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Study on optimization of process parameters for enhancing the multi-hydrolytic enzyme activity in garbage enzyme produced from preconsumer organic waste

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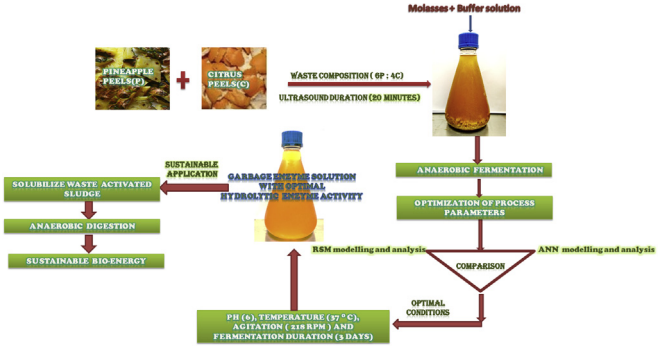
HIGHLIGHTS

- A crude mixed hydrolytic enzyme seems to be a good substitute than single enzyme.
- Garbage enzyme (GE) possesses protease, lipase and amylase activity.
- To produce GE with higher hydrolytic enzyme activity needs optimized parameters.
- Statistical model for GE production were determined.
- GE significantly utilizes for different sustainable environmental applications.

GRAPHICAL ABSTRACT



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ABSTRACT

The garbage enzymes produced from preconsumer organic waste containing multi hydrolytic enzyme activity which helps to solubilize the waste activated sludge. The continuous production of garbage enzyme and its scaling up process need a globe optimized condition. In present study the effect of fruit peel composition and sonication time on enzyme activity were investigated. Garbage enzyme produced from 6 g pineapple peels: 4 g citrus peels pre-treated with ultrasound for 20 min shows higher hydrolytic enzymes activity. Simultaneously statistical optimization tools were used to model garbage enzyme production with higher activity of amylase, lipase and protease. The maximum activity of amylase, lipase and protease were predicted to be 56.409, 44.039, 74.990 U/ml respectively at optimal conditions (pH (6), temperature (37 °C), agitation (218 RPM) and fermentation duration (3 days)). These optimized conditions can be successfully used for large scale production of garbage enzyme with higher hydrolytic enzyme activity.

1. Introduction

In the developing world, enormous amount of fruit as well as vegetables solid waste are generated primarily due to higher production, lack of appropriate preservation and transportation process (Mahmood et al., 1998). Also, these types of organic waste are resulting from peeling and trimming of fruits and vegetable in household kitchens and food industries (Bouallagui et al., 2005) The food based organic waste comprises a major fraction in increasing municipal solid organic waste due to urbanisation and increasing standard of living all over the globe. Ariunbaatar et al. (2014) stated that the generation of food waste will increase up to 44% by 2025, thus management of organic solid waste (OSW) will grow into a major issue all over the globe. OSW containing

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huge amount of organic matter which ultimately degrades to produce carbon dioxide and methane, as the conventional disposal of organic solid waste results in serious environmental pollution and health risks problems to living organisms. From an environmental perspective, there is a crucial need to develop appropriate alternate waste management technology for the utilization of organic wastes as well as to minimize the pollution problems created by them (Anto et al., 2006; Neves et al., 2008; Dhanalakshmi and Alwar, 2012). The chemical complexity, easy degradability, higher moisture content and nutrient rich composition of organic food waste (Kiran et al., 2014), made them as a useful resource for the production of higher value added products such as fuels, chemicals and biochemicals through fermentation process (Chanakya et al., 1999; Sakai et al., 2004; Wang et al., 2005; Zhang et al., 2013; Melikoglu et al., 2013) Globally, the interest of biochemical products (organic acids, enzymes, biopolymers etc.) production from organic waste is increasing day by day (Yuan et al., 2016). Among them, the enzymes play an important role to achieve zero discharge of organic solid waste from different sector by improving biological remediation process to recover valuable resources (Kavitha et al., 2013). The enzymes which are presently used in environmental applications are quiet expensive because of the cost of production and purification. Chamraj Gokul et al. (2011) deciphered that fermentation technology can be applied to effectively utilize vegetable wastes for production of industrially significant enzymes and estimated that protease specific activities for pumpkin peels is 13.44 U mg /protein/ ml, which is comparable higher than using peels of cauliflower and cabbage as raw material. Amin et al. (2014) reported that maximum activity of lipase found after 96 h of reaction in the fermentation medium with initial pH 4 using agricultural waste as raw material.

