# Bioprocessing: A Solid State Fermentation of Coffee Residues Procedure.

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***Abstract***

*Coffee residues are generated in large quantities from the processing and consumption of coffee. These residues are generally considered as waste and disposed of through non-sustainable waste management practices, which result in environmental issues. Bioprocessing is a concept which has been applied in the valorisation of agro-industrial residues, based on the conversion of available chemical constituents into useful products, by biological materials. The application of biprocessing to the valorisation of coffee residues was investigated in this work. Under basic conditions, solid state fermentation by fungal strains- Aspergillus awamori and Aspergillus oryzae; were used to determine the capacity of coffee residues to support microbial growth. Monitoring the chemical changes which occurred during fermentation showed that coffee residues contain chemical compounds, which can be utilized by micro-organisms in bioprocesses to produce value-added products. Growth of fungal hyphae was observed on coffee residues; increase in free amino nitrogen and total reducing sugars concentrations were obtained over time; and maximum protease activity of 92 U/g dry matter basis was obtained. Autolysis of fermented products indicated an increase in the protein content of coffee residues.*

***Key words:*** *Residues**;** Fermentation; Aspergillus awamori and Aspergillus oryzae*

## Introduction

Bioprocessing is a concept in biotechnology which has important application in a wide array of human related activities. It is based on the principle of using biological materials, such as enzymes and micro-organisms, to produce desired products under the right conditions. An area of increasing development is in waste management.

The issue of waste management has become one of global concern in recent times. Current waste management practices are being discouraged due to the severe impacts they have on the environment. A prevalent waste management practice is the dumping of waste in landfills, which results in environmental problems such as release of toxic gases, leachate, land use, as well as aesthetic issues. However, it has gradually come to realisation that materials considered as waste contain desirable chemical compounds and are potential raw materials for the generation of value added products. This realisation brought about the concept of transforming waste to products.
The biological conversion of agro-industrial and food processing residues has gained increasing interest in the area of valorisation, one of which is the coffee industry. A rapidly developing bioprocess employed is the solid state fermentation of these by products for the generation of

useful products such as industrial chemicals, enzymes, food additives and health care products (Ward, 1989).

Coffee is a popular beverage which is widely consumed for its refreshing and stimulatory effects in various parts of the world (Arya and Rao, 2007a); and is regarded as an important cash crop in most producing countries. It is produced in large quantities and its production involves a number of processes which result in the generation of large amounts of by-products.

Coffee pulp and husk, which are generated from the primary processing stage of coffee production, have gained considerably large attention in the area of bioprocessing. Nevertheless, large amounts of spent coffee grounds, which are henceforth referred to as coffee residues in this paper, are also produced from the secondary processing and consumption stages in the life cycle of coffee. These residues are currently being disposed of in landfills on a large scale, or used as compost on a smaller scale. The quantity of coffee residues produced, as well as the high possibility of obtaining homogenous materials, presents the potential for valorising these by-products.

Hundreds of chemical compounds have been identified in coffee beans, with qualitative and quantitative variations occurring in the various species of coffee. These variations are enhanced between green coffee beans and roasted coffee beans due to the reactions that occur during the roasting process (Viani, 1993). The basic compounds identified include carbohydrates, proteins, lipids, alkaloids and acids.

Several stages are involved in the production of coffee for end users' consumption, These include – the Planting stage, the Harvesting stage, the Field processing stage, Roasting& Extraction, packaging and consumption, as well as waste disposal stages, which is the last stage in the coffee life cycle. Various by-products which are generally regarded as waste are generated from the various stages of coffee production. These include solid materials e.g. coffee husks, coffee residues, packaging waste; and liquid effluents such as waste water and coffee pulp from the wet processing method (Coltro et al., 2006, Salomone, 2003).

The solid by-products are mainly disposed of through the conventional non-sustainable waste management practices such as land-filling and incineration, while liquid effluents are channelled into local streams; resulting in environmental impacts such as eutrophication, greenhouse gas effect, resource use, amongst others. Growing concerns about these impacts have resulted in the development of more sustainable waste management practices. Coffee residues, which are solid by-products of secondary processing and consumption of coffee, are the materials of focus in this study.

Waste can be described as a partially exploited resource which the current owner is directly or indirectly unable to exploit further; an attribute of a resource which may be obtained by further exploitation (Martin and Holmes, 2010). Waste generation is inevitable in most cases. However, materials referred to as waste, which are not useful for a particular process, have inherent properties which may be useful for other processes if exploited; thus the concept of transforming waste to value added products (Martin and Holmes, 2010). An estimated 8.3 million tonnes of household food waste is reported to be produced annually in the United Kingdom (WRAP, 2010), while 43.6 million tons is reported for food waste disposed of in America each year (Zhang et al., 2007). These waste materials contain high amounts of organic materials such as carbohydrates, proteins, lipids, as well as other materials which present the potential for generation of bioresources from bioprocesses (Kroyer, 1995). Production of soluble instant coffee from ground roasted coffee beans results in the integration of only 33.3% of the raw material in the final product (Kostenberg and Marchaim, 1993).

In Kenya, 70% of coffee husks from dry processing have been converted into briquettes and domestically burned as fuel, with better burning characteristic than wood charcoal, since the 1970s. The use of coffee residues in the removal of heavy metals from aqueous solutions has been investigated. (Fiol et al (2008) reported the successful bio- sorption and reduction of hexavalent Chromium from the effluent of various industrial processes, preventing the environmental and public health consequences of its disposal.

Fermentation is described as the process of transforming organic materials into products by micro-organisms, in the presence or absence of oxygen (Ward, 1989). The micro-organisms used in fermentation processes consist of a range of bacteria, yeasts and fungal species. The fungi commonly employed in industrial fermentation processes are of the groups Zygomycotina such as the *Mucor* and *Rhizopus* genera; and Deuteromycotina such as *Aspergillus*, *penicillium* and *Trichoderma*genera. The yeast commonly used is *Saccharomyces cerevisiae*. Enzymes, such as pectinase, protease, tannase, caffeinase, cellulases, and α-amylases, have been produced from solid state fermentation of coffee pulp and husk (Pandey et al., 2000b).

The aim of this work is to determine if coffee residues can support microbial growth in bioprocesses; and to evaluate their potential in the generation of value-added products like enzymes by the application of solid state fermentation processes on coffee residues.

**Materials and Methods**

A detailed description of all the materials and experimental procedures used in this work, the micro-organisms employed in the Solid state fermentation are described, as well as all experiments carried out, the operating conditions and the analytical methods used is discussed.

**Materials**

The coffee residues which were the principal substrate were collected from a local Starbucks coffee shop at Manchester City centre, which were generated from brewing of *Arabica* coffee beans by the espresso method. The spent residues were preserved in plastic bags at -30oC before use. Two strains of filamentous fungi were used for solid state fermentation of coffee residues. These micro-organisms are *Aspergillus awamori* and *Aspergillus oryzae. Aspergillus oryzae* is an obligate aerobic filamentous fungi, it is made up of a genome rich in metabolic genes such as secretory hydrolases, transporters, primary metabolism genes as well as secondary metabolism genes which are most prominent, and this property is believed to be important in the wide application of *A.oryzae* in biotechnology(Machida et al., 2008).

Aspergillus awamori is a strictly aerobic filamentous fungi which is well known in the food industry (Koutinas et al., 2003). It is known to synthesize large amounts of glucoamylase, as well as other enzymes such as protease; the strain employed for solid state fermentation was *A. awamori* 2B. 361 U2/1.

Various Analytical Instruments such as UV-VIS Spectrophotometer (Shimadzu 1240), Microscope (Olympus), Analytical measuring scale, Fume cupboard (Holliday Feilding), Bench pH/mV meter, Magnetic stirrer, Microflow cabinet, Micro- centrifuge etc.

**Experimental Methods**

In preparing for fermentation, frozen coffee residues and roasted coffee beans were homogenized with a domestic blender and sieved to obtain uniform particle size. The moisture content was adjusted to the desired values, after which the residues were sterilized by autoclaving.

