OPTIMIZATION OF GLUCOSE PRODUCTION FROM LIQUID PINEAPPLE WASTE USING IMMOBILIZED INVERTASE IN PVA-ALGINATE-SULFATE BEADS

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This dissertation is dedicated to my beloved family members for their endless support and encouragement.

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ABSTRACT

Pineapple (*Ananas cosmosus*) waste is known as a byproduct of the pineapple processing industry and typically consists of residual pulp, peels and skin. The liquid pineapple waste used in this study consists of sucrose (76.8%), glucose (12.6%) and fructose (10.5%). Through an enzymatic hydrolysis process, the sucrose in the waste can be converted into glucose and fructose. In this study, the conversion of sucrose to glucose was performed with the help of invertase enzyme. PVA-alginate-sulfate beads were used as an entrapment matrix to immobilize invertase using entrapment-crosslinking method. RSM comprising Box-Behnken design was successfully applied in this study as a tool to evaluate the interactive effects and to obtain the optimum operating conditions for the enzymatic hydrolysis. Four parameters were varied which was pH (4-6), temperature (40-60^{o}C), agitation rate (100-200 rpm) and amount of immobilized beads (3-6 g). Results revealed that the highest sugar content in the liquid pineapple waste was sucrose which was 79.6 g/L. While for the cations content, potassium had the highest composition which was 2195.0 mg/L whilst phosphorus was the highest anion content with the value of 79.23 mg/L. Meanwhile, the pH of the pineapple waste was 4.0. Hydrolysis of the liquid pineapple waste showed that 95% of sucrose content in the waste was hydrolyzed to glucose after 3 hours of hydrolysis and the overall glucose generated from the hydrolysis was approximately 1131% of its original content. The optimum operating conditions derived via RSM were: pH 4.74, temperature 50.11^{o}C, agitation rate of 147.68 rpm and amount of immobilized beads used were 4.45 g. The experimental yield of glucose was found to be 111.42 g/L (850.5%) and sucrose 25.70 g/L (32.3%) under optimum conditions, which correlated well with the maximum predicted value of glucose and sucrose 110.13 g/L (840.7%) and 25.93 g/L (32.6%) respectively.

ABSTRAK

Sisa nanas yang dikenali sebagai hasil sampingan daripada industri pemprosesan nanas biasanya terdiri daripada pulpa kulit, sisa dan kulit. Sisa nanas cecair yang digunakan dalam kajian ini terdiri daripada sukrosa (76.8%), glukosa (12.6%) dan fruktosa (10.5%). Melalui proses hidrolisis enzim, sukrosa dalam buangan boleh ditukar menjadi glukosa dan fruktosa. Dalam kajian ini, penukaran sukrosa kepada glukosa telah dilakukan dengan bantuan enzim invertase. Manik PVA-alginat-sulfat telah digunakan sebagai matriks perangkap untuk menyekat gerak invertase dengan menggunakan kaedah perangkap-silang. RSM yang terdiri daripada reka bentuk Box-Behnken telah berjaya digunakan dalam kajian ini sebagai alat untuk menilai kesan interaktif dan untuk mendapatkan keadaan optimum untuk hidrolisis enzim. Empat parameter telah diubah, antaranya ialah pH (4-6), suhu (40-60^{o}C), kadar pergolakan (100-200 rpm) dan jumlah manik sekatgerak (3-6 g). Keputusan menunjukkan bahawa kandungan gula tertinggi dalam sisa nanas cecair adalah sukrosa dengan nilai 79.6 g/L. Untuk kandungan kation, kalium mempunyai komposisi tertinggi iaitu 2195.0 mg/L manakala fosforus merupakan kandungan anion tertinggi dengan nilai 79.23 mg/L. Sementara itu, pH sisa nanas adalah 4.0. Hidrolisis sisa nanas cecair menunjukkan bahawa 95% daripada kandungan sukrosa dalam buangan dihidrolisis kepada glukosa selepas 3 jam hidrolisis dan glukosa keseluruhan yang dijana daripada hidrolisis adalah kira-kira 1131% daripada kandungan asalnya. Keadaan optimum yang diperolehi melalui RSM adalah: pH 4.74, suhu 50.11^{o}C, kadar pergolakan 147.68 rpm dan jumlah manik sekatgerak digunakan adalah 4.45 g. Jumlah hasil glukosa yang diperolehi melalui eksperimen ialah 111.42 g/L (850.5%) dan sukrosa 25.70 g/L (32.3%) di bawah syarat-syarat optimum, yang juga berkait rapat dengan ramalan maksimum nilai glukosa dan sukrosa 110.13 g/L (840.7%) dan 25.93 g/L (32.6%) masing-masing.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BOD	Biochemical Oxygen Demand
CCRD	Central Composite Rotatable Design
CEH	Cross-linked Ethylenic Homopolymeric
DNS	Dinitrosalicylic Acid
FESEM	Field Emission Scanning Electron Microscope
H_2SO_4	Sulfuric acid
HCl	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
IU	International Unit
IV	Intravenous
MPIB	Malaysian Pineapple Industrial Board
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
PVA	Poly (vinyl alcohol)
RSM	Response Surface Methodology
SD	Standard Deviation
SEM	Scanning Electron Microscope

