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Utilization of Whey and Permeate to Produce Single Cell Protein by Using some Yeasts

Gomaa, M. S.[1]; M. M. Abo-Srea[1]; Eman L. Mostafa[2] and Doaa M. Fathy[2]
[1] Dairy Sci. Dept., Faculty of Agriculture, Mansoura Univ.
[2] Anim. Prod. Res. Institute, Agric. Res. Cent., Giza.

ABSTRACT

The aim of work was to utilize the by-products such as whey or milk permeate to produce single cell protein and to reduce the environmental pollution from three individual yeast strains (*Kluyveromyces fraglis*, *Kluyveromyces lactis or Saccharomyces cervesiae*). All of these were cultivated at the optimum condition for 48 and 96 hr by using whey, permeate or YM as media. The obtained results indicated that *Kluyveromyces fraglis* achieved the highest growth (22 x 10^6 cfu/ml) on the whey after 96 hr, and the yield of biomass (2.5 gm/L) after 96 hr., followed by *Kluyveromyces lactis* and *Saccharomyces cervesiae*. *K. fraglis* achieved the highest value of BOD (58.1 mg/L) on the permeate after 96 hr, and the removal and consumed lactose (37 %) on permeate after 96 hr.

Keywords : Whey, Permeate, S.C.P, *Kluyveromyces fraglis*, *Kluyveromyces lactis or Saccharomyces cervesiae*, BOD.

Abbreviations :S.C.P : Single cell protein.
BOD : Biochemical oxygen demand.

INTRODUCTION

Whey is the aqueous fraction of milk generated as a by-product of cheese manufacturing which is produced in large amounts, the percentage of lactose in cheese whey about 3-8 % (Speer, 1998). While, milk permeate is a more recent by-product from ultra-filtration (UF) process of milk when membrane technology is employed to manufacture (UF) process of milk when membrane technology is employed to manufacture cheese.

Diposal of whey and milk permeate using any traditional methods could be a source for environment of pollution that increase the BOD as a result of the presence of tremendous concentration of organic substances. Also, because these by-products contain high level of nitrogen, phosphorus and some other materials, it must be managed carefully when used for land irrigation. Besides whey and milk permeate can cause serious problems even when dumped directly into municipal sewage system because of their high BOD. The use of whey for the production of yeast biomass has the advantages that it is a simple treatment process, and the final discharge of the whey and milk permeate are facilitated since the pollutant load is significantly reduced and the whey and milk permeate lactose are converted into yeast biomass by using yeasts such as kluyveromyces, candidu and trichosooron as they are naturally able to metabolize lactose (Mansour *et al*., 1993).

For this reason in our search we used their *Kluyveromyces fraglis*, *Kluyveromyces lactis* or *Sacharomyces cervesiae* to produce single cell protein (biomass) by cultivate all of them on whey, permeate or YM media.

MATERIALS AND METHODS

YM Broth : was used for the activation of different strains. It consists of: Glucose 10 g/L, Peptone 5 g/L, Maltextract 3 g/L and Yeast extract 3 g/L.Permeate of buffalo milk was obtained from Animal Production Research Institute.Whey of Mozzarella cheese being used was brought from Faculty of Agriculture, Cairo University. *Kluveromyces fraglis* (Marxians) (NRRY–1109), *Kluveromyces lactis* (DSM70800) and *Sacsharomyces cervesia* were obtained from the Egyptian Microbial Culture Collection (E.M.C.C) MIRCEN, Ain Shams University (Cairo). Ammonium sulphate (0.8 gm/ liter), Yeast extract (0.2 %) and Potassium nitrate (0.4 gm / liter) were purchased from the market. The three above source of nitrogen were added individually to the filtrate of whey or permeate to act as a source of nitrogen.

