Single Cell Protein (SCP) production from UF cheese whey by *Kluyveromyces marxianus*

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Abstract:

The aim of this study was to produce single cell protein (SCP) from ultra filtration permeate cheese whey. Cheese whey was fermented by *Kluyveromyces marxianus* under batch and aerobic condition in which pH and temperature were adjusted to 4.5 and 35°C, respectively. Ammonium sulphate as nitrogen source was added to whey to increase biomass yield. The produced biomass was analyzed for protein content in different times during fermentation. About 82% of total protein was produced in the first 18 h of 96 h fermentation, which can be an indication of the exponential phase of the yeast growth. Results of biomass yield measurements during 96 h process also correspond to this issue.

Key words: Single Cell Protein, UF cheese whey, Batch, *Kluyveromyces marxianus* (fragilis)

1. Introduction:

Whey is a yellow-green liquid by-product in cheese manufacturing, remaining after the precipitation and removal of fat and casein from whole milk and represents about 85-90% of the milk volume. The main composition of dried whey is 70% lactose, 9-14% protein, and 9% ash (3,5,7,10). The worldwide dairy industry generates over 80 million tons of whey each year. Whey represents an important environmental problem due to the high volumes produced and its high organic matter content, exhibiting a BOD of 30000-50000 ppm and COD of 60000-80000 ppm, with lactose being largely responsible for the high BOD and COD (60 g oxygen per liter) (2,4,7,11,13). Thus, whey is particularly suitable for the production of SCP using lactose-utilizing yeast (13).

SCP production technologies arose as a promising way to solve the problem of worldwide protein shortage. They evolved as bioconversion processes which turned low-value by-products, often wastes, into products with added nutritional and market value and since SCP belongs to one of the cheapest protein products in the market, its production is profitable (6,12).

As compared with plants and animals for providing proteins for food or feed, large-scale industrial production of microbial biomass for the same use has great characteristic advantages such as: Microorganisms in general have a high rate of multiplication and a high protein content (30-80% protein in terms of dry weight); They can utilize a large number of different low cost carbon sources including waste materials; Production installations occupy limited areas and give high yields and except for algae microbial production is independent of seasonal and climatic variations and therefore more easy to plan (1,8,13).

A special problem with SCP products for human consumption is the nucleic acid content. High content of nucleic acids causes no problems to animals since uric acid is converted to allatonin which is readily excreted in urine. Some practical methods for solving this problem for human use of SCP are
alkaline or acidic hydrolysis and activation of endogenous RNA-ases (usually by brief heat treatment at 64°C for 30 min) (1,8).

Microbial biomass is broadly used as fermentation starter cultures for food and beverage fermentations, waste treatment processes and agricultural inoculants; as a source of protein for human food, because it is often odorless and tasteless; as animal fodder and as functional foods, because it has flavor, fat and water binding property, dispersing action, whipping and foaming action and extrusion properties (8,13).

Several processes such as Kiel process in Germany and Vienna process in Austria have been developed for the utilization of lactose in whey to produce SCP. Some of more successful processes were operated by Bel industries in France. The Bel process was developed with the aim of reducing the pollution load of dairy industry waste, while simultaneously producing a marketable protein product (13). While most organisms do not grow on lactose as a carbon source, strains of Kluyveromyces marxianus readily grow on lactose (4). A number of plants are operated using Kluyveromyces lactis or K. marxianus (formerly K. fragilis) to produce protein, Protibel, which is used as a nutritional supplement for both human and animal consumption (13).

The aim of the present work was to investigate the potential and effectiveness of using UF whey as a substrate under batch fermentation for the production of single cell protein by the yeast Kluyveromyces marxianus. The information would be useful for the development of a cost effective process in a large industrial scale to produce protein.

