Nanomagnetic Particle Production: Effect of Carbon and Iron Sources

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Abstract. Production of nanomagnetic particles via microbial processes offers the advantage of better biocompatibility for medical applications. However, this process is not widely applied due to the difficulties in cultivating magnetotactic bacteria in the lab. This research explored the possibilities of cultivating magnetotactic bacteria in order to produce nanomagnetic particles in the lab and in particular to find the carbon and iron sources to get the optimum yield of nanomagnetic particles. Experiments were performed as semiaerobic-batch-magnetotactic-bacteria cultivations. The carbon source in the media was varied as: sodium lactate, sodium acetate, and sodium pyruvate. As for the iron source, either Fe-citrate or Fe-quinate was used. Bacterial cell growth was monitored using the turbidometry-gravimetry method, substrate concentration was measured using high performance liquid chromatography (HPLC), while the cellular iron content was measured using electron dispersive X-ray spectroscopy (EDS) and transmission electronic microscopy (TEM). It was observed that cell growth did not correlate with the production of nanomagnetic particles. The bacteria grew best on sodium pyruvate and Fe-quinate, however, the best yield of nanomagnetic particles was obtained from the cultivation with sodium acetate and Fe-quinate. The obtained TEM images confirmed the presence of nanomagnetic particles.

Keywords: *carbon source; growth; iron source; magnetotactic bacteria; yield.*

1 Introduction

Nanomagnetic particles are particles whose diameter is in the range of 20-200 nm, contain iron, and have magnetic properties. These properties offer various potential applications such as magnetic-field-based DNA or protein purification, targetted drug delivery, or as a contrasting agent for non-invasive diagnostic magnetic resonance imaging (MRI) tools. Based on the source of the nanoparticles, there are artificial nanomagnetic particles and microbial nanomagnetic particles. Artificial nanomagnetic particles are produced via chemical reaction, whereas microbial nanomagnetic particles are produced naturally by magnetotactic bacteria [1].

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Magnetotactic bacteria synthesize intracellular particles containing iron/sulphur oxide, known as magnetosome. They are mostly aquatic bacteria that move along the magnetic field lines of the earth [2] and can belong to any prokaryotic group, e.g. cocci, rod, vibrio, spiral, or multicellular microorganisms that have magnetotactic properties. Their movements are influenced by magnetic fields [3,4]. Magnetosome is postulated to function as a biological compass, which guides these bacteria to move along gradients of oxygen in water due to the influence of the Earth's magnetic field [1,4].

Microbial nanomagnetic particles offer more advantages than artificial ones because of their biocompatibility and uniformity in size, which are preferable in medical areas [2,5]. Despite these, the microbial nanomagnetic particles have not been widely applied due to difficulties in their production, particularly related to the cultivation of magnetotactic bacteria outside their natural habitat. Until recently, only a few species of magnetotactic bacteria have been isolated and cultured because of the uncommon conditions needed in cultivating them [6].

In 2002, Heyen published a study on the effect of various types of carbon sources and concentrations of carbon sources on the growth of magnetotactic bacteria [6]. His results showed that the best substrate for cultivating magnetotactic bacteria was 25 mM of lactate.

In our previous research [7], a local magnetotactic bacterial strain was isolated. This research focused on the search for optimum carbon and iron sources for the cultivation of magnetotactic bacteria and the production of nanomagnetic particles. This study gave additional understanding of the operation conditions for magnetotactic bacteria cultivation, in particular by evaluating the type of iron source and providing the quantitative aspects of the analysis, such as the calculation of biomass yield and nanomagnetic particle yield.

2 Materials and Methods

2.1 Microorganism

The microorganism used in the experiment was the previously isolated magnetotactic bacteria [7]. The strain was preserved in slant agar containing elixir vitamin 1%-v, elixir mineral 1%-v, 5 mM KH_2PO_4, 25 μM Fe-quinate, 2 mg/L resazurin, 1 g/L fumaric acid, 0.2 g/L sodium acetate, 0.1 g/L $NaNO_3$, 50 mg/L sodium tioglicolate and 15 g/L agar at 30 °C, according to [4].

