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Research Article
Utilization of Hydrolyzed UF-permeate Supplemented with Different Nitrogen Sources and Vitamins for Production of Baker's Yeast

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Abstract

Background and Objectives: Huge amounts of permeate produced from ultrafiltration of milk for cheese processing were wasted and represent a serious environmental pollution problem. Utilization of such permeates for production of added value products as yeast biomass or baker's yeast would be profitable from the economical and the environmental point of view. The aim of this work was to study the impact of permeate supplemented with nitrogen sources and some vitamins as well as some other supplements as Tween 80 and phosphorus sources on the biomass yield of baker's yeast. **Materials and Methods:** The fermentation process was carried out in shack cultures using 250 mL conical flasks at 150 rpm and 30°C for 48 h incubation period. Different concentrations of nitrogen sources (organic and inorganic), biotin, pantothenic acid, m-inositol and Tween 80 were tested for their impact on the biomass yield of baker's yeast. **Results:** Peptone was the most suitable nitrogen source yielded 8.45 g followed by malt extract and soybean extract produced 8.30 and 8.25 g L^{-1}, respectively. Marked enhancing in biomass yield was exhibited when 0.1% of K_2HPO_4, 0.15 ppm of biotin, 2 ppm of pantothenic acid and concentration of 0.4 of m-inositol was supplemented to the growth medium. The low level of tween 80 had slightly positive effect on the yeast biomass production of baker's yeast. **Conclusion:** Enzymatically hydrolyzed permeate can be used successfully as substrate for production of baker's yeast.

Key words: Baker's yeast, permeate, peptone, biotin, pantothenic acid and Tween 80

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Whey disposal is one of the most important economic and environmental problems in the dairy industry. The employing of whey and permeate as a fermentation medium seems profitable for contributing in reduction of the environmental pollution resultant from the misdisposal of this dairy wastes and serve for production of valuable products as the yeast biomass. This liquid is produced as by-product of cheese industry and consists of approximately 5% lactose, 0.7% protein, 93% water, 0.3% fat, 0.2% lactic acid, vitamins and mineral salts[1,2]. Removal of valuable whey proteins leaves whey permeate, which can contain up to 85% lactose based on dry matter[3]. Lactose waste increases biochemical and chemical oxygen demand (BOD and COD) which is in contrast with legal standards for waste water[2]. Annual world-wide cheese whey production amounts to over 160 million t/year, corresponding to approximately 6 million tons of lactose[4,5]. Whey poses significant challenges to the dairy industries environ-mental protection strategies. High production of cheese whey and whey permeate as well as their high environmental impact and nutritional content make them an important subject for careful valorization studies[6]. Lactose can be hydrolyzed by two principal methods, using acid treatment at high temperature (above 150°C) or enzymatic catalysis. The second one is preferred because of its milder operating temperature and pH. β-Galactosidase is a suitable enzyme to perform the hydrolysis, converting lactose to glucose and galactose monosaccharides[7-10]. Current whey and whey permeate utilization consumes only a small portion of lactose generated from cheese production[11]. The large surplus of whey lactose can be used as a potential low-cost substrate for industrial chemical and fuel production. *Saccharomyces cerevisiae* is one of the oldest products of industrial fermentation and is still one of the most important biotechnological products because of its several industrial applications. Currently the substrates traditionally used for producing yeast biomass are simple sugars, in particular sucrose and sugar beet molasses or sugar cane. Due to its complex chemical composition cheese whey can potentially be used as growth medium and carbon source for biomass production especially it is inexpensive and locally available. Since *S. cerevisiae* cannot utilize lactose, the main whey carbon source[12], various approaches have been suggested: conversion of lactose to glucose and galactose by free and immobilized enzymes or by chemical hydrolysis, conversion of lactose to lactic acid and genetic manipulation in order to enable *S. cerevisiae* to directly use lactose as a carbon substrate[13,14]. Utilization of permeate for production of added value products as yeast biomass or baker's yeast would be profitable from the economical and the environmental point of view. This study was aimed at investigating the production of yeast biomass using permeate in which lactose is hydrolyzed by externally added β-galactosidase as substrate. This will contribute positively for production of an important product and on the same time contribute in reducing the environmental pollution.

MATERIALS AND METHODS

This study was carried out in National Research Center in the period from June, 2017 to March, 2019.

