



Bioprocessing procedure of Waste Management using Liquid State Fermentation of Coffee Residues

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ABSTRACT: This study is aimed at utilizing the abundant coffee residue in Manchester using bioprocessing techniques to convert the raw materials into economically viable components. High amounts of carbohydrate were obtained in coffee residues, making up about 35-41% of the total solids on a dry basis; an increase of about 89.5% for total reducing sugars concentrations was observed to occur; indicating that indigenous bacterium was capable of breaking down and hydrolyzing complex polysaccharides and proteins in coffee residues. An increase of over 100% in free amino nitrogen (FAN) concentration were obtained over time, further indicating the significant synthesis of hydrolytic enzymes by the indigenous bacteria during Liquid state fermentation processes of bioprocessing. This study therefore, concludes that bioprocessing of coffee residues is feasible in obtaining optimum yield of value-added products.

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Bioprocessing is based on the principle of using biological materials, such as enzymes and micro-organisms, to produce desired products under the right conditions. The issue of waste management has become one of global concern in recent times. Current waste management practices such as landfills are being discouraged due to the severe 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 valorization, one of which is the coffee industry. A rapidly developing bioprocess employed is the Liquid state fermentation of these byproducts for the generation of useful products such as industrial chemicals, enzymes, food additives and health care products. According to an OECD report, over 4 billion tonnes of waste are generated annually by its 32 member countries, which include municipal waste and hazardous waste (OECD, 2008). Household waste and similar waste from offices, institutions and small businesses, yard and garden waste, street sweeping, markets, etc. are classified as municipal solid waste,

which are collected and treated by municipalities. The degradable fractions of municipal solid waste are referred to as bio-degradable municipal solid waste (Martin and Holmes, 2010). Waste from food processing industry is generally bio-degradable municipal waste and it constitutes a significant portion of the total quantity of waste generated annually. The characterization of waste from the food industry includes waste from the agricultural processes, food processing and from end users consumption (Kroyer, 1995). 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). Work has been carried out on obtaining value from a large variety of waste from the food industry. These include composting by micro-organisms under the right physical and chemical conditions to produce stable soil-like materials for improved plant growth (Schaub and Leonard, 1996); anaerobic digestion by micro-organisms to produce biogas (Zhang *et al.*, 2007); use of by-products like maize bran and rice husk as bio sorbents to remove heavy metals from aqueous

solutions (Hasan *et al.*, 2008, Bansal *et al.*, 2009); application of microbial solid state fermentation to produce important enzymes such as *LAsparaginase* which is an anti-cancer agent from some agro wastes (Mishra, 2006), cellulolytic and hemicellulolytic enzymes which can break down polymeric carbohydrates into fermentable sugars for further processing into liquid fuels and other chemical bio products (Leite *et al.*, 2007); use as animal feeds; amongst others. Coffee is a popular beverage with an interesting history, which is largely consumed in most parts of the world including Nigeria. The basic compounds identified include carbohydrates, proteins, lipids, alkaloids and acids (Viani, 1993). Several stages are involved in the production of coffee for end users' consumption. These stages produce waste such as 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). Coffee residues, which are solid by-products of secondary processing and consumption of coffee, are the materials of focus in this study. Bioprocessing of raw materials into products involves the fermentation of the raw materials; and it has been widely applied in the food and beverage industries, in medicine and pharmaceuticals, energy production, as well as effluent and solid waste management. The paucity of information on bioprocessing of waste and the abundance of coffee residue in Manchester has necessitated this research work. The aim of this work is therefore to determine if coffee residues can be utilized in bioprocesses; and to evaluate the possibility of obtaining value-added products from these residues by the application of liquid state fermentation processes.

