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Microbiological culture broth designed from food waste

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ABSTRACT

The current trend of increasing air, water, and soil pollution is, in part, due to inadequate management of municipal solid waste (MSW). The relationship between public health and the collection, storage and improper disposal of solid waste has encouraged several studies and the results were attributed to the spread of over twenty human and animal diseases due to this interrelationship.

The term "single cell protein" (SCP) refers to microbial biomass used as a dietary additive. It has high nutritional value because of its high content of vitamins, lipids, and proteins of biological quality (the presence of all essential amino acids) (Lal, 2005). The aim of this work was to design a culture media for microbiological assays and to produce SCP for animal feeding, using nutrients contained in organic waste. In order to compare the effectiveness of food waste (FW) and LAPTg media, different strains of *Lactobacillus*, *Enterococcus*, *Staphylococcus*, *Shigella*, *Salmonella*, *Saccharomyces* and *Schizosaccharomyces* were studied. In all cases, the growth obtained from FW and LAPTg culture media were not significantly different ($p > 0.05$). In addition, the growth of *Saccharomyces cerevisiae* was studied in order to produce SCP for animal feeding. Comparative experiments involving molasses broth, FW broth, and basal broth were carried out. The biomass yield calculated at 24 h from FW broth was 13% lower than from molasses broth. The FW broth provided a significantly lower biomass yield; however, it can be very useful in areas where molasses are not available. FW broth can be elaborated at low cost, in any populated region of the world because its ingredients are wastes generated by humans. It has great versatility, allowing the development of a wide variety of microorganisms, both Gram negative and Gram positive bacteria as well as yeasts. The production of safe protein additives, with high biological quality and low cost, is necessary due to the increasing global demand for food for humans and animals.

1. Introduction

Packaging, bags, paperboard cartons, batteries, and food waste are all included in municipal solid waste (MSW) and they represent an important source of destruction to the environment. In many Latin American cities, there are still open dumps that cause extreme pollution. The issue is serious because, after filtering, the wide range of waste components is able to contaminate the groundwater; and there is no available technology to revert this damage (Bertolino et al., 1995). At other times, solid wastes are taken to landfills, defined as the storage of waste in dug areas, where alternating layers of trash and soil are carefully compacted. For each landfill, it is important to choose a suitable area considering geology and topography conditions in order to prevent contamination of the surface or groundwater. In addition, the walls should be waterproofed with polyethylene to prevent seepage to lower layers (Mendes et al., 2003).

The anaerobic decomposition of organic waste generates a mixture of carbon dioxide and methane (CEPIS-REPINDEX 49/50: Residuos Sólidos, 1994). To prevent explosions, the landfill should have good ventilation or internal pipelines to remove gases. Furthermore, the increase of methane and/or carbon dioxide in the atmosphere is involved in the greenhouse effect (Díaz, 2000). These gases can generate electricity, heating, or fuel for vehicles. However, during combustion, they can pollute the air when the emission is directly conducted to the atmosphere. After the landfill is full, it is covered with a layer of clay (to prevent the escape of odors and filtering storm) and several layers of sand and humus (to allow vegetation growth).

Open dumps are common in Northwestern Argentina, where the average annual rainfall is 1000 mm (National Meteorological Service – Air Force Argentina – Climatology Department www.

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meteofa.mil.ar). For this reason, the location of landfills, even including a good waste treatment plant, must be carefully chosen.

According to a report from the Federation of Environmental Organizations, in Tucumán, Argentina, more than 400 open dumps are situated less than 300 m from 55% of the houses in the province (World Bank, 2006). Food waste most likely accounts for most of the MSW generated. Adhikari (2005) reported that the monthly average residential food waste production was 0.61 ± 0.13 kg/capita/day and that of a restaurant was 0.56 ± 0.23 kg/customer/day. These data were collected from Montreal, Canada, but these values could apply to all cities where the population is higher than 200,000 inhabitants.

Microorganisms useful as protein additives (single cell protein; SCP) should have the following properties: rapid growth, little nutritional requirements, an easy processing system, non-pathogenic, non-toxic, low levels of nucleic acids and good biological values. Therefore, yeasts are most commonly employed for this purpose; but bacteria, algae and fungi have also been used (NRC, 2007). The protein content of yeast cells does not exceed 60%, but the concentration of essential amino acids (lysine, tryptophan, threonine, and also methionine and cysteine) is satisfactory. These eukaryotic cells are also characterized by being rich in vitamins (group B) and the percentage of nucleic acids ranges from 4 to 10%, depending on the species. Another advantage over bacterial cells (≅1 μm) is their larger size (5–10 μm), thus facilitating separation. Species with a higher content of nucleic acids can be used without causing side effects (precipitation of urate crystals) in ruminants. However, monogastric animals (porcine) require more detailed studies related to this issue. Yeasts have been found to be suitable as food additives for pigs because they are able to exert beneficial effects such as growth promotion, stimulation of the immune system, and increased nutrient uptake (García Sedano, 2004).