2.2 Magnetotactic Bacteria Cultivation

The magnetotactic bacteria were cultivated in an-1-L-shake flask fermentor at room temperature and in micro-aerophilic condition, which was maintained by flushing the culture by air and nitrogen mixed at every sampling time so that the partial pressure of the oxygen in the fermentation headspace was 3%. The medium used in the cultivation was the same medium as used for preservation, without agar. Three different carbon sources were used: sodium-lactate, sodium-pyruvate, and sodium-acetate, each at three different concentrations: 10, 25 and 50 mM. Two different iron sources were evaluated: Fe-quinate and Fe(III)-citrate, at 100 μM. Samples were taken every 12 hours during the 5-day cultivation of each run.

2.3 Analytical Methods

Each sample was analyzed for its cell concentration using a UV/Vis spectrophotometer at a wavelength of 560 nm. The cell concentration–absorbance calibration curve was obtained using gravimetry.

The actual concentration of the carbon sources (pyruvate, lactate, and acetate) was analyzed at the beginning and at the end of the experiment by high-performance liquid chromatography (HPLC) using an Aminex HPX-87H column at 60 °C, sulphuric acid as the eluent (eluent flow rate: 0.6 mL/min) and a refractive index detector. Prior to the analysis, 15 mL of sample was centrifuged to separate the cell biomass. The supernatant was then filtered to separate the remaining particulates.

The growth of the microorganisms was parameterized as the specific growth rate (μ) was calculated from the biomass concentration data (C_X) during the logarithmic phase, following Eq. (1).

$$\frac{dC_X}{dt} = \mu C_X \tag{1}$$

The biomass yield ($Y_{X/S}$) was calculated from the concentration of cell biomass (C_X) formed and the concentration of carbon source (C_S) used during the cultivation, following Eq. (2).

$$Y_{X/S} = \frac{C_{X,t} - C_{X,0}}{C_{S,0} - C_{S,t}} \tag{2}$$

The cellular iron content was analyzed using electron dispersive X-ray spectroscopy (EDS). The structure of the magnetotactic bacteria cells was analyzed using transmission electron microscopy (TEM) to confirm the

presence of produced nanomagnetic particles. TEM and EDS analysis were conducted at Eijkman Institute, Jakarta.

The yield of nanomagnetic particles ($Y_{P/S}$) was calculated from total nanomagnetic particles formed in the culture and initial concentration of iron source during cultivation, following Eq. (3).

$$Y_{P/S} = \frac{C_{X,t} \times f_{Fe}}{C_{Fe,0}} \quad (3)$$

f_{Fe} is the cellular iron content, while $C_{Fe,0}$ is the initial iron source concentration in the medium.

3 Results and Discussions

3.1 Effects of Carbon Sources on the Growth of Magnetotactic Bacteria

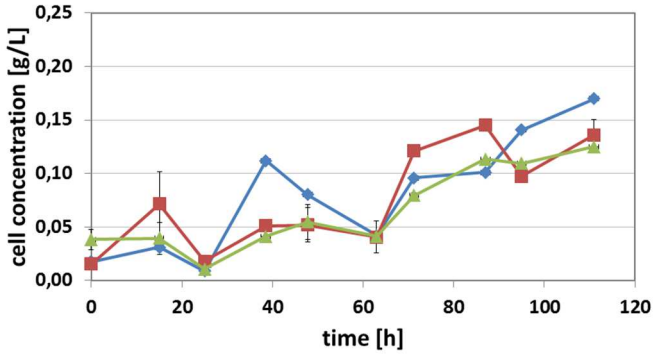
Three types of carbon source were evaluated in this research: sodium lactate, sodium pyruvate, and sodium acetate. The growth profiles of the magnetotactic bacteria on these substrates are presented in Figure 1.

The culture grew best on sodium pyruvate, regardless of the iron source used (Figures 1(c) and (e)). The highest cell concentration observed was 0.23 g dry weight/L, giving a biomass yield of 0.3 g dry weight biomass/g sodium pyruvate. At this condition, the specific growth rate was observed to be 0.03 h^{-1}. A similar trend is reported in Heyen [6]: sodium pyruvate gave the best cell growth when the type of carbon source for magnetotactic bacteria cultivation was varied.