MATERIALS AND METHODS

Sample collection and preparation: Roasted coffee beans were purchased from a local supermarket in Manchester to carry out a comparative study between coffee residues and roasted coffee beans. The packaged coffee beans were stored at room temperature prior to use. The 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. Indigenous bacteria were cultured in liquid medium to obtain inoculum for Liquid state fermentation. 5ml nutrient broth was sterilized in bottles, and inoculated aseptically with isolated bacteria. The bottles were incubated on rotor shakers at 30°C for 48 hours. Control bottles were also prepared without bacteria inoculation. The presence of indigenous bacteria in coffee residues was determined by Nutrient-Agar plating experiment,

using a solution of unsterilized coffee residues in sterilized distilled water. Nutrient-Agar plates were prepared with 13g/L nutrient broth and 2% agar. Sterilized coffee residues and distilled water were used for control experiments. Bacteria found in spent coffee residues were isolated aseptically on nutrient- agar plates by the streak plate method (Microbeid, 2010). A sterile loop was used to streak the indigenous bacteria obtained, over the surface of nutrient- agar plates to isolate colonies. The plates were incubated at 30°C for 48 hours. Liquid state fermentation was performed with a medium consisting of 20g coffee residue and 100 ml tap water in 250 ml Erlenmeyer flasks. The medium was autoclaved and cooled, then inoculated aseptically with 2ml of the prepared bacteria inoculum. The experiment was performed in duplicate with an accompanying control flask, which were incubated at 30°C on rotor shakers at 200 rpm. Fermentation was allowed to occur for 48 hours and samples were taken at intervals by sacrificing two inoculated flasks and one control flask per sampling time. An experiment was also performed to determine the presence or absence of any toxic or inhibitory compounds in coffee residues, relative to roasted coffee beans. The experiment was performed in Petri dishes, containing agar and various forms of coffee, namely roasted coffee beans, coffee beans liquid extract and coffee residues. The media used were Coffee extract + 2% Agar; 5g milled coffee beans + 2% Agar; and 5g spent coffee+ 2% Agar. Coffee extract was obtained by boiling 5g of milled coffee beans in 200ml distilled water for 20 minutes and then filtering the suspension. The media were prepared in 250 ml Duran bottles and autoclaved, after which they were allowed to solidify in Petri dishes for 30 minutes.

Sample analysis: The chemical constituents of coffee residues were 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 classic laboratory 'Loss on Drying' method which is based on evaporation of moisture was used to determine the moisture content of spent coffee residues. The pH analysis was performed by the use of a bench pH metre, to determine the change in acidity or alkalinity of coffee residues occurring during fermentation. The total carbohydrate content of coffee residues was determined using the phenol sulphuric acid procedure. The total nitrogen content of coffee residues was analysed by the Hach persulphate digestion method (FAO, 2003). The glucose concentration of coffee residues was determined with the use of a glucose analyser – Analox GL 6 Analyser. The total reducing sugars content of coffee residues and fermented samples was determined by the Miller

dinitrosalicylic acid method. The free amino acid content of coffee residues and fermentation samples was determined by the ninhydrin colorimetric method recommended by the European Brewer Convention (Chandrashekar, 2002).

RESULTS AND DISCUSSION

High amounts of carbohydrate were obtained in coffee residues, making up about 35-41% of the total solids on a dry basis. Only about 1% of the carbohydrate content obtained was available as simple sugars. Acid digestion of the total carbohydrates resulted in an increase in total reducing sugars from 1% to 38%. The result for total carbohydrate content corresponds with the quantity reported for roasted coffee beans from the literature. This indicates that brewing has little or no effect on the carbohydrate content of roasted coffee beans; and infers that these carbohydrates are water insoluble polysaccharides. Also, the increase in total reducing sugars after acid digestion confirms that the carbohydrates available for microbial growth are mainly polysaccharides, which need to be hydrolyzed to obtain carbon and energy source. From the literature reviewed, these polysaccharides are believed to be majorly celluloses, β -1, 4-mannan and hemicelluloses (Viani, 1993). 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 non protein nitrogen are reported to be water soluble by Arya and Rao (2007). The presence of bacteria in unsterilized coffee residues was indicated by the colour change observed in Plate1, in comparison to Plate2 which was performed with sterilized coffee residues. The bacteria obtained were re-isolated, as shown in Plate1c, to obtain homogenous colonies. Observation under the microscope showed rod shaped bacteria, shown in Plate1d. Inoculation of sterilized coffee residues-agar medium with the isolated bacteria resulted in bacteria growth, as illustrated in Plate5, indicating the presence of available nutrients in the coffee residues. Performance of a similar experiment on glucose-agar medium also resulted in bacteria growth, shown in Plate 6, suggesting the presence of sugars which can be utilized by bacteria in coffee residues.

