ABSTRACT

Growth of Neurospora sitophila on alkaline effluents from rutabaga, potato, and peach processing operations was studied. Submerged fermentation at 30 °C reduced COD values from 42 to 68% of initial values for peeling wastes and from 17 to 25% in rinse wastes after 4 days. This procedure for reducing COD would be of interest as a pretreatment technique for use in processing plants discharging into municipal treatment systems. The total amino acid content of potato effluent biomass was nearly quadrupled, whereas the total amino acid content of peach effluent was doubled after 1 day of fermentation.

Alkaline peeling effluents from vegetable and fruit processing operations are typically concentrated sources of suspended solids, oxidizable materials, and alkali. Disposal of these effluents is a universal problem in the food industry. If discharged with other processing wastes, the waste load from peeling can account for 86 to 98% of the total load (6, 19). Some plants use evaporation ponds; others use spray irrigation and a few are experimenting with cattle feeding after the pH is reduced by anaerobic fermentation.

Church and Nash (10) have shown that the waste load of acid effluents from vegetable processing can be reduced by fermentation with species of Fungi Imperfecti. Besides reducing the COD of corn whey by 98%, production of fungal biomass was 50 to 60 g of dry mycelium per 100 g of COD utilized. Feeding trials confirmed this material to be a beneficial source of protein for animals. This work has progressed from laboratory to pilot scale testing (9).

The nature of the waste to be used as a substrate for fungal fermentation dictates the organism to be employed. Gregory et al. (12) and Reade and Gregory (14) studied amylolytic, thermostable, filamentous fungi, since such cultures made it unnecessary to hydrolyze starch cassava substrates before fermentation.

Saccharomyces (Kluveromyces) fragilis was selected for studies involving use of coconut water wastes for production of food yeast because of its known ability to assimilate those carbohydrates present in coconut water (17). Brewery spent grain liquor has been reported to serve as an excellent substrate for growth of Aspergillus niger (13) Candida spp., Saccharomyces spp., and mushrooms (15, 16).

Previous studies in our laboratory revealed that Neurospora sitophila grows readily on a peanut substrate supplemented with tapioca starch and sodium chloride (4) and in a potato extract medium (3). The organism also was demonstrated to utilize several low molecular weight sugars (20). Since effluents from alkaline peeling operations contain substantial levels of carbohydrate and sodium chloride (after pH reduction with hydrochloric acid), N. sitophila was the organism chosen for studies to determine the feasibility of simultaneously reducing COD and increasing the protein content of biomass through fermentation.

MATERIALS AND METHODS

Neurospora sitophila NRRL 2884 was grown on potato dextrose agar (pH 5.6) at 30 °C for 20 to 25 days. Spores, along with some mycelial fragments, were dispersed in sterile water containing 0.005% Tween 80 by gently rubbing the culture surface with a glass rod. The suspension was filtered through sterile glass wool and the filtrate was used as an inoculum for all experiments. The number of colony-forming units per ml of inoculum ranged from $2 \times 10^5$ to $3 \times 10^6$.

Grab samples of rutabaga, Irish potato, and peach peeling and rinsing (washing) effluents were collected from a local vegetable and fruit processing plant. These were alkaline effluents from Magnuson peeling and scrubbing equipment which are used commercially for dry caustic peeling. Our objective was not to study high-strength dry caustic peeling wastes, however, but rather to evaluate alkaline wastes which were diluted and adjusted to a suitable fermentation pH. After diluting the peeling effluent 1:4 (vol/vol) with water and adjusting all effluents to pH 5.6 with HCl to facilitate fermentation, 100-ml aliquots of effluents were dispensed into 250-ml Erlenmeyer flasks and autoclaved at 121 °C for 15 min. One milliliter of N. sitophila spore suspensions was then added to the cooled substrate. Flasks were placed on gyratory shaker (150 rpm, 4-cm stroke) and incubation was at 30 °C for 1, 2, 3, and 4 days.

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Ferments were filtered through Whatman #541 paper and the biomass collected was air-dried at 65 C for 16-18 h and weighed. Raw and fermented samples were analyzed for COD by a standard method (1). The pH of ferments was monitored during the 4-day incubation period.

Dried biomass was analyzed for amino acid content. Samples (100 mg) were hydrolyzed in 20 ml of 6 N HCl, after flushing with high purity nitrogen, for 2 h, at 145 C. The pH was adjusted to 2.15 ± 0.05 with 12 N NaOH and the sample was diluted to 50 ml. An aliquot was centrifuged using a Beckman Microfuge and amino acids in the supernatant fluid were analyzed by the ion exchange chromatography technique of Spackman et al. (18) with a Durrum Model D-500 amino acid analyzer. A 48-cm column (1.75 mm I.D.) packed with a Durrum high resolution cation exchanger (bead diameter 8 ± 2 microns) was used. Running time was 100 min, including regeneration of the column.

RESULTS AND DISCUSSION

Changes in pH of peel and rinse fermentation media are shown in Fig. 1. Although the pH of all effluents was adjusted to 5.6 before autoclaving, some changes in pH were noted as a result of the heat treatment. Growth of N. sitophila was accompanied by marked increases in pH, especially early in the fermentation period. This may be due to autolysis and/or the production of ammonia by the organism.

