

AGROINDUSTRIAL WASTE PRODUCTS AS SOURCES OF CHEAP SUBSTRATES FOR ALGAL SINGLE-CELL PROTEIN PRODUCTION

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ABSTRACT

Four types of agroindustrial waste products were tested for their suitability as substrates for *Chlorella* single-cell protein production. Based on cell density on day 7, unsterilized rice straw filtrate, Tris-buffered inorganic medium (control), unsterilized slop (1%) and unsterilized rice hull filtrate were suitable culture media. Dry weight yield after two weeks did not vary among media. Gross protein content of algae was highest in rice straw with or without sterilization.

Substrates for algal SCP production are assessed on the basis of their nutrient content, pH, and hygienic acceptability.

INTRODUCTION

Mateles & Tannenbaun (1967) in an overview of the status of algal single-cell protein (SCP) production, proposed the establishment of national centers in the United States for studies in the production and utilization of algae. They conceptualized two types of centers operating in tandem, one to work mainly with algal biomass production using inorganic media and hygienic wastes, the other, to serve as model integrated agroindustrial complex that will produce human- and animal-consumable algae and also regenerate and recycle matter for resource and energy conservation. In the Philippines, with the biotechnological thrusts spearheaded by the National Institutes of Biotechnology and Microbiology (BIOTECH) in 1980, a significant boost to algal SCP production was realized. Recently, the United Nations Interim Fund for Science and Technology for Development approved a Philippine proposal to undertake research on microbial SCP

production, utilization, nutrition, and marketing. The organisms under study are the bacteria, yeasts, filamentous fungi, and algae. The combined financial and technological backing provided in the research grants places applied algology, at least at the University of the Philippines at Los Banos (UPLB), on a sustained basis for a minimum of three years. But this is not to say that algal SCP production in the Philippines is in its primordial stage. Noticeable strides during the early seventies include the establishment of cultural requirements, production in synthetic media and animal manure, and feeding experiments involving fish, poultry, and swine (Pantastico & Sulabo 1974; Martinez 1976; Rigor et al. 1980). These past efforts have gone up to the pond production scale.

Chlorella, whose potential as protein feed for swine (Rigor et al. 1980) and as an unconventional protein food for man (Lee et al. 1967) is firmly established, consists of from 50 to 65% protein (Endo & Shirota 1972). Mass cultures of the alga yield from 50 to 65 g (dry weight) per litre of medium under heterotrophic or mixotrophic conditions (Tamiya 1968). Local endeavors, as computed from reports, have realized yields of 0.02 g/l/day in production ponds, reflecting a maximum photosynthetic efficiency of 3.2% (Rigor et al. 1980; Martinez 1980). Evidently, much still awaits algal SCP research in terms of increasing dry matter yield under Philippine conditions.

The presently recognized primary concerns in algal SCP research in the Philippines are the following:

To increase the kinds of test materials as substrates for SCP production with emphasis on those whose product is fit for human consumption;

To establish the optimum cultural conditions with selected media necessary for a pilot-plant production scale;

To expand utilization research after a thorough testing and evaluation of reported processes;

To promote toxicological and epidemiological researches on identified SCP; and

To appraise the marketability of the algal product.

Recognizing the importance of these inextricably linked objectives, we have addressed some of our efforts on the first goal which is to widen the range of test materials for substrates. The preliminary findings here reported involved the use of rice straw, rice hull, molasses, slop, inorganic medium, and Tris-buffered inorganic medium. The general objective is to evaluate the suitability of these materials as sources of algal culture media based on their nutrient content, pH, amount of algal biomass produced, and protein yield of the algal product.

METHODOLOGY

Rice hull and rice straw filtrates were prepared from ashes of the corresponding plant materials. Rice straw, as it is burned by farmers in the field, is a mixture of rice straw and leaves and rice panicle residues. A 10% (w/v) suspension of the ash was prepared with distilled water and passed through coarse filter paper. The pH of the media was adjusted to 9 by addition of 1N KOH. No attempt was made to remove the color of the filtrates, orange-brown in rice hull and bright yellow in rice straw.

