Optimum conditions for Biomass and lytic enzyme production by *Saccharomyces cerevisiae* and removal of total solids from waste water of dairy processing

الظروف المثلى لانتاج الكتلة الحيوية والانزيمات المحللة من الخميرة *Saccharomyces cerevisia* وازالة المواد الصلبة الكلية من مخلفات تصنيع الالبان

Nadhem H. Hayder **Lamees M. R. Abbas** **Reem waled**
College of Science/ Baghdad University

ريم وليد لميس محمد رياض ناظم حسن حيدر
جامعة بغداد/كلية العلوم

Abstract

The present study was aimed to study the optimum conditions of producing yeast biomass and lytic enzymes, and removal of total solids from dairy waste water. Results showed that dairy waste water serve as a good substrate, enabling the growth of *Saccharomyces cerevisia* S4, which produced a considerable amount of yeast biomass. Maximum production of biomass was 26g/l obtained after 96h fermentation, at pH6, temperature 30°C and dairy waste concentration of 2.5% w/v. The maximum activity of α-amylase and protease were (67.7, 60.2)U/mg, respectively obtained when cultured the yeast in dairy waste water 2.5%w/v at pH6 and incubated for 120h at 30°C. The maximum reduction of total suspended solids and total dissolved solids were (44.1,53.6)% respectively observed after 96h of fermentation period at 30°C.

المستخلص

هدفت الدراسة الحالية الى دراسة الظروف المثلى لانتاج الكتلة الحيوية والانزيمات المحللة من الخمائر وازالة المواد الصلبة الكلية من مخلفات صناعة الالبان . بينت النتائج بأن مخلفات الالبان وسط جيد للنمو وانتاج الكتلة الحيوية للخميرة *Saccharomyces cerevisia* . واظهرت النتائج بأن اقصى كتلة حيوية من الخميرة الجافة 26غم/لتر تم الحصول عليها بعد فترة حضن 96 ساعة، وعند رقم هيدروجيني 6 وبدرجة حرارة 30م وباستخدام التركيز الامثل 2.5% من مخلفات تصنيع الالبان . كما بينت النتائج بأن اقصى انتاج لانزيمات الاميليز والبروتيز (60.2،67.7) وحدة/ ملغرام بالترتيب تم الحصول عليها في الظروف المثلى عند حضن الخميرة في وسط مخلفات تصنيع الالبان بتركيز2.5% وزن/حجم وعند الرقم الهيدروجيني الابتدائي 6 وفترة التخمير 120 ساعة وبدرجة حرارة 30م. وتم الحصول على اقصى ازالة 44.1% و 53.6 للمواد الصلبة العالقة الكلية والمواد الصلبة الذائبة الكلية بالترتيب بعد 96 ساعة من التخمير وعند درجة حرارة 30م .

Introduction:

Cheese whey is a by-product of dairy industries, particularly the watery portion that forms during the coagulation of milk casein in cheese making or in casein manufacture. Whey is produced in large amounts and has a high polluting charge, therefore it creating a significant environmental problem. On the other hand, whey represents about 85 – 95% of the milk volume and retains 55% of milk nutrients. Among the most abundant of these nutrients are lactose (4.5 – 5)% w/v, soluble proteins (0.6 – 0.8)% w/v, lipids (0.4 – 0.5)% w /v and mineral salts (8 – 10)% of dried extract [1].

Among the liquid industrial wastes, dairy effluents pose a serious problem to our environment. Dairy effluent has high organic loads as milk is its basic constituent with high levels of chemical oxygen demand, biological oxygen demand, oil & grease and

Keywords: Dairy waste water, *Saccharomyces cerevisia*, Amylase and protease activity, Total solids.

nitrogen and phosphorous content. Due to its high organic content with high BOD, dairy waste water dumped directly to the environment which causes serious contamination problems. Dairy industry seeks cost-effective treatment technologies to remove organic matter and nitrogen from food processing wastewater containing high levels of suspended solids and nitrogenous compounds [2]. The use of whey for the production of yeast biomass have advantages that it is a simple treatment process, and the final discharge of the whey is facilitated since the pollutant load is significantly reduced and the whey lactose is converted into yeast biomass. High production rates and protein yields as well as ease of production control makes single cell protein (SCP) more attractive as a protein source compared with conventional plant and animal sources. Since the major constituent of the whey is lactose, the selected organism must be able to readily metabolize lactose. SCP could be produced from whey, with employing of yeasts from different species including *Kluyveromyces*, *Candida*, and *Trichosporon*, which are normally capable of metabolizing lactose [3].