Untill now, vermiculture has been the primary method of reusing MSW (Adhikari et al., 2008). The aim of this work was focused on using nutrients contained in organic waste to design a culture media for microorganisms and to produce SCP for animal feeding.

2. Materials and methods

2.1. Culture media obtained from food waste

Lettuce, potato peels, cooked meat, pumpkin peel, tomato, eggshells, and bread were processed in a shredder (Biotriturador PYR) and resuspended in distilled water in order to dissolve the nutrients. The composition (g/L) of culture medium obtained, called food waste (FW), was: lettuce (37.5 g), potato peels (75.0 g), cooked meat (25.0 g), pumpkin peel (25.0 g), tomato (25.0 g), eggshells (12.5 g) and bread (50.0 g). The solid and liquid components were fractioned by filtration. After filtration, the liquid fraction remained turbid because it contained fatty components which were eliminated by centrifugation (Sorvall RC- 5B: 9000 rpm, 4 °C and 15 min). Thus, a clear culture broth was obtained. After adjusting the pH to 7, the medium was sterilized by autoclaving (121 °C, 5 min) and the bacterial growth assays were carried out. Solid medium was obtained by the addition of agar (1.2% final concentration) (Fig. 1 supplementary data).

2.2. Physical–chemical analysis of broth

2.2.1. Protein determination

The total protein concentration was determined by the Micro-Kjeldahl method (Guebel et al., 1991). Two grams of the sample were weighed and digested with 10 mL of concentrated sulfuric acid in the presence of copper (II) selenite dihydrate and potassium sulfate (catalysts). The liberated ammonia was distilled and collected in a receiving flask containing 10 mL of 1% boric acid. The amount of ammonia in the distillate was determined by titration with 0.1 N HCl.

2.2.2. Carbohydrate determination

The dinitrosalicylic acid method (DNS) (Bailey, 1988) was used for determining the total amount of reducing sugars. An aliquot of FW (500 μL) was mixed with HCl 1 M (50 μL). After 10 min of incubation at 100 °C 50 μL of NaOH and 750 μL of DNS reagent were added. The mixture was incubated in a boiling water bath for 10 min and after cooling to room temperature, the absorbance at 590 nm was measured.

2.2.3. Dry matter determination

Dry matter content was determined according to the Association of Official Analytical Chemists method (AOAC, 1984). Samples are dried to constant mass in an oven at 105 °C. The difference in mass before and after the drying process was used to determine the dry matter.

2.2.4. Ash determination

Ash content is the product resulting from dry matter incineration and was expressed as a percentage of its mass (AOAC, 1984). The crucible was heated for 30 min in the muffle furnace set at 550 °C. The crucible was allowed to cool to 200 °C. Then it was transferred to the desiccators and cooled for 30 min, and it was weighed until the two weights difference was within 0.5 mg. The crucible with dried sample was placed on an electric hot plate with low heat. The sample was heated progressively until the substance carbonized without smoke. Then the crucible was transferred to a muffle furnace with temperature of 550 °C and kept for 4 h. When it cooled to 200 °C, the crucible and the sample were removed from the muffle furnace to desiccator and cooled for 30 min. The residue was weighed until the two weights difference was within 0.5 mg. The ash content of the sample was calculated with the following formulation:

$$\text{ash}\,\% = \frac{M - m}{m'} * 100$$

M: is the weight of the crucible with the sample
m: is the weight of the empty crucible
m': is the weight of the sample

2.3. Microbial cultures in FW and in LAPTg

To study the effectiveness of FW medium, several microbiological tests were performed. The growth of different genera of bacteria was determined and the results were compared with those obtained from microbiological cultures performed in LAPTg commercial broth (Biolife Manual 3rd, 2004). To determine Colony forming units/mL (CFU/mL), the media were solidified with agar (1.2% final concentration).

2.4. Microorganisms

2.4.1. Yeasts

Saccharomyces cerevisiae and *Schizosaccharomyces* sp. (Mycology strain collection, Faculty of Biochemistry, Chemistry, and Pharmacy) were used.

2.4.2. Bacteria

Shigella sonnei S_3, *Salmonella Enteritidis* S_4, *Staphylococcus aureus* E_2, and *Streptococcus* βA E_1 (Bacteriology strain collection, Faculty of Biochemistry, Chemistry and Pharmacy); and *Lactobacillus plantarum* $ATCC_{7469}$ (CERELA-CONICET strain collection) were used.