Spores of *A. awamori* and *A. oryzae* were aseptically cultured in media containing 20% milled wheat flour and agar for seven days at 30$^{o}$C. For each fungus, 500ml of the medium was prepared and 50ml was poured into five 250ml Erlenmeyer flasks which were covered with non-adsorbent cotton wool and foil; and sterilized in an autoclave for 15 minutes at 121oC. The media were allowed to cool and solidify in a laminar flow cupboard, and then inoculated with fungal spores.

The presence of indigenous fungi was also determined by isolating coffee residues in Petri dishes for 10 days, at room temperature outside the Morton laboratory, thereby reducing the risks of growing *A. awamori* or *A. oryzae* which is present in the Morton laboratory. The moisture content of coffee residues was carefully adjusted to 60% and the Petri dishes were kept covered to prevent contamination by air borne spores as well as loss of moisture.

**Preliminary growth experiment**

This experiment was carried out to determine the ability of the fungal strains to grow on coffee residues. Unsterilized coffee slurry was prepared and inoculated with 1ml each of *A. awamori* and *A. oryzae* in Petri dishes. The experiment was performed in triplicates with accompanying control experiments, and incubated at 30oC. The Petri dishes were monitored over time for the emergence of fungal hyphae.

**Test for toxicity/inhibition** was performed to determine the presence or absence of any toxic or inhibitory compounds in coffee residues, relative to roasted coffee beans.

**Analytical procedures**

The chemical constituents of coffee residues were also analysed before and after fermentation, to characterize the carbon and nitrogen sources available for fermentation; and to evaluate the chemical changes that occurred during fermentation. The analysis performed are Moisture content, total solids, volatile solids & ash content analysis; Total Carbohydrate content analysis; Total Nitrogen content assay; Glucose content analysis, pH analysis; Total reducing sugar analysis, Free amino nitrogen assay, as well as Protease activity determination.

**Solid state fermentation**

Solid state fermentation of coffee residues was performed by A*spergillus awamori* and *Aspergillus oryzae.* 10 g of coffee residues was weighed in twenty-eight 250 ml Erlenmeyer flasks for each fungal strain, and the moisture content was adjusted to 73% by adding 6ml of distilled water in each flask. The prepared substrates were each aseptically inoculated with 50 µL of *A. oryzae,* to obtain a spore concentration of 4.8 x 106 spores/ g dry substrate; and 500 µL *of A. awamori,* to obtain a spore concentration of 33.5 x 106 spores/ g dry substrate. The experiment was performed at 30oC, which is within the 20- 40oC temperature range for the growth of filamentous fungi.

Seven samples were taken in triplicates at intervals, by sacrificing 3 inoculated flasks and one control (un-inoculated) flask for each sampling time. Solid state fermentation by *A. oryzae* was allowed to run for 4 days with samples taken at 0, 6th, 24th, 30th, 52nd, 70th and 94th hours of fermentation; while SSF by *A. awamori* was run for 6 days, with samples taken at 0, 6th, 24th, 30th, 50th, 76th, and 124th hours of fermentation.

**Sampling and extraction**

Samples of solid state fermentation were obtained by sacrificing whole flasks per given sample. The content of the flask was mixed with a spatula, and a portion of the solid material was weighed for moisture content analysis, after which 6 g of the remaining material was weighed in 100 ml Erlenmeyer flasks and dissolved with 30 ml of distilled water. Extraction was done by shaking the solution at 200 rpm for 15 minutes, and the supernatant was obtained by centrifuging at 11,000 rpm for 5 minutes. The pH of the supernatants was taken immediately after extraction and the supernatants were stored in sampling bottles at 0oC for further chemical analyses.

**Results and Discussion**

The chemical constituents of coffee residues were characterized by determining the moisture, total solids, volatile solids and ash content and the protein and free amino nitrogen content. Knowledge of the initial chemical composition provided baseline information for solid state fermentation.

**Moisture Content, Total solids, Volatile solids & Ash content**

The initial moisture content of the coffee residues obtained was in the range 52.6%- 59.9%. Coffee residues were collected in batches, thus the range in initial moisture content observed is likely due to discrepancies in coffee brewing procedure. The results obtained correspond with the 50-60% moisture content reported by Kondamudi et al. (2008) in their report.

Moisture content of the substrate is an important factor in solid state fermentation as it plays a role in nutrient diffusion, microbial growth as well as enzyme stability (Krishna, 2005). The optimum moisture content for SSF varies amongst fungal strains and substrates, and lies between 30-80% w/w, thus moisture content is not an entirely efficient parameter for determining fungal growth (Chisti, 2010). Water activity, which is the ratio of the vapor pressure of water in the substrate to the ratio of pure water at the temperature of the substrate, provides a better prediction of the optimum water requirement for microbial growth (Chisti, 2010).

Nevertheless, a moisture content of 60-70% is employed in most SSF processes, thus, considerably small quantity of water is required for moisture content adjustment when using coffee residues as substrate for solid state fermentation.

The coffee residues analyzed showed low ash content of about 1%. Low ash content in substrates is desirable for bio conversion processes because it is suitable for growth of microbial cultures (Pandey et al., 2000c, Bakker and Elbersen, 2005); thus coffee residues are suitable for bioprocesses in that regard.

**Free amino nitrogen and Protein content**

Very small amount of free amino nitrogen was measured in the coffee residues, constituting about 0.08 mg/g of coffee residue on a dry basis. The protein content of coffee residues was estimated from the total nitrogen content obtained, using the 6.25 multiplication factor discussed in chapter 4, under the method for total nitrogen content assay. Protein content was found to constitute 0.8-1.6% of the total solids present, on a dry weight basis. This value may not be accurate for the protein content of coffee residues, considering the presence of non-protein nitrogen in roasted coffee beans as reported in literature. However, most of the compounds containing nonprotein nitrogen are reported to be water soluble by Arya and Rao (2007).

**Chemical composition of Coffee residues**

| Parameter | Quantity |
|---|---|
| Moisture Content (%) | 52.6-59.9 |
| Total Solids (%) | 47.45 |
| Volatile Solids (%) | 46.56 |
| Ash content (%) | 1.06 |
| Free Amino Nitrogen(FAN)( mg/g CR db) | 0.08 |
| Protein Content (mg/g CR db) | 8-16 |

**Preliminary Growth Experiment**

Preliminary fermentation experiment was carried out in Petri dishes and the aim was to determine the growth of *Aspergillus awamori* and *Aspergillus oryzae* on coffee residues in solid state. Growth of fungi is usually indicated by the observation of hyphae on the substrate**.** Fungal hyphae play an important role in the metabolism of these micro organisms. They effectively colonize and penetrate the substrate, resulting in contact with available nutrients e.g. polysaccharides, which are broken down by hydrolytic enzymes into simple carbon and energy sources utilized by the fungi. These enzymes are released from the hyphal tips into the substrate medium.

The stoichiometric equation for microbial growth is presented as-

Carbon Source + Water + oxygen + Phosphorous

Biomass +CO2 + Metabolite + Heat

Growth of *A .awamori* and *A. oryzae* hypha was observed in the preliminary experiment, as illustrated in Figure1. However, *A. oryzae* hyphae emerged faster than *A. awamori.* Greenish *A.oryzae* hyphae were observed on the coffee residues after 24 hours of fermentation while grayish *A.awamori* hyphae were observed after 48 hours. Observation of hyphal growth indicated the presence of available nutrients in coffee residues; thus, facilitated the performance of solid state fermentation in flasks, with sampling and chemical analysis performed.

Figure1: Preliminary growth experiment with *A. oryzae*, *A. awamori* and uninoculated control (left to right).

**Toxicity/ Inhibition of various forms of coffee**

These experiments were performed on Petri dishes to determine the presence of any compounds which are toxic or inhibitory to the growth of A. awamori and/or A. oryzae in coffee residues, as well as roasted coffee beans. Similar growth patterns of A. awamori and A. oryzae were observed for coffee residues, milled roasted coffee beans and coffee extract; as illustrated in Figure 2. This suggests the absence of any compounds which are inhibitory or toxic to the growth of the fungal strains used, in any of the coffee forms. The results also suggest that coffee brewing process does not result in any major change to the chemical composition of coffee residues.

