

Production of an Ecofriendly Enzyme Biocleaner from Fruit Wastage

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Abstract - Enzyme biocleaners are an organic compounds which could be produced by fermentation process of fruit wastages by the addition of brown sugar and water in the presence of the selective microorganisms like Yeast and Bacteria. The present study was carried out for the production and analysis of enzyme bio-cleaners by using yeast and bacterial cultures in different fruit wastes, lemon collected from fresh Juice stalls in Chennai, Tamilnadu, India. The fruit wastes were analysed for its moisture content and biochemical parameters like reducing sugars, proteins, carbohydrate, pH, alcohol, acetic acid values after it being taken for fermentation. During fermentation, the enzyme production like protease, amylase, laccase, and cellulase were tested for it activity in fermented broth at different period of fermentation and found with moderate level of activity. The pH values and the microbial population including bacteria and yeast were also analysed in the fermented broth at different intervals. Our results have revealed that the produced biocleaner possess the properties as similar to enzymes and efficiently showed its activities against the hard stain and by dehairing mechanism. Therefore, our results may provide futuristic possible green approach for pollutant free earth.

Key words: Lemon, Saccharomyces cerevisiae, Enzyme, Proteins, Reducing sugar, Fermentation

I. INTRODUCTION

During the past few years, there has been serious public concern about the ecological problems arising from the use of synthetic cleaning agent which could release toxic chemicals to environment with drastic changes in the pH. 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 [1 & 2]. 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 and Bacteria. As this method of fermentation creates natural chains of proteins, mineral salts organic acids, alcohol and enzymes. The obtained bioenzymes has the capacity to breakdown, change, create and catalyse functions that make it wonderful cleaning aid in household as well as in industrial and medical applications. The enzyme bio-cleaners are referred to natural green chemicals since they become a perfect future consumer preference for miscellaneous applications and sustainable environment.

Enzyme Bio- Cleaning solution is formulated specifically to dispose the dirt safely, economically and rapidly. It 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 future. This progressive eco-friendly cleaning items depends on Bio-Bacterial innovation. This innovation comprises of billions of friendly bacteria for biocleaner takes out the wellspring of cleaning issues as opposed to veiling them. Bio cleaner guarantees a natural approach to treat normal cleaning issues with no requirements for unforgiving chemical cleaners, making cleaning hones less difficult and greener. Therefore, the main objective of the present study is a production of enzyme bio-cleaning solution by ecofriendly without any environmental hazardous using waste raw materials like fruit wastes to produce potential bioenzymes solution.

II. Materials and Method

2.1 Isolation and identification of *Saccharomyces spp* from fruit sample:

2.1.1 Sample Collection and isolation of *Saccharomyces spp* from the sample

Grape Fruits were collected from fresh juice stall near Koyembedu, Chennai, Tamilnadu, India. The fruit wastes were washed, rinsed and cut into small pieces by using sterile knife. The fruits were mashed, serially diluted and



inoculated in Yeast Peptone Dextrose broth (YPD) and incubated for 24 - 48 hrs. After incubation, 0.1ml of samples were streaked onto the YPD agar and incubated at 27°C for 24-48 hrs. After incubation period, colonies were selected and purified based on their morphology. Further, the isolate was morphologically characterized based on its shape, size and budding cells by performing by simple staining, germ tube test, hydrolysis of urea, sugar assimilation test. The isolate that was identified based on the method described by Nasir et al. (2017).

2.2. Preparation of Biocleaner

2.2.1 Collection of citrus fruit and chemical analysis of citrus fruit sample

Lemon, the citrus fruit was collected from fresh juice stall near Koyembedu, Chennai, Tamilnadu, India. The samples were brought to the laboratory using sterile plastic bags for further analysis. Inorder to remove the dirt and impurities on the fruits were scrubbed, washed and chunked into small cubes with sterile knife. The citrus fruit samples collected were analysed for the physiochemical parameters like water content, pH as well as the biochemical parameters like carbohydrates, proteins, fat and mineral content by using standard methods.

