Stabilizing High-Strength Wastes with Photosynthetic Bacteria*

D.F. TOERIEN,[1] T.E. CLOETE,[1] P.J. DU TOIT[2] AND P.J. BOTES[3]

Institute for Environmental Sciences;[1] Department of Biochemistry;[2] Department of Microbiology,[3] University of the Orange Free State, P.O. Box 339, Bloemfontein 9300

Abstract

Preliminary studies were carried out on the use of photosynthetic bacteria in stabilizing high-strength wastes such as sewage sludge and cattle feedlot effluent. Small scale units were operated under laboratory as well as outdoor conditions. Productivities ranged from 15,9 to 44,9 g m^{-2} day^{-1}, the crude protein content of the photosynthetic bacteria was 66,4 ± 12%, and the amino acid spectrum compared favourably with that of other proteins. Fatty acids were removed with an efficiency of more than 90% and a photosynthetic bacterial-algal system reduced the chemical oxygen demand of cattle feedlot effluent by more than 90%.

Introduction

The world demand (excluding China) of high quality protein meals rose from 21,2 million tons (of soya bean meal equivalent) in 1955 to 52,6 million tons in 1970 and is expected to be 120 million tons in 1990 (Taylor and Senior, 1978). The problem in meeting these demands is illustrated by the fact that the demand for fishmeal is likely to reach 15 million tons of soy bean equivalent by 1990 but the predicted supply will only be 7,5 million tons (Taylor and Senior, 1978). Against this background, it is estimated (Taylor and Senior, 1978) that by 1985 there will be a world-wide market of 5 million tons per annum for novel proteins such as single cell protein (SCP).

High strength wastes such as sewage sludge, certain cannery wastes and effluents originating from feedlot systems, contain obnoxious organic material that could possibly be converted into useful products in the form of SCP. To convert waste material as quantitatively as possible to SCP, the use of photosynthetic bacteria has an advantage above the production of aerobic organisms because less organic matter is lost in the production of photosynthetic bacterial SCP. This is because in aerobic processes as much as 60 percent of the waste matter may have to be oxidized completely to yield energy for cell synthesis, whilst the purple non-sulphur bacteria utilize substrates such as fatty acids nearly quantitatively for cell matter synthesis as their energy needs are fulfilled from sunlight (Pfennig, 1967). Using these bacteria fermentable waste material could therefore possibly be nearly quantitatively converted into SCP.

The use of photosynthetic bacteria has received some attention in waste treatment systems, particularly in Japan (Kobayashi, 1975; Kobayashi and Maki, 1978), in South Africa (Toerien, 1976) and in the USA (Shipman *et al*, 1975). Kobayashi suggested that photosynthetic bacteria can effectively be used in foul water purification and for treating high-strength organic wastes with biological oxygen demand (BOD) values as high as 10 000 mg ℓ^{-1} resulting in final effluents with BOD values of 5 to 50 mg ℓ^{-1} through the action of heterotrophic bacteria, photosynthetic bacteria and algae (Kobayashi and Maki, 1978).

Kobayashi (1975) also indicated that photosynthetic bacteria are able to remove toxic and odorous substances such as hydrogen sulphide, putrescine, cadaverine and other amines from waste water. These compounds may inhibit the growth of algae in waste waters.

Shipman *et al.* (1975) and Kobayashi and Maki (1978) suggested that photosynthetic bacteria produced on agricultural waste products may be used as feed for livestock and fish and as fertilizer for fruit and grain crops. Toerien (1976) illustrated that photosynthetic bacteria occur in South African sewage treatment plants and could effectively remove fatty acids in laboratory cultivation systems.

Since photosynthetic bacteria show promise in the treatment of high-strength wastes, aspects of such an application in the laboratory and with small-scale outdoor units using sewage sludge and a feedlot effluent, have been investigated.

Materials and Methods

Photosynthetic Bacteria

An enrichment culture of photosynthetic bacteria was obtained by using the medium of Stanier *et al.* (1963) and inoculating with water samples obtained from ponds on the campus of the University of the Orange Free State. McCartney bottles (28 mℓ) were completely filled with the medium, sterilized and inoculated with 1 mℓ each of the various water samples. The enrichment cultures were incubated at room temperature under illumination of a single tungsten lamp (50 — 100 μ Einst. cm^{-2} s^{-1}). Positive enrichment cultures were obtained from most water samples after 7 to 10 days' incubation.

