

EXTERNAL PIXE – A NOVEL APPROACH FOR DETERMINING TRACE ELEMENTS IN FERMENTATION PROCESS

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ABSTRACT: External Particle Induced X-ray Emission technique was employed in the study of submerged fermentation of ethanol from Jaggery using *Saccharomyces cerevisiae* as the organism. Effect of KNO₃ as a nitrogen source was studied and the uptake of the supplement was monitored. A Proton beam of 3MeV from the 3MV Tandem type pelletron accelerator was used for the multi-elemental analysis. Apart from K, other elements like Cr, Fe, Cu and Zn were identified. The sensitivity of the technique was also determined by varying the sample size between 2mm and 10mm.

Key words: External PIXE, Jaggery, Potassium ions, ethanol.

INTRODUCTION

Trace elemental analysis of clinical, biological, food and beverage samples established a route to suggest diagnosis, take protective measures, assure quality control and protect environment (Andrew Taylor, et al., 1999). Since the last two decades, several methods were developed to estimate trace as well as ultra trace elements. The information about sample preparation and their handling for various techniques including analytical techniques available were reviewed (Cornelis, et al., 1998; Sanz Medel, 1998). It is the nature of sample that mainly determines the technique to be used.

The various techniques used for the estimation of elements in biological samples include Atomic Absorption Spectroscopy (AAS), Mass Spectroscopy (MS), Atomic Emission Spectroscopy (AES), Atomic Fluorescence Spectroscopy (AFS), X-Ray Fluorescence (XRF) and Particle Induced X-ray Emission (PIXE) (Aucelio, et al., 1997; Kovala, et al., 1997; Rosen, 1997; Bhuloka Reddy, et al., 2002; Preoteasa, et al., 2004). Each of these techniques have a separate sample preparation method which include drying (Cabrera-Vique, et al., 1997), treatment with complexing agents (El-Brashy and Al-Ghannam, 1997), atomization (Batchelor, 1998), digestion with acids (Batel, et al., 1998) etc. Some of them require separation of sample constituents by High Performance Liquid Chromatography (HPLC) (Nowak Michael and Seubert Andreas, 1998) and Gas Chromatography (GC) (Uden, et al., 1998). Most of the techniques being used are destructive, while PIXE is a highly sensitive but relatively non-destructive technique. It has been proved outstanding in determination of the concentrations of various elements at ppm levels (Preoteasa, et al., 2004). PIXE has been used in both forms as vacuum PIXE and external PIXE. Vacuum PIXE needs minimum sample preparation in terms of biological samples, where as external PIXE requires almost no sample preparation. External PIXE is considered to be a highly sensitive non-destructive and a high speed multi-elemental technique that allows analysis of even valuable and fragile specimens. It was used in areas such as Biological and Medical analysis, Atmospheric aerosol analysis, Applications in earth sciences and Art and Archeology (Neelmeijer, et al., 1994; Lin, et al., 1994; Calligaro, et al., 1999; Hajivaliei, et al., 1999).

2. Materials and methods

2.1. Substrate:

Jaggery procured from the native makers of Anakapalli, Andhra Pradesh, India, was obtained and used as carbon source for the yeast. Its total sugars content was estimated to be 80g/100g of jaggery.

2.2. Organism:

Saccharomyces cerevisiae NCIM 3288 obtained from National Collection of Industrial Microorganisms, National Chemical Laboratory, Pune, India was used throughout the study.

2.3. Growth conditions:

Yeast strains were maintained in MGYP slants having a composition (g/l): Malt extract – 3, glucose – 10, yeast extract – 3, peptone – 5 and agar 20. PH is maintained at 7.0, and the slants were incubated at 30°C for 24h. Sub culturing was carried out once in a month and culture was stored at 4°C (P.MaryAnupama, 2001).

To prepare inoculum, a loopful of the organism was inoculated into 25ml medium taken in a 250ml Erlenmeyer flask containing same components as in the maintenance medium except that agar was not added to it. The flask was kept in an incubated orbital shaker at 30°C and 200rpm for 24hours. 5ml of the medium was then taken, centrifuged and inoculated into production medium.

2.4. Fermentation conditions:

The 100ml of basic production medium having composition as follows (g/l): jaggery-200; and KNO₃- 1 is taken in a 500ml Erlenmeyer flask. Another flask with jaggery and no KNO₃ supplementation was kept as control. It is an aerobic fermentation and the physical parameters like temperature were kept at 30 ± 1°C, pH – 5 ± 0.5, agitation – 150 rpm and the inoculum level added was 6 x 10⁶ colony forming units (cfu)/ml. samples were withdrawn every 12h and analyzed for substrate consumption and product formed.