Lactose was determined according to Perry and Doan (1950). pH values were determined using lab – pH meter (G. Schott, Germany). Titratable acidity was determined according to Ling (1963) as lactic acid percentage. Biomass was estimated by centrifuging 25 ml of the permeate, YM or whey media after proper mixing using sigma 301 centrifuge at 5000 rpm for 10 min. the supernatant was discarded; the yeast cell residue (biomass) was weighed (Capoor and Singh, 1985). This weight consider as a wet weight, then the biomass were dried in the oven at 80 ^oC for 24 hr. Biochemical oxygen demand (BOD) was determined before and after fermentation, using the method reported by APHA (1998) calculating by the equation of **:**

$$\text{BOD 5 mg/L} = \frac{D1 - D2}{P}$$

Where :
D1=D0 of diluted sample immediately after preparation, mg/L.
D2=D0 of diluted sample after 5 days incubation at 20 ^oC, mg/L.

For the preparation of the cultures YM Broth medium was distributed in 10 ml pyrex tubes and sterilized. Sterilized loup was used to transfer the strains of yeasts individually into the tubes then well stirred and incubated at 32 ^oC for 48 hr.

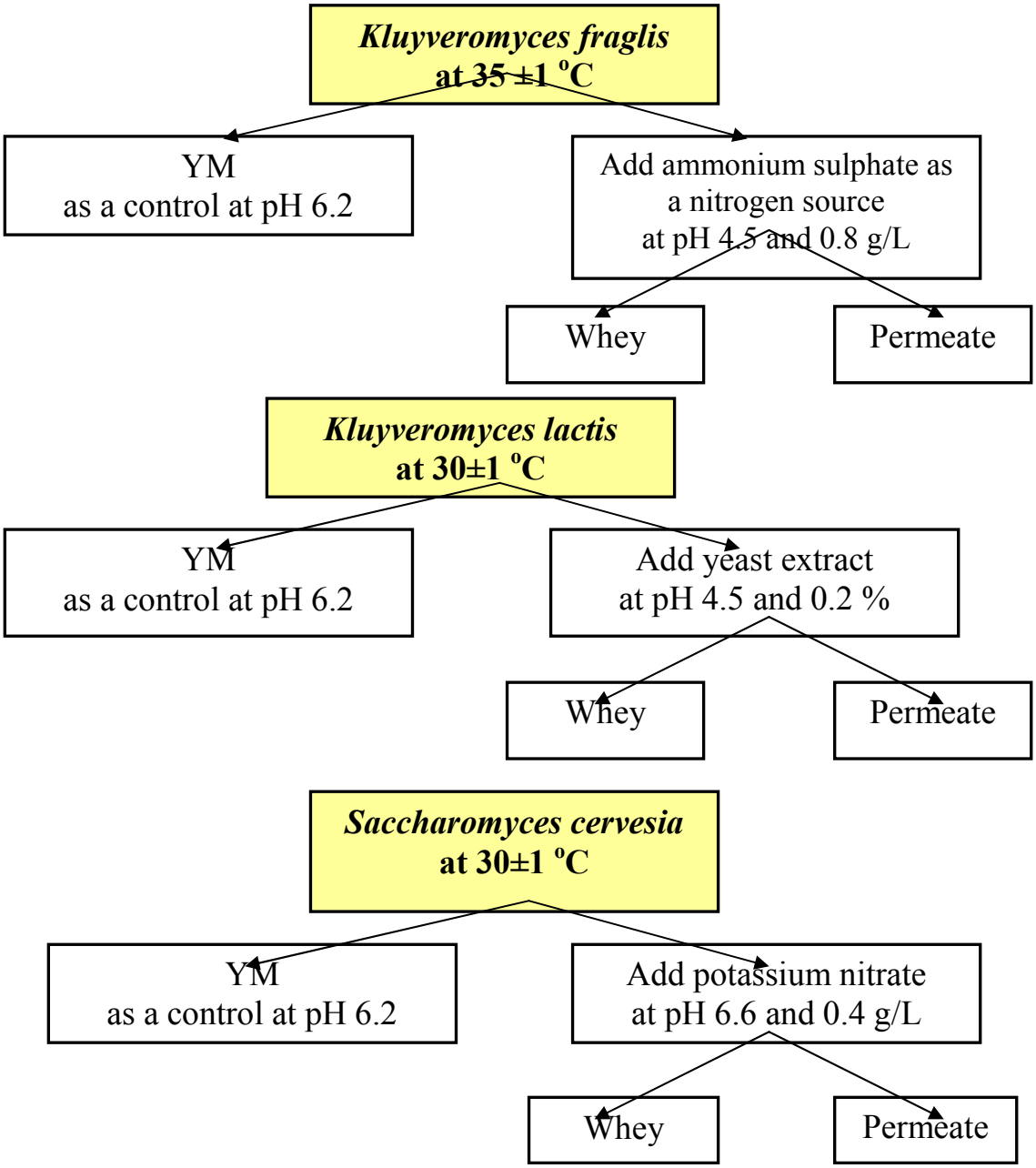
The turbidity and sedimentations refer to the activity of the yeast. The activated strains were kept at refrigerator at 4 ± 2 ^oC for using at the same day. The absorbance was measured at 650 nm as recommend by Ezzat and El- Shafei (1988) using Shimadzn (UV-visible) spectrophotometer-experiments.Malt extract agar medium was used to determine the yeast cell count as recommended by the American Public Health Association (1992).

