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Biological coagulation of skim latex using *Acinetobacter* sp. isolated from natural rubber latex centrifugation effluent

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An *Acinetobacter* sp, isolated from latex centrifugation effluent, effectively coagulated skim rubber from skim latex. After coagulation for 48 h without the addition of any nutrients, at an optimum dilution of 1:10(v/v) and with an inoculum concentration of 6.4 mg dry cell /ml, the yield of the skim rubber was 8 % (w/v) and the COD of the residual solution was only 0.4 g/l. chemical coagulation at the same dilution resulted in 7 % (w/v) yield of dry rubber content and 2.2 g COD /l.

Introduction

Skim latex, containing 2.5–10% of rubber, is obtained along with the concentrated rubber latex, as an equal fraction in volume, during centrifugation of the field rubber latex (Radhakrishna Pillai, 1980). Protein and other non-rubber constituents which have specific gravities higher than that of rubber also migrate into the skim fraction during centrifugation and not only reduce the quality of rubber but also affect the coagulation process. The usual method of recovery of skim rubber is by coagulation with sulphuric acid. In acid coagulation, the acid content of the coagulated rubber reduces its quality and shows some tendency to scorch (Naunton, 1961). Further, acid coagulation leads to generation of highly acidic latex centrifugation effluent (Jayachandran *et al*., 1994) which necessitates additional treatment before discharge to the environment in order to avoid pollution. Coagulation of the skim latex is also performed using enzymes such as trypsin where enzymatic deproteinization of the skim latex can also be achieved (Morris, 1954). However, this process is not economical as it needs large quantity of enzyme and necessitates further steps such as deammoniation and addition of sodium thiosulphate and formic acid. information on the coagulation of skim latex using microorganisms is not available in the literature and hence we studied the efficacy of whole cells of bacteria for the biocoagulation of skim latex with a view not only towards improvement of rubber quality but also to reduce environmental pollution due to highly acidic rubber effluent. To the best of our knowledge this study is the first report of this kind where whole cells of bacteria were applied for the coagulation of rubber.

Materials and methods

Sample

from a local latex centrifugation plant, skim latex was collected in clean containers from the skim latex collection tank, after it was pumped from the latex centrifugation unit. Samples were used fresh, immediately after collection.

Microorganism

Acinetobacter sp BTJR-10, isolated from highly acidic rubber latex centrifugation effluent (Jayachandran, *et al*., 1994), was grown in300 ml of nutrient broth(HI) which contained g/l : peptone,5; NaCl, 5; beef extract,1.5 and yeast extract,1.5; pH 7.4, at 28°C and 150 rpm. on a rotary shaker. After 18h cells were harvested, at 40°C, at 10000 g under sterile conditions. Cells were suspended in physiological saline, after washing with the same, and used for inoculation purposes.

Coagulation of skim latex

Coagulation studies were carried out in 500 ml conical flasks at various dilutions of skim latex. Dilution of the skim latex was made with distilled water. Inoculation of the skim latex (200 ml in each flask) was done at 1% (v/v)

level using the cell suspension prepared at various cell concentrations. After inoculation the flasks were incubated at 28°C for 48 h till a maximum quantity of the coagulated skim rubber was formed over the liquid surface. The experiment was simultaneously carried out using sulphuric acid (chemical coagulation) at the selected dilution rates. samples were withdrawn at intervals, and analysed for dry rubber content (DRC), nitrogen content and chemical oxygen demand (COD).

Analytical methods

Coagulated skim rubber was analyzed for dry rubber content (Indian Standards, Specification for natural rubber latex, 1985) and nitrogen content (Indian Standards, Specification for natural rubber latex, 1968). The residual effluent was analyzed for chemical oxygen demand (American Public Health Association, 1989).

Results and discussion

Fresh skim latex, used in the present study, had 9–10% (w/v)of dry rubber content, 9–11% (w/v)protein and a slightly alkaline pH (pH 9.1) due to dissolved ammonia. Initially the optimal dilution of skim latex and the inoculum concentration which could support maximal coagulation of dry rubber was determined by carrying out coagulation at various dilutions of skim latex and cell concentrations. Results presented in Table 1 indicated that biocoagulation at dilutions of 1:2, 1:10 and 1:20(v/v), and with cell suspensions containing 6.4 mg and 8.0 mg dry cell/ml as inoculum, at 1% (v/v), could lead to coagulation of significant amount of dry rubber content after incubation for 48 h. Results also indicated that the concentration of skim latex in the sample inhibit bacteria at lower dilutions, since the yield of rubber was comparatively less at dilutions 1:1 and 1:2(v/v) compared to 1:10(v/v) dilution.

Table 1 Estimation of rubber yield (in terms of dry rubber content) at various dilutions of skim latex and at different inoculum concentrations. Rubber yield expressed as % w/v.

Dilution factor of the skim latex (v/v)	*Inoculum concentration*				
	1.6 mgdc/ml	*3.2 mgdc/ml*	*4.8 mgdc/ml*	*6.4 mgdc/ml*	*8.0 mgdc/ml*
1:1	2.7	2.9	3.2	4.9	3.4
1:2	3.7	4.0	4.2	5.6	5.4
1:10	4.2	4.7	5.0	8.3	8.3
1:20	1.8	2.0	3.5	5.0	5.5
1:100	0.7	0.7	0.8	0.8	1.4

Inoculation at 1% (v/v); 48 h incubation; mgdc/ml is mg dry cell/ml.