2.2.2 Estimation of moisture content of fruit sample

The moisture content of the fruit samples were estimated by the loss on drying method. Fruit peels were weighed in a sterile petri dish and placed in a hot air oven. The weight of the samples was determined for every 1 hour until a constant was observed [4]. The moisture content of the samples was then calculated as follows. Moisture content (%) = [(Initial weight – final weight)/Initial weight] *100

2.2.3. Production of Biocleaning solution

The above prepared fruit wastes were weighed and mixed with 10 ml of yeast culture (*Saccharomyces spp*) and 5 ml of *Bacillus subtilis* MTCC 2274 was added to 3 parts of citrus fruit peels(300g) and add 1 part of molasses (100g} and add 10 parts of water (1000ml) in a pop bottles using wide-mouthed funnel. The bottles were shaken vigorously until the sugars get dissolved and the caps were unscrewed to vent the pressure created inside the bottle. This process was repeated at least 2 times a day for a period of 2 month. The container was incubated at 35°C for fermentation.

III. Periodical assessment of Biocleaner

The above fermented liquor was analysed for the following parameters periodically every week to record the biochemical activity and changes during fermentation. The pH of the Biocleaner was measured potentiometrically. About 5 ml of the Biocleaner sample was taken and the pH was measured using the pH electrode in a pH meter. A 2 mL aliquot of a Test solution was mixed with 1 mL of 5% aqueous solution of phenol in a test tube. Subsequently, 5

mL of concentrated sulphuric acid was added rapidly to the mixture. The test tubes were kept undisturbed for 10 min, vortex for 30 Sec, placed in water bath for 20 min at room temperature and observed color development. The absorbance was read at 490nm in spectrophotometer. Reference solutions are prepared in identical manner as above, except that the 2 mL aliquot of carbohydrate is replaced by ddH₂O. The phenol used in this procedure was redistilled and 5% phenol in water (w/w) was freshly prepared. The absorbance of test samples was plotted against the standard graph. The amount of reducing sugar was by DNSA methods as described by Miller et al. (1959) [5]. The protein present in the sample was estimated by Lowry's method [6]. The amount of alcohol content present in the Biocleaners was estimated [7]. The amount of acetic acid content was estimated as followed by the method described by Zoellner and his GROUP [8]. The total microbial load was enumerated based on the CFU/ml at the intervals of 7 days. The presence of proteases, Laccase, Amylase and cellulase in the samples were screened for further confirmation.

3.3. Antimicrobial activity

SterileMuller Hinton Agar plates were prepared. Isolates such as *Staphylococcus aureus MTCC 2490*, *Pseudomonas aeruginosa MTCC 4683*, *Escherchia coli MTCC 7410*, *Klebseilla pneumoniae MTCC 2403* were swabbed onto the agar plates. Wells were punched with sterile well puncture. The fermented samples were loaded in the wells at varying concentration and the plates were incubated at 37^oC for 24-48 hours. After incubation, the zone size (mm) was measured. Clear zone indicates antimicrobial activity of biocleaner against organisms.

3.4. GCMS Analysis

The products which are relatively small and present in even trace amount can be detected. GC-MS was performed using a THERMO GC - TRACE ULTRA VER: 5.0, THERMO MS DSQ II. Gas chromatography was conducted in the temperature-programming mode with a 100 - 250°c, RATE: 8/Min, holding time: 10 Min for 250. The initial column temperature was held at 100 °C for 8 min, then increased linearly to 250°C at 10 °C/min, and held for 4 min at 270°C. The temperature of the injection port was 275 °C and the GC/MS interface was maintained at 300°C. Helium was used as carrier gas with a flow rate of 1.0 ml/min. The injection was split less to increase sensitivity. Identification of compounds was made by comparing the retention time and fragmentation pattern with mass spectra in the library search results stored in the computer software (version 1.10 beta, Shimadzu) of the GC-MS [9].



3.5. Application of laboratory prepared Biocleaner

3.5.1 Cleaner for household things

10ml of biocleaner solution was scrubbed on the unclean toys, paintings and grimy particles. The efficiency of the cleaner was assessed on comparison with vinegar, detergent liquid and detergent cake available in the market.

3.5.2 Removal of hard stain and grease

10ml of biocleaner solution was poured on to the greasy articles /rusty floor, left for few minutes and wiped with a wet cloth. The efficiency of the cleaner was assessed on comparison with cleaners avail in the market.

3.5.3. Dehairing

Healthy goat skin with hair was washed with running tap water to remove salt and other debris. Dried n cut into small pieces and subjected for dehairing process. The skin pieces were treated in 3 ways: a) Treated only with crude enzyme (45ml); b) Treated with crude enzyme (45ml) along with 7% of sodium sulphide, calcium; carbonate (0.1%); and c) Treated with 14% of sodium sulphide, and lime (3 to 4 drops).

3.8.4. Keratin degradation:

The ability of the biocleaner to degrade Keratin was examined with Chicken feathers. 45ml of enzyme biocleaner solution was inoculated with chick feather and incubated 30°C for 2 days. After incubation, observed for color change and degradation of chick feathers.

