# BIOMASS PRODUCTION AND REDUCTION OF POLLUTION FROM WHEY. 1- USE OF LACTIC ACID BACTERIA FOR IMPROVING BIOMASS PRODUCTION BY *Kluyveromyces marxianus var. lactis*.

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## ABSTRACT

*K. marxianus var. lactis* was grown in different whey media (sweet, acid and salted (5%) whey) and permeate and tested for optimum incubation period (24-120 hr) for biomass production. The effect of adding either yoghurt starter or *L.helviticus* (at 2% rate) was also investigated. The optimum pH (3,4,5,6&7), type of supplement to enhance biomass production and the ability of the growth in salted why (0, 3, 5, 7, & 9%) in the presence of lactic acid bacteria was also studied.

The best biomass production by *K.marxianus var. lactis* was in sweet whey at incubation period of 96 hr in the presence of *L.helviticus* (2%) and yeast extract (0.2%) at pH 4 to 5. It also caused a high reduction in lactose content and BOD of whey. The biomass production decreased as the salt concentration increased, but in the presence of *L.helviticus* salt tolerance increased and biomass production increased by 14-35% in the presence of up to 9% salt.

## INTRODUCTION

In the dairy industry of today utilization or disposal of whey and/or permeate represents a problem of paramount importance as a source of pollution to water streams and sewage treatment plants. The Biochemical Oxygen Demand (BOD) of whey is extremely heavy and falls in the range of 30.000-60.000 mg/l (Kisaalita *et al.*., 1987). Another problem in whey disposal is the loss of a rather valuable product which contains about 42-44% of milk total solids. Worldwide production of whey was in the region of 130 million tons in 1992 with 3% increase per year (Zall, 1992) while in Egypt it was reported to be about 0.5-1 million ton per year (Zaid, 1997 and El-Gindy, 1997).

Although adequate technologies are available to recover fat and protein components in whey, the lactose portion (4.5 to 5% which represents about 70% of T.S in whey and 80to 85% in permeate) remains mostly unused and is often subjected to a costly waste treatment process. As an alternative, lactose can be converted to many products such as alcohol (Whalen and shahani, 1985), methane (Murthy *et al.*., 1993), single cell protein (Sandhu and Wariach, 1983), organic acids (Rekha and Gandhi, 1995), amino acids (Ko and Chipley, 1983), enzymes (Bales and Castillo, 1979), polysaccharides (Gassem *et al.*., 1997) and flavour compounds (Nieswandt, 1995).

Single cell protein was attained by the use of several types of lactose fermenting yeasts. (Barraquio *et al.,* 1981 and Capoor and Singh, 1985 & 1986*)* However, the conditions for optimizing biomass production by several types of yeasts and its effect on lactose utilization and BOD reduction is still in need for further investigations. In addition, the idea of improving biomass production through the use of some lactic acid bacteria Known for their symbiotic relationships among themselves has not been proposed. Furthermore, the use of some nutrients mainly nitrogenous compounds would also have an effect on biomass production.

Anthoer main problem in Egypt is the production of vast amounts of whey with variable amounts of salt (5-10%) as a result of the production of Domiati cheese and to investigate the effect of optimizing the conditions of growth for the yeast used on its ability to utilize lactose in salted whey would also be of great importance.

Therefore, the aim this study was to use some lactic acid bacteria in a trial to improve biomass production from whey by *K. marxianus var. lacts* and to investigate its effect on the ability to utilize lactose in salted whey.

## MATERIALS AND METHODS

**I- Materials:**

Fresh cow's skim milk was obtained from the Dairy Department, Faculty of Agriculture, Cairo University.

Pure lyophilized cultures of *Lactobacillus helviticus* and yoghurt starter (*Str. salivarius subsp. thermophilus* + *L. delbreuckii subsp. bulgariucs*, 1:1) were obtained from Chr. Hansens' Laboratories, Copenhagen, Denmark.

*Kluyvoromyces marxianus var. lactis* NRRL 1137 [formerly *K. lactis* (Law, 1997)] was obtained from Northern Regional Research Laboratory Peoria, Illinois, USA.

