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Simultaneous saccharification and cultivation of *Candida utilis* on cassava peel

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ABSTRACT

Candida utilis yeast, a rich source of proteins and vitamin B-complex was cultivated on cassava peel, a food processing waste, which was first liquefied with Termamyl 120 L α-amylase enzyme. The cultivation process was optimized simultaneously with saccharification with Novo AMG 300 L amyloglucosidase using response surface methodology approach. The design involved three duration of enzyme hydrolysis (0, 4.5 and 9 h) prior to inoculation with *Candida utilis* representing varied degrees of hydrolysis (0, 50 and 100%) and initial pH (4.5, 5.0 and 5.5). Measured responses were change in yeast protein ranging from 1.13 to 1.91 mg/mL, change in cell concentration ranging from 2.30 to 3.90 mg/mL and specific growth rate ranging from 0.21–0.51.100% hydrolysis and initial pH of 5.0 gave the highest changes in yeast protein (1.92 mg/mL) and cell concentration (3.90 mg/mL); 100% hydrolysis and pH 5.5 gave the highest specific growth rate. The optimal solution was obtained at pH of 5.5 and 100% degree of hydrolysis with a degree of desirability of 0.8. The cultivation of *Candida utilis* yeast on cassava peel is of high significance to food and agro-based industries for the production of value added products, waste disposal and valorisation.

Industrial relevance: Cassava peel is a major waste product from cassava processing industry which is faced with an enormous challenge regarding its disposal. This study revealed that *Candida utilis* can be cultivated successfully on cassava peel slurry; the cultivation of this yeast on cassava peel is of high significance to food and agro-based industries for the production of value added products waste disposal and valorisation.

1. Introduction

The food processing industry generates a great amount of wastes annually worldwide, and it is presently causing a serious disposal problem (Gomez, Pazos, & Sanroman, 2005). Such wastes are usually rich in sugars which are easily assimilated by microorganisms. This makes them very suitable as raw materials for growth of microorganisms of high significance to food and agro-based industries for the production of value added products and waste disposal.

Cassava (*Manihot* spp.), a staple food of the majority of people in Tropical Africa, Central and South America (Subrahmanyan, 1990) has been subjected to various production processes in different countries to produce similar or different products; these processes are usually accompanied with some waste products. Cassava peels, leaves and starch residues constitute 25% of the cassava plant (Iyayi & Losel, 2001). Chief among the waste obtained from cassava processing is cassava peel, a carbon-rich material, which form high mounds of heap in processing areas with the attendant smelly odors (Almazan, 1998). The peel is about 10–20% of the wet weight of the root (Obadina, Oyewole, Sanni, & Abiola, 2006) and is available all year round in Nigeria. Many high value products have been produced from cassava peel and several researches about the utilization of cassava peel have been published including the production of fructooligosaccharide (Lateef & Gueguim-Kana, 2012), bioethanol (Sivamani & Baskar, 2015), bioelectrode (Ismanto, Wang, Soetaredjo, & Ismadji, 2010), biosorbent ((Kosasih et al., 2010; Kurniawan et al., 2011), bio-oil (Ki, Kurniawan, Lin, Ju, & Ismadji, 2013), enzymes (Lateef & Gueguim-Kana, 2012; Sani, Awe, & Akinyanju, 1992), citric acid (Adeoye, Lateef, & Gueguim-Kana, 2015), biofertilizer (Ogbo & Odo, 2011), protein enriched feed by microbial technique (Aderemi & Nworgu, 2007; Lateef et al., 2008; Okpako, Ntui, Osuagwu, & Obasi, 2008; Ruqayyah et al., 2014). Acid and enzyme hydrolysed cassava peels have also been used in the production of single-cell protein (SCP) via submerged fermentation (Ezekiel, Aworh, du Preez, & Steyn, 2012). Single cell protein refers to dried cells of microorganisms such as yeasts grown in large scale culture systems for use as protein sources in human foods or animal feeds. Technology exists for recovery of food materials in waste by chemical and microbiological means either directly or indirectly for human food. Bacteria,

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yeasts and fungi can grow very well on solid substrates for production of protein-enriched products if the carbon source needed could be obtained from low-cost lignocellulosic waste. Protein enrichment of this peel for probable utilization in livestock feeding would therefore help to alleviate the problem of its disposal as a waste product and as well release some other materials exclusively as food for human consumption.

Yeasts are a rich source of proteins and B- complex vitamins; they have been used as a supplement in animal feed and as a substitute for soy bean in diets for fowls (Gohl, 1991). The yeast *Candida utilis*, are classified among the most interesting microorganisms for their protein content, which can account for up to 50% of the dry weight (Ziino et al., 1999), it is also predominantly aerobic in metabolism and follows a pentose phosphate pathway for sugar metabolism which predisposes it to carbon balance in favour of biomass production unlike other yeasts which are glucose sensitive and largely fermentative.

Utilization of plant nutrients for the growth of protein rich microbes for maximum biomass production is one of the main objectives of bioprocesses which often involve process optimization. The One Factor At A time optimization method has been observed to have a limitation of non-interaction of independent variables (Ahmed, Ahmad, & Saeed, 2010; Shojaosadati, Faraidouni, Madadi-Nouei, & Mohamadpour, 1999). Response Surface Methodology (RSM), a statistical experiment design is now being adopted in many researches to efficiently analyse effects of several independent variables and as well consider the interactions among the variables in relation to the dependent variables or responses (Myers & Montgomery, 2002). There are several reports on application of RSM for the production of primary and secondary metabolites through microbial fermentation (Balusu, Padru, Kuravi, Seenayya, & Reddy, 2005; Jargalsaikhan & Saracoglu, 2009), Tari, Gögus, and Tokatli (2007) optimized biomass, pellet size and polygalacturonase production by *Aspergillus sojae* using RSM.