IV. RESULTS AND DISCUSSION

4.1. Isolation and identification of yeast

The fruits were mashed, serially diluted and inoculated in YPD broth and streaked onto the YPD agar produced shiny and off-white in color colonies with entire margins, circular form,

convex, smooth surface. The microscopic view revealed globe, olive shape, budding cells (Figure 1(a&b)). The isolate was negative for Germ tube and hydrolysis of urea tests. The isolate was able to utilize the sugar disc like dextrose, lactose, maltose and sucrose.

4.2. Laboratory Level Production of Enzyme Biocleaning solutions

Fruit sample was inoculated with yeast (*Saccharomyces cerevisiae*) and *Bacillus subtilis* MTCC 2274 and fermented up to 90 days. The (Figure: 2(a&b)) shows as before fermentation and after fermentation.

4.3 Periodical assessment of Biocleaner

4.3.1 Chemical analysis of citrus fruit sample

The moisture content and of the fruit were found as 40%. Thirumurugan reported that the moisture content of the fruit sample of lemon was 50% respectively. The pH of the

samples was observed from 1st week to 12th week. In 1st week the pH was 4.23 in biocleaner, gradual decrease in pH was observed from 3rd week and at 12th week it declined to 3.10. The (Figure (3)) show the pH values of lemon samples from 1st week to 12th week. Our results were consistent with reported pH values by other investigators. The pH of citric acid was 3.50 as compared with other fruit such as orange (2.46), water melon (2.45), grapes (2.48), apple (2.53), banana (2.40), the pH of lemon was 3.50 as compared with 2.50and 2.48. The pH of the samples varies on storage and this phenomenon was due to oxidation of acid resulting in higher pH. One of the reasons, lower pH increases the fermentation rate is because the pH affects the shape of the enzymes (proteins) that help fermentation reactions. It has been found that at lower pH, the presence of acidic amino acids enhance the fermentation rate faster as compared to higher pH conditions. During fermentation, the pH continues to drop for a variety of reasons. Yeast cells take in ammonium ions (which are strongly basic) and excrete organic acids.

The conversion of sugar into carbon dioxide and alcohol provides energy for the yeast cells. Different sugars would release different amounts of CO2 Glucose was expected to produce more carbon dioxide than other types of sugar because of its 6-Carbon structure. Sucrose was expected to be the runner up producer of carbon dioxide after Glucose because of its formation by the combination of glucose and fructose. It has also been considered that the attachment of a 5-Carbon sugar to a 6-Carbon sugar would limit the production of CO_2 . The carbohydrate content in the sample were reduced from day 1 to day 90. The carbohydrate content ranged from 2.24 in day 20 and declined to 0.17 at day 90 on fermentation of the substrate. The graph was shown in (Figure 4) our results are consisting with report of 0.17 at 12th week according to the carbohydrates [10]. The gradual decline in reducing sugar content was observed from 1st week to 12th week, indicates the utilization of molasses as carbon source and breakdown of complex substances into simpler molecules. The value ranged from 2.12 in 1st week and declined to 0.07 in 12th week. The graph was shown in (Figure (5)) perason reported highest amount of reducing sugar content of 0.9 in biocleaner reducing sugar in all treatment decreased slightly. This indicates that the fermentation process is running optimally. Inhibition of this process of reducing sugar consumption may occur due to the influence of chemical compounds inside the compounds of molasses and yeast cell metabolism. One of the compounds derived from molasses is sulphur dioxide [11].

The protein content was measured from 1st week to 12th week. The total protein content in the sample was ranged from 2.18 in 1st week and declined to 0.30 in 12th week. The graph was shown in (Figure 6) similarly, the higher protein content were recorded in the orange and banana as 1.2 due to the proteins were indigestible due to the cellulosic wall.



The fermentation of protein content in the biocleaner consequently involved in the production of metabolic compounds by the action of bacteria and yeast. It is the process of chemical breakdown that allow absorption of nutrients by enzyme [12].

The alcohol content in the sample was increased from day 1 to day 90. The value was ranged from 0.30 in day 1 and at the day 70 it started to increase and reach the content at 0.80 in day 90. The graph was shown in (Figure 7) Thus they conclude the alcohol content in the biocleaner made form lemon was around 0.4 to 0.5%. Our study results fall in line with reports of 0.8%. During fermentation, yeast transform sugars present in the lemon into ethanol and carbondioxide. The temperature and speed of fermentation are important considerations as well as levels of oxygen present in the medium [11].

