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COD REMOVAL OF WHEY WITH MIXED YEAST CULTURE AND IMPROVEMENT OF BIOMASS YIELD

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Abstract

The aim of the research is to improve the yield of the biomass and to remove the organic matters of the whey by using a mixed culture of (*Saccharomyces cerevisiae+ Kluyveromyces lactis*) yeast at rate 1/1.

The optimal conditions of growth of the mixed culture of the (*Saccharomyces cervisiae+ Kluyveromyces lactis*) have been identified on a Rider medium and they have been as follows : pH : 5.5 , air flow : 0.5 liter air /liter whey /min, and temperature: 30 C°

The aerobic biological treatment technique of the whey has been achieved by using the mixed culture of (*Saccharomyces cervisiae+ Kluyveromyces lactis*) yeast at rate 1% and at the optimal and specified conditions of growth , So that the value of the COD can be reduced from 65000 mg / L to 514 mg / L with COD removal efficiency of 99.21% , and the forming biomass 3.53% .

Keywords : aerobic biological treatment ; COD, mixed culture; SCP; whey , Kluyveromyces lactis yeast.

1. Introduction

The whey is considered one of the most important liquid garbage produced from food factories (diary plants) because of its high content of organic matters that require very high Chemical Oxygen Demand which is about (60000-70000) mg/ L [1] , and which causes an environmental damage in the treatment stations of sewage discharged directly to industrial sewage , made it clear that the pollution resulting from (50.000) liter of this whey equals to the pollution resulting from 25.000 of inhabitants [2-3]

The international production of whey is very large , and in direct relation with the production of dairy products. It is about 170 million yearly in Syria, or 1620.000 ton according to 2009 statistics [4].

The treatment of the whey in the different bio-ways (aerobic and anaerobic) [5-6] has a great economic importance according to the following:

- Increasing the economic value of the whey by transforming it to different substances that have great importance in the national economy such as ethanol, organic acids, enzymes, vitamins, organic solvents, lipids, antibiotics and microbial proteins (used as nutritious and food additives) .
- Protecting the environment from pollution by the treatment of the whey in integrated technology without leaving any industrial waste.
- Reusing the water resulting from the treatment in different fields.

2. Experiments

We can conclude the research in four basic works:

- Preparing the necessary mixed culture of (*Saccharomyces cerevisiae + Kluyveromyces lactis*) yeast used in the aerobic biotreatment of cheese whey .
- Identifying optimal conditions of growth of the mixed culture of (*Saccharomyces cerevisiae + Kluyveromyces lactis*) on a Rider medium .
- Using the mixed culture of the (*Saccharomyces cerevisiae, Kluyveromyces lactis*) yeast according to previous growth conditions in the aerobic bio treatment of the whey and identifying the reduction rate of the COD.

- Using the *Kluyveromyces lactis* yeast as culture according to the specified treatment conditions in the treatment of whey and identifying the reduction rate of the COD.

First: Ways of Preparing the primary mixed culture of (*Saccharomyces cerevisiae* + *kluyveromyces lactise*) yeast used in the aerobic biotreatment of the whey.

The primary mixed culture is a lactic yeast of the kluyveromyces lactis type, (*K. lactis 42*) strain was brought from the physiological and microbiological institute of the Ukranian National Science Academy, with a bread yeast of the *Saccharomyces cerevisiae* type brought from the sugar factory in Homs Syria at rate 1 /1.

Lactose agar medium was prepared and sterilized for the growth of pure breed *(K. lactis 42*) and the saccharoze agar for the growth of Saccharomyces cerevisiae [7]. The mixture was put in sterilized laboratory glass inclined tubes. And shown in Table (1).

In sterilized atmosphere in the incubation room of the laboratory, every yeast was incubated on suitable nutrient agar medium existing in previous tubes and then put in incubation at 30C° for 24 hours. Enough for the yeast to be grown.

