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Pressate from Peat Dewatering as a Substrate for Bacterial Growth

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This study considered the possibility of using water expressed during the drying of fuel-grade peat as a substrate for microbial growth. Highly humified peat pressed for 2.5 min at 1.96 MPa produced water with a chemical oxygen demand of 690 mg/liter. Several biological compounds could be produced by using the organic matter in expressed peat water as a substrate. These included polymers such as chitosan, contained in the cell wall of *Rhizopus arrhizus*, and two extracellular polysaccharides, xanthan gum and pullulan, produced by *Xanthomonas campestris* and *Aureobasidium pullulans*, respectively. A very effective surfactant was produced by *Bacillus subtilis* grown in the expressed water. Small additions of nutrients to the peat pressate were necessary to obtain substantial yields of products. The addition of peptone, yeast extract, and glucose improved production of the various compounds. Biological treatment improved the quality of the expressed water to the extent that in an industrial process it could be returned to the environment.

There are vast quantities of fuel-grade peat in Canada, the United States, the Soviet Union, and other countries (13, 25). Canada alone has a virtually untapped resource of at least 5×10^{10} tonnes of dry matter with a potential fuel value of 10^{12} GJ. Since this material is 90% water, it must be pressed or thermally dried before use. During pressing a large quantity of water containing organic matter is released, providing a potential pollution hazard. A possible solution is to use the pressate as a substrate for microbial growth.

Many microbiological studies on peat bogs have been done since the microbes present in this environment are responsible for the slow decomposition of peat (4, 8, 12, 17, 18, 24, 26–30). Peat removed from the bog has been used to produce methane by anaerobic fermentations (3, 11; R. Martinelli, document distributed at the Bioenergy 1980 World Congress and Exposition, Atlanta, Ga., 1980). Peat hydrolysate (acid extract) and peat oxidate (alkali extract) have been used as substrates for the production of single cell protein, ethanol, and pullulan (15, 20; A. LeDuy, J. M. Boa, and A. Laroche, paper presented at the National Meeting of Am. Inst. Chem. Eng., Houston, Tex., 1983).

Although more dilute, the components utilized in the growth studies described above are present in bog runoff water and in the water removed from peat during pressing. Some growth experiments have been done using runoff water (30). This study, however, describes the use of expressed water as a substrate for microbial growth. Factors influencing growth will also be discussed. Several microorganisms were investigated because they were known to produce products with commercial potential.

MATERIALS AND METHODS

Growth studies. Decomposed peat (von Post scale H9) from a bog at Barrington, Nova Scotia, was pressed at 1.96 MPa for 2.5 min with a stainless steel piston. Water was collected at the bottom of the press, and all peat particles were removed by centrifugation. Water was also collected from peat which was autoclaved in a 20-liter Fisher pressure vessel at 125°C for 20 min before pressing.

Various mineral salts were added to the pressates. For *Bacillus subtilis* ATCC 21332 (5) and *Rhizopus arrhizus* (2), 0.4% NH_4NO_3, 0.4% Na_2HPO_4, and 0.4% KH_2PO_4 were added. The additives to the media for the growth of *Xanthomonas campestris* ATCC 9924 (21) and *Aureobasidium pullulans* PpKm 149 (16) are the same as described in the literature but without $MgSO_4$ and trace metals.

Other additions to the pressate included yeast extract, peptone (Difco Laboratories, Detroit, Mich.), and glucose. *Arthrobacter viscosus* ATCC 19584 (9) was grown in a medium with 0.5% peptone added to the expressed water.

All fermentations were carried out with 50-ml samples in 500-ml Erlenmeyer flasks shaken at 170 rpm. The temperature was maintained at 23°C for the growth of all cultures, with the exception of *B. subtilis*, which was grown at 37°C.

Analyses. Biomass was determined after 6 days of growth by removing the cells from the broth by centrifugation for 10 min at 36,200 × *g*. The liquid was decanted off, and the cells were washed to remove excess salts. The sample was then dried at 105°C and reweighed (10).

