IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Production of Enzyme Bio-cleaner from Fruit Waste by Yeast

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ABSTRACT

Enzyme Bio-cleaners are the organic compounds including enzymes produced by the simple fermentation of fresh fruit wastes with addition of brown sugar and water by using the selective microorganisms like Yeast. This fermentation creates natural chains of proteins, mineral salts, organic acids, alcohol and enzyme which have the capacity to breakdown, change, create and catalyse - functions that make it a wonderful cleaning aid in household as well as in industrial and medical applications. By understanding the above concept and mechanism, the present study was carried out for the production and analysis of enzyme bio-cleaners by using yeast in different fruit wastes such as *Citrus limetta* (Mosambi) and *Punica granatum* (Pomegranate) collected from various Fresh Juice Stalls in Bangalore. During fermentation, the enzyme production like cellulase, amylase and protease were tested for it activity in fermented broth at different period of fermentation and found with moderate level of activity. The results of the present investigation are presented and discussed in detail in the report.

***Key words*:** *Citrus limetta*, *Punica granatum*, cellulase, amylase

INTRODUCTION AND SCOPE

Urbanization and industrialization accompanied by population flare-up has formed a serious problem of waste generation and its disposal, treatment and management. Fruit and vegetable wastes are produced in large quantities in markets and constitute a source of nuisance in municipal landfills because of their high biodegradability (Viturtia et al., 1989). In India, Fruit and Vegetable Wastes constitute about more than 10 million tonnes annually and currently these wastes are disposed by dumping on the outskirts of cities. The main contributors of waste generation in Indian society are agriculture and municipal sectors. Industrial sectors have also not been able to handle and treat the wastes generated by them or control the emission of obnoxious gases into the atmosphere. In spite of the various environmental rules and regulations, very little has been achieved in terms of minimization of waste generations. In Indian cities total quantum of waste generation is increasing at rate of 1.33% (Srilatha *et al.*, 1995).

Enzymes are proteins, which act as catalysts. Enzymes lower the energy required for a reaction to occur, without being used up in the reaction. Many types of industries, to aid in the generation of their products, utilize enzymes. Enzymatic processes have been implemented in a broad range of industries in recent decades because they are specific, fast in action and often save raw materials, energy, chemicals

and/or water compared to conventional processes. A number of comparative environmental assessment studies have been conducted in the past 15 years to investigate whether these properties of enzymatic processes lead to environmental improvements and assess whether they could play a role in moving toward cleaner industrial production. On the other hand, enzymes have become popular in cleaning products because they are biodegradable and facilitate selective reactions. In some applications, e.g., dishes and cotton clothes, it is usual to find several cleaning detergents with some amount of lipase and cellulose enzymes in their formulations. With societal pressure to reduce the use of hazardous chemicals increasing use is being made of biological agents for industrial and medical applications.

Many industries are dependent on enzymes for the production of their goods. Fermentation is a method of generating enzymes for industrial purposes. Fermentation involves the use of microorganisms, like bacteria and yeast to produce the enzymes. Enzymes energize and catalyse biochemical reaction with high specificity and enhance the reaction rate. Enzymes are formed during growth of microorganisms (trophophase), as a result of oxidative metabolism and aerobic fermentative.

Enzyme Bio-cleaners are an organic solution produced by the simple fermentation of fresh fruit wastes with addition of brown sugar and water by using the selective microorganisms like Yeast. This fermentation creates natural chains of proteins, mineral salts, organic acids, alcohol and enzymes. This solution has the capacity to breakdown, change, create and catalyse - functions that make it a wonderful cleaning aid in household as well as in industrial and medical applications. Bio-cleaning enzyme solution was developed by Dr. Rosukon from Thailand through simple inexpensive method for various household and hospital cleaning methods. This has been developed for people to make simple cleaning solution at home or in small scale business to ease global warming because minimizing the environmental pollution by utilizing the cabbages like kitchen waste, vegetable and fruit wastes from stalls and markets. This product is natural, safe, and good for the environment and use the magic of nature to produce extraordinary removal of dirt, oil, stain etc. resulted to customer satisfaction.

During the past few years, there has been serious public concern about the ecological problems arising from the use of synthetic cleaning agent which releases toxic chemicals and unstable pH and chemicals of environment. Owing to the fact that these synthetic cleaning solutions are corrosive, toxic and exhibit a slow rate of biodegradation, their extensive usage leads to the formation of slumps, creating unhygienic conditions in the surroundings. However, the latest cleaning technologies include enzyme-containing cleaning solutions are very effective, safe and specificity in activity and environmental friendly as well as cheaper preparation methods. Enzyme Bio-Cleaning solution is formulated specifically to dispose of dirt and soils safely, economically and rapidly and work quickly and efficiently to digest chemical and organic waste with no odour or noxious gas. So suitable alternate to the synthetic cleaning solution with regard to biodegradability, low toxicity, non-corrosiveness, environment-friendliness, enhanced cleaning properties as well as increased efficiency and stability in different environmental conditions are require to develop. In this context, enzyme bio-cleaners referred to as green chemicals are becoming an ideal consumer choice for various applications.