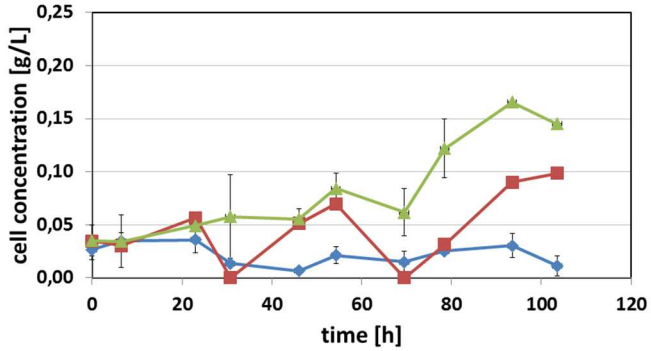
Pyruvate is an organic compound with three carbon atoms, which also happens to be one of the basic building blocks for the formation of important compounds for the growth of the bacteria. This may explain why cells grow best on pyruvate.

3.2 Effects of Carbon Source Concentration on the Growth of Magnetotactic Bacteria

The effects of carbon source concentration on magnetotactic bacterial growth were evaluated by varying the concentration of these substances in the medium at 10 mM, 25 mM, and 50 mM. The observed growth profiles of the magnetotactic bacteria are presented in Figure 1.



(a)



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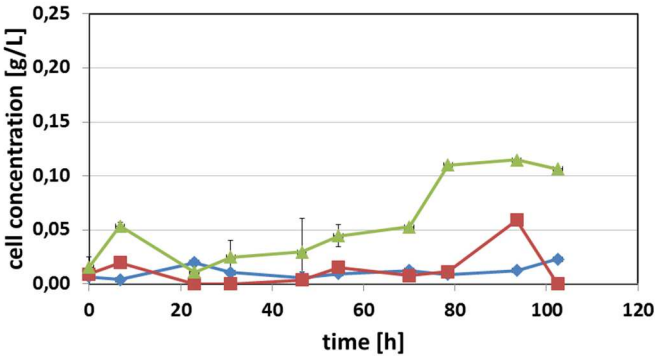
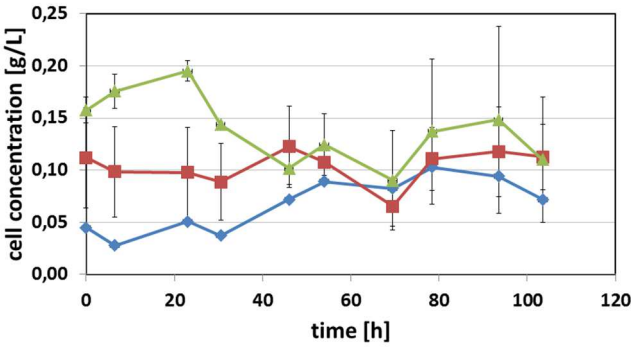
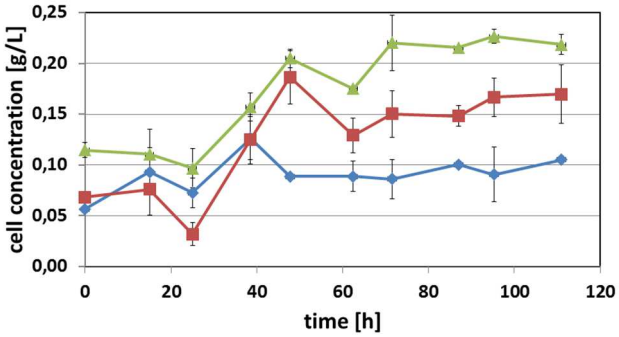


(c)

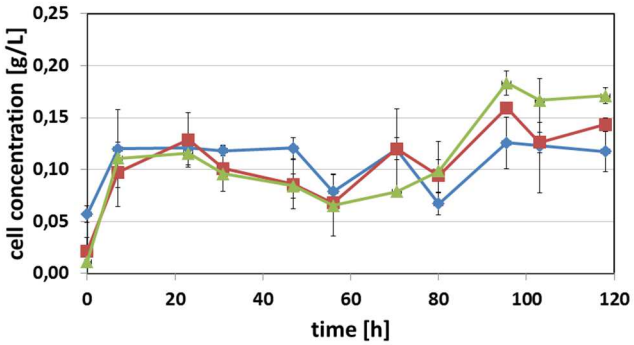
Figure 1 Growth of magnetotactic bacteria in different carbon and iron sources: (a) sodium lactate and Fe-quinate, (b) sodium lactate and Fe-citrate, (c) sodium acetate and Fe-quinate.



