40 (NP-40), 1 ml (Or 1% Triton X-100, 1 ml)

2 X SDS gel-loading buffer, 100 ml
100 mM Tris-HCl (pH 6.8) (Tris 1.21 g)
200 mM dithiothreitol
4% SDS, 0.4 g
0.2% bromophenol blue, 0.2 g
20% glycerol, 20 ml

1.5 M Tris-HCl, pH 8.8, 300 ml
Tris, 54.5 g
HCl, 12 N, 6.375 ml

0.5 M Tris-HCl, pH 6.8, 300 ml
Tris, 18.17 g

HCl, 12 N, 11.5 ml

10 x SDS-PAGE Running Buffer, pH 8.3, 1000 ml
Tris, 250 mM, 30.275 g (Tris MW = 121.1, Sigma Catalog T-1503)
Glycine, 1.92 M, 144.13 g (Glycine MW = 75.07, Bio-Rad Catalog 161-0717)
SDS, 1%, 10 g (SDS MW = 288.38, Bio-Rad Catalog 161-0301)

Western Blotting Transfer Buffer, 1000 ml, keep at 4°C before using
Tris, 25 mM, 3.03 g, MW = 121.1
Glycine, 192 mM, 14.41 g, MW = 75.07
Methanol, 20%, 200 ml

Phosphate-buffered saline (PBS), pH 7.4, 1000 ml (adjust to pH 7.4 with HCl)
NaCl, 150 mM, 8.77 g, MW = 58.44, Sigma Catalog S-9888
KCl, 2.7 mM, 0.2 g, MW = 74.55
Na_2HPO_4, 10.1 mM, 1.44 g, MW = 142.0
KH_2PO_4, 1.8 mM, 0.24 g, MW = 136.09

Blocking solution, 100 ml, in 100 ml phosphate-buffered saline (PBS, pH 7.4)
Nonfat dried milk, 5%, 5 g
Antifoam A, 0.01%, 10 ml
Sodium azide, 0.02%, 20 mg
0.2 ml Tween 20

Phosphate-free, azide-free blocking solution, 1000 m l (adjust pH with 12 N HCl about 3.35 ml)
150 mM NaCl, 8.766 g
50 mM Tris-HCl (pH 7.5), 6.057 g
5% (w/v) nonfat dried milk

Transfer Buffer (Optional), 1000 ml

20 mM Tris-HCl, pH 8.0, 2.42 g, Tris MW = 121.1

150 mM Glycine, 11.26 g, Glycine MW = 75.07

20% methanol, 200 ml

TBS (Optional), 1000 ml

125 mM NaCl, 7.3 g, NaCl MW = 58.44, Sigma Catalog S-9888

25 mM Tris pH 8.0, 3 g, Tris MW = 121.1

2.7 More description of Western blotting

2.7.1 SDS-PAGE

SDS-PAGE is the abbreviation of sodium dodecyl (lauryl) sulfate-polyacrylamide gel electrophoresis.

Agarose gels are best for isolating larger molecules (such as DNA), and SDS-PAGE is the best choice to isolate smaller molecules (such as proteins).

The common usages of SDS-PAGE could be: (1) Determining protein size; (2) Identifying protein sort; (3) Detecting protein sample purity; (4) Finding disulfide bonds in proteins; (5) Quantifying proteins; (6) Blotting applications.

The SDS portion is a detergent. The

SDS detergent makes the protein from its native shape to a denatured form. The denatured protein is a linear form that can be run by the gel depending on its molecule size.

SDS is an anionic detergent that binds quantitatively to proteins, giving them linearity and uniform charge, so that they can be separated according to the molecule weight of the proteins. The SDS has a hydrophobic tail that interacts strongly with protein (polypeptide) chains. The number of SDS molecules that bind to a protein is proportional to the number of amino acids that make up the protein. Each SDS molecule contributes two negative charges, overwhelming any charge the protein may have. So that, in this case the negative charges that the proteins have will be linearly related to their amino acid numbers. Averaged, a protein molecular weight is linearly related to its amino acid numbers (one amino acid is 110 daltons averagely). SDS also disrupts the forces that contribute to protein folding (tertiary structure), ensuring that the protein is not only uniformly negatively charged, but linear as well.

The polyacrylamide gel electrophoresis works in a similar fashion to an agarose gel, separating protein molecules according to their size. In electrophoresis, an electric current is used to move the protein molecules across a polyacrylamide gel. The polyacrylamide gel is a cross-linked matrix that functions as a sort of sieve to help catching the molecules as they are transported by the electric current. The polyacrylamide gel acts somewhat like a three-dimensional mesh or screen. The negatively charged protein molecules are pulled to the positive end by the current, but they encounter resistance from this polyacrylamide mesh. The smaller molecules are able to navigate the mesh faster than the larger one, so they make it further down the gel than the larger molecules. This is how SDS-PAGE separates different protein molecules according to their size.

Once an SDS-PAGE gel is run, we need to fix the proteins in the gel so they don't come out when you stain the gel. Acetic acid 25% in water is a good fixative, as it keeps the proteins denatured. The gel is typically stained with Coomasie blue dye R250, and the fixative and dye can be prepared in the same solution using methanol as a solvent. The gel is then destained and dried (Ji, 2006).