Figure1, shows that an increase in total reducing sugars concentration occurred within 24 hours of fermentation, which leveled out by the 48th hour of fermentation. A similar trend as obtained for the total reducing sugars concentration during bacterial liquid state fermentation was obtained for the free amino nitrogen concentration, indicating that indigenous bacterium was capable of breaking down complex polysaccharides and proteins.

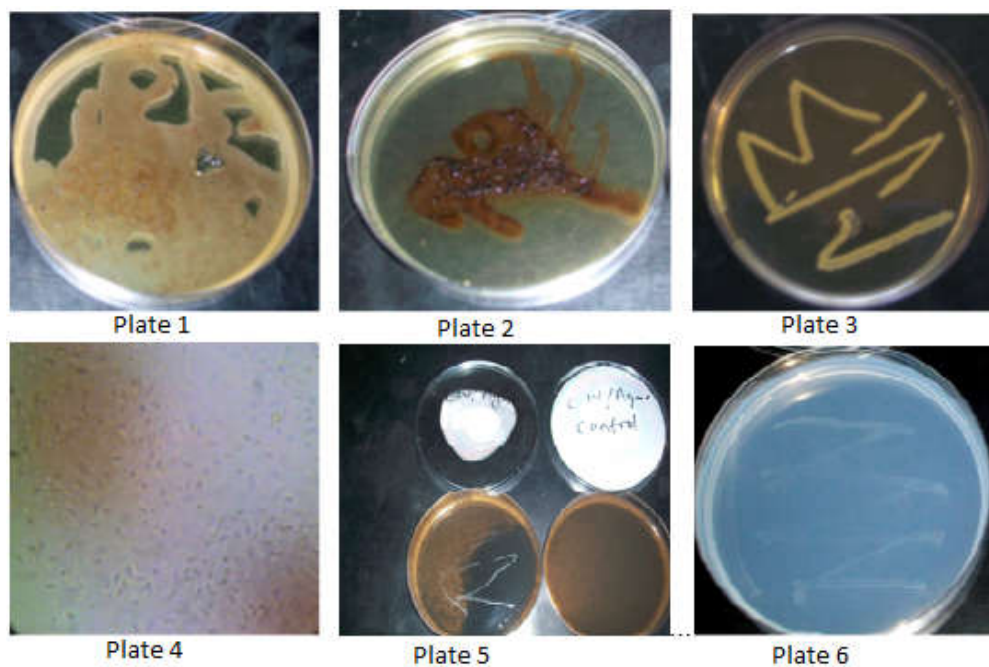


Plate 1 - 6: Test for indigenous bacteria

Plate 1 = Bacterial growth in unsterilized Coffee grounds; Plate 2 = Experiment with sterilized Coffee grounds; Plate 3 = Isolated indigenous bacteria on nutrient agar medium; Plate 4 = Microscopic picture of rod-shaped indigenous bacteria; Plate 5 = Growth of bacteria on sterilized coffee residue and agar medium; Plate 6 = Growth of bacteria on sterilized glucose and agar medium

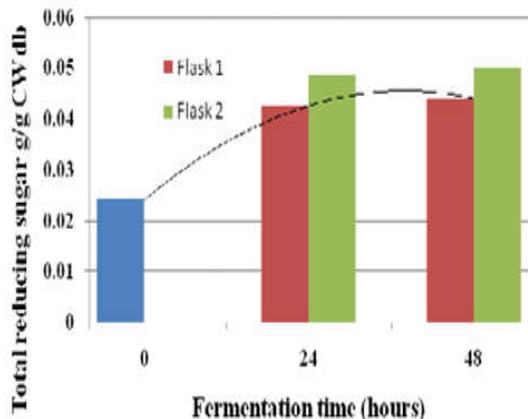


Fig 1: Column plot for change in total reducing sugars during liquid state fermentation by indigenous bacteria

