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# Economic Evaluation of *Streptococcus Pneumoniae* Culture Media

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**Abstract:** *Streptococcus pneumoniae*, a major bacterial pathogen worldwide, requires a complex medium for culture, which complicates harvesting of antigens for vaccine production. Here we present an economic evaluation of factors affecting the production cost of a chemically defined medium for the scale up of fermentation of *S. pneumoniae*, compared with the most traditional fermentation media. A basic laboratory-scale experiment of 1 L and an industrial-scale production of 100 L were assumed. The economic analysis results show that at 1 L lab-scale the Chemically Defined Medium (CDM) is the most expensive (US$35/L) compared to Tryptic Soy Broth medium (TSB) and Tryptic Soy Vegitone medium (TSBv), (US$2.9/L), Todd Hewitt (US$22.8/L) and Brain Heart Broth (BHI) (US$10.8/L). However at 100 L production-scale all the media costs decrease and CDM becomes competitive with Todd Hewitt and BHI (US$8.4, 9.6, 8.2/L, respectively) and slightly more expensive than both TSB media (US$2.9/L). We conclude that although some aspects of culture growth should be studied in more detail, the production cost of the chemically defined medium for industrial scale fermentation of *S. pneumoniae* is economically competitive on the grounds not only of price, but also taking into account other benefits such us ease of isolation, and the possibility of fine-tuning of the individual chemical species in its formulation according to the culture requirements.

**Keywords:** *Streptococcous pneumoniae*, Fermentation Media, Scale Up, Vaccine, Economic Analysis

## Introduction

The biotechnological production of medicinal products requires the use of Good Manufacturing Practices (GMPs), which comprise the part of Quality Assurance that ensures products are strictly manufactured and controlled consistent with established quality requirements for use (safety and efficacy). In particular, vaccine production must be carried out with a quality level that consistently delivers products that meet pre-established standards at all stages of the process, ensuring the efficacy and harmlessness of the finished product (WHO, 2004).

Despite their considerable importance at the production scale, the economic costs of fermentation media components are rarely quoted when a laboratory develops a new product or process, though in some processes they are obviously considerable. The cost of the various components of a production medium can have a profound effect on the overall cost of a fermentation process and of the product obtained. Stowell and Baterson (1984), for example, identified the costs of raw materials as one of the factors contributing to these costs. Usually, robust process design is desired early on, since the process experiences changes when scaling up. The culture medium used for the production of bacterial antigens is one of the main costs that have to be evaluated for scaling up. Unfortunately, the literature is scarce and out of date, or at any rate it is not openly available for the research community.

*S. pneumoniae* is a capsulated bacterial pathogen considered to be responsible for mortality in the elderly and morbidity and mortality in the infant population all over the world (PAHO, 1992; WHO 2007). The capsular polysaccharides of pneumococci have been shown to be essential for their virulence (French, 2003; Kadiouglu *et al*., 2008).

Vaccines that include a number of the most prevalent capsular serotypes have been developed in recent decades (Van Deursen *et al*., 2012). Manufacturing of

pneumococcal polysaccharide for use as an antigen in vaccines requires fermentation of *S. pneumoniae*. Consistent with the established quality requirements, the fermentation medium should contain only those essential components that allow the maximum cell density and the reproducibility of the process. Fully defined media are particularly preferred (Zhang *et al*., 1999).

The best known media for growing *S. pneumoniae* are Brain-Heart Infusion (BHI), Todd-Hewitt and Tryptic Soy Broth (TSB) media, containing supplements such as yeast extract, or other sources of carbon and nitrogen, including sources of vegetable origin. These media are inexpensive and provide ample nutrients (Restrepo *et al*., 2005).

A partially defined culture medium for *Streptococcus pneumoniae* was described in 1942 and since then, several authors (Hoeprich, 1957; Adams and Roe, 1944) have improved its formulation, but they all maintained casein or soy hydrolyzate as the nitrogen and carbon source (Van De Rijn and Kessler, 1980). In 1996, Kim *et al*. (1996) described a similar defined medium for the culture of *S. pneumoniae* but to our knowledge it was not until 2015 that a complete chemically defined medium for the cultivation of *S. pneumoniae* serotype 1 was described, by our group (Texeira *et al*., 2015). A novel culture medium free of serum and animal component requires a considerable development effort to provide a formulation capable of supporting microbial production at the desired levels. Despite the many advantages of using defined media they are still not frequently developed for industrial processes. Typically, industry takes on the challenge of separating the various components of a complex fermentation medium used to maximize fermentation performance instead of trying to develop a syntethic medium. Hence, if the required separation becomes complex and costly, the most efficient fermentation may not necessarily yield the optimum overall process.

