Production of Single Cell Protein from Hydrolyzed Pineapple (*Ananas comosus*) Peel Using Fungi

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Abstract

Production of single cell protein from hydrolyzed pineapple peels by fungi was investigated. Trichoderma viride was selected based on its high cellulase activity; diameter of clear zone on CMCagar (7.4 cm) and activity on carboxymethylcellulose (4.64 mg glucose/ml), filter paper (3.76 mg glucose/ml) and cotton wool (4.12 mg glucose/ml). Samples of pineapple peel were hydrolyzed with the solutions of HCl, H_2SO_4 and NaOH at 0.5% concentration. The NaOH hydrolysates (138 mg/ml, 298 and 9.44 mg/ml) have higher reducing sugar, soluble sugar and protein content than H_2SO_4 (129, 206l and 6.28 mg/ml) and HCl hydrolysates (131, 279 and 7.32 mg/ml), respectively. The culture of Trichoderma viride were used in fermenting the hydrolyzed pineapple peels. The protein yield in 0.5% NaOH hydrolysates (27.35 mg/ml) was significantly ($p \leq 0.05$) higher than H_2SO_4 hydrolysate (18.32 mg/ml) and HCl hydrolysate (16.48 mg/ml) after 7 days incubation. The un-hydrolyzed samples which served as control produced the lowest protein. Nitrogen sources were added to the media supplemented with ammonium oxalate [$(NH_4)_2C_2O_4$], which gave the highest protein 55.44 mg/ml for NaOH hydrolysate. The maximum weight of biomass after drying biomass was 0.66 g/100ml. This study demonstrated the potential of pineapple peel as a substrate for product recovery, waste control and management.

Key words: Single cell protein, Ananas comosus, Cellulose, Pineapple, Fungi.

Introduction

The shortage of protein and other protein rich food supplies has stimulated the effort in searching new and alternate source of protein rich food and feed (Khan and Dahot, 2010). For this reason, in 1995, sources mainly yeast, fungi, bacteria and algae were named single cell protein (SCP) to describe protein produced from biomass of different microorganisms (Parajo *et al*., 1995). Single cell protein (SCP) represents microbial cells (primary) grown in mass culture and harvested for use as protein sources in foods or animal feeds (Dhanasekaran *et al.,* 2011). The protein from microorganisms such as algae, fungi, yeast and bacteria is cheap and competes with other sources of protein with good nutritive value depending on the amino acid composition (Dhanasekaran *et al.,* 2011). The single cell protein (SCP) is a dehydrated cell consisting of proteins, lipids, carbohydrates, nucleic acids, inorganic compounds and a variety of other non- protein nitrogenous compounds such as vitamins (Dhanasekaran *et al.,* 2011).

In animal feed and nutrition, single cell protein is used in fattening of calves, poultry, pigs and fish breeding. In food, it is used as aroma carriers, vitamins, emulsified agents to improve the nutritional value of blacked food (Nwufo *et al*., 2014). There are efforts to improve the protein quantity and quality of finished food products by augmenting protein ingredients in food formulation (Nasir and Butt, 2011). Although, animals are considered best in protein quality, microbial protein known as single cell protein (SCP) grow on agricultural wastes is optional proteins because of higher protein content (Bacha *et al*., 2011).

Microorganisms can utilize a variety of substrates like agricultural wastes and effluents, industrial wastes, natural gas like methane, etc. (Nasseri *et al*., 2011). Agricultural wastes are useful substrates for the production of microbial protein, but the following criteria are required: non-toxic, abundant, renewable, non- exotic, cheap and able to support rapid growth and multiplication of the organisms to give quality biomass (Dhanasekaran *et al.,* 2011). Several studies have been conducted using agricultural waste as substrate including mango kernel meal (Diarra and Usman, 2008), Hyacinth bean (*Lablab purpureus)* (Rasha *et al*., 2007), Leaf meal (*Ipomeoea asarifolia)* (Madubuike and

Ekenyem, 2006), Breadfruit (*Treculia africana)* hulls (Nwabueze and Otunwa, 2006), Papaya (*Carica papaya L.)*(Ojokoh and Uzeh, 2005), Rice bran (Oshoma and Ikenebomeh, 2005), Banana waste peel (Sankar *et al*., 2011) and Pineapple waste (Dhanasekaran *et al.*, 2011). The use of cheap and readily available substrate is desirable to lower the cost of production, reduce waste disposal and management problems, conserve natural resources and provide feed for livestock purpose (Sanker *et al*., 2011).

The pineapple (*Ananas comosus*) is one of the most important fruits in the world and is the leading edible member of the family *Bromeliaceae*. This fruit juice is the third most preferred worldwide after orange and apple juices (Cabrera *et al*., 2000). It is short, with a narrow stout stump, fibrous and spiny leaves. It is an herbaceous perennial plant which grows 1.0 to 1.5 m tall with 30 or more trough-shaped and pointed leaves, 30 cm long, surrounding a thick stem. It is a multiple fruit, forming a single fleshy fruit (Idise, 2012). The plant develops to a cone-shaped juicy and fleshy fruit with crown at the top (Tran, 2006). According to FAO online data base, the area under pineapple plantation in 2007 was almost 920,349 hectares with an estimated production of more than 18 million tons (FAO, 2007). Commercially, it is mainly produced as canned fruits and consumed worldwide (Tran, 2006). Besides, it is also processed as juices, concentrates, and jams. Furthermore, bromelain, the proteolytic enzyme in the stem of pineapple, is finding wide applications in pharmaceutical and food (Hebbar *et al.,* 2008).