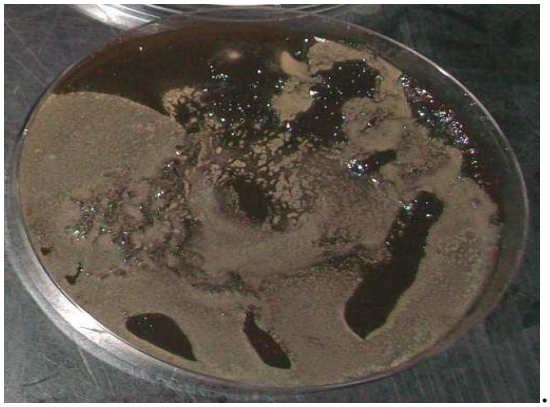
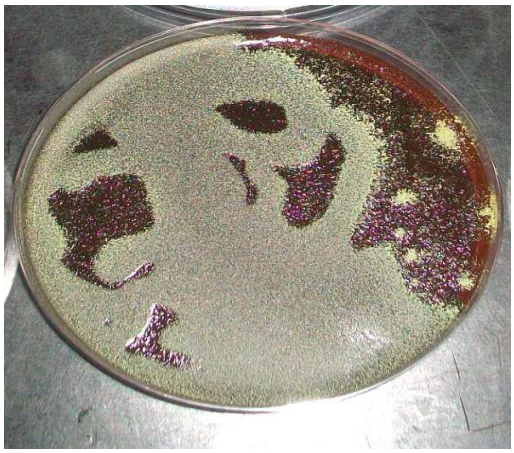


Plate2a &b: *A. oryzae* SSF on milled roasted coffee beans and agar (b). *A. awamori* SSF on milled roasted coffee beans and agar



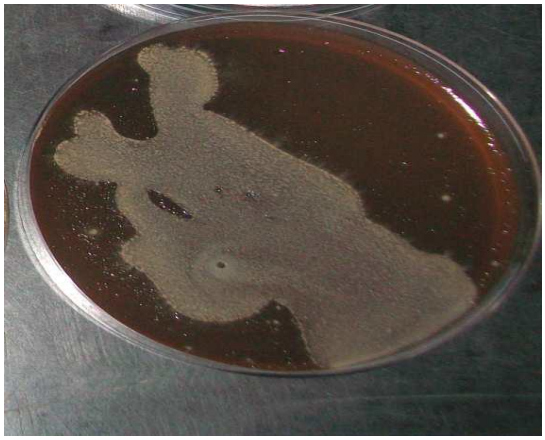


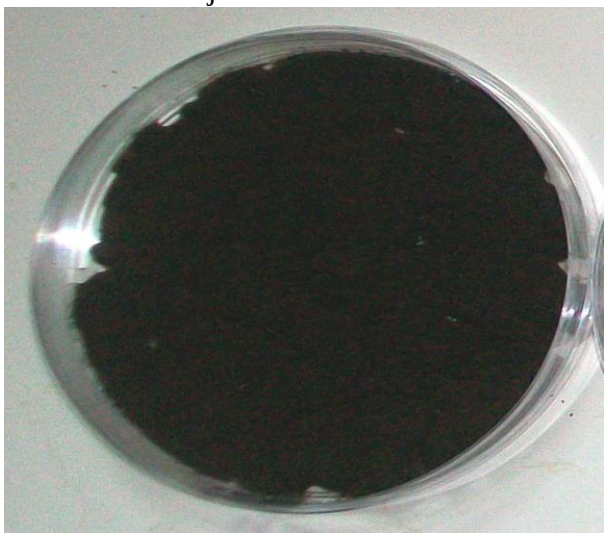
Plate2c&d (c).*A. oryzae* SSF on coffee extract and agar (d). *A. awamori* SSF on Coffee and agar extract and agar

**Fungal Isolation**

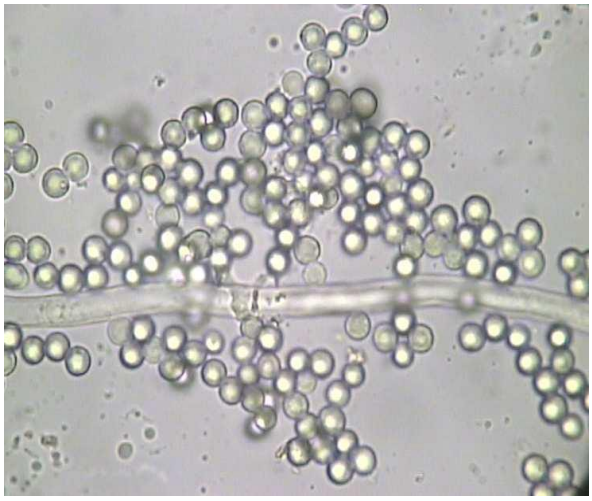
Indigenous fungi in unsterilized coffee residues were observed on covered Petri dishes after seven days at room temperature. This experiment was performed away from the Morton laboratory to prevent interference by the fungal species present in the laboratory. As shown in Figures 3 and 4 below, different fungal species were found in the coffee residues. Figure 3a has a fungal growth pattern with different defined colonies, compared to Figure 3b. This is likely due to more suitable growth condition resulting from moisture content adjustment of coffee residues in Figure 3a, unlike Figure 3b which was unadjusted. Figure 8c was uncovered for the period of this experiment, and it contained unsterilized coffee residues with the same adjusted moisture

content as Figure 3a, but no indigenous or atmospheric fungal growth was observed. This observation was likely due to an unsuitable moisture condition, which may have resulted from evaporation of moisture from the exposed surface; thus further confirms the importance of moisture to fungal growth in solid state fermentation.

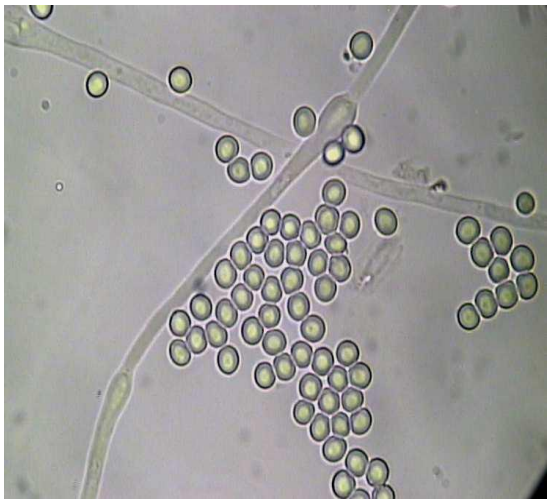
Figure3:(a)Fungal isolation on unsterilized coffee residues with adjusted moisture (b) Fungal isolation on unsterilized coffee residues with unadjusted moisture



c). Unsterilized coffee residues in opened Petri dish



A



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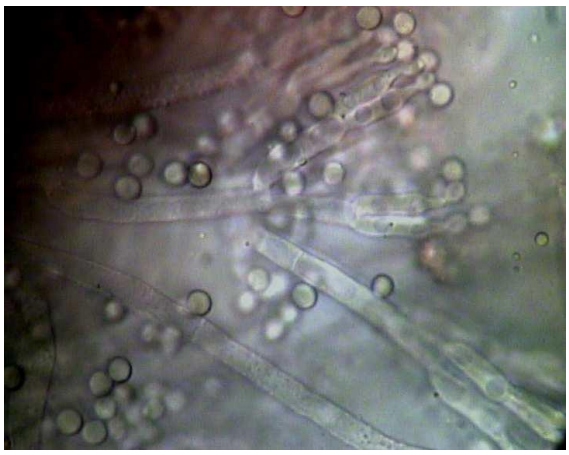