However, if the focus is on finding a new competitive chemically defined medium and minimizing the total cost of production on a large scale, a careful cost-benefit analysis of the media is necessary before starting the process.

Here we present the result of an economic evaluation of the factors affecting production cost of a chemically defined medium for the scale up of fermentation of *S. pneumoniae*. Furthermore, the results were compared with the most traditional fermentation media used worldwide. For analysis purposes a 1 L lab-scale development and an industrial-scale production of 100 L were assumed.

## Materials and Methods

The culture media utilized for this economic estimation were: Tryptic Soy Broth (TSB) (22092, Sigma), TSB vegitone (41298, Sigma), Todd Hewitt broth (T1438, Sigma) Brain Heart Broth (BHI) (53286, Sigma) and Chemically Defined Medium (CDM).

The components and preparation of CDM is described by Texeira *et al*. (2015). Briefly, a volume of one liter of defined medium was prepared as follows: 900 mL basal medium, 50 mL of vitamins, salts, and growth factor solution, 12.5 g/L of glucose and 25 mL bicarbonate/thioglycolic acid solution were mixed in distilled water. The pH was adjusted to 7.2; the solution was sterilized by filtration through a 0.22 µm pore-size membrane and placed at 37°C in 5% $CO_2$ atmosphere. The final concentration of each component is shown in Table 1.

Table 1. Components of chemically defined medium and their concentrations

| Amino acids | mg/L | Salts | g/L |
|---|---|---|---|
| L-tryptophan | 31.5 | Monobasicpotassium phosphate | 4.9 |
| Glycine | 58.5 | Monobasic sodium phosphate | 2.9 |
| L-cysteine | 149.4 | Dibasic sodium phosphate | 6.6 |
| L- tyrosine | 129.6 | Sodium bicarbonate | 1.0 |
| L-lysine | 207.0 | Thyoglycolic acid | 1.0 (mL) |
| L-valine | 156.0 | | |
| L-histidine hydrochloride | 49.5 | Vitamins and Minerals | mg/L |
| L-arginine hydrochloride | 112.5 | nicotinic acid | 4.17 |
| L-leucine | 207.0 | pyridoxal | 4.17 |
| L-isoleucine | 153.0 | calcium pantothenate | 20.83 |
| L-threonine | 108.0 | thiamine | 4.17 |
| L-methionine | 65.7 | riboflavine | 4.17 |
| L-aspartic acid | 165.6 | adenine sulfate | 41.70 |
| L-proline | 39.0 | uracil | 41.70 |
| L-phenylalanine | 112.5 | biotine | 0.01 |
| L-serine | 211.5 | ferrous sulphate 7 $H_2O$ | 21 |
| Growth factors | g/L | zinc sulphate7 $H_2O$ | 3 |
| L-glutamine | 2.08 | manganese sulphate | 1.7 |
| Asparagine | 0.41 | magnesium sulphate7 $H_2O$ | 2083 |
| Choline chlorhydrate | 0.72 | chlorhydric acid | 0.08 mL |
| Glucose | 12.5 g/L | | |

### *Culture of Streptococcus Pneumoniae*

*S. Pneumoniae* (serotype 14 strain 5287) was cultured 12 h in TSB in a 5% $CO_2$ atmosphere. Then, the bacterial mass was collected and washed twice with CDM and dispensed into flasks with either TSB or CDM. Inoculum of 300 µL of a bacterial suspension containing $7\times10^9$ cells/mL (based in McFarland's scale) were used to start the cultures in one liter flasks. Samples were taken every 3 h and at the end of the cultivation time at 16 h. Biomass growth were followed by measuring optical density at 600 nm in an Ultraspec 1000 spectrophotometer (Amersham Biosciences).