For the preparation of whey, the pH of the whey was adjusted to ph 4.5, then flasks were heated in water bath

at 90 ± 2 °C for two hours, then filtrated to get rid of whey proteins.

The pH of permeate was adjusted to 4.5 then heated in water bath at 90 ± 2 °C then cooled and filtrated, finally it sterilize at 121 °C for 15 min.

To study the effect of medium on the activity of each of individual strain, *K . fraglis* grown on either whey or permeate fortified with ammonium sulphate at optimum condition (pH 4.5, 35 ± 1 °C), *K. lactis* grown on whey or permeate supplemented with yeast extract at optimum conditions (pH 4.5 and 30 °C) while *Saccharomyces cervesia* grown on whey or permeate supplemented with potassium nitrate under optimum conditions (pH 6.6 at 30 °C). Control was done by growing *K. fraglis*, *K. lactis or S. cervesiae* on YM medium at the optimum conditions of all of them.



RESULTS AND DISCUSSION

It is clear that the acidity % ranged between (0.11 %) in YM medium and (0.42 %) in whey, and it decreased after 96 hr in all different media Table (1). The pH was parallel to acidity. These results due to *K. fraglis* produce amount of alchole which changes the acidity. These results agree with Abo-EL-Einin (2006), the growth of *K. fraglis* in permeate increased the pH during fermentation, then it decreased in the end of fermentation (Foad, 2006) and Abo-EL-Einin (2006).

The same Table and Fig. (1) show that the consumed lactose in different media ranged between (12 %) in YM medium and (23 %) in permeate after 48 hr, then it increased after 96 hr. Permeate recorded the highest value of 37 %, while YM media recorded the lowest of 24 %. These results agree with Gholson and Gough (1980), who stated that when treated the sweet Cheddar type whey with *K. fraglis*, it resulted in greater

than 90 % of the lactose, Singh and Neelakantan (1989) reported that K. fraglis 3217 and S. fraglis had higher lactose utilization than *C. pseudotropicalis*. Results in Table (1) and Fig. (2) showed considerable increase in the BOD after 48 and 96 hr, the permeate recorded the highest value (58.1 mg/L) and YM medium achieved the low value (31.3 mg/L). These results agree with Kasiem (2000); Omar (2009) and Meera Babu *et al*. (2014) who found that *Kluyveromyces fraglis* was effective in BOD removal. Reduction in BOD level was obtained by Capoor and Singh (1995), by *S. fraglis* and *K. fraglis*, respectively, while lower value was observed for *C. pseudotropicalis*.