Second: Identifying the optimal conditions of the growth of the mixed culture of (*Saccharomyces cerevisiae* + *Kluyveromyces lactis*) yeast on a Rider medium.

The most important optimal conditions for the growth of mixed culture have been specified as follows :

The temperature, pH and the relative speed of air flows, for obtaining the suitable biomass through incubation on sterilized Rider medium[7], the components of this medium are shown in the table (2) .

The amounts of the above list have been dissolved in a liter of distilled water and sterilized in the autoclave for 20 minutes at 121°C, then the yeast grown on Agric mediums incubated on this medium after cooling down to (28-30)°C.

Table 1 The components of the mixture of the nutritive agar with lactose or saccharose [7]

lactose or saccharose	30 g
$NaNO_3$	2 g
K_2HPO_4	1 g
$MgSO_4.7H_2O$	0.5 g
NaCl	0.5 g
$CaCO_3$	3 g
Agar	20 g
Distillated water	1000 ml

Table 2 The compounds of Rider medium[7]

lactose at rate 1/1 + saccharose	40 g
Ca $NO_3)_2$	0.04 g
K_2HPO_4	1 g
$MgSO_4$.7.H2O	0.7 g
NaCl	0.5 g
$(NH_4)_2SO_4$	3g
NH_2CONH_2	g1
Extract yeast	g2
Distillated water	1000 ml

2.1 Determining the growth temperature

To determine the temperature that provides maximum relative speed for the growth of the mixed culture, continuous growing processes of yeast on a Rider medium , containing 4.0 % (lactose + saccharose) at rate 1 / 1 at different temperatures within the field of (20-38) °C and fixed pH: 5.5 , have been achieved.

The relative speed for the growth under the conditions of the growing process were calculated according to the equation [8]:

$$\mu = \frac{2.3(\lg \chi_2 - \lg \chi_1)}{t_2 - t_1}, h^{-1}$$

Where: (x_2-x_1): the growth of the biomass is measured by (g absolute dried yeast / liter of the growth medium); (t_2-t_1) : the period during which the changing of the biomass was recorded is measured by (hour).

Experiments have proved that growth of the mixed culture were noticed in the thermal

field as from (22-37)°C , but the optimal temperature of the growth was noticed in a narrow field between (28-30)°C as shown in figure (1) .

2.2. Determining pH of the medium

The effect of the concentration of the hydrog iones studied through the field pH = 3.0-8.0 on the growth of the mixed culture , at optimal temperature of 30°C , Made it clear that the maximum relative speed for the growth of the mixed culture was achieved at the value of 5.5 pH as shown in figure (2) .

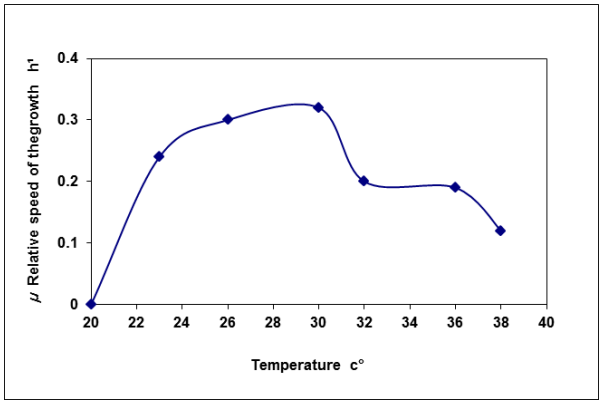

Fig 1 The effect of temperature on the relative speed for the growth of the mixed culture