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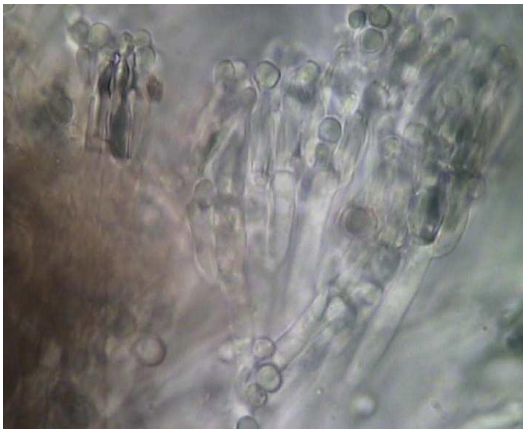

D

Figure 4a,b,c&d: Microscopic pictures of indigenous fungal spores and hypha

**Solid state fermentation**

Solid state fermentation by *A. awamori* and *A. oryzae* was carried out in 250ml Erlenmeyer flasks, in order to monitor the chemical changes occurring over the fermentation time. The parameters analysed for every sample include- moisture content, pH, total reducing sugars and free amino nitrogen. Protease activity was determined only for samples from *A. oryzae* SSF because *A. oryzae* is known to synthesize extracellular protease with ease of downstream enzyme separation (Murthy and Naidu, 2010). The results obtained for each fungus are presented below, using both column and scatter plots in some cases. The scatter plots present an average of the triplicate SSF experiment performed in separate flasks for each sampling time. However, variations occur in the separate flasks, as is typical of solid state fermentation, and so a column chart may give aclearer representation of the results obtained. Polynomial trend lines are used to obtain the trends followed by the experiment.

As obtained in the preliminary growth experiment, emergence of *A. awamori* hyphae was observed after 48 hours of inoculation. Samples were taken at intervals for chemical analysis. Figure5 below shows the presence of grey mycelia in the SSF experiment, compared to the control experiment.

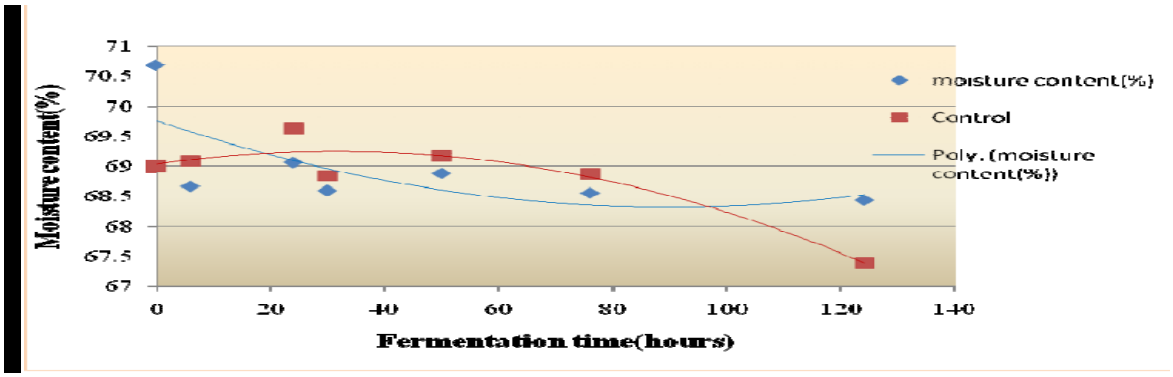


Figure 5a: Plot for change in moisture content during solid state fermentation by *Aspergillus awamori*

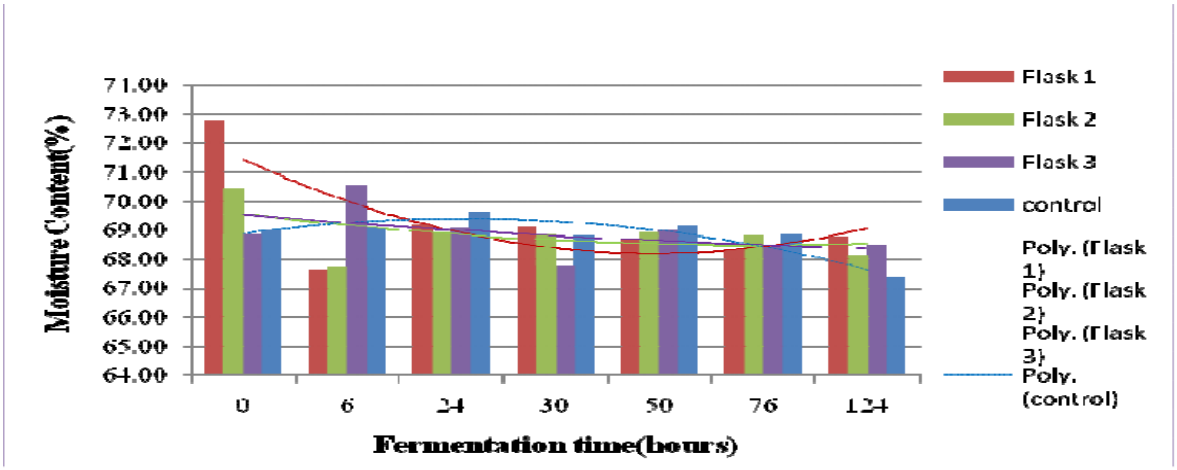


Figure 5b: Column plot for change in moisture content during solid state fermentation by *Aspergillus awamori*

**pH analysis**

The pH of each sample was taken to detect any change in acidity or alkalinity occurring with fermentation. As provided in Fig 6 below, solid state fermentation by *A. awamori* occurred in an acidic environment, with pH between 5.1 and 5.7. The slightly higher pH of the initial fermentation sample is likely due to the inoculum which was preserved in Sodium chloride

solution.

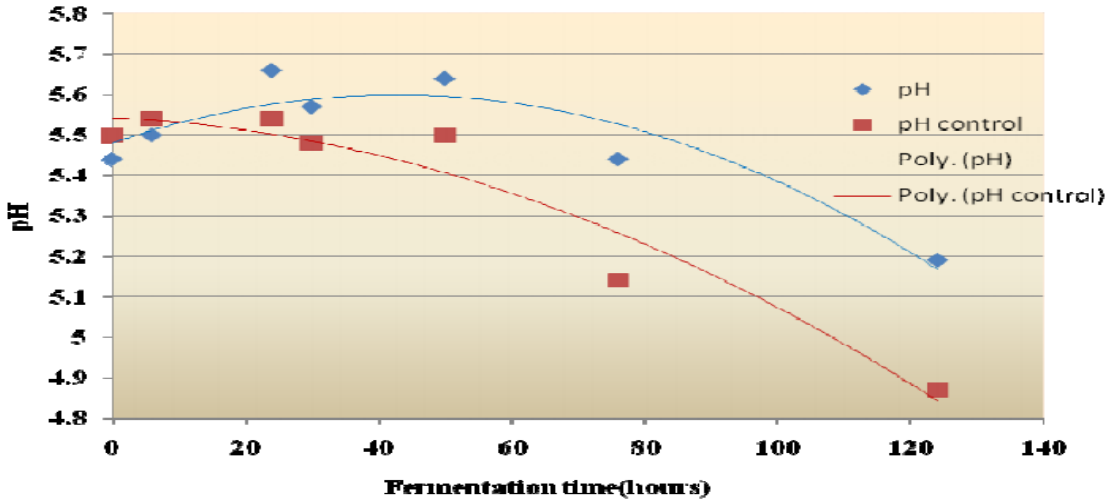


Figure 6: Scatter plot for change in pH during solid state fermentation by *Aspergillus awamori*

**Total reducing sugar analysis**

The total reducing sugar was analysed to determine the ability of *A. awamori* to hydrolyze the polysaccharides, present in coffee residues into simple sugars to obtain carbon source. From the trend depicted in Figure 7 below, a decrease in the concentration of total reducing sugar is observed until the 50th hour, after which an increase is observed. The decrease in concentration observed between 0hr and 50th hour is likely due to consumption of the available total reducing sugars. The increase in total reducing sugars observed from the 6th hour corresponds with the time of hyphae emergence, as discussed below, suggesting the release of hydrolytic enzymes from the hyphal tips produced.

A difference of 10mg/g is observed between the initial reducing sugar content of the coffee residues inoculated with *A. awamori* and the coffee residues characterised, changes occur in certain sugars when autoclaved. This may thus be responsible for this difference observed.

