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THE RAPID DEGRADATION OF SAUERKRAUT BRINE BY FREE AND IMMOBILIZED YEAST CELLS

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ABSTRACT

Industrialization of sauerkraut production in Poland will need to solve the problem associated with disposing of highly acidic waste effluents. Late sauerkraut brine present the greatest problem with respect to treatment because of their high BOD and low pH. Degradation rate of sauerkraut brine by *Kluyveromyces marxianus* yeast - depends on concentration of the brine in the medium. The removal of lactic acid in shake flask experiments varied from 98.95 to 56.75 % after 48 h at 30°C. The immobilization of yeast in sodium alginate improved the lactic acid degradation rate and allow to treat the very acid brine directly. Lactic acid was fully used in diluted brine in 24 h and the content in undiluted brine was reduced by 90.17 to 81.2 to % in the following runs after 48 h.

Key words: sauerkraut brine, biodegradation, yeast, lactic acid.

INTRODUCTION

Lactic acid fermentation is one of the most important natural methods of food and feed preservation. Properly fermented sauerkraut can be stored for a long period of time because of its high content of lactic acid. The sauerkraut effluents are rich in lactic acid and have a very low pH (3.0-3.6) and can thus pose a serious environmental problem. Additionally sauerkraut brine has a NaCl content of more than 2%. As the

environmental regulations become stricter, farmers and food processors in Poland and other European countries must find new ways to handle the fermented vegetable effluents before they can be discharged into a clean ecosystem.

The small scale production of sauerkraut which is one of the most popular fermented product in Poland is expected to be industrialized. Such situation took place in the New York State - the biggest producer of sauerkraut in the USA with 3.6 million cases of sauerkraut sold a year [5]. It is very much alike that problem of disposing of acid waste effluents from sauerkraut producing plants to local waste management systems will arise in Poland.

The data from New York State sauerkraut processing plants show that there is a problem of solids as well as liquid waste in the manufacture of sauerkraut. The solid wastes are generally returned to the growing field but the surplus sauerkraut brines present the greatest problem with respect to treatment because of their high BOD and low pH.

About 20 tons of brine are generated by fermentation of 100 tons of shredded cabbage. Early brine was estimated to 11 ton for 100 ton cabbage used and late brine for 8.5 ton. Solid waste is attributed mainly to tri losses (35.3 tons) [8]. The average yield of sauerkraut in two large New York State factories was 64.7 tons from 100 tons cabbage [5].

Other sources of waste effluents are the vat soak water and vat wash water. Because of their low BOD values, both vat soak water (BOD = 60 mg $O_2 \cdot l^{-1}$ dcm^3, pH = 10.4 should be readily biologically treatable. Early brine was reported by Hang [4] to have BOD of 11 000 mg $O_2 \cdot l^{-1}$ and late brine 24 300 mg $O_2 \cdot l^{-1}$ and pH = 3.5 (lactic acid content 0.19%).

In recent years, there has been considerable interest in the use of yeast in the treatment of acid industrial effluents [1, 2, 6]. The yeast is capable of rapidly converting the organic matter to yeast cell and thus can reduce BOD as much as 93%. Hang and Woodams [8] reported that greater than 64% BOD in sauerkraut processing wastewater was attributed to the presence of lactic acid. Therefore, the waste load for the sauerkraut industry can be significantly reduced by removal of lactic acid in the wastewater.

The treatment of sauerkraut brine with a flocculent strain of *Kluyveromyces marxianus* is simple, fast and thus may have economic value in waste disposal and the production of feed yeast [6]. Yeast biomass is also a potential source of diaecetyl reductase [9]. One of the economic advantages of using *K. marxianus* is that the yeast cells settle rapidly and are easily collected by means of flocculation and sedimentation. The dried yeast has a protein content of nearly 50% and therefore has value as animal feed [8]. However late brine (brine from the finished fermentation which stays in the fermented product up to packaging time - sometimes few month) with high lactic acid content is the most difficult for biodegradation of all sauerkraut production effluents by yeast fermentation (Hang 1977).

In this research the degradation of the lactic acid in the late sauerkraut brine by *K. marxianus* yeast was investigated both using free cells and cells immobilized in alginate beads was used. The amount of lactic acid in the average brine is 1.5-2.0% (pH = 3.2). This late was extremely high in acids – 3.06% and with very low pH = 3.0.

MATERIALS AND METHODS

Late sauerkraut brine with 3.06% of titritable acids content (as lactic acid) was obtained from Seneca Foods (Geneva , New York, USA) and stored at 4°C. It was used directly or diluted in distilled water (1:2, 1:1, and 2:1 (v/v)). The brine contained 2.40% lactic acid as detected by HPLC and 1.19 % ethanol. There was no detectable amount of glucose and other sugars.