Slop and molasses, two by-products of sugar refineries, were separately diluted to 1% concentration by addition of distilled water and their pH adjusted accordingly. For the control, Tris-buffered inorganic medium (pH 9) was used. Aliquots (290 ml) of the various media were dispensed in sterile 500-ml culture bottles and the latter plugged with cotton. Media requiring sterilization were autoclaved at 20 psi for 15 minutes. Chemical analyses of uninoculated media followed involving the following nutrients: total nitrogen by acidimetric method; available phosphorus ($\text{PO}_4^{=}$), stannous chloride method; calcium and magnesium, EDTA titrimetric method. All analyses had two replicates except for nitrogen and phosphorus with three.

Chlorella vulgaris M-3 was obtained from the BIOTECH culture collection at UPLB. Ten-day old cultures were rinsed and suspended in sterile distilled water to make an inoculum of density 12.7×10^6 cells/ml. The final volume of the medium after inoculation was 320 ml.

Cultures were maintained inside a greenhouse where temperature and light conditions fluctuated naturally. They were gently swirled and reoriented twice daily. Data on cell density were obtained at four-day intervals for two weeks using a completely randomized design. All randomly selected replicates of a treatment were discarded after cell density determination. On the 14th day, which followed the last day of cell density determination, cultures were filtered and their dry weight obtained. Then, the total nitrogen and available phosphorus contents of the harvested biomass were determined using the Kjeldahl and molybdovanadophosphoric acid methods, respectively.

RESULTS

Cell density and dry weight

The trends in growth response of *Chlorella vulgaris* M-3 measured in terms of cell density are shown in Fig. 1. Growth was minimal in all types of sterilized media during the first four days. On the other hand, all unsterilized media exhibited better algal growth than did the control. Apparently, *Chlorella* underwent a period of adaptation in sterilized media

