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ORIGINAL ARTICLE

Comparison of yeast strains for batch ethanol fermentation of cheese–whey powder (CWP) solution

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cheese–whey powder, ethanol, fermentation, *Kluyveromyces marxianus.*

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Abstract

Aims: To test the suitability of cheese whey powder (CWP) solution for ethanol fermentation and to compare performances of different *Kluyveromyces marxianus* strains for ethanol fermentation from CWP solution.
Methods and Results: Batch ethanol fermentation of cheese whey (CW), CWP and lactose solutions with the same initial sugar contents were compared by using two different *K. marxianus* strains and the CWP solution was found to be the most suitable substrate. CWP solution was fermented to ethanol using three different yeast strains and DSMZ-7239 was found to be the most suitable one yielding the highest rate and extent (3·3%, v/v) of ethanol formation.
Conclusions: CWP solution and *K. marxianus* strain of DSMZ-7239 were found to be more suitable for ethanol fermentation with the highest ethanol yield when compared with the other substrates and the yeast strains tested.
Significance and Impact of the Study: CWP can be used as a concentrated form of CW for ethanol fermentations with considerable advantages.

Introduction

Cheese whey (CW) is produced as a waste stream from cheese production facilities. Approx. 10 l CW is produced from 1 kg cheese with high carbohydrate, protein and lipid contents. Due to high lactose content (5–6%) CW has been used by many investigators for production of ethanol (Maiorella and Castillo 1984; Marhawa and Kennedy 1984; Terrel *et al.* 1984; Marhawa *et al.* 1988). Direct fermentation of CW to ethanol is not economical because of low ethanol yields (2–3%, v/v) and high separation costs. Ultra filtration process used to concentrate lactose in CW is expensive (approx. 50 USD m^{-3}) which only improves lactose concentration by a factor of 5 (Guadix *et al.* 2004). Utilization of CWP instead of CW eliminates costly ultra filtration process and provides concentrated source of lactose and other nutrients for more economical ethanol fermentation.

Different approaches were used for ethanol fermentation of CW including micro-aeration (Ghaly and El-Taweel 1995); continuous fermentation at different hydraulic residence times (Ghaly and El-Taweel 1997); use of thermo tolerant yeast strains at 45°C (Banat *et al.* 1992, 1996; Banat and Marchant 1995) immobilization of thermo tolerant yeasts on delignified cellulosic material (Kourkoutas *et al.* 2002) and alginate immobilized thermo tolerant yeasts (Brady *et al.* 1997). There are limited number of studies on ethanol fermentation from CWP (Kargi and Ozmihci 2006). CWP is a dried and concentrated form of CW and contains lactose in addition to other essential nutrients. Use of concentrated CWP solutions (200–300 g l^{-1} CWP) for ethanol fermentation yields high ethanol concentrations and reduces distillation costs for ethanol separation. For this reason, the present study was designed (i) to determine the potential advantages of using CWP solution for ethanol fermentation when compared with CW and lactose, (ii) to compare and select the most suitable *Kluyveromyces* strain for ethanol fermentation from CWP solution.

Materials and methods

Experimental system

Batch experiments were performed by using duplicate Erlenmeyer flasks and a gyratory shaker at 100 rev min^{-1}

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Table 1 Composition of cheese whey and cheese–whey powder solution (CWP = 100 g l^{-1})

(g l^{-1})	T-Sugar	T-COD	S-COD	T-TOC	S-TOC	Protein	TN	PO_4-P	Fat	pH
CW	28	59·8	42·26	28·85	21·6	9	1·58	1·28	0·545	4·4
CWP	49	114	88	39	33	20	0·33	0·615	2·6	6·2

T-sugar, total sugar; T-COD, total chemical oxygen demand; S-COD, soluble chemical oxygen demand; T-TOC, total organic carbon; S-TOC, soluble total organic carbon; TN, total nitrogen; PO_4-P, phosphate-phosphorous.