2. Materials and Methods

2.1. Whey preparation

Cheese whey was obtained from the Ramak Dairy Factory in Shiraz. It was drawn from the pipe into 2 L plastic containers. The containers were sealed and transported to the Dept. of Food Science Laboratory at Shiraz University in Shiraz, where they were stored in a freezer at -20°C until required. Some characteristics of the cheese whey used in this study are presented in Table 1. Prior to fermenting process of cheese whey it was allowed to completely thaw at room temperature for 24 h (4,5).

To reach the highest biomass yield, 0.8 g/l ammonium sulphate as nitrogen source was added to whey (9,12). Two liters of raw cheese whey was pasteurized in a bottle (4,5,13). The pasteurization technique included heating the whey to 65°C for 30 min, cooling it to 0°C for 30 min and letting it to stand at room temperature (25°C) for 24 h for any spore to germinate. The process of heating, cooling and standing at room temperature was repeated three times to destroy any vegetative or spore cells present in the whey (4,5).

2.2. Inoculum preparation

Lyophilized yeast strain K. marxianus (PTCC 5193) was obtained from the Persian Type Culture Collection, Tehran, Iran. A small amount of a pellet of K. marxianus was dissolved in a 7 mL sterilized growth medium tube containing 1% yeast extract, 2% peptone and 2% dextrose. The tube was placed at 30°C for 48 h to activate the yeasts (4,5).

A loop of this solution was streaked on an agar medium, containing 1% yeast extract, 2% peptone, 2% dextrose and 2% agar in a Petri dish (3 Petri dishes were used). The Petri dishes were then placed in an incubator at 30°C and left until visual growth appeared (after 72 h). Then they stored in refrigerator at 4°C until needed. The yeast colonies were then scooped from the surface of the agar into 80 mL pasteurized cheese whey in the sterilized Erlenmeyer flask. The Erlenmeyer flask was then capped with cotton and mounted on a shaker (4). The shaker was operated at a speed of 170 rpm for 48 h at 35°C. The number of yeast cells in inoculum was measured to insure the effectiveness of the conditions performed on the shaker.
2.3. SCP production

A 1 L flask was filled with 675 mL pasteurized whey. Then, 75 mL of the inoculum (10% by volume) were added as recommended by Ghaly et al. (4). The flask content was mixed thoroughly and then distributed in 15 flask (each 50 mL). Then flasks were shaken for 96 h. The shaker was operated at a speed of 200 rpm and 35°C temperature. The optimum temperature for *K. marxianus* propagation is in the range of 30–35°C (4). Flasks were taken out at 0, 6, 18, 24 and 96 h in 3 replicates. Immediately after the termination of the fermentation process, the flasks were put in water bath at 100°C for 10 min to deactivate the yeasts and interrupt the process. Finally samples were transferred to centrifugation unit to recover the yeast biomass from the spent medium.

2.4. Analysis of cheese whey

The chemical analysis of whey was performed using AOAC test methods. Protein content was determined by microKjeldahl method with 6.25 conversion factor.

2.5. Yeast cells number measurement of the inoculum

1 mL of the inoculum was diluted in 5 tubes each contained 9 mL saline solution consisted of 0.9% NaCl salt. The dilution rate continued to 10^{-5}. Then 0.5 mL solution of both 10^{-4} and 10^{-5} dilution tubes were differential cultured on agar yeast-peptone-dextrose (YPD) growth medium in 2 replicates. The plates were placed in incubator at 35°C for 48 h until the colonies appeared and were countable.

2.6. Biomass yield measurement

After the termination of the process, 40 mL of each sample were transferred to centrifugation tubes in triplicate and centrifugated at 4000 rpm (9). The biomass then was dried in vacuum oven at 60°C for 8 h and then weighed. The results are shown in Figure 1.

2.7. Analysis of biomass protein

The protein analysis of biomass was performed using AOAC test methods. Protein content was determined by microKjeldahl method with 6.25 conversion factor. The results are shown in Figure 1.

