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Recovery of Enzyme Alkaline Protenease from Dairy Waste by Fermentation and Membrane Separation

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Abstract: Enzyme Alkaline Protease can be produced by microbial fermentation under optimum conditions of Temperature, pH, agitation, and aeration. In this work it is propose to use microfiltration ultrafiltration membrances as an alternative methodology to carry out fermentation process producing alkaline protease enzyme and as an alternative method for the cell separation and recovery of alkaline protease from the fermentation broth because it has the potential for energy saving and higher purity. A well- known microfiltration technique for cell separation and diafiltration for enzyme recovery is used. Ultra filtration of 20,000 MWCO is used for purification and concentration of enzyme alkaline protease.

Keywords: Biotechnology, Microbial fermentation, Membrane sterilization, Ultrafiltration.

I. INTRODUCTION

The term fermentation has undergone numerous changes in meaning during the the past hundred years. Fermentation may be defined, in a broad sense, as a process in which chemical changes are brought about in an organic substrate (e.g. carbohydrate, fat, protein, hydrocarbon, etc.) through the action of the biochemical catalysis known as ‘ enzymes’ produced by specific type of living microorganisms. In general the fermentation process can be expressed by the following biochemical reaction in presence or absence of oxygen

Micro-organisms + Substrate → New Microorganisms + Fermentation Products

Products of fermentations are, in fact the metabolic products formed during the microbial growth.

II. MATERIALS AND METHODS

A. Upstream Process:

In upstream processing inoculums is prepared from Enzyme Alkaline rotease producting species of microorganism was obtained from the Dairy waste and stored at 4^0C in nutritive medium for few days. The fermentation medium was prepared using double distilled water and had following composition : NaCl, 5gm/L; Beef extract, 3g/L; Peptone, 5g/L. The pH was adjusted to 9 . The nutritive media sterilized by keeping thefermenter (covering all ports with aluminium foils) in autoclave for 1.5 hours under 15 psi. pressure.

B. Fermentation Process:

Inoculation was carried out under aseptic condition and fermentation started in 5 liters fermenter (Inceltek make) with 2.5 L working volume. The following fermentation conditions were used: agitation rate (110 rpm), fermentation temperature (37 ^{o}C), aeration rate (1 vvm i.e. volume of air per volume of medium per min), and reaction time (35 hours). All chemicals used for the fermentation were reagent grade purchased from Sigma Chemical Co. Throughout the fermentation process, the following parameters were monitored: pH, agitation (rpm) and temperature (^{o}C). After 35 hours of fementation, the reaction was stopped and the biomass was harvested, characterized and stored at 4 ^{o}C.

C. Downstream Process:

In this work Microfiltration was used to replace the uneconomical Centrifugation, pre-coat filtration methods for separation and recovery of enzyme alkaline protease as well as clarify broth for further processing.

III. RESULT AND DISCUSSION

A. Enzyme Alkaline Protease Preparation by Fermentation

Experiment for preparation of Enzyme Alkaline Protease by fermentation was carried out at the controlled temperature of 37 ^{o}C, agitation was provided at the rate 110 rpm, aeration at the rate 1 vvm. The fermentation operation was continued for 35 Hrs. and the pH variation during fermentation was from 9-7. The media can be sterilized by using membranes before fermentation starts which have the following advantages 2.5 L broth obtained at the end of fermentation , which 2.5 L broth obtained at the end of fermentation, which was stored at 4 ^{o}C for further processing.

B. Primary Isolation of Cells by microfiltration

The plate and frame microfilter module was used for cell separation. The 1600 ml fermented broth was taken for

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primary cell separation 1250 ml permeate was collected and 200 ml retenate was collected. The permeate flux Variation during microfiltration is given in Table1

After every 5 minute the samples from permeate was collected and labeled as P_1,P_2,P_3,P_4 and P_5 and retentate as R_1,R_2,R_3,R_4 and R_5 as shown in the table3. The sample from initial broth was labeled as F_0 final permeate as P_6 and final retentate as R_6 It is clear from Table 1 that the permeate flow rate decreases with time and it is due to fouling of membrane. The samples collected above (P_1 to P_6, R_1 to R_6 and F_0) are stored at 4°C and thereafter analyzed

For enzyme protease activity measuring the absorbance at 440 nM (given below) during Enzyme assay.