The composition of peeling and rinse effluents before fermentation is shown in Table 1. The reduction of COD values ranged from 42 to 68% of initial values for the peeling wastes (Fig. 2). The more dilute rinse waters from the Magna Scrubber showed reductions to 17 to 25% of initial values at the end of the 4-day fermentation. Differences in percentage reduction of COD in the two types of waste (peeling versus rinse) may have been affected by the ability of N. sitophila to grow on substrates having different compositions. Reductions in COD might be of interest as a pretreatment technique for p'ants discharging to municipal treatment systems. It is becoming increasingly common for municipalities to pass sewerage ordinances requiring industrial wastes to be reduced to the concentration of municipal wastes, which is typically 300 mg/l for Biochemical Oxygen Demand and suspended solids. Such ordinances are forcing plants to use in-plant controls, high-rate activated sludge treatment systems, and even physical-chemical treatment of concentrated waste sources. The latter approach has been advocated by Bough in his research on various food processing wastes (5, 8).

Shown in Fig. 3 are changes in biomass as a result of fermentation of peal and rinse effluents with N. sitophila. The biomass product is composed of residual suspended solids and cellular biomass of the organism. Steady increases in biomass accumulation were obtained in potato and peach peel media throughout the 4-day test period whereas a plateau was reached after 2 days in the rutabaga peel substrate. The same trends were noted in peach and rutabaga rinse media. A decrease in biomass content in the potato rinse medium after 1 day may have resulted from sampling or analytical error.

The amino acid contents of nonfermented (control) and fermented rutabaga, potato, and peach alkaline peeling effluents are listed in Table 2. Data are from 1-day ferments and controls are calculated as g amino acid per 100 g dry biomass, i.e. as a percentage of the dry sample weight. Cysteine was not detected in any of the samples; tryptophan was not determined.

Although some changes in levels of individual amino acids occurred during fermentation of the rutabaga effluent, the total amino acid content increased only slightly from 9.48 to 9.66%. The total amino acid content

<table>
<thead>
<tr>
<th>Product</th>
<th>Effluent</th>
<th>COD (mg/l)</th>
<th>Filterable materials (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rutabaga</td>
<td>Peel</td>
<td>24,850</td>
<td>838</td>
</tr>
<tr>
<td></td>
<td>Rinse</td>
<td>1,840</td>
<td>36</td>
</tr>
<tr>
<td>Potato</td>
<td>Peel</td>
<td>19,600</td>
<td>630</td>
</tr>
<tr>
<td></td>
<td>Rinse</td>
<td>12,500</td>
<td>77</td>
</tr>
<tr>
<td>Peach</td>
<td>Peel</td>
<td>62,480</td>
<td>127</td>
</tr>
<tr>
<td></td>
<td>Rinse</td>
<td>6,674</td>
<td>18</td>
</tr>
</tbody>
</table>

1. The pH of all effluents was adjusted to pH 5.6 before autoclaving.
2. Peeling effluents after dilution 1:4 (vol/vol) with water.
3. These COD values are expressed as 100% at zero time in Fig. 2.
4. Shown as biomass at zero time in Fig. 3.
of dried potato effluent biomass increased by nearly four-fold as a result of fermentation whereas the amino acid content of peach effluent biomass was doubled. Similar trends were noted for rutabaga and potato rinse effluents which increased from 10.32 to 13.19% and from 9.64 to 31.37% total amino acids, respectively. The total amino acid content of the peach rinse effluent actually increased from 16.78 to 21.39% after 1 day of fermentation with N. sitophila.

The total amino acid yield on potato effluent is comparable to that reported by Anderson et al. (2). These researchers reported yields of 31.96 to 38.5% protein in dry N. sitophila cells grown on molasses and 41.59% when the mold was grown on a starch medium.

The sulfur-containing amino acids are limiting in fermented biomass from various plant sources tested. Diets into which these products might be incorporated would have to be supplemented with these amino acids or with other proteins wherein they are not limiting. Blending fungal mycelium with other protein-containing ingredients has been shown by several researchers to improve the biological value in animal diets. For example, Doctor and Kerur (11) reported that a diet containing protein derived from dried Penicillium chrysogenum and from peanut meal performed as well as a diet containing a comparable level of protein from casein when fed to rats.

The growth rate of fungi is dependent upon both the nutritional and environmental conditions of the growth medium. In the present study no attempt was made to optimize the carbon:nitrogen ratio, pH, dissolved oxygen content, or temperature for maximum conversion of effluents to protein. It is possible that a more extensive reduction of COD within a shorter period accompanied by higher protein yields could be achieved if these growth parameters were optimized.

It is not so far in the future that food processing plants will be able to offer for sale food waste protein products consisting partly of single-cell protein (SCP). A large U.S. brewery has conducted extensive testing on a SCP product recovered from an activated sludge treatment system. Bough et al. (7) have estimated that 322 million pounds per year of dried activated sludge could be produced from food processing and brewery wastes. Containing 28 to 36% protein, activated sludge products are similar in composition to the biomass products produced in this study by fermentation of carbohydrate wastes.

REFERENCES

FERMENTING ALKALINE FOOD WASTES


