A NOVEL METHOD FOR THE PRODUCTION OF BIOETHANOL FROM PALM KERNEL CAKE THEREBY OBTAINING PROTEIN HYDROLYSATE AND OIL AS BY-PRODUCTS FOR ANIMAL NUTRITION

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Introduction/ Abstract:

Currently India produces 50,000 MT of palm oil, in this process of production the various by-products are generated such as empty fruit bunch, Mesocarp fiber, palm kernel shell, palm kernel cake, etc. The palm industry generates around 7000 MT of palm kernel cake as a by-product which is used in animal diet with lesser quantity, due to less-digestible carbohydrate and fiber. Palm kernel cake contains 50 % of hexose sugars, 14-20% of protein and also contain residue oil about 6-8 %.

In this study, palm kernel cake is used as raw material for the production of bio-ethanol. Two different methods are carried out to obtain oil, ethanol and protein. The first approach is enzymatic method, wherein in PROTAMEX enzyme from Novozymes is used to get protein hydrolysate. The residual oil and protein hydrolysate forms top layer and can easily be separated and the left out PKC biomass is utilised for ethanol fermentation. The second approach is hot water treatment and acid hydrolysis treatment, where in the oil is obtained just by heating and left slurry (water + PKC) is utilised for bio-ethanol using S. cerevisiae after dilute acid hydrolysis. After the fermentation is carried out and ethanol separated, the solid residue with spent yeast will be potential source for animal feed.

Key words: Palm Kernel Cake, Protamex enzyme, protein hydrolysate, ethanol,

Objectives:

- To extract palm kernel oil using the Commercially available Enzyme, Protamex
- To utilize protein hydrolysate for feed formulations
- Utilization of carbohydrate for Bioethanol production
Material and Methods:


Methodology:

Enzymatic hydrolysis:
The palm kernel cake was sieved twice using 1mm mesh to get fine PKC powder.

- The fine PKC powder was further mixed with distilled water in ratio 1:3 and autoclaved @ 121 °C for 20 min.
- The sterile slurry content was inoculated with 0.5 % of protamex enzyme (based on dry mass of PKC) in a sterile condition.
- The enzyme inoculated slurry was incubated in orbital shaking incubator at 55 °C, for 90 min with 250 rpm.
- The top oil and protein later was separated.
- The solid residue was taken for the fermentation process using S. cerevisiae.

Hot water treatment and Acid Hydrolysis:

- The fine PKC powder was further mixed with distilled water in ratio 1:3 and was incubated in water bath at 60 °C, for 90 min with continuous stirring.
- The oil separated from PKC was collected and stored.
- The remaining slurry were taken for the pre-treatment with 7% of H_2SO_4.
- Autoclave the slurry at 121°C for 3-4h.
- After cooling room temperature pH of the slurry were adjusted to 5 using NaOH then fermentation process were done using S. cerevisiae.

Fermentation of ethanol:

- The remained slurry were taken for the fermentation process using S. cerevisiae at a concentration of 6g per kg of PKC.
- This slurry were kept in conical flask and covered with paraffin. Holes were made on paraffin in order to release the carbon dioxide generated in the process.
- The fermentation process was carried out in orbital shaking incubator for 48 hours at 33 °C at 180 rpm.
- The sample was taken for ethanol recovery using distillation unit set up.
Results and Discussion:

- **Enzyme Hydrolysis**: Enzyme concentration was optimized and found that 0.5% of Protamex enzyme gave the highest yield of 5% oil and 20 ml of protein hydrolysate. This protein hydrolysate can be used for sport medicine, health drinks and animal nutrition. Fermentation of solid residue yielded 143 g of ethanol from 1kg of PKC.

![Three layer formed after Enzyme Treatment](image)

- **Hot water treatment and Acid hydrolysis**: 2 % of oil was obtained using simple hot water bath treatment. And the left slurry was pre-treated with 7% H$_2$SO$_4$. Fermentation of the slurry yielded 152 g of ethanol.

![Hot Water Treatment](image) ![Oil Obtained after treatment](image)

- **Spent PKC and Yeast**: After fermentation the solvent was used for the analysis of ethanol, the remaining solid residue containing palm kernel cake and spent yeast was dried and analysed for protein by kjeldahl method and results 23 % of increased protein content.
Both the methods carried out had its own advantages and disadvantages. Oil obtained from two methods shows different results that hot water treatment yield 2%, and enzymatic method yields 5% of oil, but the properties of oil were similar from both the methods. Acid hydrolysis method yielded higher ethanol recovery (152g/Kg of PKC) compared to Enzyme hydrolysis (143g/Kg of PKC). The advantage of using enzyme hydrolysis over acid hydrolysis treatment is that we get pure protein hydrolysate which can be directly used in various applications and the spent PKC and yeast is after fermentation can be used as animal feed.

Future Scope:

- Validating the extraction of oil.
- Animal trails carried out with each protein forms (Protein hydrolysate and spent residue after the fermentation).
- Validation and optimisation of pre-treatment and fermentation process.