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Pineapple waste is known as a byproduct of the pineapple processing industry and typically consists of residual pulp, peels and skin. Based on Nigam (1999), the highest concentration of sugar in liquid pineapple waste was sucrose compared to glucose and fructose. Sucrose is an organic compound which is better known as table sugar. It is sometimes also called saccharose which is odorless, white, crystalline powder that has a sweet taste and plays an essential role in human nutrition. The molecule is disaccharide which composes of fructose and glucose (Bryan, 1990).

Invertase is a yeast derived enzyme which can convert sucrose to a mixture of monosaccharides that consist of fructose and glucose via hydrolysis reaction. The official name for invertase is beta-fructofuranosidase (EC 3.2.1.26). This enzyme is commonly used in artificial honey production and food (confectionary) (Sahmetlioglu *et al*., 2006). Invertase hydrolyzes sucrose via the hydrolysis of the terminal nonreducing β-fructofuranoside residues in β-fructofuranosides. Sucrose contains a beta-D-fructose molecule and an alpha-D-glucose molecule linked by an alpha-1, 4-glycosidic bond.

When this bond is cleaved in a hydrolysis reaction, an equimolar mixture of fructose and glucose is generated (Sanjay and Sugunan, 2006).

Immobilization defines localization or confined so that it can be reused continuously. The first ever attempt to immobilize a biocatalyst was conducted in the year 1953, while in 1969 an immobilized enzyme was used for the first time in an industrial process (Malcata and Hill, 1991). Since then, this technique has gained more and more importance, and thus a wide variety of immobilized enzymes are now employed in the chemical, pharmaceutical and food industries (Malcata and Hill, 1991). Besides showing higher activity and good stability with repetitive use, enzymes immobilized in PVA also possess higher thermal stability, which contributes to the increasing of the economic viability of any biosynthetic processes (Szczęsna-Antczak and Galas, 2001).

In this study, PVA-alginate-sulfate beads will be used as an entrapment matrix to immobilize invertase. PVA is a synthetic polymer which is cheap, nontoxic to organism, and has been assigned for biocatalyst immobilization. PVA was introduced as an alternative immobilization for over three decades ago (Hashimoto and Furukawa, 1987). PVA is beneficial in various applications especially in the biomedical and pharmaceutical field because of its desirable characteristics (Hassan and Peppas, 2000). Besides that, PVA has a simple chemical structure and certain modifications are made possible by simple chemical reactions (Bruno *et al*., 2005).

1.2 Problem Statement

The pineapple industry in Malaysia produces large quantities of solid and liquid wastes. The untreated liquid pineapple waste is discharged as an effluent into the nearest stream, which eventually contributes to the decreasing of pH and BOD, causing bad odors, and consequently, the stream serves as an unsuitable place for aquatic lives. These circumstances would no doubt lead to the change in the stream ecosystem and undeniably pose serious environmental problems.

If the liquid pineapple waste could be transformed into products that would be beneficial to human lives as sugars and organic acid, this would definitely be advantageous towards sustainable technology. Thus, to overcome this problem, glucose in the liquid pineapple waste will be optimized so that it could be beneficial in many ways. Hence, with the help of immobilized invertase in PVA-alginate-sulfate beads, the conversion of sucrose into glucose and fructose can be achieved via hydrolysis process. Glucose is then optimized to serve as a useful product to human lives, environment and be advantageous towards sustainable technology.

