THE USE OF YEAST MANUFACTURE LIQUID WASTES IN THE PRODUCTION OF BIOFERTILIZERS AND PHYTOHORMONES
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ABSTRACT

The cost and composition of the nutrient medium are considered limiting factors for the production of bacterial inocula. At the same time, baker's yeast factories get rid of their liquid wastes in River Nile or water drains. The analysis of such wastes indicated their richness in various minerals, amino acids, vitamins and yeast cells. The recommended media used for *Azotobacter vinelandii*, *Azospirillum brasilense*, *Bacillus polymyxa*, *Klebsiella pneumoniae* and auxin over-producing mutant (*Azospirillum brasilense* FT) were compared with optimized yeast liquid waste (OYW) in respect to the production of both bacterial inocula and phytohormones. Data showed that *Klebsiella pneumoniae* and *Azospirillum brasilense* FT grew well in the optimized yeast liquid waste medium compared to other diazotrophs. It was also found that *Azospirillum brasilense* FT mutant produced high quantities of extracellular indoleacetic acid (IAA) and tryptophol (TOL) in both recommended medium and the optimized yeast liquid waste medium. The production of both compounds was increased at the stationary phase of the culture. These data suggested that, optimized yeast liquid waste could be used as an alternative medium for biomass and phytohormones production of associative diazotrophs.

INTRODUCTION

There is a great deal of interest in creating efficient associations between agronomically important plants, particularly cereals, and associative N₂-fixing bacteria (diazotrophs). The long-term objectives of such studies are to introduce the ability to fix N₂ into plants, which could enhance plant growth and reduce the dependence of the plants on N fertilizer applications, or to enhance the efficiency of applied fertilizer (fertilizer efficiency), resulting in saving fertilizer. These objectives could have both economic and environmental significance. Intensive application of chemical fertilizers imposes undesirable consequences of pollution besides to the ever-increasing costs of such chemicals. Many reports have been published on beneficial effects of diazotrophs inoculation on plant growth (Sumner 1990; Gunarto et al. 1998; El-Khawas et al. 2000). Diazotrophs are found to produce and release a broad spectrum of plant growth regulators such as auxins, several gibberellins, and cytokinins (Tien et al. 1979; El-Khawas and Adachi 1999). Responses of cereal plants to inoculation with associative N₂-fixing bacteria were explained as an effect of plant growth regulators released by such microorganisms (Salame et al. 1997; El-Khawas 1990). Costs of bacterial biomass production depend on prices of production medium particularly substrates used as carbon sources. Therefore, the possibility of replacing the recommended media for biomass and phytohormones...
production of various diazotrophs by yeast manufacture liquid wastes as a
cheap medium was investigated.

**MATERIALS AND METHODS**

**Bacterial strains**
Strains of *Azotobacter vinelandii* ATCC 12837, *Azospirillum brasilense* ATCC 29710, *Azospirillum brasilense* FT (an IAA-overproducing mutant of *A. brasilense* ATCC 29710, El-Khawas 1990), *Bacillus polymyxa* ATCC 842 and *Klebsiella pneumoniae* ATCC 13883 were used.

**Media**
Modified N-deficient medium of Yensen, yeast extract manitol (YEM) medium, nitrogen free glucose (NFG) medium and nitrogen free malate (NFM) medium were used as recommended media (Deutsche Sammlung von Mikroorganismen, DSM, 1977).

**Yeast manufacture liquid wastes**
The diagram (Fig. 1) shows the various stages of wastes produced during baker’s yeast production at Yeast Factory, Grand Cairo Bakers, El-Salam City. The raw waste (waste 1) and waste 2 (after first wash) were used for growing the different diazotrophs in comparison with the recommended media.

![Diagram](image)

**Figure 1:** Diagram for various stages of wastes during baker’s yeast production at Yeast Factory, Grand Cairo Bakers, El-Salam City.
Optimization of the yeast liquid wastes

Waste 1 and waste 2 were mixed with the rate of 1:1 without removing yeast cells and the pH was adjusted at 7.0. The autoclaved mixture was used as optimized yeast waste (OYW).