Permeate was obtained from Milk Technology Processing Unit, Animal Production Res. Inst., Ministry of Agric., Cairo, Egypt.

**II- Experimental procedure:**

**1- Preparation of whey media:**

Fresh cow's skim milk was heated to 80°C, cooled to 37°C and then renneted to obtain sweet whey or cooled to 42° C and then inoculated with 2% active yoghurt culture to obtain acid whey. After complete coagulation, cured was cut, then filtered through muslin cloth. Fine grade salt was added to sweet whey to obtain salted whey.

**2- Preparation of cultures:**

L. helviticus and yoghurt starter cultures were activated in sterile reconstituted skim milk (11% TS) for 16 hr at 37oC. K. marxianus var. lactis was maintained on potato dextrose agar slants (APHA, 1992). After good growth (at 30°C for 48h) the yeast culture was inoculated into sterilized malt glucose extract peptone broth (Cappor & Singh, 1985) and incubated for 48 h at 30°C. All cultures were activated four times before use.

**3- Fermentation conditions:**

All experiments were conducted in 500 ml capacity conical flasks contained 100ml of the fermentation medium. The media were heated at 75°C for about 3 min and cooled to 30°C. Active yeast culture of *K. marxianus var. lactis* was inoculated into the flasks at 7% level as suggested by Murad *et al.* (1992) and mixed well. All samples were incubated at 30°C in a shaker water bath at 120 rpm.

**4- Effect of whey media and incubation period:**

Four whey media namely sweet, acid and salted whey (5%) and permeate were tested daily over an incubation period of 5 days for titratable acidity (TA), ptt and biomass production while lactose and BOD were estimated before and after 4 days of incubation.

**5- Effect of addition of some lactic acid bacteria:**

The previous four whey media were each divided into three parts. The fist part was used as a control while to the second and third parts 2% of active yoghurt starter and *L.helviticus* cultures were added respectively.

**6- Effect of pH:**

Three 500 ml portions of sweet whey were inoculated with *K. marxianus var. lactis*, the first acted as a control while to the second and third yoghurt culture and *L.helviticus* were added at 2% rate respectively. Each portion was then derided aseptically divided into 100 ml portion in 5 flasks and the pH of each was maintained at 3, 4, 5, 6 & 7 ± 0.2 manually using 3N $NH_4OH$ or 3 N lacite acid through 4 days of incubation for the 5 flasks respectively.

**7- Effect of addition of some nutrient supplements:**

Some organic sources (peptone, malt extract and yeast extract) and inorganic sources [$KH_2PO_4$, $K_2HPO_4$) and ($NH_4$) SO4] were each added at 0.2% level to sweet whey medium containing 2% *L.helviticus* at pH 4-5 and incubated for 4 days.

**8-Salt tolerance of *K. marxianus var lactis* in the presence of starter culture:**

Salt was added in various concentrations (0, 3, 5, 7 & 9%) to sweet whey and each was divided aseptically into 3 portions, the first was inoculated with *K. marxianus var lactis* alone while the second and third were inoculated with yoghurt starter and *L.helviticus* besides *K. marxianus var lactis* respectively.

**III- Methods of analysis:**

Whey samples before and after fermentation were analyzed for pH and titratable acidity (T.A) by the technique described by Ling (1963). Lactose was enzymatically determined as a disaccharide by the method described by Nickerson *et al.* (1976). Biochemical Oxygen Demand (BOD) was determined using the method reported by APHA (1998 a & b).

Biomss production was estimated by centrifuging 25 ml of the fermented whey medium 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). The total solids of the obtained biomass was measured by the method of Murad *et al.* (1992). Total carbohydrate was determined by the method suggested by Abdel-Fattah and Hussein (1970). The protein content was estimated by kjeldahl's method (calculated as N × 6.25) as proposed by Capoor and Singh (1985). Amino acids were determined according to the method of Moore *et al.* (1958) using amino acid autoanalyzer model Beckman 118 CL. Minerals were estimated by the method recommended by AOAC (1995) using Atomic absorption spectrophotometer model Perkin Elmer 2380.