1.3 Objectives

This study aims to optimize the production of glucose by hydrolyzing sucrose in the liquid pineapple waste using immobilized invertase in PVA-alginate-sulfate beads. The overall objectives of this study are:

a) To characterize the compositions of liquid pineapple waste and to pretreat the waste.

b) To immobilize invertase in PVA-alginate-sulfate beads (using entrapment-crosslinking method).

c) To optimize the production of glucose during hydrolysis of sucrose using the immobilized invertase in PVA-alginate-sulfate beads.

1.4 Significance of the Study

Pineapple waste was characterized as a waste that is rich with glucose, sucrose and fructose. In the research conducted by Mohd Zain *et al*. (2010), immobilized invertase was used to hydrolyze sucrose in the pineapple waste.

This study will be further narrowed down to optimize the production of glucose during the hydrolysis process. The significance of this study is to make glucose a beneficial product to human lives and environment and also be advantageous towards sustainable technology.

Among the uses of glucose are it can be used as feed stock for farm livestock such as cows, goats, and sheep. Glucose can also be purified and used as artificial flavoring in food and confectionary. Besides that, it can also be used as a precursor to make vitamin C (L-ascorbic acid) in the Reichstein process. Glucose can also be used as a feed production of citric acid, bioethanol, gluconic acid, sorbitol, and polylactic acid. It can also be used in medication therapy such as a constituent in intravenous (IV) drips. Production of glucose can act as an intermediate substrate to products with a higher market demand such as ethanol and lactic acid.

1.5 Scope of Study

The scope of this study mainly covers the optimization of glucose production from liquid pineapple waste. The experiments carried out in this study composed of three stages. The first stage involved characterization of physical and chemical properties of the liquid pineapple waste and pretreatment of the waste. The second stage was immobilization of invertase in PVA-alginate-sulfate beads to be used for hydrolysis of sucrose.

The last stage involved the optimization of glucose production from liquid pineapple waste using the immobilized invertase in PVA-alginate-sulfate beads. This stage included the identification of significant factors that influenced glucose production and suggested optimal values for each variable. The predicted glucose production from statistical analysis was compared to the actual production of glucose via Design-Expert® 6.0.4 software.

Response Surface Methodology (RSM) comprising Box-Behnken design was successfully applied in this study as a tool to evaluate the interactive effects and to obtain the optimum operating conditions for enzymatic hydrolysis of liquid pineapple waste. RSM is an effective statistical technique for the investigation of complex processes.

REFERENCES

Ahmad, S., Anwar, A. and Saleemuddin, M. (2001). Immobilization and Stabilization of Invertase on *Cajanus cajan* Lectin Support. *Journal of Bioresource Technology*. 79,121-127.

American Public Health Association. (1995). *Standard Methods*. (19th ed.) Washington D. C: American Public Health Association (APHA).

Azmalisa, T., Wan Asma, I., Zulkafli, H. and Norazwina, Z. (2010). Optimization of Glucose Production from Oil Palm Trunk via Enzymatic Hydrolysis. *The 13th Asia Pacific Confederation of Chemical Engineering Congress (APCChE'2012)*. October 5-8, 2010. Taipei, 100-150.

Bosque-Sendra, J. M., Pescarolo, S., Cuadros-Rodriguez, L., Garcia-Campana, A. M. and Almansa-Lopez, E. M. (2001). Optimizing Analytical Methods using Sequential Response Surface Methodology: Application to the Pararosaniline Determination of Formaldehyde. *Journal of Analytical Chemistry*. 369, 715-718.

Bruice, P. Y. (2001). *Organic Chemistry*. (3rd ed.) Upper Saddle River, N. J.: Prentice Hall.

Bruno, L. M., Coelho, J. S., Melo, F. H. M. and Lima, J. L. (2005). Immobilization of Enzymes and Cells. *Journal of Microbiology Biotechnology*. 2, 189-192.