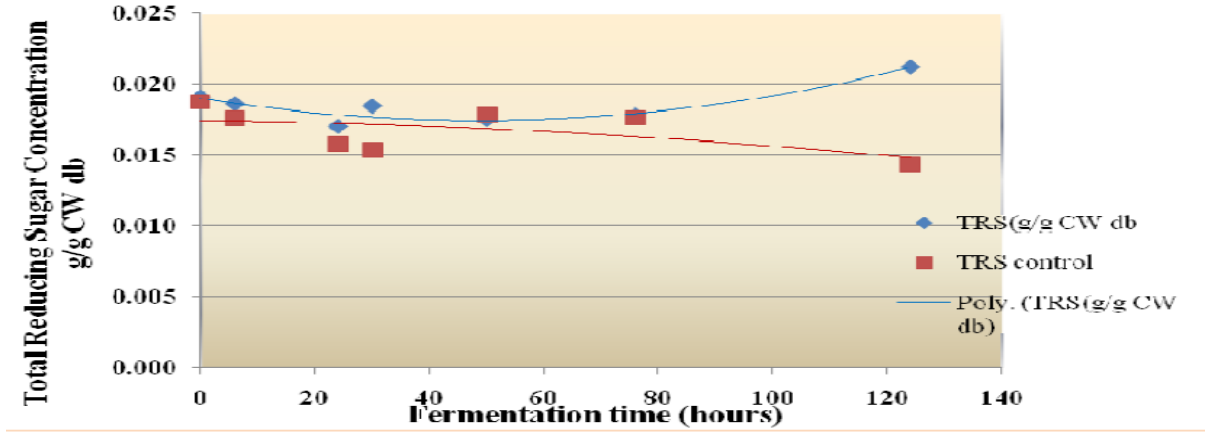


Figure 7: Scatter plot for change in total reducing sugars during solid state fermentation by *Aspergillus awamori*

**Free amino nitrogen analysis**

Similar to the trend for reducing sugar concentration, a decrease in free amino nitrogen concentration occurs during SSF by *A. awamori*. This further confirms the role of hyphae in the hydrolysis of macromolecular nutrient sources.

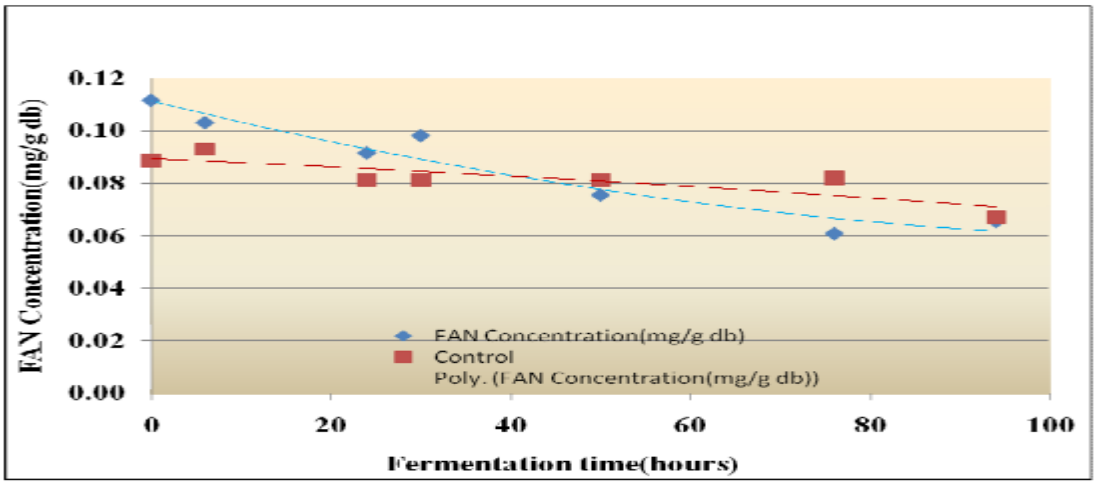


Figure 8: Scatter plot for change in free amino nitrogen during solid state fermentation by *Aspergillus awamori*

**Solid state fermentation by *Aspergillus oryzae***

SSF by *A. oryzae* was performed for 4 days and samples were taken at 0, 6, 24, 30, 52, 70 and 94 hours of fermentation. Growth of *A. oryzae* hyphae in fermentation flasks was observed after 24 hours, as observed in the preliminary growth experiment. The results obtained from the analysis performed are presented below.

**Moisture content analysis**

As performed for SSF with *A. awamori*, the moisture content of coffee residues was adjusted to 73%, then autoclaved and inoculated with 50µl *A. oryzae* spores. As depicted in Figure 9 below, the trend obtained shows a slight increase and decrease in moisture content over fermentation time for the SSF samples taken, while the control experiment shows progressive decrease in moisture content. The difference between the initial moisture content of the SSF sample and the control experiment may be due to variations in the separate fermentation flasks or due to experimental errors. The initial moisture content of both fermentation and control flasks are lower than the adjusted value, which further suggests that autoclaving may result in evaporation of moisture.

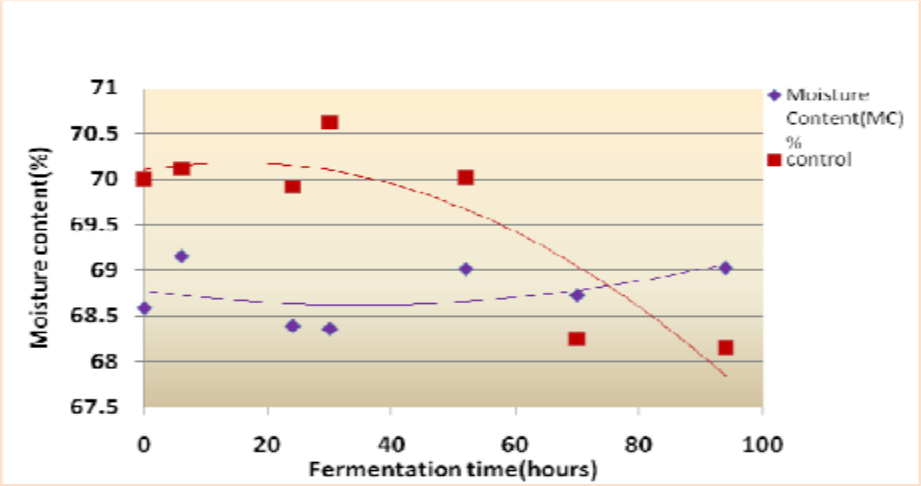


Figure 9: Scatter plot for change in moisture content during solid state fermentation by *Aspergillus oryzae*

**pH analysis**

SSF by *A. oryzae* occurred in an acidic environment with pH between 5.0 and 5.5, as shown in Figure 10 below. The trend shows an increase in pH until the 52nd hour of fermentation after which a decrease is observed, while the trend obtained for the control experiment is fairly constant.

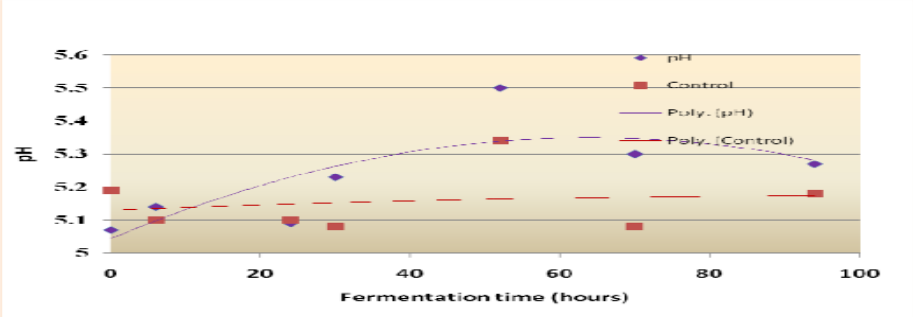


Figure 10: Scatter plot for change in pH during solid state fermentation by *Aspergillus oryzae*

**Total reducing sugar analysis**

The results obtained for total reducing sugar analysis for SSF by *A. oryzae* are illustrated in Figure 11 below. The trend shows gradual increase and decrease with fermentation, with highest concentration observed between the 30th and 60th hour of fermentation. This corresponds with the time of hyphae emergence and indicates the release of polysaccharide hydrolytic enzymes by *A.oryzae* hyphal tips after 24 hours of fermentation. The decrease observed is likely due to consumption of reducing sugars by the fungus for carbon and energy source.