Determination of auxins

Extracts from the cultured supernatant of *Azospirillum brasilense* FT for different incubation periods were partitioned into two fractions and analyzed by using high performance liquid chromatography (HPLC) according to El-Khawas and Adachi (1999). The supernatants (30 ml) of stationary phase cultures were adjusted to pH 8.6 with 1% NaOH and partitioned three times with equal volumes of ethyl acetate. The combined ethyl acetate fraction were evaporated to dryness and held for further purification. The aqueous phase was adjusted to pH 2.8 with 1% HCl and partitioned three times with equal volumes of ethyl acetate, while the remaining aqueous phase was discarded. The combined acidic ethyl acetate phase was reduced to 5 ml volume (Fraction1) and used for HPLC determination of acidic auxins. The dried basic ethyl acetate fraction was dissolved in 80% methanol, then methanol was evaporated under vacuum, leaving an aqueous phase, which was adjusted to pH 2.8 and partitioned three times with 25 ml of ethyl acetate. The ethyl acetate phase was combined (Fraction 2), reduced to 5ml volume and used for HPLC determination of neutral auxins.

Mobile phases and standards used in the study

Two different optimized mobile phases e.g., isopropanol/H2O: 12.7/87.3 containing 1.9mM citrate pH 4.35, and gradient 10-70% methanol in 10 mM aqueous acetic acid were used. The standards e.g., indole-3-acetic acid (IAA), indole-3-acetaldehyde (IAAld), indole-3-pyruvic acid (IPyA), and tryptophol (Indole-3- ethanol, TOL) were used as standards for identification of HPLC peaks.

Chemical analysis

Crude protein was determined by automated method using Kjel-Tec automatic as described in the A.O.A.C. (1998). Sodium, potassium, calcium, phosphorus and other minerals were determined according to the method described in the A.P.H.A (1992) using atomic absorption spectrophotometer, 3300 Perker-Elmer. Amino acids were determined by Beckman amino acids analyzer model 7300 and data system model 7000 according to the method of Winder and Eggum (1966). Vitamins were determined by high-performance liquid chromatography (HPLC) and post-column derivatization according to the method of Bognar (1992).
RESULTS AND DISCUSSION

Chemical analysis of the two yeast liquid wastes (W1 and W2)

Table (1) shows the chemical analysis of the two yeast liquid wastes and other recommended media used in the study. Data revealed that waste 1 contains more minerals than waste 2. When the concentrations of the elements in the two wastes were compared with recommended media, waste 1 contains high concentration of elements specially potassium, calcium, magnesium, iron and manganese. However, waste 2 contains lower concentration of elements compared with some of the recommended media. Furthermore, the concentration of carbon and nitrogen were higher in the waste 1 than waste 2 and recommended media.

Table 1: Minerals content (mg L⁻¹), carbon and nitrogen (%) in yeast wastes (W1 & W2) and other recommended media.

<table>
<thead>
<tr>
<th>Elements</th>
<th>Yeast wastes</th>
<th>Recommended media</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W₁</td>
<td>W₂</td>
</tr>
<tr>
<td>P</td>
<td>115</td>
<td>45</td>
</tr>
<tr>
<td>K</td>
<td>1450</td>
<td>152</td>
</tr>
<tr>
<td>Ca</td>
<td>110</td>
<td>9</td>
</tr>
<tr>
<td>Mg</td>
<td>1310</td>
<td>123</td>
</tr>
<tr>
<td>Na</td>
<td>75</td>
<td>6</td>
</tr>
<tr>
<td>Fe</td>
<td>65</td>
<td>8</td>
</tr>
<tr>
<td>S</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mo</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mn</td>
<td>35</td>
<td>-</td>
</tr>
</tbody>
</table>

Data in Tables 2 and 3 revealed that, the two wastes contained comparable amount of amino acids and vitamins. This is could be due to the presence of nearly the same number of viable yeast cells in both wastes. It is quite obvious that yeast manufacture liquid wastes are reaches in various minerals, amino acids, vitamins and yeast cells. The presence of yeast cells in the wastes offered an excellent source four vitamins and growth factors.
which are required for the growth of most microorganisms (Deutsche Sammlung von Mikroorganismen, DSM, 1977).