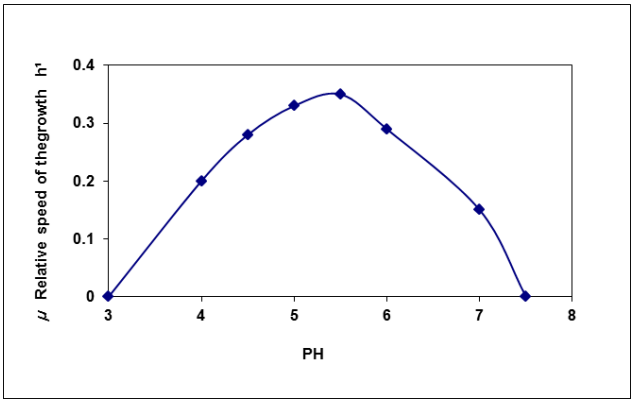

Fig 2 The effect of pH medium on the relative speed for the growth of the mixed culture

2.3. Determining the relative speed of the air consumption

The effect of changing the relative speed of the air flow within the field (0.2-2.0 air liter / treated medium liter / min) , on the growth of the mixed culture and at optimal temperature 30°C and optimal value of the pH : 5.5 was studied , it shows that the maximum relative speed for the growth of the mixed culture was achieved at the value of 0.5 air liter /treated medium liter/ min of the relative speed of the air consumption , as shown in figure (3).

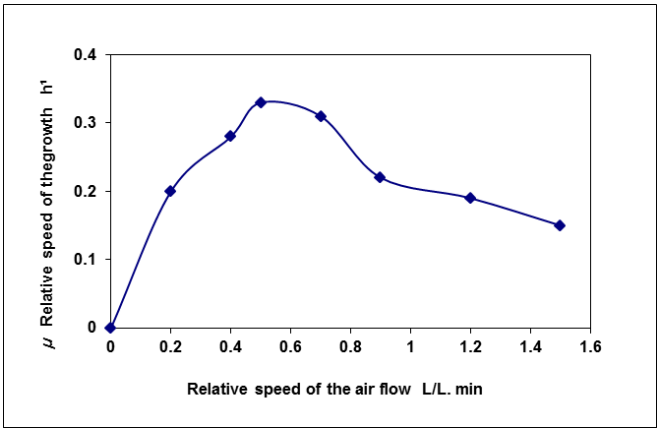

Fig 3 The effect of the changing of relative speed of the airflow on the relative speed of the growth of the mixed culture .

Third: using the mixed culture of the (*Saccharomyces cerevisiae* + *Kluyveromyces lactis*) yeast according to the previous growth conditions in the aerobic biotreatment of the whey and determining the reduction rate of the COD.

The sweet whey resulting from the diary plant in Homs in 2010 has been treated , and its characteristics are shown in table (3).

The whey was filtrated in the Ultra- filtration method to remove its protein content resulting from cheese processing and to get rid of all substances that can form suspended matters which prevent the growth of the yeast and stop its activity.

Furthermore the whey was sterilized in Autoclave at 120 C° and for (15 min) , then cooled and stored in the refrigerator until final use..

Tests were done on the whey after filtration and sterilization, and the changes occurred in the structure as shown in table (3).

Table 3 Results of tests on the whey after filtration and sterilization.

Tests	Raw whey	(After treatment) whey
Total solids %	6.97	5.92
Content of water %	93.03	94.08
Brix %	6.07	5.87
Lactose %	4.75	4.65
Protein %	0.9	0.85
Fat %	0.4	0.35
pH	6.66	5.5
(mg / L) COD	70 000	65 000

After preparing the whey , the aerobic bio- treatment used the batch cultivation method in a laboratorial experimental fermentation unit.

An amount of the whey (4 liters) were taken and put in the fermentation unit , and the mixed culture *(Saccharomyces cerevisiae* + *Kluyveromyces lactis*) yeast was added to the amount at 1% rate from the dried mass 70% .

The amount of air flow through the fermented liquid (whey) from the air pump was controlled ,as well as the speed of the stirring through a mixer inside the fermenter in order to guarantee the homogeneity of the biotreatment process and the distribution of the bubbles and the nutrient matters and cells of the yeast in the medium of the whey . The biological treatment continued for 36 hr, under the following optimal conditions:

- amount of the culture : 1%.
- temperature : 30 C° .
- pH : 5.5.
- The relative speed of air consumption : 0.5 air liter / treated medium liter / min .