Kluyveromyces marxianus NRRL Y-610 were provided by Dr. C.P. Kurtzman (Northern Regional Research Center, US Department of Agriculture, Peoria, IL). The yeast culture was maintained on YM slants and transferred to diluted sauerkraut brine (1:1) prior to use.

For immobilization, alginic acid sodium salt (ICN Biomedicals, USA) (25 $g \cdot dcm^{-3}$) was mixed with yeast cells resuspended in sterile water, added dropwise to 0.1 $mol \cdot dcm^{-3} CaCl_2$ and allow to solidify for 1 h. Gel beads (40 g) were used for one flask. Yeast cells were centrifuged after 24 h fermentation on 1:1 sauerkraut brine : water medium (10 000 min^{-1}, RC5C Sorvall Instruments, 15 min). In 40 g of alginate beads 0.34 g yeast (d.m.) were immobilized.

Experiments were conduct as follows: portion of 100 ml of medium were dispensed into Erlenmeyer flasks (500 ml) and autoclaved for 15 min at 121°C. Each flask was inoculated with 1 ml of a 24 hold culture. All flasks were incubated at 30°C on a New Brunswick C-25KC shaker operated at 200 rpm.

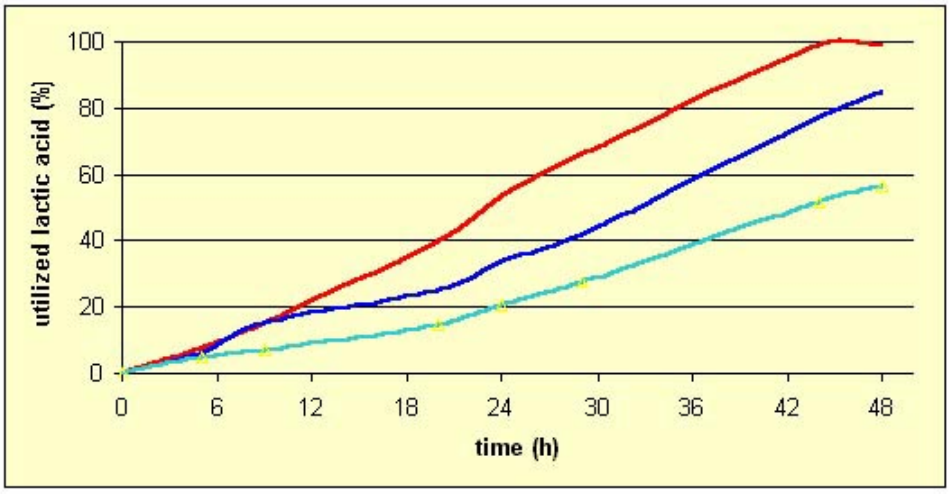
Yeast dry weight was obtained by filter through glass fiber filter circles (Fisher Scientific, G6), washing and drying at 85°C in an oven for 24 h. Glucose, lactic acid, acetic acid and ethanol were analyzed by high-performance liguid chromatography using a Bio-Rad HPX-87H column with a refractive index detector as described by Hang and Woodams [8]. Total acidity was determined by method of Stamer et al. [10] and expressed as lactic acid. The pH was measured with a Fisher pH meter (model 230).

The number of cells in the brine were counted using hemocytometer.

RESULTS AND DISCUSSION

The free cells of the yeast *K. marxianus* NRRL Y-610 in shake cultures caused a rapid decrease in the lactic acid content of the diluted waste samples (1:2, 1:1, and 2:1) (Fig. 1). The acid content of the waste diluted with 2 part of water was reduced by 53.7% after 24 and 48 h, respectively 98.9%. Value of pH was increased from 3.0 to a level of 6.5 after 48 h treatment. When the material diluted in equal volume of water was used, the yeast consumed 84.7% of the acids in 48 h. A larger amount sauerkraut brine in the medium decreased the acid consumption to 56.8% in 48 h (Fig. 1).

Fig. 1. Degradation of sauerkraut brine acids by *K. marxianus* free cells (sauerkraut brine : water, 1:2, 1:1, and 2:1, Means ±SD)