The yeast *Sacchromyces cerevisiae* is generally recognized as a safe micro-organism, lacking endotoxins and lytic viruses, being able to perform many post-translational modifications, including glycosylation, acylation and folding of proteins. *Sacchromyces. cerevisiae* has been used as a host micro-organism to produce different heterologous proteins such as β-galactosidase, glucoamylase [4].

Proteases are one of the industrially most important enzymes. These proteolytic (protein digesting) biocatalysts have been in use for many centuries, at first in the dairy industry as milk-clotting agents (rennet) for the manufacture of cheese. Proteases are enzymes that catalyze hydrolytic reactions in which protein molecules are degraded to peptides and amino acids. These constitute a very large and complex group of enzymes, which differ in properties such as substrate specificity, active site and catalytic mechanism, pH and temperature optima and stability profile. They are significant in that they not only govern proteolytic reactions, but also regulate various enzymatic cascades, which ultimately lead to all metabolic reactions involving the breakdown of fats, carbohydrates, *etc.* [5]. On the other hand, several extra cellular enzymes are commercially available. Among them, amylases are used for hydrolyzing carbohydrate and other constitutes of soy beans and wheat into simple sugar constituents. These enzymes find potential application in a number of industrial processes such as food processing, fermentation, textile, and papers industries and with the advent of new frontiers in biotechnology [6].

In recent years, however, fermentation processes have been increasingly utilized for the production of this enzyme by a variety of micro-organisms such as *Bacillus subtilis*, *Rhizopus oryzae*, *Aspergillus niger*, *Aspergillus awamori*, and attempts to investigate the potential of dates as substrate for the production of α-amylase using strains of *Saccharomyces cerevisiae* [7].

In this study, a number of yeast isolates with good ability for dairy waste fermentation were isolated and identified. The ability of the isolates for consumption of crud dairy waste, biomass production, and solids removal was investigated. Furthermore the ability of isolates to produce lytic enzymes was also studied.

Materials and methods

Collection of dairy wastewater and its characteristics

Dairy industry wastewater including whey, yoghurt and cheese were collected in 300ml plastic bottles from dairy factory of College of Agriculture/ University of Baghdad during September/2010 and they stored in refrigerator at 4°C temperature. The characteristic of Dairy Wastewater was determined in Table (1).

Table (1): Characteristics of Dairy Wastewater

Components	Concentration
Colour	Milky to greenish
Water %	86
pH	4.54
Temperature	29 °C
Conductivity	400
Total suspended solids (TSS) mg/l	55640
Total dissolved solids (TDS) mg/l	32800

Preparation of dairy waste substrate

Dairy waste water was used as sole source of carbon and energy. It was used as suspension in the culture medium. The dried powder of dairy waste was prepared by dispensing the collected waste in open tray and lifted to dry in oven at 40ºC until the crystal powder obtained and homogenized. The dried milky powder was then used for biomass and lytic enzyme production in culture media.

Sampling and isolation of yeast isolates

Five samples including whey, yoghurt and cheese were collected from dairy producing factory in College of Agriculture/ University of Baghdad. The isolates were cultivated according to the methods described by [8] with some modification. By inoculation of 1% of the sample in 50 ml of Malt Extract Broth (MEB), containing 0.1 g/l chloramphenicol. The incubation was performed at 30°C for 24 h with constant shaking of 120 rpm. The isolates were spread on plates of potato dextrose agar (PDA) after making serial dilutions. The plates were incubated at 30°C for 72h.Colonies with distinct morphological differences were selected and purified by streaking on PDA and then stored on slants at 4ºC.