Bryan, W. L. (1990). Solid State Fermentation of Sugar in Sweet Surghum. *Journal of Enzyme Microbiology Technology*. 12(6), 437-442.

Chen, K. C. and Lin, Y. F. (1994). Immobilization of Microorganisms with Phosphorylated Polyvinyl Alcohol (PVA) Gel. *Journal of Enzyme and Microbial Technology*. 16(1), 79-83.

Clarke, J. B. and Sokoloff, L. (1999). Circulation and Energy Metabolism of the Brain. *Basic neurochemistry*. (6th ed.) Siegel, G. J. (Ed.) (pp. 637-669). Philadelphia: Lippincott Raven.

Clesscery, L. S., Greeberg, A. E. and Trussell, R. R. (1989). *Standard Methods for Examination of Water and Waste Water*. New York: American Public Health Association.

Correia, T., Papayannopoulos, V., Panin, V., Woronoff, P., Jiang, J., Vogt, T. F. and Irvine, K. D. (2003). Molecular Genetic Analysis of the Glycosyltransferase Fringe in Drosophila. *Journal of Process Biochemistry*. 100(11), 6404-6409.

Degiorgi, C. F., Pizarro, R. A., Smolko, E. E., Lora, S. and Carenza, M. (2002). Hydrogels from Immobilization of Bacteria Used in the Treatment of Metal-Contaminated Waste. *Journal of Radiation Physics and Chemistry*. 63, 109-113.

Emregul, E., Sungur, S. and Akbulut, U. (2006). Polyacrylamide-gelatine Carrier System Used for Invertase Immobilization. *Journal of Food Chemistry*. 97(4), 591-597.

Gough, S., Barron, N., Zubov, A. L., Lozinsky, V. I. and McHale, A. P. (1998). Production of Ethanol from Molasses at 45°C Using *Kluyveromyces marxianus* IMB3 Immobilized in Calcium Alginate Gels and Poly(vinyl alcohol) Cryogel. *Journal of Bioprocess Engineering*. 19(2), 87-90.

Hashimoto, S. and Furukawa, K. (1987). Immobilization of Activated Sludge by PVA-Boric Acid Method. *Journal of Biotechnology and Bioengineering*. 30, 52-59.

Hassan, C. M. and Peppas, N. A. (2000). Cellular PVA Hydrogels Produced by Freeze/Thawing. *Journal of Applied Polymer Science*. 76, 2075-2079.

Idris, A., Inane, B. and Hassan, M. N. (2004). Overview of Waste Disposal and Landfills/dumps in Asian Countries. *Journal of Material Cycles and Waste Management*. 6, 104-110.

Idris, A., Mohd Zain, N. A. and Suhaimi, M. S. (2008). Immobilization of Baker's Yeast Invertase in PVA-Alginate Matrix using Innovative Immobilization Technique. *Journal of Process Biochemistry*. 43, 331-338.

Işik, S., Alkan, S., Toppare, L., Cianga, I. and Yağci, Y. (2003). Immobilization of Invertase and Glucose Oxidase in Poly-2-methylbutyl-2-(3-thienyl) Acetate/polypyrrole Matrices. *Journal of European Polymer*. 39, 2375-2381.

Kannan, N., Rajakumar, A. and Rengasamy, G. (2004). Optimization of Process Parameters for Adsorption of Metal Ions on Straw Carbon by Using Response Surface Methodology. *Journal of Environmental Technology*. 25, 513-522.

Katahira, S., Ito, M., Takema, H., Fujita, Y., Tanino, T., Tanaka, T., Fukuda, H. and Kondo, A. (2008). Improvement of Ethanol Productivity during Xylose and Glucose Co-fermentation by Xylose-assimilating *S. cerevisiae* via Expression of Glucose Transporter Sut1. *Journal of Enzyme and Microbial Technology*. 43(2), 115-119.

Kaushik, P. and Malik, A. (2009). Fungal Dye Decolourization: Recent Advances and Future Potential. *Journal of Environment International*. 35, 127–141.

Khoo, K. M. and Ting, Y. P. (2001). Biosorption of Gold by Immobilized Fungal Biomass. *Journal of Biochemistry Engineering*. 8, 51-59.