Materials: Permeate the by-product produced from ultrafiltration of milk for cheese manufacture was obtained from milk technology processing unit, animal production, Res, Inst., Ministry of Agriculture, Cairo, Egypt. β-galactosidase (activity of the enzyme at 37°C = 5000 U mL^{-1}) was obtained from Gohina company, Egypt.

All chemicals used in this study were of analytical grade.

Microorganisms (strain): *Saccharomyces cerevisiae* isolated from commercial compressed baker's yeast (Giza, Egypt) was used. After reactivation of commercial baker's yeast by overnight culture in YPD liquid medium, containing 3 g L^{-1} yeast extract, 5 g L^{-1} peptone, 20 g L^{-1} glucose, cells were sampled and plated after serial dilution on YPD solid medium. A single colony was selected arbitrarily and streaked consecutively at least 4 times to obtain pure colonies. Strain was maintained on YPD agar plates and was conformed to *S. cerevisiae* by 16s r RNA analysis[15].

Enzymatically hydrolyzed permeate was sterilized at 121°C for 15 min. Sterile permeate was cooled, maintained at 4°C and then used as medium in growth experiments, after treatment with β-galactosidase at the desired concentration and hydrolysis time for 2, 4, 6, 8, 12 and 24 h with using concentrations of enzyme 0.05, 0.075 and 0.1 mL/100 mL of permeate. The lactose concentrations of all samples were determined by HPLC.

Inoculum preparation: The inoculum of yeasts was prepared using 250 mL conical flasks contained broth medium of YPD. After autoclaving at 121°C for 15 min, the flasks were inoculated by a loop full of yeast culture and incubated in a rotary shaker (150 rpm) adjusted at 30°C for 24 h. The growing yeast was used to inoculate the experimental flasks at 1% (v/v).

Yeast biomass production: Yeast production was carried out in 250 mL conical flasks each contained 50 mL of enzymatically treated permeate contained 35 g reducing sugars per liter. The flasks were sterilized by autoclave at 121°C for 15 min, after cooling to room temperature the flasks were inoculated by above prepared inoculum then incubated in a rotary shaker adjusted to 30°C and 150 rpm for 48 h.

Optimization of the baker's yeast biomass production

Impact of nitrogen sources: Different nitrogen sources of organic (urea, soybean extract, corn extract, malt extract or peptone) and inorganic ($(NH_4)_2HPO_4$ or NH_4Cl) nitrogen sources were tested. Ground soybean and corn were extracted in whey permeate medium by modified Deak and Johnson method[16]. The others nitrogen sources were added to enzymatically hydrolyzed permeate containing 35 g L^{-1} of reducing sugar and supplemented with 0.5g magnesium sulfate and 0.1 g zinc sulfate[17] before fermentation. The fermentation experiments were carried out in 250 mL conical flasks each containing 50 mL of fermentation medium and were inoculated with 1% (v/v) with active viable culture of *S. cerevisiae* and rotated at 150 rpm on a rotary shaker 30°C for 48 h.

Impact of minerals and vitamins: Two sources of phosphorus i.e., KH_2PO_4 and K_2HPO_4 were added at 0.2% to the fermentation medium involved 35 g L^{-1} of reducing sugar supplemented with 0.5 g L^{-1} magnesium sulfate and 0.1 g L^{-1} zinc sulfate[17]. A control medium without supplementation was involved. Different concentrations of sterilized biotin (0.05, 0.1, 0.15, 0.2, 0.25 ppm), different concentrations of sterilized pantothenic acid (1, 2, 3, 4, 5 and 6 ppm), different concentrations of Tween 80 (0.1, 0.2, 0.3, 0.4 and 0.5/50 mL) and different concentrations of m-inositol (11, 22, 33, 44 and 55 µmole/50 mL) by using sterilized one use filter disc diffusion (because vitamins are sensitive to heat) were added to permeate enzymatic hydrolysate involved 35 g L^{-1} of reducing sugar supplemented with 0.5 g L^{-1} magnesium sulfate and 0.1 g L^{-1} zinc sulfate[17], besides a control without supplementation.

Analytical procedures

Fermentable sugar determination: Fermentable sugar determination was carried out according to the method of Miller[18] as fellow: About 1 mL of DNS reagent was added to 1 mL of enzymatic hydrolysate of lactose in test tube. Distilled water was used as reference blank. All the tubes were heated for 5 min in boiling water bath. About 3 mL distilled water was added to each tube. Absorbance was measured at 540 nm using UV-Visible spectrophotometer and compared with standard curve using 0.10-1.0 mg of glucose mL^{-1}.