and 28°C. CW, cheese–whey powder (CWP) and lactose solutions were used as substrate with an initial total sugar content of 28 g l^{-1} for comparison of different substrates in ethanol fermentation. Compositions of the CW and CWP solutions are summarized in Table 1. When different *Kluyveromyces marxianus* strains were compared for their sugar utilization and ethanol formation capabilities from CWP solution duplicate Erlenmeyer flasks (500 ml) were loaded with 180 ml of deionized water containing 104 g l^{-1}CWP (50 g l^{-1} total sugar) and 200 mg l^{-1} Na-thioglycolate as the reducing agent. After pH adjustment to 5·0 with dilute H_2SO_4, the flasks were sterilized at 121°C for 15 min and inoculated with 20 ml pure cultures of different *K. marxianus* strains to yield 0·8 g l^{-1} initial yeast concentration in all flasks. Inoculated flasks were placed on a gyratory shaker at 28 ± 2°C and 100 rev min^{-1}. An Erlenmeyer flask free of yeast cells containing 104 g l^{-1} CWP and 200 mg l^{-1} Na-thioglycolate was used as control.

Microorganisms

Kluyveromyces marxianus strains NRRL-1109 and NRRL-1195 were obtained from USDA National Center for Agricultural Utilization Research, Peoria, IL, USA and *K. marxianus* DSMZ-7239 from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Braunschweig, Germany) in lyophilized form.

Medium composition

Medium used for cultivation of inoculum culture consisted of yeast extract (5 g l^{-1}), peptone (5 g l^{-1}), NH_4Cl (2 g l^{-1}), KH_2PO_4 (1 g l^{-1}), $MgSO_4{\cdot}7H_2O$ (0·3 g l^{-1}), lactose (30 g l^{-1}) and 200 mg l^{-1} Na-thioglycolate as the reducing agent at pH 5·0 with an initial oxidation–reduction potential (ORP) of nearly −250 mV. The yeast culture grown on gyratory shaker in the aforementioned medium at 28°C and 100 rev min^{-1} was used for inoculation of the experimental flasks. The experimental flasks contained desired concentrations of CWP and 200 mg l^{-1} Na-thioglycolate in deionized water at pH 5·0. The CWP was obtained from Pinar Dairy Industry in Izmir, Turkey and was dried at 80°C before use. CW and CWP (58 g l^{-1}) solutions were used without addition of any external nutrients with initial total sugar concentration of 28 g l^{-1}. Lactose solution contained 28 g l^{-1} lactose, 1·54 g l^{-1} NH_4Cl and 1·66 g l^{-1} KH_2PO_4.

Analytical methods

pH and ORP of the samples were measured using a pH/ORP meter (WTW Scientific, Weilheim, Germany) with the relevant probes. The samples were removed from the flasks periodically and centrifuged at 7000 ***g*** for 20 min to remove solids from the liquid medium. Sugar and ethanol analyses were carried out on the supernatants after centrifugation. Total reducing sugar concentrations were measured by using the phenol–acid method (Dubois *et al.* 1956). The samples were analysed in triplicates and the results were reproducible within 3% deviation. Ethanol concentrations were measured using a Gas Chromatograph (Varian CP–3800, Palo Alto, CA, USA) with an FID detector and a WCOT fused silica capillary column (15 m × 0·25 mm ID, 0·25 μm film thickness). The column temperature was set at 75°C for 1 min and raised to 130°C with a rate of 20°C min^{-1} yielding a total hold time of 4·75 min. Temperatures of injector and detector were 150°C and 200°C respectively. Nitrogen was used as the carrier gas with a linear velocity of 25 ml min^{-1}. Biomass concentrations were determined by filtering the samples through a 0·45-μm Millipore filter and determining the dry weight after drying the filter paper at 105°C until constant weight.