Table 2. Amino acids (mg l\(^{-1}\)) in the two yeast liquid wastes.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Waste 1</th>
<th>Waste 2</th>
<th>AMINO ACID</th>
<th>Waste 1</th>
<th>Waste 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asparatic</td>
<td>1386</td>
<td>1120</td>
<td>Isoleucine</td>
<td>70</td>
<td>81</td>
</tr>
<tr>
<td>Threonine</td>
<td>91</td>
<td>85</td>
<td>Leucine</td>
<td>112</td>
<td>96</td>
</tr>
<tr>
<td>Serine</td>
<td>119</td>
<td>112</td>
<td>Tyrosine</td>
<td>21</td>
<td>24</td>
</tr>
<tr>
<td>Glutamic</td>
<td>1155</td>
<td>1010</td>
<td>Phenylalanine</td>
<td>42</td>
<td>38</td>
</tr>
<tr>
<td>Proline</td>
<td>77</td>
<td>84</td>
<td>Histidine</td>
<td>21</td>
<td>16</td>
</tr>
<tr>
<td>Glycine</td>
<td>147</td>
<td>122</td>
<td>Lysine</td>
<td>70</td>
<td>62</td>
</tr>
<tr>
<td>Alanine</td>
<td>210</td>
<td>198</td>
<td>Valine</td>
<td>91</td>
<td>84</td>
</tr>
</tbody>
</table>

Table 3. Vitamins and viable yeast cells of both yeast wastes

<table>
<thead>
<tr>
<th>Wastes</th>
<th>Vitamins ((\mu)g l(^{-1}))</th>
<th>Number of viable yeast cells ((X 10^4) cfu l(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waste 1</td>
<td>300 125</td>
<td>175</td>
</tr>
<tr>
<td>Waste 2</td>
<td>320 110</td>
<td>142</td>
</tr>
</tbody>
</table>

Growth curves of *K. pneumoniae* grown in the two wastes

In a primary experiment, the growth curves of *K. pneumoniae* in the two wastes and on mixture of both of them were investigated. The growth of *K. pneumoniae* was similar in both wastes and reached to \(10^8\) cells ml\(^{-1}\) culture after 48 h. of incubation (Fig. 2). However, when *K. pneumoniae* was grown in mixture of waste 1 and waste 2 at the rate of 1:1, the growth was increased and reached to \(10^{10}\) cells ml\(^{-1}\) culture after 36 h.

Figure 2: Growth curves of *K. pneumoniae* grown in yeast liquid wastes
Therefore, to optimize the liquid wastes to obtain best medium for growing the different diazotrophs, it must be mixed together at the rate of 1:1 without removing yeast cells and adjusted the pH at 7.0. The autoclaved mixture was used as optimized yeast waste medium (OYW).

**Growth curves of diazotrophs grown in optimized yeast liquid waste medium (OYW)**

The growth performance of some diazotrophs in optimized yeast waste medium (OYW) was compared with the same medium supplemented with 0.5% of carbon sources, and the recommended media of such organisms (figures 3 and 4). Generally, data showed that *Klebsiella pneumoniae* grew well in the three different media compared to other diazotrophs. When glucose was added to optimized yeast waste medium, a
slight increase in viable cell counts of K. pneumoniae was achieved (Fig. 3). The growth of Azotobacter vinelandii in OYW medium was week compared with other diazotrophs. However, when glucose was added to the medium, the growth rate was increased but still lower than the recommended medium (Fig. 3). With regard to B. polymyxa, the growth was week on OYW medium. Addition of glucose to the medium enhanced the growth until exceeded their numbers in the recommended medium (Fig. 3).

Regarding to Azospirillum, two strains (wild type of Azospirillum brasilense ATCC 29710 and its mutant Azospirillum brasilense FT) were used. The growth of A. brasilense FT mutant was better than A. brasilense wild type strain when grown on OYW medium. However, when malic acid was added to the OYW medium, the growth rate of A. brasilense wild type strain increased and reached to similar growth rate as the growth in the recommended medium (Fig. 4).