The advantages of using enzyme bio-cleaning solution are:

- It is safer for the environment and safer for human health than traditional chemical cleaners and odour control products,
- It is highly specialized enzyme producing microorganisms to clean and control odours by eliminating the soils rapidly
- It is economically cheaper and the cost of production is less
- This Bio-Cleaning solution provide residual cleaning for longer period and gives stable application and
- This Enzyme Bio-cleaning solution help to displace unknown, potentially pathogenic (disease causing) bacteria with known, healthy microorganisms and in this way contribute to better human health.

- Thus, to conclude, cleaning forms an important aspect for the maintenance of hygiene and safety of foods in the food processing industry. Due to their high efficiency and safety, it is assumed that the enzyme cleaners will eventually capture a bulk of the Indian market. Some uses recommended for this non-toxic, environmentally friendly enzyme:
- For dishes and laundry
- For washing bathrooms and toilets. grime comes off easily
- For removing stubborn stains and odours (coloured fabrics and floors)
- Clear blockages in kitchen sinks and drains (use blended pulp/sludge of enzyme)
- As a natural insect repellent (use undiluted) for ants, cockroaches
- For mopping floors
- As fertilizer for plants in garden
- Wash cars – cars will look as if they have just been polished
- Save Money, Save Space, Save Water and Save The Earth!

Based on the above scientific and technical information, the present work is proposed to carry out the production and analysis of the fermentation parameters of Enzyme Bio cleaning solution by using the residues and waste of agricultural produces like fruit with help of Yeast (***Saccharomyces sp.***) with addition of cheaper carbohydrate sources like brown sugar and water medium.

AIM & OBJECTIVES

- The main aim of the present study is Production of Enzyme Bio-cleaning solution by green method without any environmental hazardous.
- To minimize the environmental pollution by the usage of waste raw materials like fruit wastes.
- Optimize the production of Enzyme Bio-cleaning solutions provided with simple and natural media,
- To study the performance of the Enzymes, organic acids and alcohols etc. present in the fermented liquor during fermentation.
- To see the activity of the fermented liquor media on commonly found pathogens on floor, bathroom tiles hence to check the cleansing activity of Enzyme Bio-cleaning solution.

REVIEW OF LITERATURE

Parkar and his co-workers explained the cleaning strategies tested were based on biofilm biochemistry and physiology, and focused on the chemistry of the cleaners, the duration and temperature of the cleaning process and a combination of various cleaners. The success of the cleaning regimes was determined based on the removal of cells and organic debris and the elimination of viable cells. The results confirmed that a caustic (75^{o}C for 30 min) and acid (75°C for30 min) wash, relied upon heavily in most food processing industries for cleaning-in-place systems, was successful in removing these biofilms. Confirmation of these results should be carried out in a pilot plant through several use/clean cycles. Significance and Impact of the Study: Confidence in standard and alternative cleaning procedures for food manufacturing plant to prevent contamination with thermophilic bacilli that threaten product quality (Parkar et al., 2004).

Kenthorai Raman Jegannathan and his co-workers described the Enzymatic processes have been implemented in a broad range of industries in recent decades because they are specific, fast in action and often several raw materials, energy, chemical, sand/or water compared to conventional processes. A number of comparative environmental assessment studies have been conducted in the past 15 years to investigate whether these properties of enzymatic processes lead to environmental improvements and assess whether they could play a role in moving toward cleaner industrial production. The purpose of

this review is to summarize and discuss the findings of these studies and to recommend further developments regarding environmental assessment and implementation of the technology. Tradition, lack of knowledge and bureaucracy are barriers to implementation of enzymatic processes in industry. Education and streamlining of public approval processes etc. are means of overcoming the barriers and accelerating the harvesting of the environmental benefits. (Kenthorai Raman Jegannathan et al., 2013).

Microbial lipases are an important group of biotechnologically valuable enzymes, because of the versatility of their applied properties and ease of mass production explained by Fariha Hasan. Lipases of microbial origin are widely diversified in their enzymatic properties and substrate specificity, which make them very attractive for industrial applications. Enzymes can reduce the environmental load of detergent products as the chemicals used in conventional detergents are reduced; they are biodegradable, non-toxic and leave no harmful residues. Besides lipases, other enzymes are widely used in household cleaning products, in laundering, medical, agriculture, etc. This article also reviews the use of enzymes, especially lipases as detergents and different types of lipase containing detergents available in the market. (Fariha Hasan *et al*., 2010).