3. Results and Discussion

3.1. Whey characterization

The physical and chemical characteristics of ultra filtration permeate cheese whey are given in Table 1. The initial pH value of the cheese whey used in this study was 7.2, however the optimum pH for the growth and survival of *K. marxianus* is between 4.0 and 5.0 (4). It has also been recognized that keeping the pH at about 4.5 eliminates possible contamination by lethal bacteria that grow at pH above 6.0 (4). Thus, in this study, pH of the medium was adjusted to 4.5 by the addition of 1 N HCl solution (4).

Since the protein content of whey was low, no difficulties related to precipitation of protein during preparation were encountered and therefore UF cheese whey is a more suitable substrate for SCP production than other wheys.

3.2. Inoculum cell number

The colonies developed from the samples showed the elevated concave, smooth appearance and creamy color of *K. marxianus*. Direct plate count at 10^{-4} dilution was impossible since the number colonies exceeded 300. But at the 10^{-5} dilution plates were countable and yeast cells number (the
average of 2 replicates) was 142, thereby allowing to calculate cell number per mL of inoculum was as follows: 142×10^5×2= 24800000 cell/mL

This result indicates that the growth conditions were suitable and as no other kind of colony was observed, no contamination had been occurred and the inoculum was pure.

3.3. Biomass yield and single cell protein production

Data obtained from the amount of biomass yield corresponds with that of protein produced during fermentation in this study. About 82% of total protein was produced in the first 18 h of 96 h fermentation, which can be an indication of the exponential phase of the yeast growth. Total amount of protein in 96 h was measured 38.34% which is considerable and represents UF cheese whey suitable for protein production under conditions treated.

4. Conclusions

With regards to the results obtained from this work, ultra filtration permeate cheese whey is a proper substrate for single cell protein production under conditions provided in this study, however, for profitable production, interruption of the process in the first 18 h, in which 82% of total protein would be produced, can be useful. Also, further studies should be done to investigate the nucleic acid content and find some ways to reduce it to permitted levels. Finally, cost effective SCP process can be performed in an industrial scale and the product can be consumed instead of expensive proteins present in the market (12).

Table 1. The measured values of major composition of UF cheese whey

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>% ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>93.0±0.5</td>
</tr>
<tr>
<td>Lactose</td>
<td>6.1±0.03</td>
</tr>
<tr>
<td>Proteins</td>
<td>0.1±0.05</td>
</tr>
<tr>
<td>Ash</td>
<td>0.7±0.02</td>
</tr>
<tr>
<td>pH</td>
<td>7.2±0.02</td>
</tr>
</tbody>
</table>

aData is expressed as mean ± SD of three replicate samples.

Fig. 1. The protein and biomass measured during the batch culture operation
Acknowledgements

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چکیده:
هدف از این تحقیق تولید پروتئین نک سلولی از آب پنیر UF بود. آب پنیر به عنوان سویسرا در یک سیستم بسته و تحت شرایط هوازی توسط مخمر کلاپروماپیس مارکسیانوس (Kluyveromyces marxianus) تحت فرآیند تخمیر قرار گرفت. pH و دما آب پنیر به ترتیب در محدوده 5-6 و 35°C تنظیم گردید. ضمناً به منظور رسیدن به بالاترین بازدهی نمک سولفات آمونیوم به عنوان مشربی جوش به آب پنیر اضافه شد. میزان پروتئین در زمان‌های مختلف در طول تخمیر اندازه گیری گردید. حدود 72 ساعت فرآیند در طول 84 ساعت فرآیند از کل پروتئین تولیدی در طول 96 ساعت تولیدی در 18 ساعت ابداعی تولید گردید که این زمان می‌تواند نشان‌دهنده فاز نگارشی رشد مخمر باشد. همچنین نسجش بازدهی زیست توجه تولیدی نیز این مطلب را تأیید می‌نماید.

کلمات کلیدی: پروتئین نک سلولی، آب پنیر UF، مخمر کلاپروماپیس مارکسیانوس (فراجیلس) سیستم بسته