The experiment was repeated with inoculum concentrations of 6.4 and 8.0 mg dry cell/ml, at 1% (v/v), for selecting the ideal dilution of skim latex and the optimum inoculum concentration required for enhanced rubber yield. Three dilutions viz.1:2, 1:10 and 1:100 (v/v)were tried. Yield of rubber, nitrogen content of the skim rubber and COD of the residual effluent were analysed after 48 h. Maximal yield of rubber in terms of dry rubber content (8.3% w/v) was obtained at 1:10(v/v) dilution rate irrespective of the inoculum concentration compared to dilutions of 1:2(v/v) (5.6% and 5.4% w/v, respectively, for 6.4 and 8.0 mg dry cell/ml) and 1:100(v/v) (5.0% and 5.6% w/v, respectively, for 6.4 and 8.0 mg dry cell/ml). Relatively, the nitrogen content of the skim rubber was less (1.5% w/v) at 1:10 (v/v)dilution for the inoculum with 6.4 mg dry cell/ml compared to the inoculum with 8.0 mg dry cell/ml (1.6% w/v). At 1:2 (v/v) dilution, irrespective of the concentration of the inoculum tried the nitrogen content was same(1.7% w/v). Whereas at 1:100(v/v) dilution, the nitrogen content was 1.6 and 1.8% (w/v), respectively, for the inoculum with 6.4 and 8.0 mg dry cell/ml. COD of the residual effluent was 0.4 g/l for the dilution 1:10(v/v), irrespective of the concentration of the inoculum. While the dilution 1:2 (v/v) resulted in relatively a high level of COD in the effluent (10.0 and 9.5g/l, respectively, for 6.4 and 8.0 mg dry cell/ml), the 1:100 (v/v) dilution gave COD values of 1.7 and 2.0 g/l, respectively, for 6.4 and 8.0 mg dry cell/ml.

Chemical coagulation with sulphuric acid,with the same dilution and incubation time, supported only 6.8 % (w/v) yield of rubber and the nitrogen content was comparatively higher (1.7% w/v). Moreover, the residual effluent recorded 2.2 g COD /l (Table 2). The low nitrogen content of the biologically coagulated skim rubber compared to that of chemically coagulated skim rubber indicated less protein content and hence a better quality (Indian Standards, Specification for natural rubber latex, 1968). Further, the pH of the residual effluent was in the range 6.5–6.6 after biological coagulation compared to that with chemical coagulation (highly acidic pH 2.8).

Table 2 Analysis of the chemical coagulation of the skim latex.

Dilution factor of the skim latex (v/v)	*COD of the residual effluent (g/l)*	*Nitrogen content of the skim rubber (% w/v)*	*Dry rubber content of the skim rubber (% w/v)*
1:2	46.7	1.8	1.6
1:10	2.2	1.7	6.8
1:100	1.3	1.7	6.6

Incubation for 48 h.

Table 3 Comparison of the yield of rubber and COD of the residual effluent after biological coagulation with *Acinetobacter* sp. and chemical coagulation with sulphuric acid.

	Dry rubber content of the skim rubber (% w/v)		*COD of the residual effluent (g/l)*	
Period of coagulation (h)	Biological* coagulation	*Chemical coagulation*	*Biological coagulation*	*Chemical coagulation*
6	0.0	6.7	21.0	2.2
12	0.0	6.7	19.9	2.2
18	0.0	6.7	18.9	2.2
24	1.2	6.7	15.4	2.2
30	3.6	6.7	10.4	2.2
36	6.5	6.7	3.0	2.2
42	8.1	6.7	1.0	2.2
48	8.2	6.7	0.4	2.2
54	8.3	6.7	0.4	2.2

Dilution of the skim latex used, 1:10 (v/v).
* For biological coagulation cell suspension with 6.4 mg dry cell/ml was used as inoculum, at 1% (v/v).

A comparative time course study on the effect of biological and acid coagulation was also carried out and the results are presented in Table 3. From the results it was inferred that maximal coagulation of rubber in terms of dry rubber content(8.3% w/v) was effected through biocoagulation after 48 h compared to chemical coagulation. with chemical coagulation a maximum dry rubber content of 6.7% w/v was recorded after 6 h of coagulation and further incubation did not enhance the dry rubber content. Similarly, the COD levels in the residual effluent of biocoagulation process showed rapid declines with increase in incubation time and reduction in COD up to o.4g/l was observed after 42 h, unlike with chemical coagulation(2.2g/l).

From the present study it is evident that coagulation of skim latex can be effectively done with whole cells of bacteria, and *Acinetobacter* sp BTJR -10 has the potential for probable application in the rubber manufacturing industry, for recovering maximal rubber from skim latex with improved quality by virtue of reduced protein content in the rubber compared to that obtained with acid coagulation. Moreover, the biocoagulation process assures economic wastewater management in terms of contributing to a low level of COD in the effluent. In fact, chemical coagulation results in a highly acidic effluent which warrants additional neutralization of pH before the effluent is discharged to the environment, whereas, biocoagulation which resulted in very mild acidic conditions (pH 6.5) in the effluent, hardly requires intense treatment before its discharge. It is to be noted that the same strain was reported earlier as a potential strain for the treatment of latex centrifugation effluent (Jayachandran *et al*.,1994), and is now being observed to be efficient for coagulation of skim latax, the process which generates this effluent.

Considering the fact that there exists a need for improvement in the quality of rubber and safe and economic waste water management in the rubber production industries, biocoagulation process could become an effective alternative or additional process for obtaining maximal yield of dry rubber content, compared to acid coagulation, despite the fact that it takes a longer period. Of course, there is ample scope for improvement of the process through a strain improvement program. Further studies on the role of active principles involved in the biocoagulation process, identification of the genes, and overexpression of the same could definitely lead to an economic bioprocess. We conclude that biological coagulation of skim latex using whole cells of bacteria has the potential for not only recovering quality rubber from skim latex but also would facilitate economic management of effluent.

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