ABSTRACT

Lignocellulosic wastes can be repurposed in different ways for the manufacture of a wide variety of industrially important products such as bio-alcohol or specifically bioethanol. Traditionally, ethanol can be manufactured from biological substrates through the fermentation process. The industrial and market potential of ethanol is increasing day by day, due to substantial scarcity of fossil fuel globally. The crisis has become extremely evident in recent years with the increase in industrialization expansion and the introduction of advanced technologies in agriculture. The use of ethanol as an alternative fuel by valorizing various waste substances not only minimizes environmental pollution but also produces an alternative renewable resource to fossil fuel; the combustion of which results in cleaner emissions like carbon dioxide, steam, and heat.

Food wastes have been a nuisance to the environment when disposed of in untreated conditions by open dumping process. The soil surface absorbs those wastes and can transfer them eventually to water bodies causing an increased rate of water pollution. Recently, recycling of waste has become one of the major areas of research and technological development in the world encouraging sustainable technologies.

The current study experiments with the potential of a novel substrate prepared from an agro-industrial waste bagasse. The aqueous extract of sugarcane bagasse was prepared and the cooled substrate was fermented using an unconventional secondary fermenting strain *Saccharomyces cerevisiae* MTCC 180. For the selection process, different types of food waste available nearby which is generated after different processing steps were collected. They include- Potato wash water commonly called PWW, obtained after washing the peeled potatoes during chips manufacturing, spent tea that is obtained after making tea and bagasse which is obtained after the expression of sugarcane juice. All these wastes have a very high Biological

Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) value and are usually thrown or dumped into the environment which may cause serious environmental implications. These wastes can be reutilized to produce substrate for the fermentation of bioethanol. The potato wash water that was soaked for at least 15 minutes approximately, was treated in two different ways: in one method it was subjected to hydrolysis at 1% v/v of concentrated hydrochloric acid autoclaving at 121 ºC, 15 psi pressure for 15 minutes and in the other method hydrolysis was performed using 5% v/v of concentrated hydrochloric acid at 90-100 ºC for 60 minutes. The spent tea (10 g) was water extracted (150 ml) taking each extract after 2-5 minutes up to the 5^{th} extraction and mixing the extracts together one at a time to obtain combinations for preparation of fermentation broth. Bagasse was taken (5g) and cut into small pieces of approximately 1 inch length and extracted using water (200ml) boiling at 95-100 ºC. The three different potato wash water samples; Untreated, treated and autoclaved, and hydrolyzed were taken. Spent tea liquor consisted of samples; 1st extraction, 2nd extraction, 3rd extraction, 4th extraction, 5th extraction, (1st+2nd) extraction, (1st+2nd+ 3rd) extraction, (1st=2nd +3rd+4th) extraction and (1st+ 2nd+ 3rd+4th+ 5th) extraction. Bagasse aqueous extract includes all 12 extracts starting from 5 minutes to 60 minutes at an interval of 5 minutes each. All these extracts were prepared and tested for reducing sugar using the dinitrosalicylic acid method with respect to standard glucose solution. This helped us to get an estimate of the amount of fermentable monosaccharides present that may be useful in the fermentation initiation process. Then the selection was made among the three waste variants based on higher values of reducing sugar and it was found that the hydrolyzed potato wash water, first extraction of spent tea and 30 minutes extraction of bagasse scored higher than the others. The final substrate selection was made based on the fermentation of these substrates using the strain *Saccharomyces cerevisiae* MTCC 180 to get ethanol at an interval of 24 h up to 72 h. Ethanol content was estimated by performing titration using the dichromate technique and the 30-minute aqueous extract of

bagasse gave the highest value of ethanol. Therefore, this substrate was used for further experiments in fermentation. The FTIR analysis of the aqueous extract of bagasse reveals the majority of the presence of disaccharides, very small amounts of other polysaccharides, absorbed water, and other impurities.

