OPTIMIZATION OF INOCULUM SIZE AND INCUBATION PERIOD OF SOLID STATE FERMENTATION (SSF) PROCESS

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Abstract
A fourteen day study was carried out to determine the optimum initial inoculum level and incubation period of a solid state fermentation (SSF) process using filamentous fungus, Aspergillus niger (USMF 4) and cassava residual pulp (CRP) as the microorganism and substrate respectively. The study comprised four sets of experiments at room temperature; set A (un-inoculated) serving as control; and three levels of initial inoculum: B (5.55 x 10^8 spore forming units/ml, sfu/ml), C (1.11 x 10^9 sfu/ml) and D (1.65 x 10^9 sfu/ml.). Each set had eight samples in conical flasks; at every 48hours, a flask was removed from each set for analysis. It was observed that SSF was positively affected by the inoculum level and the incubation period. Generally, better values in the parameters analyzed were obtained in treatment C (1.11×10^9 sfu/ml.) and at incubation period of 12 days for cassava residual pulp (CRP). The results of this study indicated that Aspergillus niger (USMF 4) performs best at 1.11x10^9 sfu/ml. inoculum level for the improvement of the nutrient values of agro industrial by products; with optimal increment of the crude protein content of cassava residual pulp (CRP) at 12th day of solid state fermentation (SSF).

Keywords: solid state fermentation, *Aspergillus niger*, inoculum level, incubation period

Introduction

Solid-state fermentation (SSF), a fermentation that takes place on solid or semi-solid substrate has emerged as a potential technology for the production of microbial products such as feed, fuel, food, industrial chemicals and pharmaceutical products; its application in bioprocesses such as bioleaching, bio-beneficiation, bioremediation, bio-pulping, etc. has offered several advantages. The interest in SSF comes from its simplicity and closeness to the natural growth conditions of many micro-organisms, especially fungi. In recent times, concerted effort has been made to utilize agro-industrial by products (AIBPs) in their raw state in non-ruminant feed production as alternative to conventional feed ingredients which prices keep soaring and has become an albatross to animal production (Onyeonagu and Njoku 2010); but little success has been achieved, this is mainly due to fibrotic nature of these AIBPs. Other reasons attributed to this challenge include very low nutrient values of these products in their natural or unprocessed state, and also inadequate knowledge of their processing. These limitations can be circumvented by fungal fermentation of AIBPs, as it promotes digestibility and increases the utilization of the mass of these materials. Agro-industrial wastes serve as solid support, carbon, nitrogen or mineral sources, and nutrient sources in SSF processes for the production of a variety of value-added compounds. It also provides more economical fermentation processes, avoiding the use of expensive chemical components in the media formulation (Solange *et al.*, 2012 and Tobias, *et al.*, 2014). The important aspects which should be considered before starting a solid-state fermentation include the selection of suitable microorganism (identification of the organism, initial inoculum size, etc.), substrate (identification of suitable substrate for a particular microorganism, particle size, quantity, etc.), pH, temperature, moisture level of the process, and isolation and purification of the product (Manpreet *et al.*, 2005). Temperature is a very important factor of fermentation; and most fungi are greatly affected by temperature; at too low temperature they behave dormant, and at too high temperature, they indulge in an orgy of fermentation. The degradation of substrate during solid state fermentation, by fungi is not only determined by its quality but also by the particle size of the substrate; the smaller sized particles provide large surface area which helps to mix the substrate with the microorganisms and other nutrients. It is important to note the pH of the fermentation medium in order to have optimum result; culture pH strongly influences many enzymatic processes and transport of various components across the cell membranes which in turn support the cell growth and product production (Paranthaman *et al.*, 2009).

The aim of this study is therefore to determine the optimum inoculum size and incubation period for SSF of CRP using *Aspergillus niger* (USMF 4).

Materials and Method

The study was carried out in the Department of Animal science and Biological Sciences, Landmark University, Omu-Aran, Kwara State, Nigeria. The fungal species used for this study was *Aspergillus niger* (USMF4). The organism was obtained from the Laboratory stock of the Department of Biological Sciences, Landmark University Omu-Aran, Kwara State, Nigeria; while the substrate used for the study (cassava residual pulp, CRP), was collected from fufu processing plant in Omu –Aran, Kwara state, Nigeria. The substrate was sundried for 45 min daily for two weeks before pulverizing using an electric grinder to reduce particles size.

There were 4 treatments control (A), B, C, and D; each treatment has 8 conical flasks of 250ml each. 20g of the dried pulverized cassava residual pulp was weighed into each of the 250ml capacity conical flasks, cotton-plugged and sterilized in an autoclave (Shenan autoclave, Model; DSX-280) at 121°C for 15 minutes at 15 pound square inch-gauge (psi). After sterilization, flasks containing the sterile pulp were inoculated. Various inoculum level of *Aspergillus niger* (USMF4) were used for each treatment are as follow: Treatment B 5.55×10^{8} spore forming unit/ml,Treatment C 1.11×10^{9} spore forming unit/ml,, Treatment D 1.65×10^{9} spore forming unit/ml, and the control was not inoculated with organism but 40ml of water was added. All the treatments were incubated at room temperature on the laboratory bench for solid state fermentation.