Until recently, about 80% of pineapple produced in Nigeria came from small scale farms managed under mixed cropping systems and current production figures shows that Nigeria is the 6th largest producer of pineapple in the world (Fawole, 2008; FAO/World bank, 1999). The skin waste contain both carbohydrate and protein nutrients that are suitable and favourable for the growth of microorganisms, (Dhanasekaran *et al*., 2008).

Materials and Methods

Source of samples: Fresh pineapple fruits were obtained from Ogige market, Nsukka, Enugu, Nigeria.

Isolation of fungi from pineapple: Spoilt pineapple fruits (10 g) were homogenized in a sterile mortar containing distilled water. The homogenate was filtered with a Whatman No 1 filter and 1 ml transferred by a sterile pipette into a 9 ml 0.1% peptone water diluents and mixed. A serial decimal dilution in 0.1% peptone water was prepared, a dilution of 10^{-5} was attained with 0.1 ml each dilution viz., 10^{-3} to 10^{-4} spread onto Potato Dextrose Agar and incubated at 30 ± 2^{o}C for 3–4 days. Pure cultures were obtained by streaking on fresh PDA plates. The cultures was assigned arbitrary numbers and stored on agar slants at 4^{o}C.

Fungal selection based on cellulase activity: Twenty isolates that gave the best cellulase activity on CMC agar were selected and subjected to a more quantitative method of cellulase activity test. According to the method of Nwokoro *et al*., (2013), a loopful of each fungal isolate was combined with 4 ml of 0.2 M sodium acetate buffer (pH 6.5) and supplemented with : (a) 1% (w/v) carboxymethyl cellulose for the carboxymethylcellulase (CMCase) activity test; (b) 50 mg Whatman No. 1 Filter paper strip (1 x 8 cm) for the filter paper activity (FPA) test and, (c) 50 mg of well-blended, high grade commercial raw cotton wool for the cotton wool activity (CWA) test and then incubated for: 60 min. at 50^{o}C (a) and 24 h at 50^{o}C (b and c). Reducing sugars in the medium after incubation was estimated by a modification of the dinitrosalicylic acid method of Miller (1959). The fungal isolate with the best cellulase activity was selected, identified by DNA sequencing (Sanger sequencing method) and used for further works.

Preparation/pretreatment of pineapple peels: The pineapple fruits were washed with several changes of sterile water and peeled off. The peels were cleaned, cut into small pieces, rinsed in sterile water, oven dried and ground to a mesh size ~ 0.5 mm. Fifty gram samples of dried, ground pineapple peels were boiled for 1 hr in; (a) 500ml of 0.5% NaOH (b) 500ml of 0.5% H_2SO_4 and (c) 500ml of 0.5% HCl. The hydrolysates were filtered out and the slurry pH adjusted to 6.5 with sterile lactic acid (a) and 0.2M NaOH (b and c). The hydrolysates were supplemented with 10 ml each of KH_2PO_4 (5% w/v), urea (2.7% w/v) and $(NH_4)_2SO_4$ (9% w/v). A 50 g sample of dried, ground pineapple peels boiled in 500ml of distilled water was used as control. The extracts were placed into a sterile

container and carbohydrate and protein composition was determined (Sanker *et al*., 2011).

Determination of carbohydrate and protein content: Sample containing 5 ml of extract was added into a beaker, reducing sugar concentrations was determined by a modification of the dinitrosalicylic acid (DNS) method of Miller (1959). DNS (10 g) was dissolved in 200 ml of 0.2M NaOH. Potassium sodium tartrate (300 g) was dissolved in 800 ml of distilled water. The two solutions were mixed and stored in an air tight dark bottle. An aliquot (3 ml) of this reagent was added to tubes containing 1 ml of glucose solution at various concentrations and to distilled water blanks. The tubes were placed in boiling water bath for 10 minutes, cooled to room temperature and diluted by adding 3 ml of distilled water. The solutions were read in a Spectrum spectrophotometer at 540 nm. The readings were used to draw a standard curve for milligrams glucose equivalents per ml against absorbance. The total soluble sugar was estimated using the standard curve of glucose according to the method of Mustapha and Berbura (2009). The protein concentration was determined by the biuret method of Peterson (1983). Sample containing 1 ml of extracts will be mixed with 2 ml of Biuret reagent in a test tube and the mixture was shaken thoroughly and allowed for 15 minutes. Biuret reagent (2 ml) was added to tubes containing 1 ml of Bovine Serum Albumin (BSA) solution at various concentrations and to distilled water blanks. The tubes were shaken thoroughly and allowed for 15 min. The solutions were read in a Spectrum spectrophotometer at 550 nm. The readings were used to draw a standard curve for milligrams protein concentration equivalents per ml against absorbance.

Fermentation of hydrolyzed pineapple peels for production of single cell protein: The selected and identified fungal isolate was cultured on slants of Potato Dextrose Agar. Spores were harvested with 0.1% Tween 80 solution and adjusted with sterile distilled water to final concentration of 10^8 spores/ml. From the hydrolyzed samples and control, 90ml each was measured into sterile 250 ml Erlenmeyer flasks and autoclaved at 121^{o}C for 15 minutes. On cooling, 10 ml of the spore suspension (50 × 10^8) was added.

To check for effect of nitrogen sources, from the hydrolyzed pineapple extracts and control, 80ml each were measured into sterile 250 ml Erlenmeyer flasks. To each, 10 ml of different nitrogen sources i.e. sodium nitrate, potassium nitrate, ammonium nitrate, sodium nitrite and ammonium oxalate at 0.2 g/100 ml was added. The Erlenmeyer flasks were autoclaved at 121°C for 15 min. On cooling, the media in the flasks was inoculated with 10ml of the inoculum.