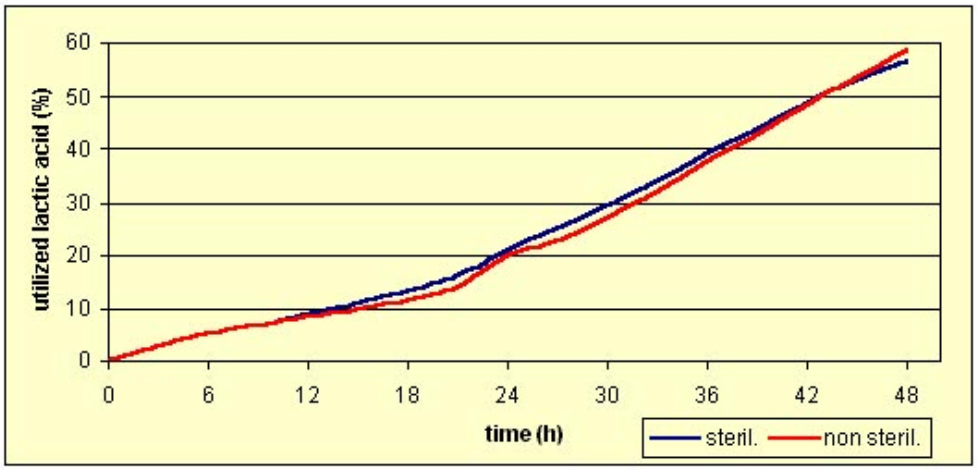


One gram of dry matter yeast biomass was produced from 5.06 to 5.9 g consumed acids.

The yeast hardly grew on undiluted sauerkraut brine and used only 14.3% of titritable acids after 48 h treatment and pH was increased to 3.2 only.

Autoclaving of diluted sauerkraut brine did not affected the fermentation. The course of lactic acid degradation in autoclaved and fresh samples were very similar (Fig. 2).

Fig. 2. Comparison of sterilized and non sterilized sauerkraut brine acids degradation by yeast (sauerkraut brine : water, 2:1, Means ± SD)



The age of sauerkraut brine has a profound influence on the yeast growth and BOD reduction. Hang [3] reported that removal of BOD decreases markedly as the brine becomes older. Up to 93% of the BOD could be removed from one-day old brine when with 49 and 98 day old brines the BOD removals reduced to 57 and 47%. The studies indicate that the older the brine is, the longer fermentation time will be required to stabilize it. Early brine is much easier to treat than is late brine and thus the dilution was performed.

The immobilization of yeast in sodium alginate medium enhanced the treatment of the sauerkraut brine. The alginate beads with immobilized yeast were used in 3 batches and they were still in very good condition (very acid medium did not destroy gel structure). No evidence of contamination was found. The average utilization of acids by immobilized cells in three runs was after 24 h as follow (2 parts of water : 1 part of sauerkraut brine – 93.8%, 1:1 - 76.6%, 1:2 – 60.3%) and after 48 h (2:1 - 100%, 1:1 - 99.6%, 1:2 – 98.8%) (Table 1).

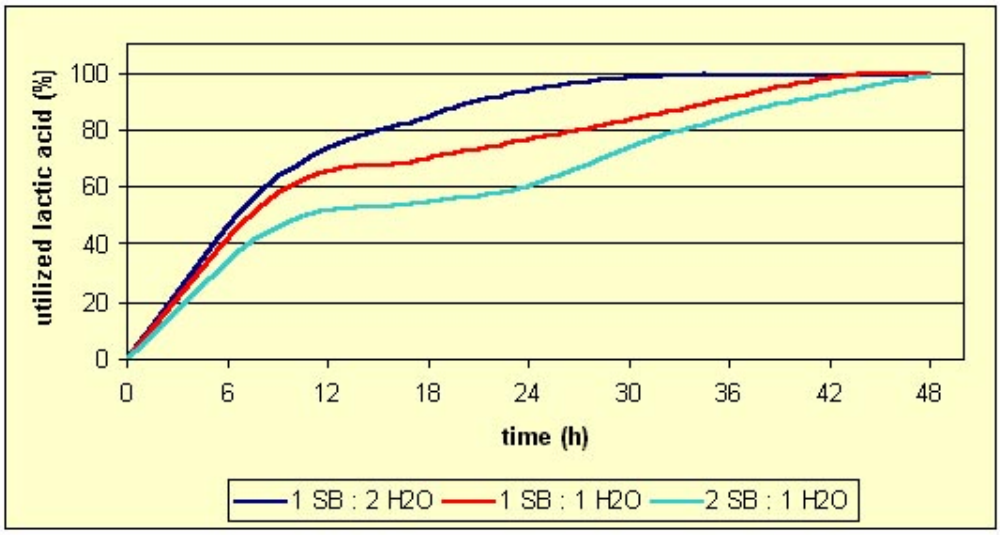
Table 1. Biodegradation of acids (%) by *K. marxianus* cells when immobilized in agar gel beads

Time (h)	Sauerkraut Brine : Water (1:2)				Sauerkraut Brine : Water (1:1)				Sauerkraut Brine : Water (2:1)				Undiluted Sauerkraut Brine			
	I run	I run	I run	Mean SD	I run	I run	I run	Mean SD	I run	I run	I run	Mean SD	I run	I run	I run	Mean SD
24	95.82	91.64	93.94	**93.80** 2.09	74.90	76.17	78.77	**76.61** 1.97	59.21	61.30	60.46	**60.32** 1.05	54.31	39.15	42.22	**45.23** 8.01
48	100	100	100	**100** 0.0	99.45	99.9	99.45	**99.60** 0.26	98.56	98.62	99.30	**98.82** 0.41	90.17	81.17	81.30	**84.21** 5.16