This liquid state fermentation by indigenous bacteria experiment indicates a marked difference in the trend for total reducing sugars (TRS) concentration. An increase of 89.5% was observed to occur within 24 hours of liquid state fermentation, which apparently remained unchanged by the 40th hour. Sampling at more frequent intervals within 24 hours of LSF may have provided more definite information about the different growth phases of the bacteria inoculated, however, it is suggested that the lag and exponential phases occurred within 24 hours and the stationary phase may have been approached by the 48th hour of fermentation. The results indicate the ability of the indigenous bacteria to better hydrolyse the polysaccharides contained in coffee residues than when fungal strains is used releasing relatively larger amount of reducing sugars. The difference in cell structure between bacteria and fungi may also contribute to the release and availability of hydrolytic enzymes.

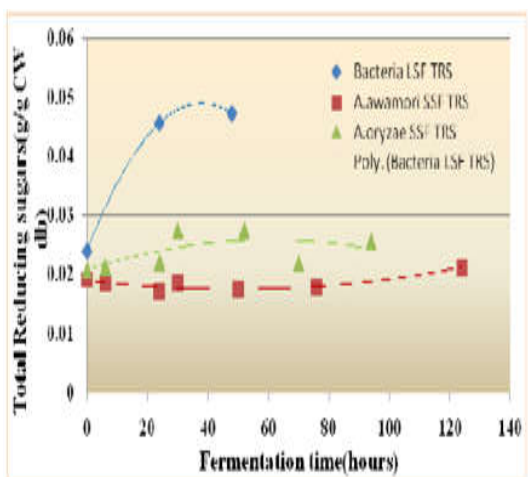


Fig 2: Scatter plot for comparison of change total reducing sugar concentration during solid state fermentation by *A. awamori* and *A. oryzae*; and liquid state fermentation by indigenous bacteria

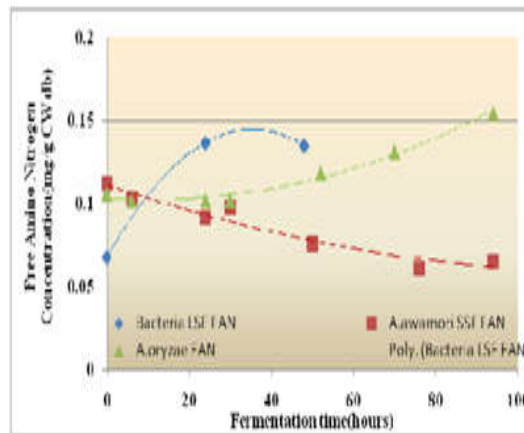


Fig 3: Scatter plot for comparison of change in free amino nitrogen concentration during solid state fermentation by *A. awamori* and *A. oryzae*; and liquid state fermentation by indigenous bacteria

The simpler cell structure bacteria may also increase the production and release of these enzymes as compare to that of the more complex cell structure of fungi. The initial TRS concentration at 0hr of fermentation is the same for both fungal experiments, as shown in figure 2 but a slight difference is observed for the LSF experiment. This is likely due to the nutrients contained in the culture medium used to grow bacteria for liquid state fermentation.

Figure 3 shows that Liquid state fermentation by the isolated indigenous bacteria resulted in an increase of over 100% in FAN concentration, thereby, further indicating the significant synthesis of hydrolytic enzymes by the indigenous bacteria during fermentation. The results obtained may have been improved by the uniformity of the liquid state medium, which is hardly obtainable in solid state fermentation media.

Conclusions: Coffee residues, which are by-products of the secondary processing of coffee are to a large extent, disposed of through a non-sustainable waste management practices. Indigenous bacteria was employed in biological valorisation of coffee residues, such as Liquid state fermentation using micro-organisms and there was increase in concentration of free amino nitrogen, total reducing sugars and useful enzymes as value-added products obtained. The study concludes that bioprocessing of coffee residues is feasible to obtain optimum yield of value-added products.

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