Results

Comparison of different substrates

Batch experiments were performed for 72 h with an initial pH of 5·0 and ORP −250 mV. The comparison of performance of two yeast strains (NRRL-1109 and 1195) with three different substrates is depicted in Fig. 1. Total sugar concentration decreased with time and sugars were depleted within 24 h as shown in Fig. 1a. Initial rate of total sugar utilization by NRRL-1109 in CWP solution was slower than that of NRRL-1195. Ethanol concentration increased with time and reached the maximum level

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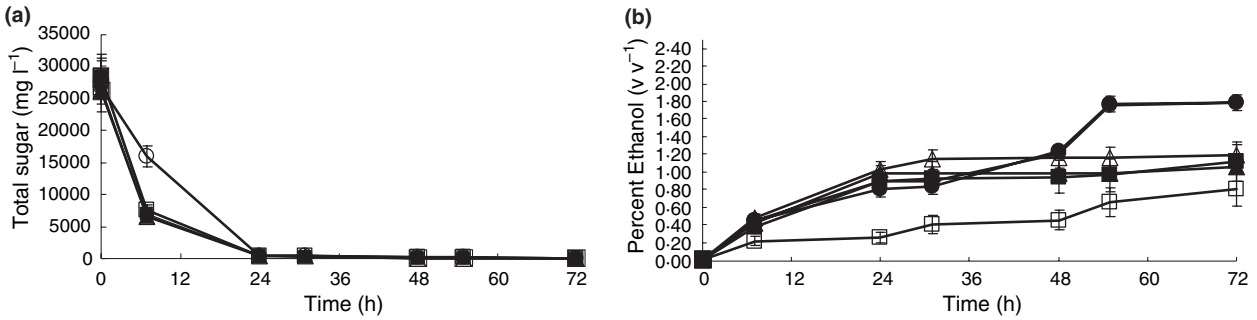


Figure 1 Variations of (a) total sugar, (b) ethanol concentrations with time for different substrates and *Kluyveromyces marxianus* strains. ○ CWP-NRRL 1109, ● CWP-NRRL 1195, □ Lactose-NRRL 1109, ■ Lactose-NRRL 1195, △ CW-NRRL 1109, ▲ CW-NRRL 1195.

after 72 h (Fig. 1b). Final ethanol concentration was 1·8% (v/v) with the CWP solution for both yeast strains while the per cent ethanol obtained from CW was 1·2% (v/v) after 72 h of incubation. The pH of lactose containing medium decreased from 5·0 to 3·6–3·2 in 7 h and remained stable while the pH of CW and CWP solutions stabilized at 4·8 and 4·6 respectively. ORP values increased from −275 ± 25 mV to approx. −100 mV in all flasks at the end of fermentation period. Standard deviations in duplicate flask data were <5%.

Ethanol yield coefficients, final ethanol concentrations, rates of sugar utilization and ethanol formation are compared in Table 2 for three different substrates and two yeast strains. Ethanol yield from CW and lactose solution were considerably lower than those obtained from the CWP solution. The ethanol yield coefficients for both yeast strains were almost equal to the theoretical yield (0·54 gE g^{-1} S) for the CWP solution. The lowest ethanol yield coefficient was obtained with the lactose solution where the yield with NRRL-1195 was higher than that of NRRL-1109. The highest final ethanol concentration was obtained with the CWP solution where performance of both strains were almost the same (1·8% ethanol, v/v). Strain NRRL-1195 yielded higher final ethanol when compared with the strain NRRL-1109 when lactose solution was used. Sugar utilization rates for the first 24 h were almost the same for CW and CWP solution (1100 mg S l^{-1} h^{-1}) which was somewhat lower than that for the lactose solution (1150 mg S^{-1} h^{-1}). Ethanol formation rate calculated over 72 h fermentation period was the highest (200 mg E l^{-1} h^{-1}) with CWP solution. On the basis of final ethanol yield, CWP was found to be more suitable substrate than CW and lactose solutions.