2.4.3. Bacteria with probiotic properties

Lactobacillus rhamnosus O_{236} (ovine origin), *Lactobacillus* sp. L_{46}, and *Enterococcus* sp. C_{999} (caprine origin) were used. These microorganisms were isolated at the CERELA-CONICET Ecophysiology and Technology Laboratory. In previous works, these lactobacilli were selected for specific beneficial characteristics.

For growth in broth, the optical density was measured at 620 nm (Metrolab RC 325 Junior Spectrophotometer), at different times, under optimum incubation temperatures. Colony counts (incubation at 37 °C for 48 h) were performed by spreading the appropriate dilutions obtained from liquid cultures on solid medium (50 μL). For successive decimal dilutions, sterile saline was used. The results were expressed as colony forming units per mL of original sample (CFU/mL).

2.5. Yeast production

For yeast production, a Bioflo model C-30 fermentor was used. The working conditions were: 30 °C, agitation at 300 rpm, aeration at 0.6 L air/L medium, a final volume of 350 mL, and pH 4.5. For *S. cerevisiae* studies, 35 mL of culture (12 h at 30 °C) was inoculated. OD_{620} and determination of dry matter (AOAC, 1984) were tested every 90 min and then at 24 h. In addition, the specific growth rate (μ) was measured. During the course of a batch culture, this parameter varies from zero (lag phase) to a maximum value (μ) in the exponential phase of growth (log phase). The specific growth rate (μ) depends on the type of microorganism and the physical and chemical conditions of the culture medium. The specific growth rate (μ) was calculated by the corresponding mathematical equation in order to determine the doubling time (the time needed for cells to double their biomass: $td = \ln 2/\mu$).

The effectiveness of the medium designed from FW was compared to that obtained when the yeasts were grown in molasses medium and basal medium. Molasses broth (1% sugar) consisted of 2 g of molasses to 50% sugar, 0.1 g of urea (N source), 0.01 mL H_3PO_4 (P source), in a final volume of 100 mL, pH 4.5.

Basal medium consisted of: 10 g/L glucose, 3 g/L yeast extract, 3 g/L KH_2PO_4, 0.6 g/L $MgSO_4 \cdot 7H_2O$, 1.5 g/L urea, and 300 mL of acetate/acetic acid buffer (pH 4.5).

2.6. Statistical analysis

All assays were performed three times and each one was carried out by duplicated.

Colonies forming units, obtained for each microorganism and for each medium, were compared by calculation of confidence intervals and *t*-test of mean difference. The average dry weights, obtained from different media (FW broth, molasses broth and basal broth), were compared by applying analysis of variance (ANOVA). To establish statistically significant pairs, Tukey's test for multiple comparisons was applied.

3. Results and discussion

The chemical composition of the FW broth is shown in Table 3 (Supplementary data). The nutritional quality (percentage values) obtained after processing the food debris suggested a good chance for microbial growth, since carbohydrate ($1.00 \pm 0.03\%$) and protein (0.77 ± 0.18) levels were not contained as limiting growth factors (Robinson et al., 1999).

The microorganisms used to compare the effectiveness of the FW and LAPTg culture media were: *L. plantarum* $ATCC_{7469}$, *L. rhamnosus* O_{236}, *Lactobacillus* sp. L_{46}, *Enterococcus* sp. C_{999}, *S. aureus* E_2, *S. sonnei* S_3, *S. Enteritidis* S_4, *S. cerevisiae* L_1, and *Schizosaccharomyces* sp. L_2. In all microorganisms assayed, after 24 h incubation under optimal conditions, the CFU/mL values obtained from FW and LAPTg culture media were not statistically significant ($p > 0.05$) (Table 1).

Streptococcus βA E_1 was unable to develop in FW medium and the growth in LAPTg was low (growth rate $\approx$ 0.09 h^{-1}). These results can be explained by the fact that *Streptococcus* strains, for optimal development, need complex media enriched with blood (Dave and Shah, 1996). Since the purpose of our study was to consider FW medium without enrichment, blood was not added even though it is known to be required for this genus of bacteria.

The performance of most microorganisms from FW broth has been very good. For this reason FW broth can be used in bacteriology laboratories as very economic culture medium. In addition, by reusing food wastes it is possible to contribute to environmental health.

The culture media commonly used in our region for the production of yeast contains molasses as the carbohydrate source, with the addition of urea and phosphoric acid (molasses broth). Therefore, to determine the behavior of *Sacharomyces cerevisiae* L_1, under the culture conditions mentioned above, comparative studies involving molasses broth, FW broth, and basal broth were carried out.