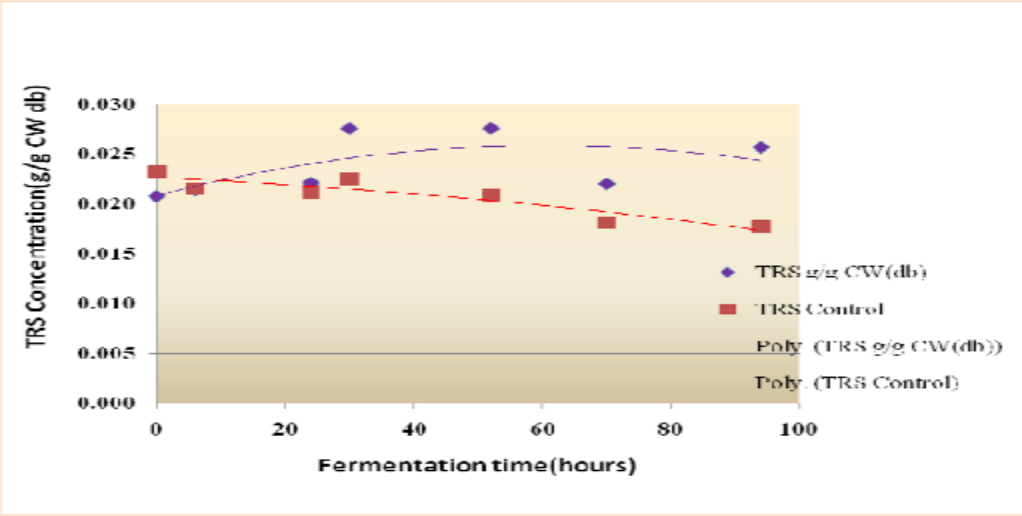


Figure 11: Scatter plot for change in total reducing sugars during solid state fermentation by *Aspergillus oryzae*

**Free amino nitrogen analysis**

Analysis of the change in free amino nitrogen concentration showed an increase from the 30th hour of fermentation depicted in Figure 12, which also corresponds with the emergence of hyphae and indicates the release of proteolytic enzymes by *A. oryzae*.

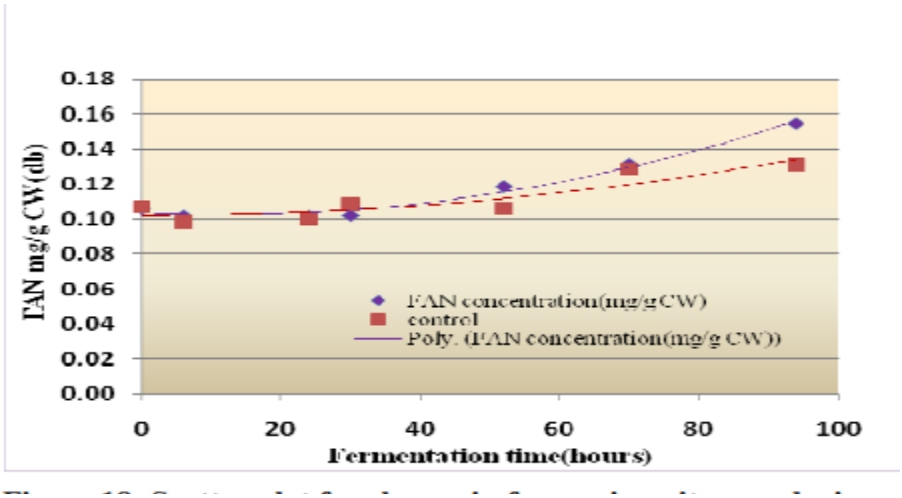


Figure 12: Scatter plot for change in free amino nitrogen during solid state fermentation by *Aspergillus oryzae*

**Protease activity analysis**

Protease activity was determined to confirm the synthesis of this enzyme during SSF by *A. oryzae*. As shown in Figure 20 below, no protease activity was obtained until after 30 hours of fermentation, after which an increase in activity with fermentation was obtained, with maximum activity of 92U/g db at the 94th hour. This result corresponds with the emergence of *A. oryzae* hyphae, as well as the increase in free amino nitrogen concentration observed; indicating the hydrolysis of the proteins present in coffee residues to amino acids by protease released.

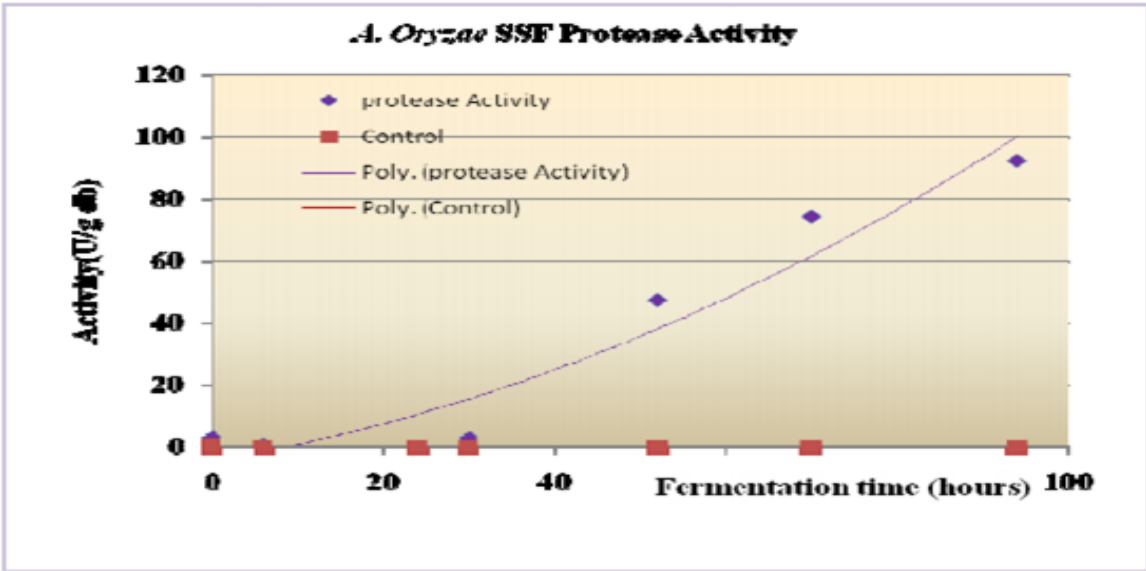


Figure 12: Scatter plot for change in protease activity during solid state fermentation by *Aspergillus oryzae*

**Autolysis experiment**

The samples obtained from the autolysis experiment carried out were analysed for the change in free amino nitrogen, as well as change in protease activity occurring during autolysis.

**Effect of Autolysis on Free amino nitrogen content**

An increase in free amino nitrogen was obtained during autolysis. As shown on Figure 13 below, the FAN concentration increased by over 90% of the initial concentration at the 16th hour of autolysis. A slight decrease is obtained by the 48th hour. The increase in FAN concentration is likely due to the breakdown of cellular proteins of *A. oryzae* and other proteins generated during fermentation. This indicates that solid state fermentation of coffee residues may be applied to increase the protein content of the residues. Also evident in the figure is the variation which occurred in the two SSF flasks which were prepared under the same conditions. This further confirms the challenge of variation and reproducibility encountered in SSF systems, however a similar trend was followed by the results obtained from both flasks, thus a conclusion may be drawn from these results.