The degradation rate of lactic acid expressed as gram of acid used in one liter of medium in one hour was higher in first 24 h of fermentation (0.54 - 0.56 g · l^{-1} · h^{-1} for more dense medium and 0.45 g · l^{-1} · h^{-1} for 2 parts of water and one part of sauerkraut in the medium). After 48 h fermentation the rates varied from the highest 0.43 g · l^{-1} · h^{-1} (1part of water +2 parts of sauerkraut brine), 0.35 g · l^{-1} · h^{-1} (1:1) to 0.27 g · l^{-1} · h^{-1} for 2:1 dilution. So from the point of view efficiency of biodegradation higher loaded medium is preferable.

Immobilization was performed for the aim to repeat the batch fermentation for several cycles with high efficiency. The 3 run with serial medium changes characterized very similar course of acid biodegradation for 3 tested media (Fig. 3).

Fig. 3. Biodegradation of sauerkraut brine acids by immobilized *K. marxianus* cells (mean % from three runs, (Means ± SD)



The immobilized cells also consumed the lactic acid in undiluted sauerkraut brine, but the process was much slower and a 48 h batch process degraded 81.2-90.2% of the acids (Table 1).

Table 2. The number of yeast cells in 1 ml medium during sauerkraut brine treatment

Time (h)	Sauerkraut Brine : Water (1:2)		Sauerkraut Brine : Water (1:1)		Sauerkraut Brine : Water (2:1)	
	Not Immob.	Immob.	Not Immob.	Immob.	Not Immob.	Immob.
24	3.57×10^{7}	5.45×10^{7}	4.01×10^{7}	3.3×10^{7}	1.82×10^{7}	1.5×10^{7}
48	7.05×10^{7}	1.44×10^{8}	1.07×10^{7}	2.04×10^{8}	8.4×10^{7}	1.65×10^{8}

The number of free cells in the brine when immobilized cells was used was higher after 48 h than in free cells culture (Table 2). The yeast cells sedimented easily and the biomass could be used as fodder yeast [7].

CONLUSIONS

Late sauerkraut brine is relatively difficult medium for the growth of free cells of yeast in shake flask cultures. There is a rapid degradation of lactic acid by *K. marxianus* when the waste is diluted with water. The use of immobilized yeast cells enhances the process for treating undiluted late sauerkraut brine.

The results of shake flask experiments show that yeast fermentation by *K. marxianus* might be a useful method of biodegradation of highly acid waste effluents especially when high amount of immobilized biomass is used as inoculum.

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REFERENCES

1. Choi M. H., Park Y. H., 1999. Growth of *Pichia guilliermondii* A9, an osmotolerant yeast, in waste brine generated from kimchi production. Biores. Technol. 70, 231-236.
2. Elmaleh S., Defrance M. B., Ghomidh C., 1999. Organic acids oxidation by *Candida utilis*: application to industrial waste water treatment. Proc. Biochem. 35, 441-449.
3. Hang Y. D., 1977. Waste control in sauerkraut manufacturing. Proc. Biochem. 12, 27-28.
4. Hang Y. D., 2003. Sauerkraut. In: Handbook of vegetable preservation and processing. Y. H. Hui, S. Ghazalaa, D. M. Graham, K. D. Murell W. K. Nip. [Eds.] Marcel Dekker Inc, New York.
5. Hang Y. D., Downing D. L., Stamer J. R., Splitistoesser D. F., 1972. Wastes generated in the manufacture of sauerkraut. J. Milk Food Technol. 35, 432-435.

6. Hang Y. D., Woodams E. E., 1981. Rapid removal of lactic acid from wastewater by a flocculent yeast. J. Food Sci. 46(5), 1498-1499 & 1513.
7. Hang Y. D., Woodams E. E., 2001. Enzymatic enhancement of citric acid production by *Aspergillus niger* from corn cobs. Food Sci. Technol. 34, 484-486.
8. Hang Y. D., Woodams E. E., Hang L. E., 2003. Utilization of corn silage juice by *Kluyveromyces marxianus*, Biores. Technol. 86, 305-307.
9. Schwarz J. G., Hang Y. D., 1994. *Kluyveromyces marxianus*: a potential source of diacetyl reductase. World J. Microbiol. Biotechnol. 10, 385-387.
10. Stamer J. R., Dickson M. H., 1975. High solids cabbage for sauerkraut production: Its effects upon brine reduction and yield of product. J. Milk Food Technol. 38, 688.
11. Stamer J. R., Dickson M. H., Bourke J. B., Stoyla B. O., 1969. Fermentation patterns of poorly fermenting cabbage hybrids. Appl. Microbiol. 18, 323.

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