Kourkoutas, Y., Bekatoroua, A., Banat, I. M., Marchant, R. and Koutinas, A. A. (2004). Immobilization Technologies and Support Materials Suitable In Alcohol Beverages Production: A Review. *Journal of Food Microbiology*. 21, 377-397.

Long, Z., Huang, Y., Cai, Z., Cong, W. and Ouyang, F. (2004). Immobilization of *Acidithiobacillus ferrooxidans* by a PVA-boric Acid Method for Ferrous Sulphate Oxidation. *Journal of Process Biochemistry*. 39, 2129-2133.

Malaysian Pineapple Industrial Board (MPIB), 2007. Wisma Nanas, No.5, Jalan Padi Mahsuri, Bandar Baru Uda, 81200, Johor Bahru, Johor.

Malaysian Pineapple Industrial Board (MPIB), 2010. Wisma Nanas, No.5, Jalan Padi Mahsuri, Bandar Baru Uda, 81200, Johor Bahru, Johor.

Malcata, F. X. and Hill, C. G. (1991). Use of a Lipase Immobilized in a Membrane Reactor to Hydrolyze the Glycerides of Butter Oil. *Journal of Biotechnology Bioengineering*. 38, 853-868.

Mansur, H. S., Sadahira, C. M., Souza, A. N. and Mansur, A. A. (2008). FTIR Spectroscopy Characterization of Poly (Vinyl Alcohol) Hydrogel with Different Hydrolysis Degree and Chemically Crosslinked with Glutaraldehyde. *Journal of Material Science and* Engineering. 28, 539-548.

Massart, D. L., Vandeginste, B. G. M., Buydens, L. M. C., de Jong, S., Lewi, P. J. and Smeyers-Verbeke, J. (2003). *Handbook of Chemometrics and Quali-metrics Part A*. Elsevier, Amsterdam.

Miller, G. L. (1959). Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Journal of Analytical Chemistry*. 31, 426-428.

Miloski, K., Wallace, K., Fenger, A. and Schneider, E. (2008). Comparison of Biochemical and Chemical Digestion and Detection Methods for Carbohydrates. *American Journal of Undergraduate Research*. 7(2), 48-52.

Mohd Zain, N. A., Suhaimi, M. S. and Idris, A. (2010). Hydrolysis of Liquid Pineapple Waste by Invertase Immobilized in PVA-alginate Matrix. *Journal of Biochemical Engineering*. 50, 83-89.

Nagadomi, H., Hiromitsu, T., Takeno, K., Watanabe, K. and Sasaki, K. (1999). Treatment of Aquarium Water by Denitrifying Photosynthetic Bacteria Using Immobilized Polyvinyl Alcohol Beads. *Journal of Bioscience and Bioengineering*. 87(2), 189-193.

Nigam, J. N. (1999). Continuous Ethanol Production from Pineapple Cannery Waste. *Journal of Biotechnology*. 72, 197-202.

Payne, A. and Persoz, J. F. (1833). A Guide to IUPAC Nomenclature of Organic Compounds. *Academic press New York*.

Pillay, V., Govender, S., Chetty, D. J., Essack, S. Y., Dangor, C. M. and Govender, T. (2005). Optimization and Characterization of Bioadhesive Controlled Release Tetracycline Microspheres. *Journal of Pharmaceutics*. 306 (1), 24-40.

Preu, M., Guyot, D. and Petz, M. (1998). Development of a Gas Chromatography Mass Spectrometry Method for the Analysis of Aminoglycoside Antibiotics using Experimental Design for the Optimization of the Derivatisation Reactions. *Journal of Chromatography*. 818, 95-108.

Rana, P., Mohan, N. and Rajagopal, C. (2004). Electrochemical Removal of Chromium from Wastewater by Using Carbon Aerogel Electrodes. *Journal of Water Res*. 38, 2811-2820.

Sahmetlioglu, E., Yűrűk, H., Toppare, L., Cianga, I. and Yağci, Y. (2006). Immobilization of Invertase and Glucose Oxidase in Conducting Copolymers of Thiophene Functionalized Poly(vinyl alcohol) with Pyrrole. *Journal of Reactive & Functional Polymers*. 66(3), 365-371.