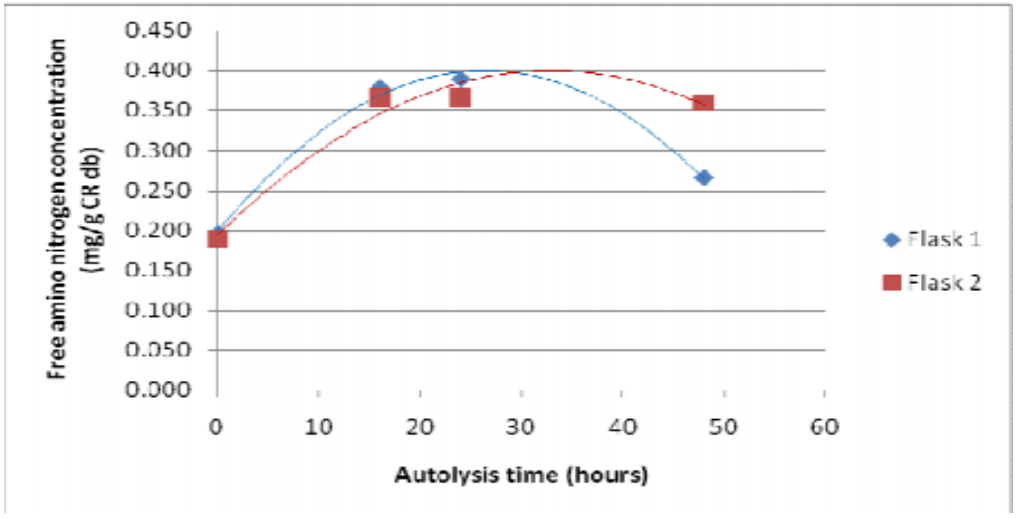


Figure 13: Plot for change in free amino nitrogen content during autolysis

**Effect of Autolysis on protease activity**

The change in protease activity with autolysis was also analysed. From Figure 14 below, it is apparent that a significant decrease in enzyme activity occurred with autolysis of the *A. oryzae* SSF product. Protease activity of between 100-120 U/g coffee residue (db) was obtained for the 0 hour autolysis sample, which was reduced to about 24 U/g db by the 16th hour of autolysis and reducing to about zero activity by the 48th hour of autolysis. This reduction is likely due to the destruction of the enzymes, which are proteins, during autolysis, thus contributing to the increase in free amino nitrogen obtained.

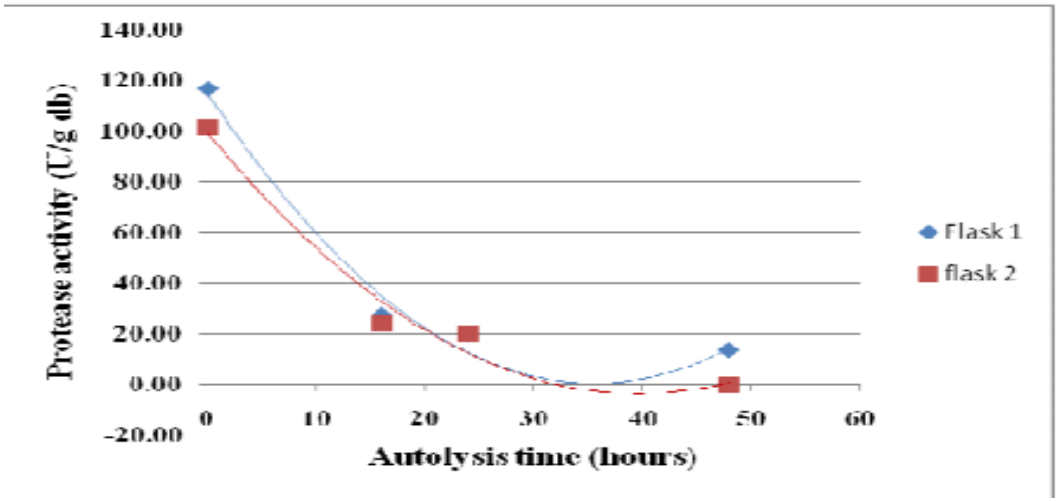


Figure 14: Plot for change in Protease activity during autolysis.

***General and comparative discussion***

The results obtained have been presented for each experiment performed in the above sections. This section provides a general discussion of the results obtained, and makes comparisons between the trends observed for the different experiments.

**Moisture content**

Solid state fermentation by *Aspergillus awamori* and *Aspergillus oryzae* gave similar trend for moisture content, as depicted in Figure 15 below. A slight decrease and increase is observed for both fungi, while both control experiments show progressive decrease in moisture content. The trend observed for both strains of fungi can be explained by the metabolic activities of these organisms. Water is used by fungi for the metabolism of available nutrient sources, as illustrated in the stoichiometric microbial growth equation, to produce metabolites, carbon dioxide, biomass as well as heat (Biesebeke et al., 2002). Mass and heat transfer occur slowly by diffusion and exchange of gases; and by conduction, free convection and evaporation of water (Mitchell et al., 1999b). These mechanisms are likely to play a role in the fluctuation of moisture content observed. The higher volume of *A. awamori* inoculum used explains the higher initial moisture content obtained.

The decrease in moisture content observed in the control experiments may be due to evaporation and condensation effects. Sterilization of the medium at 121$^{o}$C resulted in evaporation of moisture from the solid substrates into the air layer and walls of the flask. Condensation of moisture onto the solid may be responsible for the increase in moisture content observed between 0hr and 24hr of fermentation. Physical mechanisms such as capillary forces and temperature gradients may also contribute to the trend observed. As discussed above, variations in moisture content are obtained in the separate fermentation flasks, which may be due to physical dynamics or experimental errors. This confirms the challenge of homogeneity encountered with solid state fermentation and limits the ability to make definite conclusions about the moisture changes during solid state fermentation. Nevertheless, it is suggested that the fungal metabolic activities provide a counterbalancing effect on moisture content (Larroche et al., 1992), which explains the difference in the trend obtained between the control and fermentation experiments.

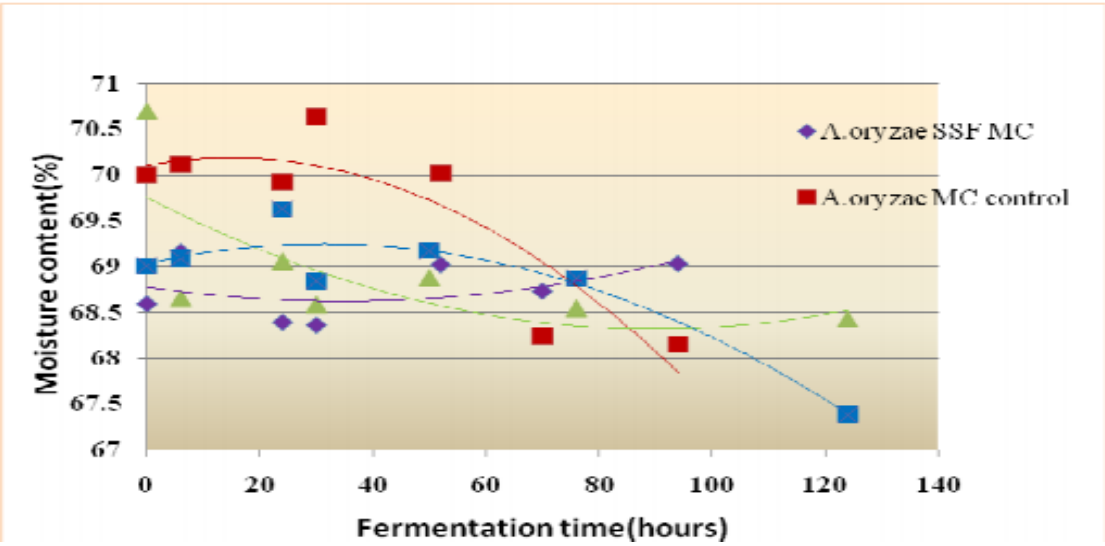


Figure 15: Scatter plot for comparison of change in moisture content during solid state fermentation by *A. awamori* and *A. oryzae*

**Conclusions and Recommendations**

Coffee residues, which are by-products of the secondary processing of coffee are to a large extent, disposed of through non-sustainable waste management practices. These byproducts contain chemical compounds which qualify them as potential raw materials for a number of

processes; bio-processing being an area of interest which presents a less expensive and more sustainable means of managing these residues.