Deusanilde J. Silva and his co - workers explained an enzymatic treatment is proposed as a preparative, cleaning protocol to remove cellulose films from resonators and sensors. Quartz crystal and surface plasmon gold sensors, coated with ultrathin films of cellulose are used in studies of molecular (for example, polymer and surfactant) adsorption. The sensors are usually recycled after removal of the film, with limited success, after one of two treatments, either hot acid or ammoniac solutions. In the proposed, improved protocol a mixture of cellulases from *Aspergillus* species, are used as a pre-treatment to facilitate the release of the cellulose film from the surfaces of the sensors. It is concluded that the use of the recycled ammoniac cleaning solution after the enzymatic treatment is a very convenient, safe and less time-consuming way to remove the cellulose films from the sensors to be recycled. (Deusanilde J. Silva *et al*., 2011).

Prasad Rao and his co-workers explained In his study pectinase enzyme was produced from Aspergillus Niger NCIM 548 under solid state fermentation (SSF) using agriculture residue and horticulture wastes. Sixteen substrates were screened for pectinase production of which jack fruit waste was found to be the best substrate. The maximum yield of pectinase 39.836U/gds was obtained with jack fruit waste 10g, particle size 152-354 μm, moisture content 70%v/w, pH 5.0, temperature 30oC, glucose 3.5%w/w, (NH)4SO4 1.0% w/w and fermentation time72 h. (Prasad Rao *et al*., 2014).

Howard and his co-workers working in related areas of lignocellulose research of the enormous economic potential of the bioprocessing of residual plant materials generally regarded as “waste”, and secondly to highlight some of the modern approaches which potentially could be used to tackle one of the major impediments, namely high enzyme cost, to speed-up the extensive commercialisation of the lignocellulose biop 8 Leather industry is facing tremendous pressure from the various pollution control bodies because of the huge amount of pollution associated with processing explained by Kanagaraj. Advancements in processing techniques and adoption of cleaner technologies have enabled the tanners to get rid of pollution from the leather processing. Though there are various cleaner technologies based on chemical methods are available but cleaner technologies based on enzymatic methods are viable, eco-friendly and form alternative to the existing technologies. Enzymes in leather industry became a part and parcel of the system to mitigate pollution problem in the leather processing operation. The enzymes find application in soaking, unhairing, degreasing and bating of leather processing operations for obtaining better leather qualities. Applications of enzymes in various stages of leather processing are discussed in this paper. (Kanagaraj *et al*. 2009).

Gareth Evans and his co-workers explained the Proteolytic enzymes are a recognised risk for respiratory and dermal allergy. Cases of asthma have been identified in health care workers using cleaning solutions containing these enzymes to decontaminate endoscopes and surgical equipment. An assessment was made of three hospitals using enzyme products to clean endoscopes. Air samples showed that approximately a third of the personal and a half of the static air samples contained protease activity at levels that may pose risk for allergic sensitisation. As a result, there were deficiencies in the application of control measures although the surface contamination levels were much lower at one

hospital where regular cleaning of surfaces was undertaken throughout the day. This report and the work it describes were funded by the Health and Safety Executive (HSE). Its contents, including any opinions and/or conclusions expressed, are those of the authors alone and do not necessarily reflect HSE policy (Gareth Evans *et al*., 2013).

Biosurfactants are surface-active substances synthesized by microorganisms having the properties of reducing surface tension, stabilizing emulsions, promoting foaming and are generally non-toxic and biodegradable described by Amalesh Samanta. Here an effort was made to screen biosurfactant activity of a protease producing bacteria isolated from municipal solid waste. Strain was identified as Pseudomonas aeruginosa by 16S rDNA based molecular 9 techniques. Biosurfactant, obtained from isolated organism was screened by hemolytic assay, drop collapsing method, oil spread method, blue agar plate method and oil spreading technique. Besides biosurfactant activity the strain also produces protease enzyme. The strain has shown maximum protease activity at pH 9.5, temperature 37°C and 48 hrs. of incubation time. So, this strain can be used in textile, leather, detergent, pharmaceutical and dairy industries for its dual ability of producing protease enzyme and biosurfactant activity (Amalesh Samanta *et al*., 2012).

Renge and his co-workers states that the enzymes are proteins, which act as catalysts. Enzymes lower the energy required for a reaction to occur, without being used up in the reaction. Many types of industries, to aid in the generation of their products, utilize enzymes. Examples of these products are; cheese, alcohol and bread. Fermentation is a method of generating enzymes for industrial purposes. Fermentation involves the use of microorganisms, like bacteria and yeast to produce the enzymes. There are two methods of fermentation used to produce enzymes. These are submerged fermentation and solid-state fermentation. Submerged fermentation involves the production of enzymes by microorganisms in a liquid nutrient media. Solid-state fermentation is the cultivation of microorganisms, and hence enzymes on a solid substrate. Carbon containing compounds in or on the substrate are broken down by the microorganisms, which produce the enzymes either intracellular or extracellular. The enzymes are recovered by methods such as centrifugation, for extracellularly produced enzymes and lysing of cells for intracellular enzymes. Many industries are dependent on enzymes for the production of their goods. Industries that use enzymes generated by fermentation are the brewing, wine making, baking and cheese making (Renge *et al*., 2012).