Submerged fermentation was carried out in Erlenmeyer flasks with three trial media of the each hydrolysates and control. The experimental set up was aerobically incubated at 30 ± 2^{o}C for a period of 7 days at static condition. Protein concentration of the each fermentation media at the start of experiment and at interval were taken. The culture broth was separated from fungal biomass after the incubation period by filtration through Whatman No. 1 filter paper (Sankar *et al.,* 2011).

Determination of weight of dried fungal biomass: At the end of incubation, the weight of fungal biomass was recorded after filtering on Whatman No. 1 filter paper and dried at 105°C in a hot air oven until constant weight was attained.

Statistical analysis: Results were expressed as mean ± standard deviation (mean ± S.D.). Statistical analysis was performed using SPSS software. One way analysis of variance (ANOVA) was used to compare differences in mean and to establish significant difference.

Results and Discussion

Seventy-two fungal isolates were gotten after 3 days of incubation. Pure cultures were obtained and the fungi were assigned arbitrary numbers IS1, IS2, IS3, IS4, IS5, IS6, IS7, IS8……..1S72. All the isolated fungi were screened for their ability to hydrolyse cellulose on CMC agar. The diameter of clear zones were measured and are shown in Table 1. No clear zones were recorded for the fungal isolates; IS4, IS9, IS17, IS18, IS19, IS26, IS27, IS32, IS34, IS35, IS40, IS50, IS52, IS53 and IS68. The highest clear zone was recorded for IS51 wih 7.4cm, followed by IS58 with 6.9cm. Some fungal isolates had lower clear zones which ranged 0.1cm – 3.6cm.

Table 1: Diameter of clear zone around fungal isolates on CMCagar

Fungal Isolate	Diameter of clear zone (cm)	Fungal Isolate	Diameter of clear zone (cm)	Fungal Isolate	Diameter of clear zone (cm)
IS1	4.0	IS25	1.1	IS49	0.3
IS2	0.1	IS26	0	IS50	0
IS3	3.2	IS27	0	IS51	7.4
IS4	0	IS28	6.3	IS52	0
IS5	0.6	IS29	0.4	IS53	0
IS6	0.8	IS30	0.8	IS54	6.1
IS7	0.7	IS31	0.1	IS55	0.7
IS8	1.2	IS32	0	IS56	0.8
IS9	0	IS33	1.8	IS57	0.3
IS10	0.1	IS34	0	IS58	6.9
IS11	5.5	IS35	0	IS59	0.2
IS12	0.9	IS36	0.9	IS60	2.1
IS13	0.7	IS37	3.6	IS61	0.1
IS14	0.3	IS38	0.2	IS62	0.6
IS15	0.2	IS39	0.7	IS63	2.3
IS16	5.5	IS40	0	IS64	0.8
IS17	0	IS41	0.4	IS65	0.7
IS18	0	IS42	5.8	IS66	0.5
IS19	0	IS43	0.5	IS67	1.9
IS20	2.7	IS44	0.9	IS68	0
IS21	0.3	IS45	4.1	IS69	0.5
IS22	0.7	IS46	0.1	IS70	0.4
IS23	4.9	IS47	0.7	IS71	1.9
IS24	0.4	IS48	0.5	IS72	0.7

Twenty fungal isolates that gave the highest clear zone diameter on CMCagar were futhur screened for their ability to hydrolyze cellulose substrates namely, carboxyl methyl cellulose (CMC), filter paper and cotton wool. The 20 fungal isolates tested produced different cellulose activities (Table 2). Culture supernatant from the fungal isolates designated IS25, IS33 and IS71 produced the lowest cellulose activities: (0.48, 0.36 and 0.42) mg/ml on CMC (0.16, 0.09 and 0.55) mg/ml on filter paper; and (0.11, 0.12 and 0.16) mg/ml on cotton wool respectively. Culture supernatant from the fungal isolate designated IS51 produced the best cellulose activities: 4.64 mg/ml on CMC 3.76 mg/ml on filter paper; and 4.12 mg/ml on cotton wool (Table 2), and was therefore selected for further work.

Result from DNA sequencing of fungal isolate designated IS51 was obtained as nucleotides. Sequence analysis from resultant nucleotides base pairs was performed with BLAST analysis by direct blasting on American data base http://blast.ncbi.nlm.nih.gov. For the fungal isolate, a read was BLASTED and the resultant top hits with minimum E-score for the BLAST result showing species name was used to name this specific organism. Sequencing result in FASTA format and corresponding Identity after BLAST analysis on NCBI website are shown in Table 3. The isolate matched *Trichoderma viride* ATCC28038.

Table 2: Fungal selection based on their cellulase activities

S/N	Fungal Isolate	CMCase activity (mg/ml)	Filter paper activity (mg/ml)	Cotton wool activity (mg/ml)
1	IS1	2.71	2.55	1.87
2	IS3	1.99	1.81	1.45
3	IS8	0.74	0.49	0.22
4	IS11	2.28	2.12	2.36
5	IS16	2.07	1.99	2.01
6	IS20	1.10	1.82	0.75
7	IS23	1.35	1.13	1.08
8	IS25	0.48	0.16	0.11
9	IS28	3.51	3.19	2.91
10	IS33	0.36	0.09	0.12
11	IS37	2.00	1.05	1.97
12	IS42	3.98	2.79	3.04
13	IS45	1.97	1.77	1.02
14	IS51	4.64	3.76	4.12
15	IS54	3.47	3.14	3.27
16	IS58	4.01	2.11	3.24
17	IS60	1.23	1.18	1.90
18	IS63	1.78	2.42	2.09
19	IS67	0.91	0.46	0.27
20	IS71	0.42	0.55	0.16