(d)



(e)



(f)

Figure 1 ***Continued***. Growth of magnetotactic bacteria growth in different carbon and iron sources: (d) sodium acetate and Fe-citrate, (e) sodium pyruvate and Fe-quinate, (f) sodium pyruvate and Fe-citrate, blue 10 mM, red 25 mM, green 50 mM).

Table 1 Biomass yield and intracellular iron particle yield in magnetotactic bacteria cultivation.

Carbon Source – Iron Source	Carbon Source Concentration [mM]	Maximum Biomass Concentration [g/L]	Biomass Yield [g-cell/ g- substrate]	Iron Concen-tration [%]*	Iron Yield [%]
Sodium	10	0.170	0.19		
lactate,	25	0.136	0.06	0.40%	9.7%
Ferri-quinate	50	0.125	0.03		
Sodium	10	0.030	0.01		
lactate,	25	0.098	0.04		
Ferri-citrate	50	0.122	0.03	0.20%	4.4%
Sodium	10	0.023	0.04		
acetate,	25	0.059	0.04		
Ferri-quinate	50	0.115	0.04	6.95%	143%
Sodium	10	0.103	0.12		
acetate,	25	0.118	0.07		
Ferri-citrate	50	0.148	0.04	1.56%	41.2%
Sodium	10	0.105	0.12		
pyruvate,	25	0.170	0.08		
Ferri-quinate	50	0.227	0.05	0.08%	3.2%
Sodium	10	0.126	0.13		
pyruvate,	25	0.159	0.06		
Ferri-citrate	50	0.183	0.04	0.09%	2.9%

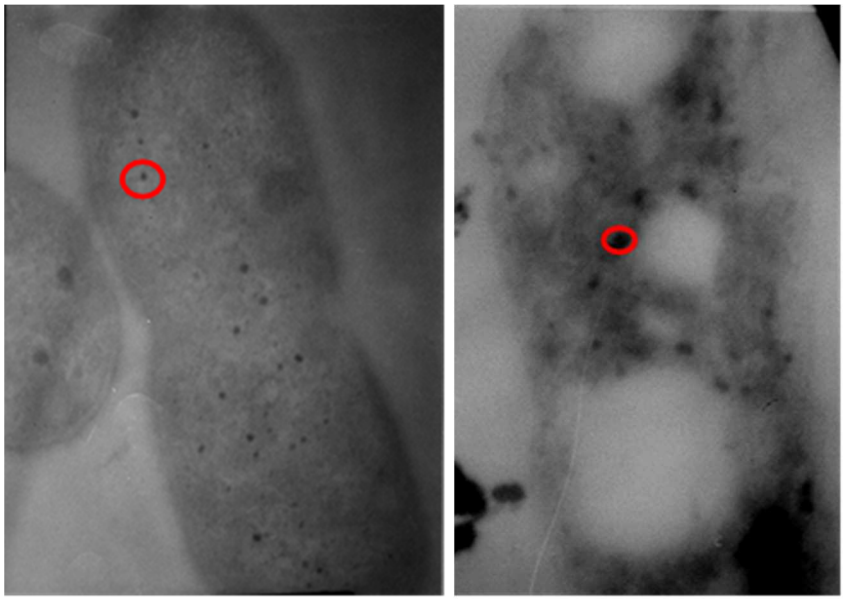
* In %-weight

A clear trend was observed in the bacterial growth on sodium-pyruvate and Fe-quinate (Figure 1(e)). A higher final cell concentration was observed at a higher substrate concentration (Table 1). At 10 mM sodium pyruvate, the final cell concentration was 0.10 g-DW/L, while at 25 mM and 50 mM, the final cell concentrations were 0.17 and 0.22 g-DW/L, respectively. On the other hand, a higher biomass yield was observed at a lower substrate concentration (Table 1).