Table 1. Effect of different media on some chemical parameters of *K. fraglis* at optimum condition for 48 and 96 hr.

Media	Parameters							
	pH		Acidity (%)		Consumed lactose (%)		BOD mg/L	
	48 hr	96 hr	48 hr	96 hr	48 hr	96 hr	48 hr	96 hr
YM medium	4.43	5.21	0.11	0.05	12	24	20.5	31.3
Whey	5.75	5.82	0.42	0.20	20	33	45.11	54.2
Permeate	3.75	5.27	0.43	0.12	23	37	55.12	58.1

BOD = Biological oxygen demand.
YM medium : (Yeast – Malt – Pepton – Glucose broth medium)

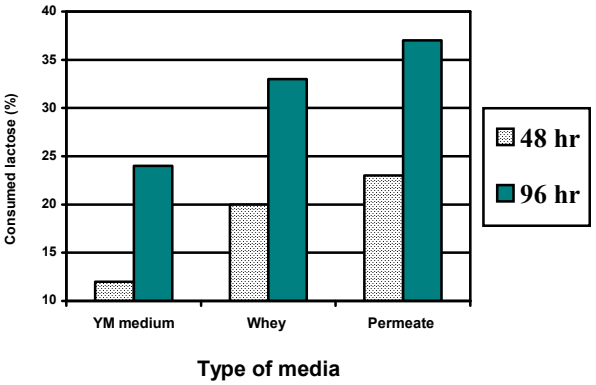


Fig. 1. Effect of different medias on decreased of lactose.

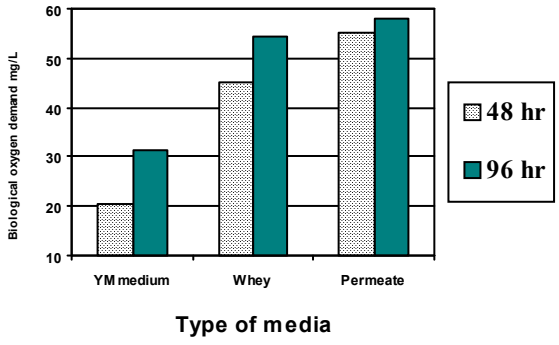


Fig. 2. Effect of different medias on BOD (Biochemical Oxygen Demand).

Table (2) shows that effect of different media on turbidity. It ranged between (0.304) in YM medium and (0.991) in whey after (48 hr). Turbidity increased in all media after 96 hr, permeate recorded the highest value (1.345) while YM medium achieved the lowest value (0.590). In the same Table and Fig. (3) showed that biomass as dry weight increased, it ranged between (0.83 gm/L) in YM medium and (1.9 gm/L) in whey after (48 hr), then increased after 96 hr in all different media. The whey recorded the highest value of 2.5 gm/L after 96 hr, but YM medium achieved the lowest of 1.9. These results agree with Capoor and Singh (1986), who found that max. biomass yield of *Kluyveromyces fraglis* and *Saccharomyces fraglis* in clarified paneer whey after incubation for 72 hr at 30 ^{o}C were 7.93 and 10.70, respectively and Demerdash and Abd EL-Ghany (1998) reported that the highest amount of cellular biomass from whey was achieved by *K. fraglis* (11.6 g/L).

Results in the same Table and Fig. (4) indicated that considerable increase in total viable count after 24 until 72 hr, then it decreased after 96 hr. Whey recorded the highest value (22 x 10^6 C.FU) at 72 hr, while the YM media recorded the lowest value (13 x 10^6) C.FU after 72 hr, this is due to consume the mineral and all nutrients in medium by the strains.

Table 2. Effect of different media on turbidity, biomass and viable count of *Kluyveromyces fraglis* for 48 and 96 hr.

