

SUMMARY

SUMMARY

Different yeast strains were screened for unicellular protein production on different growth media. The potent yeast isolates were investigated for the optimal nutritional and environmental conditions for the production of single cell protein on raw materials which are readily available, assimilable and low in cost such as cane and beet molasses, rice bran and whey. The tested yeast strains were grown in flasks, on a shaker at 30°C for 48 hours.

The obtained results can be summarized in the following:

1- Screening of yeast strains:

Sixteen yeast strains belonging to three genera (*Saccharomyces*, *Candida* and *Kluyveromyces*) were examined for the growth on different five media namely Sekeri-Pataryas *et al.* medium, Reade and Gregory medium, Alian *et al.* medium, Manilal *et al.* medium and Tsvetkova *et al.* medium. Sekeri-Pataryas medium was found to be the best medium for biomass production, protein content and total crude protein. This was generally true for most studied strains and specially for the five potent yeast strains namely *Candida scottii* NBIMCC 688, *Candida utilis* NBIMCC 608, *Candida lipolitica* NBIMCC 688, *Saccharomyces cerevisiae* (Rehab Co. Mathroh) and *Saccharomyces cerevisiae* (Alex. Co.) whereas these strains gave lower values on the other four tested media.

Since our target was to obtain yeast strains capable to grow on raw cheap material such as molasses, the five potent isolates were screened again on Sekeri-Pataryas medium in which molasses substituted glucose (Semi-synthetic medium).

Results showed that *Saccharomyces cerevisiae* (Alexanderia Co.) and *Candida utilis* NBIMCC 608 gave more biomass and protein content on the semi-synthetic medium as compared to the results of screening on the synthetic medium. On the other hand, the other three strains of yeasts gave lower values and declined in their biomass and protein content on the semi-synthetic medium. So, these two yeast strains namely *Saccharomyces cerevisiae* (Alexanderia Co.) and *Candida utilis* NBIMCC 608 were selected to be used in the subsequent experiments.

2- Factors affecting yeast protein production:

- Glucose was substituted by different carbon sources in Sekeri-Pataryas medium. Results showed that glucose was the best carbon source for *Saccharomyces cerevisiae* and *Candida utilis* NBIMCC 608 followed by maltose. The best concentration of glucose for the production of yeast protein was 2.5%.
- On studying several natural carbon sources, maximum yeast biomass and protein content were obtained with (40 : 60) mixture of clarified cane and clarified beet molasses. This was true for both yeast strains, while whey was the poorest source of carbon for the growth of both yeast strains.
- Different nitrogen sources were investigated to study their effect on growth and protein content of the tested yeast strains. Corn steep liquor was the best nitrogen source for both strains followed by ammonium sulphate and ammonium phosphate. Peptone and casein were the poorest nitrogen sources for *Saccharomyces cerevisiae*, while ammonium oxalate was the poorest nitrogen source for *Candida utilis* NBIMCC 608.
- Increasing of the level of corn steep liquor in the growth medium from 20 ml/L to 200 ml/L increased the biomass, protein content and total

crude protein for *Saccharomyces cerevisiae* and *Candida utilis* NBIMCC 608.

- pH 4.5 was the best pH degree to give the highest values of biomass and total crude protein for both strains. The best protein content was obtained at pH 5 for *S. cerevisiae* and pH 4 for *C. utilis* NBIMCC 608. Minimal biomass and total crude protein were obtained at pH 6.5.
- The optimum temperature for single cell protein production by *Saccharomyces cerevisiae* and *Candida utilis* NBIMCC 608 was 30°C.
- The best inoculum size which was added to the mixture of caltrified beet and cane molasses and corn steep liquor medium was 5% (2.5 ml inoculum/50 ml medium) which gave maximum biomass and protein content for both tested strains while the lowest values were obtained when 1% (0.5 ml/50 ml medium) inoculum size was used.
- The best shaking rate was found to be 150 rpm for both tested strains, since it gave maximum biomass and total crude protein.
- The most suitable incubation period for the maximum production of biomass was 60 hours for *Saccharomyces cerevisiae* and 72 hours for *Candida utilis* NBIMCC 608, when the flasks batch culture method was used.
- With respect to the effect of aeration using a fermentor as a batch culture technique, maximum biomass as well as total crude protein for *Saccharomyces cerevisiae* and *Candida utilis* NBIMCC 608 were obtained at the aeration rate of 2.5 liter/min (0.83 v/v).
- Effect of incubation period at optimal conditions using a fermentor as a batch culture was studied. Maximum biomass and protein content were observed for *Saccharomyces cerevisiae* and *Candida utilis* NBIMCC 608 when incubated for 60 hours with the highest values of