Comparison of different *K. marxianus* strains

Performance of three different strains of *K. marxianus* NRRL–1109, NRRL–1195 and DSMZ–7239 were compared for ethanol fermentation of CWP solution (50 g l^{-1}) and the results are depicted in Figs 2a,b. Sugar utilization was slower for the strains NRRL-1109 and NRRL-1195 when compared with DSMZ-7239 (Fig. 2a) while sugar utilization by NRRL-1195 was faster than NRRL-1109. Complete sugar utilization was achieved in 31 h with DSMZ-7239 strain and in 96 h with NRRL-1195. Clearly, DSMZ-7239 was more effective in sugar utilization when compared with the other strains tested. Ethanol concentrations increased with time and reached the highest level (3·3% v/v) after 54 h with DSMZ 7239 while NRRL-1195 yielded only 2·4% (v/v) ethanol after 72 h of incubation (Fig. 2b). Ethanol yield with NRRL-1109 was lower than the other yeast strains. pH decreased with time and reached 4·0, 4·1 and 4·5 with NRLL-1109, NRRL-1195 and DSMZ-7239, respectively at the end of

Table 2 Ethanol yield coefficients and concentrations, rates of sugar utilization and ethanol formation with different substrates and yeast strains

Substrate/*Kluyveromyces marxianus* strain	CW NRRL-1109	CW NRRL-1195	CWP NRRL-1109	CWP NRRL-1195	LAC NRRL-1109	LAC NRRL-1195
$Y_{E/S}$	0·36	0·32	0·52	0·53	0·22	0·31
Final ethanol (v/v)	1·19	1·06	1·78	1·79	0·81	1·12
Rate of sugar utilization (mg l^{-1} h^{-1})	1080	1075	1100	1100	1170	1170
Rate of ethanol formation (mg $l^{-1}h^{-1}$)	130	120	195	200	90	1

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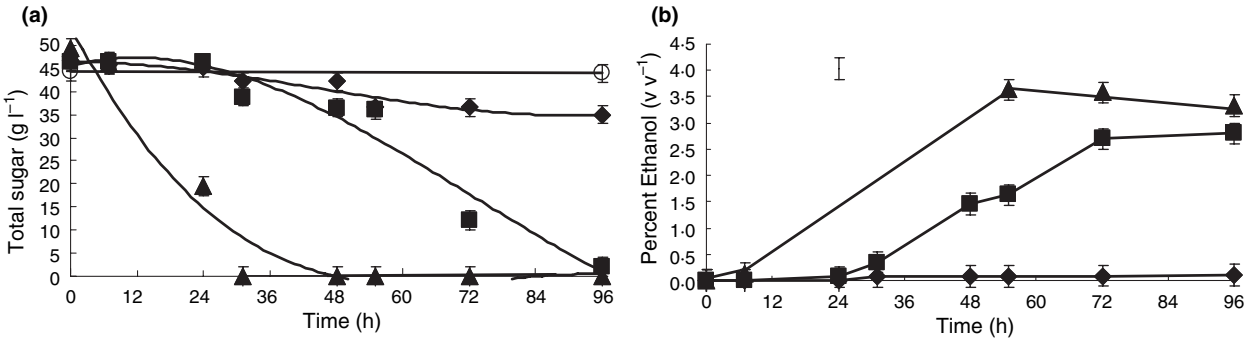


Figure 2 Variations of (a) sugar concentration, (b) ethanol concentration with time for different *Kluyveromyces marxianus* strains in fermentation of cheese–whey powder solution to ethanol. ♦NRRL 1109 ■ NRRL 1195, ▲ DSMZ 7239, □ Control.

96 h. The ORP of the media increased with time for NRRL-1109 and NRRL-1195, from −180 and −200 mV to approx. −170 and −190 mV respectively. However, ORP values decreased from −250 to −300 mV for DSMZ-7239. Lower ORP levels might be a key factor for the high performance of DSMZ culture. No sugar utilization and ethanol formation was observed in the control flask free of yeast cells.