2.7.2 Immunoassay

Transferring proteins to membranes from gels lets the proteins be more stable adhere on the membrane to be efficiently detected with various probes. Polyacrylamide is really good to separate proteins, but not suitable for the further analysis. To transfer the proteins to a stable membrane is useful for the further analysis. The most popular type of probe of immobilised proteins is an antibody. Chemiluminescent substrates have begun to be used because of their greater detection sensitivity. Other possibilities for probing include the use of fluorescent or radioisotope labels (fluorescein, ^{125}I). Probes for the detection of antibody binding can be conjugated anti-immunoglobulins; conjugated staphylococcal Protein A or probes to biotinylated / digoxigeninylated primary antibodies.

The immunoassay is normally done by blocking the transfer membrane with a concentrated protein solution (10% foetal calf serum or 5% non-fat milk powder) to prevent further non-specific binding of proteins, then incubating the membrane in a diluted antiserum/antibody solution, washing the membrane, incubating the membrane in diluted conjugated probe antibody or other detecting reagent, further washing, and the colorimetric / autoradiographic / chemiluminescent detection.

The power of the technique lies in the simultaneous detection of a specific protein by means of its antigenicity, and its molecular mass. Proteins are first separated by mass in the SDS-PAGE, then specifically detected in the immunoassay step.

It is also possible to use a similar technique to elute specific antibodies from specific proteins resolved out of a complex mixture, many of whose components react with a given antiserum: one can electrophorese a mixture of proteins, cut out a specific band from a gel or

membrane, and use this to fish out specific antibodies from a serum.

Staining of proteins in gels may be done using the standard Coomassie brilliant blue, amido black or silver stain reagents. Silver staining is more sensitive (1 ng level). The sensitivity of Coomassie brilliant blue G-250 is 300 ng level. It is possible to reversibly stain gels prior to blotting by a couple of methods (Rybicki, 1996).

2.8 A Practical Protocol for Western Blotting Handling

Reagents needed for day one:

First, prepare tissue lysis buffer and do the lysis (Table 12).

Table 12. Tissue lysis buffer for Western Blotting

Tissue Lysis Buffer for Western Blotting, all with stock solution

Chemicals	Stock (ml)	Stock (ml)	Stock (ml)	Stock (ml)	Stock (ml)	Stock (ml)	Final con.	Notes
H_2O	6.55	19.65	32.75	65.5	98.25	131	75.60%	Room Temperature
NaCl, 5 M	0.3	0.9	1.5	3	4.5	6	0.15 M	4°C, MW=58.4
Tris-HCl, 2 M, pH 7.4	0.25	0.75	1.25	2.5	3.75	5	0.05 mM	4°C, MW=121.1
EDTA (100 mM)	0.1	0.3	0.5	1	1.5	2	1 mM	4°C, MW=372.2
Sucrose (2.5 M)	1	3	5	10	15	20	250 mM	4°C, MW=342.3
Igepal (or Triton X-100)	0.1	0.3	0.5	1	1.5	2	1%	Room Temperature
Aprotinin (1.34 mg/ml)	0.07	0.21	0.35	0.7	1.05	1.4	10 ug/ml	10 mM HEPES, -20°C
Leu-peptin (10 mg/ml)	0.01	0.03	0.05	0.1	0.15	0.2	10 ug/ml	in water, -20°C
Pepstatin (1 mg/ml)	0.01	0.03	0.05	0.1	0.15	0.2	1 ug/ml	in ethanol, -20°C
Trypsin inhitor (10 mg/ml)	0.01	0.03	0.05	0.1	0.15	0.2	10 ug/ml	in water, -20°C
NaN_3, 2%	0.1	0.3	0.5	1	1.5	2	0.02%	in water, room temperature
NaF (50 mM)	1	3	5	10	15	20	5 mM	in ethanol, -20°C
PMSF 100 mM (17.42 mg/ml)	0.5	1.5	2.5	5	7.5	10	5 mM	add before use, -20°C
Sum	10	30	50	100	150	200		

Optional, 25 mM imidazole (MW=68.1) can be used as buffer, instead of Tris-HCl.

Tissue Lysis Buffer for Western Blotting, some reagents by powder

Chemicals	Stock (ml)	Stock (ml)	Stock (ml)	Stock (ml)	Stock (ml)	Stock (ml)	Final con.	Notes
H_2O	7.95	23.85	39.75	79.5	119.25	159	75.60%	Room Temperature
NaCl, 5 M	0.0876 g	0.262 8 g	0.438 g	0.876 g	1.314 g	1.752 g	0.15 M	MW=58.4
Tris-HCl, 2 M, pH 7.4	0.25	0.75	1.25	2.5	3.75	5	0.05 mM	4°C, MW=121.1
EDTA	0.0037 g	0.011 g	0.018 6 g	0.037 g	0.0558 g	0.074 4 g	1 mM	MW=372.2
Sucrose	0.8558 g	2.567 g	4.278 7 g	8.557 g	12.836 g	17.11 5 g	250 mM	MW=342.3
Igepal (or Triton X-100)	0.1	0.3	0.5	1	1.5	2	1%	Room Temperature