Biomass production efficiency: Biomass production efficiency was calculated as g biomass yield per g consumed fermentable sugars in fermentation medium divided on[19] theoretical biomass yield multiply by 100.

RESULTS

Effects of different concentrations and incubation times with the enzyme on the hydrolysis of lactose in permeate: Permeate treated with different concentrations of enzyme and incubated for deferent incubation periods. Figure 1 showed that the hydrolysis level reached its highest value after 2 h with using the lowest enzyme concentration (50 µL). Concentrations, 75 and 100 µL gave the same values. This result may indicate that 50 µL of the enzyme for 2 h hydrolysis time was sufficient for achieving the hydrolysis process effectively.

Effect of nitrogen source: Data illustrated in Fig. 2 showed that peptone (inorganic sources of nitrogen) is the best nitrogen source increasing the yield of baker's yeast to be 8.45 g L^{-1} comparing with control (7.74 g L^{-1}) followed by malt extracts and soybean extracts (8.30 and 8.25, respectively). Urea as inorganic nitrogen source gave the same yield as soybean extract reached 8.25 g L^{-1}. and comparable to that of peptone. The advantage of urea as a nitrogen source may be due to its inexpensive cost.

Consumed and residual fermentable sugars from enzymatically hydrolyzed permeate: Figure 3 showed that consumed and residual fermentable sugars from enzymatically hydrolyzed permeate contained 35 g reducing sugars per liter. The results showed that peptone supported

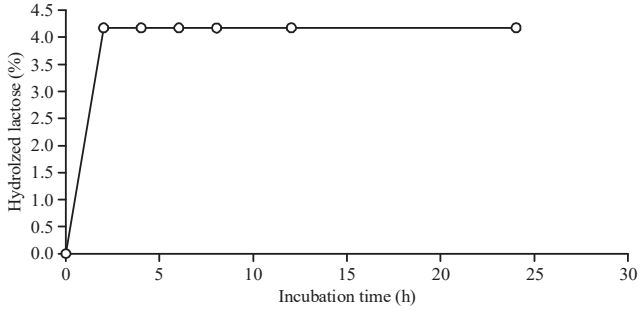

Fig. 1: Effects of incubation time on the hydrolysis of lactose in permeate by using 50 µL of enzyme

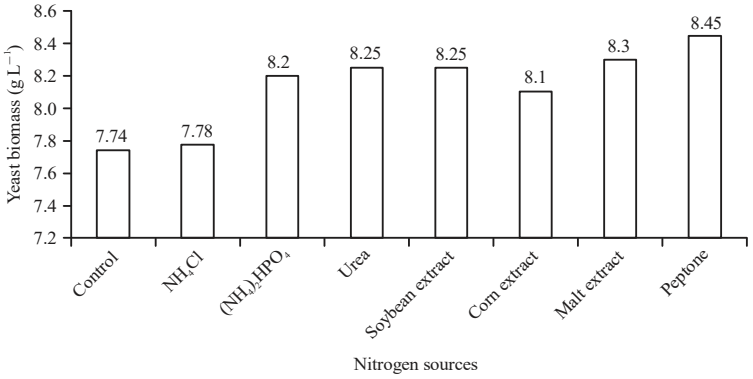

Fig. 2: Effect of different nitrogen sources (organic and inorganic) on the production of baker's yeast

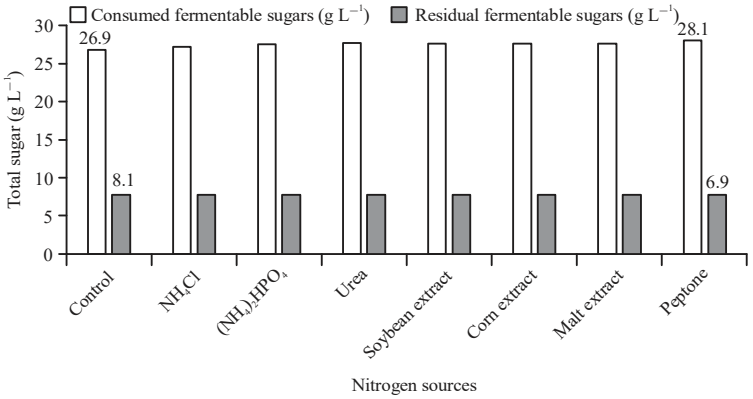

Fig. 3: Consumed and residual fermentable sugars during production of baker's yeast from permeate supplemented by different nitrogen sources