Soybean koji is an important ingredient for traditional fermented food in South-East Asia and East Asia. Koji containing 60% soybean was used as substrate to investigate the enzyme production by *A. oryzae*. T During koji fermentation, pH increase of soybean koji was caused by enzymes production. The highest protease and amylase activities were 84.38 and 200 unit/g of dry weight, respectively. Moreover, growing of enzyme activities on soybean koji correlated with the growth of this mold. Electron micrograph showed that spores of *A. oryzae* S. were formed after 48 h of cultivation period. Additionally, the highest enzymes activities were also shown in this stage (Chuenjit Chancharoonponga *et al*., 2012).

Carlos Regalado and his co-workers described Hemi cellulosic agricultural by-products such as corn Stover (CS) are highly available materials which represent an opportunity to develop value added products. Native Aspergillus niger GS1 was used for solid-state fermentation (SSF) on alkali pre-treated CS (ACS) aimed to optimize xylanolytic enzymes production, and their effect on in vitro ruminal and true digestibility of ACS. CS is a readily available by-product in different regions which after alkaline treatment and partial hydrolysis with the EE, may be advantageously used as supplement for ruminant feed (Carlos Regalado *et al*., 2010).

Toca-Herrera explained Solid-state fermentation (SSF) processes involve the growth of microorganisms (typically fungi) on a solid material in the absence or near absence of free-flowing water. Utilisation of agro-industrial residues as support-substrates in SSF processes provides an alternative avenue and value-addition to these otherwise under- or non-utilised residues. SSF processes have shown to be particularly suitable for the production of enzymes by filamentous fungi, since they reproduce the natural living conditions of such fungi. In the present chapter the production of laccase enzyme by white-rot fungi under SSF is described (Toca - Herrera *et al*., 2007).

Banana waste was used as a substrate for the production of amylase by Bacillus subtilis using solid state fermentation with various process parameters like, the incubation period, substrate concentration, pH and incubation temperature showed 24hrs, 50g, 7 pH and 35°C respectively. Peptone (0.2%) as a nitrogen sources showed maximum yield and the maximum enzyme activity showed in presence of in organic nutrients magnesium sulphate (MgSo4.7H2O), calcium chloride (CaCl2.2H2O) and di-hydrogen potassium phosphate (KH2PO4) were 0.02%, 0.04% and 0.4% respectively (Chandrashekhar Unakal *et al*., 2012).

Two strains of the food-borne amylolytic yeast *Saccharomycopsis buligera* were studied with respect to production and characterisation of their amylolytic enzymes. S. buligera KZ represents a strain synthesizing an amylolytic complex composed of amylase, 11 glucoamylase and glucosidase. *S. buligera* IFO 0111 represents a strain producing only one amylolytic enzyme glucoamylase, with a property unique among yeast amylases, namely the ability to degrade raw starch. Information on molecular-genetic aspects and enzymatic behaviour of amylolytic enzymes produced by both strains is presented (Eva Hostinova *et al*., 2002).

The influence of vitamins and zinc acetate on the synthesis of the enzyme invertase by nine yeast strains belonging to the genus Saccharomyces, namely species *S. carlsbergensis* (beer yeast), S. cerevisiae (bread yeast) and *Sacch. ellipsoideus* (wine yeast) investigated by Csilla Katalin dezsi. As invertase producer, the yeast SCHCCBM 307 (from the Biotechnology and Microbiology Research Centre at Lucian Blaga University in Sibiu) was the best on the control substrate (malt wort) and on the substrate enriched with both vitamins and acetate and the yeast SEJ 103 (from the Jidvei Centre) was the best on media enriched only with vitamins (Csilla Katalin dezsi *et al*., 2011).

Nguyen Hoang Loc and his co-workers explained the production of neutral protease (NPRC10) by recombinant E. coli BL21 (DE3) through submerged culture in 40-L fermenter with working volume of 20 L. The parameters such as cell density, pH, inoculum size, and agitation speed were investigated for the production of enzyme. The results shown that the maximum production of NPRC10 was obtained after 34 h of batch fermentation at OD600 (cell density) of 2, inoculum size of 2% and agitation speed of 500 rpm with medium pH maintained at 7. The highest total activity of NPRC10 during the course of fermentation was approximately 76 unit/mL (Nguyen Hoang Loc *et al*., 2011).

The thermostable properties of Taq DNA polymerase from *Thermus aquaticus* have contributed greatly to the yield, specificity, automation, and utility of the polymerase chain reaction method for amplifying DNA investigated by Nayak. Taqpolymerase is widely used enzyme for DNA amplification in PCR techniques and highly applicable in molecular biology and biotechnology. More than 50 DNA polymerase genes have been cloned and sequenced from various organisms including thermophiles by PCR cloning technique, whereby the gene 12 encoding this enzyme was cloned into the expression vectors that produce recombinant Taqpolymerase gene has facilitated for this enzyme production (Nayak *et al*., 2011).