Aprotinin (1.34 mg/ml)	0.07	0.21	0.35	0.7	1.05	1.4	10 ug/ml	10 mM HEPES,-20°C
Leu-peptin (10 mg/ml)	0.01	0.03	0.05	0.1	0.15	0.2	10 ug/ml	in water, -20°C
Pepstatin (1 mg/ml)	0.01	0.03	0.05	0.1	0.15	0.2	1 ug/ml	in ethanol, -20°C
Trypsin inhitor (10 mg/ml)	0.01	0.03	0.05	0.1	0.15	0.2	10 ug/ml	in water, -20°C
NaN_3, 2%	0.1	0.3	0.5	1	1.5	2	0.02%	in water, 4°C
NaF (50 mM)	1	3	5	10	15	20	5 mM	in ethanol, -20°C
PMSF 100 mM (17.42 mg/ml)	0.5	1.5	2.5	5	7.5	10		add before use, -20°C
Sum	10	30	50	100	150	200		

Optional, 25 mM imidazole (MW=68.1) can be used as buffer, instead of Tris-HCl.

To isolate the membrane protein, the membrane should be broken during the lysis, and the detergent Triton X-100 could be up to 5%.

Homogenized tissue could be centrifuged at 10,000 x g for 20 minutes (4°C).

2.8.1 Running buffer 1x

Prepare 1 day before, store at 4°C, good for 2 weeks.

(1) ➔ 100 ml of 10x running buffer (Bio-Rad, Cat # 161-0732, store at room temperature).
(2) ➔ 900 ml H_2O.

If 10x Tris/glycine/SDS buffer is not available you can prepare it as follows:

(1) ➔ 29 g Trizma base (Sigma T-1503 500 g).
(2) ➔ 144 g glycine (Bio-Rad 161-0717).
(3) ➔ 10 g SDS (Bio-Rad 161-0301 100 g).
(4) ➔ H_2O to 1 liter.
(5) ➔ Adjust pH = 8.3.

2.8.2 Cold transfer buffer

Prepare 1 day before, store at 4°C, good for 2 weeks.

(1) ➔ 100 ml of 10x transfer buffer (Bio-Rad, store at room temperature).
(2) ➔ 200 ml methanol.
(3) ➔ H_2O to 1 liter.

If 10x transfer buffer is not available you can prepare it as follows:

(1) ➔ 2.9 g Trizma base.
(2) ➔ 14.4 g glycine.
(3) ➔ 400 ml methanol.
(4) ➔ 1600 ml H_2O.
(5) ➔ Stir.
(6) ➔ Store in fridge until ready to use.

2.8.3 TBS-T 0.1%

Prepare 1 day before, store at 4°C, good for one week.

(1) ➔ 2 liter sterile H_2O.
(2) ➔ 4.84 g Trizma base.
(3) ➔ 16 g NaCl.
(4) ➔ Adjust pH to 7.6.
(5) ➔ Add 2 ml Tween 20 (last to add, remove pH meter prior to adding).

The preparation of TBS-T buffer is shown in Table 13.

Table 13. TBS-T 0.1% preparation

0.1% TBS-T for Western Blotting (Tris=0.02 M, pH 7.6)					
Chemicals	1000 ml	2000 ml	3000 ml	3500 ml	4000 ml
Tris base (g)	2.42	4.84	7.26	8.47	9.68
NaCl (g)	8	16	24	28	32
12 N HCl (ml)	1.29	2.57	3.85	4.49	5.13
Tween 20 (ml)	1	2	3	3.5	4
pH	7.6	7.6	7.6	7.6	7.6

Reagents needed for day two

2.8.4 5% non-fat milk (Prepare in the same day).
(1) ➔ 2.5 g of non-fat milk powder.
(2) ➔ 50 ml of 0.1% TBS-T.

2.8.5 1% non-fat milk.
(1) ➔ 0.5 g of non-fat milk powder.
(2) ➔ 50 ml of 0.1% TBS-T.

2.8.6 Get loading buffer (2X vial) out of 4°C and put it at room temperature for 1 hour.

2.8.7 Set heating block temperature to 95°C now.

2.8.8 Label 10 micro tubes, 1 to 10, for loading the wells:
(1) ➔ Tube 1: control.
(2) ➔ Tubes 2–9: samples.
(3) ➔ Tube 10: standard marker.

2.8.9 Get samples from deep freeze and place them on ice.

2.8.10 Loading buffer 2x (Use micro tubes):
(1) ➔ 380 ul Novex brand Tris-glycine SDS loading buffer (2x) small blue bottle, stored at 4°C. Let it warm up for one hour.
(2) ➔ 20 ul beta-mercaptoethanol (Bio-Rad 1610710), stored in hood (bad smell).

2.8.11 Loading buffer 1x (use micro tubes)
(1) ➔ 200 ul loading buffer 2x
(2) ➔ 200 ul running buffer 1x

2.8.12 Get loading instructions sheet (list of the specimens be worked with).

2.8.13 Get ice.

2.8.14 Label 10 micro tubes, 1 to 10, for loading the wells.
(1) ➔ Tube 1: control.
(2) ➔ Tubes 2 to 9: specimens.
(3) ➔ Tube 10: marker.
(4) ➔ Get specimens from deep freeze and place them in rack on ice.
(5) ➔ Prepare loading buffer 2x.
(6) ➔ Prepare loading buffer 1x.