Applying ANOVA to the dry weight data obtained from cultures of *Sacharomyces cerevisiae* L_1 in molasses broth, FW broth, and basal broth, after 24 h of incubation at 30 °C, significant differences ($p < 0.05$) were observed (Table 2). The biomass yield calculated at 24 h, from FW broth was 13% lower than that from the molasses broth (Table 2). However, FW broth, despite showing a statistically significant ($p < 0.05$) lower biomass yield, can be very useful in areas where molasses is not available.

In regions without sugar industry, a 13% reduction in biomass yield obtained in FW broth, compared to molasses broth, can overcome the unfavorable rating. The increase in cost to transport molasses from sugar cane areas may be more expensive than the diminished biomass yield.

Yeast cells are used to obtain protein paste, which has been shown to be suitable as an additive in animals food. Therefore, the growth of *Sacharomyces cerevisiae* was monitored in basal broth and FW broth. Its growth in basal broth showed biomass values, specific growth rate and doubling time significantly higher ($p < 0.05$) than values obtained from FW broth (Table 2). However, the use of food waste remains economically favorable in relation to the basal broth, due to the addition of yeast extract. The required additive does not support the advantage that should be inferred from the highest levels of biomass (Table 2).

Previously, a study of yeast strains that focused on yield performance used a whey-based medium (Petrenko, 2005). This author found *S. cerevisiae* biomass values (1.3 g/L) similar to those

Table 1
Growth of microorganisms: Comparison of effectiveness between LAPTg and FW media.

Microorganism	Log CFU/mL[a]	
	LAPTg	FW
Bacteria		
Lactobacillus plantarum $ATCC_{7469}$	8.85 ± 0.21	8.42 ± 0.48
Lactobacillus rhamnosus O_{236}	8.63 ± 0.78	8.37 ± 0.66
Lactobacillus sp. L_{46}	7.83 ± 0.04	8.53 ± 0.21
Enterococcus sp. C_{999}	8.76 ± 0.23	7.38 ± 0.34
Staphylococcus aureus E_2	8.90 ± 0.18	8.35 ± 0.33
Shigella sonnei S_3	8.84 ± 0.20	8.05 ± 0.04
Salmonella Enteritidis S_4	8.47 ± 0.09	7.58 ± 0.14
Yeast		
Saccharomyces cerevisiae L_1	8.66 ± 0.26	8.55 ± 0.42
Schizosaccharomyces sp L_2	7.72 ± 0.04	7.70 ± 0.02

[a] Incubation time: 24 h.

Table 2
Saccharomyces cerevisiae production in batch culture: dry weight, specific growth rate, and generation time.

Parameter	FW broth	Molasses broth	Basal broth
Dry weight (g/L) after 24 h of incubation	1.23 ± 0.04	1.42 ± 0.02	1.68 ± 0.04
Specific growth rate: μ (h^{-1})	0.31 ± 0.02	ND[a]	0.36 ± 0.01
Generation time or doubling time: td (h)	2.23 ± 0.10	ND[a]	1.93 ± 0.08

[a] ND: Not determined. The growth in this broth showed a decrease of optical density.

obtained in this study (1.23 ± 0.04 g/L). The whey-based culture medium contained total protein levels (0.8–0.9%) similar to those reported here for FW broth (0.7 ± 0.18%). However, some differences between both culture media were noted for carbohydrate concentration (whey broth: 4.70–5.00%; FW broth: 1.00 ± 0.03%) and ash levels (whey broth: 0.60–0.80; FW broth: 0.95 ± 0.14). Another study regarding microbial protein production from sugar cane processing waste (Ferrer et al., 2004) showed that the biomass obtained with *S. cerevisiae* (1.19 ± 0.01 g/L) was similar to that found in this work (1.23 ± 0.04 g/L) and that reported by Petrenko (2005) (1.30 g/L).

Based on the results obtained in this work, the medium obtained from organic waste (FW broth) is an easily accessible culture medium. This culture broth can be elaborated, at low cost, in any populated region because its ingredients are wastes generated by humans. It is a broth that has great versatility and allows for the development of a wide variety of microorganisms, including both, Gram negative and Gram positive bacteria as well as yeasts.

In addition, SCP yeast paste, characterized by its high nutritional value, was obtained at a competitive cost for use as a protein additive in animal feeding. The results obtained in this study may contribute to the development of protective foods that include waste recycling; and thus participate in livestock activities, which are predominantly found in the productive areas of Argentina. Large scale production could provide an additional benefit, since organic waste recycling is a better treatment of solid waste than current methods and thereby contributes to environmental conservation.

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Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jenvman.2012.10.005.

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