Penicillin acylase (EC 3.5.1.11) has been a target of study for a long time because of its pivotal role in the deacylation of the penicillin into the 6- amino penicillanic acid (6-APA) and the side-chain organic acids. In this study, sixty-five strains of E. coli were investigated for penicillin acylase activity using fluorescamine method (Sedigheh Javadpour *et al*., 2002).

Cellulase is a group of enzymes (endoglucanase, exoglucanase and betaglucosidase) required for cellulosic feedstock hydrolysis during bioethanol production by Andre L. Rodrigues. The use of recombinant cellulase is a strategy to reduce the enzyme cost. In this context, the present work describes the construction of a cellulase expression vector (pEglABglA), which allowed constitutive co-expression of endoglucanase A (EglA) from an endophytic *Bacillus pumilus* and the hyper thermophilic β-glucosidase A (BglA) from *Fervido bacterium sp. in Escherichia coli* (Andre L. Rodrigues *et al*., 2010).

Janarthanan and his co-workers described The Urbanization and industrialization accompanied by population flare-up has formed a serious problem of waste generation and its disposal, treatment and management. The solid wastes are generated more in some parts Salem city, Tamil Nadu. In the present study, the vegetable wastes were collected from various sources like vegetable market, reception halls,

hospitals, schools and market areas which were mainly from Ammapet, Hasthampatti, Suramangalam and Kondalampatti region at Salem district. In this study, there are 40 different bacterial strains were isolated and identified. Among the strains, Bacillus sp. (B17), Micrococcus sp. (C3), and Bacillus sp. (P1) were identified as efficient starch hydrolyser and those were completely composted the market waste in very short duration when compared to the normal soil micro flora. The -amylase enzyme assay also checked by Dinitrosalicylic Acid (DNS) method. In compost the NPK level was increased significantly and it could be helpful for the plant growth. In pot culture study, very lesser application of compost (2:1 - soil: compost) showed best results (Janarthanan *et al*., 2014).

Chamraj Gokul Madhumithah study was taken up to utilize different vegetable wastes as input for protease production using Aspergillus niger. Wastes like potato, pumpkin, cauliflower, cabbage and brinjal procured from local market served as substrates for the solid-state fermentation. This study presents a novel - economical approach for the bioconversion of vegetable wastes for the production of protease that is industrially significant (Chamraj Gokul Madhumithah *et al*., 2011).

Optimization of the media components for cellulase production using Trichoderma reesei was carried out by Saravanan. The optimization of cellulose production using pineapple waste as substrate was performed with statistical methodology based on experimental designs. The screening of nutrients and their influence on the cellulase production was studied using a Plackett-Burman design. Avicel, soybean cake flour, KH PO_4, and yeast extract were found to have the positive influence for the production of cellulase (Saravanan *et al*., 2012).

Olorunnisola Kola Saheed and his co-workers explained the white rot fungus are valuable class of filamentous and spore forming strains capable of use as animal feed supplements when cultivated under submerged state bioconversion. Selected *bacidiomycetes*; *Phanerochaete chrysosporium, Panus tigrinus* M609RQY (M6) and RO2 were grown solely on liquid and solid substrates of banana peel, pineapple peel and papaya peel while *P. chrysosporium* synthesized 8.16 and 10.21 mg g1. *P. chrysosporium*, M6 and RO2 produced good αamylase and cellulase enzyme activities that assisted in substrate degradation for protein synthesis (Olorunnisola Kola Saheed et al., 2013). Rahna and her co-workers explained reduced production cost of cellulase by using alternative carbon source such as lignocellulosic waste and optimized fermentation parameters for high yielding. In the present investigation, isolated the novel cellulase producing actinomycetes, *Streptomyces sp*. from decayed fruit waste and optimized the physicochemical parameters for cellulose production.It could be concluded that *Streptomyces sp.* S7 is a powerful cellulase producer strain under our tested experimental conditions using fruit waste as carbon source (Rahna et al., 2011).

Cellulase production from cellulosic pineapple waste using *Trichoderma longibrachiatum*, Aspergillus niger and Saccharomyces cerevisiae was assessed by Omojasola. The wastes were dried, pre-treated with alkali and steam, re-dried and then blended. The powdered wastes were then used as substrates in separate shake-flasks which contained mineral salts medium (MSM) and inoculum of Trichoderma longibrachiatum, Aspergillus niger and Saccharomyces cerevisiae. The results obtained from the fermentations showed that Trichoderma longibrachiatum produced the highest amount of glucose among the cultures tested (0.92mg/0.5ml). This was produced from pineapple pulp at pH 4.5 and temperature of 45ºC on Day7 of fermentation. The highest amount of glucose produced by Aspergillus niger was also from pineapple pulp (0.63mg/0.5ml) at pH 3.5 and temperature of 40ºC on Day5 of fermentation. The highest amount of glucose produced by Saccharomyces cerevisiae was from pineapple pulp (0.54mg/0.5ml) at pH 4.5 and temperature of 45ºC on Day5 of fermentation (Omojasola et al., 2008).