When you prepare the micro tubes for loading, pipet as follows:
(1) ➔ Running buffer.
(2) ➔ Homogenate specimens.
(3) ➔ 2x loading buffer last.
(4) ➔ Mix well with vortex.

2.8.15 Heat tubes on heating block at 95°C for 1 minute (except for the marker tube).

2.8.16 Centrifuge for 1 minute.

2.8.17 Cool tubes on ice for at least 3 minute.

2.8.18 Set up your electrophoresis chamber.

2.8.19 Get one Novex gel out: remove tape at bottom of gel.

2.8.20 Rinse gel with sterile water cassette without removing the comb.

2.8.21 Now remove comb and wash gel with 1x running buffer at the sink using a pipette.

2.8.22 Place gel in electrophoresis chamber.

2.8.23 Pour 1x running buffer into middle chamber, check that it is water tight. The level must be above the loading wells of the gel and above the white bar. Get rid of any air bubbles, using a pipette.

2.**8.24 Load 20 ul of samples** into each well (clean tip with 1x running buffer between specimens).

2.8.25 Fill front and back chambers with cold 1x running buffer.

2.8.26 First electrophoresis:
(1) ➔ Set constant first.
(2) ➔ Constant: AMP.
(3) ➔ Voltage: 65 volts (or up to 90

volts).
(4) ➔ AMP: 0.02 AMP.
(5) ➔ Time: 30 minute.
(6) ➔ Press run.

After 30 min, change the setting to:
(1) ➔ Constant: AMP.
(2) ➔ Voltage: 105 volts (or up to 120 volts).
(3) ➔ AMP: 0.02 AMP.
(4) ➔ Time: 90 minutes.
(5) ➔ Press run.
(It does not take the exact 90 minutes, stop when blue line reaches the bottom).

2.8.27 During electrophoresis time, cut chromatography paper and nitro membrane into 8.5x8.5 cm.

2.8.28 Soaking
(1) ➔ Soak sponges with cold transfer buffer and remove any air trapped in them (this can be started 1 day before).
(2) ➔ Soak chromatography paper in the same container. Place the container and contents in the fridge for at lease 30 minutes at 4^0C to keep it cold.
(3) ➔ When blue line reaches middle of gel (about 60 minutes before second electrophoresis): Soak the nitro membrane in sterile H_2O for 10 minutes, then place it with the sponges and the chromatography papers in the container.

2.8.29 At the end of the first get electrophoresis:
(1) ➔ Remove gel cartridge and place it in the palm of your hand, face down, and open gently with scraper. Get rid of the back of the cartridge.
(2) ➔ Take the wet chromatography paper from the transfer buffer container and place it over the gel.
(3) ➔ Flip this upside down, the front of the cartridge is now on top.
(4) ➔ Insert putty knife to separate gel from cartridge cover. Discard front cartridge cover.
(5) ➔ Place chromatography paper which has the gel over it on a flat surface that is covered with parafilm. Cut excess perimeter of gel.
(6) ➔ Place the membrane on top of the gel. Do not allow the membrane to dry.
(7) ➔ Place the other wet chromatography paper over the membrane.
(8) ➔ Transfer this "sandwich" as is using the scraper and place it in the awaiting deep end of the gray module. It contains 2 wet sponges.

2.8.30 Note: For the electronic transfer (second electrophoresis), use the deep and thin ends of the blot module that have a gray padding.
Made the following sandwich:
(1) ➔ Sponges
(2) ➔ Sponges
(3) ➔ Sponges
(4) ➔ Chromatography paper
(5) ➔ Nitrocellulose membrane (Amersham, Hybond)
(6) ➔ Gel
(7) ➔ Chromatography paper
(8) ➔ Sponges
(9) ➔ Sponges
(10) ➔ Sponges

This sandwich sits in the blot module deep component. Sqeeze tight. Wet top of sandwich with a dropper using some cold transfer buffer.

2.8.31 Place the blot module in transfer chamber.
➔ Fill middle chamber with: cold transfer buffer from tupperware.

2.8.32 Fill front and thereby back chambers with remaining cold transfer buffer from tupperware.

2.8.33 Electronic transfer (second electrophoresis)
(1) ➔ Constant: v
(2) ➔ Voltage: 75
(3) ➔ AMP: 2
(4) ➔ Time: 90 minutes.

2.8.34 At the end of the 90 minutes, pull out the blot module, and lay aside all parts of the sandwich except for the membrane (shiny nitrocellulose membrane).

2.8.35 Grab it with large hemostat, and leave it to dry.

2.8.36 The sponges are re-usable, rinse them under faucet and save them. Discard gel and chromatography paper.

2.8.37 When the shiny membrane is dry, label the front side "f" and note which is top and bottom.

2.8.38 Blocking: Always work with the membrane face up.

(1) ➔ Add 50 ml of 5% non-fat milk into small glass container.
(2) ➔ Put in membrane, face up.
(3) ➔ Store blocked membrane 1-2 hours at room temperature or overnight at 4^0C.