Examination of the cultures under the microscope revealed that *Rhodopseudomonas* spp. were present.

To build up sufficient quantities of the photosynthetic bacterial culture, the medium of Stanier *et al.* (1963) was used to fill a 5 ℓ aspirator bottle, inoculated from an enrichment culture and incubated either under the illumination of a tungsten lamp in the laboratory or outdoors in partial sunlight.

Treatment of High-Strength Wastes

Experimental Unit

The waste was fermented to produce fatty acids, followed by the growth of photosynthetic bacteria on this fatty-acid rich effluent

*Revised paper, originally presented at *Symposium on aquaculture in wastewater*, 24 26 November 1980, CSIR Conference Centre, Pretoria

and in some cases the growth of algae on the effluent from the photosynthetic bacterial system (Figure 1). The fermentation unit consisted of a 25 ℓ plastic container, provided with a funnel for the introduction of fresh wastes and an outlet system which kept the volume of the fermentation unit at 20 ℓ. No provision was made to mix the contents of this unit.

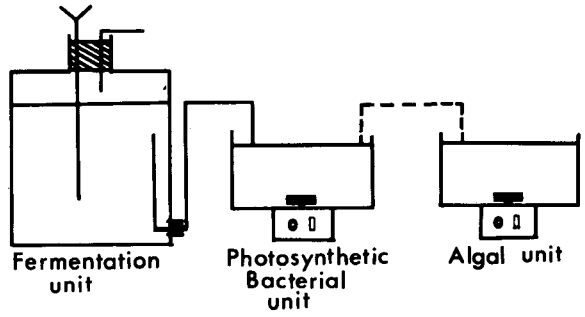

Figure 1
Schematic representation of experimental unit

The photosynthetic bacterial unit consisted of a plastic container which was filled to the 8 ℓ level and had a surface area of 830 cm^2. The contents of this unit was continuously mixed with the aid of a magnetic stirrer. A similar unit was used for algal growth.

The systems were operated on a daily draw and fill basis. For instance, a specific volume would be withdrawn from the algal unit, the same volume would then be withdrawn from the photosynthetic bacterial unit and added to the algal unit. Finally, the addition of the same volume of water or sludge to the fermentation unit would refill the photosynthetic unit (Figure 1). The average surface light intensity from an overhead tungsten light bulb was 102 μ Einst. m^{-2} s^{-1}.

The experiments were started in the laboratory using a closed system for the cultivation of the photosynthetic bacteria. However, following the suggestions of Kobayashi and Maki (1978), we changed to an open system for the cultivation of the anaerobic photosynthetic bacteria.

Several laboratory experiments were done in which raw sewage sludge from the Bloemfontein Sewage Treatment Plant was used. The fermentation unit was filled with sewage sludge diluted 1:1 with tap water. The unit was then left to ferment for about four to eight days before the feed to the photosynthetic bacterial unit (one litre per day) was initiated. Tap water was added daily to the fermentation unit which resulted in fermented liquor being added to the photosynthetic bacterial unit which was continuously illuminated with a single 100 W tungsten lamp.

Analyses

Temperature, pH and oxygen concentration were measured daily at specific times using a thermometer, a Methrom pH meter and a Yellowsprings oxygen meter respectively. Samples were withdrawn from each unit for determination of suspended solids (0,45 μm membrane filters and gravimetric determination after drying at 105°C), chlorophyll α (according to Lorenzen (1967) and Kjeldahl nitrogen, COD and BOD (according to APHA, 1975). Amino acid composition of air-dried material obtained after flocculation with a few mℓ of 5 N NaOH was determined according to Needleman (1970) on individual daily samples. Tryptophan was not determined at all, cystine was not determined on its own and corrections were made for losses. Fatty acid concentrations were determined gas chromatographically using a 2 m x 2 mm ID glass column packed with Poropak Q and a Hewlett-Packard 5830 A gas chromatograph with flame ionization detector (Botes, 1980).