### *Theory and Calculation*

For analysis purposes, two possible scales were used: A lab-scale development (1 L) and an industrial-scale production (100 L). The cost of compounds was obtained from Sigma webpage (US$ dollars, USA buyer, purity ≥ 98%, accessed March 2016). The Sigma prices available for TSB media and used in this analysis were the same at both scales, because the 500 g pack presentation is the biggest that the company offers.

The analysis was performed based on Sigma pack availability for each compound and quantity, i.e., if 0.9 mg was used, a 1 mg compound pack was quoted and if 1 mg was not available, the next size of pack available was used.

The final cost of each medium Component (CC) is represented by the equation:

$$CC = (AU / AT) * PC$$

Where:

$CC$ = Cost of each component
$AU$ = Amount used
$AT$ = Total pack amount and
$PC$ = Pack cost

## Results

Figure 1 shows the biomass growth of *S. pneumoniae* in TSB and CDM. Both media shows similar growth profiles. Figure 2 shows the cost per liter for all the fermentation media. At lab-scale, CDM is ten times more expensive than both TSB media, US$35/L andUS$2.9/L respectively. BHI and Todd Hewitt culture media have intermediate costs (US$10.7 and 22.8/L).

However at 100 L- scale production, the costs decrease and the cost of CDM (US$8.4/L) is now not only of the same order as Todd Hewitt and BHI media (US$9.6/L and 8.2/L respectively), but has also been reduced more than four-fold, approaching the cheapest TSB media (US$2.9/L).

The incidence of the components on the final cost of CDM is shown in Fig. 3. At lab-scale amino acid components have the highest incidence on the final cost at 59%, followed by vitamins and minerals with an incidence of 14%. The incidence on cost of the amino acids decreases at the 100 L-scale to 37% and the incidence of growth factors increases to 29%.

The economic contribution of each amino acid to the final cost of CDM is observed in Fig. 4. Although costs vary, it is evident that L- lysine is the most expensive amino acid with an incidence of 46% at 1 L-scale and 28% at 100 L-production scale on the total cost of amino acids.