Identification of yeast Isolates

Six isolates of yeast designated (S1-S6) was isolated from dairy waste culture and screened for their ability to biomass and lytic enzyme production. The isolate S4 which shown higher biomass and lytic enzyme production were identified on the basis of morphological and biochemical characteristics including fermentation of different sugars (glucose, lactose, sucrose and mannitol) by using the methods and identification keys for fungi [9] and hand book of soil fungi [2] respectively. This culture was grown on PDA slants for 72 h at 30ºC and then stored at 4ºC with sub culturing every three months.

Inoculums preparation

Three days old culture of the isolated yeast grown on PDA was used as inoculums. The cultures were then transferred from the surface of the agar in to 250 ml Erlenmeyer flasks containing 50ml MEB. Then plugged with cotton and the Erlenmeyer flasks were incubated at 30ºC with constant shaking of 120rpm for 16-18h.

Screening of isolates for Biomass and lytic enzyme production

The isolates (S1-S6) were separately grown on a medium developed by [10] with some modification by elimination whey's protein. The medium contained (g/l): $(NH_4)_2SO_4$, 3; Na_2HPO_4, 0.2; $MgSO_4.7H_2O$, 0.1; NaCl, 0.1; $CaSO_4.2H_2O$, 0.1; $FeSO_4$. $7H_2O$, 0.025; $ZnSO_4.7H_2O$, 0.0075; $MnSO_4.4H_2O$, 0.005; $CuSO_4.5H_2O$, 0.001 and, H_3BO3, 0.0005 (pH 6). The medium was supplemented with dairy waste powder as substrate for Biomass (SCP) and enzymes production at a concentration of 1.0 % and dispensed in Erlenmeyer flasks 250 ml each flask contained 50ml of medium. The flasks were autoclaved at 121℃ for 10min and each flask was inoculated with 1% of overnight cultures of yeast isolates (S1 –S6). The cultures were incubated in shaker incubator (Basel Switzerland) 120rpm, at 30°C. The cultures from each flask were analyzed starting from day (1 to 6) of incubation for biomass and production of amylase and protease.

Optimum conditions for biomass, and lytic enzymes production, and Total solids removal

Production of biomass was measured on the bases of weight of dry biomass (g/l) in duplicate. The isolate *Sacchromyces cerevisiae* S4 was grown in MEB broth for 16-18 h at 30˚C. This culture was used as stock culture inoculums at concentration of 1% (v/v). To determine the capability of the isolate for biomass and enzymes production, a mineral salt medium (MSM) [10] was used. Dairy powder was added separately as carbon source. Cultivations were performed in 250 ml Erlenmeyer flasks containing 50 ml mineral salt medium at 30˚C, and stirred in a rotary shaker incubator at 120 rpm.

The medium optimization was conducted in a series of experiments by changing one variable and, keeping the other factors fixed at a specific set of conditions. Four factors were optimized aiming to obtain higher productivity of the biomass and enzymes production: pH, temperature, and incubation period and carbon source. The pH used was (4-9), temperature used were (20-45)˚C. For incubation period *Sacchromyces cerevisiae* S4 was cultured at different incubation period (1-6) days at pH6 and 30˚C in a shaker incubator with 120rpm. For appropriate concentration of carbon source as energy, different concentration of dairy waste powder (0.5, 1, 1.5, 2, 2.5, 3, 3.5) % (w/v) were used, at optimized condition; pH 6, incubated at 30˚C, and 120 rpm for 96 h and 120 h for biomass and enzymes production respectively. At the end of each experiment the dry weight, amylase and protease production was measured, as well as total suspended solids (TSS) and Total dissolved solids (TDS) was measured at optimized condition.

Biomass yield measurement

At the end of each fermentation process, 40 ml of each sample were transferred to centrifugation tubes in duplicate and centrifugated at 4000 rpm [11]. The biomass then washed twice with equal volume of distilled water. The cell pellets were dried in pre-weighted tubes at 70°C to constant weights and then weighed.