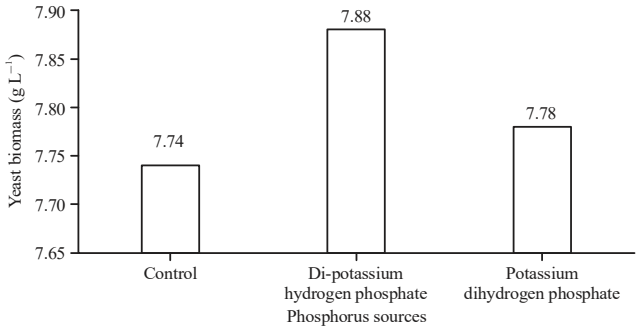

Fig. 4: Effect of different phosphorus sources on the production of baker's yeast

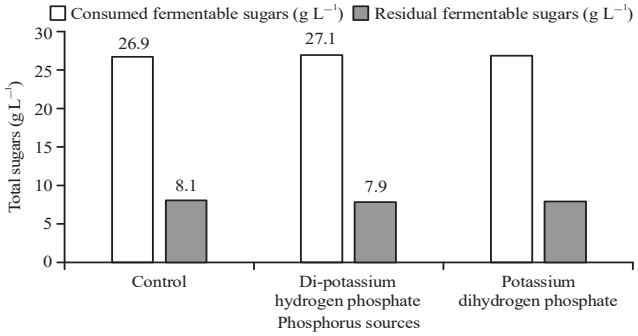

Fig. 5: Consumed and residual fermentable sugars during production of baker's yeast from permeate supplemented by different phosphorus sources

the highest yield. However the other nitrogen sources gave similar yields and quiet near to that of the highest value obtained by peptone. The nitrogen source which gave the best biomass yield has the highest consumed sugar (Fig. 3). This result is logic since a high carbon source level is required for synthesis the cells components of the higher biomass yield.

Effect of different phosphorus sources: Phosphorous sources (K_2HPO_4 and KH_2PO_4) were used as nutrient supplements for promotion of *S. cerevisiae* growth. Data illustrated in Fig. 4 showed that K_2HPO_4 supported the highest yeast biomass (7.88 g L^{-1}) than KH_2PO_4 (7.78 g L^{-1}) compared with control (7.74 g L^{-1}). Where as yeast biomass efficiency from them was the same (0.22 g g^{-1}).

Figure 5 showed the consumed and residual fermentable sugars from enzymatically hydrolyzed permeate contained

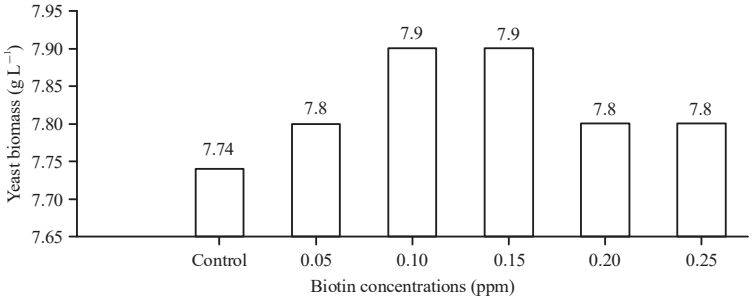

Fig. 6: Effect of different concentrations of biotin on the production of baker's yeast

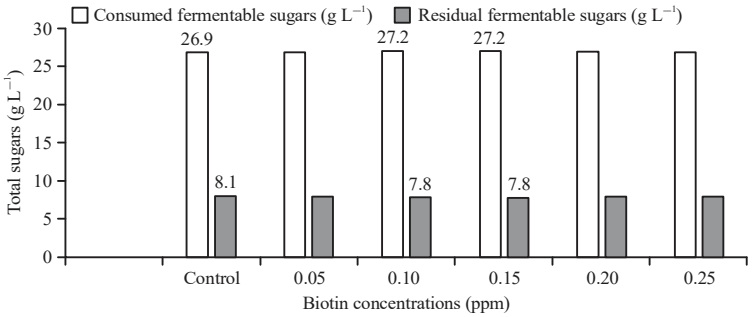

Fig. 7: Consumed and residual fermentable sugars during production of baker's yeast from permeate supplemented by different concentrations of biotin