consumed sugar, protein efficiency ratio, yield, conversion coefficient, substrate utilization efficiency and economic coefficient.

3. Chemical analysis and amino acids content of yeast biomass:

Chemical composition of *Saccharomyces cerevisiae* biomass shows high protein content (59.37%) and total carbohydrates content was (19.84%), while protein content of *Candida utilis* biomass was 51.87% and total carbohydrates content was 24.35%. Both strains were rich in phosphorus and potassium in their ash, moderate in calcium, potassium, magnesium, sodium and trace elements such as copper, ferric, manganese and zinc were also detected. With respect to nucleic acids *Saccharomyces cerevisiae* and *Candida utilis* gave high content of nucleic acids being 9.42 and 8.79%, respectively.

This threw the light on the necessity of treating the yeast to reduce its nucleic acid content to be accepted for human consumption otherwise medical disorders may take place such as stone formation in the kidney or precipitation of uric acid in tissues and joints.

Amino acids:

- Amino acid content of both yeast strains namely *Saccharomyces cerevisiae* and *Candida utilis* gave sixteen amino acids of which 9 are essential (cysteine, lysine, methionine, isoleucine, leucine, phenylalanine, tyrosine, threonine and valine).
- Both strains gave higher chemical score of cysteine relative to FAO reference protein being 100% and 128% for *Saccharomyces cerevisiae* and *Candida utilis* NBIMCC 608, respectively. It is worthy to mention that this score was higher than the scores of yeast protein recorded in other investigations.

- Essential amino acid index and biological value were 82.48 and 78.17 for *Saccharomyces cerevisiae* and 100.59 and 97.91 for *Candida utilis*, respectively.

Nucleic acids:

- *Saccharomyces cerevisiae* and *Candida utilis* NBIMCC 608 biomass was treated by heat shock to reduce its content of nucleic acids. The maximum reduction of nucleic acids for *S. cerevisiae* biomass (70%) was observed at pH 4 and 60°C for 30 seconds with 13.7% losses of its protein content, while maximum reduction of nucleic acids for *C. utilis* NBIMCC 608 biomass was 73.2% with 8.4% losses of its protein content at pH 4 and 60°C for 60 seconds.

Effect of gamma rays:

Saccharomyces cerevisiae and *Candida utilis* NBIMCC 608 were exposed to different doses of gamma rays from 5 k rad to 70 k rad aiming to obtain potent mutants for biomass and protein production or find the activation doses of radiation which may increase the biomass and protein content of the investigated yeasts.

The biomass and protein content of *S. cerevisiae* increased when exposed to 10, 30, 40 and 60 k rad. The highest stimulation was obtained at 40 k rad dose. This dose could be considered as an activation dose for *S. cerevisiae*.

For *Candida utilis* NBIMCC 608, it was found that exposure of this yeast strain to 5, 30 and 50 k rad increased the biomass and protein content. The greatest stimulation of this strain took place at 50 k rad. So, 50 k rad could be considered an activation dose for *Candida utilis*.

However, individual colonies from different gamma ray treatments showed high increase in biomass and protein content. To verify if this was temporary or permanent the selected potent yeast isolates were grown again on the optimum growth medium. It was found that these strains returned to resemble the control (untreated yeast culture) indicating the ability of the investigated yeast strains to repair their damaged DNA. This means no permanent mutants could be obtained. But an activation doses of 40 k rad and 50 k rad could be used for increasing biomass and protein content of *Saccharomyces cerevisiae* and *Candida utilis*, respectively.