Table 3: Sequencing result in FASTA format and corresponding identification of the Fungal Isolate (IS51)

Type	Query Length	Sequence nucleotides obtained	Similarity	Gene Bank /Accession Number	Identity Of Isolate Sequenced
Genomic DNA	518	>2_ITS-4-V2_B07_04 CCGAGTTTACAACTCCCAAACCCAATGT GAACCATACCAAACTGTGCCTCGGCGGG GTCACGCCCCGGGTGCGTCGCAGCCCCG GAACCAGGCGCCCGCCGGAGGGACCAAC CAAACTCTTTCTGTAGTCCCCTCGCGGAC GTTATTTCTTACAGCTCTGAGCAAAAATT CAAAATGAATCAAAACTTTCAACAACGG ATCTCTTGGTTCTGGCATCGATGAAGAAC GCAGCGAAATGCGATAAGTAATGTGAAT TGCAGAATTCAGTGAATCATCGAATCTTT GAACGCACATTGCGCCCGCCAGTATTCT GGCGGGCATGCCTGTCCGAGCGTCATTT CAACCCTCGAACCCCTCCGGGGGGTCGG CGTTGGGGACTTCGGGAACCCCTAAGAC GGGATCCCGGCCCCTAAATACAGTGGCG GTCTCGCCGCAGCCTCTCCTGCGCAGTAG TTTGCACAACTCGCACCGGGAGCGCGGC GCGTCCACGTCCGTAAAACACCCAACTT CTGAAATG	99%	AY380909.1	*Trichoderma viride* ATCC28038

The carbohydrate (reducing sugar and soluble sugar) content and protein content of the various hydrolyzed pineapple peels extracts are shown in Table 4. NaOH hydrolyzed extract gave the highest carbohydrate and protein content; 138mg/ml total reducing sugar, 298mg/ml total soluble sugar and 9.44 mg/ml protein. Extract from HCl hydrolysis had; 131 mg/ml total reducing sugar, 279 mg/ml total soluble sugar and 7.32 mg/ml protein contents, followed by H_2SO_4 hydrolyzed extract with 129 mg/ml total reducing sugar, 206 mg/ml total soluble sugar and 6.28 mg/ml protein contents. The un-hydrolyzed sample which served as the control gave the lowest carbohydrate and protein content; 83 mg/ml total reducing sugar, 107 total soluble sugar and 4.12 mg/ml protein.

Table 4: Carbohydrate and protein content of hydrolyzed and control extracts

Extract	Total reducing sugar (mg/ml)	Total soluble sugar (mg/ml)	Protein (mg/ml)
0.5% HCl	131	279	7.32
0.5% H_2SO_4	129	206	6.28
0.5% NaOH	138	298	9.44
Control	83	107	4.12

The effects of fermentation on the protein content of pineapple peel are given in Figure 1. The highest protein yield from 9.44 mg/ml to 27.35 mg/ml extract was produced when NaOH was used for hydrolysis. The control produced the lowest protein increase from 4.16 mg/ml to 15.73 mg/ml extracts (Fig. 1). The protein yield produced in 0.5% NaOH hydrolysates was significantly ($p \leq 0.05$) higher than that of H_2SO_4 from 6.26 mg/ml to 18.32 mg/ml and HCl hydrolysate from 7.32mg/ml to 16.48 mg/ml. The protein yield increased gradually from the start of the experiment till when it was terminated at day 7.

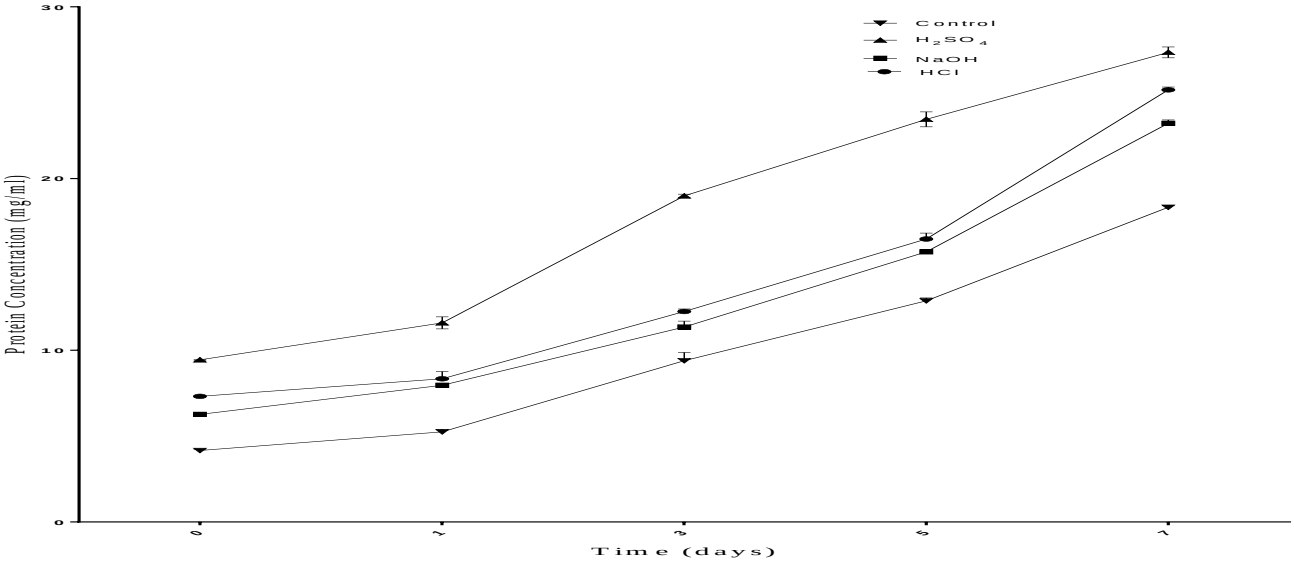

Figure 1: Protein concentration in hydrolyzed samples

Five different nitrogen sources (ammonium oxalate, sodium nitrate, sodium nitrite, ammonium nitrate and potassium nitrate) were added to the extracts and their protein yields were recorded. When ammonium oxalate was added to the samples, NaOH hydrolysate produced the highest