TABLE 1 MICROFILTRATION OF FERMENTATION BROTH

Sr. No.	Pressure (bar)		Flow rates (ml/sec)		Time min	Permeate volume collected (ml)
	Feed	Retentate	Permeate	Retentate		
1	0.3	0.1	16/15	192/15	0	0
2	0.3	0.1	12/15	190/15	5	300
3	0.3	0.1	10/15	192/15	10	600
4	0.2	0.0	8/15	190/15	15	710
5	0.2	0.0	10/15	64/15	20	1000
6	0.2	0.0	8/15	50/15	25	1250

TABLE2 PROTEASE ASSAY OF MICROFILTRATION SAMPLES

Samples	Absorbance at 440nM	Samples	Absorbance at 440nM
F_0	0.2174	F_0	0.2174
P_1	0.1664	R_1	0.2191
P_2	0.2319	R_2	0.2251
P_3	0.2333	R_3	0.2171
P_4	0.2218	R_4	0.2450
P_5	0.2201	R_5	0.2281
P_6	0.2253	R_6	0.2265

Protein estimation was carried out using Lawrys methods. The dilution factor for protein extimation was 10. The protein estimationof various samples is clear that the protein concentration of the feed is 7567.9182 microgram per ml (i.e. 1600 ml feed contains 2508.669 mg of protein) The protein concentration of final permeate is 1261.4541 microgram per ml (ie. 1250 ml of permeate contains 1576.817 mg of protein). And the final retenate concentration is 1312.1591 microgram per ml (i.e. 200 ml of retenate contains 262.43 mg. of protein) The material balance for protein if considered across microfiltration operation it is clear that 669.42 mg of protein remained in the hold up volume of microfilter. The protein separation efficiency for microfilter is about 63%. At the end of microfiltration the following concentrations were found. Concentration of Permeate, Cp, (P_5) = 1133.1052 microgram / ml. Concentration of feed, Cf. (R_6)= 1312.1591 micro gram /ml., Rejection coefficient for the microfilter, R = 1-(Cp/Cf) = 1-(1133.1052/1312.1591) = 0.14 (i.e. 14% Rejection of protein by microfiltration) Final retenate is also contain considerable amount of protein in it and need to be diafiltration of retenate again.

C. Activity Calculation:

By the same way activity of all samples is given Table 3.

That is activity of sample = = Y/[C X 150 /(1000 X 1000)] U/mg.

TABLE 3 ENZYME ACTIVITIY OF MICROFILTRATION SAMPLES

Samples	Activity (U/mg)	Samples	Activity (U/mg)
F_0	0.924	F_0	0.924
P_1	0.854	R_1	0.998
P_2	1.180	R_2	0.959
P_3	1.458	R_3	0.942
P_4	1.170	R_4	1.059
P_5	1.295	R_5	0.864
P_6	1.190	R_6	1.150

IV. RECOVERY OF ENZYMES BY DIAFILTRATION

Before starting of diafiltration the microfiltration plate was characterized for pure water permeability was considered as a feed and it filtered by adding 1000 ml of distilled water at the rate of permeate.

The various samples like P_1,P_2,P_3,P_4,P_5 form permeate line and F_1,F_2,F_3,F_4,F_5 from feed tank were collected. F_1 considered as initial feed. F_6 as final combined permeate and F_5 as a final retertate collected and labeled. The results are shown in Table 4.

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TABLE 4 DIAFILTRATION OF MICROFILTRATION RETENATE

Sr. No.	Feed Pressure (bar)	Retentate Pressure (bar)	Permeate flow rate (ml/sec.)	Time (min)	Permeate volume collected.
1	0.3	0.1	8/5	4	200 (P_1)
2	0.3	0.1	8/5	7	400 (P_2)
3	0.3	0.1	6.5/5	10	600 (P_3)
4	0.3	0.1	6/5	13	800 (P_4)
5	0.3	0.1	5/5	16	1000 (P_5)

Protein estimation of above samples was carried out and it is clear that the protein concentration of the feed is 1312.1591 microgram per ml.(i.e. 200 ml feed contains 262.4 mg of protein) The protein concentration of final permeate is 129.1944microgram per ml (i.e. 1000 ml of permeate contains 129 mg of protein). And the final retenate concentration is 430.0038 microgram per ml (i.e. 200 ml of retenate contain 86 mg. of protein) The material balance for protein if considered across diafiltration operation it is clear that 47.4 mg of protein remained in the hold up volume of the equipment. The protein separation efficiency for difiltration is about 50%.

The Figure 1 shows the graph of protein concentration in permeate with time of operation concentration in permeate with time of operation. Initially the permeate was more concentrated but after some time the permeate get diluted due to continuous addition of distilled water to feed and there by to permeate.

V. CONCLUSIONS

Microfiltration along with diafiltration operation has shown good results for the cell separation and enzyme recovery up to 93 % Ultrafiltration of proper MWCO is very effective for concentration and purification of enzyme solution.

In totality the membrane technique can made operation economical due to repeated use of membranes resulting in higher product recovery due to minimum hold up volume and elimination of contamination. It consumes less power, has low chemical and labour costs and is easy and practical to handle. Thus it has helped the process to achieve higher productivity, making it more reliable and economical.

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