2.5. Analytical Methods

Ethanol was estimated dichromate method (Neish, 1952) and also by using GLC equipped with a flame ionization detector and a stainless steel column packed with Poropack-Q (50-80 mesh, Manufactured by Nucon Engineers, India) was used. The oven is maintained at 150°C and detector and injection ports were maintained at 170°C. The carrier gas (nitrogen) flow rate is kept at 30cm³/min and the combustion gas is a mixture of hydrogen and air (Ratnam, et al., 2003). Total sugar content is measured by Anthrone method (Jose Tarquinio Prisco, et al., 1981).

2.6. Sample procurement and processing

Samples preparation for external PIXE was done by centrifuging 50ml of fermentation medium at 8000rpm for 10 minutes and the pellet was then dried at 80°C for 48 hours. The resulting dried material was scrapped and used for analysis by external PIXE. A small portion of it is placed over the target ladder which is a Mylar foil to which the applied sample gets stuck. The amount of sample applied was varied each time making sure that the increment in sample diameter is 2mm. A sample diameter range of 2mm to 10mm was maintained making sure that the centre point of the beam over the target ladder was not deviated.

2.7. Experimental details

In the present study, to the best of our knowledge external PIXE has been employed for the first time to analyze fermentation samples with the facility available at Institute of Physics, Bhubaneswar (Vijayan, et al., 2003; Sahu, et al., 2003). In this technique a 3MeV energized proton beam was obtained from a 3MV tandem type pelletron accelerator. A graphite collimator was used to collimate a beam of size 3mm diameter and the same was extracted using a KaptonTM foil at exit point of the chamber. The beam was allowed to travel 3cm in the air and about 2.4MeV proton beam was used to irradiate the targets. The targets were kept in air over a sample stand making an angle of 45° to the beam direction and a 20 nA beam current was used to irradiate the samples. A Si (Li) detector (active area 30mm², make: Canberra) with FWHM of 165eV at 5.9KeV was used to detect characteristic X-rays from the targets. To attenuate the Bremsstrahlung background a 25µm thick aluminized Mylar absorber having 6% hole was kept in front of the detector. Spectra were recorded using an S-100 Multi channel Analyzer, which was calibrated with Am²⁴¹ X-ray source.

2.8. Analysis of Data

In the present work, the analyses of external beam PIXE spectra of fermented samples were performed using GUPIX- 2004 software (the latest version of GUPIX software) package (Maxwell et al., 1989, 1995; Campbell, et.al, 2000; Rautray, et.al, 2007; SrinivasaRao, et.al, 2010). This provides a non-linear least square fitting of the spectrum, together with subsequent conversion of the fitted X-ray peak intensities into elemental concentrations, utilizing the fundamental parameter method (FPM) for quantitative analysis. The concentrations of elements were obtained by analyzing bovine liver standard reference materials NIST SRM-1577b. The absolute elemental concentrations could be obtained through normalization to reference standard at a standard error of mean less than 10%.

3. Results and Discussion

The changes in the total sugar content of the Potassium nitrate supplemented medium and the control (Figure.1) indicate that the rapidly dividing yeast cell in the KNO₃ supplemented medium have consumed more of the substrate than the cells present in the control. At the end of 3 days of fermentation KNO₃ supplementation resulted in 68g/l of ethanol, while control resulted in 53g/l. The biomass content in the KNO₃ substituted medium was found to be more by 15% (w/v), while the percentage of total sugars consumed was 70% (w/v).

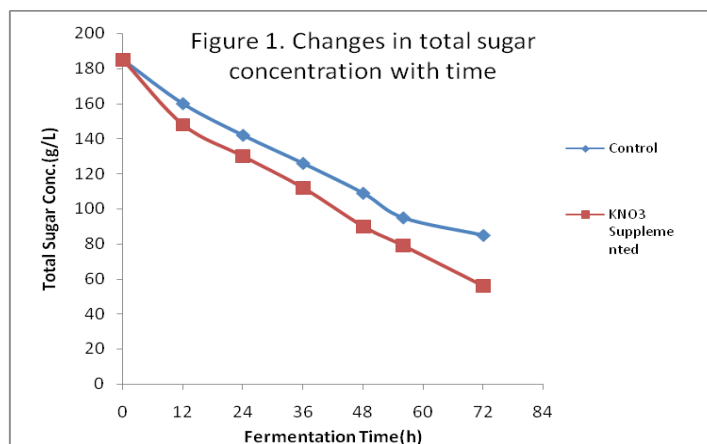


Figure.1. The rate of uptake of Potassium nitrate (KNO₃) supplemented medium and the control in total sugar concentration.