2.8.39 Prepare 0.1% TBS-T (if you have not already done so).

End of day one

Day 2

2.8.40 Prepare primary antibody (1° ab) in 10 ul 0f 1% non-fat milk, normally the dilution ratio is 1/250-1/2000 (5–40 ul), just 10 minutes before use.

2.8.41 Drain blocking solution off membrane and container.

2.8.42. Pour primary antibody solution onto membrane, incubate for 1-2 hours at room temperature or overnight at 4^0C.

2.8.43 Washing:

To remove excess primary antibody (unbound). Place membrane in tupperware container.

(1) ➔ Wash with 200 ml of 0.1 % TBS-T and shake for 5 minutes.
(2) ➔ Wash with 50 ml of 0.1 % TBS-T and shake for 10 minutes.
(3) ➔ Wash with 50 ml of 0.1 % TBS-T and shake for 10 minutes.

2.8.44 Prepare secondary antibody (2° ab) in 10 ul 0f 1% non-fat milk, normally the dilution ratio is 1/500-1/5000 (2–20 ul). Place the membrane in the glass container, face up. Add 2° ab solution. Incubate for 1-2 hours at room temperature or overnight at 4^0C. This is a good time to prepare the stripping solution.

2.8.45 Washing:

To remove excess 2^0 ab (unbound). Place membrane in tupperware container.

(1) ➔ Wash with 200 ml of 0.1 % TBS-T and shake for 10 minutes.
(2) ➔ Wash with 50 ml of 0.1 % TBS-T and shake for 10 minutes.
(3) ➔ Wash with 50 ml of 0.1 % TBS-T and shake for 10 minutes.

2.8.46 Prepare chemiluminescent solution. Lights off.

Items needed for chemiluminescence:

(1) ➔ Wooden rack
(2) ➔ One 50 ml tube and two 15 ml tubes
(3) ➔ Cassette
(4) ➔ Black dish and cover
(5) ➔ Plastic wrap
(6) ➔ Scotch tape
(7) ➔ Tweezers
(8) ➔ Timer

Get the Amersham Reagent kit (RPN 2106) in the fridge.

(1) ➔ Pour 8 ml of bottle #1 in a 15 ml tube
(2) ➔ Pour 8 ml of bottle #2 in another 15 ml tube
(3) ➔ Combine and mix in 3rd tube (50 ml tube), just prior to usage.

2.8.47 Line X-ray cassette

2.8.48 Transfer the membrane into the flat black dish. Let it sit for 1 minute with the mixed reagents, no agitation.

2.8.49 Remove membrane from solution after 1 minute. Get rid of excess fluid by touching membrane. Corner onto paper towel.

2.8.50 Place membrane squarely in X-ray cassette. Cover membrane by folding up the plastic wrap. Get rid of air bubbles. Roll finger gently over plastic wrap. Tape down with scotch tape.

2.8.51 X-ray develop. If the picture you get is too light: repeat with longer exposure time. If the picture is you get is too dark: repeat with shorter exposure time.

2.8.52 Membrane stripping- prepare stripping solution

(1) ➔ 0.2 g SDS [0.2%]
(2) ➔ 0.375 g glycine [50 mM]
(3) ➔ 100 ml H_2O
(4) ➔ 200 ul HCl (12 N) [24 mM]
(5) ➔ Adjust ph to 2.6

After finishing with X-ray, you should put nitrocellulose membrane into stripping solution (however, if you need to prepare stripping solution after X-ray, place nitrocellulose membrane in 0.1% TBS-T to keep it from drying out. Once stripping solution is prepared, place membrane into stripping solution).

2.8.53 Stripping

Incubate membrane in stripping solution for 2 minutes with shaking.

2.8.54 Washing

Wash membrane: 3 times as follows:

(1) ➔ In 50 ml 0.1% TBS-T for 5 minutes.
(2) ➔ In 50 ml 0.1% TBS-T for 5 minutes.
(3) ➔ In 50 ml 0.1% TBS-T for 5 minutes.

(Optional, you could transfer membrane into fresh 0.1% TBS-T for overnight storage at 4°C).

2.8.55 Prepare primary beta-actin antibody.

(1) ➔ Sigma A5441 (-20^{0}c)
(2) ➔ Dilution: 1/10,000 - 1/1,000 (2 ul/20 ml - 10 ul/10 ml, by 1% non-fat milk).

2.8.56. Drain blocking solution off membrane.

2.8.57. Incubation:

Pour beta-actin antibody solution onto membrane. Agitate for a few minutes and incubate for one hour at room temperature or at 4^{0}C overnight.

End of day 2

Day 3

2.8.58 Washing

(1) ➔ Wash with 200 ml of 0.1 % TBS-T for 5 minutes.
(2) ➔ Wash with 50 ml of 0.1 % TBS-T for 5 minutes.
(3) ➔ Wash with 50 ml of 0.1 % TBS-T for 5 minutes.
(4) ➔ Wash with 50 ml of 0.1 % TBS-T for 5 minutes.