The experiments carried out in this study showed that fungal strains can be employed in biological valorisation of coffee residues, such as solid state fermentation by these micro-organisms, where applicable. The proliferation of these micro-organisms on coffee residues is indicative of the presence of substances which can be exploited to produce value added products.

Increase in the concentration of Total reducing sugars and free amino nitrogen concentration were obtained during solid state fermentation with *Aspergillus awamori* and *Aspergillus oryzae*, which indicate the generation of these compounds from the metabolic activities of these micro-organisms. Although the increase observed may not be considered as significant, the ability of these micro-organisms to utilize the nutrients available in coffee residues presents the potential of obtaining various value-added products through bio-processing of coffee residues.

A value-added product which has been identified from this study is Protease, obtained from solid state fermentation of coffee residues by *Aspergillus oryzae*. This is an important enzyme which makes up about 60% of the enzymes' market, with wide application in various industries. Generation of this enzyme from this study confirms the feasibility of the concept of 'transforming waste to product', in application to coffee residues.

Optimization of the fermentation conditions may result in a more significant yield of metabolic by-products. Conditions, such as moisture content is an important factor in solid state fermentation, which has varying optimum requirements for different organisms. The basic moisture content employed in this study may have influenced the growth pattern obtained for the two strains of fungi used. Further work on factorial optimization of fermentation conditions for the different organisms may result in more significant metabolite yield. Pre-treatment of the coffee residues e.g. acid treatment, may improve the accessibility of the polysaccharides by the micro-organisms, as evident in the increased concentration of total reducing sugars measured in acid digested coffee residues from the initial chemical characterization experiment performed.

Considering that *Aspergillus awamori* and *Aspergillus oryzae* are just two out of a large variety of fungal species available, fungal solid state fermentation of coffee residues presents a large opportunity for further work, to obtain various value-products using other fungal strains. The ability of different strains of fungi to metabolize coffee residues is evident from the different colonies of indigenous fungi identified. An interesting example is the generation of mannase from the mannan component of coffee residues. Mannase is required in the manufacture soluble instant coffee for the hydrolysis of coffee mannan, in order to reduce the viscosity of coffee extract and simplify the production process (Arya and Rao, 2007b). The application of solid state fermentation by mannase producing fungi such as *Sclerotium rolfsii* (Arya and Rao, 2007c) to coffee residues, in instant coffee manufacturing sites would provide a less expensive way of obtaining this enzyme, and the impact of collecting and transporting coffee residues to other sites for valorisation, which is an important consideration in waste valorisation, may be reduced.

Autolysis of solid state fermentation products resulted in an increase in the free amino nitrogen concentration, indicating an increase in protein content of the fungi/coffee residues products. This product may serve as enhanced feed for animals. Fungal metabolism may result in the degradation of anti-nutritional compounds in coffee residues, whilst increasing the protein content, thus improving its quality as animal feed. However, the generation of toxic metabolites from fungal metabolic activities is also obtainable, thus further work needs to be done to

determine the degradation of anti-nutritional compounds, the production of toxic metabolites, as well as the effect of fermentation time on the degradation and generation of these materials.

Overall, from this study, it can be concluded that bioprocessing of coffee residues is feasible and further research should be carried out in this area to obtain optimum yield of value-added products.

## REFERNCES


Arya, M. & Rao, L. J. M. (2007a) An Impression of Coffee Carbohydrates. *Critical Reviews in Food Science and Nutrition 47: 1*, 51-67.

A., DrieL, K. G. A. V., Schutyser, M. A. I., Dijksterhuis, J., Zhu, Y., Weber, F. J., Vos, W. M. D., HondeL, K. A. M. J. J. V. D., Rinzema, A. & Punt, P. J. (2002) Aspergillus oryzae in Solid state and submerged fermentations Progress report on a multidisciplinary project. *FEMS Yeast Research*, 2, 245-248.

Bakker, R. R. & Elbersen, H. W. (2005) Managing ash content and quality in herbaceous biomass, an analysis from plant to product. *14th European biomass conference.*

Biesebeke, R. T., Ruijter, G., Rahardjo, Y. S. P., Hoogschagen, M. J., Heerikhuisen, M., Levin,

Coltro, L., Mourad, A. L., Oliveira, P. A. P. L. V., Baddini, J. P. O. A. & Kletecke, R. M. (2006) Environmental Profile of Brazilian Green Coffee. *International Journal of Life Cycle Assesment*, 11, 16-21.

Chisti, Y. (2010) Solid substrate fermentations, enzyme production, food enrichment. in

Flickinger, M. C. (Ed. *Encyclopedia of Industrial Biotechnology, Bioprocess, Bioseparation, and Cell Technology.* New York, Wiley.

Gamble, P. (2007) Characterization of food waste as feedstock for anaerobic digestion. *Bioresource Technology*, 98, 929-935.

Kondamudi, N., Mohapatra, S. K. & Misra, M. (2008) Spent Coffee Grounds as a Versatile Source of Green Energy. *Journal of Agricultural and Food Chemistry*, 56, 11757-11760.

Kostenberg, D. & Marchaim, U. (1993) Anaerobic digestion and horticultural value of solid waste from manufacture of instant coffee. *Environmental Technology*, 14, 973 - 980.

Koutinas, A. A., Wang, R., Kookos, I. K. & Webb, C. (2003) Kinetic parameters of Aspergillus awamori in submerged cultivations on whole wheat flour under oxygen limiting conditions. *Biochemical Engineering Journal*, 16, 23-24.

Krishna, C. (2005) Solid-State Fermentation Systems—An Overview. *Critical Reviews in Biotechnology*, 25, 1-30.

Larroche, C., Theodore, M. & Gros, J. B. (1992) Growth and sporulation behaviour of Penicillium roquefortii in solid substrate fermentation: effect of the hydric parameters of the medium *Applied Microbiology and Biotechnology*, 38, 183-187.

Machida, M., Yamada, O., Gomi, K. & (2008) Genomics of aspergillus oryzae: learning from the history of koji mold and exploration of its future. *DNA Research*, 15, 173-183.

Martin, A. & Holmes, S. (2010) Solid Waste Management and Valorisation. Manchester, the University of Manchester.

Mitchell, D. A., Stuart, D. M. & Tanner, R. D.(1999b) Solid-state fermentation—microbial growth kinetics. in Flickinger, M. C. & Drew, S. W. (Eds.) *The encyclopedia of bioprocess technology: fermentation, biocatalysis and bioseparation.* New York, Wiley.

Murthy, P. S. & Naidu, M. M. (2010) Protease Production by *Aspergillus Oryzae* in Solid-State

Fermentation Utilizing Coffee By-products. *World Applied Sciences Journal*, 8, 199-205**.** *Sciences Journal*, 8, 199-205.

Pandey, A., Soccol, C. R., Nigam, P., Brand, D., Mohan, R. & Roussos, S. (2000b) Biotechnological potential of coffee pulp and coffee husk for bioprocesses. *Biochemical Engineering Journal*, 6, 153-162.

Pandey, A., Soccol, C. R., Nigam, P., Soccol, V. T., Vandenberghe, L. P. S. & Mohan, R.(2000c) Biotechnological potential of agro-industrial residues. II: cassava bagasse. *Bioresource Technology* 74, 81-87.

Salomone, R. (2003) Life cycle assessment applied to coffee production: investigating environmental impacts to aid decision making for improvements at company level. *Food, Agriculture and Environment* 1, 295-300.

Viani, R. (1993) The composition of Coffee. in Garattini, S. (Ed.) *caffeine, Coffee, and Health.* New York, Raven Press Ltd.

Ward, O. P. (1989) Fermentation Biotechnology- Principles, Processes and Products, Milton Keynes, Open University Press.

Wrap (2010) Household Food Waste.

Zhang, R., EL-Mashad, H. M., Hartman, K., Wang, F., Liu, G., Choate, C. &. Kroyer, G. T. (1995) Impact of food processing on the environment--an overview. *Lebensmittel-Wissenschaft und-Technologie*, 28, 547-552.