Biomass yield was calculated as the dry yeast biomass (g) divided by the consumed lactose (g). Biomass productivity was calculated as the production of dry yeast biomass per hour.

Data were statistically analyzed as suggested by Steel and Torrie (1990).

Experiments were repeated in triplicates and each analysis in duplicates and average results were tabulated.

## RESULTS AND DISCUSSION

**Growth *K. marxianus var. lactis* in different whey media:**

Data presented in Fig. (1) indicate that after one day of fermentation the TA increased in all samples at a variable rate with the increase being at a high rate in sweet whey followed by permeate, acid whey and salted whey in a descending order. After 2 days and until the end of fermentation the TA increased slightly or fluctuated with acid whey having highest values while salted whey having lowest values. It is worth mentioning that the yeast (*K.marxianus var. lactis*) utilizes lactose as well as lactic acid and the available figures represent the difference between lactic acid formation and its dissimulation. The pH ran parallel to TA.

Regarding biomass production results in Fig. (2) show that there was a continuous increase in the biomass as the fermentation progressed to reach maximum values after 4 days and then followed by a small decrease. This decrease might be due to a higher rate of death of *K. marxianus var. lactis* than growth after 4 days. These results are in agreement with Murad *et al.* (1992) who reported an optimum biomass production by *K.lactis* after 96 hr. On the other hand, Fadel and Degheidi (1998) reported an optimum incubation period of 48 hr using *K. fragilis*, while Giec and Kosikowski (1982) and El – Samragy *et al.* (1988) reported longer incubation period (5 days) for biomass production from different types of yeast.

It might be also observed from the same results that the highest biomass yield was from sweet whey followed by permeate, acid whey and salted whey in a descending order. The lower yield from acid whey might be due to the high acidity which might retard lactic acid respiration as suggested

by Champagne *et al.* (1990), while the lower yield from salted whey might be due to the inhibitory effect of the available salt on the growth of *K. lactis*.

As for lactose utilization, results in Table (1) show that there was a considerable reduction in the amount of lactose in the different types of whey after fermentation. However, the differences were small between sweet and acid whey as well as permeate while in salted whey the rate of lactose utilization was significantly lower. This is obviously due to the effect of salt in reducing the growth of *K. marxianus var. lactis* and consequently lactose utilization. Slightly higher rates of lactose utilization were reported for *S.fragilis* and *K.fragilis* (Capoor and singh, 1995 & 1986) and *K.fragilis* (Giec and Kosikowski, 1982), while much lower figures were reported for *K.lactis* NRRLY 1118 (Giec and kosikowski, 1982) and *C. pseudotropicalis* (Capoor and Singh, 1985 & 1986).

**Table (1): Effect of growth media in lactose utilization and BOD reduction by *K.marxianus var. lactis.***

| Growth whey media | Initial lactose, % | Final lactose, % | Lactose utilization, % | Initial BOD mg/l | Final BOD mg/l | BOD reduction ,% |
|---|---|---|---|---|---|---|
| Sweet | 4.8 | 0.70 | 85.4 [a] | 73000 | 22150 | 69.4 [a] |
| Acid | 4.5 | 0.68 | 84.9 [b] | 70000 | 19000 | 72.9 [b] |
| Salted | 4.9 | 0.97 | 80.2 [c] | 73000 | 28650 | 60.8 [c] |
| Permeate | 5.0 | 0.73 | 85.4 [a] | 74000 | 24500 | 66.9 [d] |

***Growth period = 96 hr.**

*** Different superscripts at the same column are significantly different (p<0.05).**

Regarding BOD reduction, data in Table (1) indicate a considerable decrease in BOD following the fermentation of the various types of whey with the rate of reduction being highest and lowest in acid and salted whey respectively. These results are partly in agreement with Capoor and Singh (1985) who reported a rate of BOD reduction of 74.6 and 73.2% for *S.fragilis* and *K.fragilis* 3217 while lower figure (33.9%) was observed for *C.pseudotropicalis.*

**Effect of adding some starter cultures:**

The effect of adding yoghurt starter and *L.helviticus* on growth of *K.marxianus var. lactis* in different whey media and permeate are presented in Table (2). After incubation for 4 days the pH of all samples dropped although at a variable rate. Addition of yoghurt starter to all samples caused a further decrease in pH while *L.helviticus* was even more efficient. The final pH was lowest in acid whey followed by sweet whey, permeate and salted whey in an ascending order.