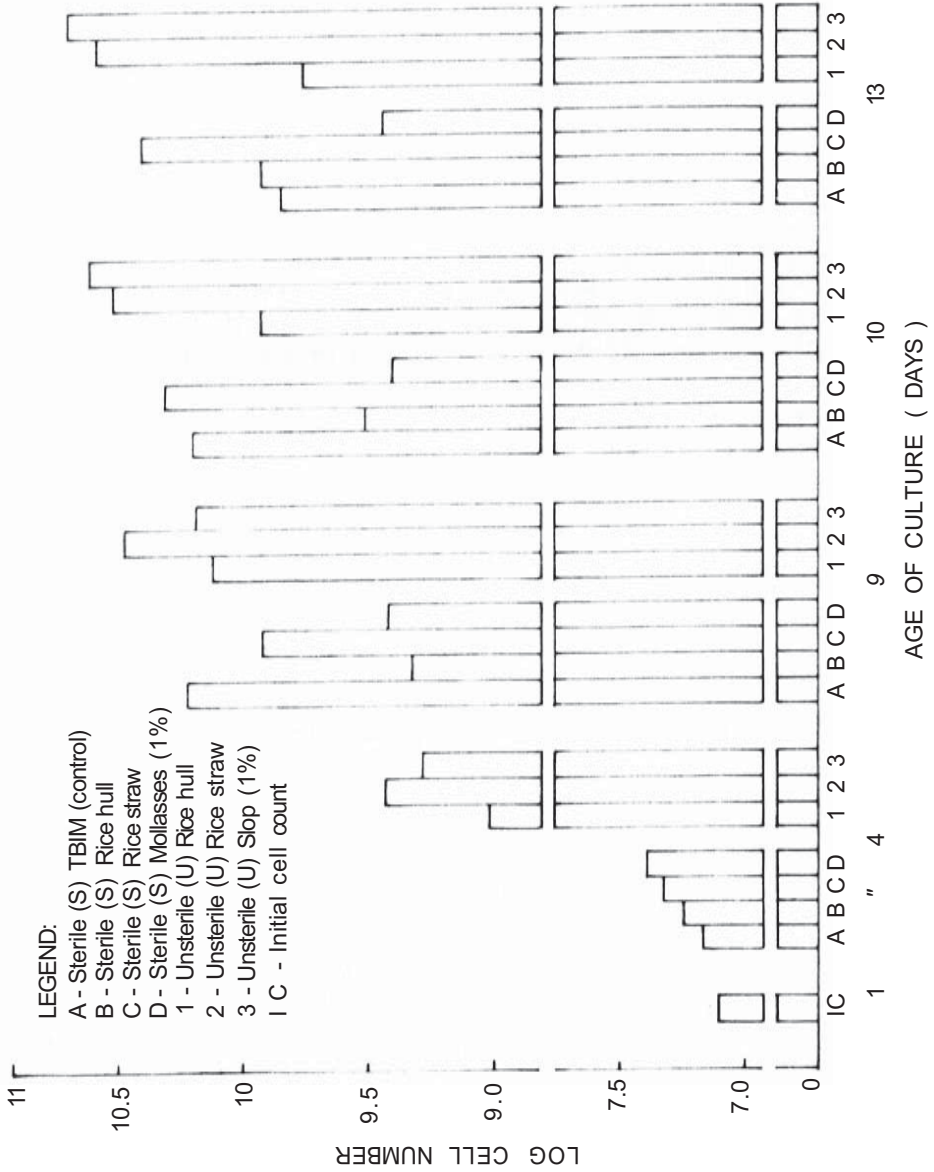


Fig. 1. Trends in growth response of *Chlorella vulgaris* M-3 in different substrates.

during the first four days which was characterized by a pronounced slowing down of growth rate. This was less expressed in unsterilized media.

Indications of substrate suitability became appreciable on day 7. Statistical analyses (Table 1) showed that around the said day, unsterilized rice straw filtrate (30×10^9), TBIM, the control (16×10^9), unsterilized slop (15×10^9), and unsterilized rice hull (13×10^9), in that order, were suitable substrates for *Chlorella* production. Results of Duncan's multiple range test also showed that in general, day 7 is the ideal time for harvest.

The cell doubling time (Stockner & Costella, 1976) of the alga in various media was shortest (7 h) in the control and longest (24 h) in unsterilized slop. An 8-h cell doubling time is normal for the alga (Kingsbury, 1968). Considering the computed averages on day 7, a desired amount of harvest may be achieved at regular intervals if a careful manipulation of the amount of inoculum is made. The possibility of this, however, depends on whether or not the adaptation period of four days as seen in some media could be shortened by preconditioning stock cultures in appropriate media.

The impressive growth behavior of the alga in the slop medium is of particular interest. Compared with molasses, slop has a lighter brown color which does not disappear in two weeks. If this brown color interfered with the degree of illumination of algal cells to an extent that was not determined, then the alga may have compensated for light limitation through heterotrophic growth which is normal in cultures supplied with organic nutrients. Another possible explanation for growth in slop is that it may have supplied certain nutritive substances, possibly organic, that promoted algal multiplication.

Statistical analysis of the dry matter yield (Table 2) showed no difference among the various substrates. This result, which runs counter to

Table 1. Growth of *Chlorella vulgaris* M-3 after seven and thirteen days; cell density at day 0, 0.013×10^9

Media	Cell density ($\times 10^9$)	
	7 days	13 days
Sterilized TBIM (control)	16 ^b	7 ^d
Sterilized molasses	3 ^f	3 ^d
Sterilized rice hull	2 ^d	4 ^d
Sterilized rice straw	9 ^c	24 ^d
Unsterilized slop	15 ^d	51 ^d
Unsterilized rice hull	13 ^{bc}	6 ^d
Unsterilized rice straw	30 ^a	38 ^b

Means with the same letter(s) are not significantly different from each other at 0.05% level, DMRT.

expectations, is attributed to the presence of bacterial contaminants and suspended particles inherent in the media. These interfering substances notwithstanding, it is well to take a cursory look at the dry matter yield data for the purpose of comparing them with those in literature.