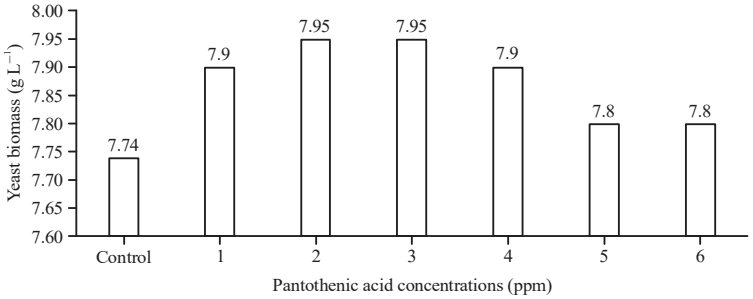

Fig. 8: Effect of different concentrations of pantothenic acid on the production of baker's yeast

35 g reducing sugars per liter. The results showed that K_2HPO_4 gave the best biomass yield have the highest consumed sugar (27.1 g L^{-1}) compared with control have a consumed sugar reached 26.9 g L^{-1}. KH_2PO_4 gave result of sugar consumption quite close to that obtained with K_2HPO_4.

Effect of different concentrations of biotin: Data illustrated in Fig. 6 showed that there is some effect of biotin concentrations on yeast biomass yield. The concentrations 0.1 and 0.15 ppm induced the same yield of yeast biomass (7.9 g L^{-1}). The higher biotin concentration gave no more yield of yeast biomass therefore a concentration 0.1 ppm of biotin was selected as the optimum concentration in further work. The consumed sugar (Fig. 7) was the same with the two concentration of biotin used in this test and this is parallel to the equal yield of yeast biomass produced by the employed strain.

Effect of different concentrations of pantothenic acid: Data illustrated in Fig. 8 showed that there are some variations in the biomass yield between the used concentrations of pantothenic acid. The concentrations 2 and 3 ppm supported the highest yeast biomass reached 7.95 g L^{-1}. Accordingly a concentration 2 ppm of pantothenic acid was the optimum concentration. Figure 9 showed that consumed and residual fermentable sugars from enzymatically hydrolyzed permeate contained 35 g reducing sugars per liter. The results showed that the concentrations of pantothenic acid (2 and 3) gave the

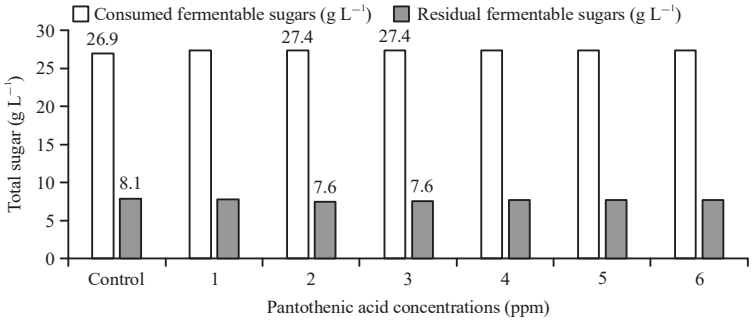

Fig. 9: Consumed and residual fermentable sugars during production of baker's yeast from permeate supplemented by different concentrations of pantothenic acid

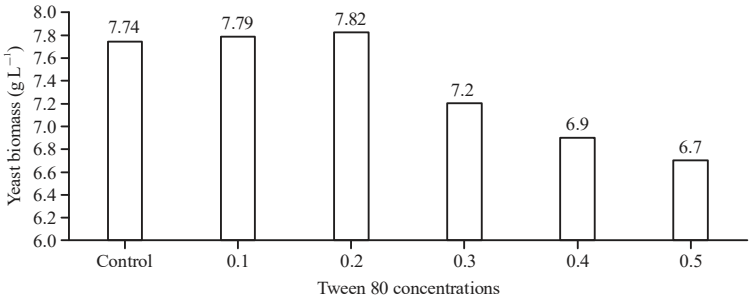

Fig. 10: Effect of different concentrations of Tween 80 on the production of baker's yeast