Acetic acid also called ethanoic acid is organic compound. Acetic acid production via fermentation pathway is conversion of glucose to ethanol and ethanol to acetic acid. The total acetic acid content in the biocleaner solution was nil from day 1 to day 20 and from day 25 production of acetic acid was observed in the biocleaner. The total acetic acid content in lemon range as 1.7 shown in (Figure 8) determined the presence acetic acid content of biocleaner range between 1.5 to 1.7 respectively [13].

4.4 Enumeration of microbial load

During the fermentation the bacteria and yeast growth was counted. In Nutrient agar plates, the bacterial count ranged from 350 x 10⁶, 1st week and gradual reduction was observed from the 5th week in the range of 210×10^4 .On 10th week the bacterial count was found to be stable in population. On 12th week, No bacterial count was reported. The yeast in YPD agar plateswere counted from day 1 to day 90. At the day 7 the yeast was found to be countable (250 x 10⁴) and from day 30, reduction was noted. The range falls between 170 x 10^3 and 50 x 10^2 till day 60. Declined growth was observed from day 80. Enumeration of microbial load was shown in (Figure (9)). As reported by Rahna et al. during the fermentation of mixer of fruit wastes, the bacterial count ranged from 100 X 10⁻⁶ in I week and declined to 800 in II week. On at 10th week and the bacterial count was found to be stable in population [14].

4.5. Screening for Enzymatic activity

In casein agar plate, the samples were loaded in various concentrations. After incubation period, the biocleaner sample showed positive result for the protease activity on 2 weeks incubation. The zone size was tabulated Table (1). As represented in Figure (10), the production of **protease** enzyme in the fermented broth of different fruit waste samples showed the activity from lower concentration as 25 μ l to higher concentration as with the zone of activity from 2.0 cm respectively. The mosambi exhibited with low

activity from 1.5 cm to 2.5 cm in the higher concentration levels. In the present study the zone size varied from 2.5 μ l to 3 μ l. The sample of fruit waste showed the laccase activity on inoculation of biocleaner at varying concentrations on the nutrient containing pyrogallol agar plate.

| Table | (1): | Screening | of Enzymati | ic activity |
|-------|------|-----------|-------------|-------------|

| S.No | Sample | Zone of Inhibition (mm) | | ition (mm) | |
|------|----------------------|-------------------------|------|------------|------|
| | | 5µ1 | 10µ1 | 15µl | 20µ1 |
| 1 | Biocleaner- Protease | 2.5 | 2.7 | 2.9 | 3 |
| 2 | Lemon- Laccase | 1 | 2.1 | 2.3 | 2.8 |

For the production of laccase enzyme in the fermented broth with different fruit waste samples, watermelon sample showed the activity from lower concentration as 20 μ l to higher concentration with the zone of activity from 1.2 mm. The mosambi and Pomegranate were with the moderate activity from 1mm in the higher concentration levels. But in the orange sample, there is no cellulose activity found which confirms there is no cellulase enzyme produced in the substrate during fermentation (Figure 11). Our results lined with the studies of laccase of 20 μ l with zone size of 2.8mm.

The amylase activity was observed in starch agar plate, the zone range falls between 1.5μ l to 2.5μ l. The zone plates was shown in (Figure (12)) the release of amylase enzyme in the fermented broth prepared from watermelon and mosambi sample showed the activity as the zoneappear around the sample. Whereas, the orange and pomegranate samples, expressed no amylase activity in the substrate during fermentation. Thus lemon sample also showed the activity by the appearance of zone respectively [4].

In Carboxymethyl Cellulose agar plate, the sample was loaded in various concentrations. The sample of fruit waste showed the cellulose activity. The cellulose activity is measured by range of zone of inhibition. The zone of inhibition was shown in (Figure 13). For the production of cellulose enzyme in the fermented broth of different fruit waste samples, watermelon and orange sample showed the activity by zone of inhibition. Thus lemon also showed the activity by zone of inhibition respectively [15].

4. 6. Enzymatic activity of a biocleaner

The protease activity was checked out by assay reading. It was carried out from 1st week to 12th week. From 1st week it ranges from 0.68 and it was stable at 10th and 11th week. On the week it ranges up to 1.36. The graph was shown in (Figure (14)). The lactase activity was checked out by assay reading. In 1st week the range 0.52 and got increased its activity in the 7th week and it got stable between 9th and 10th. On 12th week it ranges up to 1.02. The amylase



activity was checked out by assay reading. On 2nd week it ranges from 0.13 and got increased at 8th week. On 12th week it ranges upto 0,91. The cellulase activity was checked out by assay reading. On 1st week the acitivity ranges from 0.10 and on 12th week it ranges upto 0.88.