protein while control has the lowest protein yield (Fig. 2). At day1, NaOH gave the highest protein yield of 20.68 mg/ml, followed by the control 13.71 mg/ml while H_2SO_4 gave the lowest yield of 10.28 mg/ml. At the end of the fermentation, NaOH extract (55.44 mg/ml) had the highest yield, HCl (48.01 mg/ml), H_2SO_4 (34.70 mg/ml) and the control (34.16 mg/ml).

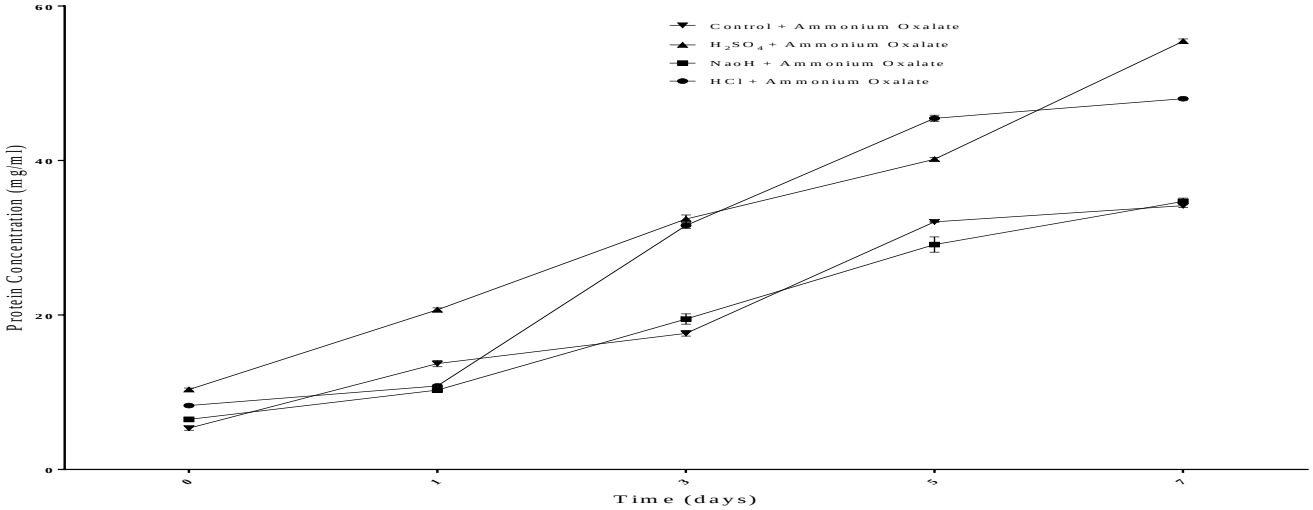

Figure 2: Protein concentration in hydrolyzed samples + ammonium oxalate

Protein yield when sodium nitrate was added to the samples were lesser than that of ammonium oxalate. However, NaOH hydrolysate also produced the highest protein yield from 9.70 mg/ml to 43.27 mg/ml and control produced the lowest from 4.40 mg/ml to 24.40 mg/ml (Fig. 3). The protein yield produced in 0.5% NaOH hydrolysates was significantly ($p \leq 0.05$) higher than that of H_2SO_4 hydrolysate (34.41 mg/ml) and HCl hydrolysate (41.66 mg/ml).

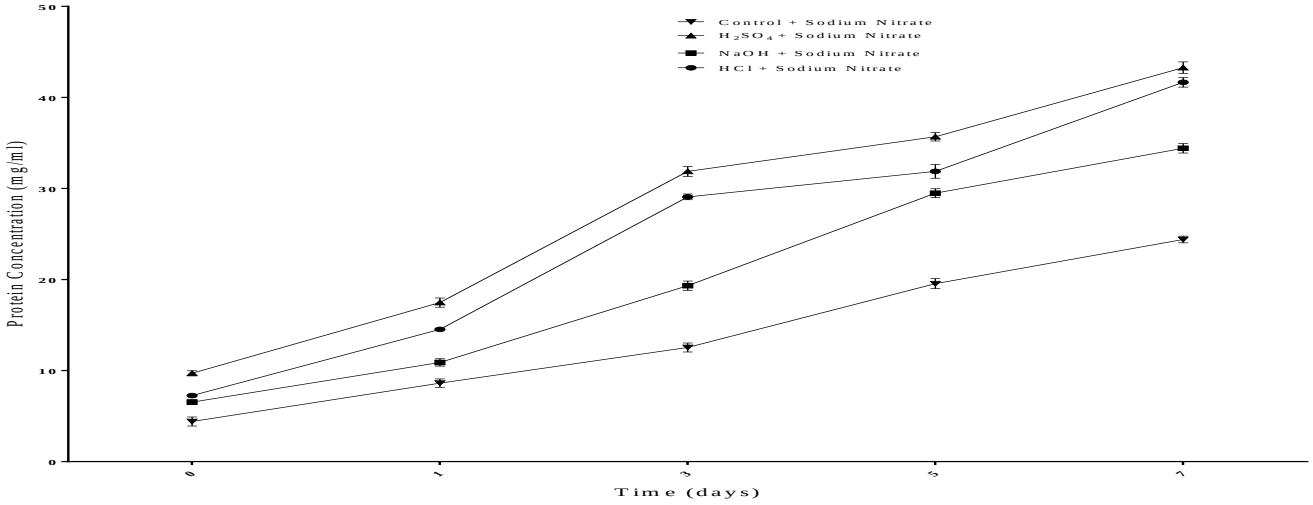

Figure 3: Protein concentration in hydrolyzed samples + sodium nitrate

Figure 4 shows the addition sodium nitrite to the samples where NaOH hydrolysate also produced the highest protein yield. At the start of the fermentation, protein concentrations of the samples were taken and they are; HCl hydrolysate (9.38 mg/ml), H_2SO_4 hydrolysate (7.13 mg/ml), NaOH hydrolysate (11.40 mg/ml) and the control had 6.35 mg/ml protein content. At