Total suspended solids and Total dissolved solids

Total suspended solids (TSS) and Total dissolved solids were estimated according to the method described in the standard methods for examination of water and wastewater [12]. TSS was measured by filtration of the sample through a pre-weighed filter paper followed by drying at 70°C for 24h to constant weight.

Enzymes assay

Alpha amylase activity was determined according to [13]. Amylase was assayed by adding 0.1 ml of enzyme fermented broth supernatant to 0.2 ml of 0.5 % soluble starch in phosphate buffer, pH 6.5 and incubated for 30 min at 37°C. The reaction was stopped by adding 0.4ml of 3.5dinitro-salicylic acid followed by boiling for 10min. The final volume was made to 10ml with distilled water and the absorbency measured at 540 nm with U.V spectrophotometer (UK/PG). One unit of amylase activity was defined as the amount of enzyme that releases 1µmol of reducing sugar as D-glucose per min under the assay conditions. Quantity of protein in the samples is estimated by the method described by [14] using serum albumin as standard protein. The results are presented as specific activity (U/mg protein).

For protease activity, a reaction mixture containing 1.0ml of 1% soluble casein in 0.05M-citrate phosphate buffer pH6.5 and 1.0ml of culture filtrate was used. The mixture was incubated for 1h at 37°C then stopped by adding 10% trichloroacetic acid (TCA), kept for another 20min at the same temperature, followed by centrifugation at 4000rpm for 20 min. Samples of 75µL were removed and tyrosine was determined. One unit of the enzyme activity was defined as the amount of enzyme required for the formation of 1.0 µM of the product / min of the reaction, under the standard assay conditions [15]. Also the quantity of protein in the samples is estimated by the method described by [14].

Results and discussion

Six different yeast isolates from dairy waste water were isolated by using Malt extract Broth (MEB) medium and Potato dextrose Agar (PDA). The isolates were examined for dairy waste fermentation ability and amylase and protease production. Four isolates were found capable of dairy waste fermentation and enzyme production Table (1). Biomass production and enzyme activity of amylase and protease was measured Table (1). Among 4yeast isolates, the S4 isolate *Sacchromyces cerevisiae* was produced the highest biomass 19.5g/l and specific enzyme activity, (32.7, 39.4) U/mg for amylase and protease respectively.

Table (1): Screening of isolate for biomass and enzymes production

Isolates	Biomass (g/l) *	Amylase specific activity U/mg ■	Protease specific activity U/mg
S1	9.0	9.7	13.6
S2	2.0	4.3	5.5
S3	17.0	30	27.8
S4	19.5	32.7	39.4
S5	14.3	18.0	25.3
S6	2.4	3.3	4.6

* Higher biomass concentration estimated at day 4 from incubation.
■ Higher enzymes activity estimated at day 5 of incubation.

The isolate S4 was identified according to its morphological and physiological properties using the standard taxonomic [2,9] and biochemical tests including fermentation of different sugars. Colonies of isolate S4 in medium grow rapidly in 3 days. They are flat, smooth, moist, and creamy in color, Cell buds are observed. They are unicellular, and globosely in shape. Multipolar budding is typical, no hyphae was seen. They multiply as single cells that divide by budding. In sexual reproduction they

produced haploid ascosporous. The results of sugar assimilation test are presented in table (2). The isolate S4 showed ability to ferment glucose and sucrose, while unable to ferment lactose and xylose.

Table (2): Sugar assimilation by isolate *Saccharomyces cerevisiae* S4

Sugar	Glucose	Sucrose	Lactose	Xylose
Isolate S4	+	+	-	-

According to the morphological and biochemical tests the isolate S4 identified *Sacchromyces cerevisiae* S4. Therefore the isolate *Sacchromyces cerevisiae* S4 was selected for detecting the optimum condition of biomass and enzymes production using dairy waste as substrate.