Recently researchers producing a mixture of crude hydrolytic enzymes through fermentation process seem to be a good substitute and perform better than expensive single enzymes (Enu et al., 2006; Wei et al., 2015). Many researchers suggested that if the crude enzymes activity of biological solution is higher, it can be used directly without any recovery process in a feasible and economical way (Parawira, 2012; Leung et al., 2012; Kiran et al., 2014). Garbage enzyme(GE) was first developed from house hold fruit and vegetable waste and this garbage enzyme functions in the same way as normal enzymes in achieving a higher degree of degradation within a shorter time at suitable environmental conditions (Joean oon, 2008).Thus it helps to promote recycling of organic solid waste back. GE can be utilized as a low-cost alternative to improve wastewater treatment processes by removing the impurities and bacteria (Khairul and shamila, 2012; Bhavani prakash, 2012; Fazna nazim and Meera, 2013).

The analysis of the above cited literature concludes that garbage enzyme was used only for mainly for wastewater treatment and no studies were reported on usage of garbage enzyme for organic solid waste treatment. In our earlier works, garbage enzyme produced by the fermentation of different preconsumer organic waste was tested for bio catalytic activity and its effects on solubilization of waste activated sludge (WAS) were reported (Arun and Sivashanmugam, 2015). The result confirms that garbage enzyme produced from pineapple and citrus peels possesses maximum protease, lipase and amylase activity, which help to increase the solubilisation of TKN(Total Kjeldhal Nitrogen), COD (Chemical oxygen demand), and TP (Total phosphorus) nearly 15–20%, 20–25%, 9–11% respectively in waste activated sludge.

The production of garbage enzyme with higher activity of lipase, amylase and protease is needed to cater for treatment of larger quantity of industrial waste activated sludge generated. This necessitates the optimizing of various parameters to improve the activity of crude enzyme mixtures to reduce their cost and cost of application. Till date no study was reported to optimize the parameters responsible for enhancing the activity of multi-enzyme in garbage enzymes solution produced from organic solid waste.

The statistical methods for optimization are gaining an increasing importance, as this methodology can save time and cost (Vitcosque et al., 2012). Currently, Response surface methodology (RSM) and Artificial neural networks (ANN) are potent mathematical methods applicable for modelling and simulation of various processes parameters in many real applications. RSM and ANN have been commonly applied for the modelling and optimization of many biochemical processes used for the production of primary metabolites and secondary metabolites (Dasu and panda, 2002; Dahunsi et al., 2016). Generally in the mathematical methods, the models are initially developed according to the functional relationships between input parameters and response (output) of the process using experimental data. Subsequently, the developed models are used to estimate the optimal values of input parameters to maximize or minimize the response (Emeko et al., 2015).

To achieve large scale production of garbage enzyme with enhanced multi- hydrolytic enzyme activity, the fermentation process parametric conditions have to be optimized and are reported in the present work. Initially the effect of fruit peel (pineapple and citrus) composition on enzyme activity of garbage enzyme was studied. The determination of ammonium sulphate maximum and minimum saturation cut off value for partial purification process for lipase, amylase and protease in garbage enzyme solution was studied. The effect of sonication time on enzyme activity of garbage enzyme was investigated to obtain the optimal duration of ultrasound required to pre-treat fruit waste to produce garbage enzyme solution with higher activity of all the hydrolytic enzymes. Subsequently, the RSM and ANN optimization methodology have been conducted and compared to determine the optimal factors (pH, temperature, and agitation and fermentation time) for producing a garbage enzyme with higher activity of lipase, amylase and protease.

2. Materials and method

2.1. Production of garbage enzyme

Pineapple and citrus fruit dregs from fruit shop were collected and mixed equally. From this mixture 90 g was taken in air tight container and 30 g of molasses and 300 ml of water were added. The container was placed in a well-ventilated, dry and cool area for fermentation for three months. After three months the garbage enzyme solution was filtered and centrifuged at 5000 rpm for 30 min. The obtained supernatant was used for further investigation.

2.2. Optimization of partial purification process

In present study ammonium sulphate precipitation methodology is used for partial purification of amylase, protease and lipase in garbage enzyme solution obtained from preconsumer organic waste. The garbage enzyme was taken in a beaker, sufficient quantity amount of ammonium sulphate was added to make the solution to 10 % saturated and stirred for 30 min. This 10% saturated solution was centrifuged at 5000 rpm for 30 min. The pellet (P) was re-suspended in a beaker with the minimal volume of homogenization buffer and labelled as 10% P and kept in refrigerator. The 10% saturated supernatant was taken in another beaker, enough quantity of ammonium sulphate was added to make the solution to 20% saturated and stirred for 30 min. This 20% saturated solution was centrifuged at 5000 rpm for 30 min. The pellet (P) was re-suspended in a beaker with the minimal volume of