the end of the fermentation the protein concentrations were; NaOH hydrolysate (50.20 mg/ml), HCl hydrolysate (45.47 mg/ml), H_2SO_4 hydrolysate (27.15 mg/ml) and control (26.19 mg/ml) (Fig. 4).

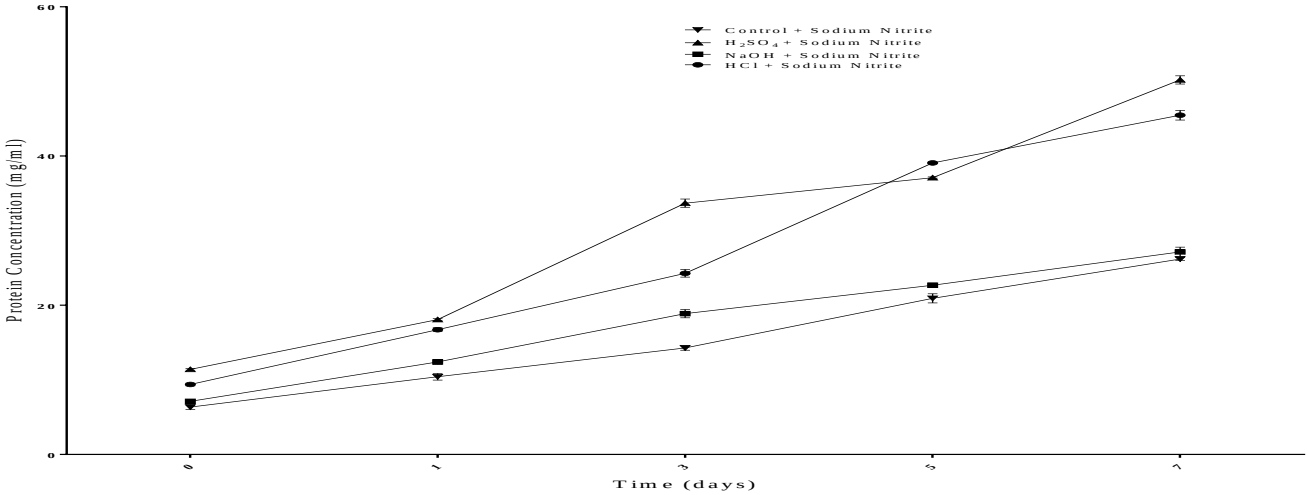

Figure 4: Protein concentration in hydrolyzed samples + sodium nitrite

The highest protein yield when ammonium nitrate was added to the samples was produced by H_2SO_4 hydrolysate from 9.66 mg/ml to 46.24 mg/ml (Fig. 5). At day1 of fermentation there was no significant difference ($p \leq 0.05$) between H_2SO_4 hydrolysate and NaOH hydrolysate (17.56 mg/ml and 17.46 mg/ml) respectively. The protein yields were measured at day5, the values were; HCl hydrolysate (34.40 mg/ml), H_2SO_4 hydrolysate (34.61 mg/ml) and NaOH hydrolysate (33.39 mg/ml) there was no significant difference among the protein content (Fig. 5).

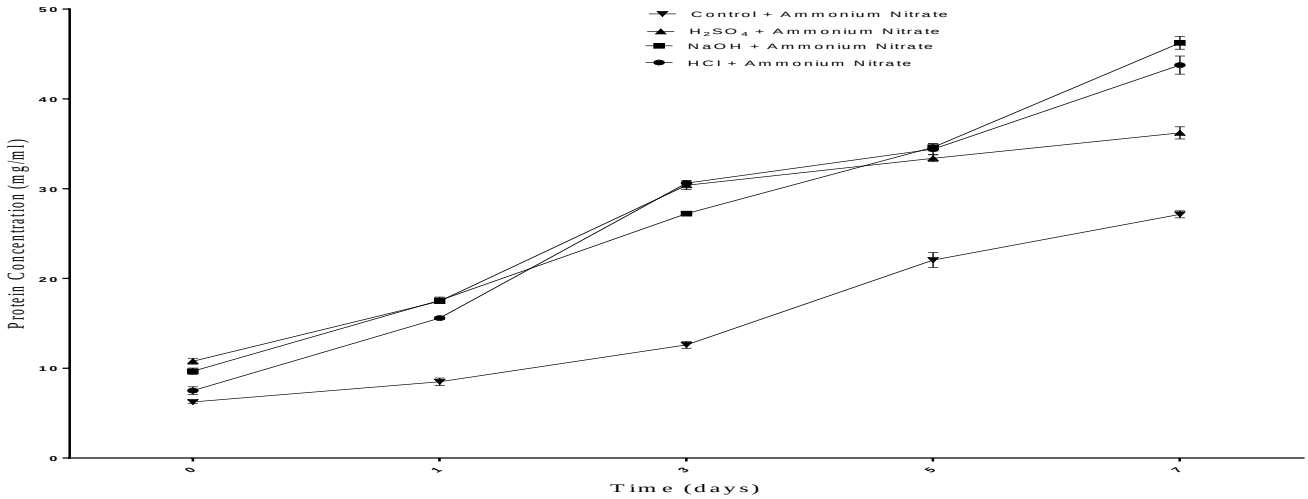

Figure 5: Protein concentration in hydrolyzed samples + ammonium nitrate

Addition of potassium nitrate to the samples produced the highest protein yield in HCl hydrolysate (Fig. 6). Among all the nitrogen sources used, potassium nitrate produced the lowest protein yield for the hydrolyzed samples but it was the best for the un-hydrolyzed sample