Regarding the biomass production, it is obvious that addition of both starters cultures casued a significant increase in biomass in all media used with *L.helviticus* being more efficient than yoghurt starter in this respect. This might be due to the effect of adding these starters on aiding the break down of lactose, production of lactic acid and production of some minute amounts of amino acids or peptides which encourage biomass production. In this respect Champagne *et al.* (1990) used *S.thermophilus*, *L.helviticus* or *L.bulgaricus* to preferment lactose in whey for the production of baker's yeast (*Saccharomyces cerevisiae*).

**Table (2): Effect of adding some starters on growth of *K. marxianus var. lactis* in different whey media and permeate.**

| Parameters | Whey media | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sweet | | | Acid | | | Salted | | | Permeate | | |
| | K.I[(1)] | K.I +Y[(2)] | K.I +H[(3)] | K.I | K.I +Y | K.I +H | K.I | K.I +Y | K.I +H | K.I | K.I +Y | K.I +H |
| Initial PH | 6.06 | 6.06 | 6.06 | 6.05 | 6.05 | 6.05 | 4.20 | 4.20 | 4.20 | 6.2 | 6.2 | 6.2 |
| Final pH | 4.18 | 4.30 | 4.50 | 4.7 | 4.85 | 5.06 | 3.5 | 3.52 | 3.94 | 3.95 | 4.1 | 4.3 |
| Biomass, g/l | 6.55 $^{k}$ | 6.22 $^{f}$ | 5.31 $^{di}$ | 5.15 $^{di}$ | 4.59 $^{h}$ | 3.92 $^{g}$ | 6.3 $^{f}$ | 5.9 $^{ae}$ | 5.2 $^{d}$ | 7.27 $^{c}$ | 6.73 $^{b}$ | 5.82 $^{a}$ |
| Initial lactose , % | 5.0 | 5.0 | 5.0 | 4.8 | 4.8 | 4.8 | 4.5 | 4.5 | 4.5 | 4.73 | 4.73 | 4.73 |
| Final lactose, % | 0.73 | 0.76 | 0.78 | 0.77 | 0.81 | 0.94 | 0.56 | 0.63 | 0.69 | 0.66 | 0.72 | 0.75 |
| Lactose utilization, % | 85.4 $^{h}$ | 84.8 $^{b}$ | 84.4 $^{g}$ | 84.0 $^{a}$ | 83.1 $^{f}$ | 80.4 $^{e}$ | 87.6 $^{d}$ | 86.0 $^{c}$ | 84.7 $^{b}$ | 86.0 $^{c}$ | 84.8 $^{b}$ | 84.1 $^{a}$ |
| Initial BOD , mg/l | 72000 | 72000 | 72000 | 70000 | 70000 | 70000 | 68000 | 68000 | 68000 | 70000 | 70000 | 70000 |
| Final BOD , mg/l | 22500 | 23000 | 24000 | 23500 | 25000 | 27333 | 18500 | 19000 | 20500 | 18833 | 20666 | 21950 |
| BOD reduction % | 68.8 $^{L}$ | 68.1 $^{k}$ | 66.7 $^{j}$ | 68.4 $^{i}$ | 64.3 $^{h}$ | 61.0 $^{g}$ | 72.8 $^{f}$ | 72.1 $^{e}$ | 69.9 $^{d}$ | 73.1 $^{c}$ | 70.5 $^{b}$ | 68.6 $^{a}$ |

**(1) K.I = *K. marxianus var. lactis*. (2) Y = Yoghurt starter. (3) H = *L.helviticus*.**
*** Different superscripts at the same raw are significantly different (p<0.05).**

As for lactose utilization and BOD reduction, data in Table (2) show that there was a significant increase in the rate of lactose utilization and BOD reduction as a result of adding of either starter cultures with *L.helviticus* having higher rate than yoghurt starter and with salted whey having generally lower values than corresponding other samples. It might be also noticed that the rate of increase in lactose utilization and BOD reduction as a result of addition of *L.helviticus* was highest in salted whey which coincides with the increase in biomass production. This might be due to the symbiotic effect of *L.helviticus*, which enabled *K. marxianus var. lactis* to withstand the unfavorable conditions of presence of salt in the whey.