Effect of cultural and environmental conditions on Biomass and Enzymes production

The effects of pH, temperature, and incubation period, dairy waste concentration on biomass yield and production of amylase and protease were investigated. As can be seen in figure (1) the maximum biomass yield (SCP) reached 23.0 g/l and enzymes production (24, 33.4)U/mg protein for amylase and protease respectively obtained at pH6. Any change in pH to lower or higher level caused drop in the biomass and enzymes production. At the optimum level of pH6, the maximum biomass and enzymes production obtained at temperature 30˚C, in which the biomass yield 22.9g/l with specific activity of 30 and 36.7U/mg protein for amylase and protease respectively were estimated figure (2). Any increase of temperature resulted in decreases in the growth and enzymes production. These results indicate that the isolate *Sacchromyces cerevisiae* S4 was favored the mesophilic condition.

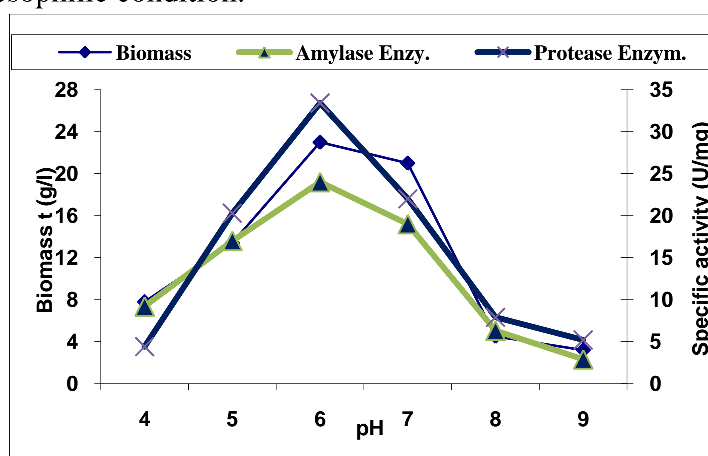

Fig (1): Effect of pH on growth rate and enzymes production of *Sacchromyces cerevisiae* S4 at 30 °C in shaker incubator at 120 rpm for 120 h.

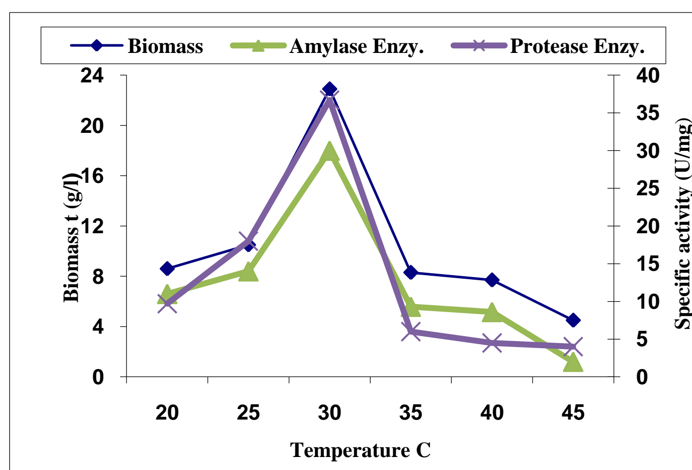

Fig (2): Effect of temperature growth rate and enzymes production of *Sacchromyces cerevisiae* S4 at 30°C, pH 6 in shaker incubator at 120 rpm for 120 h.

Furthermore, growth and enzymes production increased with incubation period and reached maximum 24.0 g/l at 96h, for biomass production, while maximum enzymes production (46.3, 58.6) U/mg was obtained at 120 h of incubation period for amylase and protease respectively. Beyond which biomass and enzymes productions were decreased figure (3).

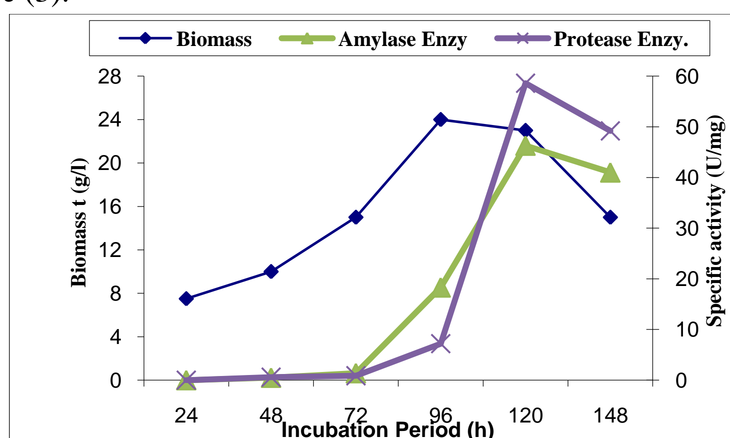

Fig (3): Effect of incubation period on growth rate and enzymes production of *Sacchromyces cerevisiae* S4 at 30°C, pH 6 in shaker incubator at 120 rpm.