Figure 4: Growth curves of A. brasilense wild type strain (W.T.) and FT mutant grown in optimized yeast waste medium (OYW), optimized waste plus malate (OYW+M), and recommended medium (NFM)

**Excretion of auxins in optimized yeast waste and recommended media**

Figure 5 represents the production of different auxins (indoleacetic acid and related compounds) by Azospirillum brasilense FT in optimized yeast waste medium as well as N-deficient malate culture medium. After the strain was grown in both culture media, extracts from the cultured supernatant were partitioned and analyzed by using high-performance liquid
chromatography (HPLC). Data revealed that both culture media contained high quantities of extracellular indoleacetic acid (IAA) and tryptophol (TOL). The production of both compounds was increased at the stationary phase of the growth (Fig. 6). On the other hand, very low levels of indoleacetaldehyde (IAAld) and indolepyruvate (IpyA) were identified in optimized yeast waste culture medium only (Fig. 5). Similar results were reported in Azospirillum lipoferum and Azospirillum brasilense (Abd El-Salam and Klingmuller 1987, Dosselaere et al. 1997, El-Khawas and Adachi 1999). They indicated that both Azospirillum lipoferum and Azospirillum brasilense could produce IAA, TOL and IpyA when these microorganisms were cultured in their respective media.

Figure 5: HPLC analysis of different auxins standard (A) and supernatants of 48 h. old cultures of Azospirillum brasilense Ft grown in N-deficient malate culture medium (B) and optimized yeast waste medium (C). IAA, indoleacetic acid; TOL, tryptophol; IAAld, indoleacetaldehyde and IpyA, indolepyruvate.

Many industrial by-products and wastes have been used as growth substrates for microbial biomass and production of economically valuable microbial products. Several investigators employed many wastes as media for diazotrophs. Bioardi and Ertola (1985) showed that a malt sprout is a cheap product, which could be an adequate component of media for rhizobia. Raw whey as well as deproteinized whey were successfully used as a cheap medium for rhizobia and azospirilla (Bissonnette, et al. 1986; Omar, et al. 2000). On the other hand, Martinez-Toledo et al. (1995) showed that alpechin (wastewater from olive oil mills) could be utilized by
Azotobacter as a cheap substrate for growth and producing poly-B-hydroxybutyrate (PHB).

![Graph](image)

Figure 6: Excretion of auxins (IAA and TOL) and growth curve of *Azospirillum brasilense* FT mutant grown in optimized yeast waste medium.

Generally, it could be suggested that, it is feasible to apply the optimized yeast liquid waste (OYW) as an alternative medium for biomass and phytohormones production of associative diazotrophs.

**ACKNOWLEDGMENT**

I would like to acknowledge Dr. M. El-Tahan for his kind help in some chemical analysis of the yeast wastes. Also, the cooperation of Mr. S. Shafik for providing the yeast wastes is very appreciated.

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استخدام المخلف السائل لمصانع الخميرة في إنتاج الأسمدة الحيوية والهرمونات النباتية

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تعتبر تكاليف وتركيب البيئة الغذائية من أهم العوامل المحددة لإنجاح الفاصوليات البكيرية. في الوقت الذي تشكل مصانع سمكًا روحيًا وماليًا لإنتاج البكتيريا في صناعة الخبز، ومواد البناء أو المجاري مما يناسب فاصلًا للاستخدام. ويتجلى هذا المخلف السائل الذي يحتوي على كميات كبيرة من العناصر الغذائية، والأحماض الأمينية، والكحوليات. لذلك كانت فكرة هذا البحث هو كيف يمكن استغلال هذا المخلف لإعداده كميخال غذائية لإنتاج الأسمدة الحيوية والهرمونات النباتية. استخدمت هذه البحث مجموعة من مصانع النسيج الحيوي (أزوسبيريا برازيليس، برازيليس بابليس، كليسيسوميا وكليسيسوميا) بالإضافة إلى مدى الطفرات العالية في إنتاج الأوكسيتال (أزوسبيريا برازيليس)

أوضحت النتائج أن كل من كليسيسوميا نيومونيا وإزوسبيريا برازيليس ينمو جيدًا على المخلف FT صيدليات A. أزوسبيريا برازيليس ينمو جيدًا على المخلف معالج. مقارنة بباقي أنواع الباكرية المستخدمة، وان درجة النمو على المخلف تقرب نسبة كبيرة من درجة النمو على البكتيريا المشتركة. وقد وجد أيضًا أن الطفرة FT تتناسب كميات كبيرة من أكسيد أتال دون أن تتحفز استخدام أي البكتيريا المختصة.

عوضاً، توكد النتائج هذا البحث إمكانية الاستفادة من المخلف السائل لمصانع سمكًا روحيًا لإنتاج الأسمدة الحيوية والهرمونات النباتية.

استخدامه في إنتاج الأسمدة الحيوية والهرمونات النباتية.