**Effect of pH:**

As can be observed from results in Fig. (3) the biomass production, lactose utilization and BOD reduction of the control in the presence of yoghurt starter increased by the increase in pH up to pH 5 and then decreased. However, in the presence of *L.helviticus* maximum values were obtained between pH 4 & 5 with these values being higher in *L.helviticus* samples followed by yoghurt and finally by the control, in a descending order. This is most probably due to the symbiotic effect of the starter present which enables the yeast to withstand unfavorable low or high pH values. These results are partly in agreement with Mahmoud and Kosikowski (1982), Shay and Wegner (1986), Fadel (1998) and Murad *et al.* (1992) who were working with *k.fragilis* while Zayed (1991) reported a pH between 4-6 for *K.lactis*, although all workers only adjusted the initial pH.

**Effect of nutrient supplements:**

From results presented in Table (3), it might be observed that biomass production, lactose utilization and BOD reduction increased considerably by the addition of organic sources particularly upon supplementation with yeast

extract followed by malt extract and peptone. On the other hand, supplementation with inorganic sources either nitrogenous or phosphorus was almost ineffective as the difference was insignificant between them and the control.

The increase in growth parameters as a result of supplementation with yeast extract and peptone might be due to their contents of various amino acids, phosphate and other growth factors as they contain 10.5 and 14.3% total nitrogen, 4.8 and 2.3% amino nitrogen and 3.2 and 1.2% P2O5 for yeast extract and peptone respectively while that due to the presence of malt extract might be due to its high contents of sugars (52.2% maltose, 19.1% dextrose, 15% dextrin and 1.8% sucrose) and protein (4.6%) (Oxiod®, 1982).

**Table (3): Effect of supplementing whey with nutrients on biomass, lactose utilization and BOD reduction by *K.marxianus var. lactis* in the presence of *L.helviticus*.**

| Growth factors | Biomass, g/l | Final lactose, % | Final BOD, mg/l | Lactose utilization, % | BOD Reduction, % |
|---|---|---|---|---|---|
| - | 7.8 $^{a}$ | 0.7 | 22000 | 85.7 $^{a}$ | 69.9 $^{a}$ |
| $KH_2PO_4$ | 7.9 $^{a}$ | 0.7 | 22500 | 85.7 $^{a}$ | 69.2 $^{b}$ |
| $K_2HPO_4$ | 8.1 $^{a}$ | 0.68 | 22000 | 86.1 $^{bd}$ | 69.9 $^{a}$ |
| $(NH_4)_2SO_4$ | 8.0 $^{a}$ | 0.7 | 22000 | 85.7 $^{ad}$ | 69.9 $^{a}$ |
| Peptone | 8.5 $^{ac}$ | 0.55 | 20500 | 88.8 $^{c}$ | 71.9 $^{c}$ |
| Yeast extract | 9.2 $^{b}$ | 0.45 | 18000 | 90.8 $^{e}$ | 75.3 $^{d}$ |
| Malt extract | 8.9 $^{bc}$ | 0.5 | 19000 | 89.8 $^{f}$ | 74.0 $^{e}$ |

*** Initial lactose = 4.9 %.**
*** Initial BOD = 73000 mg/l.**
*** Growth conditions: 96 hr, at pH 4-5.**
*** Different superscripts at the same column are significantly different (p<0.05).**

The positive effect of organic sources particularly yeast extract was previously reported by Youssef (1980) and Murad *et al.* (1992) while the inefficient effect of inorganic supplementation was reported by Mahmoud and Kosikowski (1982) and Capoor and Singh (1986). On the other hand, Murad *et al.* (1992) mentioned that supplementation with ammonium sulphate gave high biomass yield.