When applying the optimum condition of pH 6, temperature 30˚C, incubation period 96h for biomass and 120 h for enzymes production. Maximum yield of biomass 26g/l and enzymes (67.7,60.2)U/mg for amylase and protease respectively was obtained at a substrate (dairy waste powder) concentration of 2.5% (w/v) figure (4). The biomass and enzymes production increased with increase in substrate concentration. The results indicated that the production is a linear function of growth and substrate concentration.

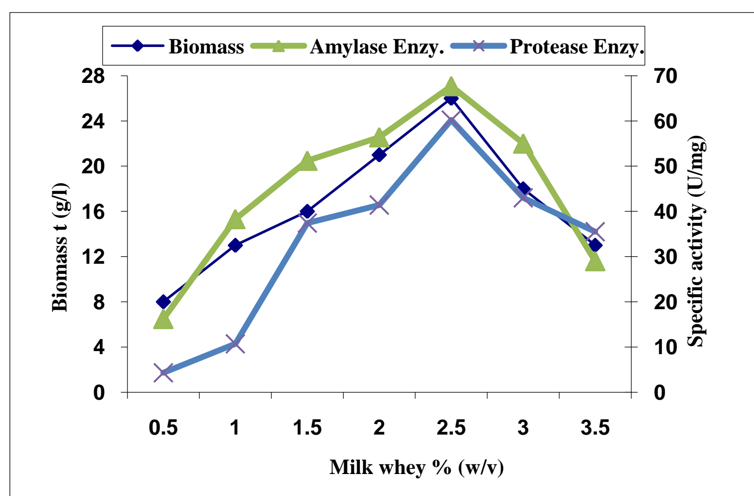

Figure (4): Effect of dairy waste concentration on growth rate and enzymes production of *Sacchromyces cerevisiae* S4 at 30°C, pH 6 in shaker incubator at 120 rpm for 96 and 120 h.

The total solids were used in present study as an indirect measurement of the soluble and insoluble organic matter in dairy waste. The difference between the initial and final values of the soluble and insoluble solids is due to the consumption of the dissolved biodegradable organic matter from the dairy waste by the yeast. Figure (5) showed that the final reduction in total suspended solids and total dissolved solids was 44.1% and 53.6% respectively, obtained at 96 h of the incubation period. The 53.6% reduction in the soluble solids shows that the soluble organic material from dairy waste was converted to insoluble material (yeast cells) in which highest biomass was observed reached 24.0 g/l.

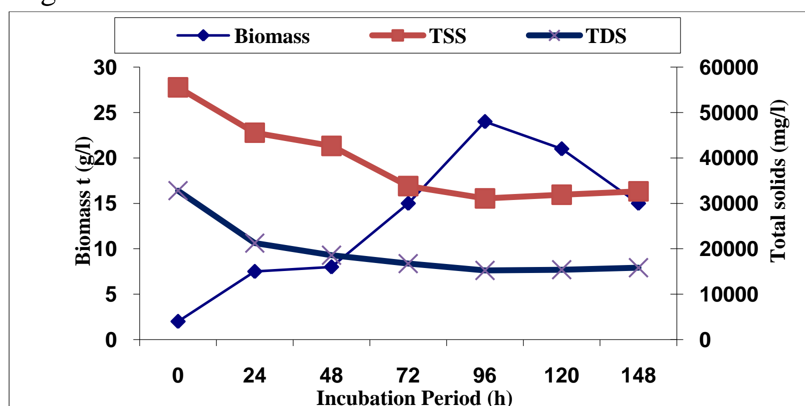

Figure (5): Total suspended and total dissolved solids removal by *Sacchromyces cerevisiae* S4 at 30, pH 6 in shaker incubator at 120 rpm for 96 h.