Tengku Norsalwani carried out the work based on Palm kernel cake (PKC) and vegetable wastes were used as a fermentation substrate for the evaluation of cellulase activity secreted by Bacillus sp. In the current work, PKC and vegetable wastes were used as substrates in order to reduce the cost of cellulase production. The aim of this study was to determine the cellulase activity by *Bacillus sp*. On lignocellulosic materials mainly on different sizes of PKC and vegetable wastes. Besides that, pH, temperature, and inoculum concentrations will also being tested for the optimum reaction of Bacillus sp. on the substrates. From this study, Bacillus sp. holds the potential of converting lignocellulosic materials

into products of commercial and industrial values such as glucose and other biofuels (Tengku Norsalwani et al., 2012).

MATERIALS AND METHODS

Collection and Preparation of samples Fruit wastes such as Mosambi (*Citrus limetta*) and Pomegranate (*Punica granatum*) were collected from various fresh juice stall presented in surroundings of Banaswadi, Bangalore. The samples were brought to the laboratory using sterile plastic bags for further analysis. The Fruit wastes were cut in to small pieces by using knife for further fermentation process.

Preparation of fermentation medium

Fermentation process

The above prepared fruit wastes were weighed to required level and mixed with following way for fermentation process

Take 3 parts fruit peels (300 g)

Add 1part molasses (100 g)

Add 10 parts water (1000 ml)

To the above 3 tea spoonful of Yeast.

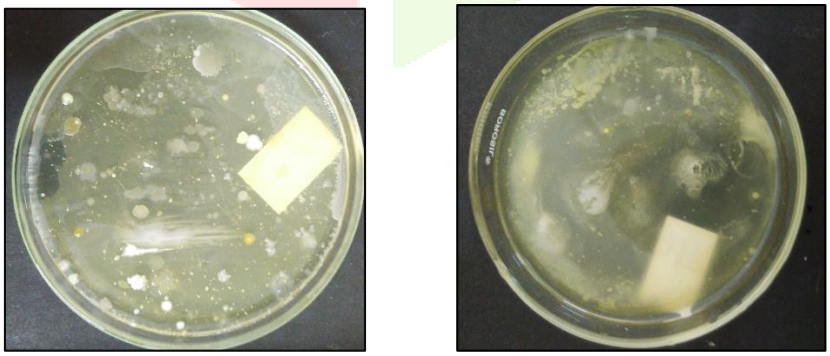
Use any multiples thereof, maintaining the same ratio.

Allow the fermentation 4 weeks to 12 weeks.

Screening of enzymes

Screening of protease

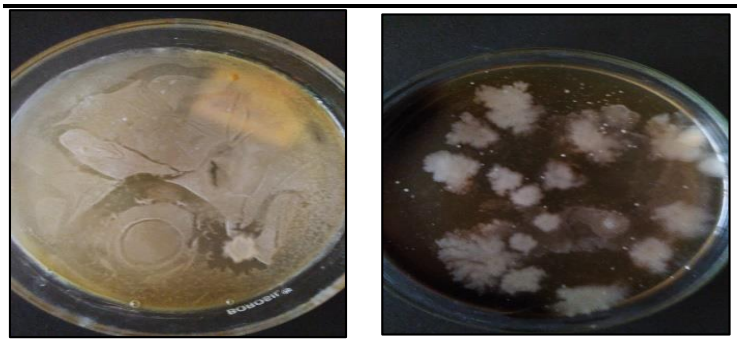
Screening of the best isolate with reference to their proteolytic activity in substrate amended medium was done. Nutrient agar containing 1% skimmed milk, casein and gelatin was sterilised, poured into sterile Petri dishes, separately. These were inoculated with young inoculum and incubated for 24-48 hours at 37°c. Zones of clearance indicating hydrolysis were measured (Emimol et al., 2012).



Zone of inhibition seen in *C.limetta* and *P.granatum* respectively

Screening of amylase

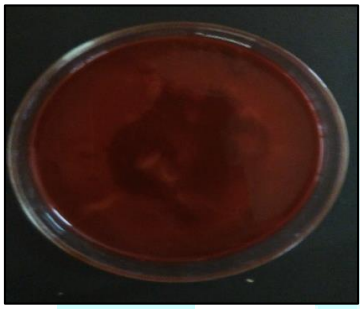
Nutrient agar was supplemented with 1% starch and sterilised. After inoculating the test organisms and incubating for 48 hours at various temperatures 30-40^0 C, plates were flooded with iodine solution. Hydrolysis of starch was visualised as clear zones around the colonies against deep blue brown staining for starch (Emimol et al., 2012).