Thin-layer (1 cm) cultures of *Chlorella*, considered by Milner et al. (1978) as impractical, yield greater than 50 g dry weight/l/da. The present yields range from 0.008 g/l/da in sterilized rice straw to 0.016 g/l/da in slop and molasses. Assuming the bacterial contaminants to be negligible, the computed biomass closely approximates local harvests from production ponds (0.02 g/l/da) (Rigor et al. 1980; Martinez 1980).

To further evaluate the suitability of the substrates for algal SCP production, the nitrogen content of the resulting biomass was also analyzed (Table 2). Algae grown in sterile or non-sterile rice hull filtrate yielded the highest nitrogen content of from 7.3 to 7.5%, this range exceeding the control by at least 62%. All other yields including that of the control had lower nitrogen content ranging from 3.8 to 5.9%. Multiplying the total nitrogen values by 6.25, the gross protein content was obtained (Table 2). *Chlorella* in rice hull filtrates had roughly 50% protein while the others had from 24 to 37%. The protein content of the former compares favorably with the normal quantities cited by Endo & Shirota (1972) for the alga.

Table 2. Dry matter yield, nitrogen and gross protein contents of *Chlorella vulgaris* M-3 grown in different substrates

Media	Dry matter yield (mg)	N content (%)	Gross protein content (%)
Sterilized TBIM (control)	27.87 ^a	4.5 ^{ab}	25.6
Sterilized molasses	34.83 ^a	3.8 ^{ab}	23.8
Sterilized rice hull	17.90 ^a	7.3 ^{ab}	45.6
Sterilized rice straw	24.80 ^a	5.9 ^{ab}	36.9
Unsterilized slop	35.17 ^a	4.5 ^{ab}	25.6
Unsterilized rice hull	32.66 ^a	7.5 ^{ab}	46.9
Unsterilized rice straw	24.83 ^a	4.1 ^{ab}	25.6

Means with the same letter(s) are not significantly different from each other at 0.05% level (DMRT).

Media Analysis

To explain the above results on cell density and protein content, mineral analysis of the uninoculated media was undertaken (Table 3). The highest level of nitrogen was in unsterilized rice straw filtrate which had an average of 2.91 ppm total nitrogen, roughly eight times that of the control (0.37 ppm). Unsterilized slop and sterilized molasses had moderate levels, 1.87 and 1.59

ppm, respectively, whereas filtrates of unsterilized rice hull, sterilized rice straw and sterilized rice hull together with the control had low levels of 0.99, 0.47, 0.37 ppm, respectively.

With respect to phosphorus, sterilized rice hull filtrate contained the highest amount at 969 ppm followed by unsterilized rice hull and unsterilized rice straw with 813 and 532 ppm, respectively. The P content of slop also exceeded that of the control by over 50%, but molasses is relatively "impoverished" in P with an average of 13.3 ppm. It is quite easy thus to appreciate the fact that rice plant remains are promising materials, nutrient-wise, for use in preparation of media for algal SCP production.

The calcium and magnesium levels of the media were also analyzed. Amounts of these nutrients are similarly abundant (Table 3).

The nutrient content of all media is apparently sizeable, even excessive, for some nutrients. More algal biomass could have been harvested had the density of inoculum been increased. This could have been realized with greater certainty if the algal inoculum had been preconditioned to the appropriate culture medium.

Correlation

Attempts to correlate cell density with the independent variables revealed two interesting relationships: nitrogen and pH positively correlated with cell density. The relationship with respect to N suggests that this nutrient may have limited algal response to various media. The proportion of P to N is tremendously lopsided (Table 3).