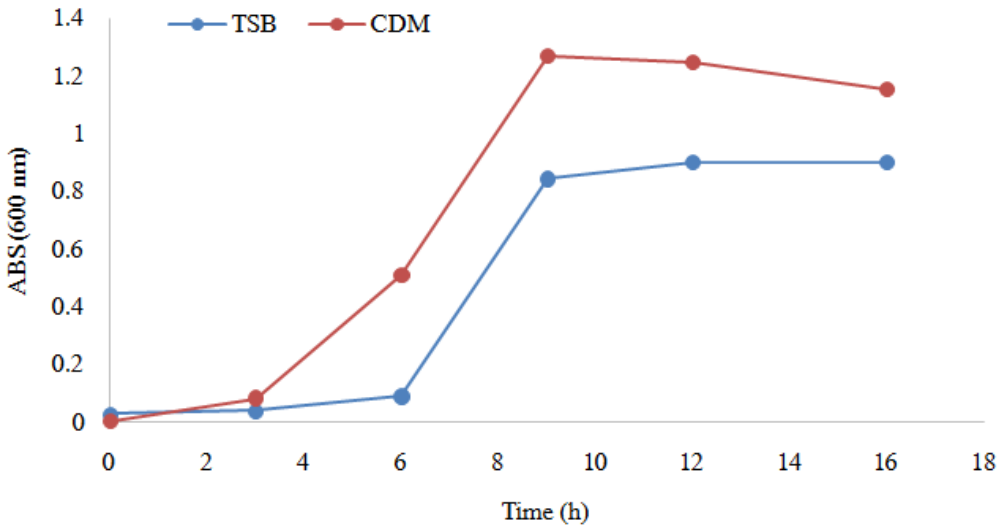



Fig. 1 Growth profile of *S. pneumoniae* in TSB and in CDM

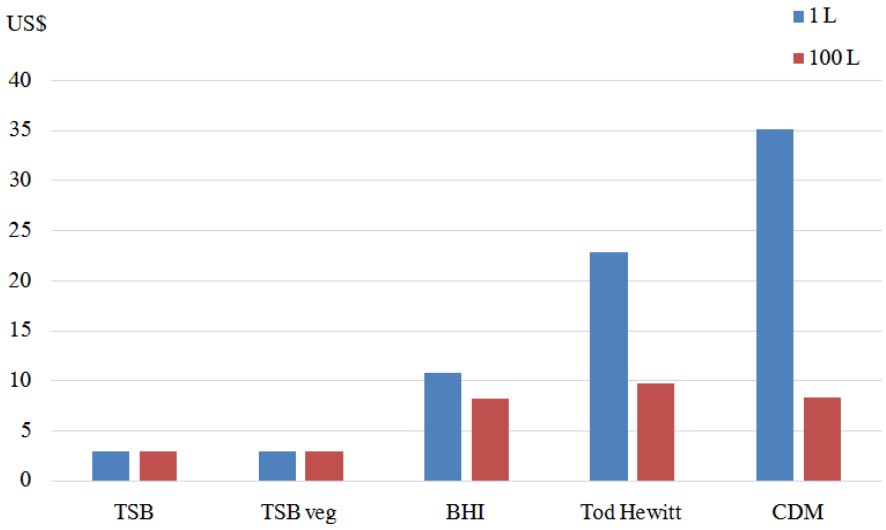
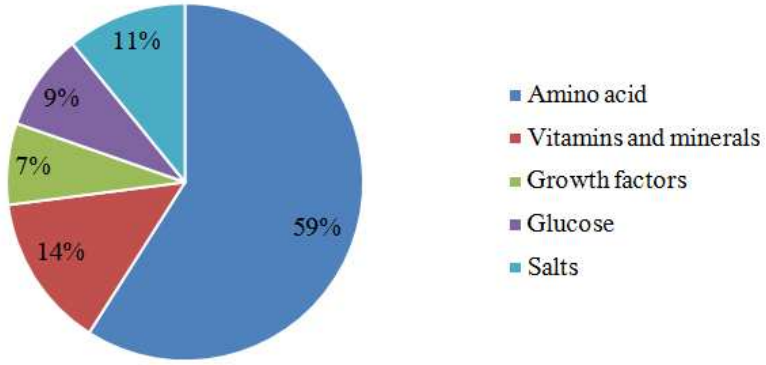
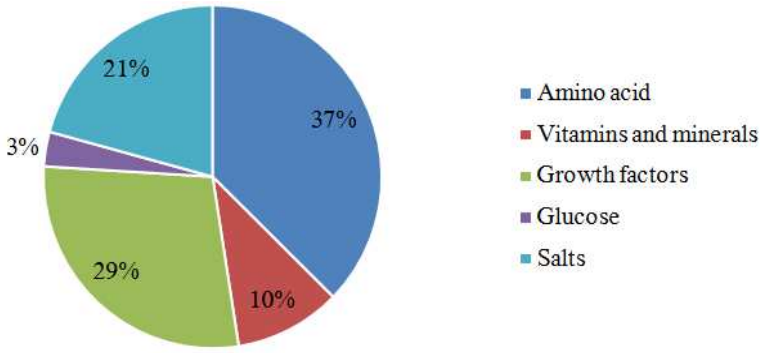



Fig. 2. Culture media cost per Liter




(A)




(B)

Fig. 3. (A) Cost incidence of components in CDM (1 L analysis); (B) Cost incidence of components in CDM (100 L analysis)

## Discussion

Various cell culture media for the growth of fastidious organisms such as *S. pneumoniae* are known. Most of the media used are available commercially and contain supplements such as animal extracts, yeast extract, or other sources of carbon and nitrogen, including sources of vegetable origin (Restrepo *et al*., 2005; Jin *et al*., 2009). These components enrich the nutritional quality of the basal media, to support the growth of the bacteria. A complete chemically defined medium for growing *S. pneumoniae* has been reported earlier by our group (Texeira *et al*., 2015).