**Salt tolerance of *k.marxianus var. lactis* grown in salted whey.**

From results presented in Table (4) & Figs. (4&5) it is noticeable that the biomass production decreased as the salt concentration increased through out an incubation period of 4 day with *L.helviticus* samples in each concentration of salt having highest amounts followed by yoghurt culture and lastly by the corresponding control. This decrease was at a slow rate in the presence of up to 5% salt. However, as the salt concentration, increased to 7% the rate of biomass production decreased at a higher rate to reach relative value to the control of 70.4 to 72.8%. As the salt concentration increased to 9% biomass production dropeed even further and relative value to the control reached between 39.4 and 46.9%. This suggest that if *k.marxianus var. lactis* is to be used, salt level have to be reduced to about 5% possibly by dilution as previously suggested by El Nimer *et al.* (1982).

These results are partly in agreement with Demeradash and Abd-El-Ghany (1998) who observed a relative growth of 90-94 % in the presence of 2% salt by few yeast isolates while in 6% salt *kluyveromyces sp.* relative growth dropped to 32.4% while the others had values ranging from 80 to 88%. At 10% salt, *C.kuresi* was the only salt tolerant with relative growth of 67.5% while the others ranged from 3.7 to 29.4% relative growth.

Regarding the growth kinetic parameters at different salt concentrations data in Table (4) show that both biomass yield (g/g) and biomass productivity (g/l/h) decreased as the salt concentration increased. The rate of decrease reached between 35.3 & 37.7% and between 52.38 & 60.81% in the presence of 9% salt for biomass yield and biomass productivity respectively. Higher rates of decrease reaching 48.3 and 79.5% for biomass yield and biomass productivity in the presence of 10% salt was reported by Demerdash and Abd-El-Ghany (1998) for *K.marxianus* which suggests that this strain was more salt tolerant.

As for lactose utilization and BOD reduction, Data in Table (4) show that ran parrallel to biomass production. They also decreased with the increase in salt concentration with the rate being slow in the presence of up to 5% salt and at a higher rate in samples containing up to 9% salt. Lactose utilization and BOD reduction reached between 52.1 to 66.7% and between 36.2 & 44.9% respectively in the presence of 9% salt. These results for lactose utilization are higher than those reported by Demerdash and Abd-El-Gahny (1998) for *K.marxianus* (the most salt tolerant strain isolated) as lactose utilization was 34.1% in the presence of 10% salt. This might be due to the strain used and /or the presence of starters and other optimal conditions selected for growth.

It might be also observed from the same results that the rate of lactose utilization and BOD reduction at each level of salt concentration was highest in the presence of *L.helviticus* followed by yoghurt starter and lastly by the control in a descending order. The relative improvement as a result of the presence of the starter increased as the salt concentration increased and reached in the presence of 9% salt 15.93 & 28% for lactose utilization and 12.15 & 24.03% for BOD reduction for yoghurt and *L.helviticus* cultures respectively. This suggests the symbiotic relationship between the added starter particularly *L.helviticus* and yeast strain used.

**Evaluation of the produced biomass:**

Results in Table (5) show some of the composition of biomass produced by *K.marxianus var. lactis* grown in sweet whey with 2% *L.helviticus*, 0.2% yeast extract at pH 4 - 5. The protein and carbohydrate contents of biomass were 44.7 and 36.3%. The results on protein content are in agreement with Barrquio *et al.* (1981) on *S.fragilis*, Sandula *et al.* (1984) on *T.candida* and Michel *et al.* (1987) on *Candida* LY49. The detected minerals included iron which represented the major type of minerals while copper, chromium and cobalt were also detected.