Results

Productivity experiments

The growth of the photosynthetic bacteria resulted in a pH rise of one unit, suggesting the uptake of fatty acids (Table 1). The productivities of the first experiment ranged from 37,8 to 44,9 g m^{-2} d^{-1} and that of the second experiment from 15,9 to 30,6 g m^{-2} d^{-1}. These are remarkable productivities since the bacteria were laboratory cultured under continuous illumination of about 102 μ Einst. m^{-2} s^{-1} compared to peak radiation levels of 1 800 to 2 200 μ Einst. m^{-2} s^{-1} in bright sunlight.

TABLE 1
RESULTS OBTAINED IN TWO LABORATORY EXPERIMENTS WITH RAW SEWAGE SLUDGE AND EACH LASTING ABOUT THREE WEEKS

	Initial sludge concentration g ℓ^{-1}	Average suspended solids PBU g ℓ^{-1}	pH PBU	O_2 PBU mg ℓ^{-1}	pH fermentation unit
Experiment No 1	23,8	3,44 ± 0,3	7,0	0,5	N.D.
Experiment No. 2	15,3	1,93 ± 0,61	7,3	0,55	6,0

PBU — photosynthetic bacterial unit, N.D. — not determined

The photosynthetic bacteria should therefore be able to grow under outdoor conditions and a similar experiment was done under outdoor conditions. The sewage sludge concentration was 17,1 g ℓ^{-1} and this yielded an average suspended solids content in the photosynthetic bacterial unit of 2,33 ± 0,16 g ℓ^{-1} with the pH of the fermentation unit at 5,2 to 5,4, that of the photosynthetic bacterial unit at 7,0 to 8,0 and the oxygen concentration of the photosynthetic bacterial unit at 0,5 to 0,9 mg ℓ^{-1}. The productivity of the system ranged from 26,1 to 30 g m^{-2} d^{-1}.

These productivities compare very favourably with that of algal mass cultivation systems (e.g. Goldman, 1979) and suggested that photosynthetic bacteria could be used in the stabilization of high-strength wastes, such as sewage sludge, whilst at the same time a potentially valuable product in the form of photosynthetic bacterial cells could be produced.

Protein content of photosynthetic bacterial cells

The average crude protein content of 22 samples was 66,4 ± 12,0%. The cells had a high protein content and the bacterial biomass showed potential as a SCP.

Amino acid composition

The amino acid spectrum of the photosynthetic bacteria compared quite favourably with that of other proteins (Table 2). Consequently, further investigations on the use of photosynthetic bacteria as a source of SCP seemed to be justified, especially since these results were obtained using sewage sludge of which the handling is a problem in South Africa (Water Research Commission, 1979).

Removal of Fatty Acids

Fermented sewage sludge liquor contained a variety of lower fatty acids including all the C_2 to C_5 forms and isomers (Table 3). Soon after the start of an experiment to stabilize a batch of sewage sludge, the fatty acid removal efficiency was 98,0% (from 1 323 mg ℓ^{-1} to 29 mg ℓ^{-1}, Table 3). As the fatty acid content of the fermented sewage sludge liquor decreased, the removal efficiency decreased to about 90% and the fatty acid content of the photosynthetic bacterial unit increased to about 80 mg ℓ^{-1}. At the termination of the experiment when only 117 mg ℓ^{-1} of fatty acids were still present in the fermented sewage sludge liquor, the removal efficiency decreased to about 52%.

Fatty acids were therefore effectively removed by the photosynthetic bacterial unit especially when it was receiving a high load and removal efficiencies of 90% or more may be achieved (Table 3).

Experiments with Cattle Feedlot Wastes

A liquid effluent from a cattle feedlot was obtained and fermented for two days prior to introducing it to an outdoor continuously stirred photosynthetic bacterial culture (8 ℓ) in an

TABLE 2
AMINO ACID CONTENT OF PHOTOSYNTHETIC BACTERIA PRODUCED IN FERMENTED SLUDGE LIQUOR AND COMPARISONS WITH OTHER PROTEINS (g 16 g^{-1} N)