Media	Parameters										
	Turbidity		Biomass g/L Wet weight		Dry weight		Total viable count X 10^6 C.F.U				
	48 hr	96 hr	48 hr	96 hr	48 hr	96 hr	Zero	24 hr	48 hr	72 hr	96 hr
YM medium	0.304	0.590	15.13	22.17	0.83	1.90	6.0	7.0	9.0	13.0	11.0
Whey	0.991	1.213	22.61	35.17	1.90	2.50	5.0	11.0	15.0	22.0	19.0
Permeate	0.309	1.345	18.17	28.60	1.70	2.00	7.0	10.0	12.0	18.0	16.0

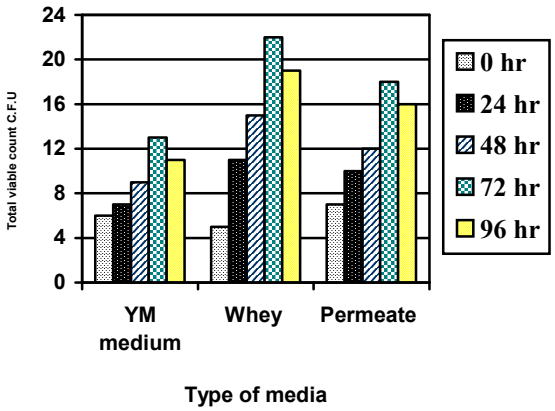


Fig. 3. Effect of different medias on total viable count cfu/ml.

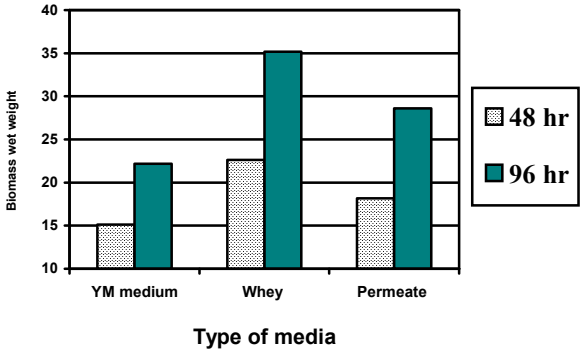


Fig. 4. Effect of different medias on biomass.

Table (3) shows that the acidity ranged between (0.03) in YM medium and (0.37) in whey after 48 hr, and decreased after 96 hr in all different media. The pH run in opposite parallel to acidity. These results due to *K. lactis* produce amount of alchole and this amount of alchole change the acidity. The same table and Fig. (5) shows consumed lactose in different media during the incubation time it ranged between (12 %) in permeate and (17 %) in whey after 48 hr then it increase after 96 hr, permeate recorded the highest value while YM medium achieved the lowest value (22 %), which agreed with Abo-EL-enin (2006). Fig. (6) showed that the BOD increased after 96 hr. It could also be observed the highest value (39.5 %) in permeate, while the lowest was recorded in YM medium (19.3 %) after 96 hr. This result agreed with Kassiem (2000).

Table 3.Effect of different media on some chemical parameters of *Kluyveromyces lactis* at optimum condition for 48 and 96 hr.

Media	Parameters							
	pH		Acidity (%)		Consumed lactose (%)		BOD mg/L	
	48 hr	96 hr	48 hr	96 hr	48 hr	96 hr	48 hr	96 hr
YM medium	5.75	5.73	0.03	0.03	16.0	22.0	13.1	19.3
Whey	4.45	5.35	0.37	0.14	17.0	25.0	28.2	35.4
Permeate	4.46	4.51	0.15	0.13	12.0	30.0	26.1	39.5

BOD = Biological oxygen demand.