3. Results

The results recorded in the table (4), and the production of the biomass reached in this way 3.53% , and the reduction rate of the COD of the whey reached 99.20 % .

Table 4 The results of the aerobic biotreatment of whey according to the batch cultivation method

Time h	Reduction rate of the COD %	COD mg/L	Lactose %	BX %	$x-x_0$ %	Biomass. X %
0	0.00	65000	4.65	5.87	0	1
1	7.54	60100	4.30	5.52	0.003	1.003
3	13.63	56142	4.02	5.23	0.125	1.125
5	33.22	43410	3.11	4.01	0.352	1.352
7	47.46	34152	2.45	3.22	0.599	1.599
9	57.60	27562	1.98	2.54	1.241	2.241
12	78.43	14022	1.01	1.42	2.362	3.362
24	86.70	8647	0.64	0.95	2.925	3.925
36	99.21	514	0.04	0.41	3.531	4.531

Fourth: Using a culture of the (*Kluyveromyces lactis*) yeast only in the aerobic biological treatment of the whey according to previous treatment conditions and determining the reduction rate of the COD.

The aerobic biological treatment of the whey done in previous laboratorial fermentation unit , by using a culture of (*Kluyveromyces lactis)* yeast only at rate 1% with its dried mass 70% , the biological treatment continued for 36 hr ,at previous treatment conditions .

The results shown in table number (5) , and the production of the biomass in this way was 3.352 % , and the reduction rate of the COD of the whey has reached 98.92% .

Table 5 The results of the aerobic biotreatment of whey according to the batch cultivation method.

Time h	Reduction rate of the COD %	COD mg/L	Lactose %	BX %	$x-x_0$ %	Biomass. X %
1	0	5.87	4.65	65000	0.00	0
1.005	0.005	5.62	4.52	61785	4.94	1
1.115	0.115	5.03	4.13	57731	11.18	3
1.332	0.332	4.40	3.81	53258	18.06	5
1.562	0.562	3.56	3.35	46828	27.95	7
2.001	1.001	2.83	2.78	38860	40.21	9
2.568	1.568	2.44	2.25	31452	51.61	12
3.460	2.460	1.01	0.82	11462	82.36	24
4.352	3.352	0.77	0.05	698	98.92	36

4. Discussion

After observing the results in tables (4-5), it is clear that :

1. The biomass apparently increases with the progress of the time .
2. The rate of the lactose of the dried matter is reduced with the progress of time until it nearly vanishes at the end of the treatment.
3. The method of the aerobic biotreatment of the whey according to the batch cultivation method by using mixed culture of the (*Saccharomyces cerevisiae* + *Kluyveromyces lactis*) yeast can reduce the rate of COD of the *Kluyveromyces lactis* yeast only until it reaches the rate of 99.20 % , and also increases the biomass to 3.53% rate .

5. Conclusions

The optimal conditions of the growth of the mixed culture (*Saccharomyces cerevisiae* + *Kluyveromyces lactis*) yeast can be applied and are : pH : 5.5, air flow : 0.5 air liter / treated medium liter / min , the temperature : 30°C , in the treatment of the whey biologically according to the batch cultivation method to reduce the value of the COD to 99.20 % .

Recommendations

1. Applying the conditions of the growth pointed out through the research in the treatment of the whey biologically in two ways (fed-batch and continuous cultivation) by using the mixed culture and comparing the results with each other and choosing the best ways in the treatment of the whey biologically and aerobically.
2. Underlining the high importance of the nutrient factor of the yeast used in the mixed culture, considered to be as a protein matter enriched in basic amines acid necessary for the healthy growth and nutrient of humans as well as a rich animal feed.

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