The physical and chemical characteristics of the dairy waste water are given in Table 1. The initial pH value was 4.5, however the optimum pH for the growth and survival of *S. ceervisiae* S4 was at pH6.0 [4]. In this context [15] mentioned that keeping the pH at 4.5 can help to prevent any possible contamination by lethal bacteria that grow at pH above 6.0.

Data obtained from the amount of biomass yield during fermentation showed that the concentration of biomass at the first day of incubation was 7.5 g/l and reached its maximum of 24 g/l on day four of incubation. During the exponential growth phase, the biomass concentration increased dramatically with the incubation period and the concentration of dairy waste was depleted rapidly figure (3). In a similar study, cheese

whey was fermented by *Kluyveromyces maxianus* under batch and aerobic conditions in which pH and temperature were adjusted to 4.5 and 30°C respectively. Approximately 82% of total protein and 32 g/l biomass were produced during the first 18h of incubation and maximum biomass of 40 g/l was obtained at 96 h of the incubation period [15].

Figure (2) showed the effect of temperature on the growth and enzyme production. In the lag phase when temperature increased from (20-25)°C the dairy waste was metabolized for maintenance of cells. In the exponential phase when temperature increased to 30°C dairy waste was metabolized for energy and growth in which maximum biomass reached 22.9g/l with maximum enzyme production of (30, 36.7)U/mg for amylase and protease respectively was obtained. In the third stage when temperature increased more than 30°C a significant decreases in microbial activity was observed, and this is an indicates that the isolate favored mesophilic condition for growth and enzyme production. In the study of [16] for single cell production by *Kluyveromyces fragils* they mention that during the lag phase when temperature increased from (20-22)°C resulted in the metabolism of lactose for maintenance of the cells. In the exponential phase during which temperature increased from (22-31)°C about 99% of the lactose metabolized for energy and growth with reduction 90.6 % of COD value. Also they pointed that the temperature above 31°C the total COD reduction continued to decline due to cell death resulting in a reduction of 42 % of COD.

Type of carbon source plays a major role in microbial production. Deferent carbon source such as cheap material (molasses, corn steep liquor, whey waste) and n-alkanes were used in byproduct and biomass production due to their low cost and availability. Agricultural and food industry wastes such as rice, starch waste liquor, whey and domestic waste also used in biomass and byproduct production [17,18]. The main composition of dairy wastewater is lactose about 70%, lactose being largely responsible for the high BOD and COD. Thus, dairy wastewater is particularly suitable for the production of SCP using lactose-utilizing microorganisms. In the present study the data in figure (4) showed higher biomass production at optimized condition obtained at dairy waste concentration of 25g/l (2.5 %), with highest biomass concentration reached to 26.0 g/l. The finding in the study [15] pointed the ability of strain *Saccharomyces Cerevisiae* to produce single cell protein (SCP) and biomass using milk whey as substrate. The results showed that the strain was found highest level of SCP production up to 11.7g/l. Also the strain showed the highest biomass production yield up to 23.38g/l dry mass cell and highest reduction in initial BOD. Together, the data showed that the isolate *Saccharomyces Cerevisiae* could be valuable application in bioconversion of whey.

Optimization of α-amylase and Protease Production:

The production of α-amylase is very sensitive to initial pH of the fermentation medium. The obtained results in the present study showed that the maximum α-amylase activity 24 U/mg was obtained at pH 6.0 figure (1). Similar results were obtained by [19] that the maximum α-amylase activity i. e., 1712.0 μmol/L/min was obtained at pH 5.5. On the other hand, [20] reported a maximum α-amylase production at pH 6.0. When the pH is altered below or above the optimum, the activity is decreased or becomes denatured.

According to [18], the growth and enzyme production were inhibited when the initial pH of the medium was above pH 10.0 or below pH 4.0.