To increase saccharification, bagasse itself can be treated with various pre-treatments before fermentation which may increase ethanol yield. Investigation on our substrate revealed that it contains a very minimum amount of approximately 0.0032g/L of furfural, a microbial inhibitor, which may increase in concentration if any acidic or alkaline treatment is performed at a higher temperature. Generally, for any secondary utilization of bagasse, it is usually sun-dried and stored. Keeping this in mind, the drying operation on the bagasse was performed using a tray dryer which was preheated and brought to a temperature of 70°C. The drying operation was carried out for 150 minutes to get dry bagasse. 5 g dried bagasse was extracted in a similar technique in water and pH, total soluble solid, and reducing sugar were analyzed. Alcohol estimation was also done after fermenting dried bagasse water extract with *Saccharomyces cerevisiae* MTCC 180. Results showed almost similar values for both dried and normal bagasse in terms of the above-mentioned quality parameters. So, drying was found to have an insignificant effect on the fermentation of bagasse aqueous extract. Depending on these experimental results, normal un-dried bagasse aqueous extract was considered for further experimentation.

Optimization of fermentation conditions and nutritional supplementation was done on the substrate using statistical tools. The fermentation conditions like pH, temperature, time, inoculum size, and inoculum age were optimized at first using one factor at a time and then the factors time and temperature were analyzed using Rotatable Central Composite Design (RCCD) where the upper and lower limits were selected based on one factor optimized values. The best conditions for fermentation were found to be at pH 4, fermentation time 22.60 h,

temperature 30.20°C, inoculum size 6% (v/v), and inoculum age of 48 h which gave 2.5 times more yield than un-optimized substrate having an absolute residual error of 6.14 %. The nutritional conditions like carbon, nitrogen, and mineral sources were selected and optimized. At first, they were selected and optimized using one factor at a time. The values were used to select the upper and lower limits in the RCCD to consider all possible combinations for the experiments. Sucrose, ammonium sulfate, and potassium chloride were selected which gave better ethanol yield at 4.06%, 0.5 %, and 0.11 mg/ml respectively. The non-supplemented medium, even under optimized fermentation conditions, gave 7.5 times lesser yield.

The fermentation was further carried out using immobilized cells of *Saccharomyces cerevisiae* MTCC 180 in 0.9% sodium chloride mixed agar matrix. On mixing and solidification, they were cut into pieces of small cubes and preserved in 0.1 (M) phosphate buffer solution of pH 5.5 in refrigerated conditions for 1 hour. For reusing the immobilized culture, it was taken out of refrigeration to achieve room temperature and washed with cold sterile water 2-3 times before they were used as inoculum for fermentation. The conditions of immobilization were optimized varying fermentation time, agar concentration, gel formation time, and cell concentration with the help of response surface methodology. The model validation of immobilization parameters gave a maximum ethanol yield of 2.056 g/100 ml at the rate of 1.025 g/L/h achieved at 20.05 h fermentation time, 3.41 % agar concentration, and 8.07 % cell concentration.

The investigation presented here is clearly problem problem-specific approach to mitigate some of the environmental pollutants produced daily as agriculture or food processing waste. An insightful conclusion of this is that an unconventional strain like *Saccharomyces cerevisiae* MTCC 180 can be utilized for fermenting a novel substrate like an aqueous extract of bagasse without any physical or chemical pre-treatment. The research can further be extended with a pilot scale study to check the yield at scale-up conditions. Different types of matrices can be

tried other than agar to check their stability and compatibility for cell immobilization. The metabolic study of the microorganism and immobilized cell reuse can be studied in more detail. The bioethanol produced can further be studied in terms of its different parameters and efficiency as a biofuel in crude as well as blended form. Other potential applications of generated bioethanol can also be investigated. Embracing technologies such as this can be a sustainable approach towards effective waste utilization serving the objective of narrowing down the pollution burden on Earth, and making it a better habitat for our descendants.

Keywords: Waste valorization, Aqueous extract of bagasse, Dichromate method, Saccharomyces cerevisiae MTCC 180, Process Optimization, Agar immobilized cell