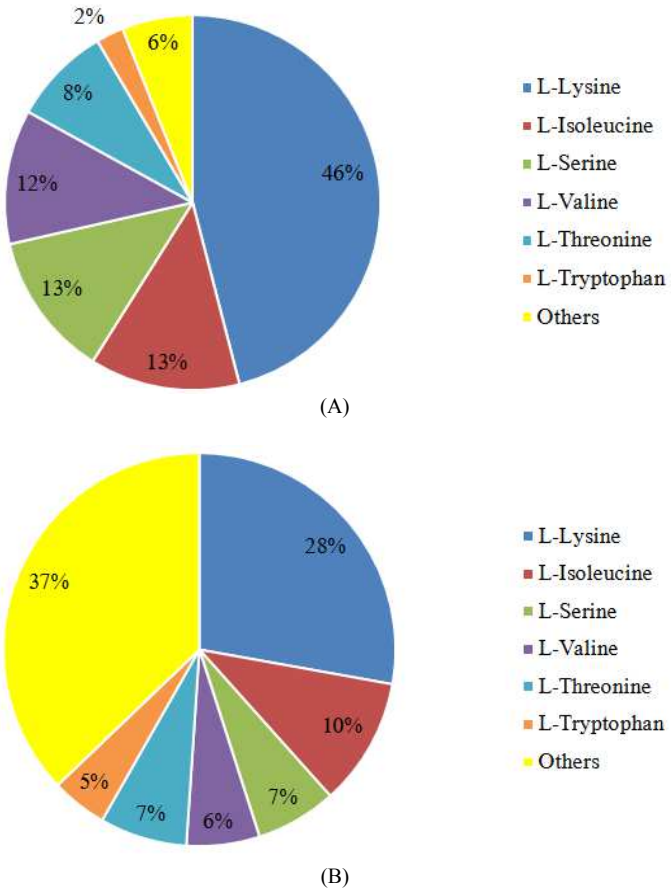



Fig. 4. (A) Cost incidence of Amino acids (1 L analysis) in CDM; (B) Cost incidence of Amino acids (100 L analysis) in CDM. "Others" indicates the incidence of the rest of the aminoacids (Glycine, L-cysteine, L- tyrosine, L-histidine hydrochloride, L-arginine hydrochloride, L-leucine, L-methionine, L-aspartic acid, L-proline, L-phenylalanine)

The results in Fig. 1 showed that the chemically defined medium is capable of supporting the growth of *S. pneumoniae* in a reproducible manner. Despite the many advantages of using defined or synthetic medium, in general the main disadvantages are related to cost. If a fermentation process is to yield a product at a competitive price, the cost of the chosen medium plays a key role. Failure to achieve a good quality product in biotechnology development is often due to scientists not taking into account the economic analysis of the process and then after a big effort the product or process lacks economic feasibility. Media development hence is a vital part of GMP manufacturing and must meet the requirements of developing a scalable, cost-effective and robust process (FDA, 1997).

Based on the medium composition provided in Table 1 our analysis shows CDM as the most expensive medium at 1 L lab-scale (Fig. 2). The comparison between the media reflects the low cost of commercial complex media, which usually contain non-defined components while the possible need for large numbers of additives to CDM (amino acids, vitamins, growth factors, etc.) increases the cost of this medium (Zhang *et al*., 1999).

It is important to notice that this reason as often discouraged attempts to develop defined media for industrial use.

However, when the analysis was performed at 100 L-scale (Fig. 2), CDM became more competitive. One of the reasons for this is the possibility of buying products on a large scale, which in turn makes the cost per liter on a large scale cheaper than at lab- scale. Furthermore, based on the results showed in Fig. 3 and 4, the cost of L-lysine represents almost 30% of final cost at the 1 L scale and 10% at the 100 L scale, suggesting that for large scale production amino acid costs could be reduced i.e. by employing the single amino acid omission method (Haslam *et al*., 1986) to determine minimal concentrations of amino acids for optimal growth.

These results show that for large scale production, a chemically defined medium is competitive compared to commercially available complex media. Even if the scaling up process may represent high raw material costs, this is compensated by a better quality product free of debris that would hinder the process of purification of the antigen (Texeira *et al*., 2015).

Furthermore, it is worth considering the tradeoff between slightly increased fermentation costs and a greatly simplified downstream process, given that fermentation is the first unit operation in the process and has a major impact on subsequent downstream operations.

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## Author's Contributions

**Victor Morais:** Participated in the conception and design of the study, carried out the calculation of the experimental work and assisted in drafting the manuscript.

**Norma Suárez:** Participated in the conception and design of the study and carried out the experimental work. Read and improved the manuscript.

## Ethics

The authors declare that they had no conflict of interest.

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