4.7 Antimicrobial activity:

MHA plates was swabbed by the organisms MTCC strain of *Staphylococcus aureus MTCC 2490*, *Pseudomonas aeruginosa MTCC 4683*, *Escherchia coli MTCC 7410*, *Klebseilla pneumoniae MTCC 2403*. The sample was loaded in a different concentration. After incubation period, the zone was observed, which indicates the sample kill the microbes present in the kitchen utensils. The 2^{nd} week of zone indicates the activity of the sample in the diameter of 1.2mm and in the 4th week the activity of the sample in the diameter of 2,1mm and in the 12^{th} week the sample were loaded in the various concentration and measure the diameter as 7cm. The zone plates was shown from 1^{st} week to 12^{th} week in (Figure 15)

Antimicrobial activity of dried fruits peel of *Citrus limon* has been evaluated. Phyto-constituents present in plants namely alkaloids, saponin, sterols, terpenoids are having an exciting set of circumstances that makes it possible to do something for more extensive of modern therapies against a wide range of microorganisms. The practical exhibition and explanation of antimicrobial activity against both Grampositive and Gram-negative bacteria and on various fungal strains may be an indication of something presence of broad spectrum antibiotic compounds in the extracts. The 100μ g/ml concentration of dried fruits peel of *Citrus limon* have an influencing antimicrobial activity [16].

4.8 GCMS Analysis:

The GCMS study was analysed to identify the presence of different compounds in the enzyme biocleaner solution. The presence of compounds in the biocleaner solution was shown in Figure (16) and Table (3) as represented below.

| Table (3): Presence of Compounds in the Biocleaner Compound Label Name DB Formula Hits (DB) | | | | | | |
|---|---|-----------------------|----|--|--|--|
| | | C8H24O4Si4 | | | | |
| Cpd 3: Cyclotetrasiloxane, octamethyl- | Cyclotetrasiloxane, octamethyl- | C8H24O4S14 C6H7O4P | 3 | | | |
| Cpd 4: Phosphonic acid, (p-hydroxyphenyl)- Cpd 8: 1,6-Octadien-3-ol, 3,7-dimethyl- | Phosphonic acid, (p-hydroxyphenyl)- 1,6-Octadien-3-ol, 3,7-dimethyl- | C10H18O | 3 | | | |
| Cpd 9: Phenylethyl Alcohol | Phenylethyl Alcohol | C10H180 | - | | | |
| 1 5 5 | | | 6 | | | |
| Cpd 10: Bicyclo[2.2.1]heptan-2-ol, 1,3,3trimethyl- | Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl- | | | | | |
| Cpd 12: Benzoic acid | Benzoic acid | C7H6O2 | 10 | | | |
| Cpd 15: Terpinen-4-ol | Terpin <mark>en</mark> -4-ol | C10H18O | 6 | | | |
| Cpd 16: Naphthalene | Naphthalene | C10H8 | 6 | | | |
| Cpd 17: LalphaTerpineol | LalphaTerpineol | C10H18O | 10 | | | |
| Cpd 18: Benzofuran, 2,3-dihydro- | Benzofuran, 2,3-dihydro- | C8H8O | 10 | | | |
| Cpd 19: 7-Octene-2,6-diol, 2,6-dimethyl- | 7-Octene-2,6-diol, 2,6-dimethyl- | C10H20O2 | 1 | | | |
| Cpd 25: Cyclohexanemethanol, 4-hydroxy.alpha.,.alpha.,4-trimethyl- | Cyclohexanemethanol, 5 4- | C10H20O2 | 3 | | | |
| | hydroxy.alpha.,.alpha.,4-trimethyl- | | | | | |
| Cpd 26: Cyclohexanemethanol, 4-hydroxy.alpha.,.alpha.,4-trimethyl- | Cyclohexanemethanol, 4- | C10H20O2 | 3 | | | |
| al for | hydroxy.alpha.,.alpha.,4-trimethyl- | | | | | |
| Cpd 29: Dodecanal | Dodecanal | C12H24O | 3 | | | |
| Cpd 32: Pentanoic acid, 5-hydroxy-, 2,4-di-tbutylphenyl esters | Pentanoic acid, 5-hydroxy-, 2,4-di-t- | C19H30O3 | 2 | | | |
| | butylphenyl esters | | | | | |
| Cpd 33: Undecanoic acid, 10-methyl-, methyl ester | Undecanoic acid, 10-methyl-, methyl ester | C13H26O2 | 5 | | | |
| Cpd 34: 1-Tetradecanol | 1-Tetradecanol | C14H30O | 10 | | | |
| Cpd 37: n-Pentadecanol | n-Pentadecanol | C15H32O | 10 | | | |
| Cpd 39: Phthalic acid, hept-4-yl isobutyl ester | Phthalic acid, hept-4-yl isobutyl ester | C19H28O4 | 10 | | | |
| Cpd 44: Dibutyl phthalate | Dibutyl phthalate | C16H22O4 | 10 | | | |
| Cpd 45: n-Hexadecanoic acid | n-Hexadecanoic acid | C16H32O2 | 1 | | | |
| Cpd 46: 2H-1-Benzopyran-2-one, 5,7-dimethoxy- | 2H-1-Benzopyran-2-one, 5,7-dimethoxy- | C11H10O4 | 2 | | | |
| Cpd 48: Hexadecanoic acid, ethyl ester | Hexadecanoic acid, ethyl ester | C18H36O2 | 1 | | | |
| Cpd 50: 9,12-Octadecadienoic acid, methyl ester | 9,12-Octadecadienoic acid, methyl ester | C19H34O2 | 8 | | | |
| Cpd 52: 13-Octadecenoic acid, methyl ester | 13-Octadecenoic acid, methyl ester | C19H36O2 | 10 | | | |
| Cpd 54: Methyl stearate | Methyl stearate | C19H38O2 | 1 | | | |
| Cpd 66: Oxiraneoctanoic acid, 3-octyl-, methyl | Oxiraneoctanoic acid, 3-octyl-, methyl | C19H36O3 | 1 | | | |
| · · · · · · · · · · · · · · · · · · · | ester | | | | | |