Analysis of dried cell pellets when subjected to external beam PIXE (Figure-2 and Figure-3) indicates that this technique reveals all the elements that are present in the samples of sizes 2mm and 4mm. As the aim of the article was to study the uptake of K ions for all the pellet sizes using external PIXE, the obtained results indicate that irrespective of the sample size that is used, K concentration present in the pellets is almost same (Table.1).

Table 1. Pellet sizes and their elemental composition (mg/l) obtained by External PIXE.

Concentration of elements(mg/l)					
Pellet Size	2mm	4mm	6mm	8mm	10mm
K	711.8	712	712.1	712.2	712.2
Cr	45.8	45.8	45.8	46	45.9
Fe	22.2	22.4	22.7	22.6	22.5
Cu	3.5	3.8	3.9	4	3.8
Zn	15.4	15.8	16	15.9	15.8

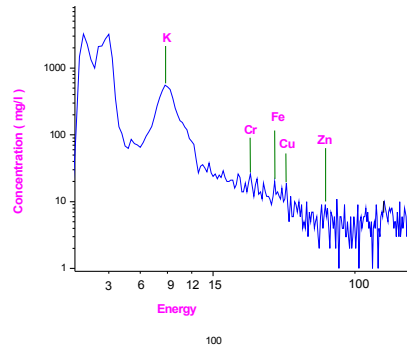


Figure.2. The spectrum of 2mm pellet fermented sample obtained by external PIXE.

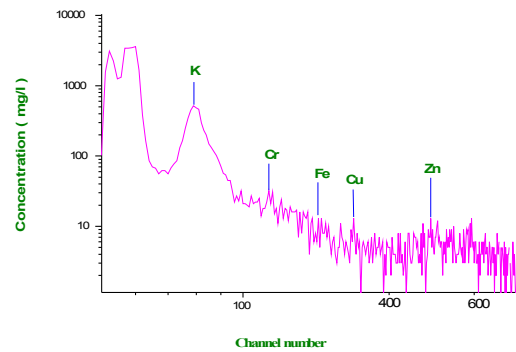


Figure.3. The external PIXE spectrum of 8mm sized pellet fermented sample.

A sample of size of 2mm indicated a K ions concentration as 711.8mg/l while the maximum sample size of 10mm was observed to contain 712.2mg/l of K ions. The increment in ions concentration was not considerable with increase in particle size. A chromium concentration of 45.8mg/l was observed to be uniform for the sample sizes 2mm, 4mm, and 6mm, and a maximum of 46mg/l was observed in the sample size of 8mm. The concentration of Fe was found to be 22.2mg/l while a maximum of 22.6mg/l was observed in case of 8mm. The concentration of Cu ions reveals that for a sample size of 2mm and 10mm the variation in the ions concentration is 0.1mg/l. And the concentration of Zn ions too had very little variation with the increment in sample size. A variation of 0.2mg/l was noticed initially till 6mm particle size, while further increase in sample size had to relative effect.

As our aim of study is to monitor the uptake of K ions, on an average 712mg of K⁺ ions are taken up by the yeast at the end of 3rd day of fermentation. Jaggery itself has approximately 0.16gm of Potassium for 100 grams of the material (Anand Sahu and Ashok Saxena, 2007) and the supplemented ions account to 1158.3mg making the net K⁺ ions content to 1190.3mg. The percentage of ions taken up by yeast comes to 59.8%. The salt also acts as a source of nitrates which act as nitrogen source and is readily available for the fermentors. Potassium ions are known to be potential stimulators for glycolytic pathway and are known to reduce the cytoplasmic concentrations of inhibitory ions (Jones, et al., 1981), while nitrate ions contribute to the nitrogen requirements of the rapidly dividing yeast cells.

4. Conclusion:

External PIXE is a highly sensitive non-destructive technique and it requires very minute quantities of sample. In the present work, the uptake of K ions by the growing yeast was estimated using external PIXE. As it allows multi-elemental analysis even the rest of the higher Z elements composition is revealed. This is enabled due to the usage of Si (Li) detector. A minimum sample size of 2mm is sufficient and the elements that were detected in the sample were Cr, Fe, Cu and Zn of which Cu which is at 3.5mg/l was also detected using this technique.

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