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Utilization of polyvinyl alcohols for the Production of Single Cell Protein by Microbial Fermentation in Enclosed Systems: Final report for the RRTTC, 1996

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Barton Bergquist, James Jurgenson, Department of Biology, University of Northern Iowa

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Utilization of polyvinyl alcohols for the Production of Single Cell Protein by Microbial Fermentation in Enclosed Systems.

Principal Investigators

Bart Bergquist, Phone (319) 273-2723, FAX (319) 273-7125; **James Jurgenson,** Phone (319) 273-2539, FAX (319) 273-7125, Department of Biology, University of Northern Iowa

This project seeks to evaluate the utility of single and two trophic level microbial systems to produce single cell protein from polyvinyl alcohols. Both shaken batch and closed fermentor cultures will employ several parameters and species (bacteria and protozoan) to identify optimal species and environmental conditions.

Summary

Single cell protein was originally defined by Professor C.L. Wilson to represent the cells of microorganisms that were cultured for their protein content (cited in Scrimshaw, 1968). Various processes have been developed to mass produce microorganisms for their food content using non-nutritive carbon sources. Industrial processes utilize such compounds as methane, propane, diesel oil, C14-C20 n-alkanes, and paraffin waxes, to produce single cell proteins for animal and/or human consumption (Litchfield, 1977). This on-going study is evaluating the feasibility of using a two trophic level microbial system to use polyvinyl alcohol (PVA - a plastic degradation product) as a substrate for production of single cell protein. The microbial systems now under study consist of a bacterium which can degrade/assimilate polyvinyl alcohols and a particulate feeding ciliate which can use the bacteria as a nutrient. Each species is being evaluated for growth characteristics as individual species and in the two trophic level (two species) closed fermentation system. Successful demonstration of the two trophic level closed fermentation system will demonstrate methodology that may be applied to other bioremediation and biodegradation systems.

Project Objectives:

1. Evaluate the utility of cultures of selected single species of bacteria or protozoa to convert polyvinyl alcohols into a single cell protein for potential animal consumption.
2. Evaluate comparable parameters and conditions using a two trophic level system, containing both a bacterial and a protozoan specie, for the production of single cell protein.
3. Demonstrate the utility and methodology of these culture systems for later implementation as a system for bioremediation and biodegradation of other environmental pollutants.

Progress to Date

The first phase of the study is in process to determine the efficacy of culturing the bacteria and Tetrahymena as single species in PVA-based media. The cells were cultured in media that contains only inorganic salts and buffer. In an effort to preclude the influence of the tris buffer, we are also examining use of a phosphate buffer. In order to reduce any small organic contaminants that may be present in the PVA substrate, the solubilized PVA was dialyzed exhaustively against water for 24 hours prior to using it in the culture media.

To date we have evaluated 15 species/strains of bacteria for their ability to grow in PVA-based media and 4 have been found to grow in the PVA-based media (see Table 1). Strain JN-1, which was derived from a crude oil contaminated soil sample from Alaska shows the fastest rate of growth using PVA. In a 18 hour period strain JN-1 utilizes 8 -10% of the PVA in the original media (5% PVA [13-24 K MW], Tris-HCl pH 8.0, M9 salts and vitamins) (Table 2). The strain JN-1 mineralized nearly 0.5 gm/liter of PVA under these conditions. Control cultures without

addition of PVA showed no growth after 36 hours. Studies using changes in culture optical density with Tetrahymena found it to also grow in the same PVA-based media as used for bacterial culture (Fig. 1).

We have developed two assays which demonstrate the utilization of PVA. One assay adapted from a colorimentric assay for PVA, involves the staining of PVA in agar media using a Borate Iodine complex which stains PVA blue black in the agar media (Finley, J.H. 1961). Colonies growing on this media show zones of clearing around the colonies analogous to starch utilization assays (Figure 2). We have been able to quantitatively demonstrate the reduction in total PVA using HPLC molecular exclusion chromatography. Quantitative measurements resulting from integration under the relevant peaks have shown reduction of PVA concentrations of nearly 10 %.

During the summer of 1996 studies were conducted to examine the process of using bacterial cultures to feed protozoa. To facilitate the development of a fermentation protocol that would allow simultaneous culturing of both bacteria and protozoa an industrial waste carbon source was used in these trials. The corn syrup industry produces a large amount of fructose which is presently being discarded. This is another potential carbon source that could be converted into single cell protein just as the PVA. The advantage of working out our two tropic fermentation protocol with this material is that the conversion of carbohydrate into cell mass is more easily measured. Culture conditions for growth of *Tetrahymena thermophila* and bacterial species in fructose rich media were first established and then the cultures were mixed. It was discovered that bacterial cultures became so acidic that the protozoa were immediately killed when they were added to the culture. Buffering the culture with MOPS buffer allowed the protozoa to grow in the presence of the bacteria. It was also determined that both bacteria and protozoa grew optimally in the presence of 1.1 % fructose as added carbon source.