Amino acid	Photosynthetic bacteria from sewage sludge	Photosynthetic bacteria from agricultural products[1]	*Acinetobacter calcoaceticus*[2]	Soybean meal[3]	Fish meal[4]	Microalgae biomass[5]
Isoleucine	5,3	3,7	5,7	5,8	4,6	3,2 — 4,4
Leucine	9,3	7,7	7,7	7,6	7,3	6,6 — 9,3
Lysine	3,9	5,8	7,0	6,6	7,0	5,0 — 5,7
Methionine	2,2	3,0	2,3	1,1	2,6	1,4 — 2,1
Cystine	n.a.	n.a.	0,6	1,2	1,0	0,7 — 1,4
Phenylalaline	6,4	4,5	4,3	4,8	4,0	3,6 — 5,0
Threonine	6,4	3,6	4,4	3,9	4,2	4,8 — 5,8
Tryptophan	n.a.	n.a.	0,6	1,2	1,2	1,2 — 1,4
Valine	6,7	6,7	6,6	5,2	5,2	6,2 — 7,2
Histidine	1,5	3,6	1,6	n.a.	n.a.	1,5 — 1,7
Arginine	5,7	n.a.	6,9	n.a.	n.a.	5,3 — 6,3
Aspartic acid	11,1	n.a.	9,6	n.a.	n.a.	n.a.
Serine	5,3	n.a.	3,5	n.a.	n.a.	n.a.
Glutamic acid	11,9	n.a.	12,1	n.a.	n.a.	n.a.
Proline	3,6	n.a.	3,9	n.a.	n.a.	n.a.
Glycine	6,3	n.a.	7,1	n.a.	n.a.	n.a.
Alanine	9,2	n.a.	6,9	n.a.	n.a.	n.a.
Tyrosine	3,0	n.a.	3,6	n.a.	n.a.	2,8 — 3,6

n.a. = not available
1 = Shipman *et al.* (1975); 2 = Du Preez (1980); 3 = Forage and Righelato (1978); 4 = Shacklady and Gatumel (1972); 5 = Soeder (1979)

open plastic container. The effluent of this unit was fed to a similar container containing a green algal suspension (8 ℓ). The units were operated on either a four or an eight day retention period. The pH increased by more than two pH units in the photosynthetic bacterial unit under both autumn and winter conditions (Table 4), suggesting that photosynthetic bacteria were able to withstand the colder temperatures associated with winter conditions. The efficiency of COD reduction through the two systems was 90% or higher whilst chlorophyll *a* concentrations in excess of 840 μg/ℓ indicated good algal growth even under winter conditions.

Discussion

The use of photosynthetic bacteria in the treatment of high-strength organic wastes such as sewage sludge and feedlot ef-

TABLE 3
THE CONCENTRATION (mg ℓ^{-1}) AND COMPOSITION OF THE LOWER FATTY ACIDS BEFORE AND DURING TREATMENT WITH PHOTOSYNTHETIC BACTERIA

	Date	Total acids	Acetic acid	Propionic acid	Isobutyric acid	*n*-butyric acid	Isovaleric acid	*n*-valeric acid	Removal ciency %
Fermentation unit	22/2	1 323	443	538	27	161	49	105	
PBU		29	29	0	0	0	0	0	98,0
Fermentation unit	26/2	1 075	372	461	27	114	35	66	
PBU		42	28	14	0	0	0	0	96,1
Fermentation unit	28/2	799	271	330	24	67	42	65	
PBU		82	59	14	0	0	9	0	89,7
Fermentation unit	12/3	117	32	35	13	0	24	13	
PBU		56	25	22	0	0	9	0	52,1

PBU = photosynthetic bacterial unit

TABLE 4
THE TREATMENT OF CATTLE FEEDLOT EFFLUENT WITH THE AID OF PHOTOSYNTHETIC BACTERIA AND ALGAE

AUTUMN CONDITIONS (12°C at 08h00, 23°C at 14h00)
Retention period 8 days in both units

Sample	pH	Oxygen mg/ℓ	BOD mg/ℓ	COD mg/ℓ	Chlorophyll μg/ℓ
Before fermentation	5,6	0,4	500	25 920	n.d.
After fermentation	5,3	0,4	480	14 634	n.d.
Photosynthetic bacteria	7,4	1,0	450	11 480	n.d.
Algae	9,6	14,0	190	1 842	1 200

WINTER CONDITIONS (0 to 3°C at 08h00, 20°C at 14h00)
Retention period 4 days in photosynthetic bacterial unit and 8 days in algal unit

Sample	pH	Oxygen mg/ℓ	BOD mg/ℓ	COD mg/ℓ	Chlorophyll μg/ℓ
Before fermentation	5,5	0,3	305	15 000	n.d.
After fermentation	5,4	0,35	290	7 680	n.d.
Photosynthetic bacteria	7,6	0,3	280	5 376	n.d.
Algae	8,8	0,4	215	1 536	840

n.d. = not determined

fluents is feasible and yields a product high in protein and with a fairly well balanced amino acid spectrum. The results obtained in this study confirm the optimism of Kobayashi (1975) about the use of photosynthetic bacteria in foul water purification.