Degree of hydrolysis of substrate prior to inoculation and pH of cultivating medium were identified as important parameters in the production of protein enriched cassava peel in Simultaneous Saccharification and Cultivation (SSC) due to their effects on substrate conversion and microbial growth. The growth of microorganism is dependent on the amount of nutrient available from the hydrolysis step as the nutrient must neither be insufficient nor be in excess, while the efficient growth of the microorganism and enzyme activity are dependent on the pH of the medium (Ruqayyah et al., 2014). The cellulose conversion option that many currently favour is the Simultaneous Saccharification and Fermentation (SSF) process, in which the cellulose hydrolysis and glucose fermentation steps are combined in a single vessel (Elumalai & Thangavelu, 2010). In this present study the technique of SSF is being applied to cultivation of *Candida utilis* for enrichment of cassava peel slurry using a response surface methodology to optimize two cultivation variables, pH and degree of hydrolysis prior to inoculation with *Candida utilis*.

The objective of this work is to study the effect of degree of hydrolysis and pH on the biomass changes and growth kinetics of *Candida utilis* using response surface methodology during its cultivation on cassava peel slurry for the production of protein-enriched cassava peel.

2. Materials and methods

2.1. Sample collection and preparation

The peel from cassava tubers, variety TME I, obtained from a farm in Ajibade village in Akinyele local government area, Ibadan, Nigeria, was used for the study.

2.2. Micro-organism and culture medium

Candida utilis NRRL Y-1084 was obtained from the culture collection of the Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, South Africa and maintained on glucose peptone yeast (GPY) extract agar slants. The micro-organism was subcultured once every three months. The yeast for inoculation was cultivated on a chemically defined mineral salts culture medium which comprised (per L.): 5 g glucose, 0.25 g citric acid, 2 g $(NH_4)_2SO_4$, 6.8 g KH_2PO_4, 0.2 g $MgSO_4{\cdot}7H_2O$, 0.01 g $CaCl_2{\cdot}2H_2O$, 0.1 g NaCl and 1 mL of a trace element solution according to du Preez and van der Walt (1983).The medium was adjusted to pH 6.0 with 3 M KOH before autoclaving.

2.3. Inoculum preparation

The *C. utilis* used as inoculum was cultivated in 500 mL Erlenmeyer flasks with cotton wool plugs containing 50 mL medium, the composition of which is given above. Each flask was inoculated with a loopful of yeast culture from a 24 h agar slant and incubated at 30 °C on a rotary shaker at 180 rpm. Growth was monitored by optical density measurement by using a Photolab S6 photometer (WTW, Weilheim, Germany) at 690 nm for 12 h at an exponential phase with a cell concentration of 2.54×10^{-3}g per mL. The amount of inoculum used was 10% of the working volume i.e. 100 mL of the inoculum was used per 900 mL of the cultivation medium.

2.4. Hydrolysis of the cassava peel slurry

The peel from fresh cassava tubers, variety TME I, were obtained from a farm in Ajibade village in the Akinyele local government area, Ibadan, Nigeria. Enzymatic hydrolysis was performed by using Termamyl 120 L α-amylase (Novo Industry A/S, Bagsvaerd, Denmark) for liquefaction, at a concentration of 0.06% on starch dry weight (equivalent to 0.036% (w/w) on dry peel weight) according to the manufacturer's dosage recommendation. Saccharification was done with Novo AMG 300 L amyloglucosidase, using a concentration of 0.15% on starch dry weight. Dried and milled cassava peel was added to distilled water to give a suspension of about 1.25% cassava peel slurry on dry weight (8% moisture content), which was subsequently adjusted to pH 4.5, 5.0 and 5.5 respectively with 3 M KOH, 8 mgL^{-1} of Ca^{2+} (0.029 gL^{-1} of $CaCl_2{\cdot}2H_2O$) added and then heated to 92.5 °C in a boiling water bath. The Termamyl was added to the slurry when the temperature approached 60 °C. After liquefaction, the temperature was decreased to 60 °C and the pH adjusted to 4.5 with 3N H_2SO_4 prior to addition of the AMG for hydrolysis taking place for 4.5 and 9 h respectively prior to inoculation. Samples, taken at 1 h intervals, were immediately boiled to inactivate the AMG whereas trichloroacetic acid was added to a final concentration of 5% (v/v) to inactivate the Termamyl.

2.5. Analytical methods

Yeast protein was determined by the Biuret method (Stickland, 1951). Samples were assayed for reducing sugar using the 3, 5-dinitrosalicyclic acid (DNSA) method (Miller, 1959).

2.6. Simultaneous Saccharification and Cultivation (SSC)

The experiment involved simultaneous saccharification of cassava peel and cultivation of the yeast *Candida utilis* in 1.25% cassava peel slurry inside a 2-litre Multigen F-2000 bioreactor with a working capacity of 1000 mL. The maximum specific growth rate of the batch culture was determined by linear regression analysis of the exponential phase of the growth curve using Microsoft Excel (Microsoft Corporation, WA and USA). The biomass yield coefficient ($Y_{x/s}$) using 1.25% cassava peel slurry as the utilized substrate was calculated as in Eq. (1).

$$Y_{x/s} = \frac{(x_t - x_0)}{s_{conc}}. \quad (1)$$