which served as the control in the entire experiment. Figure 6 shows that at the end of the fermentation (day 7), the highest protein yield was HCl hydrolysate (40.74 mg/ml), followed by H_2SO_4 hydrolysate (40.44 mg/ml), NaOH hydrolysate (38.45 mg/ml) and control (32.15 mg/ml).

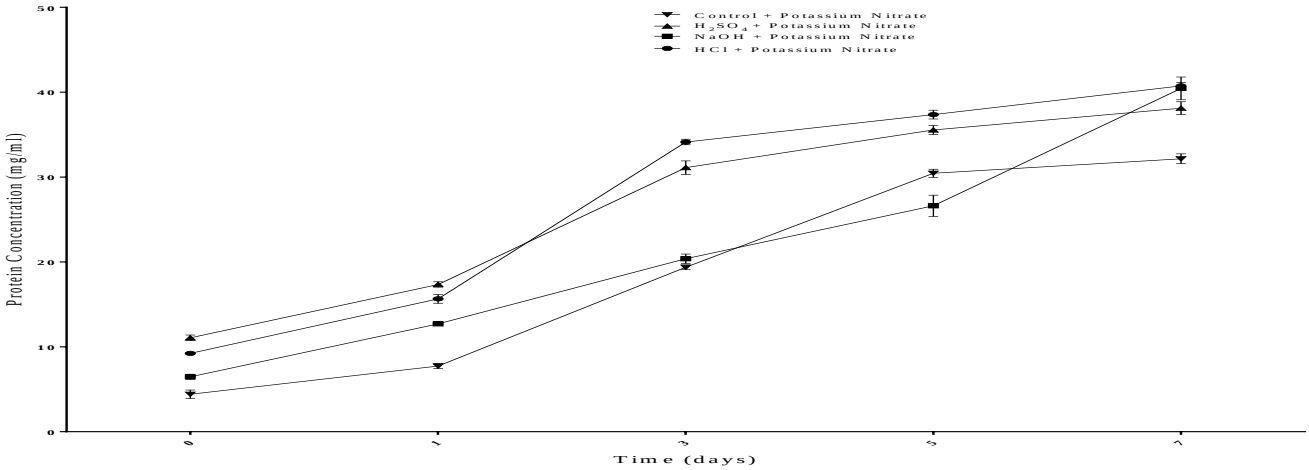

Figure 6: Protein concentration in hydrolyzed samples + potassium nitrate

After fermentation, the total weight (all trials) of the dried fungal biomass at the end of each fermentation were recorded and represented in Table 5. The highest weights recorded were from HCl + Sodium nitrate and HCl + Ammonium nitrate (0.66 g) and the lowest weights were from Control, Control + Ammonium oxalate, Control + Sodium nitrate, Control + 2% glucose and Control + 8% glucose where no biomass were harvested (Table 5).

Table 5: Weight of dried fungal biomass at the end of fermentation

Fermentation Medium	Weight (g/100ml)	Fermentation Medium	Weight (g/100ml)
HCl	0.09	HCl + Potassium nitrate	0.57
H_2SO_4	0.36	H_2SO_4 + Potassium nitrate	0.15
NaOH	0.39	NaOH + Potassium nitrate	0.06
Control	0.00	Control + Potassium nitrate	0.03
HCl + Ammonium oxalate	0.57	HCl + Sodium nitrite	0.51
H_2SO_4 + Ammonium oxalate	0.51	H_2SO_4 + Sodium nitrite	0.21
NaOH + Ammonium oxalate	0.39	NaOH + Sodium nitrite	0.09
Control + Ammonium oxalate	0.00	Control + Sodium nitrite	0.00
HCl + Sodium nitrate	0.66	HCl + Ammonium nitrate	0.66
H_2SO_4 + Sodium nitrate	0.39	H_2SO_4 + Ammonium nitrate	0.54
NaOH + Sodium nitrate	0.21	NaOH + Ammonium nitrate	0.12
Control + Sodium nitrate	0.63	Control + Ammonium nitrate	0.06

The fungal strains tested produced different cellulose activities on CMCagar (Table 1). Twenty fungal isolates with the best result were further screened for their ability to hydrolyze cellulose substrates namely, cotton wool, filter paper and carboxyl methyl cellulose (CMC) (Table 2). Culture supernatant from the fungal isolate designated IS51 produced the best cellulose activities, this can be compared to the works of Nwokoro *et al*. (2013) where several fungal isolates were able to produce cellulase activities. The isolate IS51 was identified by DNA sequencing as *Trichoderma viride* (Table 3). *Trichoderma viride* has been used to produce

single cell protein (Bhalla *et al*., 2007; Zhang *et al*., 2008; Mojsov, 2010; Mortta and Santana, 2012; Zeng *et al*., 2016).