4.9. Application of Biocleaner

(i) Household things: The efficiency of the cleaner was assessed on comparison with vinegar, detergent liquid and detergent cake available in the market. (ii) Removing of stubborn stains: The efficiency of the cleaner was assessed on comparison with cleaners avail in the market. (iii) **Dehairing** shows that the goat skin was treated as shown in the figure, from that biocleaner reacted in greater amount than the others. Keratin degradation shows the enzymatic degradation of keratin by using chicken



feathers. It was inoculated in enzyme biocleaner and incubated. After incubation period the change of biocleaner solution indicates the degradation (Figure 17).

Each type of enzyme is different and will catalyze only one type of reaction (known as a 'lock and key' mechanism). They are highly specific to the type of surface or material they can work on and are only active when conditions are correct. The produced biocleaner can be used as cleaning agent as they contain proteases which could break down protein-based stains including blood, urine, food, feces, wine and other beverages. It has which lipases could break down fat molecules like oils and grease. The presence of Amylases break down starch molecules like eggs, sugars, sauces, ice cream, gravy whereas Cellulases are used to soften the fabric and restore color to fibers made up of cellulose material. They also remove particulate soil and reduce fabric graying and pilling.

In Bio-enzymatic cleaners, both bacteria and enzymes work together to clean, relying on each other to get the job done. When applied to surfaces, soils, stains, and malodors are broken down by the enzymes, then consumed by the bacteria. As long as the soil is present and surfaces are sufficiently damp, these microscopic "cleaners" multiply, continuing to remove traces of grime and odor from surfaces hours or even days after the initial application. Enzymes can also be used without bacteria in certain cleaning products (although they are initially harvested from bacteria). Enzymes in laundry detergents, for example, work to catalyze the chemical reactions of other ingredients in the detergents. In wash water, they help to break down soils so that water can more easily wash it away. Bio-enzymatic formulations work particularly well for many cleaning applications such as carpet cleaning, laundry detergents, degreasing, drain maintenance, general surface cleaning, restroom cleaning and odor elimination [15].

V. Conclusion

The present work was 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 waste with help of Yeast (*Sacharomyces sp.*) and Bacteria (*Bacillus sp.*) with addition of cheaper carbohydrate sources like brown sugar and water medium. Thus to conclude, cleaning forms an important aspect for dehairing process in tanning industries, the maintenance of hygiene, prevention of rust formation cleaning of floors, removal of stains etc. Due to their high efficiency and safety, we believe the enzyme cleaners will eventually capture a bulk of the Indian market in future.

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

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