Zone of inhibition seen in *C. limetta* and *P. granata* respectively

Screening of cellulase

The plates were flooded with 0.3% Congo-red solution for 10mins. Then it is washed with water and flooded with 1N NaCl as a destaining solution. Cellulase production is visualized by a translucent zone around the colonies.



Zone of inhibition seen in *P. granata*

Bulk fermentation of fruit wastes for production of enzyme cleaner

All the fruit wastes were cut in to small pieces and mashed in to uniform mixer before it is being taken for fermentation. The entire contents were put into the container and closed tightly and kept for fermentation for the period of one month. The following pictures **(Fig 1)** show the setup of bulk fermentation.

(Fig 1)

Detergent properties

The Fermented liquid was tested for its microbial activity on samples collected from Washroom tiles, surface of table, surface of sink; commonly found organisms on these surfaces are *Micrococcus, Staphylococcus, Bacillus,* and *Pseudomonas*.

Micrococcus is a sphere-shaped (coccus/cocci generally means spherical), relatively harmless bacterium. It is very common on skin, and it can also be found in soil, water, and meat products. It is

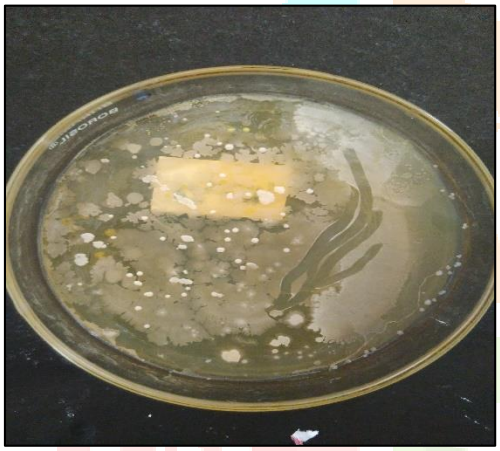
generally a saprophyte (meaning it feeds on dead and decomposing materials) and can cause spoilage of fish.

Staphylococcus is another sphere-shaped bacterium. It is much more well-known than *Micrococcus*, especially in the context of hospitals. When the medical profession refers to MRSA, they mean a particularly drug-resistant strain of this bacterium. Food poisoning and skin infections, as well as toxic shock syndrome, are among the illnesses caused by *Staphylococcus*. Unlike *Micrococcus*, *Staphylococcus* is able to grow both with and without oxygen.

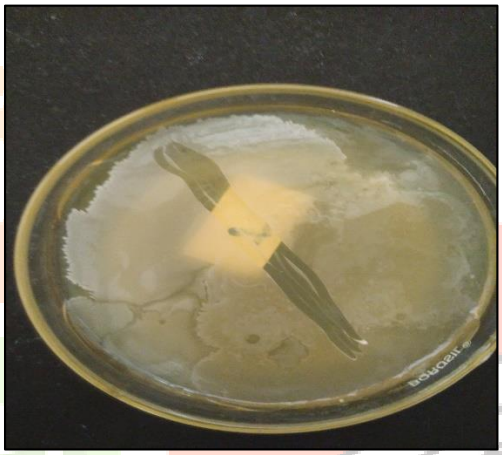
Bacillus is a rod-shaped bacterium ("*bacillus*" means "rod"). *Bacillus* is a very handy bacterium, as it has the ability to produce endospores – small, tough structures that can survive adverse conditions some species of *Bacillus* can cause food poisoning, and some can cause illness or infection.

Pseudomonas is another rod-shaped bacterium. It can be found in soil and water, and on plants. It is an opportunistic pathogen, and generally considered a nosocomial infection (gained while in the hospital), as the organism tends only to attack individuals that are immunocompromised. Along with infection, it also has the ability to produce exotoxins.

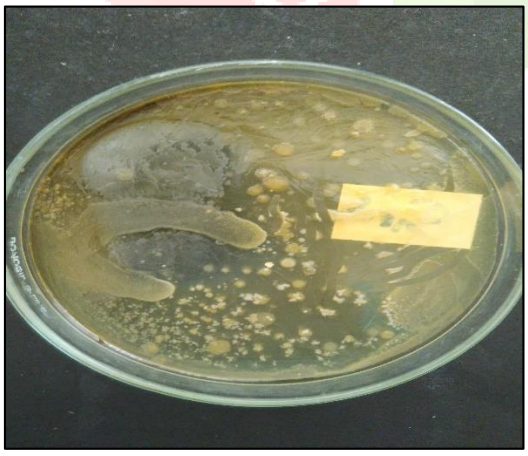
These were streaked onto sterile Nutrient Agar plates using sterile cotton swabs from various locations as mentioned above.



Pathogen from Table



Pathogen from Washroom



Pathogen from Sink

The organism from these plates were inoculated onto a Muller Hinton agar plate using pour plate method where a loop full of organism was diluted with 1mL of distilled water, poured and spread uniformly.