**Table (5): Composition of *K.marxianus var. lactis* grown under the optimum conditions.**

| Component | Concentration |
|---|---|
| Carbohydrate, % | 36.3 |
| Protein, % | 44.7 |
| Minerals, μ g/g | |
| Iron | 10.24 |
| Copper | 1.84 |
| Chromium | 1.00 |
| Cobalt | 0.32 |

- **Lead, Cadmium, Nickel and Tin were not detected.**
- **All values expressed on a solids basis**

Table (6) show the amino acid composition of *K.marxianus var. lactis* grown under optimum conditions. The acid hydrolyzate of the yeast biomass revealed the presence of 17 amino acids. Glutamic acid, aspartic acid, cystine and lysine were present in high levels. These results are partly in agreement with Murad *et al.* (1992) who found high levels of glutamic acid, aspartic acid, leucine and lysine in biomass produced from *K. lactis* NRRL 1137.

**Table (6): Amino acid profile of *K.marxianus var. lactis* grown under the optimum conditions.**

| Amino acid | Concentration, % | Amino acid | Concentration, % | Amino acid | Concentration, % |
|---|---|---|---|---|---|
| Aspartic | 23.3 | Therionine | 0.6 | Cystine | 21.3 |
| Glutamic | 24.6 | Alanine | 0.02 | Isoleucine | 0.02 |
| Serine | 1.2 | Proline | 0.2 | Leucine | 0.33 |
| Glycine | 0.3 | Tyrosine | 0.05 | Phenylalanine | 2.16 |
| Histedine | 6.04 | Valine | 1.54 | Lysine | 16.4 |
| Arginine | 1.29 | Methionine | 0.85 | | |

On the other hand, alanine and isoluecine showed the lowest level of all detected amino acids followed by tyrosine. These variations in amino acids composition of yeast biomass could be due to variation of strains and / or growing condition as suggested by Bressani (1968).

It is worth mentioning that most cereals are very low in lysine (one of the essential amino acids) (Murad *et al.*, 1992) and consequently biomass produced from *K.marxianus var. lactis* represents a good potential as supplement to cereal diet.

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## انتاج الكتلة الحيوية وتقليل التلوث البيئى من الشرش .

## 1- استخدام بكتريا حامض اللاكتيك فى تحسين إنتاج الكتلة الحيوية بواسطة *Kluyveromyces marxianus var. lactis*.

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*** قسم تكنولوجيا الاغذية والتغذية – المركز القومى للبحوث – الدقى**

فى هذا البحث تم تنمية الخميرة .*K. marxianus var. lactis* لإنتاج الكتلة الحيوية فى أنواع مختلفة من الشرش تشمل الشرش الحلو والحامضى والمملح (5% ملح) وراشح الترشيح الفوقى واختبرت لمدة التحضين المثلى (120-24 ساعة) ودرس تأثير إضافة كل من بادئ الزبادى ، *L. helviticus* بنسبة 2% على إنتاج الكتلة الحيوية بالإضافة إلى اختيار درجة الـ pH المثلى (3،4،5،6،7) وبعض المواد المغذية التى تشجع إنتاج الكتلة الحيوية. كما درست قابلية هذه الخميرة للنمو فى الشرش المملح بالنسب (0،3،5،7،9%) .

وكانت أهم النتائج المتحصل عليها :

كان افضل إنتاج للكتلة الحيوية بواسطة الخميرة .*K. marxianus var. lactis* فى الشرش الحلو خلال مدة تحضين 96ساعة فى وجود بادئ *L. helviticus* بنسبة 2% ومستخلص الخميرة بنسبة 0.2 % على درجة pH 4 – 5 . وتحت هذه الظروف المثلى من النمو زاد انخفاض محتوى الشرش من اللاكتوز وكذلك الـ BOD .

انخفض إنتاج الكتلة الحيوية بزيادة تركيز الملح ولكن فى وجود بادئ *L. helviticus* زادت مقدرة الخميرة على تحمل الملح كما زاد إنتاج الكتلة الحيوية بنسبة 14-35% وذلك فى نسبة ملح 9% .