Sanjay, G. and Sugunan, S. (2005). Invertase Immobilised on Montmorillonite: Reusability Enhancement and Reduction in Leaching. *Journal of Food Chemistry*. 6(1), 81-86.

Sanjay, G. and Sugunan, S. (2006). Enhanced pH and Thermal Stabilities of Invertase Immobilized on Montmorillonite K-10. *Journal of Food Chemistry*. 94, 573-579.

Shervan, B. and Ida Idayu, M. (2012). Optimization of Glucose Production from Oil Palm Trunk through Acid Hydrolysis. *International Conference on Biotechnology, Nanotechnology and its applications (ICBNA'2012)*. March 17-18, 2012. Bangkok, 102-220.

Souza, A., Walter, N. L., dos Santos, S. and Ferreira, L. C. (2005). Application of Box-Behnken Design in the Optimization of an Online Preconcentration System Using Knotted Reactor for Cadmium Determination by Flame Atomic Absorption Spectrometry. *Spectrochimica Acta Part B*. 60, 737-742.

Szczęsna, A. M. and Galas, E. (2001). *Bacillus subtilis* Cells Immobilised in PVA-cryogels. *Journal of Biomolecular Enginee*ring. 17, 55-63.

Tanaka, K., Zakpaa, D., Hilary, S. and Ishizaki, A. (1999). Investigation of the Utility of Pineapple Juice and Pineapple Waste Material as Low-cost Substrate for Ethanol Fermentation by *Zymomonas mobilis. Journal of Bioscience and Bioengineering*. 87(5), 642-646.

Tanioka, A., Yokoyama, Y. and Miyasaka, K. (1998). Preparation and Properties of Enzyme Immobilized Porous Polypropylene Films. *Journal of Colloid Interface Science*. 200, 185-187.

Tipton, K and Boyce, S (2000). History of the Enzyme Nomenclature System. *Oxford Journals Life Sciences & Mathematics & Physical Sciences*. 16 (1), 34-40.

Tomotani, E. J and Vitolo, M. (2007). Production of High Fructose Syrup Using Immobilized Invertase in a Membrane Reactor. *Journal of Food Engineering*. 80, 662-667.

Tümtürk, H., Arslan, F., Disli, A. and Tufan, Y. (2000). Immobilization of Invertase Attached to a Granular Dimmer Acid-co-alkyl Polyamine. *Journal of Food Chemistry*. 69, 5-9.

Uhlich, T., Ulbricht, M. and Tomaschewski, G. (1996). Immobilization of Enzymes in Photochemically Cross-linked Polyvinyl Alcohol. *Journal of Enzyme and Microbial Technology*. 19(2), 124-131.

Velaśquez, P., Leinen, D., Pascual, J., Ramos-Barradoa, J. R., Cordova, R., Gómez, H. and Schrebler, R. (2001). XPS, SEM, EDX and EIS Study of an Electrochemically Modified Electrode Surface of Natural Chalcocite (Cu_2S). *Journal of Electroanalytical Chemistry*. 510, 20-28.

Wahidin, S. (2006). *Identification of Important Factors That Influence the Production of Lactic Acid Fermentation by Immobilized Lactobacillus delbrueckii Using Waste as Substrate*. Master of Science, Universiti Teknologi Malaysia, Skudai.

Walters, F. H. and Qiu, H. C. (1992). The Use of a Box-Behnken 3 Factor Design to Study the Paper Chromatographic-separation of Several Amino-acid Hydroxamates. *Journal of Environmental Technology*. 25, 1131-1142.

Wu, K. Y. and Wisecarver, K. D. (1992). Cell Immobilization Using PVA Crosslinked with Boric Acid. *Journal of Biotechnology and Bioengineering*. 29, 447-449.

Yujian, W., Xiaojuan, Y., Hongyu, L. and Wei, T. (2006). Immobilization of *Acidithiobacillus ferroxidans* with Complex of PVA and Sodium Alginate. *Journal of Polymer Degradation and Stability. 91*, 2408-2414.

Zougagh, A., de Torres, A. G., Alonso, E. V. and Pavon, J. M. C. (2004). Automatic Online Preconcentration and Determination of Lead in Water by ICP-AES Using a TS-microcolumn, Talanta. *Journal of Analytical Chemistry.* 503-510.