2.8.59 Prepare secondary antibody (2° ab) for beta-actin

(1) ➔ Amersham anti-mouse RPN 2108 (4^{0}C).
(2) ➔ Dilution is: 1/10,000 – 1/1,000.
(3) ➔ You need: 2 ul of ab + 20 ml to 10 ul of ab + 10 ml of 1% non-fat milk.
(4) ➔ Shake for 1 hour at room temperature or over night at 4^{0}C.

2.8.60 Washing (Place membrane in tupperware container):

(1) ➔ Wash with 200 ml of 0.1 % TBS-T for 5 minutes.
(2) ➔ Wash with 50 ml of 0.1 % TBS-T for 10 minutes.
(3) ➔ Wash with 50 ml of 0.1 % TBS-T for 10 minutes.
(4) ➔ Wash with 50 ml of 0.1 % TBS-T for 10 minutes.

2.8.61 Prepare chemiluminescent solution.

(1) ➔ Lights off.
(2) ➔ Get the Amersham kit, RPN 2106 in the fridge (4^{0}C).
(3) ➔ Pour ➔ 8 ml of bottle #1 in a 15 ml tube.
(4) ➔ And ➔ 8 ml of bottle #2 in another 15 ml tube.
(5) ➔ Combine and mix in 3rd tube just prior to usage.

2.8.62 Line X-ray cassette with plastic wrap

2.8.63 Transfer the membrane into the flat black dish.

Let sit for 1 minute, no agitation.

2.8.64 Remove membrane from solution after 1 minute.

Get rid of excess fluid by touching membrane corner onto paper towel.

2.8.65 Place membrane squarely in X-ray cassette. Cover membrane by folding up the plastic wrap. Get rid of air bubbles. Roll finger gently over plastic wrap. Tape down with scotch tape.

2.8.66 X-ray develop. If the picture you get is too light: repeat with longer exposure time. If the picture you get is too dark: repeat with shorter exposure time.

For beta-actin exposure time: 1 minute.

2.8.67 You can now wrap the membrane in plastic wrap and store it in the freezer, or put the membrane in 0.1 % TBS-T for 5 minutes and store at 4^0C. You have now done with the Western blot.

2.8.68 Suggested list of reagents and chemicals for western blotting test.

(1) ➔ NaCl: Sigma, S-9888.
(2) ➔ Antibodies and controls: Santa Cruz, Sigma, Pierce, etc, store at -20°C or 4°C depending on product instruction.
(3) ➔ EDTA: Sigma, E-5134.
(4) ➔ Igepal: Sigma, I-3021.
(5) ➔ PMSF (phenyl methyl sulfonyl fluoride): Sigma, P-7626, dissolved in 100% ethanol and kept at -20°C (50 ml is enough).
(6) ➔ Aprotinin: Sigma, A-1153 (concentration 1.34 mg/ml in H_2O and stored at 4°C).
(7) ➔ Leupeptin: Sigma, 151553 (concentration 10 mg/ml in H_2O and split into tubes with 20 ul each, store at -20°C.
(8) ➔ Tris-glycine SDS Novex brand 2x sample buffer: Novex LC 2676 (20 ml, blue bottle), kept at 4°C.
(9) ➔ Beta-mercaptol-ethanol: Bio-Rad, 1610710, store at room temperature.
(10) ➔ Tween 20 (polyoxyethylene): Sigma, P-1379.
(11) ➔ Trizma base: Sigma, T-1503, 500 g.
(12) ➔ Glycine: Biorad, 161-0717.
(13) ➔ Methanol, Sigma, M-1770.
(14) ➔ Chemiluminescent solution: Amersham, ECL, RPN 2106, 1-800-323-9750.
(15) ➔ Secondary antibody, Amersham, ECL, RPN 2108, anti-mouse or anti-rabbit, 4°C.
(16) ➔ Gel: Novex, EC 6035 (4-12 % Tris-glycine gel, 1.0 mm x 10 well.
(17) ➔ Nitrocellulose memrane by Hybond, Amersham, RPN 2020d (20 cm x 20 cm, 10 sheets). 0.45 micron. 1-800-323-9750.
(18) ➔ Labelon, transparency film, Labelon, XTR-660, 4.0 mil, 8.5 x 11 inches.
(19) ➔ SDS (sodium dodecylsulfate): Bio-rad, Cat# 161-0301 (100 g bottle).
(20) ➔ Non-fat milk: from normal supermarket.
(21) ➔ Multimark: Novex, LC 5725 (-20°C).

3. Enzyme-linked Immunosorbent Assay (ELISA)

3.1 Materials and Methods

Homogenize tissue with 5 times of protein extract buffer → centrifuge 10,000 rpm 20 minutes → 0.1 ml supernatant each well → over night at 4^oC → PBS with 0.5% BSA washing 3 X 3 minutes → 0.1 ml diluted primary antibody 1-2 hour at room temperature → PBS washing 3 X 3 minutes → 0.1 ml diluted secondary antibody 1-2 hour at room temperature → PBS washing 3 X 3 minutes → dye (0.2 ml pNPP) → 0.05 ml 3 N NaOH → O.D. (405 nm) measurement.