After the lawn of organisms was seen on the Muller Hinton Agar plate, wells were made of diameter 0.5 cm. Each plate was punched with 4 wells where in the first well had the positive control i.e, alcohol; second well had the negative control i.e., distilled water; the third and the fourth wells were loaded with samples about 0.01mL in each well.

Results

Enzyme activity assay for protease in fermented broth

Sl. No	Fruit waste	Zone of Inhibition
1	***Citrus limatte***	0.4 cm
2	***Punica granatum***	0.2 cm

Enzyme activity assay for Amylase in fermented broth

Sl. No	Fruit waste	Zone of Inhibition
1	***Citrus limatte***	0.2 cm
2	***Punica granatum***	0.1 cm

Enzyme activity assay for cellulase in fermented broth

Sl. No	Fruit waste	Zone of Inhibition
1	***Citrus limatte***	-
2	***Punica granatum***	0.6 cm

Detergent properties

Table pathogens

Sl. No	Fruit waste	Zone of Inhibition
1	***C. limatte* + *P. granatum***	. cm

Washroom Pathogens

Sl. No	Fruit waste	Zone of Inhibition
1	***C. limatte* + *P. granatum***	. cm

Sink Pathogens

Sl. No	Fruit waste	Zone of Inhibition
1	***C. limatte* + *P. granatum***	0.4 cm

DISCUSSION AND CONCLUSION

Fruit wastes are produced in large quantities in markets and constitute a source of nuisance in municipal landfills because of their high biodegradability (Viturtia *et al*.,1989). Instead of taking serious measures to decompose these types of wastes in soil filling and dumping in the environment, the way of recycling the biological waste will give energy as well as prevent the environmental pollution. There are many methods of recycling of such agricultural wastes are available to reuse the waste resources. One of the such method is using the fruit wastes to produce the enzymes and organic acids in the substrate by using yeast will be better in use of replacing the chemical cleaning materials. Experiments were done in preliminary to produce such kind of product by layman method. The scientific way of doing the above enzyme cleaner production from microbial organisms in the fruit wastes is the main concept of the project.

Enzymes are proteins, which act as catalysts. Enzymes lower the energy required for a reaction to occur, without being used up in the reaction. Many types of industries, to aid in the generation of their products, utilize enzymes. Enzymatic processes have been implemented in a broad range of industries in recent decades because they are specific, fast in action and often save raw materials, energy, chemicals and/or water compared to conventional processes. A number of comparative environmental assessment studies have been conducted in the past 15 years to investigate whether these properties of enzymatic processes lead to environmental improvements and assess whether they could play a role in moving toward cleaner industrial production. On the other hand, enzymes have become popular in cleaning products because they are biodegradable and facilitate selective reactions. In some applications, e.g., dishes and cotton clothes, it is usual to find several cleaning detergents with some amount of lipase and cellulose enzymes in their formulations.

Many industries are dependent on enzymes for the production of their goods. Fermentation is a method of generating enzymes for industrial purposes. Fermentation involves the use of microorganisms, like yeast to produce the enzymes. Enzymes energize and catalyse biochemical reaction with high specificity and enhance the reaction rate. Enzymes are formed during growth of microorganisms (trophophase), as a result of oxidative metabolism and aerobic fermentative.

Enzyme Bio-cleaners are an organic solution produced by the simple fermentation of fresh vegetable wastes, fruit wastes with addition of brown sugar and water by using the selective microorganisms like Yeast. This fermentation creates natural chains of proteins, mineral salts, organic acids, alcohol and enzymes. This has been developed for people to make simple cleaning solution at home or in small scale business to ease global warming because minimizing the environmental pollution by utilizing the cabbages like kitchen waste, vegetable and fruit wastes from stalls and markets. This product is natural, safe, and good for the environment and use the magic of nature to produce extraordinary removal of dirt, oil, stain etc. resulted to customer satisfaction.

The advantage of using enzyme bio-cleaning solution are

- It is safer for the environment and safer for human health than traditional chemical cleaners and odour control products,
- It is highly specialized enzyme producing microorganisms to clean and control odours by eliminating the soils rapidly
- It is economically cheaper and the cost of production is less
- This Bio-Cleaning solution provide residual cleaning for longer period and gives stable application and
- This Enzyme Bio-cleaning solution help to displace unknown, potentially pathogenic (disease causing) bacteria with known, healthy microorganisms and in this way contribute to better human health

Thus, to conclude, cleaning forms an important aspect for the maintenance of hygiene and safety of foods in the food processing industry. Due to their high efficiency and safety, it is assumed that the enzyme cleaners will eventually capture a bulk of the Indian market.

Save Money, Save Space, Save Water and Save The Earth!

Based on the above scientific and technical information, the present work is proposed to carry out the production and analysis of the fermentation of Enzyme Bio-cleaning solution by using the residues and waste of agricultural produces like fruit wastes with help of Yeast (*Saccharomyces sp*.) with addition of cheaper carbohydrate sources like brown sugar and water medium.

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