Table 1 A list of Bacterial strains used in this study

	Bacterial Strain	Origin of strain	Growth on PVA
1.	SP-262	Alaska	-
2.	SP-282	Alaska	+
3.	SP-306	Alaska	-
4.	JAT	Alaska	-
5.	JAO	Alaska	-
6.	Wt-C	Alaska	-
7.	JAB	Alaska	-
8.	JAD	Alaska	-
9.	JDC	Alaska	-
10.	JBB	Alaska	-
11.	JAP	Alaska	+
12.	Phen-B	Iowa	-
13.	Phen-C	Iowa	-
14.	Ochrobactrum anthropi	Carolina Biological	+
15.	JN-1	Alaska	++

All bacterial strains except for the one from Carolina Biological were obtained from the laboratory of Dr. Ed Brown, Environmental Sciences program, University of Northern Iowa, Cedar Falls, Iowa 50614

Table 2

PVA containing culture media composition per liter of media

1. 5.0 gms of PVA

2. 100 mls of 10XM9 salts containing (gm/l):

    3.0 gms Potassium phosphate monobasic
    6.0 gms Sodium phosphate dibasic
    1.0 gm Ammonia chloride
    0.5 gm Sodium chloride
    1.0 ml Magnesium sulfate
    1.0 ml Calcium chloride solution

3. 10 mls of 1M Tris-Cl pH 8.0
4. 40 ul of Biotin (0.05 mg/ml)
5. 10 ul vitamin B_{12} (0.05mg/ml)
6. 800 ul Ca-pantothenate (0.5mg/ml)
7. 800 ul Niacin (0.5mg/ml)
8. 800 ul Pyridoxine (0.5mg/ml)
9. 800 ul Thiamine (0.5mg/ml)
10. 400 ul PABA (0.5mg/ml)
11. 400 ul Inositol (0.5mg/ml)

785 ml of Deionized water to make 1 liter of media.

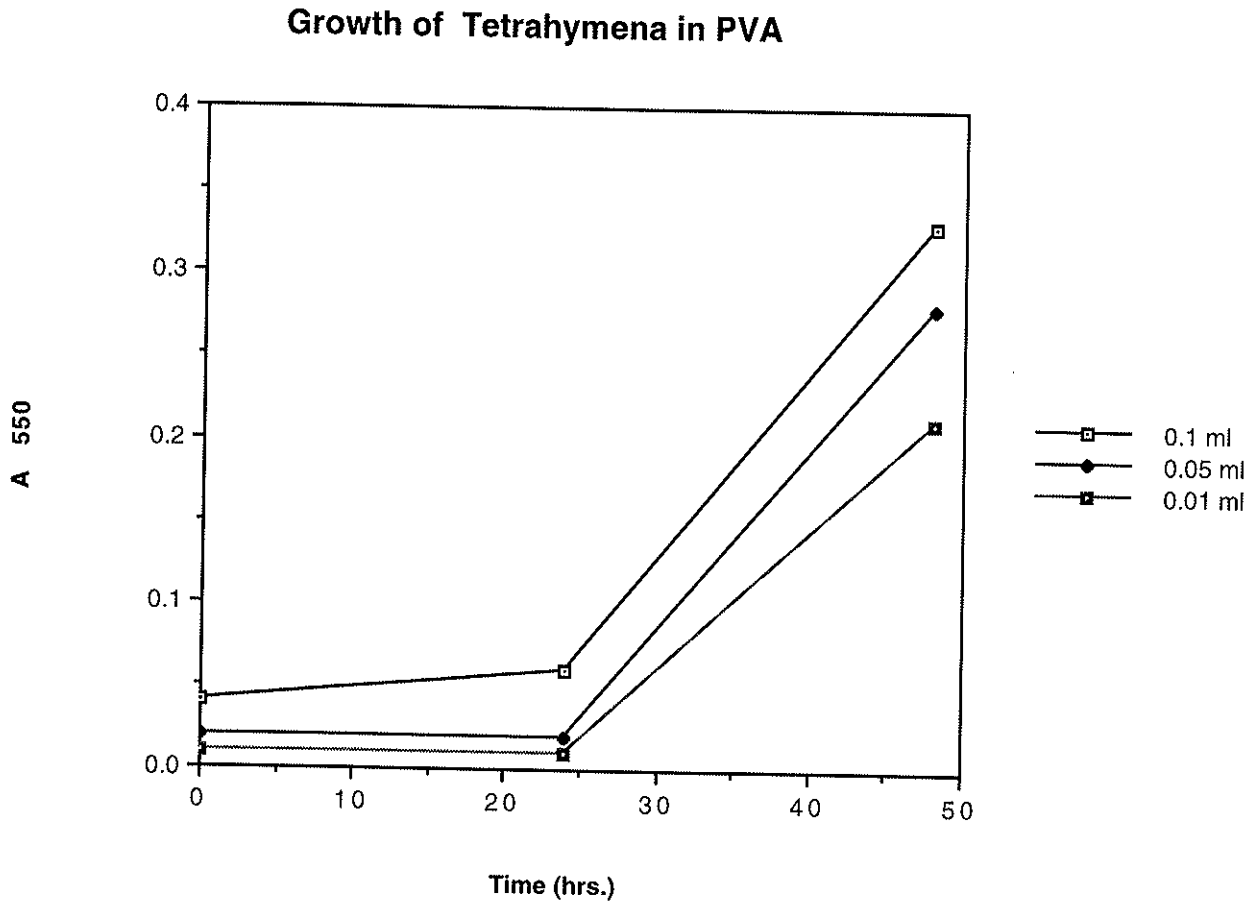

Figure 1. Growth of T. Thermophila in PVA based media. Cultures were started by inoulation with different initial volumes of stationary phase protozoan cultures.

Figure 2. Plate Staining Assay used to demonstrate PVA utilization