At 10 mM sodium pyruvate, the biomass yield was calculated to be 0.12, while at 50 mM sodium pyruvate, the biomass yield was calculated to be 0.05. A similar trend was generally observed at different carbon and iron sources used. This indicates that at a higher carbon source concentration, the provided substrate was used less efficiently.

The results are not in agreement with Heyen [6], who reported that an increase in lactate concentration until 25 mM increased the maximum cell concentration, while a further increase showed no significant effects. No growth inhibition was observed in the present research.

3.3 The Presence of Nanomagnetic Particles



(a) (b)

Figure 2 TEM images of magnetotactic bacteria from the samples of (a) sodium acetate and Fe(III)citrate, (b) sodium lactate and Fe(III)quinate.

Under a light microscope the cultivated bacteria tended to move in the direction of the magnetic field. This observation confirmed that the cultivated strain was a magnetotactic bacterial strain. To confirm this further, we performed TEM analysis to check the internal condition of the cells and the presence of nanomagnetic particles.

Figure 2 shows images of magnetotactic bacteria taken by TEM. The black granules (circled in red) indicate nanomagnetic particles. The black granule shown in Figure 2(b) has a more irregular arrangement compared to the one in Figure 2(a). This may be caused by the sample's condition, which had been stored too long at freezing temperature, causing cell lysis (shown by the white holes). Analysis of more fresh samples would provide a better picture of the nanomagnetic particles.

3.4 Effects of Carbon and Iron Sources on the Yield of Nanomagnetic Particle

The overall results, under variation of type and concentration of carbon and iron sources, are presented in Table 1. Representative samples from each type of carbon source and each type of iron source were evaluated for the production of

nanomagnetic particles. Cells with the highest iron content – in other words, those with the highest accumulation of nanomagnetic particles – were the ones cultivated on sodium acetate and Fe(III)-quinate (Table 1). The cells with the lowest iron content were the ones cultivated on sodium pyruvate and Fe(III)-quinate.

It is interesting to see that the culture with the highest cell concentration, grown on sodium pyruvate and Fe(III)-quinate, had the lowest iron content. This may indicate that there was iron deficiency in the system, so that there was not enough iron to be accumulated intracellularly. However, the calculated yield presented in Table 1 shows otherwise. In the case of cells cultivated on sodium pyruvate and Fe(III)-quinate, less than 5% of the supplied iron was accumulated as nanomagnetic particles inside the cells. There was no iron shortage. There must be another reason why the iron could not be accumulated inside the cells, for example pH or even a particular interaction between the type of carbon and iron sources used, which needs further investigation.

Overall, the best yield of nanomagnetic particles was obtained from the cultivation using sodium acetate and Fe(III) quinate. In this case, iron recovery in the form of nanomagnetic particles was calculated to be 142%. In this experiment, the iron content was obtained from single measurement EDS. More samples should be taken from each batch of experiments and more EDS analyses should be performed to give a more representative number. The second best yield was obtained from the cultivation using sodium acetate and Fe(III) citrate, with iron recovery in the form of nanomagnetic particles at approximately 41.23%.

4 Conclusion

Our experimental results showed that the previously isolated magnetotactic bacteria were successfully cultivated in the lab to produce nanomagnetic particles. In order to optimally produce nanomagnetic particles, the media should be designed in such a way that the bacteria can grow well on it and that the bacteria can accumulate as many nanomagnetic particles as possible. In this case, three different carbon sources (sodium lactate, sodium pyruvate, and sodium acetate, each at three different concentrations) and two different iron sources (Fe-quinate and Fe-citrate) were evaluated. Our results indicate that the magnetotactic bacteria grew best on sodium pyruvate and Fe-quinate, and a higher carbon source concentration led to a higher maximal biomass concentration. On the other hand, the highest cell iron content, which reflects the highest nanomagnetic particle accumulation, was observed in the culture grown on sodium acetate and Fe-quinate. This condition also gave the highest yield of nanomagnetic particles from the iron source. Overall, our data show

that cell growth does not correlate with the production of nanomagnetic particles.

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