Surface tension measurements were made by the de Nuoy method using a Fisher Autotensiomat (7). Relative surfactant concentrations were determined through the dilution of the broths to obtain the value of the CMC^{-1} (the reciprocal value of the critical micelle concentration [7]).

Polysaccharide production was determined by known methods (16). The method for chemical oxygen demand (COD) determination was devised by Knechtel (14) and was used with a few exceptions. The digestion solution was added first, followed by the COD solution and finally the catalyst solution. Digestion time was increased from 2 to 3 h. The phenol test was used for carbohydrates (22).

RESULTS

Attempts to grow various cultures in expressed water without additives were not successful. Nitrogen and phosphorous salts were added to all of the following media.

Surfactant production. When *B. subtilis* was grown in the pressate, peptone and yeast extract were found to be necessary for the production of surfactin. Surfactin production is demonstrated by a broth surface tension of 27 mN/m (Table 1). Substitution of the pressate with distilled water decreased growth substantially.

Addition of small amounts of glucose also enhanced surfactant production as shown by the increase in CMC^{-1}.

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TABLE 1. Effect of the addition of peptone, yeast extract, and glucose on the growth of *B. subtilis*

Peptone and yeast extract added (%)	Concn of added glucose (%)	Biomass (g/liter)	Surface tension of broth (mN/m)	Maximum CMC^{-1} obtained
Peat pressate				
0.00	0.00	0.21	37	≤1
0.01	0.00	0.27	50	≤1
0.01	0.04	0.33	27	4
0.01	0.40	0.51	27	8
0.10	0.00	0.58	27	2
0.10	0.00	0.18	33	≤1
Control[a] (0.10)	0.40	0.52	27	2

[a] Distilled water was used instead of peat pressate in controls.

The medium contained only small amounts (0.01%) of peptone and yeast extract.

Increasing the pressing time to 45 min increased the concentration of the organic components in the expressed peat water, as demonstrated by the dark water color. For the growth of *B. subtilis*, mineral salts, 0.1% peptone, and yeast extract were added. A significant amount of surfactant (CMC^{-1} = 20) was produced.

Polymer production. The fungus *R. arrhizus* grew well in the pressate with mineral salts, 0.05% peptone, and yeast extract. The biomass was 0.74 g/liter, of which 30% was chitosan.

Another fungus, *Aureobasidium pullulans*, was grown with mineral salts and 0.08% yeast extract added to the expressed water (Table 2). The addition of 0.08% peptone increased growth threefold. Acclimitization of the culture to the medium was an important factor. After several transfers, there was a significant improvement in pullulan production.

The effect of peptone and yeast extract addition on *X. campestris* was also investigated (Table 3). Xanthan gum production was shown to be at a concentration of these components as low as 0.05%. Increasing the concentrations increased production. Growth and xanthan gum elaboration with the pressate medium were significantly enhanced compared to the results obtained for the control experiments with no peat components added.

The effect of the addition of glucose is also shown in Table 3. A concentration of 0.04% glucose with mineral salts, 0.01% peptone, and yeast extract resulted in the production of a large amount of xanthan gum. Increasing the amount of glucose 10-fold did not significantly increase the yield of polymer. The control experiment with 0.40% gluclose did not yield as much xanthan gum as did the experiments with peat pressate.

Substitution of the standard pressate with pressate from autoclaved peat increased growth and product yield. The highest yield of xanthan gum was 2.00 g/liter when mineral salts, 0.10% peptone, and yeast extract were added.

Analyses of expressed peat water. The carbohydrate contents of the pressates from untreated and heat-treated peat samples were 560 and 450 mg/liter, respectively. The COD of both types of water were determined to be 690 and 720 mg/liter, respectively.

After this water was used as a substrate to grow microbes, the amount of soluble oxidizable carbon was greatly reduced. *X. campestris* grown on pressate with yeast extract for 3 days reduced the COD of the water to 50 mg/liter.