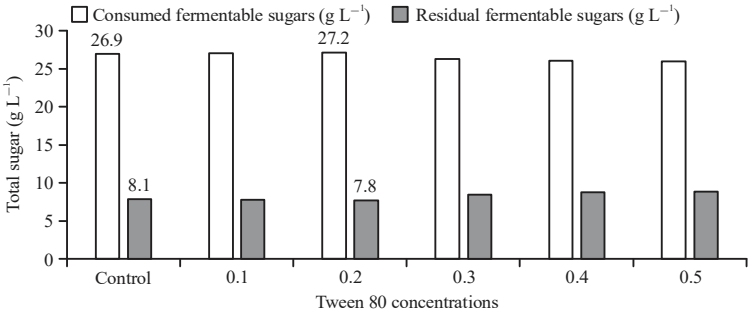

Fig. 11: Consumed and residual fermentable sugars during production of baker's yeast from permeate supplemented by different concentrations of Tween 80 (50 mL)

best biomass yield and have the highest consumed sugar (27.4 g L^{-1}) compared with control have a consumed sugar (26.9 g L^{-1}).

Effect of different concentrations of Tween 80: Data illustrated in Fig. 10 showed that the high concentrations of Tween 80 (0.4, 0.5) were reduced the yeast biomass to 6.9 and 6.7 g L^{-1}, respectively compared with control, whereas the low level of Tween 80 had slightly positive effect on the biomass yield of baker's yeast. As the previous tests the consumed sugar (Fig. 11) was positively parallel to the yeast biomass yield in the all treatments as it is increased with increasing the yeast yield.

Effect of different concentrations of m-inositol: Data illustrated in Fig. 12 showed that the concentration 22 µmole/50 mL of m-inositol yielded the highest yeast biomass (7.88 g L^{-1}) and therefore it was selected as the optimum concentration in further work. Regarding the consumed sugars (Fig. 13) it follows the same trend noted in the results of the previous experiments since it depends on the yield of the produced yeast in a positive relationship.

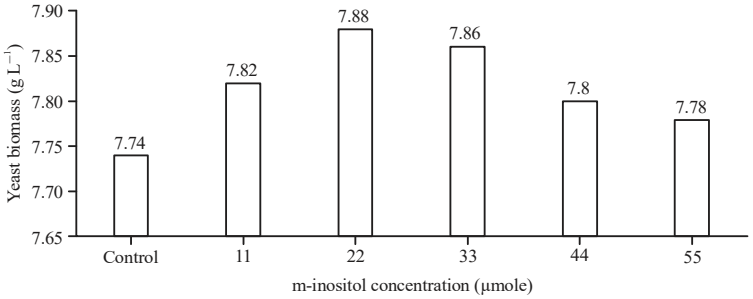

Fig. 12: Effect of different concentrations of m-inositol (µmole/50 mL) on the production of baker's yeast

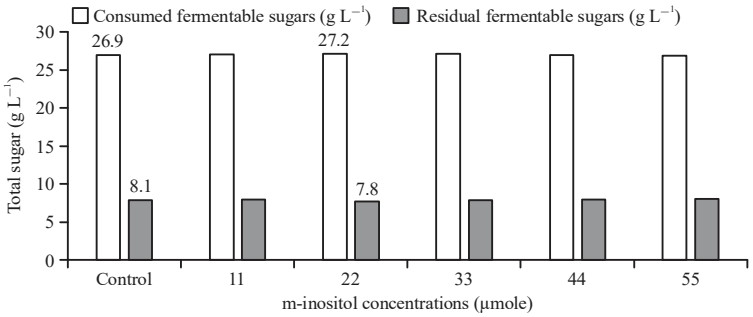

Fig. 13: Consumed and residual fermentable sugars during production of baker's yeast from permeate supplemented by different concentrations of m-inositol (µmole/50 mL)