**Table (4): Effect of salt concentration on growth of *K.marxianus var. lactis.***

| | Slat concentration (%) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Parameters** | **0** | | | **3** | | | **5** | | | **7** | | | **9** | | |
| | **K.l** | **K.l+Y** | **K.l+H** | **K.l** | **K.l+Y** | **K.l+H** | **K.l** | **K.l+Y** | **K.l+H** | **K.l** | **K.l+Y** | **K.l+H** | **K.l** | **K.l+Y** | **K.l+H** |
| Biomass, x(g/l) | 7.1[a] | 7.6[b] | 8.1[c] | 6.9[d] | 7.4[e] | 7.9[f] | 6.1[g] | 6.6[h] | 7.1[i] | 5.0[j] | 5.4[k] | 5.9[l] | 2.8[m] | 3.4[n] | 3.8[o] |
| Relative value of x,% | 100 | 100 | 100 | 97.2 | 97.4 | 97.5 | 85.9 | 86.8 | 87.7 | 70.4 | 71.1 | 72.8 | 39.4 | 44.7 | 46.9 |
| Biomass yield, Y(x/s)(g/g) | 0.173[a] | 0.181[b] | 0.191[c] | 0.170[d] | 0.176[e] | 0.189[f] | 0.156[g] | 0.165[h] | 0.177[i] | 0.139[j] | 0.144[k] | 0.153[l] | 0.112[m] | 0.117[n] | 0.119[o] |
| Relative biomass yield,% | 100 | 100 | 100 | 98.27 | 97.24 | 98.95 | 90.2 | 91.16 | 92.7 | 80.4 | 79.6 | 80.1 | 64.7 | 64.64 | 62.3 |
| Biomass productivity, rx (g/l/h) | 0.074[a] | 0.079[b] | 0.084[c] | 0.072[d] | 0.076[e] | 0.082[f] | 0.064[g] | 0.069[h] | 0.074[i] | 0.052[j] | 0.056[k] | 0.061[l] | 0.029[m] | 0.035[n] | 0.040[o] |
| Relative biomass productivity | 100 | 100 | 100 | 97.3 | 96.21 | 97.62 | 86.5 | 87.34 | 88.1 | 70.27 | 70.89 | 72.62 | 39.19 | 44.3 | 49.62 |
| Final lactose,% | 0.69 | 0.61 | 0.56 | 0.73 | 0.65 | 0.62 | 0.89 | 0.81 | 0.78 | 1.21 | 1.05 | 0.95 | 2.3 | 1.9 | 1.6 |
| Lactose utilization, % | 85.6[a] | 87.3[b] | 88.3[c] | 84.8[d] | 86.5[e] | 87.1[f] | 81.5[g] | 83.1[h] | 83.8[i] | 74.8[j] | 78.1[k] | 80.2[l] | 52.1[m] | 60.4[n] | 66.7[o] |
| Relative lactose utilization to K.I | 0 | 1.98 | 3.15 | 0 | 2.00 | 2.71 | 0 | 1.96 | 2.82 | 0 | 4.41 | 7.23 | 0 | 15.93 | 28.0 |
| Final BOD, mg/l | 23000 | 20000 | 18000 | 24000 | 21500 | 19500 | 27500 | 25000 | 23500 | 35000 | 31500 | 29000 | 44000 | 41000 | 38000 |
| BOD reduction , | 66.7[a] | 71.0[b] | 73.9[c] | 65.2[d] | 86.8[e] | 71.7[f] | 60.1[g] | 63.8[h] | 65.9[i] | 49.3[j] | 54.3[k] | 58.0[l] | 36.2[m] | 40.6[n] | 44.9[o] |

| % | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Relative BOD reduction to K.I | 0 | 6.45 | 10.97 | 0 | 5.52 | 9.97 | 0 | 6.16 | 9.65 | 0 | 10.14 | 17.65 | 0 | 12.15 | 24.0 |

**Cultivation time = 96 hrs**
**Initial lactose = 48 g/l.**
**pH= 4-5.**
**Added yeast extract at 0.2%.**
**K.I = *K.marxianus var. lactis*  Y = Yoghurt starterH = *L.helviticus***
**Different superscripts at the same raw are significantly different (p<0.05)**