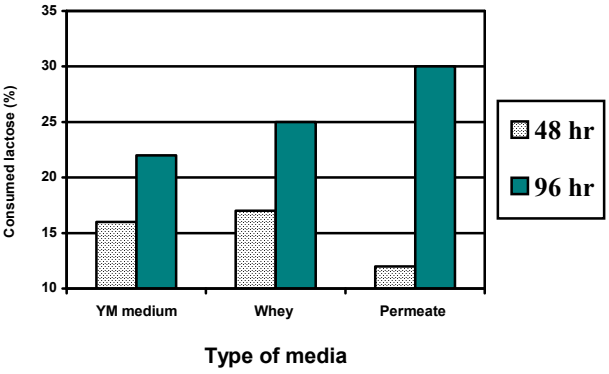


Fig. 5. Effect of different medias on decreased of lactose.

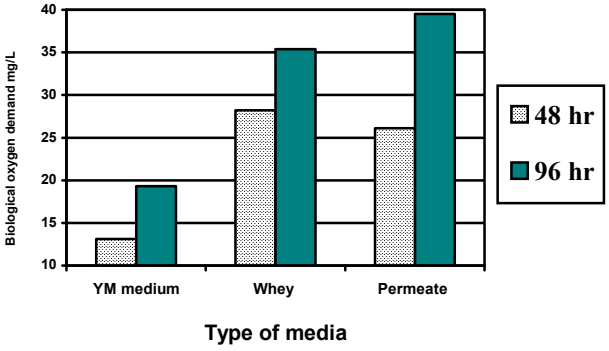


Fig. 6. Effect of different medias on BOD (Biochemical Oxygen Demand).

Table (4) shows that the turbidity ranged between 0.640 in YM medium and 1.012 in whey after 48 hr. It increased in all media after 96 hr as whey recorded the highest value of (1.038), while YM medium achieved the lowest (0.778). The biomass as dry weight ranged between 0.59 gm/L in YM medium and 1.6 gm/L in permeate after 48 hr then it increase after 96 hr in all different media. Whey recorded the highest value (2.10 gm/L) while, YM medium recorded lowest value (1.7 gm/L). These results agree with Murad *et al*. (1992) and Hassan *et al*. (2004). Also, in the same Table it could be indicated that considerable increase in total viable count after 24 hr until 72 hr, then decreased after 96 hr. The whey recorded the highest value (19 x 10^6 CFU), while the YM medium recorded the lowest value (15 x 10^6 CFU).

Table 4. Effect of different media on turbidity, biomass and viable count of *Kluyveromyces lactis* at optimum condition for 48 and 96 hr.

Media	Parameters										
	Turbidity		Biomass g/L Wet weight		Biomass g/L Dry weight		Total viable count X 10^6 C.F.U				
	48 hr	96 hr	48 hr	96 hr	48 hr	96 hr	Zero	24 hr	48 hr	72 hr	96 hr
YM medium	0.640	0.778	9.61	19.80	0.59	1.70	4.0	8.0	12.0	15.0	10.0
Whey	1.012	1.038	12.16	27.31	1.50	2.10	6.0	9.0	11.0	19.0	15.0
Permeate	0.917	1.009	14.81	25.21	1.60	1.90	8.0	11.0	15.0	18.0	17.0

Table (5) shows that (T.A) titratable acidity ranged between (0.02) in YM medium and (0.27) in whey after 48 hr, it decreased after 96 hr in all different media. The pH ran in an opposite trend to acidity. The consumed lactose in different media during the incubation time ranged between (15 %) in whey and (18 %) in YM medium after 48 hr, then it increased after 96 hr (Table 5). YM medium recorded the highest value (27 %) while sweet whey recorded lowest value (23 %). These results agree with Barraduio *et al*. (1981); Singh and Neelakantan (1989) and Compagno *et al*. (1995). The BOD after 48 hr and 96 hr whey recorded the highest value (38.5) mg/L while YM media recorded the low value (33.4) mg/L after 96 hr (Table 5).

Table 5. Effect of different media on some chemical parameters of *Saccharomyces cervesiae* at optimum condition for 48 and 96 hr.