DISCUSSION

In this study the substrate (lactose of permeate) was enzymatically hydrolyzed to simple fermentable sugars. It has been noticed that the lowest concentrations of enzyme (50 µL/100 mL) supported the best hydrolysis of lactose in the permeate. This result may indicate the high activity of the enzyme β-galactosidase used in this process and it may be also suitable for controlling the hydrolysis rate. It has been reported Pisano *et al.*[20] that the lactose hydrolysis was achieved using a low concentration of β-galactosidase in order to ensure the simultaneous use of the released glucose and galactose and avoid negative regulatory mechanisms of yeast metabolism such as the "Crabtree effect". This contributed in maximizing the yield of yeast biomass and minimizing the undesired alcohol formation. Peptone as organic nitrogen source gave the highest yield of yeast biomass. The improvement of biomass yield due to the addition of organic nitrogen source might be attributed to its content of amino acid, peptides and other growth factors[21]. Supplementation of permeate with organic sources (peptone and yeast extract) gave higher protein yield by *K. lactis* than inorganic sources[22,23]. Urea as inorganic nitrogen source gave the same yield as soybean. The advantage of urea as a nitrogen source may be due to its inexpensive cost. It has been mentioned that urea is a good nitrogen source commonly and widely used as a cheap nitrogen source in the industrial fermentation for the production of baker's yeast[24]. Replacing commercial bacteriological peptone, yeast extract and $(NH_4)_2HPO_4$ with refluxed soy meal suspension was used for baker's yeast biomass production[24,25]. In contrast some authors reported an inefficient impact of inorganic nitrogen sources compared with the organic sources[26,27]. they indicated that supplementation with ammonium sulphate and urea did not enhance single cell protein yield, while other authors mentioned that supplementation with ammonium sulphate gave high biomass yield[13,22,28,29] and in the present work urea gave a yield comparable with that supported by the organic nitrogen sources. Urea fortification led to a marked increase in protein content and a high fermenting capability for the obtained biomass[30]. This contradiction between this reports may be attributed to the using of different strains and different growth conditions. Phosphorous sources K_2HPO_4 and KH_2PO_4 used in the current research supported production of yeast biomass yield. However K_2HPO_4 gave the highest yield. Several mineral elements have been found to be essential for the growth of yeast. phosphorus, Magnesium and potassium[31]. Phosphate content of baker's yeast cells account

for around 3-5% of dry weight mostly in the form of orthophosphate[32]. Phosphate is essential nutrient as it is involved in all phases of the yeast metabolism[33,34]. Phosphorus is essential for nucleic acid synthesis and phospholipids therefore it is essential for yeast growth. Addition of KH_2PO_4 (6 g L^{-1}) led to increase in biomass production. Therefore, it was required to supplement the natural medium with this concentration of potassium phosphate[35]. Developing of accelerated strains with high protein levels (greater than 50%) cannot be efficiently achieved without the zinc addition[36]. Concentrations 0.1 and 0.15 ppm of biotin induced the same yield of yeast biomass (7.9 g L^{-1}), The higher biotin concentration gave no more yield of yeast biomass. Biotin is an essential growth vitamin for yeast and a level of 25 μg g^{-1} of yeast solids is a minimum requirement. If the molasses is deficient in biotin, it can be supplemented during the fermentation[36]. There are some variations were noted between the used concentrations of pantothenic acid on the yield they supported of yeast biomass. The concentrations 2 and 3 ppm supported the highest yeast biomass in the present research, 2 ppm of pantothenic acid was found to be the optimum. The strains of yeast differ in their capabilities in nutrient assimilation. It has been noted that the *Saccharomyces cerevisiae* strain Montrachet assimilated 50 and 95% of the pantothenic acid and biotin, respectively[37]. It has been found that the amounts of amino acids synthesized by yeast were dependent on the presence of certain vitamins[38,39]. Biotin, calcium pantothenate, inositol, pyridoxine and thiamine supplied the growth factor requirements of the yeast[40]. Biotin functions as the coenzyme transferring carbon dioxide in 4 reactions: Pyruvate carboxylase, a key step in gluconeogenesis; acetyl-coA carboxylase, the first step of fatty acid synthesis and in two other important carboxylases[41,42]. Tween 80 used as surfactant compounds that lower the surface tension between the oxygen and a fermentation medium as well as act as anti-foaming agents. Its lower concentration gave the highest yield and therefore the supplement with more concentrations not advisable.

CONCLUSION

The using Uf-milk permeates as a basal medium for production of baker's yeast serves dual purpose. It contribute for production of a value added product and on the other hand contribute in reduction of pollution resultant from the misdisposal of permeate. In this work baker's yeast were produced successfully from permeate supplemented with nutrients.

SIGNIFICANCE STATEMENT

This study discovered the suitability of employing Uf-milk permeate as a basal medium for production of baker's yeast from a cheaper substrate compared with molasses that can be beneficial for the economical production of baker's yeast. This study will help the researchers to uncover the critical areas of recycling many of food industry wastes into added value products.

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