Fungi have the ability to provide form and texture (Pogaku *et al.*, 2009), and can be harvested with ease; also, the cost of production may be reduced. Like algae, fungi generally have low nucleic acid content, can prosper on a variety of carbohydrates and are impeccably suited for agro residues (Pogaku *et al*., 2009). The direct consequence of this specificity is that various organisms, including human, cannot use cellulose to satisfy their nutritional requirement for carbohydrates. Most fungi produce cellulase that converts cellulose into monomeric glucose and the glucose generated is utilized by fungi to produce single cell protein as food for livestock and humans (Anupama and Ravindra, 2000). The choice of fungi for SCP production is governed by many factors including quality and quantity of protein in cells and its lack of toxicity (Pogaku *et al.*, 2009). Due to this specifications, three fungi namely- *A. oryzae* MTCC 1846, *A. niger* MTCC 1842 and *T. viride* NRRL 1186 were selected for the work of Noomhorm *et al*., (1992).

Hydrolyzed pineapple peel extracts contain variable ingredients with major amount of carbohydrates and small amount of protein (Table 4). The result agreed with the observation of Dhanasekaran *et al*., (2011) and Sankar *et al*., (2011) that pineapple extracts contain variable ingredients and may be used as energy sources for the growth of fungi in the production of single cell protein. The carbohydrate and protein content of pineapple peels are indication that the waste could serve as alternative substrate for cultivation of fungi (Essien *et al*., 2003). The high moisture reducing and none reducing sugar and crude protein recorded in pineapple wastes makes it a better substrate for single cell protein production (Table 4). The result agreed with the observation of Nwufo *et al*., (2014) that pineapple waste is a better substrate than other wastes products considered in the research.

From the results, there were indications that *Trichoderma viride* ATCC28038 had variability in the consumption of the different carbon sources of the hydrolyzed extracts (figure 1). The highest and the lowest protein yield of the extracts were observed, highest growth and protein yield of NaOH (27.35 mg/ml) increased significantly at ($p < 0.05$) than control extract (15.73 mg/ml), but the biomass yield were not significant. These differences were attributed to the variable nutritional values which serve as sources of nutrients for the growth of the mold in the production of single cell protein. These findings were in agreement with the observation of Yabaya and Ado (2008) that there was a higher growth in pineapple peels with higher amount of proteins, minerals, vitamins and other soluble carbohydrates which served as source of nutrients.

Several nitrogen sources were added to the samples, and the protein yields were higher than that of the media that was not supplemented with nitrogen sources. These could be as a result of nitrogenous sources which tend to supplement the nutritional status of the extracts and support the growth of *Trichoderma viride.* These findings were in agreement with the report of Emejuaiwe *et al*. (1998), that the addition of nutrient supplements provided available nitrogen source for the organism thereby enhancing its growth.

The media supplemented with ammonium oxalate [$(NH_4)_2C_2O_4$] gave the highest protein 55.44 mg/ml for NaOH hydrolysate followed by HCl hydrolysate (48.01 mg/ml) for the samples (Fig. 2), media supplemented with sodium nitrite ($NaNO_3$) and sodium nitrite ($NaNO_2$) also gave the highest protein of (43.27 mg/ml and 50.20 mg/ml) for NaOH hydrolysate followed by HCl hydrolysate with (41.66 mg/ml and 45.47 mg/ml), respectively for the samples. (Fig. 3 and 4). The media supplemented with ammonium nitrate (NH_4NO_3) gave the highest protein 46.23 mg/ml for H_2SO_4 hydrolysate followed by HCl hydrolysate (43.76 mg/ml) for the samples (Fig. 5). Lower protein yield were observed in media supplemented with potassium nitrate (KNO_3)

which gave the highest protein of 40.72 mg/ml for HCl hydrolysate followed by 40.44 mg/ml for H_2SO_4 hydrolysate (Fig. 6). This is in agreement with the work of Oshoma and Ikenebomeh (2005) where among the nitrogen sources investigated, supplementation of the medium with ammonium sulphate [$(NH_4)_2SO_4$] gave the highest biomass yield 1.95±0.03 g/l followed by 1.83±0.04 g/l when supplemented with ammonium nitrate (NH_4NO_3). But, the biomass yield was lower when media were supplemented with potassium nitrate (KNO_3), sodium nitrate ($NaNO_3$), ammonium chloride (NH_4Cl) and control (with no nitrogen supplement).

Weight of dried biomass after fermentation were taken for each medium (Table 5), Though, increase in the growth and proliferation of fungal biomass in the form of single cell protein (SCP) accounts for part of the increase in the protein content after fermentation (Raimbault, 1998), but there was no significant differences in the biomass content of extracts. The maximum amount of biomass produced 0.66g/100 ml (6.6 g/l) in this study was higher than the average reported for *Candida utilis* (5.1 g/l), and *Trichoderma viride* WEBL0702 (5.54g/ l) grown on molasses, and winery wastewater treatment, respectively (Nigam, 2000; Zhang *et al*., 2008).

Conclusion

The bioconversion effect of pineapple waste into single cell protein was evaluated using *Trichoderma viride* ATCC28038. The single cell protein production was much higher in hydrolyzed media; NaOH (27.54 mg/ml), HCl (25.17 mg/ml) and H_2SO_4 (23.22 mg/ml) than un-hydrolyzed medium (18.32 mg/ml). The supplementation of their extracts with different nitrogen sources increased significantly the growth and protein yield of *Trichoderma viride* ATCC28038. The highest protein contents of pineapple extract (NaOH hydrolysate) was recorded with ammonium oxalate (54.44 mg/ml) and sodium nitrite (50.20 mg/ml) as nitrogenous sources after incubation for 7 days. The present findings revealed that pineapple waste is an effective and alternative energy source for SCP production.

Conflict of Interest

There is no conflict of interest regarding the manuscript.

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