3.1.1 Extract buffer

50 mM Tris-HCl or 50 mM HEPES (pH 7.4)
150 mM NaCl
0.02% sodium azide
0.1% SDS
0.1 mg/ml phenylmethylsulfonyl fluoride (PMSF)
0.001 mg/ml aprotinin
1% Nonidet P-40 (NP-40) or 1% Triton X-100
(The half-life of a 0.02 mM aqueous solution of PMSF is about 35 minutes. PMSF is usually stored as a 10 mM or

100 mM stack solution 1.74 or 17.4 mg/ml in isopropanol at –20°C).

3.1.2 Homogenize sample under ice.

3.1.3 **Centrifuge sample** at 10,000 rpm for 20 minutes at 4°C.

3.1.4 **Keep supernatant at -70°C** until usage.

3.1.5 **PBS:** Phosphate-buffered saline (PBS), pH 7.4, 1000 ml (NaCl 8 g, KCl 0.2 g, Na_2HPO_4 1.44 g, KH_2PO_4 0.24 g, adjust to pH 7.4 with HCl). Add 0.5% BSA of 1% milk into PBS when washing processed. It can also use Dulbecco's PBS or try others. Instead of BSA, it can use gelatin or milk. Skim (0.5% to 1%) milk could reduce the non-specific reaction.

3.1.6 **Antibody:** Primary and secondary antibodies are normally 1:1000 to 1:2000 diluted by PBS and 0.1 ml each well.

3.1.7 **Dye:** Use alkaline phosphatase yellow (pNPP) liquid substrate as the dye for the ELISA (Derango et al. 1996). This product is supplied as a ready-to-use buffered alkaline phosphatase substrate p-nitro-phenylphosphate (pNPP). Prior to reaction with alkaline phosphatase, the substrate should appear as a colorless to pale yellow solution. It will develop a yellow reaction product when reacted with phosphatase in microwell applications. For the end-point assays, the reaction can be stopped with 0.05 ml/well of 3 N NaOH for every 0.2 ml of substrate reaction. Following the reaction with alkaline phosphatase, a yellow reaction product forms can be read at 405 nm.

3.2 Using Polyclonal Antibodies:

3.2.1 Antibody purification: Protein G column is the best for this purpose.

3.2.2 Conjugate: Making conjugate is the most important part (e.g. horseradish peroxidase).

3.2.3 96-well plate: Making the solid phase using the 96-well plate.

3.3 Buffers and other reagents:

3.3.1 Plate buffer: 0.1 M Sodium carbonate buffer, pH 9.5.

3.3.2 Reaction buffer: 0.01 M Sodium phosphate buffer, pH 7.2, 0.15 M NaCl (PBS), 0.5% BSA, 0.05% thimerosal; You can also use Dulbecco's PBS or try others. Instead of BSA, you can use gelatin. Skim (0.5% to 1%) milk could reduce the non-specific reaction.

3.3.3 Washing buffer: 0.05% Tween-20, 0.01 M Sodium phosphate buffer, pH 7.2 or 0.05% Tween-20, 0.15 M NaCl.

3.3.4 Developing buffer: 0.05 M Sodium acetate buffer, pH 5.5.

3.3.5 TMB stock solution: Tetramethylbenzidine 1 mg/ml in DMSO.

3.4 Making Conjugate:

3.4.1 Nakane's method.

3.4.2 Glutaraldehyde method.

3.4.3 Maleimide method.

3.5 Steps:

3.5.1 2 mg Horseradish peroxidase (HRP) in 1 ml water: A.

3.5.2 21.4 mg $NaIO_4$ (never to be $NaIO_3$) in 1 ml water: B.

3.5.3 100 micro-l of B into A: Color will change to the dark green!

3.5.4 Wait for 10 min at room temperature.

3.5.5 Put into the dialysis tube (such as Molecular cut off 20,000).

3.5.6 F. Put the tube into 5 mM NaAcetate buffer, pH 4.0 in a 2 to 3 l flask.

3.5.7 Dialysis overnight: Color will change to the gold.

3.5.8 Raise the pH of the HRP solution to pH = 9.0 by the addition of 0.2 M NaCarbonate buffer, pH 9.5 (try an aliquot of 0.05 ml).

3.5.9 **Mix with the antibody solution** (8 mg of IgG in 1 ml), which has been pre-dialyzed to 0.01 M NaCarbonate buffer, pH 9.0 overnight.

3.5.10 Incubate the mixture for 2 hr at room temperature.

3.5.11 Put freshly prepared 0.1 ml, 0.1 M $NaHBr_4$ in water to the solution.

3.5.12 Incubate at 4 degree for 2 hr.

3.5.13 Put the mixture into a dialysis tube and dialyze against PBS overnight.

3.5.14 Now the conjugate solution is ready for use. Add thimerosal to a final concentration of 0.02% for preservation. Add glycerol to a final concentration of 10% (optional). If you stock the conjugate solution for

a long period such as years, stock it at -80 degree. But, in this case, don't repeat freeze-thaw. You can stock the solution at 4 degree at least 6 months.