At every 48 hour period, one (1) flask was removed from each of the treatment, and also from the control, for 14 day duration. Samples from these flasks were taken immediately for the determination of pH. Fermentation process was terminated by drying the samples in an oven at 70°C for 24h, subsequently the dried fermented samples were analysed for some proximate components (dry matter, ash, and crude protein).

The pH was read using pH gas electrode; the pH scale was calibrated using buffer solution of pH 7, while the Dry matter and crude protein were determined by AOAC 1990.

Results and Discussion:

Table 1 Mean pH level of the fermented substrates

TREATMENTS	DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 12	DAY 14
Control (A)	7.23	5.90	6.00	5.91	6.14	5.70	5.50	5.60
B	7.23	7.40	6.91	6.54	6.99	5.68	5.78	6.10
C	7.23	6.13	6.03	4.85	5.68	6.29	6.12	5.98
D	7.23	6.80	5.35	5.32	6.66	5.73	5.60	8.12

Table 2. Mean values of dry matter content of the fermented substrates (%)

TREATMENTS	DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 12	DAY 14
Control (A)	70.5	77	79.5	64	87	83.5	83.5	83.5
B	70.5	72.5	73.5	64	60.5	85.5	83.5	84
C	70.5	69.5	76.5	84.5	61	83.5	83.5	83.5
D	70.5	67	79.5	83	75	83	83	83.5

Table 3 Mean crude Protein content of the fermented substrates (%)

TREATMENTS	DAY 0	DAY 2	DAY 4	DAY 6	DAY 8	DAY 10	DAY 12	DAY 14
Control (A)	1.54	1.29	1.51	0.85	0.51	3.61	0.9	0.6
B	1.54	1.29	1.47	0.88	0.5	2.15	5.36	0.58
C	1.54	1.26	1.74	3.26	0.13	2.31	6.29	1.44
D	1.54	1.22	1.25	1.1	0.13	3.74	4.44	0.61

The most acidic pH range (table 1) 4.85 was recorded at day 6 for media fermented with 1.11×10^9 inoculum level ($_C$),while highest alkaline medium of 8.12 was recorded in 1.65×10^9 inoculum level ($_D$) at14 day. Among physical parameters, pH of the growth medium plays an important role by inducing growth of microbes also affects product stability in the medium (Mienda, 2011). When the pH is highly acidic it will gives changes that will promote the preservation of the fermented substrate and increase the value of the product by enhancing the taste, appearance, texture, flavor and increase the shelf life of the food. This result also agree with those obtained by (Giese 1994) who reported increase in acidity as the incubation period of fermentation increases, and opined that this may enhance the keeping quality of millet foods, by inhibiting microbial growth and also contributing to the flavour of processed millet. The highest value of 85.5% dry matter (table 2) was obtained at day 10 (5.55×10^8 inoculum level) of fermentation process. Similar pattern have been reported for wheat and rice straw (Singh *et al.*, 1990). The loss of the dry matter (DM) after the optimum might be due to lack of preferential lignin degradation during substrate decomposition.The reduction of the moisture content of the substrate is an added advantage from the fermentation process because it leads to increase in the dry matter content of the substrates; and this is necessary for monogastrics as they are fed on dry matter basis. Also minimum moisture content of feed ingredients inhibits the growth of unwanted microbes, thereby increasing the shelf life of such ingredients. There was a rapid increase in the crude protein CP (table 3) content of the substrate from day10 of incubation with values of 2.15%, 2.31% and 3.74% and to a maximum of 5.36%, 6.29% and 4.44% respectively for the treatments 5.55×10^8, 1.11×10^9 and 1.65×10^9 respectively at day 12 of incubation; representing corresponding increase of 248.10%, 308.44% and 188.31% from the initial crude protein content of 1.54% (before fermentation). Similar report was also reported by (Aderemi *et al*, 2006) who recorded an improvement in CP from 2.09% to 7.34% representing 351% increment. Mishra *et al.*, 2007.recorded that the protein content of the treated samples increased linearly up to the 21st of the incubation and thereafter declined at day 28th .The observed trend of the crude protein is due to the metabolic activities brought about by the filamentous fungi in the substrate. Iyayi (2004) explained that the mycelia of the fungi in the substrates, degrade the cellulose and secretes enzymes which in turn degrade the non-starch polysaccharides (NSPs), converting it into soluble sugar. The growth of the fungi in the medium is as a result of the cellulose present in the substrates. Higher than optimal inoculum level may reduce protein yield, because of nutrient competition among fungal hyphae on limited substrate; at lower inoculum level lower than optimum ($5.55x10^8$), the rate of degradation is low which might be due to small size of the microbes, while at higher inoculum level than optimum ($1.65x10^9$), the number of microbes *(A. niger)* are likely more than the available nutrient for microbial growth.

Conclusion:

Optimization of parameters like initial inoculum level and incubation period is critical to the success of Solid state fermentation; in this study the optimum inoculum level was observed to be $1.1\ x10^9$ which yielded the highest crude protein of 6.29% representing 308.44% increment at optimum incubation period of 12 days.

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