The positive correlation between pH and cell density on day 7 invites attention. Among the media, rice straw with or without sterilization underwent the least departure from its initial pH of 9. Unsterilized slop

Table 3. Initial levels of nutrients and final pH of different substrates

Media	Nutrient concentration (ppm)				
	N	P	Ca	Mg	pH
Sterilized TBIM (control)	0.37 ^c	177.7 ^c	182 ^c	179.8 ^{ab}	10.0 ^a
Sterilized molasses	1.59 ^b	13.3 ^f	683 ^a	179.8 ^{ab}	4.5 ^f
Sterilized rice hull	0.37 ^c	968.7 ^a	78 ^c	212.2 ^a	7.6 ^d
Sterilized rice straw	0.47 ^c	532.0 ^c	117 ^c	185.0 ^{ab}	9.3 ^b
Unsterilized slop	1.87 ^b	230.7 ^c	455 ^b	95.6 ^{ab}	8.4 ^c
Unsterilized rice hull	0.99 ^{bc}	812.7 ^b	455 ^b	41.7 ^b	7.3 ^e
Unsterilized rice straw	2.91 ^a	417.7 ^d	390 ^b	38.6 ^b	9.5 ^o

Means with the same letters) are not significantly different from each other at 0.05% level (DMRT).

followed with pH 8.4. Rice hull dropped to around neutral, pH 7.5, while molasses became acidic with pH 4.5. The control became more alkaline, pH 10 (Table 3). These findings seem to indicate that in addition to the combined growth effects of undetermined factors in the medium, the pH factor exerts an appreciable effect on the ability of algal cells to multiply. Thus among the media, rice straw filtrate elicited better growth probably because it retains a pH level closest to that preferred by *Chlorella*, around pH 9. The same reasoning probably holds for the slop medium. There is also the possibility that unchecked pH levels had pronounced effects upon 2-week old cultures. These results emphasize the importance of a sustained pH optimum to ensure maximum sustainable yield.

DISCUSSION

The current trends in human population growth reiterate the underscored needs of the past decade for the formulation of policies that would solve the food, energy, and population problems. Single-cell proteins have time and again been turned to, reportedly as unconventional protein sources. Setting aside the issue of individual taste preferences and cultural taboos on food, it is incumbent upon the SCP researcher to recommend foodstuffs like algae which are grown under hygienic conditions. Not only must the culture be hygienically acceptable by the best of human standards; it also must generate the minimum of affront to human senses. Domestic and animal wastes clearly hold a lot of promise in terms of their ability to generate biomass fit enough for farm animal consumption. But knowing the vast areas required to meet the necessary production rate, it is not difficult to imagine the degree of pollution of the air over the production site involving animal wastes.

Rice straw and rice hull as sources of culture media have a good potential for use in algal SCP production. They are hygienic substrates in that they do not lead to fouling of the air. They have the following advantages, namely, 1) cheapness, 2) availability, 3) simplicity of preparation, 4) nutrient sufficiency, 5) pH adequacy, 6) potentially high protein yield of the algal product, and 7) recycleable residues. Slop is another medium with a high nutrient content. A cheap hygienic substrate, it has the consistency and taste of molasses, not to mention its appetizing aroma. This substrate also enables heterotrophic growth of the algal SCP, supplying as it does the energy and carbon dioxide requirements of the culture organism.

Preliminary as these findings are, some important thrusts of immediate efforts in algal SCP production become identifiable. First is the need to establish the optimum concentrations of the plant filtrates and slop used. Second is the need to undertake a complete nutrient analysis of the media in order to determine what nutrient enrichment procedures are necessary.

Residual nutrients do have to be determined to establish the optimum amount of the inoculum. Third is the need to determine what size of inoculum is optimum for the highest sustainable yield to be realized. Fourth is the need to establish what culture maintenance procedure to undertake for maximum sustainable yield. Lastly, the need is felt for a systematic enumeration of microorganisms that contaminate the algal cultures. It is only after these immediate problems shall have been solved that one may embark on the formulation of algal food preparations fit for human consumption. The next few years will see renewed efforts to accomplish these goals.

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