3.6 Preparation of ELISA Plate: This will take 2 hr to overnight. Overnight is preferable.

3.6.1 Dilute antibody (IgG) by Plate buffer: 5 to 10 micro-g/ml.

3.6.2 Put the diluted antibody solution, 0.1 ml to the wells of 96-well ELISA plate.

3.6.3 Incubate for 2 hr at room temperature or overnight at 4 degree.

3.6.4 Discard the solution and wash the plate three times by washing buffer. Put 200 micro-l into wells using micro-pipette or just put the Washing buffer using some devices.

3.6.5 Discard the Washing buffer by tapping against paper towel.

3.6.6 Put 0.15 to 0.2 ml of reaction buffer. Now, the plate is ready for use. You can stock the plate at least for 6 months. Take care not to dry up the plate.

3.7 Using Monoclonal Antibodies:

3.7.1 Antibody purification: Antibody purification step is the only special part comparing with materials and methods in using polyclonal antibody. For most monoclonals, except for IgM, Protein G column will be good for the practical use. If you failed by this method, confirm your procedure again before proceeding to the other methods such as DEAE column. When your monoclonal antibody is IgM, try Protamine column combined with molecular sieving column. Others are the same as above mentioned in "Using polyclonal antibodies.

3.7.2 Try skim milk (any kind of powdered milk such as powdered milk for babies) instead of BSA: It's really cheap! Try 1% to 3%. It will decrease the background!! Thing is stability. It will form precipitate if you keep it for a few months. If you are running many plates, it is good alternative.

4. Northern blotting and Southern blotting

Besides the Western blotting, Northern blotting and Southern blotting are also useful in the biochemical application. The following is a brief description of the Northern and Southern blottings.

4.1 Northern blotting

The northern blotting is a technique to detect RNA and it is used in molecular biology research to study gene expression. It takes its name from the similarity of the procedure to the Southern blotting procedure (named for biologist Edwin Southern). This technique was developed by James Alwine while working as a postdoc at Stanford University.

A notable difference in the procedure (as compared with the Southern blot) is the addition of formaldehyde in the agarose gel, which acts as a denaturant. As in the Southern blot, the hybridization probe may be made from DNA or RNA.

A variant of the procedure known as the reverse northern blot was occasionally (although, infrequently) used. In this procedure, the substrate nucleic acid (that is affixed to the membrane) is a collection of isolated DNA fragments, and the probe is RNA extracted from a tissue and radioactively labelled.

The use of DNA microarrays that have come into widespread use in the late 1990s and early 2000s is more akin to the reverse procedure, in that they involve the use of isolated DNA fragments affixed to a substrate, and hybridization with a probe made from cellular RNA. Thus the reverse procedure, though originally uncommon, enabled the one-at-a-time study of gene expression using Northern analysis to evolve into gene expression profiling, in which many (possibly all) of the genes in an organism may have their expression monitored.

(http://en.wikipedia.org/wiki/Northern_blot)

4.2 Southern blotting

A Southern blotting a technique to detect DNA. It is a method in molecular biology of enhancing the result of an agarose gel electrophoresis by marking specific DNA sequences. The method is named after its inventor, the British biologist Edwin Southern. This caused other blotting methods to be named similarly as plays on Southern's name (for example, Western blot, Northern blotting, Southwestern blotting, or Hawaiian

blotting).

4.3 Methods

The gel from the DNA electrophoresis is treated with an alkaline solution (typically containing sodium hydroxide) to cause the double-stranded DNA to denature, separating it into single strands. Denaturation is necessary so that the DNA will stick to the membrane and be hybridized by the probe. Restriction endonucleases are used to break the DNA strands into fragments.

A sheet of nitrocellulose or nylon membrane is placed on top of the gel. Pressure is applied evenly to the gel (either using suction, or by placing a stack of paper towels and a weight on top of the membrane and gel). This causes the DNA to move from the gel onto the membrane by capillary action, where it sticks.

The membrane is then baked (in the case of nitrocellulose) or exposed to ultraviolet radiation (nylon) to permanently crosslink the DNA to the membrane.

The membrane is now treated with a hybridization probe - an isolated DNA molecule with a specific sequence that pairs with the appropriate sequence. The probe DNA is labelled so that it can be detected, usually by incorporating radioactivity or tagging the molecule with a fluorescent or chromogenic dye. In some cases, the hybridization probe may be made from RNA, rather than DNA.

After hybridization, excess probe is washed from the membrane, and the pattern of hybridization is visualized on x-ray film by autoradiography in the case of a radioactive or fluorescent probe, or by development of color on the membrane itself if a chromogenic detection is used.

Discussion

Western blotting and ELISA have widely application in scientific researches, industry and medical practice. For example, in medical, the confirmatory HIV test employs a western blot to detect anti-HIV antibody in a human serum sample. Proteins from known HIV-infected cells are separated and blotted on a membrane as above. Then, the serum to be tested is applied in the primary antibody incubation step; free antibody is washed away, and a secondary anti-human antibody linked to an enzyme signal is added. The stained bands then indictate the proteins to which the patient's serum contains antibody. A Western blotting is also used as the definitive test for Bovine spongiform encephalopathy (BSE, commonly referred to as 'mad cow disease'). Some forms of Lyme disease testing employ Western blotting. (http://en.wikipedia.org/wiki/Western_blot).

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