Media	Parameters							
	pH		Acidity (%)		Consumed lactose (%)		BOD mg/L	
	48 hr	96 hr	48 hr	96 hr	48 hr	96 hr	48 hr	96 hr
YM medium	6.09	5.40	0.02	0.08	18.0	27.0	23.1	33.4
Whey	5.90	4.73	0.27	0.35	15.0	23.0	30.2	38.5
Permeate	5.70	5.55	0.11	0.25	16.0	25.0	22.0	34.7

BOD = Biological oxygen demoned.

Table (6) indicated the effect of different media on turbidity. It ranged between (0.305) in permeate and (0.720) in whey after 48 hr and it increased in all media after 96 hr, YM medium recorded the highest value (0.991) and permeate achieved a lowest value after 96 hr. These results were agree with Omar (2009). In the same Table the biomass as dry weight ranged between (1.6 gm/L) in permeate and (2 gm/L) in YM medium after 48 hr then it increase after 96 hr in all different media. The whey recorded the highest value (2.3) gm/L permeate recorded the lowest value (2 gm/L) after 96 hr. These results agree with Barraquio *et al*. (1981) and Compagno *et al*. (1995).

Table 6. Effect of different media on turbidity, biomass and viable count of *S. cervesia* at optimum condition for 48 and 96 hr.

Media	Parameters										
	Turbidity		Biomass g/L Wet weight		Dry weigh t		Total viable count X 10^6 C.F.U				
	48 hr	96 hr	48 hr	96 hr	48 hr	96 hr	Zero	24 hr	48 hr	72 hr	96 hr
YM medium	0.492	0.991	19.5	23.1	2.0	2.2	8.0	10.0	12.0	19.0	15.0
Whey	0.720	0.880	21.5	23.2	1.8	2.3	5.0	7.0	8.0	20.0	15.0
Permeate	0.305	0.479	18.66	20.31	1.6	2.0	7.0	9.0	11.0	17.0	11.0

In same Table we indicated increasing in total viable count after 24 hr until 72 hr then it decreased after 96 hr, the whey recorded the highest value (20 x 10^6 C.F.U) while permeate recorded the low value (17 x 10^6 C.F.U).

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الإستفادة من الشرش وراشح اللبن في إنتاج البروتين الخلوي بإستخدام بعض أنواع الخمائر

محمد شلبى جمعه[1]، متولى محمد أبو سريع [1]، إيمان لبيب مصطفى[2] و دعاء ممدوح فتحى[2]

[1] قسم الألبان – كلية الزراعة – جامعة المنصورة

[2] معهد بحوث الإنتاج الحيوانى - مركز البحوث الزراعية - الجيزة

يـهـدف هـــذا البـحث إلى الإستفـادة من مخلفات صناعة الألبان كالشرش وراشح اللبن الفائق (البرميت) لإنتاج بروتين أحادى الخلية (Single cell protein) والحد من التلوث البيئى الناتج منها وذلك بإســتخــدام 3 سلالات من الخمائر على حدى Kluyveromyces Fraglis, Kluyveromyces lactis or Saccharomyces (Cervesiae)تم تنمية كل هذه السلالات تحت الظروف المثلي لنموها لمده 48& 96 ساعة على الشرش أو البرميت أو بيئة الـYM وسجلـت Kluyveromyces Fraglis أعلـى نمـو (22 x 10 ^{6}) cfu/ml على بيئة الشرش بعد 96 ساعة وأعلـى كـم مـــن biomass (2.5 جم/لتر) على بيئة الشرش بعد 96 ساعة يـليـــه Kluyveromyces lactis ثم Saccharomyces Cervesiae كما أظهرت Kluyveromyces Fraglis أعلى قيمه لإستهلاك اللاكتـــوز (37 %) على بيئة البرميت بعد 96 ساعة والـ BOD (Biochemical oxygen demand) (58.1 ملجم/لتر) على بيئة البرميت بعد 96 ساعة.