The incubation time for achieving maximum enzyme level is depending mainly to the characteristics of the culture. The results obtained shows that the α-amylase production begins at 72h to reach to a maximum value 33U/mg at 120h, figure (2). So, the optimum time of enzyme synthesis was to be 120h after inoculation. The results were agreement with that reported by [6]. They mention that the α-amylase production begins at 48 h i.e., 760µmol/L/min to reach to a maximum value at 96h, i.e., 1680.66µmol/L/min. Incubation beyond 120h was undesirable as this resulted in decreased α-amylase activity. The decrease in enzyme yield after optimum level may be due to the denaturation or decomposition of α-amylase due to interaction with other components in the medium or could have started producing secondary metabolites, resulting in a lower α-amylase activity [20].

Variation in initial substrate concentration showed that the α-amylase synthesis was related to the availability of sugars content in the dairy waste. The obtained results showed that the α-amylase activity increased with increases dairy waste concentration. The maximum yield 67.7U/mg was obtained at 25g/L (2.5 % w/v) of dairy waste concentration figure (4). Our finding are comparable for the reported results by [6] they observed maximum α-amylase activity at 20 g/l (2% w/v) date sugar concentration.

It has been reported that the production of extracellular protease by different microorganism can be strongly influenced by the culture conditions. So it became necessary to understand the nature of protease and their catalytic potentiality under different conditions [21].

The results obtained in figure (1- 4) found that the maximum level of extracellular protease and biomass production by *Saccharomyces Cerevisiae* S4 was at optimum pH6, temperature 30°C and 120h incubation period. The obtained results was in agreement with that observed by [22] they found that the maximum level of extracellular protease and biomass production by *Saccharomyces Cerevisiae* was at optimum pH 6, temperature 25°C and 120 h incubation period. Also the study investigation showed the ability of soil yeasts, *Geotrichum candidum, Geotrichum capitatum, Williopsis californica* and *Sacchromyces cerevisiae* to produce extracellular enzymes (amylase, cellulose and protease. Another finding by [23], reported that the maximum protease enzyme production occurred during 7th day of incubation by using *Aspergillus flavus* at pH5, temperature 30°C. In another study [24], they pointed that protease and amylase production with both cultivation method had a similar optimal pH between (6,7) and optimal temperature between 35°C and 45°C.

Total suspended and dissolved solid removal

The initial concentration of the total and dissolved solids in the dairy waste was 55640 mg/l and 32800 mg/l respectively. The total solids decreased with time due to decreases in concentration of degradable material in the waste. The results in figure (5) showed decreasing in concentration of TSS from (55640-31100)mg/l and TDS from (32800-15250)mg/l at optimized condition pH6, temperature 30°C, incubation period 96h and at substrate concentration of 25g/l (2.5 % w/v) dairy waste. These results indicated that the

fermentation process achieved a final reduction of 44.1% in TSS, and 53.6% in TDS. Results found in the present study was closely to that found by [16], they pointed that the concentration of the total solids in the raw whey was 66830 mg/l (56730 mg/l volatile solids (VS) and 10100 mg/l ash). The total solids decreased with time (due to decrease in VS). The results also indicated that the fermentation process achieved a final reduction of 47.48% in total solids, and 55.91% in VS. The reduction in total solids corresponded to the reduction in the total COD which represented the conversion of lactose to energy, CO2 and H2O. The remaining solids in the medium represent the SCP (yeast biomass) and other useful products.

Conclusion

Results obtained showed that dairy waste water serve as a good substrate, enabling the growth of *Saccharomyces cerevisia* S4, which produced a considerable amounts of yeast biomass. Maximum production of yeast biomass was obtained when culturing *S. cerevisiae* S4 in production media containing dairy waste water 2.5% w/v as sole source of carbon and incubated at 30°C for 96h.

As for the production of amylase and protease, the optimum conditions were obtained at an incubation period of 120h, temperature of 30°C, initial pH6, and dairy waste concentration of (2.5% w/v). Maximum reduction of TSS and TDS 44.1% and 53.6% respectively were obtained at fermentation period of 96h.

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