TABLE 2. Growth of *Aureobasidium pullulans* in peat pressate

Additives (%) to the pressate	Transfer no.	Biomass (g/liter)	Pullulan produced (g/liter)
K_2HPO_4 (0.5) NaCl (0.1) $(NH_4)_2SO_4$ (0.06) Yeast extract (0.08)	1	0.29	None
K_2HPO_4 (0.5) NaCl (0.1) $(NH_4)_2SO_4$ (0.06) Peptone (0.08) Yeast extract (0.08)	1	0.95	None
	2	0.85	0.96
	3	1.21	1.81

DISCUSSION

This study demonstrated that peat pressate can be used as a substrate for microbial growth. Since large quantities of water which contains significant amounts of organic matter will be released during a large-scale pressing operation, it is important that the quantity of pollutants be reduced (19). Microbial growth reduces potential pollutants so that the water can be returned to the environment.

Several useful products, including chitosan, pullulan, xanthan gum, and surfactin, were produced with the expressed water. Xanthan gum and pullulan are beneficial in the chemical, petrochemical, and plastics industries (23). Surfactants can be used as foaming agents, emulsifiers, and soaps. Chitosan is a good flocculating agent (1).

Some of these products can also be used as dewatering agents (6). When added to the peat before pressing, these compounds enhance the amount of water released. Chitosan from *R. arrhizus* and surfactin produced by *B. subtilis* are two examples. They can be produced on the expressed water and then added to the peat for further dewatering, resulting in a cyclic treatment process.

Small additions of nutrients were made to the water. Mineral salts and peptone were necessary to increase the low levels of nitrogen and phosphorus and to raise the pH from 5 to 6.5. Vitamins in the yeast extract also enhanced growth. These factors plus the correct temperature and aeration lead to significant growth in peat (17), as well as in peat pressates as shown by the present study. Harsh treatments such as acid or base catalysis accompanied by 2 h of

TABLE 3. Effect of the addition of peptone, yeast extract, and glucose and type of pressate used on the growth of *X. campestris*

Type of peat used to obtain pressate	Peptone and yeast extract added (%)	Concn of added glucose (%)	Biomass (g/liter)	Xanthan gum produced (g/liter)
Heat treated	0.00	0.00	0.37	0.40
Untreated	0.01	0.00	0.46	None
Heat treated	0.01	0.00	0.41	0.56
Untreated	0.01	0.04	0.41	2.98
Untreated	0.01	0.40	1.99	3.08
Untreated	0.05	0.00	0.65	0.76
Heat treated	0.10	0.00	1.16	2.00
Untreated	0.10	0.00	1.00	1.70
Control[a]	0.10	0.00	0.47	0.44
Control	0.10	0.40	1.06	1.80

[a] Distilled water was used instead of peat pressate in controls.

autoclaving (15, 20; LeDuy et al., Natl. Meet. A.I.Ch.E.) were not necessary.

Analysis of the pressates indicated that a significant amount of the dissolved solids were carbohydrates. They would most likely contain glucose, xylose, and galactose with some amino acids similar to those contained in peat hydrolysate (22). These components are not toxic and can be used as a carbon source by the microbes.

The composition of the water will vary according to the type and decomposition of the peat from which it is extracted and will affect the growth results. Pressing the peat increases the organic matter in the water compared to bog runoff water. Increasing pressing time from 2.5 to 45 min increased the amount of extracted matter and resulted in better yields of the surfactant.

Heat treatment of the peat breaks down the peat and increases the dissolved carbon as shown by a higher COD. This increased growth and xanthan gum production. However, temperatures higher than 125°C might increase the amount of toxic substances released into the water, such as phenols which inhibit growth and make water treatment difficult (21).

Small amounts of glucose were also found to enhance surfactin and xanthan gum production. Increasing the amount of glucose to 0.4% did not dramatically improve yields. The control experiments with 0.40% glucose did not produce yields as high as those obtained in the experiments with peat pressate and 0.04% glucose. The effect of added glucose was greater than just the addition of a carbon source, but it is not clear what the effect was.

This study has shown that various microorganisms can be grown in the pressate obtained from peat dewatering. In addition to the production of useful products, improvement in the water quality is also accomplished. Only small amounts of added nutrients were necessary. Future work will involve the optimization of the production of these biological compounds.

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