A removal of more than 90% of the COD from a waste such as a feedlot effluent occurred, yielding at the same time material high in protein (photosynthetic bacteria and algae), and suggesting that material of negative value (such as high-strength wastes) could be used to produce valuable products.

Since photosynthetic bacteria-algal production systems would not be operated under sterile conditions, these systems may be less costly to construct and operate compared to other SCP processes envisaged for use on waste products (e.g. Romantshuk, 1975). As a consequence an economic advantage might be derived by producing photosynthetic bacteria and algae in non-sterile systems operating on high-strength wastes.

References

AMERICAN PUBLIC HEALTH ASSOCIATION (APHA) (1975) *Standard Methods for the Examination of Water and Wastewater*, 14th Ed., Washington, USA.

DU PREEZ, J.C. (1980) *Growth Kinetic Studies of Acinetobacter calcoaceticus with Special Reference to Acetate and Ethanol as Carbon Sources*, Ph.D.-thesis, University of the Orange Free State, Bloemfontein.

FORAGE, A.J. and RIGHELATO, R.C. (1978) Microbial protein from carbo-hydrate wastes, pp. 59–94. *In: Progress in industrial Microbiology*, Volume 14, M J Bull, Editor, Amsterdam, Elsevier Scientific.

GOLDMAN, J.C. (1979) Outdoor algal mass cultures. I. Applications. *Wat. Res.* 13 1 19.

KOBAYASHI, M. (1975) Role of photosynthetic bacteria in foul water purification. *Progr. Water Technol.* 7 309–315.

KOBAYASHI, M. and MAKI, T. (1978) Production of useful materials by photosynthetic bacteria. *Abstracts XII Internat. Congr. Microbiol., Abstract no S16,5, Munich*, August 1977.

LORENZEN, C.J. (1967) Determination of chlorophyll and phaeopigments: spectrophotometric equations. *Limnol. Ocean.* 12 343–346.

NEEDLEMAN, S.B. (1970) Protein sequence determination. *In: Molecular Biology, Biochemistry and Biophysics*, Vol. 8, Springer Verlag.

PFENNIG, N. (1967) Photosynthetic bacteria. *Ann. Rev. Microbiol.* 21 285–324.

ROMANTSHUK, H. (1975) The Pekilo-process: protein from spent sulfite liquor, pp. 344–356. *In: Single Cell Protein II.* S.R. Tannenbaum and C.I.C. Wang, Editors, Cambridge, MIT Press.

SHACKLADY, C.A. and GATUMEL, E. (1972) The nutritional value of yeast grown on alkanes, pp. 27–52. *In: Proteins from Carbohydrates*, H Gounelle de Pontanel, Editor, New York, Academic Press.

SHIPMAN, R.H., KAO, I.C. and FAU, L.T. (1975) Single cell protein production by photosynthetic bacteria cultivation in agricultural byproducts. *Biotechnol. Bioeng.* 17 1561–1570.

SOEDER, C.J. (1979) Chemical composition of microalgal biomass as compared to some other types of single cell protein (SCP). Contribution at the *Seminar on Algae Production*, NRC Cairo-Dokki, Egypt, 13 November 1979.

STANIER, R.Y., DOUDOROFF, M. and ADELBERG, E.A. (1963) *General Microbiology*, Second Ed., London, MacMillan.

TAYLOR, I.J. and SENIOR, P.J. (1978) Single cell proteins: a new source of animal feeds. *Endeavour* (New Series) 2 31–34.

TOERIEN, D.F. (1976) Occurrence and possible use of non-sulphur purple bacteria in effluent treatment processes. *S. Afr. J. Sci.* 72 148–194.

WATER RESEARCH COMMISSION (1979) *Current sludge treatment and disposal practices at major South African Centres.* Situation statement, Report, Pretoria.