Ethanol yield coefficients and final ethanol concentrations are compared in Table 3. The highest ethanol yield (0·54 gE g^{-1} S) was obtained with DSMZ-7239 which is equal to the theoretical ethanol yield coefficient (0·54 g EtOH g^{-1} lactose). The ethanol yields of NRRL-1109 and NRRL-1195 were 0·08 g EtOH g^{-1}sugar and 0·5 g EtOH g^{-1}sugar respectively. The results indicated that both the DSMZ-7239 and NRRL-1195 strains effectively fermented CWP solution to ethanol. The final ethanol concentrations were 2·8% (v/v) for NRLL-1195 and 3·33% (v/v) for DSMZ-7239. The biomass concentration in all flasks was nearly 0·8 g l^{-1}.

Sugar utilization and ethanol formation rates are compared in Table 4. The highest sugar utilization rate (1030 mg S l^{-1} h^{-1}) was obtained with DSMZ-7239 followed by NRRL-1195 (460 mg S l^{-1} h^{-1}). Ethanol formation rate obtained with DSMZ-7239 (550 mg E l^{-1} h^{-1}) was also higher than those of the other strains. The lowest ethanol formation rate was obtained with NRRL-1109. The specific sugar utilization rate for DSMZ-7239 (1285 mg S $g^{-1} \times h^{-1}$) was considerably higher than that of NRRL-1195 (570 mg S $g^{-}1 \times h^{-1}$) and NRRL-1109 (150 mg S $g^{-}1 \times h^{-1}$). Specific ethanol formation rate of DSMZ-7239 was also higher (685 mg E $g^{-}1 \times h^{-1}$) than the others. On the basis of sugar utilization and ethanol formation rates, DSMZ-7239 was found to perform better than the other yeast strains tested.

Table 3 Comparison of ethanol yield coefficients ($Y_{p/s}$) and final ethanol concentrations in cheese–whey powder fermentation to ethanol with different *Kluyveromyces marxianus* strains

Kluyveromyces marxianus strains	NRRL-1109	NRRL-1195	DSMZ-7239
$Y_{p/s}$	0·08	0·50	0·53
Per cent final ethanol (v/v)	0·10	2·80	3·33

Table 4 Comparison of volumetric and specific rates of sugar utilization and ethanol formation from cheese–whey powder solution with different *Kluyveromyces marxianus* strains

Kluyveromyces marxianus strains	NRRL-1109	NRRL-1195	DSMZ-7239
Volumetric rate of sugar utilization (mg l^{-1} h^{-1})	120	465	1030
Specific rate of sugar utilization (mg g^{-1} h^{-1})	150	567	1290
Volumetric rate of ethanol formation (mg l^{-1} h^{-1})	10	230	550
Specific rate of ethanol formation (mg g^{-1} h^{-1})	10	285	690

Discussion

Three different media (CW, CWP and lactose solutions) and two different *K. marxianus* strains were used to determine the most suitable substrate for ethanol formation. CWP solution was found to be a more suitable substrate when compared with CW and lactose solutions with higher ethanol yields for both yeast strains tested. Concentrated CWP solution was probably nutritionally richer than the other substrates resulting in higher ethanol yields. When performances of three different *K. marxianus* strains (NRRL-1109, NRRL-1195 and DSMZ-7239) were compared for ethanol formation from CWP solution, DSMZ-7239 strain of *K. marxianus* was found to be more suitable yielding higher ethanol

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concentrations (3·3%, v/v) and faster sugar utilization. The highest sugar utilization (1030 mg S l^{-1} h^{-1}) and ethanol formation rates (550 mg E l^{-1} h^{-1}) were also obtained with DSMZ-7239 strain. The results indicated that CWP solution is a suitable substrate and DSMZ-7239 is an effective yeast strain for ethanol formation from CWP solution.

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